Effects of bakery by-products inclusion in the broiler’s diet on growth performance, carcass yield and gene expression profiling

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ASPA 25th Congress Book of Abstract

Pasquale De Palo

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Italian Journal of Animal Science

The *Italian Journal of Animal Science* is an international peer-reviewed open access journal publishing original scientific papers, reviews and short communications.
The journal serves as essential reading for animal scientists, technicians and all those who research animal production.
The journal encourages submissions of international relevance on the following subjects:

- Animal derived food quality and safety
- Animal genetics and breeding
- Aquaculture, poultry, companion and wild game animals
- Livestock systems, management and environment
- Non-ruminant or ruminant nutrition and feeding
- Production physiology and functional biology of farmed, companions and wild game animals.
- Animal behavior
- Animal welfare
- In vitro studies that have an application to farmed livestock

Manuscripts must address topics based on research at molecular, cellular, organ, whole animal and production system levels. Manuscripts discussing milk or meat analysis and compositions must show a direct link to either livestock production system, product quality, animal feeding/nutrition, animal genetics or breeding. Manuscripts describing laboratory animal models will be considered where the study highlights a potential benefit to farmed livestock.

Submissions discussing epidemiology, parasitology, infective diseases, food-borne diseases do not fit with the aims and scope of the journal.

Meeting reviews, book reviews and conference supplements are also published, as well as news and guidelines from the Animal Science and Production Association (ASPA). We welcome submissions from ASPA members and non-members alike.

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ASPA 25th Congress

Monopoli (BARI – ITALY), June 13–16, 2023

Guest Editors

**D'Alessandro Angela Gabriella, De Palo Pasquale, Maggiolino Aristide,
Mele Marcello**

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Monopoli (BARI – ITALY), June 13–16, 2023

#ASPA2023

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The 25th congress of the Animal Science and Production Association

"Animal Production Science: Innovations and sustainability for future generation" is under patronage of Loghi patrocini

Monopoli (BARI – ITALY),
June 13–16, 2023

Venue
Torre Cintola Natural Sea Emotions
Località Capitolo – Monopoli (BARI – ITALY)

The 25th Congress of the Animal Science and Production Association
“Animal Production Science: Innovations and Sustainability for Future Generation”

Under the auspices of

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SYNTHESY

The 25th Congress of the Animal Science and Production Association (ASPA) is hosted in Monopoli (Puglia) by the University of Bari.

The Congress is entitled "Animal Production Science: innovations and sustainability for future generations" and returns to Puglia after its second edition which was held in Bari 47 years ago.

The congress is hosted at the charming Torre Cintola resort in Monopoli (Bari) from the 13th to the 16th June 2023, a special location also for celebrating the 50th anniversary of our association.

This edition of the ASPA congress has received a total of 467 scientific contributions: 297 oral presentations and 170 posters have been selected. This is a very great result! The highest number of contributions of ever, according to our best knowledge. Moreover, 24 invited lectures will be presented.

The congress has implemented concrete actions for improving its sustainability, like the dematerialization of the posters (available through a smartphone app and on touch screen desks), the increasing of the use of public transportations, the donation of leftover food to charities, as well as the increased the use of zero-mile food. Moreover, the Congress opens a focus also on the role of women in Science, dedicating the congress rooms to neglected scientists poorly celebrated but fundamental for the progress of knowledge and societal development. Finally, a disseminated photo exhibition in the Congress rooms on Marginal Areas is a further opportunity for attendees to deepen the knowledge of Southern Italy landscapes and their relationships with livestock industry.

The scientific program is enriched by 23 main lectures, covering all the main topics. Many thanks for the job to the President of the Organizing Committee, Prof. Pasquale De Palo, and to all the components. I also wish to thank all the members of the Scientific Committee, starting from the President Prof. Angela Gabriella D'Alessandro.

I would like to congratulate and to thank all people that have contributed to the organization of the meeting and that have collaborated in reviewing the summaries. A special thanks to them for what they have done for ASPA and for the Italian Animal Science. A special thank also to Prof. Marcello Mele, Editor-in-Chief of the Italian Journal of Animal Science, for having contributed to the edition of the proceedings.

Nicolò Pietro Paolo Macciotta
ASPA President

ASPA 25th Congress

Monopoli (BARI – ITALY), June 13–16, 2023

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Program at a glance

Tuesday June 13th

	Room Daunia	Room Peucetia	Room Messapia	Room Apulia
	14:30-17:00 **Chicken biodiversity** *Session 01* Invited: **Cerolini Silvia** Chairs: **Iaffaldano N., Schiavone A.**	14:30-16:00 **Environmental footprint** *Session 02* Chairs: **Sevi A., Zucali M.E.**	14:30-16:30 **Advances in dietary feed supplementation** *Session 03* Chairs: **Calabrò S., De Palo P.**	/
17:00 – 17:30	*Coffee break*			
	/	/	/	**Opening Ceremony**
20:00	*Welcome cocktail*			

Opening Ceremony

Chairs: **Nicolò Pietro Paolo Macciotta, Giuseppe Pulina**

17:30	Institutional Addresses
18:00	Book launch and presentation "Carni e salumi: le nuove frontiere della sostenibilità" by Bernardi E., Capri E., Pulina G., Franco Angeli Ed. **Giuseppe Pulina** *President of "Carni sostenibili" Association and Full Professor of "Livestock farming ethics and sustainability" University of Sassari*
18:15	Policies and perspectives of European livestock industry: which role is played by the Animal Science Community? **Paolo De Castro** *Member of the EU Committee on Agriculture and Rural Development and Professor of Agricultural Economics*
18:45	National Policies and future perspectives for livestock and animal food supply chain in Italy: is there a role for Animal Science Community? **Giuseppe Blasi** *Head of Department of European and International Policies and Rural Development, Ministry of Agriculture, Food Sovereignty and Forestry*
19:15	ASPA and its first 50 years **Nicolò Pietro Paolo Macciotta** *ASPA President*
20:00	**Official Opening of the 25th ASPA Congress**

Wednesday June 14th

	Room Daunia	Room Peucetia	Room Messapia	Room Apulia
	08:15 - 11:00 **Environmental footprint** *Session 04* Invited: **Kuipers Abele** Chairs: **Bernabucci U., Sepe L.**	08:30 - 11:00 **Advances in dietary feed supplementation** *Session 05* Invited: **Salem Abdelfattah Z.** Chairs: **D'Alessandro A.G., Formigoni A.**	08:30 - 11:00 **Precision livestock farming: production efficiency in dairy cows** *Session 06* Invited: **Giordano Julio O.** Chairs: **Abeni F., Pugliese C.**	08:30 - 11:00 **Development of genomics in biodiversity** *Session 07* Invited: **Hanotte Olivier** Chairs: **Cecchinato A., Ciani E.**
11:00 – 11:30	*Coffee break*			
	11:30 - 13:30 **Environmental footprint** *Session 08* Chairs: **Claps S., Sandrucci A.**	11:30 - 13:30 **Sustainable feeding strategies in livestock systems** *Session 09* Chairs: **Bonanno A., Tufarelli V.**	11:30 - 13:30 **Animal welfare and health** *Session 10* Invited: **Chebel Ricardo C.** Chairs: **Trevisi E., Gottardo F.**	11:30 - 13:45 **Advances in meat quality** *Session 11* Invited: **Gagaoua Mohammed** Chairs: **Marino R., Mele M.**
13:30 – 14:30	*Lunch*			
	14:30 - 16:30 **Advances in rabbit and poultry products** *Session 12* Chairs: **Maiorano G., Trocino A.**	14:30 - 16:15 **Genomic tools for ruminant resiliency** *Session 13* Invited: **Miglior Filippo** Chairs: **Pilla F., Crepaldi P.**	14:30 - 16:30 **Heat stress and climate resilience in livestock** *Session 14* Invited: **Dahl Geoffrey E.** Chairs: **Braghieri A., Cozzi G.**	14:30 - 16:30 **Metagenomic approaches in animal science** *Session 15* Invited: **Lourenco Jeferson Menezes** Chairs: **Trevisi P., Castiglioni B.**
16:30 – 17:00	*Coffee break*			
	17:00 - 18:30 **New perspective in bees' production** *Session 16* Chairs: **Fontanesi L., Minozzi G.**	17:00 - 18:05 **Breeding for our future** *Session 17* Invited: **Cassandro Martino** Chairs: **Campanile G., Martelli G.**	17:00 - 19:30 **Advances in meat quality** *Session 18* Chairs: **De Marchi M., Serra A.**	17:00 - 18:30 **Metagenomic approaches in animal science** *Session 19* Chairs: **Buccioni A., Conte G.**
20:30	*Typical Dinner*			

Thursday June 15th

	Room Daunia	Room Peucetia	Room Messapia	Room Apulia
	08:30 - 10:30 **Animal welfare and health** *Session 20* Chairs: **Albenzio M., Tarantola M.**	08:15 - 11:00 **Sustainability and innovation in aquaculture** *Session 21* Chairs: **Centoducati G., Parisi G.**	08:15-11:15 **Advances in milk and dairy products quality** *Session 22* Chairs: **Di Trana A.C., Martini M.**	08:15 - 11:00 **New paradigms in animal breeding** *Session 23* Invited: **Lourenco Daniela** Chairs: **Ciampolini R., Landi V.**
11:00 – 11:30	*Coffee break*			
	11:30 - 13:15 **Advances in rabbit and poultry products** *Session 24* Invited: **Stadnicka Katarzyna** Chairs: **Bovera F., Soglia F.**	11:30 - 13:45 **L.E.O. project: the Italian research commitment for the future livestock strategies** *Session 25* Invited: **Burke Martin** Chairs: **Donda M., Negrini R.**	11:30 - 13:30 **Advances in milk and dairy products quality** *Session 26* Chairs: **Maggiolino A., Summer A.**	11:30 - 13:15 **Genomic tools for ruminant resiliency** *Session 27* zoetis Invited: **Di Croce Fernando** Chairs: **Lasagna E., Sacchi P.**
13:30 – 14:30	*Lunch*			
	14:30 - 16:30 **Recent advances in mammary gland biology and health** *Session 28* Chairs: **Caroprese M., Lacetera N.**	14:30 - 17:15 **Sustainable feeding strategies in livestock systems** *Session 29* MIGNINI&PETRINI ACADEMY Invited: **Pulina Giuseppe** Chairs: **Pinotti L., Masucci F.**	14:30 - 16:45 **Precision Livestock Farming: production efficiency and environmental impacts** *Session 30* Invited: **Rose Guilherme** **O'Brien Bernardette** Chairs: **Mattiello S., Neglia G.**	14:30 - 16:30 **Genomic tools for ruminant resiliency** *Session 31* Invited: **Lecchi Cristina** Chairs: **Bozzi R., Pasquini M.**
16:30 – 17:00	*Coffee break*			
17:00	/	/	/	**ASPA Assembly**
20:30	*Social Dinner*			

Friday June 16th

	Room Daunia	Room Peucetia	Room Messapia	Room Apulia
	08:30 - 10:30 **Animal welfare and health** *Session 32* Chairs: **Di Palo R., Santillo A.**	08:30 - 11:00 **Development of genomics in biodiversity** *Session 33* Invited: **Laloë Denis** Chairs: **Biffani S., Cipolat-Gotet C.**	08:15 - 11:00 **Companion animals** *Session 34* Invited: **Switonsky Marek** Chairs: **Cutrignelli M., Stefanon B.**	08:00 - 11:00 **Sustainable feeding strategies in livestock systems** *Session 35* Invited: **Atzori Alberto Stanislao** Chairs: **Di Francia A., Bailoni L.**
11:00 – 11:30	*Coffee break*			
	11:30 - 13:30 **Research and sustainability in horse production** *Session 36* Invited: **Vial Cèline** Chairs: **Salimei E., Capomaccio S.**	11:30 - 13:15 **Insects' production for animal feeding** *Session 37* Chairs: **Chiofalo B., Gasco L.**	11:30 - 13:30 **Advances in dietary feed supplementation** *Session 38* Chairs: **Trabalza Marinucci M., Nudda A.**	11:30 - 13:15 **Genomic tools for animal resiliency and susceptibility** *Session 39* Invited: **Vergani Andrea Mario** Chairs: **Bagnato A., Sartori C.**

ASPA 25th Congress

Monopoli (BARI – ITALY), June 13–16, 2023

Italian Journal of Animal Science
2023; volume 22, supplement S1

ORAL SESSIONS

Tuesday, June 13th – Room Daunia

Session 01 – Chicken biodiversity

Chairs: Iaffaldano Nicolaia – Schiavone Achille

14:30 *INVITED LECTURE*
Cerolini Silvia
Advancement in conservation programs of Italian poultry genetic resources for rural development

15:00 Marelli Stefano Paolo, Zaniboni Luisa, Madeddu Manuela, Mangiagalli Maria Grazia, Tognoli Cristina, Cerolini Silvia
Behavioural characterization of two local Italian chicken breeds **O369**

15:15 Cendron Filippo, Cassandro Martino, Penasa Mauro
Copy number variants in 23 Italian local chicken breeds **O481**

15:30 Di Iorio Michele, Iaffaldano Nicolaia, Rusco Giusy, Antenucci Emanuele, Madeddu Manuela, Zaniboni Luisa, Marelli Stefano Paolo, Cerolini Silvia
Phenotypic characterization of semen production and quality in Italian chicken and turkey breeds **O090**

15:45 Madeddu Manuela, Zaniboni Luisa, Marelli Stefano Paolo, Di Iorio Michele, Iaffaldano Nicolaia, Mangiagalli Maria Grazia, Cerolini Silvia
Variability in semen freezability within an Italian chicken breed **O115**

16:00 Cappone Eleonora, Soglia Dominga, Sartore Stefano, Zambotto Valeria, Maione Sandra, Profiti Margherita, Bongiorno Valentina, Gariglio Marta,
Cerolini Silvia, Schiavone Achille
Millefiori Piemontese, genetic characterization of an endangered local chicken breed **O203**

16:15 Piscitelli Raffaela, Zampiga Marco, Cartoni Mancinelli Alice, Brugaletta Giorgio, Castellini Cesare, Petracci Massimiliano, Sirri Federico
Comparison of productive traits and egg quality characteristics of two Italian chicken breeds and their crossbreeds reared in enriched-cage and free-range systems **O359**

16:30 Angelucci Elisa, Mattioli Simona, Cartoni Mancinelli Alice, Dal Bosco Alessandro, Ciarelli Claudia, Guarino Amato Monica, Failla Sebastiana, Castellini Cesare
Assessment of muscle metabolism in differently kinetic chicken genotypes using the Activity Index ... **O500**

16:45 Perini Francesco, Wu Zhou, Cartoni Mancinelli Alice, Mattioli Simona, Soglia Dominga, Schiavone Achille, Mugnai Cecilia, Castellini Cesare, Smith Jacqueline, Lasagna Emiliano
RNAseq reveals modulation of genes involved in fatty acid biosynthesis in chicken liver according to genetic background, sex and diet **O354**

17:00 Coffee Break

Tuesday, June 13th – Room Peucetia

Session 02 – Environmental Footprint

Chairs:	Sevi Agostino – Zucali Maddalena Enrica	
14:30	Romanzin Alberto, Braidot Matteo, Sarnataro Chiara, Fabro Carla, Spanghero Mauro *In vitro rumen methane production is not affected by the feeding efficiency of donor bulls*	**O160**
14:45	Primi Riccardo, Ripert Stefano, Viola Paolo, Rossi Carlo Maria, Danieli Pier Paolo, Ronchi Bruno *Trend in management scenarios of smallholder livestock farming systems affects ecosystem services: a landscape perspective from Campo di Segni (Lazio, Central Italy)*	**O126**
15:00	Marchesini Giorgio, Fossaluzza Davide, Andrighetto Igino *Discriminating between limiting and reducing factors in dairy farms through yield gap analysis*	**O522**
15:15	Xavier Vergé, Paul Robin, Nadège Edouard, Valentina Becciolini, Adam Cieslak, Malgorzata Szumacher, Lena Fehmer, Paul Galama, Violeta Juškiene, Gitana Kadziene, Diana Ruska *Development of a screening method for GHG and NH3 measurements – First results based on a European study*	**O460**
15:30	Vaiani Sergio *An agronomic-food strategy to improve the environmental impact in terms of water saving and food efficiency*	**O505**
15:45	Karatosidi Despoina, Michailidis Georgios, Avgeris Efthimios, Symeon Georgios, Tarricone Simona *Meat quality assessment of raw meat from two Mediterranean autochthonous pig breeds reared in sustainable conditions*	**O559**
16:00	Fatica Antonella, Giovanditti Anna Concetta, Fantuz Francesco, Salimei Elisabetta *Carbon footprint of intensive vs. extensive dairy farming in Gargano area*	**O243**
16:15	Finocchiaro Raffaella, Galluzzo Ferdinando, van Kaam Jan-Thijs, Cassandro Martino *Predicted methane emission a new breeding value for the Italian Holstein*	**O477**
17:00	*Coffee Break*	

Tuesday, June 13th – Room Messapia

Session 03 – Advances in dietary feed supplementation

Chairs:	Calabrò Serena – Trabalza Marinucci Massimo	
14:30	Frazzini Sara, Reggi Serena, Pavlovic Radmila, Canala Benedetta, Torresani Claudia Maria, Resca Rita, Rossi Luciana *Characterization of major algal species and their prebiotic potential*	**O355**
14:45	Negrini Clara, Luise Diana, Correa Federico, Amatucci Laura, Virdis Sara, Roméo Agathe, Manzke Naiana, Trevisi Paolo *Implication of authorized level of Zn provided from different sources on the performance and health of low and normal birth weight piglets post-weaning*	**O347**
15:00	Dell'Anno Matteo, Frazzini Sara, Ferri Irene, Scaglia Elena, Reggi Serena, Rossi Luciana *Dietary supplementation of Ascophyllum nodosum and Lithothamnium calcareum as functional feed additives for weaned piglets*	**O339**
15:15	Correa Federico, Luise Diana, Negrini Clara, Virdis Sara, Amatucci Laura, Dalcanale Simone, Olia Alessandro, Del Zozzo Francesca, Trevisi Paolo *Effect of the administration of two phytoextracts pre-mixes compared to the pharmacological dose of ZnO on the health, growth performance and faecal microbial profile of weaned pigs*	**O343**
15:30	Ferlisi Flavia, De Ciucis Chiara Grazia, Fruscione Floriana, Mecocci Samanta, Cappelli Katia, Razzuoli Elisabetta, Trabalza Marinucci Massimo *Antimicrobial and immunomodulatory evaluation of Olive Mill Wastewater Polyphenols for dietary supplementation in an in vitro swine intestinal model*	**O132**
15:45	Lanzoni Davide, Rebucci Raffaella, Cheli Federica, Baldi Antonella, Giromini Carlotta *Functional in vitro characterization of hemp seeds for nutritional applications*	**O537**
16:00	Sandrini Silvia, Perricone Vera, Cremonesi Paola, Castiglioni Bianca, Biscarini Filippo, Parra Titos Eva R., Vittorio Francesco, Verdile Nicole, Brevini Tiziana, Savoini Giovanni, Agazzi Alessandro *Effect of a combination of three yeasts on growth performance and gut health of weaning piglet*	**O097**

16:15 Bee Giuseppe
Effect of nutrient self-supply through choice feeding on growth performance, feeding behavior **O473**

17:00 *Coffee Break*

Wednesday, June 14th – Room Daunia

Session 04 – Environmental footprint

Sponsored by IGreen Innovation

Chairs: Bernabucci Umberto - Sepe Lucia

08:15 *INVITED LECTURE*
Kuipers Abele, Galama Paul
Innovative practices and techniques dealing with the environmental impact of livestock farms

08:45 Lunesu Mondina Francesca, Caratzu Maria Francesca, Nudda Anna, Battaccone Gianni, Pulina Giuseppe
Net carbon footprinig of Sardinia Beef Cow-Calf system **O496**

09:00 Gislon Giulia, Zucali Maddalena, Bava Luciana, Marusi Maurizio, Ferrari Valentina, Finocchiaro Raffaella, Cassandro Martino, Sandrucci Anna
Modelling GWP of milk production using technical efficiency indicators **O479**

09:15 Finocchi Matteo, Moretti Michele, Mantino Alberto, Ripamonti Alice, Mele Marcello
GIS model application for the assessment of nitrogen emissions from cattle **O141**

09:30 Grossi Giampiero, Vitali Andrea, Evangelista Chiara, Rossi Chiara, Cesarini Francesco, Basiricò Loredana, Turriziani Giovanni, Lacetera Nicola, Bernabucci Umberto
Implementation of water footprint assessment methodology on buffalo mozzarella cheese **O209**

09:45 Vitali Andrea, Grossi Giampiero, Rossi Chiara, Cesarini Francesco, Evangelista Chiara, Basiricò Loredana, Turriziani Giovanni, Lacetera Nicola, Bernabucci Umberto
Cradle-to-grave life cycle assessment of buffalo mozzarella cheese **O214**

10:00 Bragaglio Andrea, Romano Elio, Cutini Maurizio, Claps Salvatore, De Palo Pasquale
Sustainability of Life Cycle Assessment (LCA) for the estimation of donkey milk environmental impact: a case study **O585**

10:15 Caratzu Maria Francesca, Lunesu Mondina Francesca, Nudda Anna, Battacone Gianni, Correddu Fabio, Massidda Andrea, Gamberoni Alberto, Pulina Giuseppe
Suckling lamb as net-zero product in Sardinian dairy sheep farms **O527**

10:30 Correddu Fabio, Cadau Giordana, Carta Silvia, Cesarani Alberto, Congiu Michele, Macciotta Nicolò Pietro Paolo
Predicting enteric methane emission of dairy sheep using milk fatty acid profiles **O293**

10:45 Cavallini Damiano, Buonaiuto Giovanni, De Matos Vettori Julio, Fusaro Isa, Formigoni Andrea
Methane emission pattern in dairy cows evaluated in automatic milking system **O307**

11:00 *Coffee break*

Wednesday, June 14th – Room Peucetia

Session 05 – Advances in dietary feed supplementation

Sponsored by Prosol

Chairs: D'Alessandro Angela Gabriella - Formigoni Andrea

08:30 *INVITED LECTURE*
Salem Abdel Fattah Zeidan, Vazquez Mendoza Oscar Vicente, Elghandour Mona M.M.Y., De Palo Pasquale, Maggiolino Aristide
Feed additives in ruminants feeding: Update knowledge with focusing on natural additives

09:00 Greco Roberta, De Palo Pasquale, Maggiolino Aristide, Mulè Giuseppina, Ferrara Massimo
Effects of nucleotides and yeast supplementation on gut microbial population of calves **O587**

09:15 Sgarro Maria Federica, Vaiani Sergio, Palazzo Clemente, Di Marziantonio Pietro, Maggiolino Aristide, De Palo Pasquale
Effect of a diatomaceous-based dietary additive on milk production, antioxidant status and immune response during peripartum in buffaloes **O591**

09:30 Foggi Giulia, Rocchetti Gabriele, Amarie Roxana Elena, Campidonico Luca, Agnolucci Monica, Gallo Antonio, Tognocchi Monica, Silvi Alina, Buccioni Arianna, Mele Marcello
Can olive cake by-products be employed to produce silage with enhanced nutrition characteristics?........ **O148**

09:45 Salimei Elisabetta, Castoria Raffaello, Tremonte Patrizio, Lombardi Silvia Jane, Caturano Costantino, Ianiri Giuseppe, Palmieri Davide, Conte Thomas, Fatica Antonella
Ensiled tobacco cv. Solaris biomass: chemical, microbiological, phytopathological and nutritional characteristics........ **O264**

10:00 Fumo Valentina, Meli Giovanna, Scicutella Federica, Invernizzi Guido
Camelina sativa and Cynara cardunculus cakes supplementation on performance and milk composition in dairy goats during the transition period........ **O419**

10:15 Scaglia Elena, Reggi Serena, Ferri Irene, Frazzini Sara, Dell'Anno Matteo, Rossi Luciana
Effect of milk replacer supplemented with Ascophyllum nodosum on gut health of Holstein calves.... **O536**

10:30 Sfulcini Marta, Cattaneo Luca, Lopreiato Vincenzo, Berelli Marco, Cagnassi Enrica, Castellani Elena, Piccioli Fiorenzo, Trevisi Erminio, Minuti Andrea
Effect of yeast supplementation with Saccharomyces Cerevisiae fermentation products (SCFP) on performance and metabolic profile in suckling Holstein calves........ **O524**

10:45 Amato Annalisa, Oteri Marianna, Cavallo Carmelo, Bonacci Sonia, Chiofalo Vincenzo, Liotta Luigi
Evaluation of fatty acids profile of milk produced by mid-lactating Holstein cows fed with enriched olive cake diet........ **O151**

11:00 *Coffee break*

Wednesday, June 14th – Room Messapia

Session 06 – Precision Livestock Farming: production efficiency in dairy cows

Sponsored by MSD Animal Health

Chairs: Abeni Fabio – Pugliese Carolina

08:30 *INVITED LECTURE*
Giordano Julio O.
Improving dairy herd monitoring and management using automated monitoring technologies

09:00 Fiorbelli Erica, Atzori Alberto Stanislao, Tedeschi Luis, Cattellani Alessandro, Gallo Antonio
A dynamic mechanistic model to forecast the oscillatory feeding behaviour of lactating dairy cows... **O407**

09:15 Zucali Maddalena, Bianchi Maria Cecilia, Gislon Giulia, Bonizzi Serena, Sandrucci Anna
How much is the environmental benefit of using Precision Livestock Farming in Italian dairy farms?........ **O241**

09:30 Confessore Andrea, Aquilani Chiara, Fuchs Patricia, Probo Massimiliano, Pauler Caren M., Schneider Manuel, Argenti Giovanni, Pugliese Carolina
Does age affect learning capacity and grazing activities of Holstein cows managed with Virtual Fencing collars?........ **O313**

09:45 Abeni Fabio, Marino Rosanna, Petrera Francesca, Canevaro Greta, Richichi Mariantonietta, Carminati Domenico, Giraffa Giorgio, Barzaghi Stefania, Benedino Dario, Tidona Flavio
Effects of different automated feeding management on milk traits and rumination time of dairy cows during hot season........ **O418**

10:00 Giovinazzo Simone, Brambilla Massimo, Lazzari Andrea, Bisaglia Carlo
NIR spectrophotometric investigation to highlight early signs of ketosis in dairy cows........ **O189**

10:15 Celozzi Stefania, Calcante Aldo, Pavesi Martina, Mattiello Silvana, Mazzocchi Chiara, Corsi Stefano, Zannotti Michele, Zucali Maddalena, Battini Monica
From tie-stall to loose house with Automatic Milking Robot in dairy cow systems: economic, production and animal welfare implications........ **O530**

10:30 Ranzato Giovanna, Aernouts Ben, Lora Isabella, Ben Abdelkrim Ahmed, Adriaens Ines, Gote Martin Julius, Cozzi Giulio
Comparison of different methods to estimate dairy cows' production potential........ **O451**

10:45 Costa Angela, Cavallini Damiano, Mammi Ludovica, Visentin Giulio, Formigoni Andrea
Assessment and validation of individual water intake of dairy cows from reticular boluses........ **O274**

11.00 *Coffee break*

Wednesday, June 14th – Room Apulia

Session 07 – Development of genomics in biodiversity

Chairs: Cecchinato Alessio – Ciani Elena

08:30 *INVITED LECTURE*
Hanotte Olivier
The known and unknown African cattle genomes

09:00 Chessari Giorgio, Criscione Andrea, Crepaldi Paola, Marletta Donata, Portolano Baldassare, Biscarini Filippo, Mastrangelo Salvatore
Mapping of Heterozygosity-Rich Regions in Italian and Worldwide goat populations........ **O064**

09:15 Moscarelli Angelo, Chessari Giorgio, Tolone Marco, Criscione Andrea, Zumbo Alessandro, Rizzuto Ilaria, Riggio Silvia, Macaluso Vito, Portolano Baldassare, Sardina Maria Teresa, Mastrangelo Salvatore
Genomic tools for the characterization of the not officially recognized livestock populations: a case study in Mascaruna goat and Pecora Nera sheep from Sicily **O513**

09:30 Bionda Arianna, Cortellari Matteo, Lopreiato Vincenzo, Crepaldi Paola, Chiofalo Vincenzo, Liotta Luigi
Genomic characterization of the Comune di Sicilia goat, a local Sicilian genetic resource **O285**

09:45 Gomez Proto Guido, Mancin Enrico, Tuliozi Beniamino, Sartori Cristina, Mantovani Roberto
Genome-wide association analysis of productive traits in grey alpine cattle breed **O556**

10:00 Di Civita Marika, Senczuk Gabriele, Rillo Luigina, Macciocchi Alessandra, Occidente Mariaconsiglia, Saralli Giorgio, D'Onofrio Valentina, Galli Tiziana, Persichilli Christian, Di Giovannantonio Claudio, Pilla Fabio, Matassino Donato
Genomic profiling of several Lazio Indigenous goat breeds in the Italian context........ **O371**

10:15 Barbato Mario, De Nardo Floro, Bigi Daniele, Vajana Elia, Eufemi Elisa, Crepaldi Paola, Ajmone Marsan Paolo, Colli Licia, Orozco-terWengel Pablo, Bruford Michael W., Russo Isa M.
Assessing signatures of selection and climate adaptation in European and African livestock **O427**

10:30 Somenzi Elisa, Partel Erika, Barbato Mario, Chero Osorio Ana Marìa, Colli Licia, Franceschi Niccolò, Pilla Fabio, Komjanc Matteo, Achilli Alessandro, Hauffe Heidi Christine, Ajmone Marsan Paolo
Genetic diversity and runs of homozygosity in Rendena Cattle........ **O507**

10:45 Senczuk Gabriele, Di Civita Marika, Persichilli Christian, Destro Bisol Giovanni, Anagnostou Paolo, Pilla Fabio
On the road to domestication: a preliminary comparative genomics approach to reconstruct human and livestock correlates........ **O568**

11:00 *Coffee break*

Wednesday, June 14th – Room Daunia

Session 08 – Environmental Footprint

Chairs: Claps Salvatore – Sandrucci Anna

11:30 Martinic Oliver, Magrin Luisa, Dante Simone, Cozzi Giulio, Gottardo Flaviana
Product Environmental Footprint (PEF) as evaluation tool of environmental sustainability in beef cattle farms **O337**

11:45 Meo Zilio David, Steri Roberto, Iacurto Miriam, Cenci Francesco, La Mantia Maria Chiara, Chiariotti Antonella
Study of Methane Emission in the Mediterranean Buffalo Through the Use of Laser Methane Detector and its Correlation with Microbial Population from Different Matrices whitin the Agridigit Project........ **O361**

12:00 Mantino Alberto, Ripamonti Alice, Tranchina Margherita, Villani Ricardo, Finocchi Matteo, Mele Marcello
Agroforestry to support carbon-neutral dairy productions in Tuscany **O553**

12:15 Salimei Elisabetta, Colombo Claudio, Di Iorio Erika, Manzo Alessio, Miraglia Nicoletta, Fatica Antonella
A holistic characterization of pasture area of Centre-South Italy **O267**

12:30 Berton Marco, Schiavon Stefano, Gallo Luigi
Environmental footprint of heavy pig production system........ **O032**

12:45 Benedetti Del Rio Elena, Ramanzin Maurizio, Raniolo Salvatore, Sturaro Enrico
The value of sheep transhumance for rural landscape: a GPS tracking approach **O062**

13:00 Biagini Davide
Evaluate, drive and communicate the sustainability of complex systems: a proposal **O269**

13:15 Ghiaccio Francesca, Federiconi Alessia, Colleluori Riccardo, Canestrari Giorgia, Palmonari Alberto
Assessment of individual dairy cows in vitro rumen methane emissions **O306**

13:30 *Lunch*

Wednesday, June 14th – Room Peucetia

Session 09 – Sustainable feeding strategies in livestock system

Sponsored by Plantamura

Chairs: Bonanno Adriana - Tufarelli Vincenzo

11:30 Mazzoleni Sharon, Tretola Marco, Luciano Alice, Ottoboni Matteo, Manoni Michele, Bee Giuseppe, Pinotti Luciano
The use of sugary and salty food industry leftovers as alternative to cereal grains does not affect the growth performance in growing and finishing pigs **O107**

11:45 Virdis Sara, Correa Federico, Luise Diana, Negrini Clara, Amatucci Laura, Trevisi Paolo
Different formulations of benzoic acids as strategy to replace therapeutic dose of ZnO on health and performance of weaned pigs **O357**

12:00 Luise Diana, Correa Federico, Virdis Sara, Negrini Clara, Cestonaro Giulia, Nataloni Luigi, Titton Greta, Sattin Eleonora, Costanzo Enrico, Trevisi Paolo
Effect of different doses of camelina cake as soybean meal substitution of growth performance and gut health of weaned pigs **O353**

12:15 Evangelista Chiara, Basiricò Loredana, Galli Tiziana, Bruni Gianpaolo, Bernabucci Umberto
Relationship between feeding management and milk characteristics in dairy buffalo **O112**

12:30 Silvi Alina, Conte Giuseppe, Turini Luca, Foggi Giulia, Tognocchi Monica, Castagna Antonella, Mantino Alberto, Mele Marcello
Effect of increasing concentration of Salicornia on in vitro degradability of dietary dry matter and neutral detergent fibre **O060**

12:45 Kiatti Dieu donné, Vastolo Alessandro, Koura Bossima Ivan, Cutrignelli Monica Isabella, Vitaglione Paola, Calabrò Serena
Cashew (Anacardium occidentale L.) apple by-products: a promising feed in animal nutrition **O093**

13:00 Lambiase Claudia, Serrapica Francesco, Braghieri Ada, Riviezzi Amelia, Di Matteo Roberto, Masucci Felicia
The inclusion of hemp (Cannabis sativa L.) cake in laying hen diets influences yolk fatty acid profile and sensory quality during the shelf life of eggs **O154**

13:15 Fatica Antonella, Fantuz Francesco, Sacchetto Carlo, Ferro Antonio, Zullo Tullio, Salimei Elisabetta
Tobacco cv. Solaris seed cake in the diet of growing beef calves **O238**

13:30 *Lunch*

Wednesday, June 14th – Room Messapia

Session 10 – Animal Welfare and Health

Sponsored by A.R.A. Puglia

Chairs: Trevisi Erminio – Gottardo Flaviana

11:30 *INVITED LECTURE*
Chebel Ricardo C., Schuenemann Gustavo M., Shearer Jan
US Welfare Standards: Investing in Animal Welfare Pays Off

12:00 Cecchinato Alessio, Toledo-Alvarado Hugo, Macedo Mota Lucio Flavio, Giannuzzi Diana, Trevisi Erminio, Ajmone Marsan Paolo, Pegolo Sara, Bittante Giovanni
Milk infrared-derived predictions of blood metabolites as a tool to assess fertility in dairy cattle **O494**

12:15 Cattaneo Luca, Piccioli-Cappelli Fiorenzo, Lovotti Giorgia, Lopreiato Vincenzo, Trevisi Erminio, Minuti Andrea
Impact of decreased nutrient density at dry-off on inflammatory conditions in dairy cows.................. **O452**
12:30 Catellani Alessandro, Minuti Andrea, Trevisi Erminio, Gallo Antonio
Transition period for Brown Swiss and Holstein dairy cows: feeding behavior and metabolic status... **O521**
12:45 Fossaluzza Davide, Marchesini Giorgio
Management of dairy heifers: can operant conditioning decrease stress and ease animals' monitoring and manipulation?.. **O523**
13:00 Pulido-Rodriguez Lina Fernanda, Secci Giulia, Tignani Maria Vittoria, Medeiros Adja, Faccenda Filippo, Parisi Giuliana
Cold shock by immersion in ice salty water is a suitable method to stun Campione del Garda (Salmo carpio) both considering animal welfare and flesh quality during storage.............................. **O543**
13:15 Florit Eleonora, Romanzin Alberto, Spanghero Mauro
Could eating time be a useful indicator in dairy farm management?.. **O215**
13:30 Lunch

Wednesday, June 14th – Room Apulia

Session 11 – Advances in meat quality

Sponsored by Siciliani

Chairs: Marino Rosaria – Mele Marcello
11:30 *INVITED LECTURE*
Gagaoua Mohammed
Building better knowledge on meat quality determination through integrated data mining and curation of proteomics studies
12:00 Bordini Martina, Soglia Francesca, Zappaterra Martina, Davoli Roberta, Sirri Federico, Meluzzi Adele, Petracci Massimiliano
Evaluation of the expression level of genes coding for Collagen type 4 in Pectoralis major muscles belonging to meat-type chickens selected for different growth-rates **O345**
12:15 Forte Lucrezia, Calzaretti Giovanna, Landi Vincenzo, Aloia Alessandra, De Palo Pasquale, Maggiolino Aristide
Dry vs wet aging in the enhancement of culled goat meat quality.. **O586**
12:30 Aquilani Chiara, Confessore Andrea, Sirtori Francesco, Pugliese Carolina
Effect of immunocastration on quality traits of fresh and dry-cured loin in Italian heavy pigs production.. **O139**
12:45 Valenti Bernardo, Roscini Valentina, Bolletta Viviana, Fodaroni Chiara, Mercati Valentino, Morbidini Luciano, Pauselli Mariano
Monitoring the meat quality of grass-fed Angus beef during different seasons...................................... **O265**
13:00 Mattioli Simona, Cartoni Mancinelli Alice, Castellini Cesare, Angelucci Elisa, Dal Bosco Alessandro
Application of the Healthy Fatty Index to discriminate the meat nutritional quality of different slow-growing chickens.. **O026**
13:15 Castro Ndong Ncogo Nchama, Saccà Elena, Brunner Ilario, Sepulcri Angela, Corazzin Mirco, Piasentier Edi
Pork characteristics of Mangalitza pigs reared outdoors... **O538**
13:30 Piscopo Nadia, Di Paolo Marika, Casalino Loriana, Matera Roberta, Balestrieri Anna, Marrone Raffaele, Esposito Luigi
Physico-chemical and rheological differences of wild boar muscle in post-mortem time in Campania region: Preliminary study.. **O550**
13:45 Lunch

Wednesday, June 14th – Room Daunia

Session 12 – Advances in rabbit and poultry products

Chairs: Maiorano Giuseppe – Trocino Angela

14:30 Serra Valentina, Castrica Marta, Agradi Stella, Curone Giulio, De Bellis Roberta, Pastorelli Grazia
Antioxidant enzyme activity of rabbits fed dietary bovine colostrum supplementation **O312**

14:45 Pitino Rosario, Simoni Marica, Danese Tommaso, Mantovani Giorgia, Kyriakaki Panajota, Tsiplakou Eleni, Righi Federico
Use of natural antioxidants in poultry diet balanced for antioxidant capacity **O441**

15:00 Birolo Marco, Trocina Angela, Gratta Francesco, Zuffellato Andrea, Xiccato Gerolamo
Feed restriction strategies in growing rabbits fed post-weaning diets with different protein levels **O578**

15:15 Mannelli Federica, Daghio Matteo, Scicutella Federica, Nannucci Lapo, Falce Michele, Brajon Giovanni, Galigani Ilaria, Buccioni Arianna
Cardoon meal as an alternative sustainable ingredient in broiler feeding **O028**

15:30 Perricone Vera, Sandrini Silvia, Irshad Nida, Comi Marcello, Lecchi Cristina, Stella Simone, Tirloni Erica, Savoini Giovanni, Agazzi Alessandro
Effects of hydrolyzed yeast on growth performance, meat quality, and intestinal immune status of broiler chickens **O051**

15:45 Guerrini Alessandro, Zago Massimo, Tedesco Doriana E.A.
Effects of Citrus aurantium dulcis essential oil and Yucca schidigera saponins on broiler performance and health **O070**

16:00 Srikanthithasan Karthika, Gariglio Marta, Fiorilla Edoardo, Giorgino Andrea, Dellepiane Lucrezia, Diaz Vicuna Elena, Sola Dario, Bongiorno Valentina, Bergagna Stefania, Schiavone Achille, Raspa Federica, Profiti Margherita, Stoppani Nadia, Soglia Dominga, Forte Claudio
Effects of bakery by-products inclusion in the broiler's diet on growth performance, carcass yield and gene expression profiling **O029**

16:30 *Coffee break*

Wednesday, June 14th – Room Peucetia

Session 13 – Genomic tools for ruminant resiliency

Chairs: Pilla Fabio – Crepaldi Paola

14:30 *INVITED LECTURE*
Miglior Filippo, Baes Christine
Leveraging genomics to achieve dairy net zero

15:00 Cesarani Alberto, Lourenco Daniela, Degano Lorenzo, Vicario Daniele, Macciotta Nicolò Pietro Paolo
Different strategies for selecting core animals in the APY for single-step GBLUP **O076**

15:15 Giovannini Samira, Negro Alessio, Lasagna Emiliano, Giontella Andrea, Grande Silverio, Sarti Francesca Maria
The ability of udder linear morphological evaluation to predict milk production in Massese and Comisana sheep **O181**

15:30 Tiezzi Francesco, Biffani Stefano, Fabbri Maria Chiara, Crovetti Alessandro, Bozzi Riccardo
Genomic selection for stay-ability in Italian Limousine and Charolais **O185**

15:45 Pugliano Mariagiulia, Nowacka-Woszuk Joanna, Longobardi Valentina, Stachowiak Monika, Peretti Vincenzo, Szczerbal Izabela, Ciotola Francesca, Switonski Marek, Albarella Sara
MBL2 gene polymorphisms in Mediterranean Italian River Buffalo (Bubalus bubalis) in relation to milk production traits **O436**

16:00 Colli Licia, Correddu Fabio, Palazzo Mariantonietta, Somenzi Elisa, Eufemi Elisa, Cesarani Alberto, Zedda Marco, Macciotta Nicolò Pietro Paolo
An ancient DNA perspective on the genomic variation of present day Sardinian Cattle **O374**

16:30 *Coffee break*

Wednesday, June 14th – Room Messapia

Session 14 – Heat stress and climate resilience in livestock

Chairs: Braghieri Ada – Cozzi Giulio
14:30 *INVITED LECTURE*
Dahl Geoffrey E.
Late gestation heat stress programs reduced performance and survival phenotype in dairy cattle
15:00 Sirtori Francesco, Fabbri Maria Chiara, Parrini Silvia, Crovetti Alessandro, Messeri Alessandro, Mancini Marco, Bozzi Riccardo
Precision livestock farming to control changes in behavior and production in dairy cows based on variations in environmental THI **O528**
15:15 Laloë Denis, Biscarini Filippo, Mastrangelo Salvatore, Senczuk Gabriele, Persichilli Christian, Conte Giuseppe, Finocchiaro Raffaella, Van Kaam Jan Thijs, Benzoni Lorenzo, Ciampolini Roberta, Cassandro Martino
Integrative factorial methods to explore the relationships between genotypes, phenotypes and climate in Holstein cows **O470**
15:30 Cresci Roberta, Atamer Balkan Büsra, Tedeschi Luis Orlindo, Atzori Alberto Stanislao
Modeling heat flows in heat-stressed dairy cows using System Dynamics techniques **O394**
15:45 Magrin Luisa, Fabbri Giorgia, Prevedello Paola, Concheri Gianmaria, Gottardo Flaviana
Testing of a pilot system for the automatic monitoring of activity and body temperature of beef cattle **O335**
16:00 Martinez-Marin Gustavo, Bittante Giovanni, Toledo-Alvarado Hugo
Modelation of the effect of heat stress on milk yield and milk composition in purebred and crossbred cows **O542**
16:15 Carta Silvia, Correddu Fabio, Peana Ilaria, Fois Giuliano, Cesarani Alberto, Macciotta Nicolò Pietro Paolo
Effect of THI on milk composition and fatty acid profile of Sarda dairy sheep **O234**
16:30 *Coffee break*

Wednesday, June 14th – Room Apulia

Session 15 – Metagenomic approaches in animal science

Chairs: Trevisi Paolo – Castiglioni Bianca
14:30 *INVITED LECTURE*
Lourenco Jeferson Menezes
Unravelling the Complexity of Animal-Microbe Interactions
15:00 Maltecca Christian, Tiezzi Francesco
Microbial and genomic information synergistically contribute to predicting swine performance across production systems **O487**
15:15 Floridia Viviana, Giuffrè Letterio, D'Alessandro Enrico
Influence of genetic background and the effect of liquid whey supplementation on the faecal microbiota composition in Nero siciliano and commercial crossbreed pigs **O146**
15:30 Amarie Roxana Elena, Campidonico Luca, Tognocchi Monica, Tinagli Sara, Casarosa Laura, Correa Federico, Serra Andrea
A comparison of waxy versus regular maize in diets for post-weaning piglets: effect on growth performance, colon volatile fatty acids concentration, and gut microbiota **O506**
15:45 Toscano Alessandro, Giannuzzi Diana, Malgwi Isaac H., Deb Saptarathi, Squartini Andrea, Stevanato Piergiorgio, Schiavon Stefano
Characterization of dry-cured ham microbiome by 16S rRNA profiling during seasoning **O124**
16:00 Raspa Federica, Chessa Stefania, Moretti Riccardo, Ferrocino Ilario, Corvaglia Maria Rita, Cocolin Luca Simone, Bordin Clara, Bergero Domenico, Valle Emanuela
Effect of two feeding managements on the microbiota of different intestinal compartments of the horse digestive tract **O297**
16:15 Tiezzi Francesco, Maltecca Christian
Use of gut microbiota as a selection tool in swine **O579**
16:30 *Coffee break*

Wednesday, June 14th – Room Daunia

Session 16 – New perspective in bees' production

Chairs: Fontanesi Luca – Minozzi Giulietta

17:00 Lazzari Filippo, Cresta Eleonora, Carbonari Fausto, Kasiotis Konstantinos M., Shairra Souad A., Dipasquale Daniele, Manganello Federico, Danieli Pier Paolo
Honey quality and traceability: a preliminary investigation on Citrus honeys physio-chemical traits from three Mediterranean Countries **O205**

17:15 Danieli Pier Paolo, Lazzari Filippo, Cresta Eleonora, Carbonari Fausto, Petrocchi Jasinski Francesca, Manganello Federico
Pollen quality: a study on the elemental profile of pollen sampled in a suburban environment **O200**

17:30 Coppola Francesca, Sagona Simona, Tafi Elena, Casini Lucia, Piana Lucia, Dall'Olio Raffaele, Felicioli Antonio
Glucose oxidase and carboxypeptidase A-like activity as new potential freshness markers for Royal Jelly **O166**

17:45 Manganello Federico, Cresta Eleonora, Carbonari Fausto, Danieli Pier Paolo, Lazzari Filippo
Ex-hive trials on the efficacy of hop beta acids on the control of the honey bee parasite Varroa destructor **O134**

18:00 Coppola Francesca, Sagona Simona, Nanetti Antonio, Tafi Elena, Felicioli Antonio
Substitution of pollen source with commercial protein diet in honey bees nutrition: effects on survival rate and health **O165**

18:15 Taurisano Valeria, Ribani Anisa, Utzeri Valerio Joe, Sami Dalal, Johnson Kate, Fontanesi Luca
An over-time distribution map of Apis mellifera mitotypes in Italy constructed using an environmental DNA approach **O225**

Wednesday, June 14th – Room Peucetia

Session 17 – Breeding for our Future

Sponsored by FEDANA

Chairs: Campanile Giuseppe – Martelli Giovanna

17:00 *INVITED LECTURE*
Cassandro Martino
Rethinking Breeding Strategies in Livestock species

17:10 Benzoni Lorenzo, Tiezzi Francesco, Finocchiaro Raffaella, Galluzzo Ferdinando, Cassandro Martino
Genetic parameters of growth, feed efficiency and greenhouse gases emissions in Italian Holstein young bulls **O457**

17:15 Benzoni Lorenzo, Finocchiaro Raffaella, Niero Giovanni, Invernizzi Guido, Savoini Giovanni, Galluzzo Ferdinando, Cassandro Martino
Animal breeding sustainability: the Italian Holstein experience **O458**

17:20 Biffani Stefano, Goméz Mayra, Cimmino Roberta, Rossi Dario, Zullo Gianluigi, Negrini Riccardo, Cesarani Alberto
Experiences with a single-step genome evaluation in Italian Mediterranean Buffalo **O236**

17:25 Bertolini Francesca, Bovo Samuele, Schiavo Giuseppina, Ballan Mohamad, Cappelloni Manolo, Tinarelli Silvia, Gallo Maurizio, Fontanesi Luca
Signatures of selection in Italian heavy pigs: how genetic programs for PDO ham productions are shaping the genomes of cosmopolitan breeds **O255**

17:30 Schiavo Giuseppina, Bovo Samuele, Bertolini Francesca, Bolner Matteo, Ribani Anisa, Taurisano Valeria, Dall'Olio Stefania, Bonacini Massimo, Fontanesi Luca
Reconstructing pedigree information in the Reggiana cattle breed based on high density SNP data **O244**

17:40 Degano Lorenzo, Vicario Daniele, Romanzin Alberto, Cesarani Alberto
Selection for feed efficiency in Italian Simmental breed **O580**

17:45 Ablondi Michela, Asti Vittoria, Capomaccio Stefano, Sartori Cristina, Giontella **Andrea, Cappelli Katia, Mantovani Roberto, Silvestrelli Maurizio, Sabbioni Alberto**
Inbreeding and genetic diversity in Italian horse heritage **O379**

17:50 Ablondi Michela, Cipolat-Gotet Claudio, Stocco Giorgia, Crepaldi Paola, Cortellari Matteo,
Negro Alessio, Summer Andrea, Biffani Stefano
How to combine microsatellite and SNP for parentage verification in sheep? **O377**

17:55 Callegaro Simone, Biffani Stefano, Tiezzi Francesco, Fabbri Maria Chiara, Bozzi Riccardo
Impact of heat stress on growth of Italian Limousine and Charolais **O195**

18:00 Sbarra Fiorella, Quaglia Andrea, Bittante Giovanni, Mantovani Roberto
Genetics of udder volume and maternal ability in Italian beef cattle **O019**

Wednesday, June 14th – Room Messapia

Session 18 – Advances in meat quality

Chairs: De Marchi Massimo – Serra Andrea

17:00 Tavaniello Siria, Zejnelhoxha Sanije, Viegas Olgas, Pinto Edgar, Ferreira Isabel M.L.P.V.O,
Maiorano Giuseppe
*Effect of different doses of Vitamin E added to beef patties on the formation of Polycyclic
aromatic hydrocarbons* **O569**

17:15 Berardi Giovanna, Di Taranto Aurelia, Vita Valeria, Iammarino Marco
*Study of nitrite and nitrate residual levels in meat products after different types of
cooking treatment* **O082**

17:30 della Malva Antonella, Gagaoua Mohammed, Santillo Antonella, Priolo Alessandro, di Corcia Martina,
Marino Rosaria, Ciliberti Maria Giovanna, Caroprese Mariangela, Sevi Agostino, Albenzio Marzia
Plasma proteome, a non-invasive biofluid to monitor lamb meat quality **O116**

17:45 Soglia Francesca, Babini Elena, Mazzoni Maurizio, Petracci Massimiliano
*Collagen and extracellular matrix composition of chicken breast meat affected by growth-related
abnormalities* **O229**

18:00 Baldi Giulia, Zampiga Marco, Soglia Francesca, Gagliano Mara Antonia, Petracci Massimiliano,
Sirri Federico
*Microalgae as alternative protein source to soybean: effects on the main quality traits of
broiler breast meat* **O109**

18:15 Huerta Almudena, Trocino Angela, Pirrone Fabrizio, Bordignon Francesco, Xiccato Gerolamo,
Birolo Marco
Technological and sensorial meat quality of broiler chickens: effect of genotype and heat stress **O454**

18:30 Polidori Paolo, Cammertoni Natalina, Vincenzetti Silvia
Mule carcass characteristics and meat quality **O577**

18:45 Frongia Andrea, Acciaro Marco, Manca Carla, Pintone Andrea. Picconi Stefano, Sitzia Maria
Heavy lamb production as a way to differentiate dairy sheep livestock system **O583**

19:00 Nardelli Valeria, Ingegno Mariateresa, Della Rovere Ines, Chiappinelli Andrea, Casamassima Francesco,
Tomaiouolo Michele, Iammarino Marco
*Development of a highly sensitivity analytical method for the determination of
PAHs in baby food (meat puree) by optimized QuEChERS extraction and determination
by GC/MS-MS* **O272**

19:15 Iammarino Marco, Berardi Giovanna, Ferrara Alfredo, Di Taranto Aurelia
High levels of nitrate in fresh meats: Does nutrition play a role? **O081**

Wednesday, June 14th – Room Apulia

Session 19 – Metagenomic approaches in animal science

Chairs: Buccioni Arianna – Conte Giuseppe

17:00 Palmonari Alberto, Federiconi Alessia, Ghiaccio Francesca, Buonaiuto Giovanni, Cavallini Damiano
Impact of dietary lipid source on fecal microbiota composition in dairy cows **O263**

17:15 Giagnoni Lucia, Salza Sara, Melillo Rita, Piras Gabriella, Tondello Alessandra, Stevanato Piergiorgio,
Cecchinato Alessio, Squartini Andrea, Tedde Tiziana, Mudadu Alessandro Graziano, Spanu Carlo
*Study of microbial communities, pathogenic and emerging microorganisms in sheep's milk cheese
processing facilities of Sardinia using a DNA metabarcoding approach* **O085**

17:30 Ferrari Carolina, Evangelista Chiara, Basiricò Loredana, Luziatelli Francesca, Ruzzi Maurizio, Bernabucci Umberto
Effects of environmental factors on milk microbiome **O099**

17:45 Congiu Michele, Lourenco Jeferson, Cesarani Alberto, Macciotta Nicolò Pietro Paolo, Dimauro Corrado
Can we predict cattle future feed efficiency from faecal microbiome data collected at weaning? **O065**

18:00 Federiconi Alessia, Ghiaccio Francesca, Cavallini Damiano, Palmonari Alberto
Changes of rumen microbiota composition in dairy cows fed with different lipid source **O202**

Thursday June 15th – Room Daunia

Session 20 – Animal welfare and health

Chairs: Albenzio Marzia – Tarantola Martina

08:30 Di Vuolo Gabriele, Scali Federico, Romeo Claudia Rosa, Capelli Giovanna, Serrapica Maria, Ambra Chiara Denise, De Carlo Esterina, Alborali Giovanni Lorisi, Bertocchi Luigi, Vecchio Domenico
Linking Animal Welfare and Antibiotic Use in buffalo farms **O259**

08:45 Bianco Emanuele, Trabacchin Veronica, Arango Sheyla, Guzzo Nadia, Simonetti Emilio, Zerbinati Elena, Rainis Simona
Evaluation of fecal and urinary excretion and behavior of Italian Simmental dairy cows during the access to exercise pasture for 2 and 4 hours per day **O403**

09:00 Salzano Angela, Bifulco Giovanna, Piscopo Federica, Fioriniello Salvatore, Kosior Michael, Fiorino Ferdinando, Campanile Giuseppe
Influence of kisspeptin on buffalo superovulation **O484**

09:15 Ciliberti Maria Giovanna, Valasi Irene, Bouroutzika Efterpi, Makri Sotiria, Kouretas Demetrios, Albenzio Marzia, della Malva Antonella, di Corcia Martina, Santillo Antonella, Marino Rosaria, Sevi Agostino, Caroprese Mariangela
Melatonin administration in heat stressed ewes: redox and immunity status of their offspring **O576**

09:30 Mezzetti Matteo, Bencetti Fabio, Vercesi Alessandro, Minuti Andrea, Cattaneo Luca, Piccioli-Cappelli Fiorenzo
Effect of omitting dry period in Saanen and Alpine goats on metabolic profile and performance in the subsequent lactation **O540**

09:45 Ripamonti Alice, Mantino Alberto, Casarosa Laura, del Tongo Alessio, Silvi Alina, Goracci Jacopo, Turini Luca, Mele Marcello
Growth rate and hair cortisol variation of beef cattle in pastoral and silvopastoral systems **O074**

10:00 Ambra Chiara Denise, Lorenzi Valentina, Serrapica Maria, Di Vuolo Gabriele, Cappelli Giovanna, Fusi Francesca, Bertocchi Luigi, De Carlo Esterina, Romeo Claudia, Scali Federico, Vecchio Domenico
Linking Animal Welfare and Biosecurity, a case study in buffalo farms **O247**

10:15 Chiattelli Diletta, Birolo Marco, Cartoni Mancinelli Alice, Menchetti Laura, Castellini Cesare
Assessing the motivation of Leghorn hens to access outdoor space and pasture resources **O501**

11.00 *Coffee break*

Thursday June 15th – Room Peucetia

Session 21 – Sustainability and innovation in aquaculture

Chairs: Centoducati Gerardo – Parisi Giuliana

08:15 Rimoldi Simona, Di Rosa Ambra Rita, Terova Genciana, Oteri Marianna, Saroglia Marco, Hasan Imam, Chiofalo Biagina
Effects of insect meal-based diets on growth, intestinal well-being and microbiota in Gilthead seabream (Sparus aurata) **O010**

08:30 Prete Andrea, Sist Paola, Tramer Federica, Centoducati Gerardo, Maggiolino Aristide, Deflorio Michele, Passamonti Sabina
Stress assessment in juvenile seabass (Dicentrarchus labrax) during transport density simulation ... **O590**

08:45 Roncarati Alessandra, Galosi Livio, Arcangeli Giuseppe, Vetri Alessia, Tosi Federica, Gennari Lorenzo, Magi Gian Enrico
Growth performances in Cupped oysters (Crassostrea gigas) during prefattening stages in the Middle Adriatic Sea **O208**

09:00 Foglio Luciano, Proietti Lorenzo, Galli Andrea, Aidos Lucia, Di Giancamillo Alessia, Mirra Giorgio, Paolacci Simona, Maguire Julie, Markou Giorgos, Alarcón Francisco Javier, Parati Katia
A promising mix of protein sources coming from biorefinery as partial replacement of dietary ingredients in aquafeed for Gilthead Sea Bream (Spaurus aurata) broodstock **O593**

09:15 Bordignon Francesco, Fanizza Cecilia, Nicoletto Carlo, Maucieri Carmelo, Trocino Angela, Birolo Marco, Xiccato Gerolamo
Growth and quality of fish reared in haloponics **O404**

09:30 Fanizza Cecilia, Bordignon Francesco, Jover-Cerdá Miguel, Martìnez-Llorens Silvia, Sánchez-Peñaranda David, Trocino Angela, Tomás-Vidal Ana
Digestibility of raw materials candidate for sustainable feed formulation in Mediterranean yellowtail (Seriola dumerili) **O405**

09:45 Lira de Medeiros Adja Cristina, Faccenda Filippo, Pulido Rodriguez Lina Fernanda, Bruni Leonardo, Secci Giulia, Parisi Giuliana
Quality of fillet from trout (Oncorhynchus mykiss) fed diets supplemented with olive pomace at two different levels **O414**

10:00 Centoducati Gerardo, Deflorio Michele, Specchiulli Antonietta, Fanelli Giovanni, Fortunato Nicola, Mossa Michele, Ungaro Nicola, Carlucci Roberto, Albenzio Marzia, Grassi Giuseppe, Goffredo Elisa, Roma Rocco, Seccia Antonio, Petrosillo Irene
Towards sustainable marine spatial planning of aquaculture in Apulia Region **O493**

10:15 Daniso Enrico, Uboni Costanza, Cardinaletti Gloriana, Garlatti Nicolas, Tibaldi Emilio, Luzzana Umberto, Tulli Francesca
Finishing diets to modulate flesh fatty acid composition and skin colour in gilthead seabream (Sparus aurata) **O514**

10:30 Di Rosa Ambra Rita, Accetta Francesca, Armone Rosangela, Aliquò Doriana, Chiofalo Biagina
Effect of Hermetia illucens meal on gilthead seabream (Sparus aurata) flesh sensory profile. An innovative approach using electronic senses and data fusion **O071**

10:45 Rusco Giusy, Di Iorio Michele, Esposito Stefano, Antenucci Emanuele, Lerza Letizia, Roncarati Alessandra, Iaffaldano Nicolaia
The supportive breeding of native Mediterranean brown trout (LIFE Nat.Sal.Mo project, Molise region): the effect of ovarian fluid on frozen sperm motility parameters during artificial fertilization **O048**

11:00 *Coffee break*

Thursday June 15th – Room Messapia

Session 22 – Advances in milk and dairy products quality

Sponsored by Consorzio per la Tutela del Formaggio Mozzarella di Bufala Campana D.O.P

Chairs: Di Trana Adriana Carmen – Martini Mina

08:15 Sepe Lucia, Lovallo Carmela, De Martino Laura, De Feo Vincenzo
Cosmetics based on goat milk whey enriched by essential oils from Mediterranean bushes **O509**

08:30 Salari Federica, Altomonte Iolanda, Sodi Irene, Martini Mina
Fatty acids and sterols composition of three commercial cow's milks **O021**

08:45 Cappelli Giovanna, Di Vuolo Gabriele, Vecchio Domenico, Grassi Carlo, Bufano Francesca, Noschese Rosario, Capacchione Roberta, De Carlo Esterina
Buffalo milk in Italy and the traceability system **O212**

09:00 Amalfitano Nicolò, Secchi Giorgia, Tagliapietra Franco, Lante Anna, Dettori Maria Luisa, Pazzola Michele, Vacca Giuseppe Massimo, Bittante Giovanni
Influence of single protein fractions on goat milk coagulation ability **O260**

09:15 Franceschi Piero, Malacarne Massimo, Cipolat-Gotet Claudio, Evangelista Chiara, Basiricò Loredana, Bernabucci Umberto, Summer Andrea
Distribution of Zn, Fe and Cu and their relationships with milk quality in individual cow milk samples **O310**

09:30 Malacarne Massimo, Franceschi Piero, Cipolat-Gotet Claudio, Evangelista Chiara, Basiricò Loredana, Bernabucci Umberto, Summer Andrea
Influence of content and distribution of Ca, P and Mg on rennet coagulation properties in individual cow milk samples **O315**

09:45 Tognocchi Monica, Giannerini Fabiola, Villani Fabio, Silvi Alina, Macchi Mirco, Guidi Ilaria, Durani Cinzia, Serra Andrea, Mele Marcello, Conte Giuseppe
Effect of bactofugation on milk and Pecorino cheese quality **O057**

10:00 Ponte Marialetizia, Maniaci Giuseppe, Pipi Marianna, Gannuscio Riccardo, Busetta Gabriele, Gaglio Raimondo, Settanni Luca, Alabiso Marco, Todaro Massimo, Bonanno Adriana, Di Grigoli Antonino
Dehydration preserves the ability of tanniferous sulla forage (Sulla coronaria (L.) Medik.) to improve nutritional, microbial and sensory traits of sheep cheese **O551**

10:15 Nudda Anna, Guiso Maria Francesca, Sanna Gavino, Deroma Mario, Tsiplakou Eleni
Essential and Toxic Mineral Content of Colostrum and Milk in Dairy Sheep **O548**

10:30 Di Paolo Marika, Casalino Loriana, Mazzocca Roberta, Bettua Giuseppe, D'Anza Emanuele, Peretti Vincenzo, Ciotola Francesca, Marrone Raffaele
Effect of ripening time on chemical-nutritional quality of the traditional Pecorino Bagnolese cheese **O552**

10:45 Niero Giovanni, Penasa Mauro, De Marchi Massimo
Quantification and variation of lactoferrin in cow milk assessed through radial immunodiffusion technique **O033**

11:00 Varricchio Marialuisa, Barone Carmela Maria Assunta, Scalera Grazia, Grasso Fernando, Sarubbi Fiorella, Pacelli Corrado, Di Francia Antonio
Does the use of ensiled forage affect the sensory properties of fresh cheese? **O159**

11:15 *Coffee break*

Thursday June 15th – Room Apulia

Session 23 – New paradigms in animal breeding

Chairs: Ciampolini Roberta - Landi Vincenzo

08:15 *INVITED LECTURE*
Lourenco Daniela
Are there benefits in using sequence data for genomic predictions?

08:45 Cimmino Roberta, Goméz Carpio Mayra, Rossi Dario, Gombia Yuri, Zullo Gianluigi, Biffani Stefano
Genetic aspects of longevity in Italian Mediterranean Buffalo **O252**

09:00 Carta Antonello, Usai Mario Graziano, Casu Sara, Salaris Sotero
Implications on Heritability Estimates of Blending the G Matrix with Different Levels of the Pedigree Matrix in a Nucleus Flock of Dairy Sheep **O217**

09:15 Punturiero Chiara, Bernini Francesca, Milanesi Raffaella, Strillacci Maria Giuseppina, Bagnato Alessandro
The Use of Genomics in Dairy Cow Farming – results from the GENORIP project **O411**

09:30 Negro Alessio, Biffani Stefano, Cortellari Matteo, Bionda Arianna, Cesarani Alberto, Fresi Pancrazio, Macciotta Nicolò Pietro Paolo, Grande Silverio, Crepaldi Paola
Estimation of Breeding Values in Italian dairy goats: from BLUP to ssGBLUP **O334**

09:45 Altieri Damiano, Biffani Stefano, Goméz Carpio Mayra, Carnovale Francesca, Rossi Dario, Gombia Yuri, Di Palo Rossella
Preliminary investigation on stayability in Italian Mediterranean Buffalo **O571**

10:00 Callegaro Simone, Tiezzi Francesco, Fabbri Maria Chiara, Bozzi Riccardo
Different methods to handle preferential treatment in slaughter age in Italian Limousine **O180**

10:15 Macciotta Nicolò Pietro Paolo, Degano Lorenzo, Vicario Daniele, Cesarani Alberto
A transgenerational study on the effect of birth month of female ancestor on lactation curves of Italian Simmental cattle **O584**

10:30 Persichilli Christian, Karaman Emre, Finocchiaro Raffaella, Pilla Fabio, Fabris Anna, Visentin Giulio, Cassandro Martino
Study of fertility traits in Italian Jersey Cattle **O563**

10:45 Sartori Cristina, Tuliozi Beniamino, Mancin Enrico, Gomez-Proto Guido, Mantovani Roberto
How mum and pen mates affect my growth: maternal effects and indirect genetic components on growth traits in young bulls at performance testing station **O399**
11:00 Coffee break

Thursday June 15th – Room Daunia

Session 24 – Advances in rabbit and poultry products

Chairs: Bovera Fulvia – Soglia Dominga
11:30 *INVITED LECTURE*
Stadnicka Katarzyna
Technologies applied in embryonated eggs
12:00 Peng Meng, Açar Samil, Biesek Jakub, Banaszak Miroslaw, Tavaniello Siria, Maiorano Giuseppe
Influence of rearing system and hen age on fatty acid composition of egg yolk **O574**
12:15 Trabalza-Marinucci Massimo, Ranucci David, Galarini Roberta, Branciari Raffaella, Acuti Gabriele
Egg production and egg quality in free-range laying hens as affected by dietary polyphenols from olive mill wastewaters **O397**
12:30 Biasato Ilaria, Bellezza Oddon Sara, Cerutti Gian Luca, Renna Manuela, Xiccato Gerolamo, Trocino Angela, Perano Danilo, Caimi Christian, Brugiapaglia Alberto, Gasco Laura
Mulberry leaf meal as alternative feed ingredient in rabbit nutrition: preliminary results about productive performance and meat quality **O121**
12:45 D'Amico Francesco, Casalino Gaia, Schiavitto Michele, Dimuccio Michele Maria, Camarda Antonio, Romito Diana, Circella Elena
In vitro efficacy of Phyto-L against Entero-Pathogenic Escherichia coli strains of rabbits from industrial farms **O581**
13:30 Lunch

Thursday June 15th – Room Peucetia

Session 25 – L.E.O. PROJECT: the Italian research commitment for the future wild stock strategies

Sponsored by A.I.A.

Chairs: Donda Mauro – Negrini Riccardo
11:30 *INVITED LECTURE*
Burke Martin, Roalkvam Tone, van der Linde Rene
ICAR Harmonisation of Sustainability related Milk Traits
12:00 Milanesi Marco, Pietrucci Daniele, Vignali Giovanni, Chandramouli Balasubramanian, Mancini Marco, Renzi Francesco, Valentini Riccardo, Santini Monia, Negrini Riccardo, Chillemi Giovanni
Contrasting climate change effects on dairy cattle through Machine Learning approach **O300**
12:15 Pascarella Lorenzo, Fioretti Mauro, Melilli Caterina, Biffani Stefano, Negrini Riccardo
Results from Italian National Breeders Association (AIA) nationwide collection of setting and efficiency parameters in milking systems of Italian dairy farms **O113**
12:30 Luisi Federica, Fioretti Mario, Moliso Mesfin Mekonnen, Tondo Alessia, Negrini Riccardo
Estimating enteric methane emission in dairy cows exploiting longitudinal data measured on single animal and at farm level to refine IPCC equations **O143**
12:45 Tolone Marco, Mastrangelo Salvatore, Sadina Maria Teresa, Scatassa Maria Luisa, Rizzuto Ilaria, Riggio Silvia, Moscarelli Angelo, Portolano Baldassare
Differential somatic cell count as indicator of intramammary infection in dairy sheep **O072**
13:00 Riggio Silvia, Di Gerlando Rosalia, Tolone Marco, Rizzuto Ilaria, Moscarelli Angelo, Portolano Baldassare, Mastrangelo Salvatore, Sardina Maria Teresa
*Maedi Visna virus infection and TMEM***154** *genotypes in Valle del Belice sheep breed* **O510**

13:15 Morelli Daniela, Di Domenico Marco, Marcacci Maurilia, Cammà Cesare, Averaimo Daniela, Vincifori Giacomo, Iannetti Simona, Ricchiuti Luciano, Radomsky Nicolas, Costarelli Silva, Pezzotti Giovanni
Illumina Ampliseq approach for multi-pathogen detection **O588**

13:30 Pascarella Lorenzo, De Renzis Maurizio, Melilli Caterina, Fioretti Mauro, Luisi Federica, Negrini Riccardo
LEO livestock national bio-repository: a tool for the conservation of Italian livestock genetic diversity **O589**

13:45 *Lunch*

Thursday June 15th – Room Messapia

Session 26 – Advances in milk and dairy products quality

Chairs: Maggiolino Aristide – Summer Andrea

11:30 Lopez Annalaura, Moretti Vittorio Maria, Ratti Sabrina, Rossi Raffaella, Bellagamba Federica
A comparison of the nutritional quality and sensory properties of commercial milk with different labeled production systems **O191**

11:45 Cipolat-Gotet Claudio, Summer Andrea, Ceresa Paolo, Molle Arnaud Paul J., Mariani Elena, Stocco Giorgia
Fourier transform infrared spectroscopy of milk as a tool for monitoring efficiency in the Parmigiano Reggiano PDO production **O273**

12:00 Stocco Giorgia, Mariani Elena, Ablondi Michela, Biffani Stefano, Grande Silverio, Summer Andrea, Biagini Domenico, Cipolat-Gotet Claudio
The role of Comisana and Massese breeds on cheese-making efficiency and daily cheese production **O299**

12:15 Rocchetti Gabriele, Ghilardelli Francesca, Carboni Eliana, Atzori Alberto Stanislao, Masoero Francesco, Gallo Antonio
Milk metabolome reveals pyrimidine and its degradation products as the discriminant markers of different corn silage-based nutritional strategies **O009**

12:30 Natrella Giuseppe, Maggiolino Aristide, De Palo Pasquale, Pedota Giuseppina, Faccia Michele
Survey on milk quality at late lactation and presence of ancient Zebu whey protein marker in Podolic cattle of Basilicata region **O483**

12:45 Natrella Giuseppe, Gambacorta Giuseppe, Faccia Michele
Effect of aS1 casein level on stretchability of goat curd **O485**

13:00 Giannuzzi Diana, Vanzin Alice, Toscano Alessandro, Bisutti Vittoria, Pegolo Sara, Cecchinato Alessio
Associations between the detailed milk mineral profile and total and differential cell count in Holstein Friesian dairy cows **O488**

13:15 Capitanio Fabian, Cascone Ilaria, Pugliano Mariagiulia, Luongo Flavio, D'Anza Emanuele, Rossetti Alberto, Albarella Sara, Ciotola Francesca, Peretti Vincenzo
Livestock biodiversity and endogenous area development: The POD "Provolone del Monaco" model **O447**

13:30 *Lunch*

Thursday June 15th – Room Apulia

Session 27 – Genomic tools for ruminant resiliency

Sponsored by Zoetis

Chairs: Lasagna Emiliano – Sacchi Paola

11:30 *INVITED LECTURE*
Di Croce Fernando, Fessenden Brenda, Layfield Michael, Weigel Daniel, Pisoni Giuliano, Wijma Robert
Precision Ag comes to dairy: Genomics tools to improve profit, environmental footprint, and cow wellbeing

12:00 Mancin Enrico, Sartori Cristina, Gomez Guido, Mantovani Roberto
Environmental variation of milk production: an effective indicator of genetic and genomic animal resilience **O409**
12:15 Ferrari Valentina, Visentin Giulio, van Kaam Jan-Thijs, Penasa Mauro, Marusi Maurizio, Finocchiaro Raffaella, Cassandro Martino
Genetic aspects of heifer fertility in Italian Holstein population **O036**
12:30 Fabris Anna, Tiezzi Francesco, Finocchiaro Raffaella, Marusi Maurizio, Cassandro Martino
Preliminary genetic analysis for survival in Italian Jersey **O037**
12:45 Buonaiuto Giovanni, Lopez-Villalobos Nicolas, Costa Angela, Formigoni Andrea, Visentin Giulio
Effect of morphological characteristics and productive traits on the survival of dual-purpose Simmental cows **O041**
13:00 Pegolo Sara, Giannuzzi Diana, Bisutti Vittoria, Ramirez Mauricio Marco Aurelio, Macedo Mota Lucio Flavio, Ajmone Marsan Paolo, Trevisi Erminio, Cecchinato Alessio
Genetic parameters for blood indicators of metabolic distress and milk traits in dairy cattle **O066**
13:30 Lunch

Thursday June 15th – Room Daunia

Session 28 – Recent advances in mammary gland biology and health

Chairs: Caroprese Mariangela – Lacetera Nicola
14:30 Zappaterra Martina, Zanna Martina, Nanni Costa Leonardo, Padalino Barbara
Application of infrared thermography for detection of udder health disorders in dairy cattle **O015**
14:45 Bobbo Tania, Matera Roberta, Neglia Gianluca, Gomez Mayra, Cimmino Roberta, Manunza Arianna, Biffani Stefano
What do we know about differential somatic cell count in buffaloes? **O329**
15:00 Turini Luca, Bonelli Francesca, Senatore Elena, Roncoroni Cristina, Sgorbini Micaela, Silvi Alina, Conte Giuseppe, Mele Marcello
Relationships between on-farm animal welfare assessment, milk yield and quality in dairy cows **O069**
15:15 Vanzin Alice, Battisti Ilaria, Franchin Cinzia, Bisutti Vittoria, Toscano Alessandro, Giannuzzi Diana, Masi Antonio, Arrigoni Giorgio, Gallo Luigi, Cecchinato Alessio, Pegolo Sara
Mass spectrometry-based characterization of the bovine milk peptidome upon Streptococcus agalactiae and Prototheca spp. infection **O123**
15:30 Bisutti Vittoria, Mach Nuria, Giannuzzi Diana, Vanzin Alice, Capra Emanuele, Cecchinato Alessio, Pegolo Sara
Transcriptomic and phenomic data integration to identify markers of subclinical mastitis in Holstein cattle **O125**
15:45 Mondini Sara, Tamburini Alberto, Palladini Nicola Maria, Bava Luciana
Differential somatic cell count during lactation in dairy cattle herds from northern Italy **O482**
16:00 Matera Roberta, Conte Giuseppe, Cotticelli Alessio, Pascarella Lorenzo, Tondo Alessia, De Luise Luca, Campanile Giuseppe
Milking parameters related to mammary gland health and milk characteristics in Italian Mediterranean buffalo **O431**
16:15 Mecocci Samanta, Pietrucci Daniele, Milanesi Marco, Capomaccio Stefano, Evangelista Chiara, Basiricò Loredana, Bernabucci Umberto, Chillemi Giovanni, Cappelli Katia
Transcriptomic characterization of water buffalo's Extracellular Vesicles from colostrum and milk for their Immunomodulatory Potential **O130**
17:00 Coffee break

Thursday June 15th – Room Peucetia

Session 29 – Sustainable feeding strategies in livestock system

Sponsored by Mignini & Petrini

Chairs:	Pinotti Luciano – Masucci Felicia	
14:30	*INVITED LECTURE* Pulina Giuseppe, Correddu Fabio, Caratzu Maria Francesca, Lunesu Mondina Francesca *Livestock sustainability, the four slices of the same pie*	
15:00	Oteri Marianna, Scordia Danilo, Armone Rosangela, Costale Annalisa, Meineri Giorgia, Gresta Fabio, Chiofalo Biagina *A sustainable alternative grain legume for livestock: agronomic and nutritional traits of three lupin (Lupinus spp.) species*	**O030**
15:15	Braidot Matteo, Sarnataro Chiara, Fabro Carla, Spanghero Mauro *In vitro evaluation of sodium nitrate as a rumen methane reducer*	**O358**
15:30	Battelli Marco, Colombini Stefania, Crovetto Gianni Matteo, Galassi Gianluca, Manfredi Maria Teresa, Rapetti Luca *Quebracho condensed tannins fed to lactating goats: effect on methane production*	**O325**
15:45	Vastolo Alessandro, Formato Marialuisa, Piccolella Simona, Calabrò Serena, Pacifico Severina, Cutrignelli Monica Isabella *Evaluation of the effect of leaf extracts from deciduous trees (Castanea sativa, Fagus sylvatica and Quercus robur) on ruminal fermentation*	**O178**
16:00	Scicutella Federica, Mannelli Federica, Daghio Matteo, Azim Khalid, Toni Elisabetta, Viti Carlo, Buccioni Arianna *Mediterranean agro-industrial by-products as ingredients in a sustainable feeding strategy for dairy sheep: an in in vitro trial to evaluate rumen ecosystem*	**O489**
16:15	Piccioli-Cappelli Fiorenzo, Moschini Maurizio, Froldi Federico, Bani Paolo, Müller Isabel, Tamassia Luis, Bulgarelli Paolo, Trevisi Erminio *Effects of 3-nitrooxypropanol (3-NOP, Bovaer®10) on methane emission and productive performance of mid-lactating dairy cows fed a typical diet for Grana Padano cheese-making*	**O491**
16:30	Massaro Selene, Andersen Jonas Bylov Hedegaard, Franciosi Elena, Tagliapietra Franco *Alpine herbs affect rumen in vitro degradability and methane emissions*	**O079**
16:45	Caturano Costantino, Tremonte Patrizio, Lombardi Silvia Jane, Fatica Antonella, Sorrentino Elena, Coppola Raffaele, Salimei Elisabetta *Use of lactic acid bacteria in forage production of Alto Sannio area*	**O245**
17:00	*Coffee break*	

Thursday June 15th – Room Messapia

Session 30 – Precision livestock farming: production efficiency and environmental impacts

Chairs:	Mattiello Silvana - Neglia Gianluca	
14:30	*INVITED LECTURE* Rosa Guilherme, Hernandez Laura, Dorea Joao R. R. *Digital technologies and Machine learning: A new way to look at novel traits at spatial and temporal dimensions*	
15:00	Casu Sara, Usai M. Graziano, Salaris Sotero, Caredda Marco, Addis Margherita, Carta Antonello *Phenotypic Analysis of Ovine Milk Infrared Spectra*	**O193**
15:15	Cartoni Mancinelli Alice, Chiattelli Diletta, Menchetti Laura, Bernacchia Gianmaria, Castellini Cesare *Preliminary validation of Ultra-Wide Band device as an innovative tool to assess chicken behaviour*	**O499**
15:30	Pietrucci Daniele, Milanesi Marco, Chandramouli Balasubramanian, Vignali Giovanni, Negrini Riccardo, Macini Marco, Santini Monia, Chillemi Giovanni *Identification of the most impacting environmental variables on dairy cows milk yield using Machine Learning methods*	**O324**

15:45 Lazzari Andrea, Brambilla Massimo, Giovinazzo Simone, Calcante Aldo, Giannini Alessandro Antonio, Bisaglia Carlo
Automation to support rationing of dairy and beef cattle: comparisons and practical examples of application **O133**

16:00 Caré Sara, Migliorati Luciano, Abeni Fabio, Pirlo Giacomo
Expectation from precision livestock farming (PLF) in reducing environmental impact of dairy farms **O424**

16:15 *INVITED LECTURE*
O'Brien Bernadette
Precision technologies and smart dairy farming in extensive pasture based systems

17:00 *Coffee break*

Thursday June 15th – Room Apulia

Session 31 – Genomic tools for ruminant resiliency

Chairs: Bozzi Riccardo – Pasquini Marina

14:30 *INVITED LECTURE*
Lecchi Cristina
Bovine extracellular vesicles: a new challenge for animal science

15:00 Gómez Mayra, Cimmino Roberta, Rossi Dario, Zullo Gianluigi, BIffani Stefano
Genomic regions associated with Somatic Cell Score in Italian Mediterranean Buffalo **O220**

15:15 Salaris Sotero, Usai Mario Graziano, Casu Sara, Carta Antonello
Genetic Relationships between Milk Production and Impacting Diseases in breeding Sheep **O221**

15:30 Ramirez Diaz Johanna, Manunza Arianna, Rincon Florez Juan Carlos, Alvarez Luz Angela, Ciappesoni Gabriel, Peraza Pablo, Barreto Mourao Gerson, Arranz Jose, Freire Fernando, Cozzi Paolo, Stella Alessandra
Looking for the genes involved in local adaptation in sheep **O342**

15:45 Capomaccio Stefano, Milanesi Marco, Vajana Elia, Bomba Lorenzo, Chillemi Giovanni, Ajmone-Marsan Paolo, Colli Licia
BITE v.2: a user friendly R package for genomic analysis **O372**

16:00 Raspa Federica, Stoppani Nadia, Soglia Dominga, Perini Francesco, Fiorilla Edoardo, Profiti Margherita, Maione Sandra, Schiavone Achille, Sacchi Paola, Lasagna Emiliano, Mugnai Cecilia
Evaluation of the effect of the breeding system on the expression of liver genes in local slow-growing chicken breeds **O204**

16:15 Ben Jemaa Slim, Tolone Marco, Sardina Maria Teresa, Chessari Giorgio, Di Gerlando Rosalia, Portolano Baldassare, Mastrangelo Salvatore
A genome-wide comparison between unselected and selected Valle del Belice sheep reveals selection signatures related to production traits **O415**

16:30 Rossoni Attilio, Nicoletti Chiara, Vevey Mario, Santus Enrico
Integration between genomic and microsatellite analysis for the parentage verification **O442**

17:00 *Coffee break*

Friday June 16th – Room Daunia

Session 32 - Animal welfare and health

Chairs: Di Palo Rossella - Santillo Antonella

08:30 Ciarelli Claudia, Pillan Giulio, Bordignon Francesco, Xiccato Gerolamo, Pirrone Fabrizio, Ghiro Franco, Trocino Angela
Space use by laying hens in a cage-free system: effect of genotype and enrichment with perches **O453**

08:45 Poulopoulou Ioanna, Zanon Thomas, AlRhmoun Mousaab, Katzenberger Katja, Holighaus Louis, Gauly Matthias
Assessment of dairy farm welfare using a benchmarking tool **O464**

09:00 Crosetto Riccardo, Sartore Stefano, Ponzio Patrizia, Macchi Elisabetta, Mioletti Silvia, Miniscalco Barbara, Sicuro Benedetto, Tarantola Martina, Mugnai Cecilia
Grey Piedmont's rabbit welfare evaluation in three housing systems **O526**

09:15 Benitez Mora Maria Paz, Longobardi Valentina, Piscopo Federica, Esposito Riccardo, Bifulco Giovanna, Salzano Angela
Effect of pomegranate extract on post-thawed sperm quality **O433**

09:30 Torsiello Benedetta, Giammarino Mauro, Battaglini Luca, Battini Monica, Mattiello Silvana, Quatto Piero, Renna Manuela
Evaluation of inter-observer reliability of animal-based welfare indicators in the case of trichotomous variables with two or more observers **O008**

09:45 Lanzoni Lydia, Waxenberg Kaia, Ramsey Rachael, Rees Robert Martin, Bell Julian, Vignola Giorgio, Atzori Alberto Stanislao
The environmental cost of impaired welfare in dairy sheep farming **O043**

10:00 Zanon Thomas, Gauly Matthias
Walking distance and maintenance energy requirements of sheep during mountain pasturing (transhumance) **O054**

10:15 Colombini Stefania, Menni Giorgio, Pavesi Martina, Battelli Marco, Xiaoxia Dai, Galassi Gianluca, Crovetto Gianni Matteo, Rapetti Luca
Effect of a blend of essential oil on in vitro rumen fermentation for SARA prevention **O295**

11:00 *Coffee break*

Friday June 16th – Room Peucetia

Session 33 – Development of genomics in biodiversity

Chairs: Biffani Stefano – Cipolat Gotet Claudio

08:30 *INVITED LECTURE*
Laloë Denis, Jaffrézic Florence, Zerjal Tatiana, Rau Andrea
Old wine in new bottles: Factorial analyses in the age of multi-omics

09:00 Strillacci Maria Giuseppina, Bernini Francesca, Punturiero Chiara, Vevey Mario, Blanchet Veruska, Bagnato Alessandro
Structural Variation in the Aosta cattle breed **O410**

09:15 Bernini Francesca, Punturiero Chiara, Bagnato Alessandro, Milanesi Raffaella, Vevey Mario, Blanchet Veruska, Strillacci Maria Giuseppina
A Genome Wide Association Study in the Aosta Cattle population **O413**

09:30 Pauciullo Alfredo, Versace Carmine, Gaspa Giustino, Cosenza Gianfranco
A functional polymorphism influencing the promoter activity of alpaca α-lactalbumin gene (LALBA) **O188**

09:45 Cortellari Matteo, Negro Alessio, Bionda Arianna, Fresi Pancrazio, Grande Silverio, Crepaldi Paola
Effect of artificial insemination on genomic population structure in Camosciata delle Alpi dairy goat breed **O240**

10:00 Dadousis Christos, Ablondi Michela, Cipolat-Gotet Claudio, van Kaam Jan-Thijs, Finocchiaro Raffaella, Marusi Maurizio, Cassandro Martino, Sabbioni Alberto, Summer Andrea
The effect of ancestral and SNP panel genotyping on genomic inbreeding coefficients from imputed SNP in Holstein-Friesian dairy cows **O283**

10:15 Manunza Arianna, Ramirez Diaz Johanna, Zamora Bustillo Roberto, Ciappesoni Gabriel, Peraza Pablo, Lenstra Johannes A., Estrada Reyes Zaira, Arranz Santos Juan José, Revelo Cuaspud Herman Alberto, Rincon Flòrez Juan Carlos, Stella Alessandra
About the origin of traditional sheep breeds from South America: a comprehensive investigation **O346**

10:30 Biscarini Filippo, Cozzi Paolo, Ramirez-Díaz Johanna, Stella Alessandra, Manunza Arianna
Detection of heterozygosity-rich regions (HRRs) in the genome of domestic ruminants: which parameters for which breed **O031**

10:45 Ribani Anisa, Taurisano Valeria, Rakaj Arnold, Fianchini Alessandra, Grosso Luca, Pensa Davide, Buttazzoni Luca, Capoccioni Fabrizio, Pulcini Domitilla, Utzeri Valerio Joe, Fontanesi Luca
Mitochondrial DNA based diversity over several Mediterranean regions in holothurian species **O251**

11:00 *Coffee break*

Friday June 16th – Room Messapia

Session 34 – Companion animals

Chairs:	Cutrignelli Monica – Stefanon Bruno
08:30	*INVITED LECTURE* Switonsky Marek, Szczerbal Izabela, Nowacka-Woszuk Joanna, Stachowiak Monika *Cytogenetic and molecular background of canine and feline disorders of sex development*
09:00	Delsante Costanza, Vecchiato Carla Giuditta, Sportelli Federica, Cabri Giulia, Pinna Carlo, Cavazzoni Marco, Biagi Giacomo *Blood and clinical parameters of obese and lean cats, and the effects of a caloric restriction* **O011**
09:15	Pinna Carlo, Vecchiato Carla Giuditta, Delsante Costanza, Sportelli Federica, Parazza Paola, Dall'Olio Massimo, Biagi Giacomo *Effects of oral supplementation with coconut oil on fecal microbiota of healthy dogs* **O012**
09:30	Moretti Riccardo, Chessa Stefania, Profiti Margherita, Uva Anna Maria, Tarducci Alberto, Zatelli Andrea, Sacchi Paola *Resistance versus susceptibility to Leishmania: genetic differences in a population of English Setter dogs* **O150**
09:45	Balouei Fatemeh, Sandri Misa, Scarsella Elisa, Sgorlon Sandy, Stefanon Bruno *Reconstruction of functional features of fecal microbiome in dogs fed different diets* **O197**
10:00	Crepaldi Paola, Bionda Arianna, Cortellari Matteo, Lopreiato Vincenzo, Liotta Luigi *Selection signatures in Italian hunting dogs* **O286**
10:15	D'Anza Emanuele, Bionda Arianna, Liotta Luigi, Ronchese Mauro, Cortellari Matteo, Albarella Sara, Ciotola Francesca, Crepaldi Paola *English and American Golden retrievers differentiation: from sperm morphometry to genomic data* . **O301**
10:30	Atuahene David, Costale Annalisa, Martello Elisa, Chiofalo Biagina, Sandri Misa, Stefanon Bruno, Meineri Giorgia *Nutraceuticals: a promising alternative to the use of drugs for intestinal wellness in dogs* **O052**
10:45	Vastolo Alessandro, Ruggiero Alessio, Calabrò Serena, Kiatti Dieu Donné, Cutrignelli Monica Isabella *Influence of grain-free and cereal-based diets on postprandial glycaemic response* **O490**
11:00	*Coffee break*

Friday June 16th – Room Apulia

Session 35 – Sustainable feeding strategies in livestock system

Chairs:	Di Francia Antonio – Bailoni Lucia
08:00	*INVITED LECTURE* Atzori Alberto Stanislao, Atamer Balkan Busra, Tedeschi Luis Orlindo, Cannas Antonello, Gallo Antonio *Dynamic modelling in ruminant nutrition: background, applications and perspectives for the sustainability of livestock productions*
08:30	Serrapica Francesco, Di Francia Antonio, Barone Carmela Maria Assunta, Genovese Alessandro, Balivo Andrea, Masucci Felicia *Can sprouted barley grains replace maize silage in dairy buffaloes' diets? Effects on production efficiency, milk traits, and mozzarella cheese quality* **O149**
08:45	Gannuscio Riccardo, Vastolo Alessandro, Maniaci Giuseppe, Lucia Caterina, Todaro Massimo, Cutrignelli Monica Isabella *Novel feedstuffs obtained mixing different by-products: nutritional evaluation* **O554**
09:00	Bolletta Viviana, Ruggero Menci, Pauselli Mariano, Servili Maurizio, Taticchi Agnese, Morbidini Luciano, Valenti Bernardo *Dietary hazelnut skin and addition of phenolic extract from olive mill wastewaters: Effects on pork salami quality* **O512**
09:15	Tinagli Sara, Silvi Alina, Casarosa Laura, Turini Luca, Tognocchi Monica, Serra Andrea *Effect of a Sulla-based diet on Massese sheeps' milk and cheese* **O292**

09:30 Maniaci Giuseppe, Ponte Marialetizia, Gannuscio Riccardo, Giosué Cristina, Di Grigoli Antonino, Bonanno Adriana, Alabiso Marco, Pipi Marianna
Effect of the inclusion of Opuntia ficus-indica cladodes in the feeding of Cinisara cows on Caciocavallo Palermitano cheese produced in summer **O406**

09:45 Iommelli Piera, Sarubbi Fiorella, Musco Nadia, Lombardi Pietro, Morittu Valeria Maria, Moniello Giuseppe, Totakul Pajaree, Infascelli Federico, Tudisco Raffaella
Effect of fennel seeds as a dietary supplement in grazing goats: preliminary results **O462**

10:00 Piccioli-Cappelli Fiorenzo, de la Maza-Escolà Victor Sainz, Grilli Ester, Berelli Marco, Benedetti Lorenzo, Lovotti Giorgia, Trevisi Erminio
Effects on feed efficiency and resilience in the transition period of Holstein dairy cows fed with different sources of rumen-protected choline **O463**

10:15 Reggi Serena, Guagliano Marianna, Pedrazzi Simone, Allesina Giulio, Spalletta Ambra, Scoranelli Simona, Cristiani Cinzia, Rossi Luciana
In vitro evaluation of biochar from chestnut and vine residues gassification as possible feed additive: antioxidant and antimicrobial activities **O279**

10:30 Trevisi Paolo, Correa Federico, Virdis Sara, Negrini Clara, Dalcanale Simone
Efficacy of low protein diets with different amylose/amylopectin ratio on growth performance and health of weaned pigs **O356**

10:45 Danieli Pier Paolo, Manganello Federico, Fiore Leonardo, Natoli Vincenzo, Ronchi Bruno, Primi Riccardo
Nutritional profile of hay from tef (Eragrostis teff) genotypes grown in South Italy **O128**

11:00 *Coffee break*

Friday June 16th – Room Daunia

Session 36 – Research and sustainability in horse production

Chairs: Salimei Elisabetta – Capomaccio Stefano

11:30 *INVITED LECTURE*
Vial Céline
Equine production and activities in France: what contributions to the sustainability of local areas?

12:00 Asti Vittoria, Sabbioni Alberto, Stocco Giorgia, Summer Andrea, Ablondi Michela
The background of novel phenotypes in a native horse breed **O314**

12:15 Bordin Clara, Raspa Federica, Roggero Angela, Palestrini Claudia, Harris Patricia, Ellis Andrea Dorothea, Bergero Domenico, Defilippi Silvia, Valle Emanuela
A new-designed feeding device for sustainable hay feeding management in ponies **O302**

12:30 Zappaterra Martina, Felici Martina, Nanni Costa Leonardo, Padalino Barbara
Welfare on arrival at the slaughterhouse: handled versus unhandled horses **O014**

12.45 Zoratti Aloma, Pividori Isabella, Comin Antonella, Piasentier Edi, Prandi Alberto, Peric Tanja
Assessment of the allostatic load in horses at the slaughterhouse **O294**

Friday June 16th – Room Peucetia

Session 37 – Insects' production for feed and food

Chairs: Chiofalo Biagina - Gasco Laura

11:30 Ferri Irene, Dell'Anno Matteo, Reggi Serena, Canala Benedetta, Petrali Beatrice, Rossi Luciana
Innovative growing substrates for the production of functional Tenebrio molitor meals **O547**

11:45 Renna Manuela, Coppa Mauro, Lussiana Carola, Le Morvan Aline, Rastello Lara, Gasco Laura, Maxin Gaelle
Rumen lipid biohydrogenation of insect meals: results of an in vitro study **O039**

12:00 Mancini Simone, Mattioli Simona, Fratini Filippo, Cacchiarelli Chiara, Tuccinardi Tiziano, Dal Bosco Alessandro, Paci Gisella
Comparison between lesser mealworm, mealworm and superworm larvae reared on the same substrate for their use as feed/food **O100**

12:15 Bellezza Oddon Sara, Biasato Ilaria, Resconi Andrea, Loiotine Zaira, Caimi Christian, Zaniboni Luisa, Gasco Laura
Effect of different iso-waste-based diets on black soldier fly larvae growth and chemical composition **O520**

12:30 Sogari Giovanni, Tzompa-Sosa Daylan Amelia, Moruzzo Roberta, Riccioli Francesco, Menozzi Davide, Liu Aijun, Li Jie, Mancini Simone
Consumers' acceptance toward edible insects: A cross-country study in Belgium, China, Italy, Mexico, and the US **O163**

12:45 Addeo Nicola Francesco, Vozzo Simone, Alleva Rosanna, Tancredi Federica, Piccolo Giovanni, Bovera Fulvia
Growth performance and morphometric characteristics of Hermetia illucens larvae reared on four different substrates **O213**

13:00 Pascon Giulia, Cardinaletti Gloriana, Messina Maria, Daniso Enrico, Tibaldi Emilio, Randazzo Basilio, Tulli Francesca
Effect of dietary chitin on growth performance, nutrient utilization and metabolic response in rainbow trout (Oncorhynchus mykiss) **O516**

Friday June 16th – Room Messapia

Session 38 – Advances in dietary feed supplementation

Chairs: De Palo Pasquale – Nudda Anna

11:30 Mammi Ludovica Maria Eugenia, Ghiaccio Francesca, Bonfante Elena, Vezzani Claudio, Colleluori Riccardo, Cortese Martina, Formigoni Andrea
Precision feeding: amino acid balance in dairy cows fed two different levels of total crude protein ... **O278**

11:45 Lopreiato Vincenzo, Chiofalo Vincenzo, Cavallo Carmelo, Amato Annalisa, Trevisi Erminio, Liotta Luigi
Effect of feeding enriched-olive cake on metabolic and milk performance response of mid-lactating Holstein cows **O281**

12:00 Lora Isabella, Tolasi Cecilia, Ranzato Giovanna, Zanoli Yadav, Cozzi Giulio
Field study on the effects of pasteurization on colostrum quality of Holstein dairy cows **O308**

12:15 Mastroeni Carmelo, Ghilardelli Francesca, Sigolo Samantha, Mosconi Martina, Gallo Antonio
Oxygen barrier plastic films: an opportunity to improve silage quality and reduce the use of plastic . **O386**

12:30 Arango Sheyla, Massaro Selene, Guzzo Nadia, Tagliapietra Franco, Bailoni Lucia
Effect of hemp leaves on milk yield and quality in dairy cows: preliminary results **O402**

12:45 Meli Giovanna, Fumo Valentina, Scicutella Federica, Invernizzi Guido
Impact of maternal milk from goats fed with Camelina sativa and Cynara cardunculus cake on goat kids' growth performance and health status **O420**

13:00 Marchetti Luca, Rebucci Raffaella, Giromini Carlotta, Lanzoni Davide, Cremonesi Paola, Castiglioni Bianca, Biscarini Filippo, Perricone Vera, Sandrini Silvia, Bontempo Valentino
Evaluation of essential oils from natural extracts and medium chain fatty acids on piglets growth performance, salivary stress markers and gut health **O087**

13:15 Righi Federico, Pitino Rosario, Pozza Marta, Goi Arianna, De Marchi Massimo, Simoni Marica
Relationship between diets characteristics and milk composition in lactating dairy cowsfed silage- or hay-based diets **O440**

Friday June 16th – Room Apulia

Session 39 – Genomic tools for animal resiliency and susceptibility

Chairs: Bagnato Alessandro - Sartori Cristina

11:30 *INVITED LECTURE*
Vergani Andrea Mario, Bagnato Alessandro, Masseroli Marco
The use of Machine Learning in the Genomic Era of Livestock Farming

12:00 Fabbri Maria Chiara, Tiezzi Francesco, Lozada-Soto Emmanuel, Crovetti Alessandro, Consortium TREASURE, Bozzi Riccardo
Loss of autozygosity in QTL regions in crossbred pigs **O168**

12:15 Ballan Mohamad, Bovo Samuele, Schiavo Giuseppina, Bolner Matteo, Bertolini Francesca, Cappelloni Manolo, Tinarelli Silvia, Gallo Maurizio, Fontanesi Luca
Mining high density SNP chip data and whole genome sequencing information to identify putative unfavorable alleles in pig breeds **O223**

12:30 Bovo Samuele, Schiavo Giuseppina, Fanelli Flaminia, Ribani Anisa, Bertolini Francesca, Gallo Maurizio, Galimberti Giuliano, Dall'Olio Stefania, Martelli Pier Luigi, Casadio Rita, Pagatto Uberto, Fontanesi Luca
Whole metabolome and genome analyses provide information on the genetic variability affecting the metabolism in pigs **O248**

12:45 Albarella Sara, Zappaterra Martina, D'Anza Emanuele, Piegari Giuseppe, Costanza Dario, Meomartino Leonardo, Peretti Vincenzo, Davoli Roberta, Ciotola Francesca
Perosomus Elumbis in a family of Casertana pigs: case description and identification of candidate genes **O173**

13:00 Anzalone Antonietta, Gallo Daniela, Fulgione Andrea, Capuano Federico, Pauciullo Alfredo, Cosenza Gianfranco
An association analysis between CSN3 genotype and milk yield in Italian Mediterranean river buffalo **O472**

ASPA 25th Congress

Monopoli (BARI – ITALY), June 13–16, 2023

Italian Journal of Animal Science
2023; volume 22, supplement S1

POSTER

ACQUACOLTURE

ANIMAL BREEDING AND GENETICS

ANIMAL NUTRITION AND FEEDING – Monogatrics

ANIMAL NUTRITION AND FEEDING – Ruminants

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Floridia Viviana, D'Alessandro Enrico, Lopreiato Vincenzo, Cavallo Carmelo, Chiofalo Vincenzo, Randazzo Cinzia, Russo Nunziatina, Liotta Luigi
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Calabrò Serena, Primi Riccardo, Kiatti Dieu Donné, Manganello Federico, Tiberi Cristiano, Sestili Francesco, Palombieri Samuela, Ronchi Bruno, Danieli Pier Paolo
Nutritional characteristics of innovative feed containing by-products from high-amylose wheat **P333**

Sini Matteo, Fulghesu Fabio, Ledda Antonello, Porcu Maria Angela, Atzori Alberto Stanislao, Cannas Antonello
Effect of two different forage sources (oat hay vs. alfalfa hay) fed during pregnancy on the performances of Sarda ewes during early lactation **P382**

Sini Matteo, Fulghesu Fabio, Ledda Antonello, Atzori Alberto Stanislao, Cannas Antonello
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Microplastics contamination in dairy cows' total mixed ration **P492**

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Effects of an electrolyte, antioxidant and osmolyte blend supplement on milk yield and cheesemaking properties in Holstein lactating cows during warm season **P498**

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ANIMAL PRODUCTION SYSTEMS

ANIMAL WELFARE, HEALTH AND BEHAVIOUR

BIODIVERSITY AND CONSERVATION OF GENETIC RESOURCES

COMPANION ANIMALS

ENVIRONMENTAL IMPACT OF LIVESTOCK

EQUIDS PRODUCTION

FREE COMMUNICATIONS

INSECTS

MEAT PRODUCTION

MILK PRODUCTION

POULTRY AND RABBIT PRODUCTION

PRECISION LIVESTOCK FARMING

QUALITY OF ANIMAL PRODUCTS AND FOOD PROCESSING

REPRODUCTION

SUSTAINABLE LIVESTOCK FARMING

GENERAL INFORMATION

Congress dates

13th June (Tuesday) – 16th June (Friday) 2023

Venues

Opening ceremony and Welcome Cocktail on the 13th June
Location: Torre Cintola Resort
Oral sessions from the 13th to the 16th of June 2023
Location: Torre Cintola Resort

Parking

Congress participants will have the opportunity to park free of charge inside the Torre Cintola Resort.

Secretariat and Registration Desk times during the Congress

Tuesday 13th June	14.00 – 19.00
Wednesday 14th June	08.00 – 19.00
Thursday 15th June	08.00 – 19.00
Friday 15th June	08.00 – 12.00

Registration

The registration fee includes:
Admission to the Scientific Sessions and the Exhibition Area
Conference kit
Abstract collection, available online as a supplement of the "*Italian Journal of Animal Science*"
Opening Ceremony and Welcome Cocktail on Tuesday 13th June
Apulian typical dinner on Wednesday 14th June at "Torre Cintola Resort"
Coffee breaks and lunches as shown in the programme
Certificate of attendance, to be downloaded from our informatic system after the congress

Badge

All participants will receive a badge, which they are requested to wear during all congress activities to be admitted to the scientific sessions and to the social programme.

Tyvek bracelets

All participants and all the registered accompanying persons will dress the bracelet for getting access to coffee breaks, welcome cocktail, lunches and Apulian dinner.

Language

The official languages of the Congress are English and Italian. Slides for oral presentation in English only.
Simultaneous translation will not be provided.

WiFi

Free WiFi connection at the congress venues: Torre Cintola
Password: torrecintola

Mobile phones, photo and video

Participants are requested to keep their mobile phones switched off (or set to silent mode) in the session rooms.
Photography and video recordings are strictly prohibited during all scientific sessions.

Opening Ceremony

The Opening Ceremony will take place on Tuesday 13th June 2023 at the "Torre Cintola Resort" and will be followed by a Welcome Cocktail.

Working lunches

On Wednesday 14th and Thursday 15th June working lunches will be served from 13.30 to 14.30 at Torre Cintola Resort.

Social dinner

The Social Dinner will be held on the evening of Thursday 15th June at "Tenuta Monacelle"
A transfer service by private bus will be organized, with departure at 20.00 from Torre Cintola Resort

Provided services

A biberon-feeding service is guaranteed during the congress half an hour before lunch.

3rd edition of the "ASPA Young Researchers Competition – Oral Presentations"

To promote and stimulate young researchers, the Animal Science and Production Association (ASPA), in collaboration with the Italian Journal of Animal Science (IJAS) and with the economic support of the Organizing Committee of the 25th ASPA Congress (2023), organizes the third edition of the "ASPA Young Researchers Competition - Oral Presentations" with the aim to select the best 5 papers as oral communications at the 25th ASPA Congress. The awarding of the best communications is scheduled for the 15th June at 18.00.

3rd edition of the "ASPA Young Researchers Competition – Posters"

To promote and stimulate young researchers, the Animal Science and Production Association (ASPA), in collaboration with the Italian Journal of Animal Science (IJAS) and with the economic support of the Organizing Committee of the 25th ASPA Congress (2023), organizes the third edition of the "ASPA Young Researchers Competition - Poster Presentations" with the aim to select the best 5 papers as Poster communications at the 25th ASPA Congress. The awarding of the best communications is scheduled for the 15th June at 18.00.

APP

Photo Exhibition

Photos of marginal areas, rural environments, native breeds of Southern Italy (Apulia and Molise) will be displayed in the conference rooms. Ph: Luigi Geraci, Rocco Giorgio, Fabio Pilla.

Monopoli

Monopoli (from the Greek "*monos"* and "*polis"*) means unique city and certainly it is still faithful to its name.
Indeed, the city retains intact the charm that comes from its history and traditions.
A city of nearly 50,000 inhabitants, 43 km southeast of Bari on the Adriatic coast. It borders with the towns of Polignano a Mare, Conversano, Castellana-Grotte, Alberobello and Fasano.
Monopoli stretches along 15 km of low, indented coastline, with numerous coves with evocative names and long sandy shores that are a safe landing place for tourists for their vacations.
The medieval center, characterized by churches and convents, rises again today thanks to major restoration works, such as the

complex of fortifications (walls and castle), the Romanesque Church of Santa Maria Amalfitana and the sumptuous Baroque Cathedral.
The rural territory, consisting of as many as 99 districts, covers an area of more than 150 square kilometers of great natural, scenic and cultural interests.
There are in fact numerous fortified “Masserie” (centers for agricultural activities) also dating back to Roman times, rock churches, “trulli”, beautiful neoclassical villas and farmhouses.
Monopoli's major tourist attractions include Roman (Egnatia), Byzantine (rock frescos), Longobard, Norman (farmhouses, masserie, fortresses) and Hispanic (Castle of Charles V) monuments.

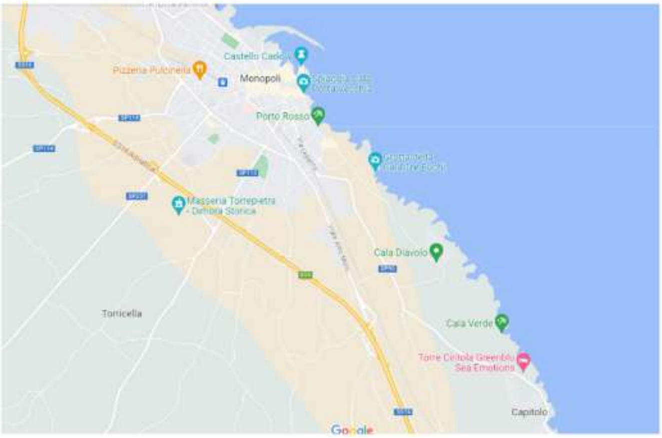
Shuttle bus to and from airport

From Airport to Monopoli:
Monday 12th at 21.00
Tuesday 13th June at 12.00 and 18.00
Wednesday 14th June at 12.00

From Monopoli to Airport:
Thursday 15th June at 16.00
Friday 16th June at 14.00

Please reserve until the 10th June

Where to have dinner



It is possible to reserve the dinner for Tuesday and Thursday night, directly in Torre Cintola Resort. The resort is located in “Capitolo”, a locality in the municipality of Monopoli, in the metropolitan city of Bari. It is known to be one of the main tourist areas of Monopoli, as well as its seaside resort per excellence.
During the summer period, it is a place full of clubs and beach lidos, active even in the evening, which can offer tourists the opportunity to have lunch and dinner directly on the beach.
It is located 3 km far away from Monopoli City center which is also full of restaurants and clubs that can offer lunches and dinners of all kinds, types and tastes.
For participants interested to dinner on Monday night or for the lunch of Friday, it is possible to reserve directly in Torre Cintola Resort

Bus Service

To reach Monopoli during the ASPA Congress it will be possible to get on the city bus just at the entrance of Torre Cintola Resort from 8.00 to 23.00 every 45 minutes. On the registration desk it is possible to have the exact city bus timetable.

Dynamic modelling in ruminant nutrition: background, applications and perspectives for the sustainability of livestock productions

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Nutrition modelling was largely based on empirical or mechanistic approaches, as firstly shown by the well-known INRA, NRC and Cornell Net Carbohydrate and Protein System (CNCPS) animal nutritional feeding systems. These approaches allowed to merge algorithms and equations and to find the nutritional outputs from analytical solutions. In particular, mechanistic models increased the degree of understanding of biological systems, proposing the description of the nutritional dynamics at steady state conditions. It must be noticed that biological systems are characterized by a high degree of complexity, which include non-linear feedback dynamics with multiple loops that cannot be solved solely with analytical approaches. Dynamic approaches have been proposed for specific focuses and often without association with systemic perspectives or feedback loop thinking. Otherwise, systemic perspectives and system dynamics modelling have increased in the last decades in many scientific areas, underlining the need for higher complexity understanding to drive decision support. Different dynamic approaches have been already proposed to describe ruminal dynamics, and the modeling applied to ruminant nutrition could take advantage of including feedback mechanisms. Thus, the aim of this work is to present the system dynamics approach for nutritional modelling, based on systemic perspectives and dynamic calculation methods. System dynamics modelling is a descriptive simulation modelling technique, aiming to explicitly embed the cause-and-effect relationships of variables within the model. It follows a modelling process emphasizing mental model development and dynamic hypothesis identification, to map the mathematical relationships describing the nutritional processes, phenomena and patterns of behavior. It aims to synthesize all that is known about the problem into an endogenous (i.e. feedback-based) theory, on which to evaluate the quantitative model expressed in mathematical terms. As a demonstrative outcome, three specific examples of this approach are provided through the presentation of nutrition models. They include ruminal degradation dynamics, maintenance requirement calculation for dairy cows, and feed intake behavior modelling description and calculations. This work allows exploring an alternative modelling methodology for the development of future nutritional models.

Nutrition modeling has large implications and multiple roles in addressing livestock production sustainability can be declined in technical, environmental and economic directions and aspects. Empiric algorithms and mechanistic sub-models have been successfully integrated to several feeding systems calculate technical performances and nutritional indicators, to estimate environmental impact of livestock and to compute economic indicators of feeding economics. Nutritional indicators with relevant environmental and economic implications have been included in several feeding systems and feeding formulation software, to enhance the decision making power of the nutritionists at farm level. Black box and empiric approaches left the place to mechanistic modeling, allowing to increase understanding and awareness of livestock nutrition and the effectiveness of formulated diets, and in driving animal biological response in terms of productivity and welfare, especially in ruminants. From a scientific and technical point of view, as demonstrated by the CNCPS in the past decades, nutritional mechanistic modeling has been prioritized to reduce nitrogen and phosphorus excretion and pollution, and to target technical and economic efficiency in optimized diets, introducing the concepts of precision feeding techniques.

With the high emphasis on climate change contribution of livestock sector and on its mitigation, recently many models have been developed to estimate methane emissions, with the double focus to quantify emissions and increase nutrient harvesting at ruminal and gastrointestinal level. Mechanistic and dynamic models are every day more diffused to improve understanding of nutritional processes at organ, tissue, animal or farm levels. Models of ruminal dynamics have been published and are under development, with the aim to mimic biological relations among substrates, microbial populations and host, in order to predict and enhance feed utilization efficiency. Feed efficiency has been recognized as the most important parameter of sustainable production in ruminants being associated for 50% to technical/environmental efficiency and energy use and for another 50% to the economic performances of dairy farms. From this point of view, mathematical models can help to identify and optimize key factors of productivity, to increase effectiveness of feeding techniques and reach more sustainable and environmentally-friendly farm practices. Currently, dynamics models are under development to cope with the most relevant environmental challenges. They include nitrogen and phosphorus pollution, greenhouses gas emissions (N_2O and CH_4), particulate matter 2.5 (NH_3), and water consumption.

The passage from mechanistic to dynamic models opens space to new cutting edge research in nutritional evaluations of diets and animal performances since it allows to move, in the decision making process of nutritionists and farmers, from static considerations to over time scenarios. By using dynamic models, we can improve the understanding of trade-offs and potential unintended consequences of management decisions. This is surely relevant in the estimation of animal productivity, in emission estimation and pollution mitigation, but can be even more beneficial for studies on climate change adaptation. In fact, considering the small relevant contribution of each single livestock specie to the climate change, the reduction of emissions by livestock cannot ensure the reduction of climate change risks, since the anthropogenic emissions from other sectors might contribute to impair the global equilibrium, with detrimental effects on agriculture and livestock productions. Thus, a climate adaptation of livestock is undoubtedly needed. In this sense, dynamic modeling brings opportunities to study, describe and predict the future performances. Some examples can be shown for animal responses to heat stress, variation of animal requirements, and adaptation and tolerance to heat stress as biologic delayed process. Several modeling techniques, such as system thinking, hybrid approaches based on age-based modeling, and artificial intelligence based on deep learning and machine learning are being used to rapidly reach the goals of having more productive and sustainable livestock productions trough the enhancement of decision making in nutritional processes.

ICAR harmonisation of sustainability related milk traits

Martin Burke[a], Tone Roalkvam[b], Rene van der Linde[c] and as representatives of the ICAR Sustainability Task Force

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[b]ICAR Board Member, TINE, Norway; [c]ICAR, Utrecht, The Netherlands

ICAR has the role of developing and providing animal recording guidelines to support the dairy industry and farmers in their efforts to collect quality data for use in herd management decision making, and to deliver proven and validated information for genetic evaluations. Policy, science, consumer expectations and farmer practices all point to the same paradigm: optimisation not productivity, for land, animals and energy. Taking on this challenge, we need to reset and take ecology and the ecological boundaries as our starting point. This means a paradigm shift towards circularity, as part of a larger bio-based economy. Following the successful ICAR 2021 conference in Leeuwarden, where 'Circularity in Agriculture' was the main theme, the ICAR Board appointed Board Member Tone Roalkvam and their CE, Martin Burke to form a Sustainability Task Force (STF) with the goal of developing a coordinated approach to Sustainability across ICAR Working Groups and Sub Committees.

As well as ICAR Board and staff being represented, the Sustainability Task Force also includes ICAR's key Working Chairs ; Birgit Gredler-Grandl (Feed & Gas WG) and Christa Egger-Danner (Functional Traits WG).

In subsequent meetings in 2022 and 2023, the STF worked to develop a '*Table of Sustainability Related Traits currently recorded in Member Milk Recording Organisations*'. Traits were identified, grouped based on herd management area, and assessed based mainly on their 'Impact' on Sustainability as well as to their 'Ease' of implementation. This exercise identified over 35+ traits currently being recorded by ICAR Members. As ICAR's key role is harmonisation of recording guidelines we recommend the next steps;

a. ICAR provides **Standard Definitions** of the key Milk Recording Traits
b. ICAR provides a standard comparable reference on **how to Measure important sustainability-related traits**.

This presentation offers a progress report including an overview of the recommendations of the ICAR Sustainability Task Force.

KEYWORDS: ICAR; sustainability; milk recording traits; trait harmonisation; collaboration

Rethinking breeding strategies in livestock species

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Food and Agriculture Organization of the United Nations (FAO) estimates that annual consumption of meat and milk will increase by approximately 50%, in 2050 forecast. As the number of farms is decreasing, the demand for more livestock production will be met through intensification, resulting in larger farms. Furthermore, as consumers require animals being raised respecting more ethical criteria, animal welfare and health are likely to add pressure on livestock management practices. Ensuring that sufficient care attention is paid to animals respecting an economical viable business, there is an increasing need for modern livestock facilities and technological devices to monitor environmental conditions but also animal behavior and health status.

Today's available smart sensors, detectors, cameras, and microphones can assist integrated management systems for animal husbandry that are based on continuous, real-time monitoring and control of production, animal welfare and health, as well as environmental conditions. Such detailed devices enable farmers to suddenly detect thermal stress, illness, or air quality problems so that they can take immediate action. Such issues have recently been placed under the precision livestock farming (PLF) definition, whereby livestock production systems are viewed as a set of interconnected processes, including animal growth and behavior, product yield, endemic disease, and more recently the physical environment of livestock buildings which includes their

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thermal microenvironment and the emission of gaseous pollutants (CH_4 and NO_2 mostly).

Animal breeding in the third millennium has been swept up to tensions between the ever-increasing demands of world population nutrition, animal welfare and environmental consequences. The National Breeders' Associations, coordinated by FedANA (National Federation of Breeds and Species Breeders' Associations) have grasped the upcoming change and all together we are responding with a strong effort in research and development, as well as with projects and organization of daily activities by gathering the demands and needs of breeders, animals, and more recently consumers, citizens and society. We have actually started to change selection goals of each animal population, by matching criteria that are increasingly holistic and sustainable in different environments and markets. Targets for the coming years call for preserving natural resources such as air, water and soil, protecting human and animal health, reducing the number of pharmaceuticals used in animal husbandry and preserving genetic variability that is increasingly valuable for the challenges of the future.

However, these goals are not the only interest that FedANA and associated ANAs are pursuing, as after huge steps forward in functional genome analysis and molecular biology we are now focusing on a gap related on understanding the interactions between hereditary factors and environmental impact that ultimately shape an animal's phenotype. In the animal genome area time has come to clarify how genetic variation shapes different individuals, breeds, phenotypes and stages of development. As this knowledge deserves to be deeper investigated, FedANA and the associated ANAs have therefore started a real 'RETHINKING of the ANIMAL BREEDING MODEL' or better said a rethinking of the whole breeding strategy, with various actions and activities such as better control of inbreeding rates, increase in genotyping the female population, strengthen collaboration between the various players in the industry (e.g. POD or protection consortia, ARA-AIA, ANA-FedANA, AI Studs, companies working on animal and human health, specialists in the agri-food sector, etc.). Moreover, FEDANA and ANA Associates are defining new studies and new services about animal resilience on heat stress, interaction between the rumen microbiome and animal DNA, but also early researches on epigenetic, reconstruction of pedigrees and the verification of the documented family tree. Last but not least, we are monitoring and improving models for predicting genetic values, indeed, in the genomic era, over-estimation due to the pre-selection of young reproducers identified through genomic screening, seem to be due for selecting not only the best pedigree but also the most favorable mendelian sampling. In animal breeding, parents of the next generation are typically chosen in multiple stages, with the initial steps of selection are called preselection. Candidates that survive preselection are called preselected animals, and those that do not pass the threshold are called pre-culled animals. Aim of preselection is reducing costs and efforts required for animals that are not of interest to the breeding program, and it can be achieved by avoiding phenotyping or further testing of pre-culled animals. Due to the introduction of genomic prediction, preselection is now mostly based on genomic estimated breeding values (GEBV) of young animals even before they have records for any trait. This type of preselection is called genomic preselection (GPS). GPS popularity is due to genotyping becoming cheaper day by day, and also to acceptable reliabilities of GEBV. As genomically pre-culled animals have neither progeny nor records for some or all breeding goal traits, they are generally not included in subsequent genetic evaluations (i.e. genetic evaluations that come after preselection). Thus, GPS decreases the amount of information available for subsequent genetic evaluations of preselected animals. This may not only lead to loss of accuracy, but may also result in bias in the GEBV that can lead to incorrect estimates of genetic trends, as well as mistakes in ranking animals across generations. Properly assessing the impact of preselection on subsequent genetic evaluation of preselected animals requires comparison of scenarios that take different approaches into account, including a scenario without preselection.

FedANA and its associated ANAs are in fact rethinking an animal breeding model that can make an innovative animal husbandry sustainable based on a wisdom deeply rooted in the cultural and typical values of the agricultural world. The holistic and sustainable selection pursued foresees that livestock are irreplaceable in maintaining a circular flow of materials in agriculture, recycling in various ways the large quantities of inedible biomass that are generated as side products during the food production for human consumption. In addition, well-managed livestock systems can generate many other benefits, including carbon sequestration, improved soil health, biodiversity, watershed protection and the provision of important ecosystem services. As a matter of fact, the drastic reduction in the number of animals in livestock farming, as desired by the opponents of world animal husbandry, combined with the support of laboratory food or commonly called artificial food suppliers, could actually cause environmental problems on a large scale. For thousands of years livestock and agriculture has nourished humanity, not to mention clothing, energy, manure, employment and income, as well as goods, guarantees, insurance and social status. Knowledge in animal science and related technologies are currently improving animal husbandry performance along all of the aforementioned health, environmental and socio-economic dimensions faster than even before in human history.

Advancement in conservation programs of Italian poultry genetic resources for rural development

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Italian poultry breeds were reared for productive purposes until the '40s in the last century and then were rapidly replaced by commercial strains selected for improved performance. Since then, local breeds were totally discarded by the food producing poultry system and underwent a progressive constant reduction in population size putting their survival at risk and, in worst cases, leading to actual extinction. In 2021, Italian chicken (n. 21) and turkey (n. 7) breeds have been still surveyed in farming conditions; however, only 5 chicken breeds had a population size classified not at risk, whereas the majority of chicken and all turkey breeds were classified at risk according to FAO risk categories.

Local breeds still represent a wide reservoir of genetic diversity and their conservation has become a priority at global and regional level as an insurance to face the current and future changes in animal production, including poultry systems. Breeding of poultry breeds was for many years the exclusive interest of fancy farmers and only in the last decades local public Institutions developed actions providing technical and economic support for conservation programs of Italian poultry genetic resources. The herd book of Italian poultry breeds was established in 2014 by the Ministry of Agriculture (MASAF) and represented a key initiative to organize a national system aimed to safeguard poultry biodiversity and support the development of an advanced national conservation strategy with the concomitant consideration of genetic and productive goals.

In 2017, the first national network of Breeding Poultry Centres (BPC) was organized and supported by MASAF within the 'National Plan for Rural Development – Support for conservation and sustainable use of animal genetic resources in agriculture'. The poultry network has been developing a wide conservation program (TuBAvI project, www.pollitaliani.it) on poultry Italian breeds with the aim to provide tools and knowledge to implement both *in situ* and *ex situ* conservation techniques. Several activities have been considered with the overall long-term purpose to re-introduce Italian poultry breeds into the productive system. The poultry network includes academic partners having the peculiarity to combine research studies on conservation techniques with the direct *in situ* management of nucleus populations.

Genetic diversity and population structure were studied after bird genotyping using specie-specific high density SNP array in 17 chicken and 7 turkey breeds. The patterns of genetic differentiation showed that most breeds formed non-overlapping clusters and were clearly separate populations. The level of genomic inbreeding was markedly different among the breeds and ranged from 0.121 (*Valdarnese*) to 0.607 (*Siciliana*) in chickens, and from 0.126 (*tacchino di PR e PC*) to 0.401 (*Ermellinato di RO*) in turkeys. Genotyping of chickens with a set of 14 microsatellite loci was also used to study the level of genetic diversity, molecular kinship, inbreeding, contribution to overall genetic diversity and rate of extinction in Italian breeds. *Bianca di Saluzzo* and *Bionda piemontese* provided the greatest and *Livorno bianca* the lowest contribution to global biodiversity. *Livorno bianca* was also the breed with the lowest genetic variability and higher extinction risk index. In general, low genetic diversity and high self-inbreeding was found in Italian chicken breeds; therefore, the introduction of different genetic lines and the use of mating schemes was strongly recommended to limit the increase of inbreeding and improve the conservation program.

Breeding management of nucleus populations has been improved in order to preserve genetic diversity, control inbreeding and monitor reproductive performance. Bird genotyping with microsatellite molecular markers has been implemented in nucleus populations to identify familiar lines and study mating plans in order to prevent the loss of biodiversity and the increase of inbreeding. The conservation breeding management is currently applied to 8 chicken breed populations (*Ancona, Bianca di Saluzzo, Bionda Piemontese, Livorno, Mugellese, Mericanel della Brianza, Siciliana, Valdarnese bianca*) reared within BPC and is expected to be extended to other chicken and turkey populations in the future.

Many efforts have been implemented to study a detailed phenotypic characterization of the chicken and turkey breed populations today available in order to overcome the current lack of knowledge and support the economic potential of breed farming. So far, the morphological traits and performance of 15 chicken breeds (*Ancona, Bianca di Saluzzo, Bionda Piemontese, Ermellinata di Rovigo, Livorno, Millefiori di Lonigo, Mugellese, Mericanel della Brianza, Padovana, Pepoi, Polverara, Robusta lionata, Robusta maculata, Siciliana, Valdarnese bianca*) were recorded for the first time or updated and comprehensive breed forms are going to be published online in the site www.pollitaliani.it. Data related to a few most known chicken breeds will be presented.

The combination of *in situ* and *ex situ* techniques is recognized to improve the conservation of biodiversity and is recommended, in particular, for the management of populations very limited in size. Therefore, beside activities related to the *in situ* technique, as before described, tools for the implementation of the *ex situ in vitro* technique in the conservation of Italian poultry breeds have been considered. The semen cryobank of autochthonous chicken and turkey breeds was planned in the period 2018–2019, started in 2020 and is currently in progress until 2024. Species-specific cryopreservation procedures were studied and implemented to store semen doses in several chicken and turkey breeds, even if the number of doses is highly variable according to the availability of birds and the variability in semen production among breeds.

TuBAvI project provided relevant results and furthers are expected within 2024. The poultry network BPC may play a fundamental role in the organization of a rural productive system. The involvement of local farmers and the valorisation of poultry products are key factors affecting the future development of the system.

Are there benefits in using sequence data for genomic predictions?

Daniela Lourenco

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Increasing the prediction accuracy in animal populations requires a certain number of genotyped individuals and SNP, which depends on the effective population size. As the increase in prediction accuracy in real populations has never achieved the levels observed in real populations, one hypothesis was that insufficient SNP were used for genomic evaluations. Then, the quest for sequencing started because one of the assumptions with the whole-genome sequence is an increased likelihood of having the true causative variants in the data. Based on that, genomic evaluations could benefit from using whole-genome sequences if data are large enough to represent the variability within the population. Additionally, this type of data would greatly help multi-breed (MB) and across-breed predictions. However, the prediction machinery should be able to account for sequence properly. Although tens of millions of SNP are called from the sequence, using all of them is not beneficial; therefore, a preselection step is required where important variants are chosen. We investigated the impact of using preselected variants from sequence data for genomic predictions in dairy cattle and pigs using 27k to 105k sequenced/imputed individuals in single-breed (SB) populations. Multi-breed evaluations were also investigated in pigs with up to 207k sequenced/imputed individuals. We examined three genotype sets: regular SNP chip, top markers in each 40k genomic window, and significant SNP based on GWAS combined with the chip. In the MB evaluations, selected SNP were from a MB GWAS. Three terminal pig breeds were evaluated for five traits, namely average daily feed intake (ADFI), average daily gain (ADG), backfat (BF), ADG recorded in crossbred animals (ADGX), and BF recorded in crossbred animals (BFX). All three terminal breeds were combined for the MB analyses. Maximum gains of 0.01 and 0.03 in GEBV accuracy were observed for MB and SB with selected sequence variants. Regarding MB evaluations, breeds with fewer sequenced animals had a decrease in accuracy when models involved the top markers in each genomic window of 40k SNP. This might be because those top SNP selected from MB GWAS could be related to causative variants in the largest breed but not in the small ones. The benefit of using sequence data depends on the breed, size of the sequenced population, and how the whole-genome sequence variants are preselected. However, even when sequence is available on hundreds of thousands of animals, the advantage of this data is limited. In this talk, I will review the recent applications of sequence data for genomic predictions and summarize why no benefits have been observed so far. Finally, I will provide perspectives on when sequence can be helpful for genomic evaluations.

Late gestation heat stress programs reduced performance and survival phenotype in dairy cattle

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Over the past decade, extensive evidence has accumulated to support the concept that heat stress in the dry period alters the trajectory of maternal mammary development, immune status and ultimately reduces milk yield and health in the next lactation. Of perhaps greater interest, studies now indicate that heat stress in utero programs the developing calf for a lower productivity and lower survival phenotype. Compared with herdmates born to cooled dams, calves from heat stressed dams are born at a lower bodyweight, and that reduction in growth that persists through puberty. In utero heat stress also reduces the uptake of immunoglobulin G from colostrum, which was associated with accelerated gut closure from birth. At maturity, those heifers born to heat stressed dams produce 2–4 L/d less milk than herdmates from cooled dams throughout the first three lactations. That yield drag results from gross and cellular structural shifts in mammary gland development that are apparent from birth, and reductions in alveolar number are present in the mammary gland in the first lactation, which suggest a compromised capacity for milk secretion. Metabolically, calves that experience in utero heat stress have altered responses to insulin and glucose challenges that suggest a greater uptake of glucose at peripheral tissues and increased adipose accumulation. Other systems that are compromised include the reproductive axis, wherein lower AMH and follicular reserve are noted following in utero heat stress. The heat stress insult alters the development of skin structures related to heat dissipation, which may explain the improved capacity for heat loss in calves that are heat stressed in utero relative to cooled herdmates. Thus, a number of whole animal, tissue and cellular level observations indicate a profound negative impact of in utero heat stress. Further investigation has revealed that heat stress in utero shifts the methylation patterns across a number of tissues, and therefore offers an epigenetic mechanism to explain the observed phenotype. Ultimately, the lower performance and health observed in these calves born to heat stressed dams leads to a reduction in the survival time within the herd, and there is evidence that they pass on that lower performance phenotype to their offspring. There is no evidence that these observed effects of in utero heat stress can be reversed after birth, thus, appropriate management interventions during late gestation must be considered to avoid long term reductions in performance and survival.

Precision Ag comes to dairy: genomics tools to improve profit, environmental footprint, and cow wellbeing

Fernando Di Croce, Brenda Fessenden, Michael Layfield, Daniel Weigel, Giuliano Pisoni and Robert Wijma

Genomic selection has revolutionized dairy cattle breeding and transformed dairy genetics more than any other innovation since artificial insemination. Genomic information improves selection capabilities and provides an unprecedented opportunity to help improve health, fertility, and production efficiency in dairy cattle. Since 2016, a comprehensive suite of dairy genomic predictions for economically relevant health and wellness outcomes have been developed with methods and associated validation studies documented in the scientific literature. Furthermore, these health and wellness traits have been integrated into economic selection indexes designed to help producers make more profitable decisions. In a recent Zoetis study, the efficacy of the genomic economic selection index (DWP$) was evaluated to predict observed lifetime profit in US Holstein animals. The results of this study indicated that genomic selection indexes including wellness traits predicted significant differences in observed lifetime profit, IOFC, ECM, health disorders incidence, and days in milk of Holstein animals. Using a discount rate of 10.5%, every 1-point increase in the genomic selection index was associated with $1.84 more in observed lifetime profit. In the same study, cows in the best genomic quartile for DWP$ showed 55% less metritis, 33% less mastitis, and 42% less lameness than the worst genomic group. This study demonstrates that in well-managed herds, using well-designed genomic selection indexes, genetic progress may result in demonstrable profit, improved cow health, and cow welfare. Importantly, the genetic improvement derived from the use of genomic indexes has also been associated with a reduction in the dairy cow's environmental footprint. As dairy cows become healthier and more productive, efficiency improves via the dilution of maintenance effect, and both resource use and enteric methane emissions are reduced per unit of milk. In another Zoetis study analyzing genomic data and on-farm records, the top 25% of cows with superior genetics based on DWP$ demonstrated 10% less enteric methane emissions, 44% less antibiotic usage for their lifetimes and 5% less feed for maintenance purposes as compared to the inferior 25% group. These reductions were achieved while also producing 35% more milk and generating an average of $869 more lifetime profit per cow than those cows in the inferior genetics group. Therefore, healthy and efficient cows are both profitable and contribute significantly to long-term sustainability outcomes. Additionally, the demonstrated value and accuracy of genomic predictions have driven (and enabled) adoption of strategies including heifer culling/reducing replacement inventories, strategic semen allocation, mate allocation, embryo programs, and changes in herd management following analysis of observed response relative to genetic potential. These breeding strategies using genomic information can be used to inform selection, breeding, and management decisions in an overall precision animal health approach to optimize production efficiency, reproduction, health, and overall profitability. Furthermore, genomic predictions are key for algorithm development using sensor technology and leveraging new traits such as feed and water efficiency, methane intensity, and heat tolerance. Genomic information provides the opportunity for farmers and veterinarians to identify and select cows genetically predisposed for better health, and proactively install differential management strategies for those with greater risk of undergoing health disorders, both resulting in reduced dependency on antibiotic usage and improved animal welfare.

Improving dairy herd monitoring and management using automated monitoring technologies

Julio Giordano

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Health disorders (HD) affect a substantial proportion of dairy cows negatively impacting their health, welfare, and performance. In response, dairy farms spend substantial amounts of time and money to identify, treat, and care for cows that suffer from HD. For example, a systematic, once- or twice-daily evaluation of health is typically performed for the first 1–3 wk after calving in most dairy farms. Such clinical exams disrupt natural behavior and are time-consuming, labor intensive, and inherently subjective. The burden of cow health monitoring continues to growth as qualified personnel becomes scarce and labor costs rise limiting the amount of personnel and time available to evaluate individual cows in dairy herds that continue growing. Moreover, interest in improving cow care, cow performance, and dairy-producer quality of life, are driving automation of management tasks. Automated or semi-automated milking, estrus detection, and feeding are all being rapidly adopted by numerous dairy farms in the U.S. and the world. In this regard, there has been an explosion in automated monitoring systems that use sensors to measure behavioral, physiological, and performance parameters such as rumination, activity, lying time, body temperature, milk volume and components, milk conductivity, body condition score, and body weight.

Automated health monitoring technologies can only be effective if cows affected by HD present alterations of the patterns of sensor-monitored parameters. Moreover, the deviations from expected patterns and temporal shifts around clinical manifestation of HD must be of sufficient magnitude to be detected through visual inspection, statistical methods, or data analytics techniques. Recent studies with lactating dairy cows demonstrated that cows affected by health disorders manifest alterations of sensor-monitored parameters of sufficient magnitude to be detected through specific algorithms or visual inspection of data. For example, cows fitted with accelerometer-based neck- or ear-attached sensors and affected by HD presented different trajectories of rumination time, eating time, physical activity, and lying time immediately before, during, and after clinical diagnosis of HD in the early postpartum period. The magnitude and temporal dynamics of changes for sensor-monitored parameters is also relevant because alterations in the pattern of a parameter of sufficient magnitude to be detectable before clinical manifestation of disease is critical to the ability of automated health monitoring systems to flag cows for clinical examination. Although there is substantial variation across automated health monitoring systems and types of parameters monitored, some studies have shown promising results. Data support the notion that there is an association between the degree of alteration of the pattern of behavioral parameters monitored by sensors and the clinical status of cows. Specifically, rumination, activity, and lying behaviors were affected considerably more in cows with multiple and potentially more severe clinical signs of disease. Therefore, automated health monitoring systems that monitor and use behaviors such rumination time, physical activity, and lying time either directly or indirectly to generate health alerts might be more effective for identifying cows affected by HD that cause more severe alterations to cow behavior and multiple HD because these cows manifest multiple and more severe clinical signs of disease at the same time or within a timespan of a few days. The observed temporal shifts of sensor data patterns, the magnitude of the absolute and relative changes, and the timing of the nadir and peaks for different parameters such as rumination time, physical activity, and lying time suggested that monitoring these parameters might be useful for screening cow health in early lactation. In this regard, some studies evaluated the ability of automated health monitoring systems including rumination and physical activity to identify cows with HD based on automated alerts and the interval between these alerts and the timing of clinical diagnosis disease. This research demonstrated that most cows affected by metabolic-digestive disorders had health alerts at the same time or before clinical diagnosis, whereas the ability to detect cows with metritis and mastitis was moderate. For uterine and udder health disorders, cows were identified through automated alerts only in cases that affected cows systemically or if cows were affected concomitantly by another health disorder.

More recently, some experiments have also explored if implementing health monitoring strategies that rely primarily on automated health alerts can be as effective as intensive traditional health monitoring programs that rely heavily on extensive clinical examination of cows. For example, in an experiment conducted by our group, we evaluated a fresh cow health monitoring program for which cows were selected for clinical examination based primarily on automated health monitoring systems alerts. We were interested on the effect of this novel monitoring program on herd performance and the ability to identify cows with HD when compared with a more traditional monitoring program based primarily on compulsory clinical examination of cows. The only difference between experimental treatment groups was the method used to select cows for clinical examination. For the traditional monitoring strategy, all cows underwent an extensive clinical examination up to 10 d after calving. Thereafter, cows with a reduction in milk yield, or suspected sick by visual observation during a walk-through of the fresh pen underwent clinical examination. Conversely, for the program designed to rely primarily on automated health alerts, cows only underwent extensive clinical examination if cows had alerts from a health index score created based on rumination and activity, milk yield reduction alerts, or detected visually if missed by the alerts. Data from this experiment supported the hypothesis that implementation of a program designed to rely primarily, but not exclusively, on the identification of cows for clinical examination based on alerts from automated health monitoring systems would not have detrimental effects on herd exit dynamics, productivity, and reproductive performance outcomes. Notably, similar herd performance was observed despite a 30% smaller risk of diagnosis of health disorders and a 4 percentage point smaller total cows diagnosed with HD for the program using automated alerts. Collectively, results of this first of its kind experiment, indicated that dairy farms may be able to successfully implement a health monitoring program that relies primarily but not exclusively on automated health

The known and unknown African cattle genomes

Holivier Hannotte

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In this presentation, I will review our current knowledge about the genome diversity of indigenous African cattle in relation to their origins, migration, dispersion, crossbreeding and natural adaptation. It is now well established that the first cattle to reach the African continent were of the taurine type. Subsequently, the continent saw the arrival of humped cattle (indicine). It was followed by centuries of crossbreeding and hybridization, which shaped today's African cattle genomics landscape. At the roots of these events were trading networks (within the continent and between Africa and Asia), natural selection (climatic, vector-borne diseases) and ancient human dispersion events. Here, the role and importance of African civilisations and societies remain underrated despite the socio-cultural importance of cattle pastoralism across the continent. European colonisation saw the arrival of exotic improved germplasm, adding a new layer of cattle diversity. Still, several unknowns remain, such as possible African domestication or introgression from African auroch and the eventual presence of multiple landing waves of Asian indicine. The known and the unknown of African cattle diversity will be put in the broader context of today's needs for improving cattle productivity in a continent witnessing climatic turmoil.

Unravelling the complexity of animal-microbe interactions

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Animal-microbe symbiosis plays a key role in animal health and productivity, yet the true nature and complexity of these interactions remain largely unknown. One of the reasons for that is the fact that most microorganisms are unculturable, making them difficult to study. Fortunately, in recent years, culture-independent technologies have become more widely used, enabling the study of previously unknown microbial species. This new scenario has allowed scientists to produce vast amounts of microbiome data, making it possible to better understand the animal-microbe relationship. This symbiotic relationship involves microorganisms such as bacteria, fungi, protozoa, and viruses, which live in and on the bodies of animals, and in many instances provide essential services to the host animal. These services can include aiding digestion, combating disease-causing organisms, and providing essential vitamins to the host. The animal, in turn, provides the microorganisms with a safe environment to live and reproduce. This relationship is therefore essential to the health and well-being of both the animal and the microorganisms. In summary, there are two main metagenomic approaches applicable to animal science studies: (1) marker gene studies and (2) whole-genome shotgun metagenomics. Marker gene studies rely on sequencing a specific region of the gene, and have as main example the 16S rRNA gene (to analyze archaea and bacteria), the 18S rRNA gene (for eukaryotes), and the internal transcribed spacer (ITS) region (to study the composition of the fungal community). On the other hand, whole-genome shotgun metagenomics attempts to sequence all the genomes present in a sample, in order to analyze its biodiversity and microbial function capabilities. Since the entire genetic material of a sample is theoretically being studied, whole-genome shotgun metagenomics makes it possible to characterize a more complete diversity of a habitat, including bacteria, archaea, eukaryotes, and viruses. Both the marker gene and whole-genome shotgun approaches have strengths and limitations, and are becoming increasingly more available to animal scientists, allowing the field to gain a better understanding of the importance of the animal-microbe relationships at different levels.

Innovative practices and techniques dealing with the environmental impact of livestock farms

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In Western Europe, in last 30 years, emphasis of environmental public and policy debates shifted gradually from nitrate and phosphorus leaching to groundwater and rivers, to emissions to the air and maintaining biodiversity. Reduction in antibiotic use was another challenge on the way. Presently, ammonia emission from livestock manure is the hot topic in the Netherlands and Belgium, while the entering of wild animals in rural areas is becoming another hot topic. Ammonia deposition threatens certain vegetation in the Natura 2000 areas. Therefore, the level of nitrogen precipitation needs to be reduced. Besides this, focus on methane is increasing. Methane (GHG) emissions are related to climate change. Methane comes partly from manure, but mostly from the ruminating animal.

Complying with the intensive societal discussions, the Dutch Dairy chain has set goals for 2030 to increase sustainability on the topics antibiotic use, welfare, grazing, climate, biodiversity, new business model and land based farming. In addition, a coalition of several Dairy organizations have set management goals about dilution of manure, grazing and protein in ration together with the Ministry of Agriculture to reduce the nitrogen losses, especially ammonia emission. There is potential to reduce ammonia and methane emissions with these management measures and investment in housing systems like floor types to separate feces and urine, daily removal of manure from the barn, different freewalk housing systems and cow-toilet. Injection of manure (slurry) into the fields to prevent emissions was already introduced 20 years ago.

The challenge is to design a cow barn that improves animal welfare, manure quality and reduces emissions. These indicators were studied in case control studies with groups of 16 cows at research station Dairy Campus. The Cow-toilet is an automatic urinal that cows use voluntarily in a concentrate feeder. It collects 35% of the urine production and reduced the ammonia emission by around 35–45%. A permeable plate on a slatted floor improves the walkability of the cows and collects all the urine underneath the floor. The ammonia emission can be reduced between 35% and 50% by acidification of the urine, flushing the plates with the urine or by spraying 20-liter water per cow per day on the floor in combination with a urease inhibitor. A freewalk housing system with woodchips bedding material decreased the ammonia emission with 32% but did increase methane emission with 30%. A new development is a freewalk system with sand bedding that separates the urine by drains at the bottom of the bedding. The feces are picked up by a bedding cleaner behind the tractor. Primary separation of manure with a different floor type in a cubicle stable or with an artificial floor or sand bedding in freewalk housing can make a dairy farm more sustainable and may offer arable farmers more opportunities to select the right fertilizer or soil improver. In addition, the reduction potential of ammonia and methane emissions by grassland management and lower protein feeding is being studied. The data of 12,000 dairy farmers using the Annual Nutrient Cycle Assessment tool (ANCA) were analyzed and show the importance of fertilizing, feeding and housing systems on the emissions of ammonia and GHG.

Various strategies can be applied to reduce methane emissions. One strategy is to adapt the animal to the environment and the other is to adapt the environment to the animal. Practices of the 1st strategy are feeding practices, e.g. adding methane blockers to the feed or lowering protein in feed, and genetics, i.e. selecting for low methane animals. Recently, we study the filtering of methane and ammonia from air, a practice belonging to the 2nd strategy.

In some intensive European livestock areas, air washers (scrubbers) are common in pig housings to filter ammonia from the air. The air is sucked into a water basin where ammonia reacts with acid to dissolve into the water phase. This technique was not adopted in cattle housings, mostly because those facilities have an open structure, making capturing the airstream difficult. Moreover, capturing of methane from air in cattle houses is complicated due to the very low concentration in barns (in 60 farms spread over Europe ranging from 5 to 80 ppm at 2 m height) and low solubility of methane in water. In a dairy housing about 30% of methane comes from the manure and 70 % from the mouth of cows through the physiological process of rumination. In practice, air washers are recently also introduced in dairy housings, in which the ammonia is filtered from the air of manure storages.

We studied the challenging possibility of simultaneously filtering methane and ammonia from the air in the housing and/or manure storage facilities. Three separate processes were examined: (1). Air circulation: trying to combine the air containing methane and ammonia in one flow; air is sucked both from the manure storage and from close to the animals to capture the methane originating from rumination. The effectivity of capturing methane and ammonia from barns with varying degrees of openness and by use of cow hood samplers, and the potential of increasing the methane concentration by recirculating air (concentrations of about 200–500 ppm can be achieved). (2) Filter techniques: study of the effectivity of absorbing material, bio-bed and land/soil filters for oxidation or conversion of methane and ammonia in the captured air from the dairy house (bio-beds are a viable route). (3) Potential re-use of filtered N and C materials (leads to precision farming). It became clear that filtering of methane from the air of barns forms the biggest challenge to solve.

In coming years, environmental issues will continue to impact the livestock sectors. The EU Green Deal and Farm to Fork Strategy policies emphasize this development. The route to a sustainable livestock sector is a big challenge affecting farm families, service organizations and industries around the farming community and the rural areas.

Old wine in new bottles: factorial analyses in the age of multi-omics

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Factorial analyses are used across a wide variety of modern scientific disciplines, but they have a long and rich history. Principal Component Analysis (PCA), which is arguably the best known among them, can be traced back over a century to work published by Karl Pearson in 1901. However, the full potential of these methods could only be fully explored more recently with the advent and the rapid rise in power of computers.

Roughly speaking, factorial analyses aim at reducing the dimensionality of a dataset while retaining as much of the variation present in the dataset as possible. This is achieved by performing a singular value decomposition of the dataset, occasionally following an appropriately chosen transformation.

Initially, the use of these methods was restricted to the analysis of small-sized single data tables, with only a few variables. However, from the 1960's onward, advances in both data collection and computing power led to a renewed interest in these methods, with the so-called 'Exploratory Data Analysis' approach ('Let the Data speak'), championed by John Tukey, and the development of multivariate descriptive analysis tools, mainly in France with Jean-Pierre Benzécri, but also in other countries including Japan and Italy.

Their scope has gradually been extended to the analysis of two or more linked data tables (multi-table analyses, i.e. Multiple Factorial Analysis), as well as to approaches to explicitly model covariates (Redundancy Analysis).

More recently, technological advances have enabled the generation of high-throughput biological data, corresponding to large amounts of heterogeneous, complex and high dimensional data at multiple molecular levels (so-called multi-omics data, including the genome, transcriptome, methylome, etc). Comprehensive exploratory, descriptive and predictive analyses for these multi-omics data are critical for extracting their full potential.

The framework of factorial analyses provides a variety of flexible methods for this purpose, as it can integrate a variety of types of data (binary, qualitative, quantitative) and diverse data structures. For instance, multi-block analyses (e.g. Multiple Factor Analysis and Co-inertia Analysis) provide a synthetic view of the relative influence of each data block and provide a consensus representation of the full dataset. Partial Triadic Analysis is a special case of multi-block analysis that deals with so-called data cubes, where omics data are repeated (e.g. across tissues or across time). Interestingly, unlike predictive methods, these descriptive methods do not include a matrix inversion step, implying that they can be used without constraint on the number of samples (observations) with respect to the number variables, thus obviating the need for regularization. This represents a considerable advantage in the context of multi-omics data, where data blocks (e.g. transcriptome-wide expression in a given tissue) are often available for a limited number of observations (e.g. animals) measured on a huge number of variables (e.g. genes).

Finally, Redundancy Analysis (RDA), which corresponds to the supervised (constrained) version of factorial analysis methods, can be used to model omics data with respect to a set of covariates, such as sanitary, geography, climate, or herd system. One limitation is that only a small number of covariates with respect to the number of observations can be accounted for in such an approach.

We will illustrate the broad interest of different factorial analysis methods in the context of multi-omics data through three specific examples:

a) Quantification of the structuring impact of geography on genetic diversity in cattle and goats: an RDA on Italian bovine and ovine genomic data was used to quantify and compare the effect of geography on the genetic structuring of both species.
b) Identification of covarying gene expression and metabolite levels in layer chickens: a Co-inertia Analysis identified significant covariation between metabolomic and transcriptomic data of layer chickens subjected to different abiotic stresses.
c) Identification and characterization of individuals with atypical multi-omic profiles in a large-scale human cancer study: a Multiple Factor Analysis was extended in the *padma* approach to characterize global sources of variability from multi-omic data, identify individuals with atypical profiles within a population, and highlight genes and omics with a strong contribution to these profiles.

These examples illustrate the versatility of factorial analysis methods in the multi-omics era, and notably their strengths for tackling a variety of questions and structures and dealing with high-dimensional, complex, and heterogeneous data.

Bovine extracellular vesicles: a new challenge for animal science

Cristina Lecchi

Extracellular vesicles (EVs) are a heterogeneous group of cell-derived structures characterized by a phospholipid bilayer reflecting the donor cells. EVs include exosomes and microvesicles with a size from 30 to 150 nm and 150 to 1000 nm, originating from the endosomal system or being shed from the plasma membrane, respectively. EVs are involved in pathophysiological processes and are a mechanism for cell-to-cell communication, delivering bioactive cargoes including metabolites, lipids, DNA, and small RNA, which can induce phenotypic reprogramming of recipient cells. Physiological challenges like injury, inflammation, infection, disease, or application of exogenous abiotic stress like hyperthermia can alter EVs' molecular contents and their function. EVs can be isolated from several biofluids, including blood and milk, and can modulate the host immune response and the molecular pathway of several types of cells. In particular, milk EVs attract the scientific and industrial communities' interest thanks to their ability to protect cargo against degradation in the gastrointestinal tract and during industrial processing. They are absorbed following oral administration and cross barriers such as intestinal mucosa, placenta, and blood-brain barrier. Other salient features of EVs include their long half-life in the body, low immune response, good biocompatibility, ability to target cells through their surface biomarkers, and capacity to carry macromolecules. Thus, EVs may be used for the diagnosis and treatment of several disorders and as a targeted delivery system of drugs or bioactive molecules for veterinary and human purposes.

Knowledge of the cellular processes that govern EV biology is essential to disclose the pathophysiological functions of these molecular shuttles as well as on applications involving their use and analysis.

Leveraging genomics to achieve dairy net zero

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Together with other major international dairy organizations, Dairy Farmers of Canada has pledged to reach net-zero greenhouse gas (GHG) emissions from farm-level dairy production by the year 2050. Consequently, Canada is building capacity to measure and/or predict enteric methane (CH4) emissions for both herd monitoring and genetic tools. Data collection of CH4 emissions started in 2016 in two research herds using the GreenFeed System (750 cows as of December 2022). This data has been used to predict CH4 production for first parity Holstein cows using milk mid-infrared (MIR) spectra data between 120 and 185 DIM, and serves as input for a new genomic evaluation system launched in April 2023.

Furthermore, through regional and national initiatives, the recording of methane emissions will soon start in commercial farms equipped with milking robots using an emission sniffer device prototyped in Italy (Tecnosens.it, Brescia, Italy) with German-made CH_4 and CO_2 sensors (WiTek-sensorik.de, Wesel, Germany). Approximately 50 sniffer devices will be installed in up to 25 herds across Canada, which will enable a higher throughput of CH4 measurements across lactation, parities and from multiple breeds.

In addition, a new large proposal is under review with the overall goal to deliver a roadmap for GHG management in ruminant species. Specific objectives include development of scientific protocols that quantify impact and uncertainty surrounding GHG mitigation strategies (genetic, nutrition and management); estimation of individual animal and herd-level emissions; quantification of the benefits of reducing GHG emissions through genetic selection, nutrition and herd management strategies, as well as genotype by nutrition interaction; optimization of existent methane (CH4) emission data consolidation (including beef); enhancement of genomic evaluations for CH4 emission through mid-infrared (MIR) predicted CH4; and quantification of the value of CH4 emission reductions. The involvement of Agriculture Canada and Environment Canada will ensure that the results of this project will be relevant for inclusion in Canadian policy decisions and GHG inventories. Our target is to reduce GHG by more than 55% (-6.7 Mt CO_2eq directly in dairy. At a current carbon price of $50/tonne, this represents a value of $338M/year nationally. An additional saving of $102M/year will be realized due to improved production efficiency and animal welfare.

Precision technologies and smart dairy farming in extensive pasture based systems

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From both business and societal viewpoints, when we consider livestock farming, we need to focus on economic performance, environmental impact, well-being of the livestock unit and well-being of the of the manager to ensure the sustainability of farm and food production into the future, and also the consumer. Currently, these aspects of livestock production are being increasingly challenged on many levels. Precision technologies can help farmers to become more effective and help consumers to source products that satisfy all of these requirements through using data to enhance resource management, to inform the decision making process, and ultimately increase the precision of the operations.

From the dairy production system and farmer perspectives, precision technologies have potential in addressing two important areas, i.e. reducing farm labour requirements and increasing efficiency of production. For this potential to be met, a user need-driven approach has to be applied by the development companies, and a focus on how outputs arising from such technologies can be delivered to users to maximise their value is required. But even if these criteria are met, the farmer must be able to see that the technology represents a good investment and must be comfortable in using the technology.

There is significant overlap between pasture based and indoor systems with regard to technological development in the area of fertility and heat detection, body condition score, cow lameness, etc. However, implementation of technology in grass based systems does bring unique opportunities and challenges. The mobility of animals and the areas they graze in pasture based systems often have connectivity challenges; this together with the smaller market size and perceived demand relative to indoor milk production systems, may in part, explain the comparatively reduced use of precision technologies in those systems. Dairy farmers that rely on pasture as their primary source of feed do require accurate real-time measurement of pasture biomass to optimise cow nutrition and grazing management. This is more complex than some international dairy systems, where cows are primarily fed a mixed ration (with accurate mixing of feed ingredients) in confined housing. But there is now a clear need on pasture based farms for effective automated technologies to measure and provide decision support on pasture quantity and quality, animal health, heat detection, milking, and to gain some control over animal location and mobility.

Much work has been done in recent years at Teagasc and other research institutes pertaining to the development of precision grass measurement technologies. GrassQ represented a European project which encompassed both ground based and remote sensing grass measurement technologies; a prototype grass measurement tool that generated grass measurement protocols, which were optimised for both precision and labour efficiency, was developed within this project. Furthermore, a decision support tool (DST), PastureBase Ireland (PBI) was also developed at Teagasc and is becoming more frequently used by grassland farmers. PBI assists farmers in determining appropriate actions around grassland management, mainly by processing uploaded herbage mass (HM) cover estimations to determine appropriate herbage allocations in accordance with on farm growth rates. Recent data from PBI indicates that farmers using this system are utilising more grass than the national average (8 t DM year^{-1})

and are growing between 11–15 t DM year^{-1}. Further studies have utilised online DST databases to combine grassland management factors with measurement and meteorological data from local weather stations to forecast HM growth rates. Online tools similar to PBI have been developed in other European countries and an increasing amount of grassland data is being stored on cloud computing platforms. Such DSTs can also perform as national databases for research which is a significant advantage.

Significant work was also carried out on successfully integrating automatic milking with a pasture based system, where almost 100% of the cows' diet was supplied by grass and relatively high milk yields were sustained during the main grazing period. Different collar sensors which were originally designed for health and fertility monitoring of dairy cows and later incorporated additional behavioural traits have also been examined, e.g. the RumiWatchSystem was validated to record cow activity and feeding behaviour in a pasture-based system. Further work with collar sensors was conducted to identify possible indicators of insufficient or sufficient grass allocation by behaviour measures. The parameter of bite frequency was significantly different between cow groups having sufficient grass allowance or not; consistent differences were also observed between the groups for rumination time per day, rumination chews per bolus and frequency of cows standing or lying. Two automated body condition score technologies were also assessed; these technologies have a significant potential role, not only in feeding management but also in animal welfare evaluations at pasture. A further study examined an ear tag with an acceleration sensor integrated with a localization system. This was tested in Minnesota and in Ireland, and results suggested that there was potential for its use on pasture-based dairy farms, but it required further development. Some topics investigated proved intractable, e.g. virtual fencing. However, this was partly due to study design and early learning; a different approach to the virtual fence in recent research has shown it to be a valuable tool for maintaining animals in conservation grazing areas. It can support farmers through improved lifestyle (less requirement for manual labour) and reduced capital costs and it can also add to the safety and welfare of animals.

A limitation was observed across the research, i.e. each technology had their own databases and user interfaces. There is potential for systems to provide a database platform where data from multiple sensors and companies could be centrally located. A trusted, non-commercial stakeholder could deliver this, which would reduce the number of places a farmer would need to go to access their information. The need for robust testing of equipment before deployment in a commercial setting was also noted. While many companies claim to have developed solutions for different issues, if they have not been independently tested, many of these claims have little value. This is important because the initial period of use of a technology is generally the period where a farmer/user will gain or lose confidence.

There is limited information on the level of adoption of precision technologies and the factors associated with their adoption in pasture-based systems. Recent research at Teagasc examined the uptake of technology across the areas of reproductive, grass, milking and calf management on a representative survey of Irish pasture-based dairy farms from 2018. Adoption levels varied depending on the technology, with the most adopted being those related to the milking process. The results also suggested that herd size, proportion of hired labour, agricultural education, and discussion group membership were positively associated with a high intensity of adoption, whereas, age of farmer and number of household members were negatively associated with it.

It is important for research, technology companies and practitioners to develop and target strategies that would improve future adoption of precision technologies in pasture-based settings. It is also crucially important to focus on the considered view of farmers regarding the use of technologies to allow progress of precision farming within farming communities.

Livestock sustainability, the four slices of the same pie

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Sustainability has now become such a pervasive word that it now labels any human activity. Although polysemous, sustainability is generally understood in the environmental sense and, when it comes to livestock farming, in the component of greenhouse gasses (GHG) impacts produced by livestock supply chains. IPCC estimates, very similar to FAO's, place GHG emissions at around 14% of total anthropogenic emissions, including LULUCF, with methane being the largest source of impact. Agricultural and livestock science and technologies have developed applications that can reduce the climate-changing impacts of livestock production through four areas that we could consider as slices of the same pie called livestock sustainability: increased productivity, reduction of direct and indirect emissions, carbon sequestration, adoption of new metrics.

The increase in production per head reared was the most important driver of the reduction in emissions per functional unit. The phenomenon is explained by the decrease of requirements (and related emissions) for maintenance in favour of those for production per unit obtained. With constant production, the increase in productivity leads to fewer animals being reared with an overall reduction in emissions as well. This trend is common to all livestock sectors and, in the case of the Italian dairy cattle industry, has generated a drop in global emissions of 31% CO_2e from 1990 to 2020. Genetic improvement, the evolution of feeding and breeding technologies, animal welfare and the longevity of animals and the prevention and treatment of diseases all contribute to this.

Mitigation strategies able to reduce GHG impacts in the livestock sector focus on reducing both direct and indirect emissions. Mitigation options targeted towards the reduction of enteric CH_4 are of great relevance and are merely based on the modulation of methanogenesis (by using chemical inhibitors, electron receptors, ionophores, and dietary lipids) and on the decrease in emission intensity (by increasing diet digestibility, improving health and welfare, raising reproductive efficiency and promoting selective breeding for higher productivity). The inclusion of agro-industrial by-products and certified deforestation-free soy in animal diets provide a range of environmental benefits including carbon footprint reduction associated to concentrates use and, in general, to land use change. Finally, renewable energy generation designed to reduce CO_2 emissions could be identified as another solution to promote farm sustainability. Carbon sequestration by soil and biomass appears to be an opportunity of mitigation and can effectively contribute to the climate ambition of sustainability. In this context, the implementation of carbon farming practices is globally of growing interest. Carbon farms can involve measures to increase the carbon storage in soil, such as the adoption of minimum and zero tillage practices, the use of cover crops, and the improvement of crop rotation. Expanding the agroforestry, improving forest management, and reducing deforestation has an important global mitigation potential. Extensive livestock with pasture-based systems positively contribute to the storage of carbon in the soil. There is, also, a close link between carbon sequestration and emission reduction practices, especially in livestock systems, where the optimization of nutrients, manure and grazing management can contribute to reduce GHG emitted at farm level. When the environmental impacts related to animal products, such as milk and meat, are assessed, the contribution of carbon sequestration can be a key strategy to compensate part of GHG emissions associated to livestock production.

The adoption of new metrics is needed because the Global Warming Potential (GWP), the standard and widely adopted method proposed by the IPCC for estimate the GHG emissions reported as CO_2 equivalent (CO_2e), does not capture adequately the different behaviour between short- and long-lived gases impact on global temperature. For the livestock sector, where the emissions of short lived GHG (i.e. methane) play a crucial role in the definition of their environmental impact, these new metrics can serve to better assess the specific contribution to global warming. Among the proposals, the GWP* represents a new way to use GWP_{100} and describes the relative warming as a function of the timeline of a short lived GHG emission, giving more accurate information about the impacts, when scenarios of increasing or decreasing emissions are considered. Interesting results of the application of this new metrics have been recently published, highlighting that reducing methane emissions results in a cooling effect on the global temperature, rehabilitating in some way the livestock sector in the contribution to the climate change.

KEYWORDS: Livestock sustainability; productivity; reduction of emissions; carbon sequestration; new metrics

Digital technologies and machine learning: a new way to look at novel traits at spatial and temporal dimensions

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The advancement of livestock research necessitates precise measurement of target traits in animals or groups of animals exposed to various environmental conditions such as different diets, disease treatments, and management practices. Experimental studies on research farms ensure internal validity of results, while observational studies on commercial farms provide external validity. Both types of studies require longitudinal follow-up of animals and individual identification for monitoring their well-being, production, reproduction, and disease traits. Advances in digital technologies, including wearables and remote sensors, coupled with efficient data management and data mining techniques, now allow for accurate monitoring of animals and their environment in both spatial and temporal dimensions. These technologies enable investigations of novel traits related to product quality, animal behavior and welfare, individual feed intake, and gas emissions. This presentation highlights our research efforts in developing and adapting tools and methods for livestock applications, including specific research projects that implement such tools. For instance, we present a case study that predicts dairy cows future performance based on their growth and mammary gland development during calf-heifer stages.

Feed additives in ruminants feeding: update knowledge with focusing on natural additives

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As the demand for animal products increases along with the expansion of the human population, the need for feed additives in ruminants' diet also increases to increase animal productivity, maximize feed resource efficiency, and maximize gain for farmers. Increased production of ruminants to meet the growing need for nutrients from animals, animal production necessitates a steady expansion of the base of feed additives and components. This is done to preserve the balance between environmental exploitation and production. However, the aim of this presentation was to discuss the impacts and mode of actions of some feed additives such as probiotics, prebiotics, certain organic acids involved in metabolic pathways, herbs, and plant extracts which are used few amounts in ruminant diets and can offer some of the benefits than antibiotics supplementation.

Several feed additives are utilized in diverse animals for varied purposes. The use of feed additives including antibiotics, hormones, and others that pose a substantial risk to consumers or the environment is prohibited in many nations. As a result, the ruminant feed sector is growing more interested in other excellent substitute feed additives that may be preferred by the public without posing any health risks. Choosing the appropriate feed additive for ruminant feeding has become essential because of today's tight business margins and rapidly rising environmental contamination. Reducing the workload of technical employees that oversee herd health by using prompt feed additive at the appropriate time would increase overall profitability. Rumen feed digestion and ruminant productivity can be positively impacted by probiotics, dicarboxylic acids, enzymes, and substances derived from plants. The numerous ways by which these additives work is varied. Regardless of their differences, they all have an impact on the metabolic pathways involved in fermentation and/or the microbial community in the digestive tract. These compounds usually have several, refined ways of action, in contrast to antibiotics, which have a definite microbiological target. The type of feed and physiological condition of the animal has an impact on their effectiveness. To better comprehend the interactions that take place between hosts, feeds, and additives, further research is required.

Natural plant products are a special example among additives, due to the variety of bioactive components in these natural and unpurified preparations, its difficulty to determinate their effective quantities, and the effects on animal physiology and health are not well measured. Plant extracts must therefore comply by the same general regulations as non-natural products when used as feed additives, including that they must be safe for both the animal and the person managing the product, cannot be found in animal products as residues, and cannot pose a threat to the environment. Use of plant essential oils (EO) as feed additives for ruminants, only a small number of preparations methods have been standardized and commercialized. Many of the biologically active compounds in EO have antibacterial properties that shield plants from herbivores and diseases. Since EO are lipophilic, they interact with bacterial cell membranes and have toxic and antimicrobial effects, especially on Gram positive bacteria. The amount of EO varies significantly between different cultivars of the same species or between different plant organs (leaves, stems, roots, flowers, and fruits), depending on growth conditions, the age or physiological stage of the plant, and the methods used for extraction and processing. Chemotypes are different plants of the same species or genus that superficially look the same but exhibit a change in their chemical components, frequently because of climate, altitude, or soil circumstances. The EO are becoming more significant in ruminant nutrition as a means of lowering enteric methanogenesis and enhancing animal health and production. Most of the biologically active compounds in EO have antibacterial properties that shield plants from herbivores and diseases. Since EO are lipophilic, they interact with bacterial cell membranes and have toxic and antibacterial effects, especially on Gram-positive bacteria. Moreover, several plants contain saponins, which are glycosides. A hydrophobic aglycone called 'sapogenin', which may be terpenoid or steroid in origin, is glycosidically connected to a sugar moiety in these substances. The many different aglycone structures, the types of side chains already present, and the locations where the sugar fractions are connected on the aglycone are what give saponins their enormous variety. The non-polar sapogenin unit and the water-soluble sugar work together to give a saponin its capacity to foam. The most popular commercial sources of saponins are active ingredients isolated from either *Yucca shidigera* (steroid saponins also known as sarsaponins) or *Quillaya saponaria* and *Sapindus sp*. (triterpenoid saponins). The two main saponin-rich plant foods that are frequently found in ruminant diets and may play a role in certain facets of animal nutrition are lucerne and soybeans. Two methods have been proposed to explain how saponin administration in ruminants improves the absorption of nitrogen from feed by animals because less NH_3 is formed in the rumen and less urea is excreted in urine. In addition, NH_3 produced by microbial protein degradation can be bound by saponins in a balanced chemical reaction that is controlled by NH_3-N concentration.

However, a wide variety of secondary metabolites that are not essential to a plant's growth, development, or reproduction are produced by plants. These substances help plants fight off predators, parasites, and diseases. They also shield them from interspecies competition and can draw insects that help plants reproduce. Before the discovery of antibiotics, they were widely utilized as medications. Tannins are one of these substances. Condensed tannins or proanthocyanidins, which are polymers of flavonoid units connected by highly stable covalent carbon-carbon bonds, and hydrolysable tannins, which contain a carbohydrate with hydroxyl groups partially or completely esterified with phenolic acids like gallic acid (gallotannins) or ellagic acid (ellagitannins), fall into two different chemical categories. The ability of tannins to chemically bond with proteins has historically been extensively exploited to transform fresh animal skin into rotproof leather. The same feature was investigated as a means of reducing ruminal NH_3-N production and nitrogen excretion in urine as well as slowing dietary protein breakdown in the rumen and improving amino acid bioavailability in the small intestine. The production of ruminants and feed digestion in rumen can both be favorably influenced by feed additives such as probiotics and products derived from plants. These additives come in a variety of forms and work in several ways. Despite their diversity, they all eventually have an impact on the metabolic pathways for fermentation and/or the microbial community in the digestive tract. The kind of feed used, and the animal's physiological condition have an impact on its effectiveness. A fully comprehend complicated interactions that find and take place between hosts, feeds, and additives. This knowledge will allow us to identify the potential applications of a particular feed additive, hence minimizing inconsistent field responses.

KEYWORDS: Feed additives; ruminants; secondary metabolites; mode of action

Building better knowledge on meat quality determination through integrated data mining and curation of proteomics studies

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Meat quality is very important for consumers, processors, and producers. The traditional methods for assessing meat quality are subjective and time-consuming, and they do not provide a comprehensive understanding of the factors that affect meat quality. Proteomics, a high-throughput technology for the analysis of entire proteins, has emerged as a powerful tool for investigating the molecular mechanisms underlying the conversion of muscle into meat, meat quality variability, and the discovery of biomarkers. However, the large amount of data generated by meat research proteomics experiments in the last two decades can be overwhelming, and the integration of published meat proteomics datasets through data mining approaches and bioinformatics is a promising way to expand our scientific knowledge. Furthermore, mining such data in their biological context may allow the extraction of novel and more robust biomarkers of meat quality traits. Data reuse is a leading, active, and evolving field suggested and very recently applied in the meat research field to rediscover and reshape the public proteomics data for a better understanding of the unknowns related to meat quality determination and refinement of the list of biomarkers so far proposed. In this talk, the application of integrated data mining and curation approaches to improve our understanding of the molecular basis of meat quality will be presented. The focus will be on important meat quality traits and their defects such as tenderness, color and post-mortem processing treatments (e.g. electrical stimulation). The most updated databases, patterns, knowledge and relationships between different factors and meat quality gained thanks to the integromics studies will be presented. Overall, integrated data mining and proteomics studies curation allowed for providing a comprehensive view of the biochemical mechanisms and molecular signatures underlying meat quality, which all together allowed better insights of their affecting factors. These novel approaches further allowed proposing robust biomarkers for the development of more accurate and efficient methods/tools for assessing meat quality. Integrated data mining and curation of proteomics studies will have important implications for the meat industry, as they will provide new insights into the factors that affect meat quality.

Technologies applied in embryonated eggs

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Implementation of precise solutions to develop natural and sustainable ‘immunity’ in the animal is an element of a systemic approach defined by World Health Organization, and referred to as the One Health. Stakeholders of poultry production seek solutions to eliminate and/or control the pathogens transmitted with food and animals. Intentional stimulation of embryonic development and determination of the future post-embryonic health of the organism is possible by *in ovo* application of natural antioxidants and prebiotics, gut stabilizers like probiotics and other immunological enhancements, including vaccines. In light of the above, advancements in biotechnology and robotization have led to a fast development of several break-through practices in poultry hatcheries: *in ovo* vaccination, *in ovo* feeding, stimulation of embryonic development using prebiotics, probiotics and synbiotics, thermal manipulations and embryo sex determination. The work flow of a modern hatchery is almost fully automatized, and as such serves as an excellent, controlled space for implementation of novel *in ovo* techniques with elimination of human error and potentially adverse environmental factors. A post-embryonic life of a broiler chicken is limited to about 42 days. Therefore, considering the complete period from egg fertilization to animal maturity, the embryonic development in egg (21 days) constitutes a one third of a total life span of the animal. This biological feature enables poultry production to be highly transformative and allows for a shift of some of certain treatments from post embryonic phase (vaccination and synbiotic supplementation), to the pre-hatch period. Since the first successful immunization of chicken embryos with HVT (Turkey's Herpes Virus) in 1980's, the *in ovo* vaccination systems have been implemented in 90% hatcheries in United States. In Europe, the vaccination in hatcheries has expanded over the last several years, especially in Spain, Portugal, Italy, France, Germany and Poland. Over the last decade, a portfolio of *in ovo* multivalent vaccines available for the control of Marek's and Gumboro' diseases, and recombinant vaccines to control Newcastle disease has been constantly enriched. There are new *in ovo* vaccines under development to control avian influenza and Salmonella infections. A long lasting protection of a bird is ensured after a minimal single dose of a vaccine (50 microliters) injected into the amniotic sac or intra-embryo between 18.5 and 19 days of egg incubation. There are several vaccinating machines available on the market that allow for *in ovo* immunization at an average capacity of 70000 embryos per hour. Another early strategy applied in embryonated eggs to combat the first post hatch challenges (hatching window and late onset on farm), is *in ovo* feeding applied between 17 and 19 days of embryo development. However, the concept of *in ovo* feeding is well known, it is too early to measure the scale of it's effects in practice. No nutrients or other formulations (vitamins, minerals, and amino acids preparations) have been commercially registered specifically for *in ovo* feeding yet. The European Food Safety Authority (EFSA) has recently added a new category of feed additives called 'zootechnical additives' including: digestibility enhancers (enzymes), gut flora stabilizers (typically probiotics), environmental enhancers, immunity enhancers, and animal welfare improvers. A major technical challenge that limits the use of majority of the implemented vaccination systems for the purpose of *in ovo* feeding is due to a necessity of a precise injection directly to the amnion. Whereas the vaccine can also be administered to the embryonic body, subcutaneously. There is a proven concept for efficacy of a strategy to modulate the immune system and gut development on day 12 embryo development, by injecting an optimized dose of a prebiotic or synbiotic (200 microliters) to the egg air cell. Previously, such a stimulation showed to accelerate the development of immune organs and reactivity of gut associated lymphoid tissue. It also leaded to establishment of beneficial profiles of gut microbiota in mature chickens and improved metabolic processes through liver function. Moreover, the favorable meat composition was observed and evidences for stimulated muscle development through a better microvascularization. There has been a research ongoing to verify hypotheses, that a stimulation with synbiotic on day 12 of embryonic development can be considered an epigenetic factor, and that the epigenetic impact caused be in ovo synbiotic stimulation could be heritable. This format of evidences would allow to set fundaments for development of new nature-based epigenetic breeding strategies. Another novel line of research has been initiated based on an implication that the probiotics may have a potential to modulate (enhance) the immune response to vaccination and can work as *in ovo* vaccine adjuvants. In a pilot study, the synbiotic added to *in ovo* vaccine against Gumboro disease led to an early production of IBD antibodies (accelerated immunization effect) and improved the gut bacteriological profile in adult chickens. Based on previous evidences, such an effect can be explained by a synergistic mode of function of the synbiotic. In human studies, the probable adjuvant role of probiotics was identified for oral vaccines. The probiotic compound, apart from sensitizing the enterocytes receptors, was expected to modulate polarization of dendritic cells that influences the lymphocytes responses. This assumption could be extrapolated to *in ovo* treatments referring to a fact that the vaccine with a probiotic/synbiotic must be delivered to the amnion (therefore, ingested in a similar way as oral administration). The above strategies are now subject of an extensive investigation leaded in parallel by several research groups, globally. To summarize, mass production of poultry meat and eggs have an undisputable impact on nature, but due to the unique biology of birds that allows for a full management of embryo development, it is possible to implement transformative strategies in hatcheries to produce environmentally friendly and healthy food while minimizing the use of energy and environmental impact. Poultry industry plays a key role to deliver affordable animal products (eggs and white meat) of undisputable high nutritive value recommended for all consumer groups, including the infants, elders, and patients with special dietary needs. Moreover, the OECD/FAO (2022) predicts that in the context of the Zero Hunger initiative, consumption of poultry meat would increase globally to 154 Mt by 2031 (which is 27% higher than the growth in consumption predicted for pork and twice as higher than for beef). The new common agricultural policy (CAP) and the European Green Deal indicated aspiring targets concerning the sustainability and safety of food production to be achieved by innovation driven agricultural research. In that context, development of early life strategies in poultry seem to take an important position in the action plan to accelerate food production systems transition.

KEYWORDS: In ovo programming; poultry; health; chicken embryo

Cytogenetic and molecular background of canine and feline disorders of sex development

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Classification of disorders of sex development (DSD) is based on sex chromosome complement. There are three major DSD categories: (1) caused by an abnormality of sex chromosomes, (2) cases with normal XX complement, and (3) cases with normal XY complement. Sex development is controlled by dozens of genes and the *SRY* is a major gene responsible for the triggering development of the undifferentiated fetal gonads into testicles. Thus, the first step of genetic diagnosis of DSD cases is cytogenetic analysis, followed by molecular detection of the *SRY* gene.

Cytogenetic diagnosis of DSD dogs usually shows a normal XX or XY sex chromosome complement, however, there are also reports on X monosomy, X trisomy, XXY syndrome, leukocyte XX/XY chimerism and X/autosome reciprocal translocations. In DSD cats, the incidence of sex chromosome abnormalities seems to be much higher. Until now we identified 9 DSD cats with abnormal sex chromosome set, including X monosomy, XXY syndrome, XX/XY leukocyte chimerism (2 cases), X/Y translocation (2 cases), X/XY mosaicism and 37,X/38,X,r(Y) mosaicism. Among these cases, the most interesting is X/Y translocation, which caused a transfer of the *SRY* gene from Y to X chromosome and the presence of XX^{SRY+} karyotype, which in humans is called de la Chapelle syndrome (XX males). These two cats are the only such cases diagnosed in domestic animals until now.

Searching for gene mutations causing DSD in dogs and cats with a normal set of sex chromosomes is not advanced. A single common mutation and two rare mutations were found in XY DSD dogs. In Miniature Schnauzers, a missense substitution in the *AMHR2* gene is quite distributed. On the contrary, a 2-bp deletion in the *HSD17B3* gene and a large deletion of 4 exons in the *NR5A1* gene were identified in single XY DSD dogs. In XY DSD cats, two rare mutations in *CYP11B1* and *TAC3* genes and one risk variant in the *LHCGR* gene, were reported.

In female dogs with normal XX chromosomes, the testicular or ovotesticular DSD is quite common, and until now, it was diagnosed in numerous breeds (>40), however, its incidence in French Bulldogs, American Staffordshire Terriers and Pugs seems to be higher than in other breeds. Searching for the molecular background of this form of DSD did not bring clear results. There are several cases with duplication of the entire *SOX9* gene. Moreover, associations with CNV (copy number variation) upstream of the *SOX9* or SNP in the *PADI6* gene were also reported.

The most common type of DSD in dogs and cats is cryptorchidism, which is a polygenic disorder. There are two SNP polymorphisms in *RXFP2* and *HMGA2* genes, which are potentially associated with the predisposition of dogs to this disorder. Recently, we performed a comparative analysis of gene expression in descended and undescended testicles of dogs with unilateral inguinal cryptorchidism. This analysis showed that over 8000 genes are differentially expressed, however, the comparison between descended testicles of unilateral cryptorchid dogs and testicles of control dogs showed no difference. This result suggested a lack of DNA variants responsible for the altered gene expression in cryptorchids.

Hypospadias is another polygenic form of DSD. Its incidence in dogs and cats was not precisely estimated, however, it seems that at least in dogs, it is not a very rare condition, as it was suggested in the literature. Molecular studies of this DSD are not advanced, in spite of the fact that sequences of several candidate genes (e.g. *MAMALD1, SRD5A2*, *AR* and *DHH*) were analyzed.

In conclusion, genetic studies of canine and feline DSD recently have brought new insights, however, the progress is still limited. It is mainly caused by a small number of laboratories which are specialized in cytogenetic diagnostics of these species. One can expect, that the use of new molecular techniques and tools (whole genome sequencing, SNP microarray, etc.) will enable the involvement of molecular labs in such studies.

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The use of machine learning in the genomic era of livestock farming

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The availability of genotypic and longitudinal environmental data in dairy cattle farms offers interesting perspectives in phenomics. Indeed, the direct analysis of quantitative traits can be conducted by coupling the genomic information, nowadays obtainable at low costs on all the individuals of a herd, with the environmental conditions collected by farmers and by sensors (up to multiple times a day). This large amount of available data within herd opens the possibility for the use of artificial intelligence techniques in livestock farming, especially focusing on phenomic prediction.

In this context, we present a machine learning approach to forecast the lactation curve, exploiting within-herd data collected by automatic milking systems (AMS, i.e. milking robots) and genotypes. The choice of the phenotype is motivated by the availability of daily collected milk production records in the analysed farm, as well as by the importance of lactation curve predictions for herd management, animal monitoring and early disease detection; however, the methodological approach we propose can be extended to any quantitative trait, such as fertility and resiliency ones.

The study is conducted on a Holstein Friesian herd of 533 cows situated in Lombardy; milk production and environmental records were collected daily for 26 consecutive months (between February 2020 and March 2022), resulting in about 168000 available observations from over 900 lactations; genotypes of all females were obtained with the Neogen GGP Bovine 100k SNP chip and the genomic EBVs were calculated by the breeders' association.

Our model uses genotypic and environmental variables to predict the individual daily milk yield. We consider days in milk, lactation number, month of calving and age at calving (in months) as environmental features; moreover, we may integrate some additional factors, namely the concentrate supplementation provided in the AMS and the number of daily milkings (averaged on the last seven days), which are not traditionally considered in the literature. Regarding the genotypic features, we find out that the estimation of each SNP additive genetic value in the problem formulation is not beneficial, due to dimensionality issues and the negligible effect that every individual SNP has for the quantitative trait under analysis. For these reasons, our approach envisages the estimation of the genomic EBV (i.e. genomic prediction step, in our case performed by the breeders' association) prior to the milk production forecasting model. This methodology reduces the dimensionality issue using the overall additive genomic value and shows to be the most accurate in phenomic forecasting. Moreover, the genotypic feature proves to have a considerable impact in the final prediction, as well as the additional environmental variables that are considered, i.e. concentrate supplementation and number of milkings: as a matter of fact, the inclusion of the genomic EBV contributes to a 0.0771 increase (from 0.6282 to 0.7053) in the cross-validation Pearson correlation coefficient (r) between predicted and true milk yield, with respect to the problem formulation only considering environmental inputs; moreover, the cross-validation r measure rises to 0.8549 when also considering the concentrate supplementation and the number of milkings.

Both the results outperform a linear model with repeatability and genomic information and, to the best of our knowledge, the state-of-the-art in daily milk yield prediction approaches. The main innovation introduced by this work is the integration of explicit genotypic information in the individual bovine milk production forecasting task, through a suitable representation based on genomic EBV.

Our solution may support dairy cattle breeders in their herd and reproduction management decisions, completely leveraging information available in farms with AMS (i.e. without requiring further investments for data collection); indeed, the prediction of the lactation curve can be performed even before first calving and for newborn calves, as the SNP genotype is available after birth; additionally, as some input variables are partially controllable by breeders, our model may be extended to support and optimize farmers' decisions in a proactive manner. Finally, the idea to include the genomic EBV in machine learning-based phenomic prediction is general and applicable to other traits and domains, with the goals of improving livestock farming sustainability, herd conditions and animal welfare.

KEYWORDS: Phenomics; genomic estimated breeding value; machine learning; dairy modelling; individual prediction

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Equine production and activities in France: what contributions to the sustainability of local areas?

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In France as in the rest of the European Union, little is known about the equine industry, despite its recent evolution. These changes generate the development of new kinds of activities and the growth of the whole equine industry. From livestock to sporting athletes, through landscape managers and leisure partners, equines can be used in various ways. These activities can be developed within farms specialized in equines or in addition to other agricultural productions. Consequently, the equine industry is today firmly rooted at the intersection of various sectors: agriculture, tourism, sport/leisure, industry and consumer.

The evolutions of the equine industry generate new questions about the role of equines in economic vitality, culture, social connections, the environment, and rural development - questions which reflect major changes in society. This communication aims to provide an overview of economic, but also social, and environmental issues related to equine production and activities, and to illustrate them through examples of research projects carried out in France.

The equine industry impacts regions in a variety of ways.

First, it has an economic significance. The French Institute for Horse and Riding (IFCE) estimated the number of equines in France at 1 million in 2021, which represents a density of 15 equines per 1000 inhabitants and of 18 equines per 1000 hectares. The French equestrian sector generates a turnover of 11 billion euros, including 1 billion for equestrian sports and leisure activities. Equines contribute particularly to the attractiveness of deserted rural areas that look for levers to attract transient or permanent populations. Moreover, it is estimated that the equivalent of 66,000 full time jobs are provided in France by the sector. Many commercial interactions exist with different companies for food, equipment, accommodation, training of practitioners or of horses. Equine-related business activities also generate an increasing share of income in rural economies, thanks to the diversification of farmers, the use of equines in modern agriculture and forestry, the production of equine feed, etc.

Second, equines generate social impacts, obviously through horse riding, which is a popular leisure activity among children and young adults – particularly female ones. In addition, we can note the development of the use of equines for therapy or social rehabilitation, as well as riding for the disabled. Equines are also a source of new social links around riding and equine ownership, and between urban and rural inhabitants. Equines benefit from a positive image in the collective imagination and their presence contributes to the attractiveness of local areas, and to connect urban populations to nature.

Third, the equine industry has environmental impacts, some of them being negative, but others positive thanks to equine environmental advantages. Consequently, equines are not only an animal production, but also ecosystem service providers, especially for land use and the conservation of biodiversity. For example, equines are herbivores which contribute to the maintenance of the landscape. They occupy at least 3 million hectares (ha) of land in France. They are thus significant actors in the use of suburban and rural areas. For instance, as a link between urbanization and rurality, equines are present in transitional areas which have been abandoned by agriculture but have not yet been developed by urbanization. Moreover, the specificities of horse behavior allow them to use grasslands that are unsuitable for cattle grazing, or to use the same areas in a complementary way. Equines therefore contribute to the preservation of natural resources and biodiversity. Equine tourism and equine work also show environmental assets, as an ecological source of energy and a green means of transport.

The various impacts of the equine industry generate issues of interest for research. During this communication, while exploring the diversity of the equine industry impacts, examples of research carried out in France will be highlighted. For example, the project ‘horse and territory’ questions the role and place of equines in land use in France. This work highlights the link between the development of equestrian activities over the past twenty years, and the new residential, recreational and environmental evolutions of rural areas. These activities allow the maintenance of spaces that would be abandoned without their presence, they can represent a source of additional income for local business activities, and they participate in the development of a residential economy and in the attractiveness of the regions. Another example is a collaborative research program that aimed to study the economic, social, and environmental impacts of different kinds of equestrian sporting events, in the short and long term. Results show how these events could participate in the local economic development, but also in social utility, and how their environmental impacts can be optimized.

The ‘equine green assets’ project illustrates the importance of equines as ecosystem providers. Five major green assets are identified, linked (i) to equine intrinsic specificities that for examples lead to a particular way of grazing, or to a large domestic biodiversity of equine species, (ii) to their geographical distribution and their land use, and (iii) to some uses by human beings, in tourism and work. Equines impact landscape, biodiversity, greenhouse gas emissions, soil and water quality in many ways: creating ecosystem niches in pastures, producing renewable energy through traction, creating and maintaining bridle paths for their use in tourism, maintaining sensitive areas thanks to the adaptation of local breeds to their natural environmental conditions, etc. Practical recommendations are proposed to help owners and stakeholders to promote these assets in their projects and debates.

Finally, thanks to its wide range of productions: meat, work, leisure, sport, or races; and the diversity of possible activities: breeding, teaching, horse rental or boarding; equine activities are particularly a source of economic, social and environmental amenity value. Consequently, the equine industry can provide diversification opportunities to agricultural activities, contributing to their resilience and sustainability and to the vitality of the regions. In the future, it would be interesting to support more equine research, thus making this industry more visible and understandable. A better knowledge of the economic, social and environmental impacts of equine productions and activities in the agricultural sector should increase their readability by professionals and public actors. This could lead to a better integration of the equine industry in the agricultural policies and rural development towards more sustainability.

US welfare standards: investing in animal welfare pays off

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Welfare is a broad term and its definition reflects that. Although animal scientists often focus on ethology, the science of animal behavior, when discussing welfare, behavioral responses may be short lived and may not necessarily impact other welfare-related responses. This approach may be detrimental for the discussion of welfare because dairy producers ultimately need to generate profit to be sustainable. The World Organisation for Animal Health (WOAH) defines animal welfare as ‘the physical and mental state of an animal in relation to the conditions in which it lives and dies’. Furthermore, good animal welfare is present when animals are healthy, comfortable, well nourished, safe, able to express innate behavior, and are not suffering from pain, fear, and distress (WOAH). This is in line with the three major areas of animal welfare concerns: biological functioning, natural living, and affective states. Welfare standards were developed to assure customers and consumers of dairy products that their expectations of dairy cattle husbandry, stocksmanship, management, and health are met. In the US, nearly all milk produced comes from dairy farms that participate in the National Milk Producers Federation’s F.A.R.M. (Farmers Assuring Responsible

Management) program. Areas evaluated relate to the proof of training of personnel, availability of clear standard operating procedures, and specific areas of management and animal condition. Literature exists that demonstrates the association of welfare responses such as passive transfer of immunity (colostrum feeding), stocking density and availability of resources, hygiene score, body condition score, and locomotion score and survival, production, and reproduction of dairy cattle. In short, animal welfare standards may be perceived as an unnecessary burden by dairy producers, but economic gains are realized when welfare standards are met by improving animal comfort, longevity and performance, which are generally easily justifiable and support sustainability.

O377

How to combine microsatellite and SNP for parentage verification in sheep?

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Microsatellite markers (MS) have been widely used for parentage verification in most of the livestock species mainly due to their high polymorphic information content. In the genomic era, the spread of genotype information as Single Nucleotide Polymorphism (SNP) has raised the question to eventually use SNPs also for parentage testing. Despite the clear advantages of SNP panels in terms of cost, accuracy, and automation, the transition from MS to SNP markers for parentage verification is still very slow and, so far, only routinely applied in cattle. A major drawback is the need for the same genotyping technology for parents and offspring which results in additional costs. To overcome this issue, in this study, we aimed to assess the feasibility of developing an MS imputation pipeline from SNPs in two Italian local sheep breeds: Comisana ($N = 331$) and Massese ($N = 210$). Those animals were genotyped for eleven MS which are part of the standard ISAG panel and with the Ovine SNP50 Bead Chip. Prior to imputation, a quality control (QC) was performed, and SNPs located within a window of 2 Mb from each MS were selected. The core of the developed pipeline was made up of three steps: (a) storing both MS and SNP data in a Variant Call Format file, (b) masking MS information in a random sample of individuals (10%), (c) imputing masked MS based on non-missing individuals (90%) using an imputation program. The procedure was repeated 100 times randomly selecting a balanced number of animals per breed. The accuracy of the MS imputation was assessed on the genotype concordance which was defined as 0 if none of the imputed alleles matched the true allele, 0.5 if only one of them matched, and 1 if both alleles matched the true alleles. A total of eight MS passed the QC, and 505 SNPs were located within the ±2 Mb window from each MS, with an average of 63 SNPs per MS. The overall imputation accuracy was 92.84% and 94.10% in the Comisana and Massese, respectively. The MS that performed best was the MAF214 in the Comisana with an accuracy of 99.0% and the FCB304 in the Massese (97.8%). Our findings suggest that the proposed imputation approach can reach high accuracies which is a prerequisite for parentage verification based on imputed MS genotypes. Additional studies are needed to test if MS imputation in other more distantly related breeds could also be performed.

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MBL2 gene polymorphisms in Mediterranean Italian River Buffalo (Bubalus bubalis) in relation to milk production traits

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Mediterranean Italian River Buffalo (MIRB) is mainly reared for milk production intended for mozzarella cheese production. Thus, the selection is focused on the improvement of milk yield and its quality, as well as on the management of technopathies like mastitis. Recent studies have shown that milk somatic cell count (SCC) combined with differential somatic cell count (DSCC) could be used as an indicator of udder inflammatory reactions. The mannose binding lectin 2 (*MBL2*) gene encodes protein involved in innate immune system and several studies demonstrated that some haplotypes of this gene are associated with *Brucella Abortus* infection in MIRB. The aim of this study was the identification of DNA variants in the *MBL2* and the analysis of their association with milk quality traits. Blood and milk samples were collected from 121 female buffaloes: 82 healthy and 39 affected with mastitis. The *MBL2* (NC_059179) was sequenced using the Sanger method. Altogether 33 SNPs were identified: 12 in exons (including 7 missense ones), 8 in promoter region, 8 in 3'UTR or 3'flanking region, and 5 in introns. Distribution of DNA variants in healthy and affected females have been studied using the odd ratio statistical test to check for any correlation. Finally, an association study was performed between all the SNPs found and different milk parameters: 3 SNPs located in non-coding sequences (2 in introns and 1 in 3'UTR), were significantly associated with SCC ($p < 0.05$). This study showed that *MBL2* is a highly polymorphic gene, however, association of its variants with resistance to mastitis and milk production traits needs further studies on larger cohorts of healthy and affected females.

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O173

Perosomus elumbis in a family of Casertana pigs: case description and identification of candidate genes

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Genetic selection relies on the screening of candidate breeders and on the exclusion from the selection schemes of males carrying negative traits. This objective becomes a priority in schemes applied to autochthonous breeds, where high inbreeding rates may cause the accumulation of harmful alleles leading to the emergence of severe abnormalities. Casertana pig is an autochthonous breed reared in Southern Italy particularly appreciated for its rusticity, frugality and muscularity. Currently, the herdbook comprises 1280 Casertana pigs (47 boars, 262 sows, and 961 piglets). A Caserta pig farm recently reported the born of piglets affected by a rare anomaly incompatible with life. The aim of this study was to characterize the congenital abnormality and find possible associations with candidate genes. The eight affected piglets belonged to 2 different litters; in the first litter, 3 out of eight newborn piglets showed dysplasia of the caudal part of the spine and spinal cord, and of the hind limbs; in the second litter, all piglets were born dead or died soon afterward, and 5 of them were malformed. The agenesis or malformation of the caudal spine and the observed macroscopic lesions led to a diagnosis of Perosomus elumbis (PE), which is a rare, lethal, congenital disorder characterized by agenesis or malformation of the caudal spine (lumbar, sacral and coccygeal vertebrae), often associated with musculoskeletal alterations of the pelvic bones and hind limbs. Malformations affecting other organs or systems have been described over time in various animal species.

The 8 PE piglets, 9 full-sibs, 20 paternal half-sibs and 54 unrelated pigs (all enrolled in the breed herdbook) were genotyped with Porcine GeneSeek® Genomic Profiler (GGP) 70K array, and the association analysis was tested with a case/control model on PLINK software v.1.07. Based on Identity by State analysis results, the boar and the gilt were unrelated. After the Bonferroni adjustment, 15 markers located on chromosomes 2, 4, 9, 12, 13, and 15 were found significantly associated with the occurrence of PE in piglets. Among the candidate genes located in these regions is the *DNAH11* gene, which codes for a primary cilia structural protein, and whose mutations has been previously implied in the occurrence of urogenital malformations and *situs inversus*-like phenotypes in humans. Along with this gene, other peaks were identified suggesting that PE was caused by an accumulation of lethal recessive variations.

O314

The background of novel phenotypes in a native horse breed

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Nowadays there is an increasing interest in conserving local breeds to maintain biodiversity. Genomic characterization is an essential tool to preserve local breeds, monitoring inbreeding level and supporting selection at the same time. Bardigiano horse is an Italian native horse breed, that counts about 3000 horses, traditionally used for agricultural purposes and nowadays for sport and leisure activities. Breeding strategies, such as optimal contribution selection, are used to control inbreeding and promote this conversion simultaneously. Thanks to the advent of genomic tools, this progress could be even faster. The aim of this study is to detect the genetic background of novel phenotypes that could be under selection to help the conversion from agricultural to riding purposes. Biometrical measurements of 185 horses were provided by the Italian Equine and Donkey breeders' association (ANAREAI). The horses were measured at 36 months of age by official judges. GGP Equine70k® was used to genotype all the horses and a total of 6 different traits were analyzed in this study. Biometrical measurements (height at withers, shoulder length, chest circumference and cannon bone circumference) were used to calculate two additional phenotypes, called anamorphosis index (chest circumference^2/height at withers) and dactylo-thoracic index (cannon bone circumference*100/chest circumference). A Genome wide association study (GWAS) was performed considering a suggestive *p*-value of 4 due to the limited number of animals. A total of 27 markers have been discovered as potentially associated to height at withers, shoulder length, chest circumference, cannon bone circumference, anamorphosis and dactylo-thoracic traits. A window of 250 kb has been taken in consideration around the significant markers and most of the genes founded in those regions were involved in bone density and structure, body mass, fat deposition and adipogenesis. Thus, it seems that genes responsible for body conformation in other species appear to be related to the biometrical measurements in horses. In the future, those genes could be targeted for directional selection. This could be a good opportunity to help the conversion from agricultural to riding purposes, although in small breeds like the Bardigiano horse, inbreeding and typicity are as well key elements to consider for breed conservation.

O413

A Genome Wide Association Study in the Aosta cattle population

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Aosta cattle has a core role in the valley economy for meat and milk production, the importance in the maintenance of landscape and mountain environment during summer pasture and the cultural value of the 'Battailes des Reines'. The milk is mostly entirely used to produce the PDO cheese Fontina. For this reason, milk fat and protein contents are important in the breed selection plans. To help both breeders and farmers to select individuals for milk production and quality, relying on their genomic information, it's important to understand their genomic bases. Genome Wide Association Studies (GWAS) are the gold standard to identify genomic regions harbouring QTL associated with complex traits. For this study, 3191 female genotypes and EBVs for production traits of Aosta cattle have been used. Genotypes have been produced with the GGP bovine 100K SNP chip by Neogen. EBV's of each animal for milk, fat and protein yield and contents were provided by the national breeders' association. Genotypes have been filtered obtaining a total of 70,674 SNPs for the analyses. All the GWAS have been performed with the Mixed Linear Model Analysis module of *SNP and Variation Suite* by Golden Helix®, using a single locus mixed model, including the genomic relationship matrix. The FDR threshold of 5% has been used to identify SNPs significantly associated with QTL. The positions of significant SNPs have been used to identify genes and functional elements within a ±200 kb window. For fat and protein contents some interesting QTL have been identified. For the fat percentage, QTL have been found on chromosomes 3, 5 and 14, and for the protein percentage we identified QTL on BTA 5, 6 and 27 with significative markers that laid in the TBC1D22A, CSN1S1, CSN2, HSTN and ZNF385D genes. Since these analyses have been carried out on the actual females in production, the results represent what is in segregation currently in the population. QTL for the fat percentage have an intragenic SNP lying in the MGST1 gene, which has been shown to be associated with milk fat in other studies. Moreover, in the QTL region on BTA 14 harbouring many candidate genes, two of them, CYHR1 and VPS28, had intragenic significant SNPs that are only 100 kb away from the DGAT1 gene. Furthermore, among the QTL identified for the protein percentage, the one on BTA 5, harbouring the TBC1D22A gene, has been already identified in other association studies for milk protein content. Funded by PSRN-DUALBREEDING_2.

O255

Signatures of selection in Italian heavy pigs: how genetic programs for PDO ham productions are shaping the genomes of cosmopolitan breeds

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Breeding and selection programs designed for Italian heavy pigs have enhanced specific meat quality and carcass traits, in addition to several other traits, in line with the needs of the Protected Designation of Origin (PDO) dry-cured ham production systems. These programs have specific breeding goals for each of the breeds (Italian Large White, Italian Landrace and Italian Duroc) that compose the terminal pigs used for ham production. Selection programs may have produced signatures of selection left in the genome of the improved breeds, where relevant QTL or genes are located. In this work, we have dissected the genomic architecture of these three breeds by combining signature selection analyses and genome-wide association studies of the most relevant production traits. A total of 9089 pigs (4626 Italian Large White; 3253 Italian Landrace; and 1210 Italian Duroc) have been genotyped with high-density single nucleotide polymorphism (SNP) chip. Several population genomic parameters and features were defined or calculated in these breeds and, where, relevant, in the pairwise comparisons: runs of homozygosity (ROH) islands, Integrated Haplotype score (iHS) and extended haplotype homozygosity (XP-EHH). Genome-wide association studies have been also performed for several production traits. Breed-specific ROH islands (i.e. shared by at least 50% of the animals) were detected on three, three and seven different chromosomes in the Italian Large White, Italian Landrace and Italian Duroc breeds, respectively. Several ROH signals were confirmed with the iHS patterns. Common overlapping signals between breeds were identified, including genes for coat colour such as OCA2. Some signatures of selection regions, therefore in genomic regions of low variability, fell within QTLs. Some of the overlapping QTL were Visible intermuscular fat in Large White and Ham weight at first salting in Duroc. The obtained results provide a landscape genomic picture obtained by years of breeding and selection in Italian heavy pig breeds.

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O236

Experiences with a single-step genome evaluation in Italian Mediterranean buffalo

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Thanks to its ability to combine traditional (i.e. pedigree and phenotypes) and innovative information (i.e. genotypes), single-step genomic evaluation is becoming a common methodological approach to implement genomic selection in livestock breeding. This is particularly true for species other than dairy cattle like, for example, the buffalo species. The objective of this study was to evaluate the potential benefits of the implementation of a single-step genomic evaluation (ssGBLUP) for production and conformation traits in the Italian Mediterranean Buffalo population. Data were 270-day milk, protein and fat (kg and %), mozzarella yield, and two composite traits feet and legs (FL) and mammary system (MS). Production records included 743,904 lactations from 276,451 buffalo cows born from 1984 to 2019. Morphological traits were from 91,966 buffalo cows from 2004 to 2022. A total of 2017 buffalo cows and 133 bulls were genotyped with the Axiom Buffalo Genotyping Array 90 K. Data were analysed fitting two multi-trait animal models, a 6-trait model for production data and a 2-trait model for morphology data. According to the relationship matrix used, two models were fitted: (i) the pedigree-based (BLUP) with the numerator relationship matrix

(A); (ii) the single step genomic BLUP (ssGBLUP) where A and the genomic relationship matrix (G) are blended into H. Breeding values were estimated with BLUP and ssGBLUP models and results were compared by the LR method. Three different scenarios were used, according to the cut-off year used to create the partial datasets, namely 2012, 2015 and 2017. In each scenario correlation, accuracy, dispersion, and bias statistics were calculated. Both bulls ($N=49$) and cows ($N=1288$) were used for validations. On average, correlation between EBVs from partial and whole dataset estimated with BLUP and ssGBLUP increased from 6 to 49% and from 14 to 17% for production and type traits, respectively. Accuracy increased from BLUP to ssGBLUP and the most affected traits were protein/fat content and mozzarella yield as well as AM, whose accuracy increase was above 20 %. All LR statistics improved also for non-genotyped females. Results of the present study showed that the inclusion of genotypes can improve breeding values accuracy in the Italian Buffalo.

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O31

Detection of heterozygosity-rich regions (HRRs) in the genome of domestic ruminants: which parameters for which breed

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The detection of heterozygosity-rich regions (HRR) in livestock genomes is a recent concept: HRR promise to be a useful tool to estimate heterozygosity and to identify regions of the genome that are under balancing selection or are the result of introgression and admixture events. Very little is known on how sensitive results are to the detection parameters. We used data on three domestic ruminant species-cattle (Holstein), sheep (Lacaune) and goat (Saanen)- to explore the effect on HRR detection of: (i) minimum number of SNP in the HRR (range 10–20); (ii) minimum length of the HRR (range 150–350 kb); (iii) maximum number of homozygous SNP in the HRR (range 0–5); (iv) maximum number of missing SNP in the HRR (range 0–5). These parameters were tweaked around the base scenario with minimum 15 SNP, minimum 250 kb long, maximum 3 homozygous SNP and 2 missing SNP. Before the analysis, data were filtered for MAF >5%, SNP missing-rate <5%, individual missing-rate <10%; only SNP on autosomes (cow: 1–29; sheep: 1–26; goat: 1–29) were used. The filtered data consisted of 43,737 SNP for 104 sheep, 40,025 SNP for 63 cows and 44,624 for 163 goats. HRR were detected using the consecutive methods with the R package detectRUNS. The following evaluation metrics were used: the number of HRR detected (total and per sample), the average length of HHR and the average number of SNP inside HRR. The minimum number of SNP and the maximum number of homozygous SNP in a HRR showed the largest impact on the results. With minimum 10 SNP, over 600 HRR per animal were detected in all species, which dropped to below 100 and below 10 HRR per animal when the minimum number of SNP was increased to 15 and 20. The number of HRR showed an inverse relationship with their length, doubling from 588 kb (sheep) – 670 kb (cow) to 1057 kb (goat) – 1261 kb (sheep) with a 20-SNP threshold. When no homozygous SNP were allowed, only 1–2 HRR per animal were detected on average, which jumped to 295 (sheep) – 384 (goat) when as many as 5 homozygous SNP were allowed. This was in a direct relationship with the size of HRR, which increased from 830 kb to 996 kb in cows and from 751 kb to 836 kb in goats. Interestingly, in sheep this relationship was reversed, with the longest HRR found when no homozygous SNP were allowed (986 kb vs 882 kb). These results represent a fundamental basis to better understand HRR and to define guidelines for the detection parameters in domestic ruminants.

O329

What do we know about differential somatic cell count in buffaloes?

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Besides total somatic cell count (SCC), the knowledge of the different type of leucocytes that compose it could provide precious information to better establish the udder health status. Differential somatic cell count (DSCC) is a novel phenotype representing the proportion of neutrophils and lymphocytes on the total SCC. Whereas several studies have been performed on DSCC in dairy cattle, little is known about this novel trait in dairy buffalo. In the present study we investigated the phenotypic sources of variation and the genetic aspects, i.e. variance components and heritability, of DSCC in the Italian Mediterranean Buffalo. A total of 14,571 test-day (TD) records of 1501 animals from 6 herds, as well as climatic information of the sampling location, were considered for the analysis. A filter of at least 3 TD/lactation and exclusion of outliers beyond 4 standard deviations were applied. Fixed effects included in the model were: herd (6 levels), days in milk (10 classes of 30 days each, with the last being an open class till 360 days), parity (6 levels, from 1 to 6+), year-season of calving (11 levels, from Summer 2019 to Winter 2021/2022), year-season of sampling (9 levels, from Spring 2020 to Spring 2022), production level (4 classes, based on quartiles of average milk production by herd), and THI (4 classes based on quartiles, with THI calculated using the average temperature and relative humidity of the 5 days prior to sampling). Different averages DSCC across herds were observed, ranging from 43.8% to 60.5%. Increased DSCC levels around the lactation peak and with increasing parity were reported. Year-season calving and year-season sampling only slightly affected DSCC variation. Buffaloes grouped in the highest THI classes and with greatest milk yield showed higher DSCC percentages. Genetic results, obtained via a Gibbs sampling, revealed that DSCC is a heritable trait ($h^2 = 0.086$; 95% confidence interval =0.040 – 0.126). Findings of the present study represent a preliminary necessary step for the possible future inclusion of DSCC in breeding programs aimed to improve resistance to mastitis.

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O345

Evaluation of the expression level of genes coding for collagen type 4 in pectoralis major muscles belonging to meat-type chickens selected for different growth-rates

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Collagen type IV (COL4) is one of the essential components of the basement membrane of several tissues, especially vascular endothelium. The two genes coding for the COL4 protein (*COL4A1* and *COL4A2*) are highly conserved across species, thus suggesting their biological importance. In humans, anomalies concerning COL4 folding have been demonstrated to be involved in muscular abnormalities having microscopic features similar to those of the growth-related abnormalities affecting *Pectoralis major* (PM) muscles of fast-growing (FG) chickens. Since recent studies hypothesized a potential involvement of COL4 in the cascade of events leading to these defects, the present study aimed at quantifying levels of COL4 chicken genes to test likely associations between their expression and the chickens' susceptibility to manifest these defects. Considering the high prevalence of these defects in FG compared to broilers having a slower growth rate, and that the defect progression is related to birds' age/growth, the present research focused on evaluating the *COL4A1* and *COL4A2* gene expression by looking at multiple steps of PM development in broilers belonging to both FG and medium-growing (MG) genotype farmed and slaughtered under controlled experimental conditions. Thus, PM samples (5/each genotype) have been collected at 28, 35, and 42 days of age (d) and used to perform Quantitative Real-Time PCR of *COL4A1* and *COL4A2* mRNA using *RPL4* and *GAPDH* as normalizing genes. At each sampling time, differences between genotypes in *COL4A1* and *COL4A2* mRNA quantification were assessed by using the non-parametric Mann-Whitney U test. Concerning the *COL4A1* normalized gene level at 28, 35, and 42 d, no significant differences have been detected between the FG and MG. As for *COL4A2*, significant differences ($p < 0.05$) have been found between FG and MG at 28 d. Considering that the first signs of muscular abnormalities in FG are macroscopically detected at 28 d, the potential involvement of *COL4A2* in the initial progression of physiological and biological alterations characterizing modern broilers' breast muscles could be assumed. Also, in view of the higher amount of *COL4A2* mRNA in FG at 28 d, a resulting increase in its protein level could be supposed. This could support the hypothesis already reported in the literature suggesting that an increased intracellular accumulation of COL4 (e.g. at the endoplasmic reticulum level) might have a role in the onset of growth-related abnormalities.

O248

Whole metabolome and genome analyses provide information on the genetic variability affecting the metabolism in pigs

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Pig production and reproduction traits are complex phenotypes demanding novel strategies for their genetic improvement. Complexity arises from the interplay within and between different biological layers encompassing the genome, proteome and metabolome spaces. Metabolites are simple and intermediate phenotypes that upon genetic regulation, establish molecular routes resulting in the expression of complexity. As such, the deconstruction of complex phenotypes in their single biological components may be useful to describe the genetic factors affecting economically relevant traits. Here, we obtained the metabolomics profile of about 1300 heavy pigs, including 900 Italian Large White and 400 Italian Duroc pigs. Targeted and untargeted metabolomics was applied to plasma samples to recover abundance levels of about 1000 metabolites. Pigs were also genotyped with the Illumina PorcineSNP60 BeadChip. Metabolomics profiles were initially used to study the metabolite-metabolite relationships and to reconstruct metabolic routes. A Gaussian Graphical Model approach was used for this purpose. Metabolomics profiles were then coupled with genotype data to study the effect of genome variability over the metabolome via genome-wide association studies (GWAS). Association of both single metabolite abundances and metabolites ratios were tested. For each trait, genomic heritability was also estimated. Whole genome sequencing data from hundred animals were than used to identify putative causative mutations. Reconstructed metabolic networks resulted quite similar though differences emerged, pointing out putative breed specific metabolic routes. Networks were characterized by poorly interconnected modules representing the specific metabolism of the different metabolite classes. Several associations were recovered from GWAS; as expected, most significant associations were between an enzyme-encoding gene and a metabolite constituting its specific substrate or final product. Overall, we obtained a first catalogue of genes and variants affecting the pig metabolism and that represent a novel source of information for explaining, indirectly, complex traits. This information gives the possibility to include metabolites and novel genetic markers for fine tune breeding and selection programs, to improve sustainability of the pig production sector.

O41

Effect of morphological characteristics and productive traits on the survival of dual-purpose Simmental cows

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In recent years, dairy farmers have observed a substantial decrease in cow survival, with a direct negative consequence on the profitability. Shorter lifespan raises questions about animal welfare and farming conditions at which cows are exposed to. Traditionally, the cows' productive life length is affected by voluntary and involuntary culling, e.g. sale, slaughter, salvage, or death. Culling risks are affected by animal-related features such as calving events, lactation stage, energy balance, reproduction, and aging, but also external factors, namely management and season. The present study aimed to investigate the morphological and productive traits affecting the survival of Italian Simmental dual-purpose cattle. Data available belonged to 2656 Italian Simmental dairy cows from 324 dairy herds (Emilia Romagna region, Italy). Cows involved in the study were linear classified once, as primiparous, between the 2002/2003 to 2019/2020 dairy seasons. Kaplan-Meier survival analyses were performed with the LIFETEST procedure of SAS software v 9.4 using milk yield, muscularity, and body condition score (in classes) as independent variable and survival at the subsequent lactation up to the 6th as dependent variable. As expected, the culling risk increased with parity, i.e. as the age of cows progressed. In general, animals with a low production level and medium body conditions have the highest probability of survival compared to high-producing cows. This trend was more evident in later parities: in fact, cows in parity 5 with low milk production and medium muscularity were more likely (+20.11% of probability) to survive at the subsequent lactation compared to others (13.99 vs 10.49, for medium and high production levels, respectively). Moreover, high-producing cows with medium body conditions were those with the lowest probability to survive at the subsequent lactation. The reasons that could explain these findings may be linked to the metabolic stress experienced by the cows during lactation. Indeed, especially during the peripartum period, lactating cows undergo a state of negative energy balance and a reduction of immune competence. Results from this study indicated that other than productivity level, morphological traits are important for making culling decisions in the Italian Simmental cattle.

O217

Implications on heritability estimates of blending the G matrix with different levels of the pedigree matrix in a nucleus flock of dairy sheep

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When historical records of phenotypes or deep pedigrees are not available, the heritability (h^2) estimates could benefit of expected higher accuracies of genomic inbreeding and relationship coefficients with respect to the traditional pedigree matrix (A). For this kind of trait, phenotypes and individual genotypes with commercial SNP arrays are often available in experimental populations. The aim of this study was to evaluate the implications of using the A matrix, the genomic relationship matrix (GRM) and different levels of their blending into the realised relationship matrix (H) on the h^2 estimates in an experimental nucleus of Sarda dairy ewes. Milk traits were used as an example. In 2000, a nucleus of 900 Sarda × Lacaune backcross was generated, then the 25% replacement ewes were yearly generated with rams from the Herd Book. A total of 4489 ewes born from 2000 to 2020, 272 sires and male ancestors were genotyped with the OvineSNP50 Beadchip. Data were 15,008 lactation records of milk yield (MY), protein (PC) and fat contents (FC). Estimates of h^2 were obtained by applying repeated animal models using different weights of A matrix into H matrix (from 0 to 1 by 0.2) using single-step Genomic BLUP. The highest h^2 estimates were obtained at 0.80 A weight (H0.80A) for MY (0.44), PC (0.72) and FC (0.58). The lowest estimates were obtained at 0.05 A weight (H0.05A) for MY (0.32), PC (0.61) and FC (0,51). The total individual variance (genetic plus permanent environment) remained constant across models for all traits *i.e* H0.80A matrix attributes a larger portion of the total individual variance to the genetic component at the expense of the permanent environmental one. This result relies on the correlations between the diagonal and off-diagonal elements of H0.80A with H0.05A (0.63 and 0.95 respectively). This study suggests that H matrix strongly affects h^2 estimates. In our population, the blending of GRM with a high level of A matrix provides higher h^2 estimates for all the analysed traits. The most likely explanation is that in populations where SNP have different origins (Lacaune breed and different Sarda subpopulations in our nucleus) the A matrix adjusts for genomic relationships which are partly due to identical by state (IBS) rather than identical by descent (IBD) genome sharing.

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O193

Phenotypic analysis of ovine milk infrared spectra

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Fourier Transformed Mid Infrared (FT-MIR) spectroscopy has commonly been used for the estimation of macro and micro components in milk. Energy absorptions recorded in spectra are due to the interaction of infrared radiation with the chemical bonds of milk constituents, making building prediction equations possible. MIR absorption in milk spectra at each wavenumber has also been considered as a trait in its own right and directly used for association studies and genetic parameters' estimation, especially in dairy cattle. This work aimed to estimate the repeatability of individual spectral wavenumbers of sheep milk to identify those potentially showing a genetic component. From January to July 2021, 13,160 milk samples were collected during morning and evening milking from 696 Sarda ewes. MIR spectra were recorded using the MilkoScan FT + equipment (Foss, Hillerød, Denmark) and consisted of absorbance (A) values at 1060 wavenumbers, from 925.92 to 5011.54 cm^{-1}. Spectral data were analyzed with single trait repeatability models including the ewe within date ($n = 6958$) and the ewe ($n = 696$) as random effects. Within lactation repeatability (r) was calculated as the ratio between the ewe and the sum of ewe, ewe within date and residual variances. Thirty-nine percent of wavenumbers showed r higher than 0.3 and three percent higher than 0.5. The highest r were estimated in the absorbance regions of the main milk components: [1095.67–1145.83] cm^{-1} containing information on lactose; [1222.99–1253.85] cm^{-1} and [1450.61–1462.18] cm^{-1} harboring information on milk proteins, such as the absorption of amide III at around 1250 cm^{-1}; [2962.94–2974.52] cm^{-1} which is associated with alkyl C-H stretching, that is abundant in fat. Indeed, A values at these wavenumbers showed the highest raw correlations with lactose content, protein (but also fat) and fat contents, respectively. Low (<0.3) r were estimated between 2299.37 cm^{-1} and 2712.17 cm^{-1} and between 3676.67 cm^{-1} and 5011.54 cm^{-1} wavenumbers. Both regions are often discarded when setting up prediction equations for milk composition since they do not contain valuable information. Finally, r estimates were close to zero from 1624.22 cm^{-1} to 1670.51 cm^{-1} and from 3094.11 cm^{-1} to 3657.38 cm^{-1} wavenumbers, the latter being a region strongly influenced by water absorption. These findings suggest the feasibility of directly using spectral data to investigate the genetic architecture of milk composition even in dairy sheep.

O494

Milk infrared-derived predictions of blood metabolites as a tool to assess fertility in dairy cattle

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Fertility is one of the major factors affecting the efficiency of dairy herds. Various factors influence reproductive performance, including the nutritional and metabolic status of animals, especially in the post-partum stage when the ovarian activity begins and cows are subjected to insemination. Variation in this status can be monitored through blood metabolites. Therefore, the objective of our study was to infer the associations between milk infrared-derived predictions of blood metabolites and fertility-related traits in a population of 83,056 dairy cows. The hematochemical parameters comprised 28 metabolites related to energy metabolism, liver function, oxidative stress and the inflammation/innate immunity and blood minerals. Blood metabolites were predicted using the milk Fourier transform mid-infrared (FTIR) spectroscopy and equations previously developed using machine learning algorithms (R^2 ranged from 0.48 to 0.87) on an independent calibration database of 1300 lactating cows reared in 5 herds. Calibration equations were then applied to a population database of 159,151 test-day records and milk spectral data, which were merged with fertility data collected by the Breeders Federation of Alto Adige from the northeast of Bolzano province in Italy. The response variable was the days open (DO), which was analyzed with a Cox's proportional hazards model. Besides standard nuisances, models included the independent effect of FTIR-based predictions of metabolites (pMET), discretized on the basis of 25th, 50th, and 75th percentiles, and tested one at the time. The inflammatory and oxidative stress metabolites had a large impact on DO. Specifically, increasing levels of predicted serum ceruloplasmin (CP), total reactive oxygen metabolites (ROMt) and advanced oxidation protein products (AOPP) have shown to be associated with an impairment of DO. Indeed, the hazard ratio (HR) of becoming pregnant after calving at time t linearly decreased from the 1st quartile (always taken as reference, hence set to 1) compared to the 4th quartile (the extreme group), for CP with HR =0.60, for ROMt with HR =0.72 and AOPP with HR =0.46. Among the liver function metabolites, the increase of serum paraoxonase concentrations was associated with a linear increase of probability to get pregnant (4th quartile HR =1.33). Results of effects of pMET on DO are consistent with expected physiological patterns and they appear to be promising tools to assess fertility in dairy cattle.

O76

Different strategies for selecting core animals in the APY for single-step GBLUP

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The animal breeding programs are moving towards the genomic selection worldwide. The most adopted method, the single-step genomic BLUP (ssGBLUP) requires the construction of the H matrix, which is a blend of the pedigree-based (A) and genomic-based (G) matrices. This method involves the inverse of the G matrix, that is computationally feasible for up to 150 thousand animals. However, in the last years, the number of genotypes rapidly increased: some populations (e.g. US Holsteins, American Angus) have now more than one million genotypes. Thus, the implementation of the ssGBLUP for these populations require the use of the Algorithm for Proven and Young (APY), which divide the genotyped animals in two groups, core and noncore. Since only the portion of **G** corresponding to core animals is directly inverted, ssGBLUP with more than 4M genotyped animals has been implemented for the US dairy cattle. The optimum size and choice of core animals in the APY are still under debate, even if some studies demonstrated that randomly select a number of animals that represent the number of independent chromosome segments provide the same accuracy of the standard ssGBLUP. In the present study, different criteria to select core animals based on their genetic contributions (GC) have been tested. A total of 4100 genotyped animals, of which 3000 had three phenotypes (mimicking milk yield, fat yield and percentage), were simulated. GC scores for all genotyped animals were computed using the principal component analysis of the G matrix; animals were then selected randomly or based on low or high GC values. Four different core sizes (5, 10, 20, and 30%), which reflected from 95.3% to 99.9% of the total variance of G, were tested and validated based on the correlation between the true breeding value of the 1000 youngest animals and the corresponding estimated breeding values GC values were not correlated with the phenotypes, and negatively correlated with pedigree inbreeding and number of offspring. As expected, the prediction accuracies increased as the core size increased, and they ranged from 0.81 to 0.99.

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Selecting the animals with the largest GC values led to lower prediction accuracies, whereas the inclusion in the core of animals with the lowest GC showed the best results.

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O252

Genetic aspects of longevity in Italian Mediterranean buffalo

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Functional longevity estimates the animal's ability to not be culled due to low production, low fertility or impaired health status. In the past, longevity was not considered as a breeding objective because such an information was available only when the animal was culled. However, longevity in livestock has been experiencing a growing economic importance over the past two decades, including the Italian Mediterranean Buffalo (IMB). The aim of this study was to present results of the first genetic evaluation of functional longevity in the IMB. Data from 178.619 buffalo with first calving from 1992 to 2019 were used. A Weibull mixed survival model was used and functional survival was defined as the number of days from the first calving until culling or until the last available date of milk recording (censored animals).

The model included time-dependent effects of herd-year-season, the year and season of calving, parity, milk production and yield, and morphological traits, as well as time-independent effects the age at first calving, year of birth, herd size and as covariate the milk production within the first 60 days.

Results indicated that the average duration of productive life was 1225 days. Among fixed effects which have shown a higher risk of culling (RC) there are: calving in autumn (RC =1.101), being too old at first calving (RC =1.102). Regarding the morphological traits, females with lower scores for udder and feet and legs composite traits showed the greater culling risk. Moreover, the effect of the milk production level showed a strong impact on the longevity of IMB with a significant risk of elimination ($p < 0.01$) when milk production decreased. The heritability on the original scale was 0.12. Despite not having selected for this trait, the genetic trend of the bulls showed a favourable trend for longevity since 2009.

In conclusion, these results showed that it is possible to implement a genetic evaluation for longevity in the IMB. A next step further would be to economically quantify the value of survival traits and their possible inclusion in the IMB selection index breeding value. To avoid bulls with unfavourable breeding values for longevity, may improve productive life in the population and prevent hidden economic losses.

O240

Effect of artificial insemination on genomic population structure in Camosciata delle Alpi dairy goat breed

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The use of artificial insemination (AI) is becoming a consistent reality for the Camosciata delle Alpi goat breed, especially in the dairy farms of northern Italy. It is therefore very interesting to understand the impact of the diffusion of AI in our autochthonous populations also in light of the future implementation of new genomics indexes that have been developed by ongoing projects.

Inside one these projects, the CHEESR project, we analyzed pedigree, genotyping data (obtained with a 50k chip) and more than 23000 production records of 1231 subjects of Camosciata delle Alpi belonging to 64 farms.

We classified all the individuals into three different groups based on the use of the AI in their lineage: subjects deriving from direct use of artificial insemination (FA), from parents deriving from direct use of AI (SO) and from natural mating (MN). For each of the 3 groups, we calculated the average values of 5 production parameters (Milk Kg, Protein Kg and %, Fat Kg and %) and tested their difference between groups, the F_{ROH} values and the classification of Runs of Homozygosity into length classes. All analyses were performed using PLINK1.9 software and R-base package functions.

As regards the production data, we found significantly higher values ($p < 0.001$) in the FA group than the other two for all five traits. Instead, no statistical differences were found between MN and SO. In terms of F_{ROH} values and distributions of ROH classes, SOs showed the highest mean F_{ROH} (0.07), FAs the lowest (0.057) and MNs the middle values (0.065); the distribution of the different ROH classes was homogeneous in all three groups.

These results highlight differences in the productivity levels between the FA group and the other two groups (MN and SO) but not between the latter two as one would expect. The most interesting finding is the unexpected slightly higher level of

inbreeding in the second-generation offspring of AI (SO), which might be due to the unbalanced use of parents deriving from the AI suggesting the need of an optimal contribution evaluation of the mating plans. In conclusion, AI could represent a very important tool for improving the Italian Camosciata delle Alpi population both from a production and an inbreeding management point of view.

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O472

An association analysis between CSN3 genotype and milk yield in Italian Mediterranean river buffalo

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Buffalo milk is characterized by the presence of all 4 casein fractions (αs1, α, αs2 and κ) encoded by 4 genes (*CSN1S1*, *CSN2*, *CSN1S2* and *CSN3*, respectively) mapped on chromosome 7. In particular, κ-CN plays a crucial role in the formation of stable casein micelles and has a key influence on milk-clotting. The *CSN3* gene is divided into 5 exons of which the exon 4 codes for 160 out of 169 aa of the mature protein. Mutations in this exon are responsible for the quali-quantitative differences of gene expression in cattle. Different studies have been focused on the identification of polymorphisms also in buffaloes. Particularly investigated is the SNP HQ677596:c.536C > T at the nt 377 of the exon 4 that leads to the aa change p.Ile135 > Thr of the mature κ-CN. This replacement changes the theoretical MW of the buffalo κ-CN from 19,114.7 to 19,102.6 Da; increases hydrophobicity, with the hydropathicity average increasing from −0.526 (Thr) to −0.495 (Ile); and causes the loss of an O-glycosylation site in the variant 135Thr. However, despite the existence of several studies, nowadays the association of *CSN3* SNPs with buffalo milk quantity has not still deeply identified. The aim of this study was to evaluate possible effects of the SNP c.536C > T on milk yield (MY) in Mediterranean river buffaloes. A total of 7601 records for MY measured monthly on 1141 lactations of 753 buffaloes belonging to different farms located in Campania region (Italy) were analysed. To estimate the frequencies of this SNP a method based on ACRS-PCR has been adopted. The major allele (c.536C) had a relative frequency of about 0.6 and χ^2 values showed that there was no evidence of departure from the HWE ($p \le 0.05$). Association between *CSN3* polymorphism and MY was investigated with a mixed linear model that included effects of parity, calving season and month of production. A significant association between c.536C > T and MY was found ($p < 0.05$). In particular, the CC genotype showed an average daily MY approximately 0.62 kg higher than TT buffaloes. Although such results need to be confirmed with large-scale studies in the same and other buffalo populations, they might offer useful indications for the application of MAS programmes in buffalo and, in the future, they might be of great economic interest for the buffalo dairy industry. Infact, increases in average MY and, consequently, in mozzarella PDO production may be expected.

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O283

The effect of ancestral and SNP panel genotyping on genomic inbreeding coefficients from imputed SNP in Holstein-Friesian dairy cows

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Imputation of whole genome single nucleotide polymorphisms (SNP) data is routinely applied in dairy cattle breeding because of the reduction of genotyping costs. The central idea is to genotype few core animals (i.e. animals whose genome is represented in the entire population) with high density (HD) SNP panels, many animals with low or medium density (LD/MD) SNP panels, and impute the LD/MD genotypes to HD. Imputation success depends on: (i) the relationship between the core animals genotyped in HD and the animals to be imputed from LD/MD to HD (for e.g. parent-offspring), (ii) the distribution along the genome and the number of SNP in the LD panels and (iii) the linkage disequilibrium between SNP in the LD/MD and in the HD.

The aim of this study was to evaluate the combined effect of ancestral genotyping and SNP imputation on the estimation of genomic inbreeding coefficients in dairy cattle. We analyzed 68,127 Italian Holstein dairy cows registered to the official herd book of the Italian National Association of Holstein, Brown and Jersey Breeders (ANAFIBJ). Cows were genotyped with the GeneSeek Genomic Profiler 3 and 4, GeneSeek MD and the Labogena MD SNP panels imputed, and the HD Illumina Infinium BovineHD BeadChip and GeneSeek Genomic Profiler HD-150K degraded to 84k preselected SNP used in the ANAFIBJ genomic evaluations. Genomic inbreeding coefficients were obtained with SNP-by-SNP estimators (four PLINK v1.9 and two estimators based on genomic relationship matrix) and runs of homozygosity. Information on the presence or absence of genotypic information from the sire, dam and maternal grandsire during the imputation was also investigated. Results evidenced that cows genotyped with MD SNP panels, whose SNP were poorly represented on the final imputed SNP set, and without having their parents genotyped, are likely to have inflated genomic inbreeding coefficients. In those cases, the correlation of genomic inbreeding coefficients estimates with genotyped vs. imputed SNP varied between 0 and 0.8 depending on the SNP panel and estimator. Both parameters should be carefully considered for designing imputation strategies in dairy cattle.

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O580

Selection for feed in Italian Simmental breed

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Improvement in feed efficiency has the potential to increase cattle farms' profit and decrease environmentalfootprint. Residual feed intake (RFI) is a measure of feed efficiency related to the greenhouse gases and pollutant emissions. Since august 2018, the Italian Simmental Association started to collect data about the feeding behavior of young male candidates in Perfomance test; 601 records of individual RFI were available. Variance and covariance components for Residual feed intake (601 records of individual RFI were available) were estimated using a model implemented in a Bayesian framework using the software GIBBS3F90; 500,000 iterations were run discarding the first 200,000 samples as burn-in and storing samples every 100 iterations (i.e. 3000 chains were available for marginal posterior density analysis). Pedigree counted 15.389 animals Genetic variance of RFI resulted 0.114 ± 0.0060 kg^2/d^2 while heritability was 0.293 ± 0.147.

A procedure for the genomic evaluation of RFI has been developed. Despite the large marginal posterior standard deviation of heritability, this preliminary analysis confirmed a genetic component that affects RFI. Validation parameters of GEBV for RFI had values of reliability comparable to the parent average; this is due to the little number of observations currently available. Feed efficiency trait has been introduced in the new selection index (Indice Duplice Attitudine Sostenibile) with a weight of 2%

O571

Preliminary investigation on stayability in Italian Mediterranean buffalo

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Animal longevity, as productive life, is an essential functional trait in the economic management of dairy herds, as well as in dairy buffalo breeding. Dairy buffalo herds have always had a lower recovery rate than dairy cows due to a higher longevity, which could be justified, among other factors, with their lower productive level. Selection criteria adopted for Italian Mediterranean Buffaloes from the National Association of Buffalo Breeders (ANASB) include several morphological traits aiming to preserve and to increase buffalo longevity. One way to quantify longevity in the herd is through the evaluation of stayability which has been defined as the aptitude for which an animal remains in the farm to a given time point.

This approach uses calving date to calculate the time spent by each animal in the farm and to define stayability, assigning a value of 1 if a calving date was present or 0 otherwise. The aim of this preliminary work is to assess the stayability from the first parity up to 10 in Italian Mediterranean Buffaloes (IMB).

Data from 276.415 buffalo cows registered in the ANASB studbook starting from 1989 were extracted and merged with the official milk test-day recording datasets from the Italian Breeder's Association (AIA). The first 10 parities were retained, and the variables used were: date of birth, calving dates and culling dates.

The stayability frequencies (stayability =1) were calculated to a given parity (e.g. Bstay2 = stayability at parity 2 until Bstay10 = stayability at parity 10) and between two successive calving (Bstay2–3, Bstay3–4, Bstay4–5, Bstay5–6, Bstay6–7, Bstay7–8, Bstay8–9, Bstay9–10). Stayability to a given parity was 77.2% for Bstay2; 57.9% for Bstay3; 42.4 for Bstay4; 30.6% for Bstay5; 21.4% for Bstay6; 14.5% for Bstay7; 9.90% for Bstay8; 5.7% for Bstay9 and 3.2% for Bsty10. Values of stayability between two successive calving events decrease linearly from 77.2% of Bstay1–2 to 56.6 of Bstay9–10.

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O65

Can we predict cattle future feed efficiency from faecal microbiome data collected at weaning?

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The rumen microbiome is one of the biological factors associated with variation in cattle feed efficiency. Several authors have reported noticeable differences in the rumen microbial populations between animals with negative or positive residual feed intake (RFI). In the present study, the faecal microbiome of 28 Angus steers were evaluated at weaning, and 100 bacterial families (BF) were identified. After RFI was evaluated, animals were divided in two groups: one with negative (NRFI) and the other with positive (PRFI) RFI. Collecting rumen microbiome information is expensive and require special equipment. A valid alternative could be the use of faecal microbiome information, which is simpler and cheaper to obtain. The objective of this study was to test the ability of the canonical discriminant analysis (CDA), developed by using faecal BF as variables, in correctly discriminating the two RFI groups. First, a stepwise discriminant procedure was applied to select, among the 100 BF, those most discriminant. This resulted in 23 BF, which were submitted to a CDA that significantly separated the two groups. Having only two groups, the CDA derived a canonical function (CAN), i.e. a linear combination of the original 23 BF, for which canonical coefficients (CC) were computed. CC indicate the partial contribution of each original variable in composing the CAN; thus, the higher the absolute value of a CC, the higher the weight of the corresponding variable in composing the CAN. Animals belonging to the NRFI group had a greater abundance of BF with negative CCs, whereas those belonging to PRFI were characterized by BF with positive CCs. The highest CC was estimated for Atopobiaceae (0.339), whereas the lowest for Fibrobacteraceae (−0.325). The ability of CDA in correctly assigning new observations to NRFI and PRFI was tested with the leave-one-out cross validation procedure. All animals were correctly assigned to their groups. In conclusion, CDA could be used to determine, at weaning, whether an Angus steer will have a positive or negative RFI by using faecal microbiome information.

O180

Different methods to handle preferential treatment in slaughter age in Italian Limousine

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The aim of this study was to investigate the different methods and models to handle preferential treatment for slaughter age in Italian Limousine, and its impact on genetic evaluations for body weight (BW). Slower-growing animals are usually slaughtered at older age, leading to biased genetic evaluations. The animals were 867 bulls, raised on a performance test between 1992 and 2022 and assigned to 28 contemporary groups (CG). The duration of the performance test was 5 months. Age and BW of the bulls averaged 350.20 ± 61.29 and 455.91 kg ±90.76, respectively. The final dataset included 867 animals with multiple records, for a total of 5197 observations. A repeatability animal model including the fixed effects of CG and age at phenotyping was used as reference method. This model showed that body weight was heritable (0.51 ± 0.07).

Then, a subset of weight records was used as realistic measures of body weight at slaughter. This included a single record per individual, being chosen as the closest one to a target weight (519 kg, third quartile of the weight distribution). Seven different animal models were implemented, to be compared to the reference one for the estimated breeding values they produced. Four were univariate models, with: (i) BW as the phenotype, (ii) age as the phenotype; (iii) BW as the phenotype and age was included as linear covariate; (iv) age as the phenotype and BW was included as linear covariate. One bivariate model: (v) combining age and BW. Two multivariate models using 3 weights in different periods: (vi) without age effect, (vii) including age as linear covariate. All models included the fixed effect of CG. These models provided lower estimates of heritability than those from repeatability model ranging from 0.15 ± 0.04 to 0.41 ± 0.13. Correlation among solution of the repeatability used as reference model and the seven model was 0.35, 0.54 for BW (models i and iii) and −0.52, −0.63 for age (models ii and iv) for univariate analyses, −0.58 for age and 0.32 for BW considering bivariate analyses, 0.38, 0.40, 0.37 (vi model) and 0.23, 0.41. 0.33 (vii model) for multivariate model. Model iv showed higher correlation respect the other six models, for this reason this analysis resembles better the repeatability model, suggesting that age can be used as a good predictor of potential growth in beef cattle.

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O168

Loss of autozygosity in QTL regions in crossbred pigs

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In local European pig breeds, the high level of consanguinity and small population size threaten conservation efforts. However, the loss of within-breed genetic diversity increases the possibility of planning and exploiting of crossbreeding. This study included 1133 individuals belonging to 23 pig breeds: 20 European local breeds ($n = 978$) reared in 9 countries (Croatia: Black Slavonian, Turopolje; France: Basque, Gascon; Germany: Schwabisch-Hällisches Schwein; Italy: Apulo Calabrese, Casertana, Cinta Senese, Mora Romagnola, Nero Siciliano, Sarda; Lithuania: Indigenous Wattle, White Old Type; Portugal: Alentejana, Bísara; Serbia: Moravka, Mangalitsa; Slovenia: Krškopolje pig; Spain: Iberian, Majorcan Black), and 3 cosmopolitan breeds ($n = 155$, Duroc, Landrace and Large White). All individuals were genotyped with GGP-70K HD porcine chip containing 68,516 SNPs. Twenty mating pairs drawn from each combination of breeds (both in pure-breeding and cross-breeding) were simulated after phasing genotypes with Beagle software (v.5.4). All simulated individuals ($n = 5520$) were assembled into a single dataset, then Runs Of Homozygosity (ROH) were detected using the DetectRUNS R package. The proportion of the genome in autozygosity (F_{ROH}) was calculated for each simulated individual. Two purebred pairs and their crossbreds (Cinta Senese – Large White and Alentejana – Duroc) were chosen to investigate potential regions interested in the loss of autozygosity. Each genomic region was tested for systematically losing autozygosity between purebreds and crossbreds.

The F_{ROH} was higher in purebred animals, namely 0.21 in Cinta Senese and 0.19 in Large White, while their crossbreds showed a F_{ROH} of 0.06. Alentejana showed an F_{ROH} of 0.18, Duroc reached an estimate of 0.31, and their crossbreds showed a very low value (0.03). Regions with F_{ROH} significantly different between purebreds and crossbreds (for Cinta Senese × Large White and Alentejana × Duroc, respectively) were mapped. The lost autozygosity focused on regions containing 26 and 57 QTLs (for Cinta Senese – Large White and Alentejana – Duroc, respectively), these were mainly related to body weight, carcass and fatty acid composition traits. The severe decrease in autozygosity with the non-complete loss of production traits in the simulated crossbred populations could suggest a potential use in mating plans.

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O37

Preliminary genetic analysis for survival in Italian Jersey

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The Associazione Nazionale Allevatori della Razza Frisona, Bruna e Jersey Italiana (ANAFIBJ), with the LATTeco2 project (National Rural Development Programme – PSRN), has been working on improving animal welfare, biodiversity and sustainability in a wider approach. Focusing on animal welfare, ANAFIBJ is developing a genetic evaluation for longevity in the Italian Jersey population. This study aims to determine which parity might evidence a critical point in herds' life, if there is a sire genetic effect and an environmental effect given by the herd of calving. Therefore, cattle survival has been analysed as the ability of cows to survive at specific lactation. The dataset included 21,724 cows belonging to 1369 herds and related to 755 sires, all of them showing at least the first calving event. Calving dates from 2000 to 2019 were included. Lactations from 1 to 5 have been considered, developing 4 traits per individual. Bulls' and herds' effects were estimated using a threshold-liability model that included the sire and herd of calving (random) effects. Sire's variance (as a proportion of total phenotypic variance) increased from 0.428 for survival at first parity to 0.552 for survival at fourth parity. On the contrary, herds' variance decreased from 0.216 for survival at first parity to 0.196 for survival at fourth parity. These results showed that, as the parity order increases, sire effect becomes more relevant while the herd effect decreases in relevance. Sires' and herds' solutions were then converted from the liability to the probability scale (0–1). Considering the whole set of sires and herds, correlations between bulls' EBVs across parities ranged from 0.608 to 0.656, while correlations for herds' solutions ranged between 0.597 and 0.718. Considering sires and herds with at least 50 records, correlations between bulls' EBVs range between 0.756 and 0.798, while herds' solutions range between 0.789 and 0.832. In conclusion, it is shown that on cow's longevity there is a genetic impact of sire and herd.

O36

Genetic aspects of heifer fertility in Italian Holstein population

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Fertility is fundamental to enhance the production efficiency of the dairy herd and thus it is a contributor to annual farm profitability. Cow fertility has been included in Italian Holstein breeding objectives since 2009. Heifer fertility is another key trait that deserves attention as it has direct connection with overall efficiency. In general, the main goal is to improve conception and daughter pregnancy rates, favour shorter calving interval in lactating cows, and reduce failure of conceiving in heifers. The advantages of heifer over cow fertility traits are the early availability in life and, overall, the moderate to strong genetic correlations with fertility of lactating cows. The aims of the present study are to assess genetic parameters of Italian Holstein heifers and develop an aggregate selection index to improve heifer fertility. Data (ANAFIBJ, Cremona, Italy) included information on insemination, calving, and pregnancy diagnosis dates of Italian Holstein heifers. The investigated traits (mean ± standard deviation) were age at first insemination (AFI, mo; 17.25 ± 2.89), nonreturn rate at 56 d from the first insemination (NRR56, binary; 0.78 ± 0.41), conception rate at first insemination (CR, binary; 0.61 ± 0.49), and interval from first to last insemination (IFL, d; 26.09 ± 51.85). Genetic parameters were estimated using a 4-trait animal model that included the fixed effects of herd-year of birth and month of birth for AFI, and herd-year-season of birth and month-year of insemination for IFL, NRR56, and CR. The animal additive genetic effect was included as random term. An aggregate index was developed from the estimated additive genetic (co)variance matrix by considering CR as the breeding goal and AFI, NRR56, and IFL as selection criteria. Heritability ranged from 0.012 (CR) to 0.015 (IFL), except for AFI (0.071). Conception rate at first insemination was strongly correlated with both IFL (−0.730) and NRR56 (0.668), and weakly to AFI (−0.065). The relative emphasis placed on each selection criteria in the aggregate index was 10%, 47%, and 43% for AFI, IFL, and NRR56, respectively. Results of the present study suggest that heifer fertility should be considered as an additional trait in the breeding objectives of Italian Holstein.

O146

Influence of genetic background and the effect of liquid whey supplementation on the faecal microbiota composition in Nero siciliano and commercial crossbreed pigs

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The genetic background of the host, together with several other biotic and abiotic factors, including feeding, plays a crucial role in modulating the gut microbiota composition of many animal species. Furthermore, several authors have reported that the microbiota of native pig breeds reflects distinctive traits that commercial crossbreeds have lost. Nero Siciliano pig is an important autochthonous Sicilian pig breed that has a marked lipolytic capacity. The aim of this study is to assess the composition of the faecal microbiota of two different genetic types of pigs reared within a farm where liquid whey was integrated in the feeding as dairy by-product. The faecal bacterial composition has been investigated at three time points, T0, T1 (after 30 days) and T2 (after 60 days), in two pig groups (Control and Treatment). Microbial genomic DNA has been extracted from stool samples collected directly from the rectal ampoule of 20 crossbreed (Large White × Landrace) pigs (10 Control and 10 Treatment) and 10 autochthonous (Nero Siciliano) pigs (5 Control and 5 Treatment). The 16S rDNA gene has been sequenced using an Illumina MiSeq platform and the clean reads (Phred-score ≥20) have been classified at phylum, family, and genus level, with an identity threshold of 75%, 87% and 95%, respectively, by using QIIME2. In both groups and population sets, *Firmicutes* (51%) was the most abundant phylum followed by *Bacteroidetes* (36%) whereas the most abundant genera were *Prevotella, Treponema* and *Lactobacillus*. The two pig genetic types have showed a different attitude towards liquid whey diet. In fact, as shown by beta diversity analysis, measured by Bray-Curtis distances, the microbiota of crossbreed samples has been significantly affected by liquid whey diet (PERMANOVA $p < 0.001$). The global microbial composition of Nero Siciliano samples has not been significantly affected by liquid whey diet. Despite this, the beneficial effects of liquid whey diet were present in both pig genetic types. In fact, based on differential abundance analysis, *Bifidobacterium* and *Ruminococcus* were more prevalent at T2 in all treated samples; several species of these genera can promote intestinal health, and this could be a positive consequence of liquid whey administration. In conclusion, this metagenomic study is a first step in understanding how the breeding system affects the composition of intestinal bacterial communities in different genetic types of pigs.

O223

Mining high density SNP chip data and whole genome sequencing information to identify putative unfavorable alleles in pig breeds

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Deleterious and usually recessive alleles are present at very low frequency and in heterozygous state in all livestock populations. Their frequency could increase if these alleles are in linkage disequilibrium with favorable QTL alleles or if heterozygous animals show heterosis for some production traits under selection. The homozygous state of these alleles is usually not compatible with life and therefore it is not possible to observed homozygous adult animals. Consequently, carrier animals might have reduced reproduction performances, depending on the frequency of these alleles in the breeding population. In this study, we mined high density single nucleotide polymorphism (SNP) chip data from three Italian pig breeds (Italian Large White, $n = 5528$, Italian Landrace, $n = 3470$; Italian Duroc, $n = 1354$) to identify genome regions carrying putative unfavorable and deleterious alleles. The strategy was based on SNP allele frequencies and the expected occurrence of homozygous genotypes following population genetic rules over the absence of these genotypes in the three pig breeds, after filtering SNP data with stringent criteria for genotyping errors and other genotyping biases. Then, we compared SNP chip data with whole genome sequencing information obtained from 160 pigs of the three targeted breeds to identify loss of function variants in the candidate genome regions. Overall, we detected five genome regions carrying putative unfavorable alleles. Their estimated allele frequency ranged from 0.07 to 0.17, suggesting that some of them might be linked to favorable alleles affecting production traits under selection. The identification of loss of function variants in key genes located in these regions suggests some biological mechanisms that would explain their deleterious effect. These results provide genomic markers that can be used to design targeted breeding and selection programs aimed to reduce the frequency of unfavorable alleles in the Italian pig populations and reduce their negative impact on reproduction performances by avoiding carrier-carrier mating.

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O220

Genomic regions associated with Somatic Cell Score in Italian Mediterranean Buffalo

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Genome-wide association studies are a powerful tool to identify genomic regions and variants associated with phenotypes. Mammary infections in livestock are a major problem that damages animal health, eventually translating into significant economic losses in the dairy industry and the Somatic Cell Score (SCS) is commonly used as a predictor in selection for mastitis resistance.

The aim of this study was to identify genomic regions as well as genes and pathways associated with SCS in Italian Mediterranean Buffalo cows.

Phenotype of 1,302,332 test-day record corresponding to 96,589 buffalo cows with first calving from 2010 to 2018 and a pedigree that included 148,493 animals, were considered in this study. The SCS was defined as the geometric mean in the first 150 days of lactation (SCS150) and its mean and standard deviation was 2.72 ± 1.24. Regarding the genotypes, information from 2150 animals with 46,904 loci was used.

Effects of SNPs were estimated by a single-step GWAS (ssGWAS), which back-solved the genomic breeding values predicted using single-step genomic BLUP (ssGBLUP) fitting a single-trait animal model with repeated observations.

The largest genetic variance (0.63%) was explained by an SNPs window located on chromosome 2. However, most windows explained less than 0.5%, and these low-contributing regions were spread across the entire genome. These results suggest that SCS is a moderate to highly polygenic trait, where many regions across the genome contribute to its genetic variation. Overall, a total of 23 informative windows that explained at least 0.5% of additive genetic variance were identified, explaining 12.22% of the observed genetic variance.

These regions harbour genes with biological functions that are related to the analysed trait. The identification of these regions and genes will contribute to a better understanding of the relationship between mammary infection and SCS, eventually supporting the latter as a proxy for genetic improvement of mastitis resistance in the Italian Mediterranean Buffalo.

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O556

Genome-wide association analysis of productive traits in grey alpine cattle breed

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Genome-wide association (GWAS) studies are a powerful tool that allows the identification of genomic regions and variants associated with phenotypes. Many studies used this approach to identify candidate genes related to milk production. Despite this, that approach was rarely exploited in local cattle breeds. The aim of the study was to detect associations between genomic regions and milk production and quality traits, in Grey Alpine cattle. We used a 110K single nucleotide polymorphism (SNP) markers panel to profile the genotype of more than 1100 individuals, to investigate milk, fat, and protein yields (MY, FY, PY); fat, protein, percentage (FP, PP); and somatic cell score (SCS). SNP regression model corrected by population structure was used to test the associations of each SNP. GWAS was performed by using the de-regressed EBV of the target phenotypes. Deregreesed EBV was calculated on 16,335 animals using test-day random regression models for a total of 1,046,249 records. We inspected the position of each SNP via UCSC Genome Browser and we determined if it was located within or close to a gene or another functional DNA element. The genomic regions associated with milk production traits contained more than 50 candidate genes spread on 18 chromosomes [Bos taurus autosome (BTA) 1–7, 9, 11–16, 20–21, 26–27, and 29]. The number of associated genes for FP and PP was the highest, while for SCS the number of candidate genes was by far the lowest (only 6 candidate genes). It was remarkable the abundance of candidate genes in BTA14 (including the DTAG1 gene), which is a very long region of the genome of cosmopolitan dairy cattle breeds. The results from our GWAS led to the detection of a variety of genes both well-known and novel for dairy production. Based on the results obtained, we argue that continuing to expand genomic research at the local breeds level, may help to understand part of the genetic architecture worldwide. Therefore, it would also be an input for making appropriate breeding decisions.

O470

Integrative factorial methods to explore the relationships between genotypes, phenotypes and climate in Holstein cows

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Understanding the complex relationship between animal genotypes, phenotypes and climate is a topic of increasing relevance in animal breeding, given the rapidly changing climatic conditions. In this work, we used 15,545 first lactation daily records on Italian Holstein dairy cows from ANAFIBJ. Recorded traits were milk production, fat and protein content, as well as climate data such as the Temperature Humidity Index (THI), maximum daily temperature (MaxTemp) and average daily percent humidity (%Hum). In addition, herd characteristics such as geographical coordinates, were available. Moreover, pedigree and genomic (Bovine 50k SNP chip) data were available for each recorded cow. Since THI is highly correlated with MaxTemp ($r = 0.99$), THI was not further considered in the study.The first step of the study consisted of the analysis of the lactation curves for milk, fat and protein through a functional Principal Component Analysis (fPCA). The aim of fPCA is to summarize longitudinal data in a few synthetic variables (the eigenfunctions). For the three traits, three eigenfunctions were retained by the fPCA. The first eigenfunction describes the average curve, and the following ones are related to the features of the curve. For instance, for milk production, the three eigenfunctions describe the production level, the production persistency and the peak production. They explain, respectively: 89.6%, 8% and 2.4% of the total variation for milk. Similar results were observed for protein content (85.2%, 11.7 % and 3.1 %), and fat content (89.2 %, 7.4%, 3.4 %). Eigenfunctions scores were then analysed in relationship with the average climate variables MaxTemp and %hum, with a model that also includes a Herd effect. For instance, climate variables are significant for the milk production level (First eigenfunction of milk production level) Following steps of the study will consist in the joint analysis of climate, production traits and genotypes by combining milk production and climate records with the SNP genotypes of dairy AI sires that have lactating daughters across different regions of Italy. This will add a genetic and a geographic component to the analysis of the relationships between phenotypes, climate and genotypes.

O409

Environmental variation of milk production: an effective indicator of genetic and genomic animal resilience

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The objective of this study was to identify an indicator of resilience in the Rendena breed. A good resilience indicator must be easy to measure and analyze, with good heritability (h^2) and favorable correlation with other traits. With this in mind, we studied the potential role of the standard deviation of the total amount of milk produced between lactations (SDMilk) as a potential indicator of resilience. The underlying hypothesis was based on the idea that greater sum of square is related to a decline in the target phenotype, which in turn is due to some external factor that has adversely affected animal's production. The suitability of this indicator was validated through (i) h^2 estimate, (ii) analysis of genetic correlation with other production and fitness traits, and (iii) through genome-wide association analysis (GWAS). Specifically, SDMilk among lactations were calculated from whole lactation data using a database containing individual information on total milk production per lactation standardized to 305 days (134,287 lactations belonging to 34,825 animals). Pre-corrected milk yields by fixed effect (lactation number, herd year season) where obtained and residuals were used to calculate the SDMilk for each animal. An animal model in a Bayesian framework was applied to estimate h^2 with heterogeneous residuals according to the different lactations used to calculate SDMilk. Genetic correlations with other fitness and productive traits were then calculated. Finally, the ssGWAS algorithm was used in GWAS analysis to better match animals without phenotype but genotyped and vice versa. A total of 10,538 animals presented a phenotypic SDMilk value, and 2499 were genotyped (100,000 SNPs). Only 630 animals had both phenotype and genotype, but ssGWAS was performed in all genotyped animals with reasonable accuracy. The target trait had an h^2 of 0.17 (±0.05), showing that SDMilk present a moderate h^2. Genetic correlations showed a positive correlation with other productive traits such as milk, protein, and fat production ($r = 0.25$, on average) and a negative genetic correlation with other fitness traits like somatic cell score (0.15), udder health (−0.22), longevity (−0.35) and fertility (−0.25). Last, the GWAS identified 6 significant signals that combined with pathway analysis demonstrated a connection between these traits and biological processes related to the immune and nervous systems.

O415

A genome-wide comparison between unselected and selected Valle del Belice sheep reveals selection signatures related to production traits

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The Valle del Belice is a sheep breed reared in Sicily for milk production. About three decades of breeding and selection in this breed are expected to have left various genomic footprints related to dairy traits. We have assembled a dataset with 451 individuals, 267 old samples (collected before 2000s) and 184 new samples (collected between 2018 and 2020), and 40,660 SNPs to identify genomic regions potentially under selection, using five different approaches within (iHS and ROH) and between (F_{ST}, Rsb and XP-EHH) groups. Population structure analyses were able to separate all individuals belonging to the two considered groups. There were no overlapped genomic regions jointly identified by the five statistic methods, since each approach tends to detect different signals. However, a total of 7 genomic regions on four different chromosomes were jointly identified by at least two statistical approaches, which can provide clues to potential new targets of selection. In particular, we found a congruence among the EHH-based methods (iHS, Rsb and XP-EHH), but also betweeen iHS and ROH approaches. Several candidate genes for milk production were identified within the genomic regions of the samples under selection, corroborating the polygenic nature of this trait. We also found that some of our candidate regions putatively under selection spanned several genes related to growth traits and innate immunity. Overall, the identified genes may explain the effect of selection to improve the performances related to milk production traits in the breed. The genomic regions here identified corroborate with previously reported studies carried out in other livestock species. The different approaches did not detect genomic regions known to contain strong functional candidate genes for milk production traits, such as casein clusters or *DGAT1*. This could be a consequence of a low density of the adopted SNP array in these regions. Therefore, further studies using the high density array data, would be particularly relevant to refine and validate these results.

O130

Transcriptomic characterization of water buffalo's extracellular vesicles from colostrum and milk for their immunomodulatory potential

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Recently, much interest has been raised in the characterization of signaling molecules carried by extracellular vesicles (EVs), which are particularly enriched in milk (mEVs), for their capability to cross biological barriers, resist acidification in the gastric environment and, exert modulation of the immune system, mainly through their microRNA (miRNA) content. This work aimed to characterize the small-RNA cargo of colostrum EVs (colosEVs) and mEVs of Mediterranean buffalo through next generation sequencing (NGS).To this purpose, 7 subjects reared in central Italy were chosen, collecting two different samples: colostrum (from the first milking after the parturition) and milk 50 days after. ColosEVs and mEVs were isolated through differential centrifugations, an EDTA treatment and ultracentrifugations to recover vesicles in the pellets. A morphological characterization through transmission electron microscopy for shape and contamination assessment, and Exoview technology for concentration, dimension and positivity to EV-markers testing confirmed the EV isolation. Total RNA was extracted and the small-RNA libraries were sequenced through the Illumina® technology. In both cases, most of the small-RNAs referred to miRNAs (95% for colosEVs and 96% for mEVs) and, out of these 350 were shared, 17 colosEV-specific and 73 mEV-specific. The differential gene expression analysis showed 1504 differentially expressed genes (DEGs, log2 Fold Change – log2FC > |1| and adjusted $p < 0.05$), 961 up-regulated and 543 down-regulated, in colosEVs compared to mEVs. The RNA types with a highest number of DEGs were protein coding (918 up-regulated and 281 down-regulated) and miRNAs (28 up-regulated and 193 down-regulated). For DE miRNAs, targets were retrieved and a protein-protein interaction (PPI) network was build. On these targets, a gene ontology (GO) enrichment analysis was carried out highlighting, for targets of up-regulated miRNAs, enriched terms related to the innate immune response, miRNAs, transmembrane receptor protein kinase activity, mitochondrion, DNA methylation or demethylation, smooth muscle cell proliferation and nitric oxide metabolic process. For targets of down-regulated miRNAs, cellular response to cytokine stimulus and innate immune response, I-kappaB kinase/NF-kappaB signaling, DNA methylation and organization, signal transduction, stress-activated MAPK cascade, posttranscriptional regulation of gene expression and RNA splicing emerged as enriched terms.

O334

Estimation of breeding values in Italian dairy goats: from BLUP to ssGBLUP

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Genomic evaluations are routinely used in most livestock breeding programs and Single–Step Genomic Best Linear Unbiased Prediction (ssGBLUP) is the most popular methodology. Saanen and Camosciata delle Alpi are the most important dairy goats in Italy and they have similar breeding programs in which estimated breeding values (EBVs) for productive traits are calculated using BLUP. Developing a new genomic evaluation in these breeds using ssGBLUP is one of the aims of CHEER and SHEEP&GOAT projects, managed by the Italian Sheep and Goat Breeders Association (Asso.Na.Pa.) and funded by the Italian Rural Development Plan (sub-measure 10.2). In this study, we present the first results of this activity, for which genomic breeding values were estimated using BLUP, GBLUP, and ssGBLUP methods. The following data were included for Saanen and Camosciata delle Alpi breeds, respectively: 1139 and 2472 animals genotyped with the Illumina GoatSNP65 Bead Chip; 8881 and 2589 lactations of 210 days belonging to them and used within official genetic evaluations, with milk (MY), protein (PY) and fat (FY) yields and protein (PP) and fat (FP) percentages; and three generations of parents loaded from the official herdbooks, checked, and corrected with genomic data using seekparentf90. Variance components estimated with blupf90+ on the corrected pedigrees were used for all the three EBV's estimation methods. Furthermore, we calculated the correlation between EBVs and genetic trends for all the three methods. Results were validated with the linear regression method (LR).

Moderate heritabilities were estimated for all the traits: 0.30 for MY, 0.33 for PY, 0.28 for FY; 0.33 and 0.52 for PP, and 0.20 and 0.34 for PP in Saanen and Camosciata delle Alpi, respectively. EBVs estimated with BLUP, GBLUP, and ssGBLUP showed very high correlations (>0.90); the genetic trends were very close for all methods and generally increasing from 2015 to 2020. EBVs were more accurate under ssGBLUP than BLUP and GBLUP (around +9% and +1% across traits).

In conclusion, our results show that ssGBLUP improves the accuracy of EBVs, especially when animals do not have phenotypes; thus, it is likely to be the best method to improve genetic gain in Saanen and Camosciata delle Alpi goats.

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O188

A functional polymorphism influencing the promoter activity of alpaca α-lactalbumin gene (LALBA)

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Alpha-lactalbumin (α-La), encoded by *LALBA* gene, is a Ca^{2+} binding whey-protein whose key function is to facilitate lactose synthesis by the galactosyltransferase component, serving as a regulatory subunit. Other biological functions have been demonstrated including immune modulation, cell growth regulation, antimicrobial activity, etc. Gene promoters have transcription factor (TF) binding sites necessary for gene expression regulation. Mutations in the promoters may modify the transcription rates or the mRNA stability, thus affecting the protein yield.

This study aims to sequence the *LALBA* promoter in alpacas, identify putative TFs and detect genetic diversity affecting gene expression.

A DNA fragment (800 bp) spanning the gene promoter until the exon 1 was amplified and sequenced for 20 alpacas. Multiple alignments and SNP discovery were accomplished by DNAsis software, whereas Transfact 7.0 was used for the TF sites search. Three independent gene reporter assays were achieved by pGL3 specific contructs to test luciferase expression in HEK 293T cells. Data elaboration was performed using JASP software ($p < 0.05$, students's *t*-test).

TF binding sites analysis evidenced 16 putative consensus sequences, including 3 C/EBPα, 3 Sp1, one NF-1, etc. Seven polymorphic sites were found. Taking as reference the first nucleotide of the exon 1, one SNP (g.15C > G) was found in the signal peptide, but it is a silent mutation. The other 6 SNPs were detected in the promoter (g.-553A > G, g.-428C > T, g.-308C > G, g.-236A > T, g.-73C > G, g.-51A > G). The SNP g.-553A > G creates a putative binding site of the TF Sp1. This motif is a well-known enhancer element for the basal expression of many genes, including milk proteins.

To assess the SNP effect on the *LALBA* promoter, we amplified and cloned a DNA region of 178bp in the pGL3-basic vector from four homozygous individuals (two g.-553AA and two g.-553GG). The two different constructs (g.553A and g.-553G), with the pGL3 vector as a control were used to transiently transfect HEK293T cells. After 48 h, the reporter activity of the variants was measured using the luciferase assay system. The G variant of this SNP enhances the promoter activity of the alpaca *LALBA* ($p < 0.01$). Therefore, we suppose an effective role of this binding site in the expression of the α-La in alpaca milk that, consequently, may affect the functional roles of the protein.

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O66

Genetic parameters for blood indicators of metabolic distress and milk traits in dairy cattle

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Changes in blood biochemical pattern reflect animals' health status, which is strictly related to the productivity. In this framework, our hypothesis is that the blood metabolic profile should not only be considered an indicator of disease, but also an indicator of animal productivity. Therefore, aim of our study was to investigate the genetic basis of blood metabolites (including energy-related metabolites, indicators of liver function or hepatic damage, oxidative stress, inflammation, innate immunity, and mineral status) and to estimate the genetic associations between them and a set of milk phenotypes related to milk yield and composition in Holstein cattle. Milk and blood samples were collected from 1353 Holstein cows belonging to five herds. A set of Bayesian univariate and bivariate animal models (using H matrix) was implemented via Gibbs sampling, and statistical inference was based on the parameter marginal posterior distributions. Glucose had moderate positive genetic correlations with milk fat (0.32 ± 0.024) and negative with lactose (−0.29 ± 0.022). β-Hydroxybutyrate had moderate negative genetic correlations with protein (−0.30 ± 0.001) and casein proportions (−0.27 ± 0.001). Among inflammation/innate immunity, ceruloplasmin had moderate negative genetic correlations with lactose proportion (−0.29 ± 0.025), and weak negative genetic correlations with milk yield (−0.22 ± 0.025). In the case of liver function or hepatic damage indicators, aspartate aminotransferase and γ-glutamyl transferase had moderate negative genetic correlations with milk fat proportion (−0.39 ± 0.026 and −0.36 ± 0.025, respectively) and moderate positive with milk yield (0.39 ± 0.024 and 0.42 ± 0.024, respectively). For oxidative stress metabolites, we found only weak genetic correlations except for thiol groups and total reactive oxygen metabolites which had moderate to strong negative genetic correlations with milk yield (−0.31 ± 0.021 and −0.47 ± 0.024). This study shed light on the shared genetic basis of blood biochemical indicators and milk traits.

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O354

RNAseq reveals modulation of genes involved in fatty acid biosynthesis in chicken liver according to genetic background, sex and diet

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Chicken products are the most consumed animal-sourced foods globally, spanning diverse cultures, traditions, and religions. Indeed, in the last twenty years, chicken meat has seen a major increase in production, and is expected to grow further in the coming years. In chickens, increases in production are mainly due to specialised breeds. These breeds have been selected for fast growth and products. Nowadays, of increasing importance are the local breeds, which are known for their ability to adapt to the environment and for their particular phenotypes. Conventional poultry meat contains low levels of n-3 fatty acids, and so the aim of this study was to determine the genes involved in LC-PUFA biosynthesis pathways, and to evaluate the modulation of their expression according to genetic diversity, diet and sex.

Birds from two local breeds (Bionda Piemontese and Robusta Maculata) and a commercial line (Ross708) were fed different diets: control and experimental diet (10% linseed supplementation). For each breed and diet group, both males and females were reared. RNA was extracted from a total of 36 liver samples and were subjected to RNAseq. Bioinformatic analysis was carried out to find differentially expressed genes (DEGs) from different comparisons between experimental groups. Results showed low impact of diet on DEGs related to fatty acid biosynthesis. Concerning sex comparisons, the female groups showed genes up-regulated and involved in fatty acid metabolism respective male groups. The genetic background also determined the expression of genes related to LC-PUFA biosynthesis. Specifically, in females of both local breeds compared to commercial birds, 23 genes were found to be commonly up-regulated. Amongst these, there was significantly more expression in two genes having a role in *de novo* triglyceride biosynthesis (*MTTPL* and *GPAM*) and two genes involved in *de novo* FA biosynthesis (*ACACA* and *SCD*). In conclusion, female sex and local genetic background appear to have significant influence on expression of genes associated with LC-PUFA pathways.

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O563

Study of fertility traits in Italian Jersey Cattle

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Selection Index of Italian Jersey cattle is composed by breeding values (BVs) of production traits, type traits and somatic cell score. Knowing selection for milk yield has been proved to deteriorate fertility performances, aim of the Italian Holstein, Brown and Jersey Breeders Association (ANAFIBJ) is to develop a fertility index inspired to the model they use for Italian Holstein breed. The model used considers different phenotypes for heifer and cow evaluation, but while for Holstein only the first three parities are considered, for Jersey all parities are considered to overcome the lower size of the population. Starting from two different datasets, containing data on inseminations and lactations we derived several fertility traits. From the inseminations dataset we harvested information on the dates of services, which we grouped in baches of a maximum length of 300 days. The groups of inseminations have then been attached to the calvings happened in an interval of 260–300 days from at least one of the services in the group. Phenotypes estimated for both heifers and cows are Non-Return Rate at 56 days (NRR56) and Interval from First to Last service (IFL); Age at First Insemination (AFI) has been computed only for heifers, while Days from delivery To First Service (DTFS) and Equivalent Milk Yield 305 (EMY305) only for cows. The values of the phenotypes have been filtered to be in line with biological and economic criteria of cattle farming. The final dataset is composed of 35081 records for heifers and 100285 records for cows. Correlations and Co-Variances between the phenotypes have been calculated. For Heifers we observe a slight positive correlation between AFI and NRR56 ($\rho_{xy} = -0.061$) and a negative correlation between NRR56 and IFL ($\rho_{xy} = -0.349$); IFL and AFI are negatively correlated ($\rho_{xy} = -0.048$). For cow fertility traits NRR56 resulted to be positively correlated with DTFS ($\rho_{xy} = 0.088$) and negatively correlated with EMY305 ($\rho_{xy} = -0.092$) and IFL ($\rho_{xy} = -0.293$); DTFS resulted negatively correlated to IFL ($\rho_{xy} = -0.015$) and EMY305 ($\rho_{xy} = -0.045$), while IFL and EMY305 resulted positively correlated ($\rho_{xy} = 0.083$). The next step is to calculate genetic parameters and EBVs for fertility traits for Italian Jersey.

O352

Analysis of longevity performance in the different bull lines of Busha cattle in Croatia

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Longevity is a complex trait; the longevity performance of the sire is a measurement of the time it's produces in a herd and is determined by various factors such as fertility, health, and workability. The sire fulfilling an active working life in the herd increases the average herd production and efficiency by reducing input costs. Busha is a native breed of cattle reared in an extensive system and classified as an endangered population – maintained. From the establishment of the Herd Book, breeding systematization defined 24 lines of bulls (LB). The aim was to analyze the herd life of different bull lines of the Busha cattle breed in Croatia. The data on 83 breeding sires born in 1995–2022 with at least one offspring were obtained from the Veterinary Information System of the Ministry of Agriculture, Directorate for Veterinary Administration. Herd life was defined as the total number of days from the calving date of first offspring to the last (culling) date of the bull. Testing of differences in the number of days spent in the herd between different lines was carried out taking into account as a minimum criterion 4 breeding bulls for each line. The difference in the days spent in the herd between the lines was carried out with one-way ANOVA at $p < 0.05$. The average age of the bulls in the herd was 1549.84 ± 974.14 days with a coefficient of variability (CV) of 62.85 %. The most represented line of bulls were LB3 (13.25 %), LB4 (22.89 %), LB5 (10.84 %), LB6 (4.82 %), LB7 (4.82 %), LB8 (4.82 %), LB9 (9.63 %), LB11 (8.43 %) and LB12 (7.22 %), with the most significant number of male progenies.

whereby no statistical significance was recorded. Results show a small number of certain bulls from the different lines in the breeding and relatively high use of natural service sires. Furthermore, considering the current living bulls, some bull lines are no longer in breeding. Increasing the number of productive bulls and improving longevity aid in the herd's average production enhancement, aiming to develop sustainable breeding strategies and maintain genetic diversity in the Busha cattle population.

O577

Mule carcass characteristics and meat quality

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Donkey carcass parameters and donkey meat quality characteristics have been determined in previous studies, while mule carcass and meat quality traits have scarcely been evaluated. The aim of the present study was to compare the carcass data and meat quality parameters obtained from 10 mules slaughtered, respectively, at an age of 5 ± 1 years and at an age of 10 ± 1 years. All the animals were weighed before their arrival at abattoir; the carcasses were transferred to a cold room at a temperature of 4 °C and stored suspended by the hind legs. 24 h after slaughtering, the cold carcass weights and the dressing percentages were recorded. From each carcass, 24 h after slaughtering samples of the muscle *Longissimus thoracis* (LT) were collected. Chemical composition was determined 24 h after slaughtering, while colour parameters were measured 48 h after slaughter using a Minolta CM-3600 D spectrophotometer in order to determine the L* (lightness), a* (redness), and b* (yellowness).

Meat samples for tenderness determination, weighting approximately 30 g, were stored in the cold room at 4 °C for 7 days post slaughter before evaluating the shear force values, determined using a Warner-Bratzler device. The shear force values were expressed in kg/cm^2. Analysis of variance was used to determine the significant differences in the values determined in this study; significant differences were shown when $p < 0.05$. Carcass characteristics determined in both groups of animals did not show significant differences. Meat sampled in animals slaughtered at an age of 10 years showed significant ($p < 0.05$) higher content of fat (5.22% *vs* 4.09%) and cholesterol levels (0.88 mg/100 g *vs* 0.52 mg/100 g) compared to younger mules. Meat produced by older mules was significantly ($p < 0.05$) darker (L* 31.09 *vs* 39.71, a* 13.8 *vs* 17.1) compared to the values determined in younger mules. Meat tenderness evaluation found not significant differences in both the groups of mules, obtaining values of shear force not particularly appreciated by the consumers. The results obtained in this study confirmed the need of adequate feeding strategies and good farm practices in mules breeding in order to produce a more tender mule meat.

O411

The use of genomics in dairy cow farming – results from the GENORIP project

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GENORIP (GENOmic tool for the management of REProduction in dairy cattle and for the control of inbreeding) is a project funded by Lombardy Region. The aim was to release an innovative tool for the control of inbreeding, the maintenance of genetic variability in cattle breeding and to facilitate the choice for animal replacement based on genomic information of herd females. In recent years, thanks to reduced genotyping costs, genomic selection has become attractive for breeders and farmers. Current genotyping techniques make it possible to determine at low cost the genotype of an animal in hundreds of thousands of markers known to be associated with phenotypic variability and to use this information to select animals. GENORIP's partners are 7 farms of the Lombardy region, ARAL and INSEME S.p.a, together with the University of Milan. More than 6000 females of Italian Friesian cows were sampled and genotyped with the Neogen GGP Bovine 100K SNP chip in the 7 partner farms and in other 50 farms involved in the dissemination phase. Principal component analysis (PCA) was applied for analyzing the genetic diversity within and among farms using the SVS 8.9 software of Golden Helix. Run of Homozygosity (ROH) and the genomic inbreeding (FROH) were obtained using the detectRUNS package of the R software. Genotype frequencies for mendelian disease, fertility and production mendelian traits were also obtained. PCA showed clustering of cows within herds according to sires used in each farm, whereas when analyzed jointly herds overlap except one due to the selection goal pursued by this farmer. ROH were found in all chromosomes with an average length of 2,703,811 bp covering 12.7% of the genome. The ROH analysis showed several genomic regions that appears under selection at the population level, while a specific region on BTA4 is under selection in one herd, harboring genes related to the specific breeding strategy of the farmer. The F_{ROH} values obtained, considering ROH greater than 16 Mb, varied from 0.004 to 0.325, with the highest average value for the F_{ROH} of 0.136. Among mendelian heritable diseases, the Haplotype Cholesterol Deficiency was the one with the largest of carrier animals, i.e. 5.6%. A process to assist farmers in genomic management of reproduction was released tailored to each farm.

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O510

Maedi visna virus infection and TMEM154 genotypes in Valle del Belice sheep breed

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Maedi visna virus (MVV) referred to as small ruminant lentiviruses (SRLVs), belong to the genus *Lentivirus* of the Retroviridae family. MV infection causes pneumonia, arthritis and progressive paralysis, and chronic mastitis not always clinically detected. The key role in the spread of the virus is attributed to the vertical transmission through colostrum and milk from infected mothers, as the infectious agent is present in mammary secretions. Since there are no vaccines or treatments against the virus, the available control strategies are laboratory diagnosis with ELISA test to find out the infection presence, control of environmental conditions and selection of genetically resistant individuals. The presence of a polymorphism in the TMEM154 gene modulates the resistance/susceptibility of sheep to MV infection. The polymorphism is G→A substitution in exon 2 of TMEM154 gene which involved the glutamate amino acid residue at position 35 (E35) of the protein encoded by this gene, changing in lysine residue (K35). Animals carrying KK genotype were resistant to MV infection while EK and EE genotypes were susceptible ones. The purpose of this study was to verify the prevalence of MV infection within Sicilian herds of Valle del Belice sheep using an ELISA serological test (IDvet) to detect animals affected by the virus and a molecular test to estimate frequencies of the resistant/susceptible genotypes to the TMEM154 gene. A total of 1083 animals (both ewes and rams) from 9 flocks of Valle del Belice sheep located in different provinces of Sicily, were serologically tested, and genotyped for E35K polymorphism. The ELISA method showed 15.33% ($n = 166$) of infected individuals. Analyses of the obtained sequences showed the presence of both K and E amino acid with frequencies of 0.151 and 0.849, respectively. On the total individuals, only 23 carried KK genotype while 280 were heterozigous EK and 780 homozygous EE. The locus was in Hardy-Weinberg equilibrium in the breed (p-value <0.05) and observed and expected heterozygosity values were 0.258 and 0.256, respectively. In conclusion, this study allowed us to know the prevalence of MV infection in Sicilian flocks of Valle del Belice breed and could be helpful in establishing selection programs aimed at controlling and eradicating this virus.

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O442

Integration between genomic and microsatellite analysis for the parentage verification

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Parentage verification is one of the crucial points of cattle breeding selection, since pedigree errors have a negative impact on variance component estimation, EBV reliability, and breeders' selection choice. In a genomic evaluation based on single-step approach, an imprecise pedigree matrix can cause convergence issues when that matrix is integrated with genomic relationships. Before 1999, parentage verification was done with blood groups, then microsatellite markers (MS) were employed. With the advent of genomic tools, SNP arrays are widely used for secure parentage analysis and for parentage discovery. However, it is not always possible to use SNP array information directly since we often only have access to microsatellite analyses of the parents. We developed a robust and cost-effective method for imputing MS alleles from SNP haplotypes. Reference haplotypes were generated for over 17000 Brown Swiss genotypes and over 4000 Valdostana genotypes. Densities of SNP arrays vary greatly between 7K and 777K. To phase the genotypes, BEAGLE was used, as it was one of the few software that could handle both bi- and multi-allelic data. The MS region was imputed based on McClure et al.'s 2012 publication. For cattle parentage, this approach was used to impute the recommended panel of 12 MS by ISAG (International Society of Animal Genetics). The results show that, depending on the breed and microsatellite combination, the error in MS imputation varies from 0.001 to 5%. With the implementation of this method, producers and breed associations can continue to utilize SNP-based parentage verification even when SNP genotypes are missing by analysing MS genotypes from historical data. A broader application of this approach may be possible for cattle breeds and other species wishing to migrate from MS-based to SNP-based parental verification.

O221

Genetic relationships between milk production and impacting diseases in breeding sheep

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Genomic tools are crucial for the efficiency of the breeding program of the Sarda sheep breed. A female population in a nucleus flock (FRP) has been set up to trigger genomic selection for health traits costly to measure on large scale. The inclusion of resistance to Gastrointestinal Nematodes infestation (GIN), Paratubercolosis (PTB) and Maedi Visna (MV) infections as selection objectives needs to estimate their heritabilities (h^2) and genetic correlations with milk yield (MY). The FRP ewes were genotyped (Illumina 50K chip), pedigree and MY recorded from 2000 to 2022 (16,249 lactations of 4940 ewes). Moreover, 17,679 individual fecal egg counts *i.e.* the number of GIN eggs per g of faeces (FEC), of 4681 ewes were available. FEC was log-transformed (lnFEC) for genetic analyses. The infection status for PTB of 3032 ewes (1 for infected and 0 for not infected) was determined by 14,349 ELISA screening tests from 2001 to 2012. A ewe with at least one positive test along the recording period was considered affected by PTB. From 2016 to 2019, 770 ewes were first tested at 1 year age to determine the infection status for MV (1 for infected and 0 for not infected). ELISA tests were repeated every six months until 36 months of age just for ewes with negative previous test. Means and standard deviations were 216 ± 46 litres for MY, 4.82 + 1.43 for lnFEC. The ewes considered affected by PTB and MV were 906 and 740 respectively. The infection status along the lifetime for PTB and MV and repeated measures for MY and GIN were used to estimate h^2 with single trait analyses considering the genomic relationship matrix. Genetic correlations were estimated with bi-trait animal models using pseudo-phenotypes derived from the single trait models: the sum of genetic and residual predictions for MV and PTB and the sum of the genetic, permanent environment predictions and the average of residuals for MY and GIN. Heritabilities were 0.36 for MY, 0.22 for GIN, 0.22 for PTB and 0.20 for MV. The genetic correlation between MY and resistance to GIN was moderate and unfavorable (0.24). The genetic correlations between MY and resistance to PTB and MV were negligible (0.08 and −0.01). This study confirms that selection for GIN implies a moderate impact of the improvement on MY. Results for resistance to PTB and MV did not give evidence of any genetic relationship with MY.

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O181

The ability of udder linear morphological evaluation to predict milk production in Massese and Comisana sheep

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The aim of the present study is to validate the morphological evaluation applied on the udder traits of two Italian dairy sheep breeds: Comisana (COM) and Massese (MAS). These breeds are studied within CHEERS and SHEEP&GOAT projects, managed by the Italian Sheep and Goat Breeders Association (Asso. Na.Pa.), funded by the Italian Rural Development Plan (sub-measure 10.2). The aim was to evaluate the relationships between 4 udder traits estimated through a nine-point linear scales (teat placement TP; udder depth UD; degree of suspension of the udder SU; degree of separation of the 2 halves DS), 1 udder trait measured in cm (teat length TL), 2 morphological traits (body condition score BCS, the chest circumference CC), and the milk production. For this purpose, the milk yield expressed as Mature Ewe Equivalent (MEE) of 451 COM ewes and 216 MAS ewes and the quality traits (fat content – FC, protein content – PC, somatic cell count – SCC) were analyzed and associated to the udder evaluation score. Correlations with very low magnitude were observed between CC and MEE, ranging from −0.15 for COM and −0.24 for MAS. FC and PC were found to have both a low negative correlation with MEE in COM breed ($r = -0.30$). Instead, a negative correlation between FC and MEE ($r = -0.41$) was found and no correlation was observed for PC and MEE in MAS. Regarding udder evaluation scores and MEE no correlations were found in both breeds, even if a low positive correlation ($r = 0.21$) resulted between UD and MEE in MAS. Furthermore, SCC resulted were not correlated with the udder linear scores. The results highlight the possibility to implement the measurement of the udder and its morphological traits for both breeds. In literature, the possibility to focus the selection for milk yield on the udder morphological characteristics has been confirmed. The low correlations observed in this study suggest us to develop a better morphological evaluation in both breeds, in order to identify the best udder traits that might be useful for the selection and for prediction of milk production in Comisana and Massese ewes.

O399

How mum and pen mates affect my growth: maternal effects and indirect genetic components on growth traits in young bulls at performance testing station

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Individuals can influence the expression of each other's phenotypes through indirect genetic effects (IGEs) – the effect that the genotypes of others have on the phenotype of an animal – that can belong to various sources. Two are the most important sources of IGEs: the maternal effects of the mother on the calf and social genetic effects, i.e. those of the pen-mates. Certain individual characteristics as aggressiveness, or competitiveness can lead to a reduced growth in individuals. For this reason, social genetic effects often are negatively correlated with the additive genetic component. Including in a quantitative genetic model any potential IGE can help increasing selection accuracy for animals raised in groups. Few studies have been performed in situations where maternal and social genetic effects could be tested at the same time. Moving from these considerations we aimed to analyse both maternal and social IGEs in young bulls at performance testing station (PTS). Rendena cattle, a dual-purpose local breed from the Northeast of Italy was the study subject. A number of 11 monthly weighting realized along the whole permanence at PTS was considered for about 1300 candidate young bulls tested over 27 years. Each individual that reached the PTS at about one month of age, was individually stabled for the first three months and then was kept in pens of 8–15 individuals. We considered a time-specific phenotype (weight, divided in 11 weightings) measured on the young bulls, and obtained estimates of heritability, maternal and social genetic effects for each weight. We discovered that the maternal effect was strongest at the first two weigh-ins and became negligible at the following weightings. The social genetic effect was also time-specific, but in the opposite way, as the genetic correlation with the direct genetic component linearly moved from positive to negative values during the weightings. The social-direct genetic correlation showed the expected negative relationship only from the last weigh-ins. Result agreed with theory, that would predict that both effects could impact the phenotype of the animal, but in different timeframes. In fact, the maternal effect is stronger soon after birth, whereas the social effect increases the longer the animal spends time with other individuals. Results shed light on the importance of considering the complex interactions among conspecifics in the genetic evaluations of traits expressed under a social environment.

O244

Reconstructing pedigree information in the Reggiana cattle breed based on high density SNP data

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Reggiana is an autochthonous cattle breed, mainly reared in the North of Italy. Milk of Reggiana cows is almost exclusively used for the production of a mono-breed branded Protected Designation of Origin (PDO) Parmigiano-Reggiano cheese. Reggiana population size experienced a dramatic reduction in the past, which was then reverted after 1980'. As in many local breeds, Reggiana pedigree records are not always complete and partial recording may increase error rate in several applications, such as inbreeding coefficient calculation and definition of selection plans. High-throughput genotyping data could help to identify and then correct pedigree errors. A few approaches have been proposed to identify pedigree inconsistencies using DNA markers. Opposing homozygotes (OH) define parentage inconsistencies in candidate parent-offspring pairs based on homozygous single nucleotide polymorphisms (SNPs) for alternative alleles. In this work, available Reggiana pedigree information has been used as a starting point for reconstructing problematic records using OH evaluation. A total of 2730 Reggiana cattle, including commonly used 50 sires, have been genotyped with the GGP Bovine 150K SNP Chip. PLINK software and ad hoc scripts have been used to filter and to obtain all pairwise comparisons of the genotyped cattle based on OH. The genotyping error rate was estimated and then used to define the threshold of the number of inconsistent OH SNPs to declare an incorrect pedigree. Parentage information from pedigree records was not correct for about 10% of the animals. The routine application of this method will improve both the managing and conservation of Reggiana cattle breed and will improve accuracy of estimated breeding values.

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O204

Evaluation of the effect of the breeding system on the expression of liver genes in local slow-growing chicken breeds

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The production of poultry meat is based on fast-growing strains (Ross 308) reared in intensive system with concerns on animal health and welfare. The free-range system of local poultry breeds appears crucial to safeguard welfare, sustainability and biodiversity. The aim of the present study was to evaluate the effect of two breeding systems – intensive (I) *vs* free-range (F) – on the expression of liver genes related to stress and lipid metabolism. Two local chickens' breeds (Bionda Piemontese, BP and Robusta Maculata, RM), their cross-breeds (BPxSassò, RMxSassò) and Ross 308 were used. A total of 300 one-day-old male chickens were randomly allocated into two groups (3 replicates/treatment/genotype; 10 chickens/replicate).

The intensive group (I), received a standard diet and the free-range group (F) received a low input diet until slaughtering (84 d). At slaughter, liver samples were collected in RNAlater and stored at −80 °C. Eleven genes (*Acox1, FABP1, HSPA2, CASP6, CAT, FADS2, LPL, SOD1, SREBF2, ACTB* and *GAPDH*) were studied in liver. RNA expression levels were normalized using *GAPDH*. The laboratory protocol was characterised by the following steps: RNA extraction, reverse-transcription, multiplex PCR, library preparation and NGS sequencing (Miseq Illumina platform). R software (version 4.2.2) was used for statistics by applying R packages to perform reads count (FeatureCounts) and differential gene expression (Deseq2). The results obtained showed differences in gene expression related to lipid metabolism. Chickens reared in I breeding system showed a higher expression in the lipoprotein lipase gene (*LPL*) ($p < 0.001$; log2 Fold change 1.35). *LPL* is involved in liver fatty acid metabolism and the result suggested that I may increase lipid synthesis and accelerate lipolysis by upregulating the *LPL* expression. Instead, in F breeding system, a higher expression of the sterol regulatory element binding transcription factor 2 (*SREBF2*) ($p < 0.001$; log2 Fold change +1.96) was observed. *SREBF2* regulates the transcription of genes related to cholesterol biosynthesis and its expression is higher when the sterol concentrations are low, likely as a consequence of the low-input feeding management. The different breeding systems showed an effect on the expression of liver genes related to lipid metabolism. Further studies should be carried out to identify poultry genotypes more suitable for free-range breeding systems in order to promote sustainability and biodiversity.

O410

Structural variation in the Aosta cattle breed

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Copy Number Variants (CNVs) are structural variants affecting genetic diversity that influence phenotypic expression. Scientific community has underlined relationship between CNV and adaptation to different environmental conditions (e.g. high altitude and harsh climate). Aosta cattle (Aosta Red Pie – ARP; Aosta Black Pied/Chestnut – ABC and Mixed Chestnut-Herèn – ACH) are autochthonous dual-purpose breeds, well-adapted to be reared in the Alps mountain area. The aim of this study was to characterize Aosta population through the CNVs detection and investigate their relation to the adaptative selection to the mountain farming system. ANABORAVA provided the LogR Ratio (LRR) and the B allele frequency of 3195 females (2108 –ARP, 929 – ABC, 158 – ACH), obtained with the NEOGEN's GGP Bovine100K, that were used to call CNV on autosomes using the SVS 8.9 (Golden Helix®) CNAM module. Taking into account the genetic similarity between ABC and ACH found using SNP genotyping data, all the statistics were calculated considering these two breeds as a unique one (ABCH). CNVs were aggregated into CNV regions (CNVRs) based on at least 1 bp overlap; only CNVRs identified in at least 2% of the cows were considered to infer statistics at breed level (ABCH vs ARP). After quality control performed on the obtained calling, a total of 87,488 CNVs and 2328 CNVRs were identified. Cows of the ABCH and ARP showed similar relationship existing between the CNV count and the CNVs mean length. In the PCA performed using the called CNVs, ABCH and ARP also appear overlapping, without any defined cluster, suggesting that these markers do not segregate because of the directional selection occurring at breed level, but highlight the adaptive selection to the Alpine environment shared by all breeds. In fact, about 67.2% of the CNVRs identified in at least 2% of samples (n. 314 regions) resulted in common between the two breeds. Instead, only 18.2% (ABCH) and 14.6% (ARP) CNVRs were proper of each breed. Annotated genes (n. 730) and QTL (n. 411) overlapping the CNVRs associated with productive, functional and health traits. Funded by PSRN DUAL BREEDING_Fase_2.

O185

Genomic selection for stay-ability in Italian Limousine and Charolais

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The objective of this study is to propose an optimal genetic evaluation modeling for survival in the Italian Limousine and Charolaise cattle populations. Parity records were available for both populations, covering 37,518 and 7818 cows, respectively. Survival was expressed as stay-ability (binary, i.e. 0/1) at a given parity x, i.e. the ability of a cow to survive from parity x to parity $x+1$, with the parity being from 1 to 6 (e.g. STAY1 for surviving from parity 1 to parity 2).

Gaussian and threshold models were tested with pedigree-BLUP and Single-Step GBLUP. Genomic information (about 14,000 SNP markers) was included for 3039 Limousine and 700 Charolais individuals. Variance components were estimated using pedigree information, breeding values were estimated with all combinations of models. Spearman rank correlation was used as a measure of re-ranking.

Heritability estimates showed a decline from STAY1 to STAY6. For Charolais, it went from 0.156 for STAY1 to 0.056 for STAY5. For Limousine, it went from 0.187 for STAY1 to 0.096 for STAY5. In Limousine, results showed a moderate re-ranking (with both pedigree-BLUP and Single-Step GBLUP) for phenotyped cows between the Gaussian and the Threshold model, with correlations from 0.98 for STAY1 to 0.89 for STAY5. The trend was similar for bulls. In Charolais bulls, the re-ranking was almost null for STAY1, but was stronger for other traits, reaching correlation of 0.84 for STAY5 when using pedigree- or Single-Step GBLUP. The re-ranking for cows showed a similar trend and magnitude. Genomic information helped limiting the re-ranking, the lowest value of correlation was 0.89 for cows on STAY5. The use of Single-Step GBLUP, as opposed to pedigree-BLUP, had a stronger impact on re-ranking. Correlations ranged between 0.85 and 0.75 for Limousine cows, 0.95 and 0.81 for Limousine bulls, 0.90 and 0.87 for Charolaise cows, 0.95 and 0.80 for Charolais bulls.

The model used did not impact the rank of the individuals remarkably. The inclusion of genomic information did, and the better quality of genomic-enabled breeding values should be validated with a cross-validation.

Acknowledgements
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O487

Microbial and genomic information synergistically contribute to predicting swine performance across production systems

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Microbial composition is a promising tool in precision farming as an environmental indicator and direct target for selection. We compared the ability of microbiota and genomic information to predict swine performance in two production settings. Terminal (TE, 1141) and Nucleus (NU, 769) pigs, sired by 28 founders, were available. Microbial information was collected at three time points during performance test: weaning (S1), mid-test (S2), and test-end (S3). 3001 common operational taxonomical units were used for analyses. Genomic information (43,515 SNP) was available for all individuals. Measured traits were: daily gain (ADG), fat deposition (BF), loin area (LEA) and depth (LD), and intramuscular fat (IMF). Three different models were implemented using Bayesian Ridge Regression. A model including genomic information (G), a model including microbial information (M), and a model including both. Accuracy of prediction was evaluated across systems, predicting from nucleus to terminal (NU-TE) and vice-versa (TE-NU). Predictions were obtained through cross-validation using solutions for the appropriate *omic* in training to predict traits in validation. Microbiome was predictive for all traits in both systems for information collected at S2 and S3. Highest accuracies were achieved for BF, with correlations of 0.08 and 0.04, and 0.30 and 0.23 for S2 and S3, for NU-TE and TE-NU. Similarly, ADG accuracies were 0.05 and 0.04 and 0.18, and 0.15 for the same time points and scenario combinations. Including genomic information yielded prediction accuracies ranging from low for LEA (0.06 TE-NU, 0.07 NU-TE) to moderate for BF (0.19 NU-TE, 0.16 TE-NU). M had higher prediction accuracies than G for BF (+0.11, +0.07; NU-TE, TE-NU) and ADG (+0.08, +0.02; NU-TE, TE-NU) at S3. Lower accuracies were obtained for IMF (−0.13, −0.14; NU-TE, TE-NU). Including genomic and microbial information produced higher accuracies than M or G alone at S3 for BF (0.37 and 0.29 for NU-TE, TE-NU) and ADG (0.19 and 0.21 for NU-TE, TE-NU). Results for other traits differed for different scenarios. We investigated the effectiveness of genomic and microbial composition in predicting phenotypes production systems. We found that microbial information can predict performances for most growth and carcass composition traits, particularly for growth and fat deposition. Results from the current study can increase information flow from diverse production systems used for selecting individuals in swine.

O579

Use of gut microbiota as a selection tool in swine

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Traits such as meat quality and composition are becoming valuable in modern pork production; however, they are difficult to select because of the high phenotyping costs. Combining genomic information with multi-trait indirect selection with cheaper indicator traits is an alternative for continued cost-effective genetic improvement. In addition, gut microbiome information is becoming more affordable to measure using targeted rRNA sequencing, and its applications in animal breeding are becoming relevant.

Phenotypic information included meat color, meat tenderness, marbling, loin muscle depth, and backfat depth. Gut (rectal) microbiome was characterized using 16s rRNA sequencing at three different time points: weaning, mid-test, and off-test.

Estimation of genetic progress and cross-validation were used to assess the value of using host genomic and gut microbiome information to select for expensive-to-record traits measured on crossbred individuals. First, variance components estimation for multi-trait models was carried out. Here, for each meat quality trait, the 25 OTU were included as correlated traits. The set of OTUs was specific for each trait and time point combination. OTUs were selected based on the correlation between their estimated genomic breeding values and those for the trait of interest, all obtained from single-trait models. In a second step, the inclusion of 1, 2, 5, 10, and 25 OTU into multiple-trait models was compared to single-trait models in its ability to predict unobserved phenotypes. A pedigree-derived relationship matrix also replaced the genomic relationship matrix to assess the value of including (host) genomic information.

Results confirmed the advantage of including genomic information for some traits, but the inclusion of gut microbial information was, in some cases, advantageous. Some traits showed an advantage from including the microbial information rather than the host genomic one.

The impact of the shared genetic architecture between the microbial features and the traits of interest is probably at the basis of the increase in prediction accuracy and should be further studied with statistical methods that account for the compositional and high-dimensional nature of the microbial data. This study proposed a simple method to include gut microbiome information into swine breeding programs to improve expensive-to-record traits, such as meat quality.

O72

Differential somatic cell count as indicator of intramammary infection in dairy sheep

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With the introduction of a new high-throughput flow-cytometry-based analyzer (Fossomatic 7 DC, FOSS, Hillerød, Denmark), a means for identifying individual immune cell populations became available for commercial milk-testing laboratories. Specifically, the differential somatic cell count (DSCC) parameter indicates the sum of polymorphonuclear leukocytes combined with lymphocytes as a percentage of total somatic cell count (SCC). To the best of our knowledge, DSCC is not yet extensively discussed in dairy sheep. Some of the findings indicated that DSCC and SCC used in combination could lead to increased sensitivity to detect IMI in udder health monitoring programs through monthly dairy testing. This study considered ewe milk test day records from twelve Sicilian herds. A total number of 1893 milk samples were collected aseptically after routine cleaning and disinfection of the udder and discarding of the first streaks of milk. Samples were cooled and transported in iceboxes directly to the milk laboratory for SCC and DSCC determination and to the microbiology laboratory for bacteriological culturing. The IMI status of each animal was defined according to pathogens identified with bacteriological analyses and classified in minor (*Streptococcus spp.*, *Streptococcus pyogenes*, *Pseudomonas spp.*, *Prototheca spp*), and major (*Trueperella pyogenes*, *Escherichia coli, Mannheimia haemolytica*, *Pausterella spp*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Staphylococcus intermedius*, *Streptococcus dysgalactiae*, *Streptococcus uberis*). The response variable used in the model corresponds to the binary disease status, coded as 0 or 1 to represent uninfected or infected individuals, respectively. DSCC was normalized using asin(sqrt(DSCC/100)), ranging from 0 to 1. Receiver-operating characteristics (ROC) analysis was performed using the OptimalCutpoints-Package with maximize Se and Sp method. Considering infection due to minor pathogens, the optimal cut-off for DSCC was 57% (95% CI: 0.544–0.597) with sensitivity and specificity of 52% (95% CI: 0.479–0.553) and 61% (95% CI: 0.580–0.636). In conclusion, our study provides for the first time an indication on use of DSCC to detect intramammary infection in dairy ewes; however, more investigation using a larger sample and considering a higher incidence of major pathogens will be conducted.

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O213

Growth performance and morphometric characteristics of Hermetia illucens larvae reared on four different substrates

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Reusing and enhancing organic waste and converting them into new and nutritious food or feed is possible by using it as a substrate for insect growing (Addeo et al., 2021). In this study, 5-days-old *Hermetia illucens* larvae were raised on: (1) broiler feed (CON, control diet), (2) cauliflower waste diet (CAU), (3) apple waste diet (APP), and (4) carrot waste diet (CARR), having an initial larval weight (ILW) of 10.7, 10.5, 10.6 and 10.8 mg, respectively. The larvae were placed in plastic containers (60 × 40 × 15 cm) with a feeding rate of 0.1 g/d of substrate per each larva, considering a growing period of 20 days. The substrates were placed in plastic containers one day before the deposition of the larvae; each container was a replicate and each substrate had 9 replicates. At the end of the trial, when 25% of the larvae developed into prepupae, the larvae were separated from the substrate, frozen at −80 °C and then freeze dried. Data were analyzed by a one-way ANOVA, using the GLM procedure of SAS (2002) and considering the substrate as main effect. Focusing on the larval growth performance, the final larval weight (LFW) was higher in the CON group (0.22 g) characterized by a prepupal development (end of trial) of 15 d only, while in the experimental groups the final larval weight was 0.14, 0.06, 0.04 g ($p < 0.01$) in the CAU, CAR and APP with the trial ending at 21, 25, and 23 d respectively. The percentage of larval mortality (LM) was highest in CAU 16.55% and lowest in APP group 1.77% ($p < 0.01$). The larval yield (LY) was higher in all three experimental groups and lower in the CON group (27.9, 25.8, 22.7, and 15.2% in CAR, APP, CAU and CON group, respectively, $p < 0.01$) probably due to the short time of larvae growth in the CON group. The larval percentage of dry matter was the highest in the CAR group 44.14% and the lowest in the APP group 30.39% ($p < 0.01$). The growth rate (GR) was higher in the APP and CON groups (13.93 and 11.01 mg/d) than in the CAR and CAU (4.56 and 3.55 mg/d, $p < 0.01$). Furthermore, the morphometric characteristics showed that the larvae of the CON group had a length (1.90 mm ±0.17) and a thickness (0.42 mm ±0.06) higher than the experimental groups even if no differences were recorded for the larval shape index. *Hermetia illucens* larvae can growth on different kinds of substrate but, probably, it is important to define a specific feeding rate for each substrate.

O520

Effect of different iso-waste-based diets on black soldier fly larvae growth and chemical composition

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Black soldier fly (BSF) is known as bio-converter of a wide range of waste. This variety generates a diversity in the nutritional composition of the substrates and research results on larvae growth are, in some cases, difficult to compare. In this perspective, 21 waste and by-products were used as ingredient (from 8 to 13) of six iso-nitrogenous, iso-lipidic and iso-energetic diets (13.5%, 7.6%, 18.2 MJ/kg on DM). Diets (D) 1 and 5 were composed mainly by brewer's spent grain, vegetable and fruit waste, D2 by glaze waste and buffalo milk whey, D3 by vegetable and water, D4 by fruit waste (two different origins) and D6 by fruit and vinegar waste. To evaluate growth performance, 3600 6-day-old larvae were divided in homogeneous groups of 100 individuals (6 replicates/treatment) and 30 larvae per replicate were randomly sampled every 4 days until the 40% reached the prepupae phase, in which survival rate, larva-prepupae time duration and bioconversion indexes were calculated (substrate reduction, waste reduction index and bioconversion efficiency corrected for residue). To obtain larvae samples for the descriptive chemical analysis, 3000 larvae per treatment were estimated (2 replicate/treatment). Larvae were inactivated at −80 °C, freeze-dried and analysed for DM, CP, EE and ash content. Data were analysed using the IBM SPSS Statistics software (V20.0.0). The larvae weight was analysed by generalized mixed model with a gamma probability distribution (two fixed factors: diet/time, and their interaction; replicate considered as random effect), while no statistical analysis was performed for chemical composition. Considering survival rate (>92%), all diets were suitable for BSF breeding. Despite the nutritional comparability of the diets, variable results were obtained in all the BSF history traits and larvae chemical composition. D1 and D5 overall performed the best larvae growth and development time, while D2 displayed the worst results (0.144, 0.146 and 0.060, respectively, $p < 0.01$). Moreover, growth and development time were strongly correlated to the substrate pH (positive and negative correlation, respectively; *p*

< 0.05). The bioconversion indexes followed the trend of the larvae performance. The results obtained allowed to discriminate the nutritional variable of the substrates on BSF life history traits and observe other factors of influence (such as waste interaction and water holding capacity) which will need to be evaluated with future research.

O547

Innovative growing substrates for the production of functional Tenebrio molitor meals

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Due to the predicted growth of the world population, food demand is expected to increase exponentially in the next years, and it will be necessary to find alternative solutions to conventional protein sources for animal and human nutrition. In this regard, *Tenebrio molitor* larvae represent a sustainable alternative protein source for food and feed. Several studies have shown that larval growth and nutritional composition may be modulated by growing substrate used. The aim of this study was to evaluate the inclusion of innovative ingredients in growing substrates on growth performance of *T. molitor* larvae and on nutritional and functional characteristics of the obtained meals. Briefly, thirty-two cassettes, each containing 100 g of 7-week-old larvae, were randomly divided into 4 experimental groups ($n = 8$ cassette/group) differentiated by substrate characteristics for 14 days: control group (CTRL) fed wheat bran; treatment group 1 fed wheat bran supplemented with 12.5% chestnut shell (TRT1), treatment group 2 fed wheat bran supplemented with 25% chestnut shell (TRT2) and treatment 3 fed wheat bran supplemented 40 mL at day 0,4,8, and 12 with soluble lactoferrin solution (TRT3). Larvae weight per cassette, substrate consumption, and larvae mortality were recorded weekly to evaluate the growth performance. At the end of the trial, the larvae were harvested for meal production and substrates were collected for analyzing bromatological characteristics, *in vitro* simulated digestibility using enzymatic method, antioxidant properties through colorimetric test and antibacterial activity against *E. coli* O138 by microdilution method in 96-well plate for 6 h. Our results showed lower larval mortality in TRT1, TRT2 and TRT3 compared to CTRL group over 14 days (79, 50, 45 and 148 n° of dead larvae respectively; $p < 0.05$). The nutritional composition of the obtained meals showed no significant differences among groups, and digestibility was not impaired. A higher antioxidant activity was recorded in growing substrates of TRT1, TRT2 and TRT3 compared to CTRL ($p < 0.05$). Meals of TRT1 and TRT2 showed higher *E. coli* inhibitory activity compared to CTRL group after 2 h of incubation, and TRT3 after 4 h compared to CTRL group ($p < 0.05$). In conclusion, the use of innovative ingredients in growing substrates enhanced functional characteristics of insect meals, suggesting that their inclusion in animal feed could support animal health thus reducing the use of antibiotics.

O100

Comparison between lesser mealworm, mealworm and superworm larvae reared on the same substrate for their use as feed/food

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In the last decade, great attention was placed on insects to increase feed-food production without negatively affecting the environment. Indeed, insects could be reared on several different organic materials, called substrates, with a valuable conversion factor. Several research studies were conducted in the last decade to evaluate how substrates could affect insects' live performances and chemical composition. To determine the different outcomes that the insect sector could produce to respond to the increased request for feed and food in this research study we compare three different insects of the Tenebrionidae family (*Alphitobius diaperinus*, lesser mealworm; *Tenebrio molitor*, mealworm; *Zophobas morio*, superworm) fed the same substrate. The insect larvae's chemical composition, fatty acid profile, antioxidant content, and microbiological loads were determined. Superworm larvae show higher dry matter (38.06%) and ether extract (17.65%) contents followed by mealworm (32.77% and 13.86%) and lesser mealworm larvae (27.11% and 10.78%). No differences were detected in the crude protein and ash content. Superworm larvae showed the highest content of SFA (41.43% total fatty acids), while mealworm larvae were the richest in MUFA (40.61%) and PUFA (29.05%). Anyhow, in all the larvae the three more representative fatty acids were oleic acid, linoleic acid, and palmitic acid (mean of 34.43%, 23.04% and 25.26%). The worst ratio of n-6/n-3 was detected in *Alphitobius diaperinus* larvae, while *Zophobas morio* larvae showed a good amount of n-3 fatty acids. The antioxidant contents determination revealed a linkage to the fatty acids content. Low differences were determined in microbiological loads of the larvae, and no foodborne pathogens were detected. Differences in chemical-compounds metabolism shown by the three species reared on the same substrate highlight the insects' potential to respond to different questions derived from the food and feed sectors. Results point out the high plasticity of the larvae and the potential to tailor the final outcomes in relation to the required characteristics need in the final products, also in relation to their utilization as food or feed. This feature plays a key role in the insects' sector as rearing and also processing steps can meet the marketing strategies and requirements unleashing the insect potential.

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O163

Consumers' acceptance toward edible insects: a cross-country study in Belgium, China, Italy, Mexico, and the US

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Given their advantages in terms of sustainability and nutritional value, edible insects are becoming more and more popular as food in both traditional and non-traditional insect-eating countries. Only a few studies, meanwhile, have looked at the acceptance of eating processed or whole insects across the country. Therefore, the purpose of this study was to determine the amount of consumer approval of eating whole and visible mealworms and/or processed mealworms, and to investigate the factors influencing the level of acceptance of eating mealworms in nations with and without an entomophagy heritage. Using a quota sampling technique, an online survey was used to gather 3006 responses from five countries: Belgium, China, Italy, Mexico, and the US. Moreover, an information treatment was included with about half of the participants receiving information about the advantages of edible insects as food (ingredient) and the presence of food safety regulations. Across countries, gender was the main factor affecting acceptance level as men accepted mealworms more than women. As expected, the disparities across nations were mostly accounted for by entomophagy traditions. Comparatively to nations with no entomophagy traditions (i.e. Belgium, Italy, and the US), countries with entomophagy traditions (Mexico and China) shown increased approval of consuming whole or processed mealworms. While information and age did not affect differently the acceptance of including processed mealworms in countries with entomophagy traditions showing that consumer acceptance was affected by information in Mexico and by age in China. Whereas it was found that younger people (below 42 years old) in countries without entomophagy tradition were more open to accepting processed mealworms in their diet. Moreover, across countries, the acceptance of including processed mealworms was higher compared to whole mealworms. These findings provide insights into which consumer segments to target and the potential impact of information when introducing new insect-based foods in countries with and without entomophagy traditions. Future studies should also consider how the knowledge, previous experience towards entomophagy, and the drivers in each market (i.e. traditional value, food security, sustainability, curiosity, health) could influence acceptance to consume insects.

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O516

Effect of dietary chitin on growth performance, nutrient utilization and metabolic response in rainbow trout (*Oncorhynchus mykiss*)

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Chitin is the second most abundant biopolymer in nature as a component of the exoskeleton of arthropods. Despite the dietary inclusion of chitin-containing ingredients in the diet has been claimed to be beneficial to fish health, controversial results on growth performance and nutrient availability have been reported in literature. The present study aimed at assessing the response of rainbow trout (*Oncorhynchus mykiss*) in terms of growth, metabolic status, liver functionality, *in vivo* nutrient digestibility and *in vitro* protein degradability when fed graded levels of chitin (0, 1.5, 3.0 and 4.5% as fed) included in a semi-purified basal diet. Two-hundred and twenty-five juvenile rainbow trout (27.4 ± 2.4 g body weight) were randomly distributed in twelve tanks and fed in triplicate the test diets over ten weeks. At the end of the trial, 6 h after the last meal, fish were euthanized for the evaluation of growth performance, blood serum metabolites, intestine brush border enzyme activity, gene expression of gastric enzymes and liver functionality and from twenty spared specimens (57.4 ± 2.07 g body weight) the intestinal tract was quickly removed to collect the enzymatic extract to test *in vitro* protein degradability of the diets. Chitin inclusion up to 3% in the diet did not affect fish growth and levels of most plasma enzymes and metabolites. The diet including 4.5% chitin significantly reduced fish final body weight, nutrient apparent digestibility, feed utilization, brush border leucine aminopeptidase activity in pyloric caeca, while alkaline phosphatase activity increased in the anterior intestine indicating a possible inflammatory status. Molecular analyses highlighted an upregulation of gastric pepsinogen and chitinase in fish fed more than 3% dietary chitin, indicating a higher effort in diet digestion compared to the other groups. Data obtained *in vitro* did not show major changes in protein degradability due to the dietary treatments.

The results observed in the present study demonstrate a good tolerance of rainbow trout to diets including up to 3% chitin inclusion, on the contrary, a higher inclusion level affected growth and several indicators of fish metabolic status, thus suggesting a limit to chitin inclusion in diets for rainbow trout.

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0260

Influence of single protein fractions on goat milk coagulation ability

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Milk proteins are known to be the main actors in the coagulation process, but compared to bovine milk little is known about their actual role in goat milk. For this reason, the present study aimed to analyze the effect of the single protein fractions on the clotting, curd firming, and syneresis phases of goat milk. This study is part of the GOOD-MILK project (D.M. 9367185 – 09/12/2020). The detailed milk protein profile of 820 goats belonging to 6 different breeds (Camosciata delle Alpi, Saanen, Murciano-Granadina, Maltese, Sarda, and Sarda Primitiva) was obtained using the reverse-phase high-performance liquid chromatography technique. The protein fractions were expressed quantitatively as grams per liter of milk (g/L) and qualitatively as percentage of total casein content (%CN). Traditional single-point milk coagulation properties (RCT, k_{20}, a_{30}, a_{45}, a_{60}) were obtained using the lactodynamography technique. All the measurements of curd firmness recorded with this analysis were examined using a mathematical model, which yielded new parameters of coagulation time (RCT_{eq}), curd firming and syneresis rates (k_{CF} and k_{SR}), potential curd firmness (CF_P), and maximum curd firmness (CF_{max}) at t_{max} time. All the coagulation traits were analyzed fitting a first linear mixed model where the fixed effects were breed, lactation stage, parity order, daily milk yield, and single protein fractions in g/L, while the flock was included as random. A second model was also applied, where milk production was substituted by the casein content and the protein fractions were expressed in %CN. The results show that the quantitative (g/L) variation of β-CN, the most abundant fraction, improves the curd firmness without affecting the clotting time. The α_{S1}-CN improves the curd firmness as much as the β-CN even being one-third in concentration. The κ-CN, which is the smallest fraction, improves the curd firming rate and slightly the curd firmness. The α_{S2}-CN has only a limited positive effect reducing the clotting time. When expressed qualitatively (%CN), the increasing proportion of the α_{S1}-CN at the expense of the other caseins improves the curd firmness, while the increase of β-CN has no effect. The κ-CN exerts a similar positive effect as in g/L, while α_{S2}-CN in %CN is detrimental for the curd firmness. In both models, the whey proteins seem to not affect the process. This information can be used as animal selection guidelines for the improvement of goat cheese production.

0551

Dehydration preserves the ability of tanniferous sulla forage (*Sulla coronaria* (L.) Medik.) to improve nutritional, microbial and sensory traits of sheep cheese

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Sulla is a forage legume species appreciated for the positive impact on small ruminants' dairy production, due to its high protein content and moderate level of phenolic compounds, consisting mainly of condensed tannins (CT). Fresh sulla forage (FSF) is exploited by grazing ewes during early spring, and then ungrazed to constitute hay stocks. Dehydration showed to be a valid alternative to haymaking for reducing the losses of

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biomass and nutrients and preserving the properties of bioactive components. This investigation was aimed to evaluate the potential of sulla in relation to its seasonal utilization, as fresh or stored forage. Two experiments were carried out, in spring (SPR) and autumn (AUT), both involving Valle del Belice ewes at 60 days in milk. In SPR, diets consisting of FSF or fresh barley forage (FBF) provided *ad libitum* were compared using 12 ewes divided into 2 groups and fed the 2 diets in a 2 × 2 Latin square design (LQD). In AUT, pellets of dehydrated sulla forage (DSF) obtained in 2 cutting times and differing in crude protein (CP) (April, 15.1% dry matter (DM); May, 11.3% DM) were compared with sulla hay (SH, CP 11.8% DM) using 9 ewes divided into 3 groups and fed the following 3 diets in a 3 × 3 LQD: ADSF, 2 kg/d per ewe of April pellets and SH *ad libitum*; MDSF, 2 kg/d of May pellets and SH *ad libitum*; SHL, SH *ad libitum*. Concentrate feed was supplied to all ewes (600 or 800 g/d in SPR and AUT). Manufactured cheeses were sampled at 15 and 30 d of storage. Compared to FBF, FSF improved milk yield (1460 vs 1774 g/d; $p < 0.001$), milk casein and milk conversion ratio (DM feed intake/milk yield), while in cheese increased the polyunsaturated fatty acids (PUFA), and reduced redness index (a*) and fat. In AUT, both ADSF and MDSF induced higher milk yield than SH (1661, 1538, 1374 g/d; $p < 0.001$), and in cheeses enhanced total PUFA, especially due to linoleic, α-linolenic and rumenic acids, while ADSF improved the cheese antioxidant capacity, regardless storage time. The microbiological profile of milk and cheese was similar among all diets, indicating no negative effect of stored forages on fermentation process. At triangle tests, the panel was able to distinguish cheeses from different diets for both seasons and storage times, without recording differences in their acceptance degree. The results confirmed the potential of DSF in ensuring adequate dairy productions in periods of unavailable fresh forage, especially when derives from early-cut forage.

O212

Buffalo milk in Italy and the traceability system

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Milk is often referred to as the ‘perfect food', produced by the mammary glands of all periparturient female mammals. Cattle and buffaloes are the first and second source of milk worldwide. Buffalo milk is known for its high quality, with a higher fat content than cow's milk (6.4–8.0% versus 4.1–5.0%). Further studies on buffalo milk dairy products in the Mediterranean region and the variability of their technologies are needed, as they represent an important part of global biodiversity. Buffalo milk, like cow's milk, can be used for the production of a wide variety of dairy products. It has long been valued for its chemical composition, which determines its nutritional properties and suitability for the production of both traditional and industrial dairy products. Given the importance of buffalo milk in Italy, which to date is estimated at a total production of 25,000 tonnes and is mainly produced in the southern regions where Mozzarella di Bufala Campana with Protected Designation of Origin (PDO) is produced, the need arose to trace the path of the milk from the farm to the dairy. The aim was to verify the applicability of a national buffalo traceability system, following the Ministerial Decree of 9 September 2014. Through the use of the online computer system, control operators can monitor production and sales in real time through alerts and access registers. In Italy, the most representative number of registrations was registered in Campania, the region with the highest concentration of dairy buffalo farms. Currently, 2475 breeders, 816 non-PDO dairies, 102 PDO dairies, 119 non-PDO intermediaries and 17 PDO intermediaries are registered in Italy. The system shows that the non-PDO circuit absorbs 35% of the total milk produced, while 65% of the milk is processed in PDO dairies. Analysing the data extrapolated from the traceability system of the buffalo chain from 2016 to 2019, it was highlighted that following the application of the Ministerial Decree of 9 September 2014, the traceability of the chain has increased the price of buffalo milk at the barn from 1.37 euro/kg to 1.55 euro/kg, which allows the control of milk production throughout the territory and facilitates the control of milk that is frozen or can only be used in non-PDO productions; this increases the transparency of the chain.

O310

Distribution of Zn, Fe and Cu and their relationships with milk quality in individual cow milk samples

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The cow milk mineral content is mainly due to milk concentration of macro minerals such as K, Ca, P, Na, and Mg. However, in addition to these macro elements, there are also traces of micro elements, mainly divalent cations such as Zn, Fe, Cu. In milk, these elements are distributed between the soluble and insoluble phases. Insoluble fractions of Zn, Fe and Cu interacts with both proteins and fat globules. Several studies have focused on the effect of Ca, P and Mg on the rennet coagulation properties (RCP) of milk. For example, high concentration of Ca, P and Mg is associated with improved milk coagulation aptitude. However, there are no information on the effect exerted by microelements on milk RCP. The aim of this study was to evaluate the cow milk content and distribution – between soluble and insoluble phases – of Zn, Fe and Cu. Moreover, their relationship with rennet coagulation properties was also investigated. Inside to the AGER project FARM INN and the MIPAAF project INNOVALAT, a total of 359 samples of individual cow milk were collected. On each milk samples basic chemical composition and the rennet coagulation properties were assessed. Furthermore, total (in milk) and soluble (in ultrafiltered whey) contents of Fe, Zn and Cu were determined as well. Moreover, the Pearson correlation moment among the total, insoluble and soluble content of Zn, Fe and Cu and the rennet coagulation properties were calculated. Total contents of Zn, Fe and Cu resulted 4.56 ppm (*min.-max.* 2.27–6.75 ppm), 1.77 ppm (0.66–2.82 ppm) and 0.35 ppm (0.18–0.58 ppm), respectively. The soluble contents of Zn, Fe and Cu resulted 5.26, 64.21 and 67.78 % of their respective total values. The remaining quota (insoluble fraction) of these microelements is bound to the proteins or fat globules. This observation is confirmed by the weakly positively correlation between the content of total Zn and Fe with the milk casein content ($r = 0.199$; $p \leq 0.001$ and $r = 0.150$; $p \leq 0.01$ respectively) which indicates that a part of their content is bound to casein. Finally, the soluble contents of Fe and Cu resulted weakly negatively correlated with the curd firming time ($r = -0.150$; $p \leq 0.05$ and $r = -0.162$; $p \leq 0.01$ respectively) and the soluble content of Cu was also weakly negatively correlated to the curd firmness ($r = -0.136$; $p \leq 0.05$).

O315

Influence of content and distribution of Ca, P and Mg on rennet coagulation properties in individual cow milk samples

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In bovine milk Ca, P and Mg are distributed between the soluble and colloidal phases. In the latter, these elements interact with caseins to form the casein micelle. Bulk milk samples classified as optimal on the basis of their rennet coagulation parameters (RCP) showed high levels of colloidal Ca, P and Mg. Little information about the effect of Ca, P and Mg on milk RCP at individual milk level is available. Therefore, this study is aimed to evaluate the effect of colloidal contents (mg/100 g) and distribution (as % of their total contents) of Ca, P and Mg on milk RCP at individual milk level.

A total of 359 individual milk samples from Italian Friesian cows reared in 3 herds were collected within the MIPAAF project INNOVALAT and within the AGER project FARM INN. On each sample the chemical composition and the RCP (r, k_{20} and a_{30}) were assessed. Total contents of Ca, P and Mg were assessed in milk whereas their soluble contents in ultrafiltered whey. Colloidal contents were calculated by the difference between total and soluble fractions. Data were analysed by ANOVA mixed model using two different models. The fixed factors considered in the first model were the sampling period, days in milking, n. of parities and the colloidal contents of Ca, P and Mg (5 classes for each mineral). Random factors were the day of sampling and the herd. In the second model the colloidal contents were substituted by their colloidal % of their total values (5 classes for each mineral) and the total contents of Ca, P and Mg were used as a covariate. Each of the 5 classes of minerals in the two models was determined on the basis of the distribution of the variables.

The first model evidenced as colloidal contents of Ca and P affected a_{30}. The values of a_{30} tended to decrease with increasing amounts of colloidal Ca ($p \leq 0.05$). On the contrary, the same parameter showed an irregular increasing trend with the increasing of content colloidal P ($p \leq 0.05$). The second model evidenced as all RCPs were influenced by the % of colloidal Ca ($p \leq 0.05$). Furthermore, k_{20} was affected by the % of colloidal P ($p \leq 0.05$). In particular, r and k_{20} increased and a_{30} decreased with increasing % colloidal Ca. The values of k_{20} increased from class 1 (52.96%) to class 3 (53.89%) of colloidal P. Then it showed a decreasing trend with an increasing % of colloidal P. The distribution of Ca, P and Mg affect the RCPs of milk. In particular increasing % of colloidal Ca and P are associated with better RCP.

O482

Differential somatic cell count during lactation in dairy cattle herds from northern Italy

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Somatic cell count (SCC), the most used tool to identify udder inflammation, is composed for 98% by leucocyte cells: neutrophils (NEU), lymphocytes (LYM) and macrophages (MAC). There is an increase of the sum of the percentage of NEU and LYM on the total SCC during an intramammary infection. The aim of this study is to describe the trends of the three differential somatic cells fractions (NEU, LYM, MAC) in combination with somatic cell count during the first 240 days of lactation in cows involved in different lactations. A total of 210 cows, in the first 60 days of lactation, from 6 farms in Northern Italy was selected and udder milk samples were collected at regular interval of 62 ± 7 days (4 samples per cow during lactation). Milk samples were analysed using Vetscan DCQ Milk Analyzer (AAD Advanced Animal Diagnostics, NC, USA) to provide the concentration (cells/mL) of Total milk Leucocyte Count (TLC), defined as SCC without epithelial cells, and NEU, LYM and MAC, expressed as percentage of TLC. GLM procedures with repeated measures was performed with SAS 9.4 version. The average TLC was $4.94 \pm 0.61 \log_{10}$ cells/mL (<100,000 cells/mL), underling good mammary health conditions in the herds. Considering parity, there was an increase of TLC from milk of first lactation cows to multiparous cows, as described in literature. In this study no different values of NEU, LYM and MAC percentages were observed in milk of cows in different parity (respectively $p = 0.48$, 0.14 and 0.46). The major component of TLC was NEU (around 59%), followed by MAC and LYM. During lactation there was a significant increase of TLC ($p < 0.0001$), from $4.76 \log_{10}$ cells/mL at the first sample (2–60 d) to $5.10 \log_{10}$ cells/mL at the last one (180–240 d). However, the NEU percentage significantly decreased, from 64% after calving to 56% at the last sample time ($p = 0.0018$). MAC percentage increased and, simultaneously LYM percentage kept the same value (approximately 16%) during the first 240 days of lactation. In this study, there was a good correlation between NEU and MAC percentage ($R^2 = 0.65$), with the decrease of NEU at MAC increasing. On the other hand, the expected correlation between TLC and NEU percentage was not obtained ($R^2 = 0.14$). The results show an increase of TLC without a concomitant increase of NEU percentage, further investigations are requested to better understand the evolution of differential somatic cell in milk during lactation.

O483

Survey on milk quality at late lactation and presence of ancient Zebu wheyprotein marker in Podolic cattle of Basilicata region

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More than 800 milk samples from 13 farms were collected in Basilicata region (South-Italy) to make a survey on milk quality of Podolic cattle. The gross composition showed fluctuating variability, influenced both by sampling and endogenous parameter of the animal. The fat content had the highest variability, probably as a consequence of manual milking. Protein ranged from a minimum of 3.4 to a maximum of 3.8% (±0.13 sd), while lactose ranged from 4.9 to 5.2% (±0.12 sd); in contrast to the fat content, these parameters had very low variability. Somatic cell counts (SCC) and differential somatic cell count (DSCC) showed good udder health for all farms (except 1 farm), having a SCC mean value of 318.3 Colony Forming Unit (CFU)/mL value, but DSCC below 66.3%. The urea content fell within the range of 13.9 and 36.9 mg/dl, 6 farms fell out the normal range (20–46 mg/dl) meaning that animals were supplied with unbalanced protein food, which in turn could affect the milk production (if low) or have impact on cow fertility (when high). Acetone and β-hydroxybutyric acid (BHB) are considered useful indicators of subclinical ketosis (SCK), 0.15 mM for milk acetone and 0.1 mM for milk BHB have been proposed as threshold for SCK. Our results showed only 1 farm subject to SCK. Considering the casein content, the mean value represents the 78.4% of the total protein content, which is within the normal range. The coagulation properties of milk showed mean value of coagulation time slightly above the normal range, this implies a slow reaction of milk to rennet action ($r = 23.6$ min vs normal range $11 < r < 17$ min); similarly, the coagulation rate (k_{20}) was slight high (5.1 min), it reflects in a slower rate of aggregation of micelles. On the other hand, A30 mean value (39.7 mm) showed good curd firmness at 30 min after the addition of calf rennet on milk. According to the correlation matrix SCC had negative correlation with urea content ($r = -0.587$, $p < 0.05$); then, acetone had high significative correlation with BHB ($r = 0.877$, $p < 0.0001$). The research of the ancient marker of Zebu whey protein was based on the detection of the α-lactalbumin variant A by RP-HPLC analysis of 239 individual milk samples. The variant was found only in the 13.8% of the population studied. The most of them were heterozygotes (both containing variant B, the most common in taurine breeds, and variant A), while only the 6.1% were, surprisingly, homozygotes containing only the variant A.

O33

Quantification and variation of lactoferrin in cow milk assessed through radial immunodiffusion technique

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Lactoferrin (LF) is a multifunctional protein produced by mucosal epithelial cells of mammalians. It is present in different biological secretions including tears, mucus, saliva and milk. The antibacterial activity of LF is associated with two different mechanisms. First, LF has the ability to bind iron, an essential element in the replication of microorganisms, thereby indirectly acting as a bacteriostatic agent. Second, LF promotes peroxides generation affecting bacterial membrane permeability and bacterial cell lysis. The present study aimed to evaluate analytical performances of radial immunodiffusion (RID) technique for the quantification of milk LF and to investigate the main sources of variation of LF in individual milk samples of Holstein Friesian ($n = 1516$) and Simmental ($n = 230$) cows.

Repeatability (RSD_r) and reproducibility (RSD_R) of RID assay were assessed as relative standard deviation of 15 measurements within the same day and 45 measurements across 3 days, respectively. Sources of variation of milk LF were investigated through a mixed linear model which included the fixed effects of classes of stage of lactation, classes of parity, their interaction, cow breed and cow udder health status (UHS), and the random effects of the herd-test day nested within breed and the residual. The UHS was defined combining somatic cell count (SCC) with differential somatic cell count (DSCC), which allowed to allocate cows to 4 UHS groups: 'healthy' (≤200 cells/µL and DSCC ≤66.3%), 'susceptible' (SCC ≤200 cells/µL and DSCC >66.3%), 'mastitic' (SCC >200 cells/µL and DSCC >66.3%) and 'chronic' (SCC >200 cells/µL and DSCC <66.3%).

Method performances were satisfactory in terms of repeatability ($RSD_r < 9\%$) and reproducibility ($RSD_R < 8\%$), suggesting that the RID is a reliable cow-side test for the quantification of milk LF. Lactoferrin was positively associated with somatic cell score ($r = 0.40$) and negatively with lactose content ($r = -0.33$). Lower concentration of LF was observed in milk of 'healthy' and 'susceptible' (13.03 and 12.87 mg/dL, respectively) than 'mastitic' and 'chronic' cows (17.91 and 17.33 mg/dL, respectively). Results supports that milk LF has the potential to be a useful biomarker to detect early mastitis. Further research is needed to deepen the association between milk LF content and observed clinical mastitis.

O21

Fatty acids and sterols composition of three commercial cow's milks

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Milk contains several components important for human nutrition and health. Although extensive literature is available on several cow milk components such as cholesterol and saturated fatty acids which should be limited in a healthy diet, only a few information is available on the content of other milk minor sterols of animal and plant origin which could be beneficial for health. The aim of this study was to investigate and compare the presence of some lipid components (fatty acids and sterols) in commercial cow milks of three different types: conventional, high quality (D.M. 185/1991) and organic (in compliance with European Regulation). Raw and pasteurized bulk milk for each type of products were weekly sampled within a month from a dairy factory, for a total of 24 samples. Gross, mineral, fatty acid and sterol composition was evaluated in duplicate for each milk sample. The results were analyzed by ANOVA with the product type and the heat treatment as fixed effects; the effect of the sampling time, the interaction between the type of milk and the heat treatment were not significant and were excluded. No differences were highlighted for gross, mineral and sterols compositions between the three types of milk. Cholesterol was the main sterol for all the types of milk (ranging from 271.37 to 278,76 mg 100 g^{-1} of fat) while lanosterol was the main minor sterol of animal origin, (ranging from 3.41 to 4.37 mg 100 g^{-1} of fat), followed by desmosterol (ranging from 0.54 to 1.13 mg 100 g^{-1} of fat). Regarding phytosterols, the brassicasterol was the main sterol of vegetal origin (2.6–2.93 mg 100 g^{-1} of fat), lower levels of beta-sitosterol, campesterol, and stigmasterol were also recorded. The only significant difference between the three commercial milks was found in the fatty acid profile; in particular omega-3 content was higher ($p \leq 0{,}05$) in high-quality milk than conventional and organic milk (0.77, 0.65, and 0.67 g 100 g^{-1} of fat, respectively). Regarding the effect of heat treatment, no differences for any of the parameters investigated were found. These findings demonstrate that the investigated product categories and labels have minimal effect on the sterol and fatty acid profile of commercial cow's milk.

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O149

Can sprouted barley grains replace maize silage in dairy buffaloes' diets? Effects on production efficiency, milk traits, and mozzarella cheese quality

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Grains sprouting has been proposed as a promising technique for producing high-quality fresh forage in areas, such in the case of the Italian PDO Mozzarella di Bufala production district, characterized by declining arable land availability and changing weather patterns. However, no data are currently available to support the sprouting technology as a viable option for feeding dairy buffalo. In this study, we assessed the effect of replacing maize silage (MS) in buffalo diet with sprouted barley forage (SB) on feed intake, milk production, and milk quality. Thirty-three lactating buffaloes (parity 2.85 ± 1.86, days in milk 40.42 ± 21.83 d, milk yield 13.0 ± 2.85 kg/cow) were assigned to three balanced groups according to the following dietary treatments: G0 (control, 100% MS), G50 (50 MS and 50% SB on a fed-basis) and G100 (100% SB). All diets were at a 60:40 forage-to-concentrate ratio on a dry matter basis. The 6 week-experimental period consists of a 2-week of adaptation followed by a 4-week sampling phase. The cows were weighed and scored for body condition at the beginning and the end of the trial. As per farm routine, the diets were *ad libitum* provided as a total mixed ration, and the animals were milked twice daily (05:00 and 15:30 h). Milk yield and feed intake were recorded daily and weekly, respectively. Individual milk samples along with diets and refusals were collected weekly. Milk samples were assayed for fat, protein, and lactose content. In addition, milk clotting ability parameters, in terms of the rennet clotting time, curd-firming time, and curd-firmness, were evaluated. Milk data were analyzed using ANOVA for repeated measures, with diet, sampling time, and diet × sampling time as factors. Feed intake, body weight (BW), and body condition score (BCS) were analyzed by an ANOVA GLM model with diet as the factor. The substitution of MS for SB did not alter ($p > 0.05$) dry matter intake, BW, and BCS, had no effect on milk fat, protein, and lactose content ($p > 0.05$) and, accordingly, on milk coagulation characteristics and estimated mozzarella cheese yield ($p > 0.05$). By contrast, the incremental replacement of MS with SB forage in the diet enhanced ($p < 0.05$) daily milk yield. Based on our findings, SB can successfully replace MS in the diet of dairy buffaloes possibly enhancing production potential, which could lead to significant benefits in terms of feeding management differentiation and in increasing home-grown forage production potential.

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O139

Effect of immunocastration on quality traits of fresh and dry-cured loin in Italian heavy pig production

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Immunocastration has been proposed as an alternative to surgical castration. It involves the use of an anti-GnRH vaccine (Improvac®, Pfizer) which induces an immunological suppression of testis function, thus preventing boar taint in meat and improving piglets' welfare by avoiding stress and pain. Immunocastration has been largely studied for light pork production, but few research has been carried out for Italian heavy pig production. The aim of this work was to evaluate the effects of immunocastration on carcass and meat quality traits of Italian heavy pigs intended for the production of dry-cured products. For this purpose, a group of 22 male pigs of the same age was selected after weaning and divided into two groups of 11 pigs each. The first group (SUR) was surgically castrated, while the second group (IMM) was given three doses of Improvac® (140, 203, 301 days of age). Animals were slaughtered at 9 months of age and the following parameters were recorded: carcass weight, main cut percentages, tissue composition (lean, fat, and bone percentages) of a sample joint between the 2nd and 5th lumbar vertebra, and meat quality traits (color, texture profile analysis (TPA), chemical composition, fatty acids (FA) profile) of fresh *Longissimus dorsi* muscle (LD) and dry-cured loin. Data were analyzed using GLM procedure.

Results showed that the castration techniques did not affect animal growth since the two groups reached similar weights at slaughter (180 kg for SUR and 185 kg for IMM). Also, the main cuts percentage and sample joint's tissue composition were not affected, except for the LD area, which resulted significantly greater in IMM (101 mm^2) than in SUR (88 mm^2) animals. Meat from IMM pigs had higher protein level and lower fat percentage than SUR meat. TPA and FA profile were also affected. Indeed, IMM meat resulted in greater chewiness and lower content of MUFA, while PUFA percentage was higher than in SUR meat. For dry-cured loin, IMM products resulted in higher a_w and in lower fat content, whereas differences in TPA and FA profile disappeared along the curing process. In conclusion,

immunocastration mainly affected the parameters related to fresh meat quality, whereas it had few impact on dry-cured loin. This makes this technique still interesting for Italian heavy pig industry which is based on the production of cured meat products.

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O538

Pork characteristics of Mangalitza pigs reared outdoors

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Eating and ethical aspects, such as animal welfare, biodiversity and environmental protection, increasingly guide the consumers' purchasing choices. The autochthonous Hungarian Mangalitza breed is characterized by late maturity and low fertility, slower growth rate, higher adiposity, and lower lean tissue deposition than other pig breeds. In addition, the results of previous studies showed a lean meat percentage of less than 40% on the sides of the carcass, but sufficient for the production of high-quality dry cured and processed products. Recently, the growing interest towards autochthonous breeds led to the rediscovery of the Mangalitza breed. The aim of this research was to evaluate the meat quality of Mangalitza pigs reared outdoor as affected by sex. Fifteen animals, 10 castrated and 5 females, were considered. The animals had an average age of 22 months at slaughter, and the average hot carcasses weight, 141.4 ± 5.72 kg, was similar between experimental groups ($p > 0.05$). Samples of *longissimus lomborum* were collected, ultimate pH (pHu) and colour were measured, and the analyses of proximate composition, cholesterol, drip loss, cooking loss, warner bratzler shear force (WBSF) and fatty acids (FA) contents were performed. pHu, measured at 24 h after slaughter, was within normal range 5.48–5.58. Sex did not influence meat characteristics ($p > 0.05$). Regarding the colour, the meat from Mangalitza had 39.1 ± 1.02, 7.2 ± 0.51 and 12.4 ± 0.40 of L* (lightness), a* (redness) and b* (yellowness), respectively. The lipid and the cholesterol content were 7.6 ± 0.69% and 66.8 ± 1.81 mg100 g^{-1}, respectively. Drip loss was 4.8 ± 0.42%, cooking loss was 26.9 ± 1.18% and WBSF was 42.6 ± 2.70 N. Regarding the FA content, the meat showed a values of 39.0 ± 0.37%, 52.5 ± 0.25%, and 8.5 ± 0.31% for saturated FA, monounsaturated FA and polyunsaturated FA, respectively. In conclusion, the meat from Mangalitza had beneficial characteristics from a human health perspective, such as low cholesterol content and moderate lipid saturation level, with no differences between castrated and females.

O550

Physico-chemical and rheological differences of wild boar muscle in post-mortem time in Campania region: Preliminary study

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Wild boar meat has conquered many local European markets. It is possible to find it in specialized fairs as well as in many restaurants. Despite the request, it is still not possible to supply homogeneous foodstuffs when the animals come from hunting. Our study aims to provide information on the quality of meat from wild boars captured during selective control period. Study was carried out on 30 female wild boars (10 of each group) classified in three age groups, housed for 3 days after capture at a stop facility of Campania Region. Animals were taken to an authorized slaughterhouse. They were stunned electrically and jugulated. The carcasses were scalded in a tub of boiling water, dehaired and eviscerated. All the carcasses were divided at the midline into two halves. The 'capocollo' meat cut (500 grams on average) was excised from the right side and subjected to analysis. For 30 'capocollo' samples were measured: pH, activity water (aw), moisture, protein, fat, color (CIE L*a*b*) and fatty acid profile (FA). The pH and aw values were different ($p \leq 0.01$) among the three groups. The moisture and fat content did not show significant differences between age groups. Fat content seems to be very low in all animals (4.78% ± 1.88). Colour results did not highlight significant differences among groups, as well as the effect of age on the FA. Environmental factors and diet have an important role on the meat caracteristics, more evident on wild animals. This condition is also true for the 'capocollo' cut of meat, for which the color and nutritional traits are variable. The fatty acid and protein composition falls within the same range independently to the studied groups. The results obtained demonstrate that there are no significant differences between the meat of wild boars belonging to the three age classes. Based on what has been obtained, the authors suggest the possibility of promoting the meat of young animals, captured and not hunted as a means to contain wild boar's populations. These meats would therefore find a correct place in the context of agritourism catering and, although not able to compete with the classic pig farming market, going to increase a specific traditional niche market.

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O265

Monitoring the meat quality of grass-fed Angus beef during different seasons

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Ruminant meat provides high-value protein and essential micronutrients (B group vitamins, iron and zinc). However, recent studies consider meat production and consumption as major causes of GHG emissions and occurrence of cancer and chronic diseases, respectively. These statements do not distinguish among meat from different farming systems. Indeed, a wide literature shows the environmental benefits resulting from grass-fed systems as compared to conventional ones. Also, grass-fed meat is richer in favorable fatty acids such as ω-3 polyunsaturated fatty acids (PUFA), rumenic and vaccenic acid, and in bioactive compounds such as carotenoids, vitamin E and phenols that improve meat nutritive and technological properties.

Grass-fed animals graze during spring and summer and receive preserved forages in winter when grazing is not available due to weather. The variable nutritive value of the diet due to change in pasture composition and hay proportion in the diet could affect the quality of grass-fed meat throughout the year. Considering the lack of knowledge on this, we monitored the meat quality of grass-fed beefs slaughtered during different seasons.

To this aim, 33 Black Angus castrated males were reared in grass-fed oriented farm from 12 months of age until commercial maturity and slaughtered between March and October 2021. The animals were subdivided into 3 groups (winter, spring and summer) according to the diet received in the last 4 months. Intramuscular fat and fatty acid profile (gas-chromatography), antioxidant capacity (FRAP and TEAC) and color (CIELAB) and oxidative stability (TBARS) during refrigerated storage were investigated as meat quality parameters.

Intramuscular fat, antioxidant capacity, total saturated FA and PUFA were comparable between groups. However, winter meat had the highest percentage of monounsaturated FA and lowest percentage of 18:3 ω-3, 20:3 ω-3 and total PUFA ω-3 ($p < 0.05$). Nevertheless, ω-6:ω-3 ratio was comparable between groups and always below 4. As for color, winter meat had the highest a*, but it decreased faster than in spring and summer meat reaching the lowest value after 7-d storage ($p < 0.05$). Similarly, b* lowered with storage only in winter meat ($p < 0.05$). TBARS progressively increased with storage ($p < 0.05$) but did not differ between seasons.

Concluding, investigated meat quality traits may vary in grass-fed beef slaughtered in different seasons but show better characteristics if compared to data on conventional meat.

O500

Assessment of muscle metabolism in differently kinetic chicken genotypes using the Activity Index

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In recent years, there has been an increased interest towards poultry meat coming from extensive rearing systems (ERS), whose characteristics are strongly appreciated by consumers. In ERS, is important to rear animals able to exploit outdoor space, with the aim of improving animal welfare and products quality. In this context, slow-growing (SG) poultry strains are more adapted than fast growing ones. The oxidative muscles (constituted by red fibers) of chicken thigh is the main muscle involved in the movement, which mobilizes a great quantity of fatty acids to produce energy by β-oxidation. The aim of this study is to estimate the *Activity index* between red and white thigh muscles of SG chickens characterized by different kinetic activities. One hundred chicks of Nacked neck genotype were reared indoor (I) or outdoor (O), according to the EU Regulation. The animals were fed *ad libitum* the same diet (starter 1–21 d, grower 22 until slaughter 81 d). A kinetic monitoring system, constituted by chips and antennas for recording the times that chicks pass through the area (LUNA GERB, Italy), outlined 2 outdoor groups with high (OH) and low (OL) kinetic activity ($n = 10$/groups). At 81 days of age, 30 chickens were selected and slaughtered. The left thigh was excised from each carcass/group, dissected, deskinned and M. *iliotibialis lateralis postacetabularis* (PIL, glycolytic) and M. *puboischiofemoralis pars medialis* (PIFM, oxidative) muscles were removed and stored at −20 °C for fatty acid evaluation by GC-FID. The *Activity index* was calculated as follows: [n-3 High Unsaturated Fatty Acids (n-3 HUFA)/α-linolenic acid (ALA) of PIL] – [n-3HUFA/ALA of PIFM]. The *Activity index* resulted significantly different in I group with respect to the O ones (2.25 ± 1.57 vs 3.59 ± 1.20; 3.31 ± 1.58; in C, OH and OL, respectively). Glycolytic muscle had the same PUFA profile in all the experimental groups whereas oxidative muscles of OH chickens showed higher levels of n-3 HUFA and ALA than OL ($p > 0.05$). The lack of significance was probably due to the consumption of these FA for the high activity of OH chickens. In conclusion, the *Activity index* could be a *post mortem* marker of kinetic activity in a given genotype also assessing the adaptability to ERS and giving indication of n-3HUFA metabolism.

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O109

Microalgae as alternative protein source to soybean: effects on the main quality traits of broiler breast meat

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The sustainability concerns regarding the increasing request for animal proteins along with the massive production of conventional feedstuffs has elicited the necessity to find environmentally sustainable protein sources. Among them, microalgae are a promising feed protein ingredient whose production is characterized by a lower environmental footprint compared to conventional feedstuffs. This study aimed at evaluating the effects of the inclusion of dehydrated microalgae protein meal (MPM; *Arthrospira spp.*) in broiler diets, as partial replacement for soybean, on the main breast meat quality traits. A total of 1000 broilers (Ross 308, males) were divided into 5 groups according to both MPM dietary dosage (3 and 6%) and administration period (finishing-F: 29–41 d and growing + finishing-GF =14–41 d): commercial soybean-based diet (CON), F3 (3%, 29–41 d), F6 (6%, 29–41 d), GF3 (3%, 14–41 d) and GF6 (6%, 14–41 d). At 41 d, 15 carcasses/group were selected for the evaluation of meat pH, color, drip and cooking losses, shear force, lipid and protein oxidation, while plasma amino acids content was assessed by ^{1}H-Nuclear Magnetic Resonance. Data were analyzed using the One-Way ANOVA option of the GLM procedure of SAS software and means were separated by Tukey's HSD test at a level of $p < 0.05$. Results showed that, regardless of the dosage and feeding period, the use of MPM significantly changed ($p \leq 0.001$) meat color parameters, with a special reference to b* which was found more than tripled in the group fed the highest dosage of MPM and for the longest period compared to CON. Feeding MPM-supplemented diets did not alter meat water holding capacity and shear force, with the only exception being drip loss, which was significantly reduced in GF6 group compared to CON ($p < 0.01$). The inclusion of MPM in broilers diets did not affect the oxidative status of chicken meat, regardless of the dosage and the duration of inclusion. The dietary use of MPM significantly ($p < 0.01$) reduced plasma levels of histidine, arginine and creatine, while did not affect the main markers of *in vivo* energy metabolism, as also confirmed by the similar meat pH_u values observed among experimental groups. In conclusion, no major changes on *in vivo* energy metabolism and meat quality were observed, except for yellowness which was greatly increased in meat from broilers fed MPM. Thus, MPM can represent an effective strategy to increase skin and meat pigmentation by means of a sustainable feed source.

O578

Feed restriction strategies in growing rabbits fed post-weaning diets with different protein levels

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The present study evaluated the effects of the feeding system (AL: *ad libitum* vs. R1: monophasic feed restriction vs. R2: biphasic feed restriction) and the dietary crude protein (CP) level (HP: 16% CP vs. LP: 14% CP) on growth performance and slaughter yield of 336 crossbred growing rabbits reared from weaning to slaughtering (33–78 d of age) in 48 collective pens (7 rabbits/pen; 8 pen/group). The access time to feeders decreased from 16 to 9 h/d in the first week of trial in both R groups; it was 8 h/d given in a single slot (R1 group) or in two slots (R2 group: 4 h + 4 h separated by 4 h without feed access) during the 2nd and 3rd week. Then, access time increased to 12 h/d during the 4th week and, thereafter, it was 12 h/d in a single slot of access for both R groups until the end of the trial. The HP and LP diets were administered from 33 to 60 d of age, then the same fattening diet (14% CP) was provided to all groups. Data were analysed by ANOVA using PROC GLM procedure of SAS software. In the first period (33–61 d), both R groups showed lower daily weight gain (DWG) and feed intake (DFI) and better feed conversion (FC) compared to AL group ($p < 0.001$). In the second period (61–78 d), R rabbits maintained a lower DFI ($p = 0.02$) compared to AL rabbits but showed a higher DWG ($p < 0.001$) and a better FC ($p < 0.001$). At the end of the trial, there were no differences on growth performance among groups, whereas both R systems improved FC compared to AL one ($p < 0.001$). The R2 group had better FC than R1 during the 2nd and 3rd week. However, the differences

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between R1 and R2 groups disappeared in the following weeks with the return to the monophasic system. At slaughtering, R rabbits had decreased carcass yield ($p < 0.001$) compared to the AL ones. The use of LP diet decreased DWG ($p < 0.001$) and increased FC ($p < 0.001$) in the first period. However, in the second period, LP rabbits achieved a higher DWG ($p = 0.01$) and a better FC ($p = 0.01$) compared to HP rabbits. At the end of the trial, LP and HP rabbits showed similar growth performance and slaughter results. Finally, no significant interaction was found between feeding system and dietary protein level. In conclusion, the application of feed restriction systems improved rabbit feed efficiency but significantly decreased slaughter yield compared to the AL system. The dietary CP level can be reduced until 14% in the post-weaning diets without negative effects on growth performance and slaughter yield.

O581

In vitro efficacy of phyto-L against entero-Pathogenic Escherichia coli strains of rabbits from industrial farms

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Colibacillosis is one of the most common diseases in rabbit farms. It more frequently occurs in post-weaning rabbits, and it is due to the proliferation of Entero-pathogenic *Escherichia coli* (EPEC) in the ileocecal contents, resulting in weight loss, diarrhea, mortality and economic losses. The frequent use of antimicrobials occurred until recently to treat and prevent the disease in farms lead to the increase of the risk of the onset of antimicrobial resistance (AMR) and the selection of multidrug-resistant (MDR) bacteria. Therefore, the interest in natural substances as alternative treatment has increased in the scientific community. The garlic (*Allium sativum*) has many properties, such as antimicrobial, anti-inflammatory, antioxidant, immunomodulatory activities. The aim of this study was to evaluate the *in vitro* antimicrobial efficacy of Phyto-L (Pro Tech s.r.l.), a commercial product containing garlic, against *E. coli* strains isolated from rabbits coming from 19 farms. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Phyto-L were evaluated. One hundred and eight strains of *E. coli* were tested. Bacterial suspensions with charge of 10^8 CFU/mL, corresponding to those found in pathologic conditions in rabbit gut, were prepared and tested with different concentrations (2%, 1%, 0.5%, 0.25%, 0.125%, 0.06%, 0.03, 0.015%) of Phyto-L. For each strain, the MIC and the higher concentrations were examined by plating the corresponding bacterial suspensions on Tryptic Soy agar (TSA) without Phyto-L, to assess the MBC values.

MIC of the tested strains corresponded to 0.125% (34/108–31.5%), 0,25% (73/108–67.6%) and 0.5% (1/108–0.9%). MBC was 0.125% (15/108–13.8%), 0.25% (49/108–44.9%), 0.5% (8/108–7.3%), 1% (19/108–17.4%), 2% (9/108–8.3%) and higher than 2% for 9 (8.3%) strains.

The data highlight the greater bacteriostatic activity of Phyto-L than the bactericidal effect against EPEC strains from rabbits. *In field* application, Phyto-L should be used to prevent the enteritis due to EPEC in rabbit farms. Therefore, further investigations are in progress testing bacterial charges of 10^4–10^5 CFU/mL, which correspond to those usually found per gr of the intestinal content of rabbits in physiological conditions, to assess the possibility to use lower doses of Phyto-L *in field* application.

O574

Influence of rearing system and hen age on fatty acid composition of egg yolk

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In recent years, the use of cages in egg production has decreased and free-range system has increased, driven by the societal sensitivity for animal welfare. The aim of the study was to evaluate the effect of housing system (cage, C vs free-range, FR) and hens age on total lipid, cholesterol and fatty acid composition of egg yolk. Eggs were collected from Lohmann Brown Classic hens, after the 68th week of age every two weeks (at 70th, 72th and 74th week) for both housing systems (C and FR). The production technology was in accordance with the technological standards for this type of laying hens. Total lipid, cholesterol and fatty acid (FA) composition of 15 eggs/age/system were determined. Data were analyzed by GLM where housing system and hen age were the main factors. Differences among the means were determined with Scheffé's test. Total lipid (from 32.04 to 33.45%) and cholesterol (from 9.20 to 9.98 mg/g) contents of egg yolk were not affected (p > 0.05) by both factors. Housing system had a significant effect on the single fatty acids ($p < 0.01$). Egg yolk from FR group showed similar total saturated fatty acid (SFA) content, lower ($p < 0.01$) monounsaturated fatty acids (−6.3%; MUFA) and higher ($p < 0.01$) polyunsaturated fatty acids (+ 6.5%; PUFA) of both n-3 and n-6 series compared to that of C. From the nutritional point of view, the PUFA n-6/n-3 ratio was favorably lower ($p < 0.01$) and the PUFA/SFA ratio was higher in FR (0.63) group as compared to C (0.45) one. On the contrary, hens age did not affect ($p > 0.05$) the fatty acid composition of yolk, except for the PUFA n-6/n-3 ratio ($p = 0.052$). Significant interactions ($p < 0.01$) were found between factors for both n-3 and n-6 PUFA and their ratio n-6/n-3. In conclusion, FR system produced a better FA profile from the nutritional point of view, while age is of little importance in terms of egg quality in hens over 68 weeks of age.

O359

Comparison of productive traits and egg quality characteristics of two Italian chicken breeds and their crossbreeds reared in enriched-cage and free-range systems

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Egg production is essentially based on the use of highly selected commercial hybrids, which show greater laying performance and feed efficiency than local breeds (LB). However, the reduced diffusion and interest in LB has led to a progressive decrease in poultry biodiversity besides increasing the risk of their extinction. Crossbreeding with more productive genotypes could be considered as a potential strategy to improve the LB utilization allow the preservation of genetic variability while achieving acceptable levels of efficiency. This study aimed to evaluate the productivity and egg quality traits of two Italian LB (Bionda Piemontese and Robusta Maculata) and their respective crossbreeds (CB) with a hybrid strain (Sassò) compared to Lohmann brown as well as the effect of the housing system (enriched-cage *vs*. free-range). A total of 300 laying hens (18 wks of age) were raised in enriched cages (EC) and free-range (FR) pens (3 replicates of 10 birds/genotype/housing system) and fed the same commercial feed. Individual body weight (BW) was recorded at 25, 34, 50 and 66 wks of age, while egg deposition, egg mass, feed intake and feed conversion ratio (FCR) were evaluated every fourteen days from 25 to 66 wks of age. Also, 100 eggs/genotype/housing system were collected at 32, 47 and 66 wks of age and used for assessing Haugh index (HI), eggshell breaking strength (EBS) and eggshell-yolk-albumen/egg proportions. Lohmann brown showed higher egg deposition, egg mass and better FCR than other genotypes ($p < 0.01$). CB increased BW compared to LB ($p < 0.01$), improving parameters related to productivity (egg deposition, egg weight, egg mass) and FCR ($p < 0.01$). Considering the housing system, hens kept in EC showed better performance than those raised in FR. At 66 wks, hens in EC had greater BW than those in FR (2649 *vs*. 2517 g, respectively; $p < 0.01$). Eggs from LB had a lower weight and albumen proportion compared to others, whereas CB showed a lower eggshell to egg ratio ($p < 0.01$). LB and CB laid eggs with a higher yolk to egg ratio and a lower EBS than the commercial hybrid ($p < 0.01$). In addition, Lohmann brown's eggs showed the highest HI ($p < 0.01$). Surprisingly, EBS was higher in eggs obtained in FR (4.17 *vs*. 3.93 kg, respectively; $p < 0.01$). Further investigations are ongoing to detect the content of vitamin D and related metabolites in plasma and yolk to establish if sunlight exposure in FR boosted vitamin D synthesis, thereby positively affecting calcium metabolism.

O397

Egg production and egg quality in free-range laying hens as affected by dietary polyphenols from olive mill wastewaters

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Among plant secondary metabolites, phenols and polyphenols are known for their antimicrobial and antioxidant activities. Olive mill wastewaters (OMWW), produced in massive quantities by olive mills in many Mediterranean countries, are rich in several phenolic compounds. A phenolic extract obtained from OMWW was used as dietary supplement (60 mg/kg feed) in an experiment conducted with a total of 96 Barrata Italiana hens aged 18 months. Hens were allocated into 2 treatment groups (control and OMWW-enriched feed) with 4 replicates and each replicate containing 12 hens. The trial lasted 54 days and included an adaptation period of 21 days. Hens were housed in outdoor paddocks (10 m^2/hen) and had access to wooden houses with nests (1 per 3 hens). Egg production was recorded daily. Egg characteristics, feed intake and feed conversion ratio were measured weekly. All measurements were repeated after a follow-up period of 21 days, during which both groups received the control diet. Dietary supplementation with OMWW improved ($p < 0.05$) laying performance (+10.2%) and decreased feed conversion ratio (4.09 vs. 4.34 for treated and control groups, respectively). Egg weight was increased ($p < 0.05$) in the treated group (53.0 vs. 49.1 g) and egg components followed a similar trend. At the end of follow-up, production performances decreased in both groups, but those recorded in the OMWW supplemented hens tended ($p = 0.07$) to be higher compared to control. Protein and lipid contents of yolk and albumen and their pH values did not differ between groups. The yolk colour score was unaffected by dietary treatment. Low concentrations of major phenolic compounds hydroxytyrosol and tyrosol were detected in OMWW yolks by LC-HRMS (range: 0.9–1.09 µg/kg), while they were not detected in the albumen. The concentration of several hydroxytyrosol and tyrosol sulphate metabolites was increased ($p < 0.05$) in yolk by dietary treatment with OMWW. Except for hydroxytyrosol-4-sulphate, all other sulphate metabolites observed in the albumen matrix were close to the detection limit. It can be concluded that the dietary supplementation with OMWW extracts may have beneficial effects on egg production, egg weight, and feed conversion ratio. The presence and role of phenolic compounds and their sulphate derivatives in yolk needs to be further investigated.

O454

Technological and sensorial meat quality of broiler chickens: effect of genotype and heat stress

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New challenging environmental and geopolitical scenarios ask for sustainable productions based on animals resilient to climate changes and alternative feeds where slow-growing genotypes can fit on the possible diverse scenarios. The present study aimed to evaluate the effect on meat quality and sensorial properties of different poultry genotypes reared under alternative environmental conditions. A total of 240 broiler chickens were reared in a $3 \times 2 \times 2$ factorial arrangement with three genotypes (Ross 308, Bionda Piemontese, and Robusta Maculata chickens), two environmental temperatures (normal and high), and two sexes. Chickens were reared in pens until slaughtering (42 and 99 days of age for Ross 308 and local breeds, respectively). Right and left breast fillets were dissected from 72 carcasses (6 per experimental group) and submitted to rheological and sensorial analyses.

At the *Pectoralis major* muscle, Ross chickens showed the highest pH, lightness, yellowness and cooking losses ($p < 0.001$), while Bionda Piemontese showed the highest redness ($p < 0.001$). Meat from Ross chickens had higher water and lower crude protein contents, while higher ether extract content compared to the other chickens ($p < 0.001$). At the sensory analysis, Ross breasts had a higher juiciness compared to Bionda Piemontese while intermediate values for Robusta Maculata ($p < 0.05$). As for the flavour, meat of Ross chickens received a lower score for 'brothy' ($p < 0.05$) and 'chickeny/meaty' ($p < 0.001$), and a higher score for 'wet feathers' ($p < 0.001$) compared to that of local breeds. The environmental temperature did not affect meat quality traits and flavour attributes, whereas only the 'toothpack' texture increased ($p < 0.05$) when chickens were reared under high temperature. Males' breasts presented higher pH ($p < 0.001$) than females which corresponded to lower lightness ($p < 0.01$) and yellowness ($p < 0.001$). Moreover, in males, breasts had higher water and lower crude protein content ($p < 0.05$), and scored higher hardness ($p < 0.001$), juiciness ($p < 0.001$) and chewiness ($p < 0.01$), and a higher salty flavour ($p < 0.01$) than females.

In conclusion, while meat traits in slow growing local chickens, such as Bionda Piemontese and Robusta Maculata, were different from those of fast-growing genotypes, sensorial quality was comparable from nutritional and sensorial point of view for the consumers. The effect of environmental temperature was negligible.

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O379

Inbreeding and genetic diversity in Italian horse heritage

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Horse local breeds traditionally used in agriculture have lost their original breeding goals and are currently being challenged to meet current market demand; otherwise, extinction is a solid possibility. Unfortunately, what emerged from the latest FAO report is that 12% of the horse populations worldwide is extinct and over 28% of the remaining ones are either at risk or endangered. The Italian equine gene pool is rich in numbers and diversified in more than 20 local breeds. Although none of them are yet extinct, over 90% are in a critical or endangered status. Therefore, the aim of this study was to calculate inbreeding based on genotype data of 1308 horses from 22 breeds genotyped with the GGP Equine70k SNP chip. Genomic inbreeding coefficients were derived by means of Runs of Homozygosity (ROH) assessment. The ROH segments were detected using the DetectRUNS package in R and defined as follows: at least 15 SNPs in a run, a minimum length of a run equal to 500 Kb, a lower density limit of 1 SNP per 100 kb and allowing for a maximum of one missing and one heterozygous SNP in a run. The genomic inbreeding coefficients (FROH) were calculated as the proportion of individual genome size covered by ROH. Based on the hypothesis that ROH length reflects the chronological time points at which inbreeding occurred, the FROH was expressed separately for five length ROH categories (0.5–1 Mbp, 1–2 Mbp, 2–4 Mbp, 4–8 Mbp, >8 Mbp). Finally, ROH island discovery defined as ROH shared in over 80% of the horses within breed were retrieved as possible signatures of selection. The overall FROH mean was 0.18 (SD =0.05) with a maximum of 0.24 (SD =0.03) in the Lipizzaner and a minimum of 0.11 (SD =0.05) in the Monterufolino breed. In all breeds the majority of the ROH were relatively short (86.3% were less than 2 Mbp long), highlighting the occurrence of older inbreeding, rather than a more recent one. However, all breeds except the Cavallo Pentro showed individuals with ROH longer than 8 Mbp which might be interpreted as a more recent reduction of genetic variability. A total of 16 breeds showed ROH islands with an average of 5.1 ROH island per breed (SD =4.47), highlighting the potential presence of selection in those regions.

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O405

Digestibility of raw materials candidate for sustainable feed formulation in Mediterranean yellowtail (Seriola dumerili)

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With the aim of increasing the efficiency and sustainability of recirculating aquaculture systems (RAS), this study compared the digestibility of cutting-edge ingredients and aquafeed, the physical characteristics of feed pellets and faeces and the rate of ammonia excretion in Mediterranean yellowtail (*Seriola dumerili*). The fish were fed 5 experimental diets which were prepared by mixing a fishmeal-based control diet (crude protein: 51.8% DM; crude lipid 13.6% DM) with one of the alternative ingredients in a ratio of 7:3, i.e. porcine haemoglobin (diet PH), soy protein concentrate (diet SPC), hydrolysed wheat protein (diet HWP), potato protein (diet PP), and beet pulp (diet BP). A total of 20 fish (271 g ± 9.5 g) were randomly distributed into 5 RAS tanks (4 fish per tank) equipped with a Guelph system and fed for 12 weeks the diets. A Latin square experimental design was used based on 5 diets ×5 tanks (2 weeks per diet per tank). Regarding digestibility of raw materials, soy protein concentrate and porcine haemoglobin showed the highest protein digestibility compared with other ingredients (85.5% vs. 68.1%; $p < 0.05$); beet pulp and porcine haemoglobin showed the highest lipid digestibility compared with potato protein (96.0% vs. 90.3%; $p < 0.05$). As for diets, apparent protein digestibility was higher in diet BP than in diets PP and HWP (94.1% vs. 86.4%; $p < 0.05$) whereas other diets performed in between. Apparent lipid digestibility was lower in diet PP compared with other diets (96.8% vs. 98.0%; $p < 0.05$). With regard to physical properties of feed pellets, no differences in oil leakage (%) were found between the experimental diets ($p > 0.05$), while the lowest water turbidity at 15 min after feed administration was recorded for diets PH and HWP (3.7 vs. 4.4 mg L-1; $p < 0.05$). Considering faeces characteristics, no differences according to diets were found in fine (≤0.5 mm), middle (0.6 –1.2 mm) and large (>1.2 mm) faeces particles and furthermore, either the volume (%) of the three classes of faecal particles did not differ. Regarding ammonia excretion, a maximum (N-NH4+ mg kgfish-1× h) was reached between 4 and 6 h after feed ingestion without differences among diets ($p > 0.05$). Overall, the tested ingredients are worth of further investigation in order to make sustainable choices of alternative ingredients that satisfy the dietary requirements of Mediterranean yellowtail and provide best performance in RAS.

O404

Growth and quality of fish reared in haloponics

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The study evaluated the effects of three water salinity levels (0.5‰, 3.0‰, and 6.0‰) on fish growth and fillet colour, pH, lipid peroxidation (TBARS), proximate composition and fatty acid profile of black bullhead catfish (*Ameiurus melas*) and rainbow trout (*Oncorhynchus mykiss*) reared in a brackish-water aquaponic (haloponic) system during a 9-month production cycle. A total of 261 black bullhead catfish (initial weight 147 ± 22 g) were randomly distributed among the three experimental treatments (three units per treatment; initial stocking density 8.53 kg m^{-3}) and reared for 268 days (September-June). In December, 150 rainbow trout (initial weight 153 ± 22 g) were added to the system (initial stocking density 5.10 kg m^{-3}) and reared for 103 days in polyculture with catfish (December-March). During the trial, two cultivation cycles of Swiss chard of 'Nostrana' (1st cycle) and 'Pugliese' (2nd cycle) varieties were carried out (12 plants m^{-2}, 24 plants per unit). As for rainbow trout, water salinity did not affect fish final weight (348 g, on average), survival (89.7%) and fillet yield (52.0%), as well as colour, TBARS (0.30 mg kg^{-1} of malondialdehyde), proximate composition, and fatty acid profile (total saturated fatty acids, SFA, 15.6%; total monounsaturated, MUFA, 49.8%; total polyunsaturated, PUFA, 34.6%). On the other hand, fillet pH was higher (6.38 vs. 6.31; $p < 0.05$) in trout reared at 6.0‰ than at 0.5‰ salinity. As for catfish, water salinity did not affect fish final weight (193 g) and survival (75.8%). Carcass yield was higher (+3%; $p < 0.05$) in fish reared at 0.5‰ than at 3.0‰ salinity, whereas fillet yield was higher (+6%; $p < 0.05$) in fish reared at 3.0‰ than at 6.0‰ salinity. No differences according to water salinity were found for fillet colour, pH (6.08), TBARS (0.44 mg kg^{-1} of malondialdehyde), and proximate composition. As for the fatty acid profile of catfish fillets, MUFA were higher (+7%; $p < 0.05$) in fish reared at 3.0‰ than at 0.5‰ salinity, whereas PUFA were higher (+7%; $p < 0.05$) in fish reared at 0.5‰ than at 3.0‰ salinity; intermediate values of MUFA and PUFA were found in fish reared at 6.0‰ salinity, while no changes were found for SFA. In conclusion, catfish and trout farming in haloponics is feasible until 6‰ salinity. The quality of trout fillets was not affected by water salinity, whereas changes were found in the fatty acid profile of catfish fillets, which warrant further investigations.

O493

Towards sustainable marine spatial planning of aquaculture in Apulia Region

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The existing conflicts between human uses in the coastal and offshore zone and the conservation of marine habitats are reconnected to the indications of the EU Marine Spatial Planning Directive. In this context, targeted and efficient measures of spatial planning may avoid potential conflicts as well as create positive synergies between activities and interests. In particular, the topic of marine and maritime sustainable development has been a focus of attention since 2012 and the EU Commission recognises aquaculture as a crucial sector with great innovation potential for a smart, sustainable and inclusive growth.

The identification of suitable areas for aquaculture requires the integration of multiple socio-economic and environmental factors that can be different for fish and shellfish farming and therefore the use of an approach based on the EU Marine Spatial Planning can guarantee the allocation of adequate maritime spaces for the sustainable development of both aquaculture systems.

The present study was carried out along the Apulia Region coastal zones with the aims to propose a conceptual model suitable for marine spatial planning of aquaculture; to collect and integrate indicators useful for the characterization of the study area in terms of socio-ecological-economic sensitivities and pressures; and to identify and map the suitable areas for the development of new fish and shellfish farming, taking into account the carrying capacity of natural resources and the effect of aquaculture farms on the quality of the marine ecosystem.

The maps that were produced from the zoning process show the marine-coastal areas classified as *potentially very suitable*, *potentially suitable* and *potentially unsuitable* areas for new aquaculture activities or for the expansion of existing ones. In the first two areas, there are no interferences with other users and the environmental conditions can guarantee the well-being of farmed organisms, the minimization of environmental impacts and the sustainability of the productions.

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O71

Effect of Hermetia illucens meal on gilthead seabream (Sparus aurata) flesh sensory profile. An innovative approach using electronic senses and data fusion

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Use of insects as alternative protein source for aquaculture feed production is an excellent example of circular economy. With the aim to evaluate the effect of fishmeal (FM) replacement with *Hermetia illucens* meal (HIM) in the diet of *Sparus aurata* farmed offshore on the organoleptic quality of fillets, two isolipidic (18%, as fed) and isoproteic (42% as fed) diets were tested at farm scale: a control diet with no HIM, and an experimental diet with 35% of HIM in substitution to FM. At the end of the trial, the organoleptic properties of fresh fillets from 12 fish per each group were evaluated using a sensor-based instruments platform consisting of E-eye, E-nose with 18 MOS sensors and a potentiometric E-tongue with 7 chemical sensors. The artificial senses platform was used to detect the organoleptic *fingerprint* of the fillets of the 2 groups (HIM vs. FM) in terms of color, volatile fraction and taste. For each instrument, all sensors data was analyzed by principal component analysis (PCA) to highlight similarities and differences between the groups; then, the pattern discrimination index and organoleptic distance (based on Mahalanobis distance), were calculated. PCA graphical representation of the E-eye and E-nose results showed nearly overlapping color and odor profiles of the 2 groups. The organoleptic distance and the pattern discrimination index between the 2 groups were for the color 5.39 and 3.88% ($p = 3.78$) and for the volatile profile 0.08 and 2.36% ($p = 0.08$), respectively. Instead, regarding the taste, the PCA of the E-tongue showed a clear distinction between the fillets of the FM and HIM groups and the first two principal components accounted for 94.61% of the variance. Specifically, the vector that mostly identified the fillets of the HIM group was the AHS sensor, particularly sensitive to astringency, while for the fillets of the FM group, the PKS, NMS and CTS sensors, respectively metallic, umami and salty taste indicators. The organoleptic distance and the pattern discrimination index were also significantly high (837.15 and 55.64% respectively; $p = 0.001$). The results highlighted that the diet with 35% of HIM meal in substitution to FM influenced the taste of fillets confirming E-senses as powerful instruments for organoleptic evaluation.

Acknowledgements

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O543

Cold shock by immersion in iced salty water is a suitable method to stun Carpione del Garda (Salmo carpio) both considering animal welfare and flesh quality during storage

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The effect of the method of stunning/killing both on animal welfare and on quality of the final product is one of the most critical process that influences adenosine 5-monofosphate (ATP) depletion but also quality-related parameters such as pH, colour and water holding capacity (WHC). Despite the request of the European Commission to identify species-specific stunning systems, many works need to be done. Carpione del Garda (*Salmo carpio*) is a salmonid endemic of Lake Garda whose meat are highly appreciated. However, scarce are the studies aimed to investigate the effects of stunning methods on flesh quality. Seventy-two *Salmo carpio* (347.4 ± 68.9 g) specimens were randomly and equally sorted into three experimental groups (24 fish per treatment) based on the stunning method: (i) lethal dose of anaesthetic (AN, 400 mg/L MS-222), used as control; (ii) electrical stunning (ES, 24 V applied for 4 s); (iii) cold shock by immersion in iced salty water (ICE, 30 s at −3 °C; NaCl 35 ppt). ATP and related catabolites in muscle immediately after death were analysed to obtain ATP to inosine monophosphate (IMP) ratio; colour parameters, pH, water holding capacity (WHC), fatty acid profile, conjugated dienes and thiobarbituric acid reactive substances (CD and TBARS, respectively) were determined immediately after death and after a storage of 30, 60, and 90 days at −20 °C. The results obtained from the statistical analysis (two-ways ANOVA) highlighted that the AN and ICE groups had higher ATP/IMP ratio (57.3 and 41.4, respectively), than ES (10.7). pH value of AN samples (6.5) was higher than the value found in ES fillets (6.32;

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$p < 0.05$); a pH drop ($p < 0.001$) was observed after 30 days of storage irrespective to the stunning method applied. WHC resulted unaffected by stunning and storage. The ICE fillets were brighter (L*: 54.9) than the ES ones (52.48), whereas redness (a*) and yellowness (b*) indexes were modified by the storage. Noteworthy, the AN group showed a high lipid oxidation level ($p < 0.01$), since a higher ($p < 0.01$) TBARS content was observed after 60 days of storage (>0.10 mg MDA/100 g fillet) compared the values found in the other groups, despite the fatty acid profile of the fillets at the beginning of the trial did not differ. In conclusion, with the attempt to find the best compromise between fish welfare and flesh quality, cold shock by immersion in iced salty water could be suggested for *Salmo carpio* stunning.

O251

Mitochondrial DNA based diversity over several Mediterranean regions in holothurian species

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Sea cucumbers are deposit feeding members of marine benthic communities which play a key role in sea floor dynamics by processing and bioturbating the sediment. Many of these species are considered economically relevant as luxury food and for preparations in traditional medicine in Asia. This importance has caused an over-exploitation of many natural stocks, especially in the Mediterranean area where the excessive fishing has caused a biodiversity loss among populations with a negative impact on the marine ecosystem. For these reasons, few initiatives are recently evaluating sea cucumbers as novel aquaculture species. The aim of this study was to evaluate the level of genetic diversity within two sympatric *Holothuria* sea cucumber species, *H. polii* and *H. tubulosa*, across different Mediterranean areas, before considering the implementation of conservation actions and as a first step to establishing farmed stock populations.

We sequenced informative portions of *cox1* and *16S* mitochondrial genes in a total of 240 holothurians (120 *H. polii* and 120 *H. tubulosa*). Sea cucumbers have been sampled from three sites across Mediterranean Sea on different seabed typologies (sand, rock, *Posidonia* meadows). Genetic divergence (*p-distance*) within and between species has been calculated and two Neighbour Joining (NJ) phylogenetic trees have been generated from reduced datasets, including six samples per species per site and three outgroups (*H. scabra*, *H. forskali* and *H. mammata*). While the average *p-distance* was similar between and within species (0.10 and 0.09, respectively), the phylogenetic analyses showed a clear differentiation between the two species. Moreover, the outgroup *H. mammata* clustered as a sister group of *H. tubulosa* clade, as expected. Evidences of heteroplasmy in mtDNA in few holothurians were detected, suggesting that some hybridization events might have occurred between these sympatric species. The NJ trees showed no differentiation between site populations, indicating active gene flows among Mediterranean areas.

The results provide genetic markers useful for species identification and an overview of the genetic variability in three Mediterranean populations of *H. polii* and *H. tubulosa*. Further studies are needed to disentangle the origin of the mtDNA heteroplasmy reported in a few samples. These data will be useful to establish farmed stocks with high level of genetic variability.

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O208

Growth performances in Cupped oysters (Crassostrea gigas) during prefattening stages in the Middle Adriatic Sea

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In the last decade, demand for bivalves has increased substantially worldwide. In the Adriatic Sea, offshore shellfish farming is almost entirely based on mussel farming although some companies started to diversify their production. Growth performance and interaction between pathogens were analysed in different batches of cupped oysters (*Crassostrea gigas*), during the years 2018 and 2019. Our results showed satisfactory mean weights, ranging from 3.979 ± 5.377 g to 8.860 ± 5.224 g, at the end of the pre-fattening phase, but an increase of mortality occurred in summer months. Histological analyses recorded regressive lesions in summer sampling, with the presence of rod-cell bacteria, immunolabelled as *Vibrio* spp. The molecular research of OsHV-1 and *V. aestuarianus* showed negative results in all the timepoints, whereas *V. splendidus* was detected. The role of *V. splendidus* remains unclear as, during 2018 summer mortality, it showed a significant increase in load but, in contrast, through the whole 2019 the bacterial load was low even during the mortality peak. Oysters farming seems to be a feasible activity in the Adriatic Sea, although other trials need to be performed.

O414

Quality of fillet from trout (Oncorhynchus mykiss) fed diets supplemented with olive pomace at two different levels

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The increasing importance of sustainable aquaculture productions has generated a rising interest for the development of functional diets with benefits both for fish and quality of fish products. Rainbow trout (*Oncorhynchus mykiss*) can be fed diets rich in pigments, such as astaxanthin, which gives the reddish color to fish flesh. However, the oxidation of both astaxanthin and polyunsaturated fatty acids (PUFA), fish products' flagship, can spoil feed or fillets. The present trial aimed to evaluate the antioxidant benefits of dietary olive pomace in terms of trout growth performance and quality of fresh and frozen fillets. To reach these goals, 600 rainbow trout (311.3 ± 48.8 g) were randomly distributed in 12 tanks and fed one of either 4 experimental diets formulated to be isoproteic (41.4 g/100 g feed), isolipidic (23.75 g/100 g feed) and isoenergetic (22.4 MJ/100 g feed): (i) control diet (CTRL); (ii) CTRL added with olive pomace at 1% (T1); (iii) CTRL added with olive pomace at 1.6% (T1.6) on the feed as is; (iv) CTRL added with a commercial blend of polyphenols (CTRL+). Polyphenols and astaxanthin feed contents were analyzed. After 10 weeks, feed conversion ratio (FCR) and specific growth rate (SGR) were calculated, and 8 fish/tank (24 fish per diet) were slaughtered. Flesh color, fatty acid (FA) profile and lipid oxidation (thiobarbituric acid reactive substances, TBARS) of 15 fish/diet were analyzed immediately and after 5 months at −20 °C. In addition, other 9 fish/diet were allotted to a descriptive sensory evaluation. Data were analyzed with the PROC GLM of SAS with diet as fixed effect. FCR and SGR did not differ among groups. Diets did not significantly modify fillet color values (lightness, yellowness, and redness) in line with the muscle content of astaxanthin (from 6 mg/kg in CTRL to 7.2 mg/kg in T1.6). However, the trained panelist described the color of cooked fillets as more intense ($p < 0.05$) in T1.6 and CTRL group than in the others. Besides, the panelist described the aroma of T1.6 fillets as more intense ($p < 0.05$) than the other groups, with algae, damp/earth and acidity attributes. Despite T1.6 diet was richer than the others in OH-tyrosol, tyrosol, and ferulic acid, lipid oxidation tended to increase after storage, being TBARS values 0.16, 0.15, 0.16, and 0.19 mg MDA-eq./100 g in CTRL, CTRL+, T1, and T1.6, respectively. Interestingly, at the end of the storage, the highest content of the saturated FAs was found in T1.6 and CTRL + groups ($p < 0.05$).

O10

Effects of insect meal-based diets on growth, intestinal well-being and microbiota in Gilthead seabream (Sparus aurata)

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The current study aimed to evaluate the effect of fish meal (FM) replacement with insect meal from *Hermetia illucens* (Hi) in the diet of *Sparus aurata* farmed offshore on fish growth, intestinal health, and microbiota composition. Two isolipidic (18%, as fed) and isoproteic (42% as fed) diets were tested at farm scale: a control diet (FM) with no Hi meal, and an experimental diet (Hi) with 35% of Hi meal in substitution to FM. At the end of 25 weeks of feeding trial, final body weight (Hi: 244 g *vs* FM: 246 g, $p = 0.707$), specific growth rate (Hi: 0.21% *vs* FM: 0.22%, $p = 0.720$), feed conversion rate (Hi: 1.32 *vs* FM: 1.44, $p = 0.138$) and hepatosomatic index (Hi: 1.20% *vs* FM: 1.15%, $p = 0.512$) were not influenced by the diet.

The gross morphology of the gastrointestinal tract and liver were preserved with no evident signs of inflammation. However, increased intestinal villi length and lamina propria width were observed in the distal intestine of fish fed Hi meal.

High-throughput 16S rRNA gene amplicon sequencing (MiSeq platform, Illumina) utilized to characterize the gut microbial community profile showed that Proteobacteria, Fusobacteria, and Firmicutes were the dominant phyla of seabream gut microbiota, regardless of the diet. Dietary inclusion of Hi meal changed gut microbiota by significantly reducing the amount of *Cetobacterium* and increasing the relative amount of *Oceanobacillus* and *Paenibacillus* genera.

Our findings clearly indicated that dietary inclusion of 35% of Hi meal, as an alternative animal protein source, positively affected seabream gut microbiota, by increasing the amount of beneficial genera thus improving gut health, and maintaining the growth performance of *Sparus aurata* farmed offshore.

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O514

Finishing diets to modulate flesh fatty acid composition and skin colour in gilthead seabream (Sparus aurata)

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Cultured finfish species contribute substantially to the overall seafood supply therefore their nutritional quality, particularly the muscle fatty acid composition becomes a primary goal to be achieved. Moreover, it is well known that the external appearance of farmed fish greatly affects its marketability. Besides the shape, the colour of the skin is one of the first features that guides consumer choices. Consequently, the modulation of the meat composition and the skin appearance through specific finishing diets assumes a growing interest in farmed fish species. This study was aimed at evaluating the skin colour pattern and muscle fatty acid compositions of gilthead sea bream (*Sparus aurata*, GSB) fed diets supplemented with carotenoids or LC-w3PUFA during the finishing phase. To this end, three isoproteic (41%) and isolipidic (18%) aquafeeds were compared. The diets coined COLOR and PUFA were fortified with carotenoids (+35%) or LC-w3PUFA (+240% FAMEs), relative to a standard commercial diet (CTRL). Nine groups of GSB (IBW: 402.2 ± 5.4 g) were reared in a RAS system under optimal rearing conditions (T, 24 ± 0.5 °C; Salinity 30–35 ppt) and were fed the test diets to satiety over 84 days. Three additional fish groups per diet were also set and used for intermediate measurements (days 0, 20, 40, 60 and 84). Fish fed diets COLOR and CTRL were individually photographed with a digital camera on the left side, and subjected to colour measurement with a Minolta spectrophotometer in the dorsal and opercular area. Six fish fed diet PUFA and CTRL were sacrificed to analyse muscle fatty acid composition.

At the end of the trial, the dietary treatment did not affect the growth performance. Only the red component in the opercular area was significantly affected by the dietary treatment (a* 2.14 vs 6.54, $p < 0.05$; CTRL vs COLOR). The analysis of the extension of the opercular spot gave opposite results compared to those related to the colour intensity. In fact, diet COLOR actually enhanced the intensity, but the extension of the opercular spot tended to shrink over time, regardless of the dietary treatment.

As expected, feeding diet PUFA significantly increased the concentration of LC-polyunsaturated fatty acids in the muscle lipid composition (10.54 vs 4.98 % FAMES; $p < 0.002$). Such an improvement started to be clear 21 days after feeding.

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O512

Dietary hazelnut skin and addition of phenolic extract from olive mill wastewaters: effects on pork salami quality

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Processed pork products are specialties in many countries, but minced meat is prone to lipid oxidation, which may result in a reduction of shelf-life and generates toxic molecules. Hazelnut and olive oil industry by-products are largely available in the Mediterranean. Due to the high content of bioactive compounds, they could be profitably used as feed ingredients or food additives to improve meat products quality and oxidative stability.

In this study, 20 barrows were divided into two groups of 10 and fed a conventional feed (CTRL) or a feed containing 11% of hazelnut perisperm (HAZEL) during a 78-day finishing period. Two types of salami were obtained from each barrow by adding E250 and E252 additives (N salami) or an olive milling wastewaters phenolic extract (OWP salami) in the salami mix. We hypothesized that fatty acids (FA) and antioxidant capacity (FRAP and TEAC) as well as lipid oxidation (TBARS) and colour (CIELAB) stability could be affected by dietary treatment, salami recipe or their interaction. A panel test assessed sensory properties and consumer acceptability of the different salami types.

Feeding strategy had a minimal effect on investigated parameters. As for FA, CTRL salami showed a lower ω-6/ω-3 ratio ($p = 0.035$) due to the higher content of PUFA ω-3 ($p = 0.008$). Antioxidant capacity did not differ between the two recipes or diets, whereas OWP salami had a higher percentage of ω-3 and ω-6 FA than N salami ($P \leq 0.05$). Despite the higher polyunsaturated FA and similar antioxidant capacity, OWP salami showed lower TBARS than N salami during 7-day refrigerated storage ($p < 0.001$). Colour analysis showed that nitrites exert a greater colour-stabilizing action than phenolic extract ($p < 0.001$). A dietary treatment × recipe interaction was observed for the lipid oxidations ($p = 0.040$), with TBARS higher in N salami than OWP salami when processed meat was obtained from barrows fed the CTRL diet. The untrained panel expressed comparable general satisfaction between conventional and OWP salami during blind tasting, whereas OWP salami was preferred after the informed tasting ($p < 0.001$), which indicates that consumers appreciated the use of an alternative to nitrites and nitrates.

Concluding, providing a diet with hazelnut perisperm to pigs and using OWP as an additive during pork processing could be viable strategies to produce salami with acceptable sensorial quality, with the added value of reducing the use of conventional feeds and providing a cleaner label.

O273

Fourier transform infrared spectroscopy of milk as a tool for monitoring efficiency in the Parmigiano Reggiano PDO production

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This study aimed at exploring the feasibility of Fourier-transform mid-infrared (FTIR) spectroscopy on vat milk in Parmigiano Reggiano PDO production for predicting cheese making traits [3 measures of cheese yield (%CY): fresh, solids and retained water ($\%CY_{CURD}$, $\%CY_{SOLIDS}$, $\%CY_{WATER}$); 4 recovery traits (%REC): milk fat, protein, solids and energy in the curd ($\%REC_{FAT}$, $\%REC_{PROTEIN}$, $\%REC_{SOLIDS}$ and $\%REC_{ENERGY}$)]. In total, 134 vats from 34 dairy industries (2 vats/dairy industry, double sampled in summer and winter, respectively) were monitored during cheese making. For each vat, the weight of milk and the cheese after 24 h from cheese making was measured, while milk and whey composition traits (protein, fat, lactose and total solids) were analyzed. Two spectra from each vat milk sample were collected in the range between 5011 and 925 cm^{-1} and averaged prior the data analysis. The calibration models were developed with a Bayesian approach by using the BGLR (Bayesian Generalized Linear Regression) package of R software. The feasibility of the equation models was assessed in two different scenarios: (i) a random cross-validation (CV) [80% calibration (CAL); 20% validation (VAL) set; 10 replicates], (ii) a stratified CV [(SCV), 33 dairy industries used as CAL, and the remaining one as VAL set]. To assess models' performance, the coefficient of determination (R^2_{VAL}) and the root mean square error of validation ($RMSE_{VAL}$) were used. The results obtained from the CV showed that R^2_{VAL} ranged between 0.06 and 0.67. The most accurate predictions were obtained for $\%CY_{CURD}$ and $\%CY_{SOLIDS}$, which exhibited R^2_{VAL} and $RMSE_{VAL}$ values of 0.67 and 0.50, and of 0.38 and 0.32, respectively. The worst prediction performance was obtained for $\%REC_{PROTEIN}$ (R^2_{VAL} and $RMSE_{VAL}$ values of 0.06 and 0.61, respectively), probably because of the very low variability of this trait. When the SCV was used, the R^2_{VAL} increased for all the investigated traits (from 0.48 to 0.79), due to the great variability that characterizes dairy industries in terms of milk quality and cheese making procedures adopted (i.e. milk natural creaming). This study demonstrated that FTIR spectroscopy could be a valid tool for monitoring cheese making efficiency at the dairy industry level for Parmigiano Reggiano PDO production. Moreover, our findings highlighted the importance of considering the differences among dairy industries to propose tailored and reliable tools applicable along the Parmigiano Reggiano chain.

O166

Glucose oxidase and carboxypeptidase A-like activity as new potential freshness markers for Royal Jelly

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Royal Jelly (RJ) is a nutrient secretion of nurse bees for larvae and a high interest functional food in human nutrition due to its several health-promoting properties. As functional food there is a growing interest in consumers and food industry towards this bee product and its shelf life. Among several potential markers for RJ freshness previously suggested, enzymatic activity has been poorly investigated and it is still a neglected topic despite it may be a useful tool for quality product assessment during shelf life. In this study the enzymatic activity of glucose oxidase (GOX) and five proteases (i.e. chymotrypsin, trypsin, N-aminopeptidase, carboxypeptidase A and B) in RJ under different storage condition (i.e. refrigeration and freezing) and times (i.e. two-months, 1, 2, 3 and 4 years) were preliminary investigated.

Enzymatic activity was measured on RJ samples collected from 2017 to 2021 and refrigerated from production to analysis. In 2020 and 2021 an aliquot of samples was also frozen.

Refrigeration determined a higher GOX activity in RJ after two months of storage that it significantly decrease after one year. Higher carboxypeptidase-like A activity was also recorded in refrigerated RJ at two-months compared to other investigated time points. Freezing did not determined variation in both GOX and carboxypeptidase-like A activity in RJ stored for two months rather than 1 year. Conversely, a higher GOX and carboxypeptidase A-like activity was recorded in frozen RJ after 1 year compared to refrigerated one. No variation in the activity of the other proteases investigated were recorded in both refrigerated and frozen RJ.

Therefore, GOX and carboxypeptidase A-like activity resulted suitable RJ freshness markers within one year at refrigeration condition and freezing may be a valid alternative storage method to ensure a higher preservation of their activity.

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O116

Plasma proteome, a non-invasive biofluid to monitor lamb meat quality

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Monitoring meat quality characteristics with accurate and non-invasive tools to detect, before slaughter, animals with desired quality characteristics, is a prerequisite of meat industry. Plasma, as a readily available biofluid, represents a potential biological source of information about the animal's physiological and pathological condition, as well as its underlying biological mechanisms.

In this regard, a proteomics approach on plasma and muscle proteomes were applied to assess the effect of two different dietary treatments on in vita biological processes and meat quality. Twenty-two Valle del Belice male lambs were randomly assigned to two dietary treatment groups. Control group (C) received a maize-barley without any supplement, whereas the hazelnut group (H), received a hazelnut skin by-product as a maize partial replacer in the concentrate diet. The results revealed that hazelnut skin by-product dietary supplementation impacted meat quality characteristics, with meat from lamb of the H group displaying greater values of lightness, redness, yellowness, and chroma color parameters together with greater myofibril fragmentation index. Proteomic and bioinformatics approaches applied to plasma proteome revealed twenty protein spots (18 unique gene names) belonging to ‘enzyme regulator activity’ including serpins, ‘identical protein binding’, ‘antioxidant activity’, ‘steroid binding’ and ‘cholesterol transfer activity’ as differentially expressed due to hazelnut skin by-product supplementation. Twenty-three proteins from the muscle proteome of known roles in post-mortem processes, likely ‘muscle contraction, structure, and associated proteins’, ‘energy metabolism’, ‘heat shock proteins’, ‘oxidative stress’ and ‘immunity, binding & transport proteins’ could act as potential predictors of lamb meat quality. To clear up the complex relationships between plasma and muscle proteomes of the two dietary groups, comparative bioinformatics revealed APOA1, PHB, ACTG1, and ALB as common proteins, hence suggesting sophisticated biological crosstalk and potential use as candidate biomarkers to monitor lamb meat quality production. Our findings underline the important role of plasma as a challenging biofluid protein source to predict in a non-invasive manner the lamb meat quality.

O552

Effect of ripening time on chemical-nutritional quality of the traditional Pecorino Bagnolese cheese

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Pecorino Bagnolese is a typical ripened cheese of Irpinia area in Campania Region (Italy) made with at least 70% of Bagnolese ewe’s milk. It is included in the 22th revision of the national list of Traditional Agrifood. The traditional cheesemaking, as such as the feeding system (pasture in the cheese production area) make cheese strongly linked to the territory. To increase the diffusion of traditional products and preserve the sheep breed, strategic and operational activities aimed at improving the value creation of the product are necessary. To improve the competitiveness of Pecorino Bagnolese cheese, the aim of study was to deepen the knowledge of the production process and to create an added value to the dairy product, assessing the effect of ripening on its chemical-nutritional properties. Cheesemaking were performed in a small cheese plant, located in Bagnoli Irpino. Filtered raw ewe’s milk was gently heated at 36–38 °C in a copper vat;

then, kid paste rennet was added. At curd formation, the coagulum was cut by a wooden stick to rice size, cooked at 42 °C for approximately 5 min, transferred into perforated plastic molds and pressed by hand to allow the whey drainage. Molded curd was left to acidify overnight and then dry salted by hand; finally, cheeses were ripened in cool farm cellars for 8 months with frequent rotations and cleaning of the surface and then sent to the laboratory for analyses. Raw milk, curd (day 0), and cheeses at specific sampling times (1 d; 2, 4 and 8 months) were collected and submitted to the physicochemical and nutritional analyses. The average raw milk fat content was of 7.6 ± 1.3% and protein content of 5.6 ± 0.8%. Results showed that moisture and a_w content decreased, resulting in a relative increase in protein content during the ripening time. The moisture content showed a negative correlation with the pH value due to the syneresis rate of the cheese which increases as the pH decreases. After 8 months of ripening, lipolysis increased while the concentration of oxidation products was low and constant throughout the ripening time without negatively affecting the flavor of the cheese. Few changes have occurred in the acidic profile of the cheeses, demonstrating how the nutritional properties are preserved. Overall, the traditional production system combined with the typical quality characteristics of the cheese can strengthen the identity of Pecorino Bagnolese and represents a strategy for creating value for the product.

O99

Effects of environmental factors on milk microbiome

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In the context of the ‘INNOVALAT’ project, whose goal was to enhance the quality of dairy cattle’s feed to guarantee animals’ health and performance using new-generation technologies, a study about the effects of environmental factors on the milk microbiome was carried out. The trial was conducted in 4 dairy cattle farms (3 in the Province of Viterbo, 1 in the Province of Rome, Lazio Region). In all farms, physical and chemical analysis of diets were carried out as well as the eating behavior of cows. Bulk milk samples were collected monthly from March 2021 to April 2022 by each farm, directly from the tanks after accurate mixing, and placed in plastic bottles containing Bronopol® as a preservative. Fifty-two milk samples were obtained at the end of the trial. All samples underwent a chemical-rheological analysis to assess fat, protein, lactose, casein, and urea content, freezing point, pH, and fatty acids composition. The following parameters were determined for sanitary hygienic analyses: total bacterial count and somatic cells. Cheesemaking attitude was evaluated determining rennet coagulation time (r), firming time (K20), and clot consistency at 30 min (a30). For the metagenomic analysis, the samples were taken in duplicate. Not all the samples were available for this analysis, obtaining a total of 2 × 46 (=92) sequenced samples at the end. Milk was treated for DNA extraction and amplification and then sequenced by the NGS Illumina platform by the MiSeq sequencer, and subsequent bioinformatics analysis was carried out. The most abundant phyla detected in the samples were *Proteobacteria, Firmicutes, Actinobacteria,* and *Bacteroidetes*, in line with the literature. Their fluctuation across different time points can be determined by different farm management practices. Taxa belonging to the phylum *Bacteroidetes* whose presence above a defined threshold could be evidence of subclinical mastitis represented up to 17% of the total OTUs. Several taxa which are associated with environmental contamination were also revealed. OTUs belonging to genera associated with potential probiotic functions or that could play an important role in cheese-making and ripening were differentially represented both in the different samples of the different farms and of the different seasons. The use of the NGS technique for sequencing the milk metagenome could be considered a valuable analysis tool for managing dairy farms, given the vast amount of information obtainable.

O85

Study of microbial communities, pathogenic and emerging microorganisms in sheep's milk cheese processing facilities of Sardinia using a DNA metabarcoding approach

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Microbial contamination from the surfaces of food processing environment can severely affect the quality and safety features of the final products. Environmental conditions occurring in Sardinian sheep's milk processing facilities, such as humidity, condensation, residues of organic matter, presence of spatial niches for biofilm formation, could allow the survival and growth of spoilage and pathogenic microorganisms, such as *Listeria monocytogenes*, *Listeria* spp., *Salmonella* spp., *Bacillus cereus*, *Pseudomonas* spp., *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Escherichia coli*. Monitoring environmental bacteria contamination represents a fundamental strategy of a food safety management system to identify the presence of sites of contamination and the persistence of microorganisms in the cheese manufacturing facilities.

The objective of the present study was to evaluate the presence, abundance and taxonomical identity of the bacteria occurring in different processing areas of eight Sardinian cheese-making plants. Samples were collected with sponges from food contact- and non food-contact surfaces from different processing areas and analyzed using the culture-independent DNA sequencing-based 16S metabarcoding method.

Preliminary data from two facilities yielded 5,041,784 different sequence variants, sorted into 28,763 taxa. While alpha diversity values were similar between the two dairies, differences occurred among processing areas. Communities mainly showed trends of separation splitting the areas of the washing-processing operations from those of salting-maturation ones, which in turn were more similar to the cheese surface itself.

The three most abundant taxa were Acinetobacter, Streptococcus and Pseudomonas. Only 2 samples out of 36 contained *L. monocytogenes* and *Listeria* spp., while Salmonella spp were present in 6 specimens. 2 sequence variants for Bacillus cereus and 8 for Escherichia coli were found. An abundant number of variants for Pseudomonas spp., 10 for Pseudomonas aeruginosa and none for Pseudomonas fluorescens were found.

Overall, a remarkably high diversity was found. A generally acceptable hygiene standard was observed, although the abundance of Pseudomonas spp. is to be taken with particular care. Knowledge of the complex microbial ecology of the processing environments is essential in order to be able to adopt adequate risk management measures and provide safety guarantees for products to be placed on the market.

O82

Study of nitrite and nitrate residual levels in meat products after different types of cooking treatment

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The inorganic salts of nitrate and nitrite anions are common chemical compounds added in meat products. In these products they act as flavouring, safety and preservative agents, playing their role in inhibiting microbial growth (also the high concern bacterium *Clostridium botulinum*), maintaining freshness, improving product attractiveness by enhancement of the characteristic red-pink colour and retarding the rancidity process by inhibiting the lipid peroxidation. Many studies demonstrated the correlation between cured meat consumption and increased incidence of cancer. This is due to the reaction between nitrites and secondary amines which leads to the formation of nitrosamines. The nitrate intake is also important, since this compound may be reduced to nitrite in the stomach under acidic conditions, or through the action of commensal bacteria enzymes. Other health effects due to the presence of these additives in food are the oxidation of haemoglobin to methaemoglobin (*Methemoglobinemia*) and several adverse reactions in susceptible people. In this view, the continuous evaluation of risk exposure to nitrite and nitrate from food, especially meats, is essential for assuring food safety.

Meat cooking can modify the residual levels of these 2 compounds then modifying the actual intake.

In this study, meat products containing nitrite and nitrate, usually consumed after cooking, such as wurstel, bacon and sausage, were submitted to 3 types of cooking: frying, oven and stewed cooking.

Overall, cooking leads to nitrite decrease and nitrate increase in the final products. Steewed cooking causes nitrite decrease up to 43.5% in wurstel samples. No significant change was registered for bacon samples, probably due to the low initial amount of this additive. Regarding nitrate, grilling always leads to significant increase, up to 94.4 mg/kg in sausage. This increase caused the limits of 150 mg/kg as $NaNO_3$ to be exceeded in 13 samples out of 45 analysed (concentration range: 176.2 – 293.8 mg/kg). This is a very important finding in the view of food safety and 'Total Diet Studies'.

In conclusion, given the decrease of both nitrite and nitrate after treatment, stewed cooking resulted the best option, while considering the increase of nitrate and low decrease of nitrite (if compared to other cooking types), grilling can be identified as a cooking type which substantially worsens the food safety of meat products added with nitrite and nitrate.

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O205

Honey quality and traceability: a preliminary investigation on Citrus honeys physio-chemical traits from three Mediterranean countries

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Honey from *Citrus* spp. (CH) is a highly valuable monofloral hive product that as other animal food products, must meet the request of consumers of quality, safety and traceability and the regulatory frame as well. The purpose of this research, as part of the European project Plant-B, funded by PRIMA Foundation (grant number 1812/PLANT-B), is to evaluate the quality and the geographical traceability by measuring some chemical-physical traits of CH samples from three Mediterranean countries. The study was performed on 41 samples of the 2020 beekeeping season of CH from project case studies in Italy (Sicily, Campania and Sardinia) and Greece (Peloponnesus) and from the market, and 40 samples collected during the season 2021 from the same Italian and Greek regions and from and Egypt. The parameters analyzed were moisture (%), free acidity (meq/kg), pH, colour (mm Pfund) and electrical conductivity (mS/cm). A bibliographic search was also carried out which led to the creation of a dataset related to this parameter studied in CH from the Mediterranean basin area. The results showed that the data obtained are similar to what has been found in the literature as regards citrus honeys from the Mediterranean basin. Whever, significant differences were found between the different geographical origins of the samples. Italian and Greek samples differed as far as the electrical conductivity (0.3 ± 0.1 mS/cm vs. 0.2 ± 0.01 mS/cm, $p < 0.05$) and for moisture (17 ± 1 *vs.* 16 ± 0.7 %) and colour (32 ± 9 *vs.* 25 ± 9 mm Pfund) ($p < 0.01$). At the same time, as regards the samples of the 2021 harvesting season only, significant differences were found between the Greek samples and the Egyptian ones for colour (27 ± 10 *vs.* 41 ± 17 mm Pfund, $p < 0.05$). The Egyptian samples were lower in free acidity (4.1 ± 1.1 meq/kg) with respect the Greek and Italian samples (5.8 ± 0.9 and 5.6 ± 2 meq/kg, respectively) ($p < 0.05$), but the Italian samples (0.36 ± 0.15) differed from the Egyptian and Greek samples (0.21 ± 0.01 and 0.17 ± 0.05 mS/cm, respectively) as far as the electrical conductivity ($p < 0.05$). Further investigations are in progress on 2022 CH samples, coming from the same areas, to validate the discriminant power of such interesting physico-chemical traits.

O200

Pollen quality: a study on the elemental profile of pollen sampled in a suburban environment

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The curbicolar bee pollen (CBP) is a basic feed for good growth and development of honey bees (HB). It is also a food that is increasingly receiving interest from human consumption. However, it can be expected that locally environmental conditions can play a role on the nutrition of HB colonies through the diversity pollen, but, at the same time, environmental conditions can lead to an accumulation of toxic elements in pollen. The aim of this work as part of the European project Plant-B, funded by PRIMA Foundation (grant number 1812/PLANT-B), was to appraise the nutritional quality and safety through the content of some macro and trace elements in CBP collected in the suburban area of Viterbo, Central Italy. From March to August 2021 CBP was collected monthly from the PLANT-B apiary located in the Experimental Farm 'Nello Lupori' (Tuscia University, Viterbo, Italy). One-day bee pollen samplings were performed from 10 HB colonies by means of commercial pollen traps. After each harvest, the pollen samples were combined, packed and stored frozen, and then dried before analysis. Two pollen samples were pooled on the monthly basis, mineralized and analyzed by Atomic Absorption Spectrometry (AAS) with graphite furnace atomization (GFA, for Cd, P, and Pb), or air-acetylene flame atomization (FL, for Ca, Cu, Fe, K, Mg, Na, and Zn). The results obtained from the mineral profiling for Ca 1351.7 ± 123.9, Cu 5.02 ± 1.12, Fe 4.35 ± 0.88, K 11325.2 ± 3041.9, Mg 1055.8 ± 266.3, Na 96.1 ± 61.8, P 1749.8 ± 488.0, Zn 50.7 ± 15.0, Cd 0.009 ± 0.002 and Pb 0.071 ± 0.013 mg/kg, showed that during the monitoring period, there were variations in the concentrations of the individual elements based on the month of collection, even though significant differences were recorded only for Na, that showed a decreasing trend from May to June ($p < 0.05$), and for Zn that showed a reduction step between the months of July and August ($p < 0.05$). As far as the content of undesired elements Cd and Pb, it was in line or lower with respect to the values obtained from literature (0.29 ± 0.24 mg Pb/kg, 0.020 ± 0.030 mg Cd/kg). These preliminary results suggest that the value of the CPB from the mineral nutrition standpoint changes according to the period of sampling, but that the surrounding environment does not seem affecting its safety. It is planned to extend the analysis to more elements in order to get insights about a more exhaustive mineral profile of the pollen gathered by the HB in the same apiary.

O191

A comparison of the nutritional quality and sensory properties of commercial milk with different labeled production systems

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In the current marketplace, different quality seals exist for commercial milk to support producers in (a) sustaining the additional costs related to the practice of unconventional and less intensive production systems and (b) endorsing consumers requirements and expectations toward superior milk quality, including inner (healthiness, taste) and external (sustainability, origin, production method, animal welfare) quality features. The aim of this research was to verify the nutritional and sensorial properties of commercial milk collected on the retail chain in the plain area of Lombardia (Italy). Four different types of retail milk were considered, including conventional 'High Quality' fresh milk (HQ), fresh milk labelled as 'Mountain Product' (MM), conventional milk with 'Extended Shelf Life' (ESL), and STG-certified Haymilk (HM). One sample for each type of whole milk was collected weekly during March, May, July, and September 2022, for a total of 80 samples (20 per type). Fatty acids (FAs) were quantified by gas chromatography. Milk sensory profile was assessed according to ISO 13299:2010 recommendation. Comparisons among groups were performed by means of parametric and non-parametric statistics. Results showed significant differences among the different labels for the content of many FAs that are biomarkers of origin and/or functional compounds. Particularly, HM was evidenced as the quality seal associated with the most favourable FAs profile, represented by the highest content of branched chain fatty acids (BCFA, 79.11 mg/100 mL), rumenic acid (RA, 35.83 mg/100 mL) and the lowest n-6/n-3 ratio (3.46), followed by MM (69.98 mg BCFA/100 mL milk; 16.92 mg RA/100 mL milk; n-6/n-3 ratio equal to 5.46) and the two conventional types of milk, HQ and ESL (52.71–59.23 mg BCFA/100 mL milk; 14.36–15.07 mg RA/100 mL milk; n-6/n3 ratio equal to 7.31–7.56) ($p < 0.001$). No significant association with the quality mark was evidenced for the sensory attributes, with the exception of aroma intensity, which showed a lower score for MM (5.03) if compared to HM, HQ, and ESL milk (6.32, 6.94, and 7.47, respectively) ($p < 0.001$). The outcomes obtained in this research provided useful knowledge for all the stakeholders involved in the milk supply chain, from producers to consumers, supporting the existence of a relationship between the claims on milk label and its nutritional quality and indicating a superior quality of commercial milk obtained by certified production systems.

O569

Effect of different doses of Vitamin E added to beef patties on the formation of polycyclic aromatic hydrocarbons

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The increasing concern of consumers regarding the impact of red meat on human health, has bring to the attention of the scientific community the study of polycyclic aromatic hydrocarbons (PAHs), toxicants generated in grilled meat undergoing high temperatures. Considered potential carcinogenic agents to humans, the European Union has established PAH4 (sum of benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene and benzo[*a*]pyrene) as the most appropriate indicator for the occurrence and carcinogenic potency of PAHs in food. In the light of this, the emerging issue of health risk from exposure to PAHs has leaded to mitigation strategies consisting in the use of antioxidants, such as vitamin E. This research work aimed to evaluate the effect of different added concentrations of DL-α-tocopheryl acetate (2, 3, 4.5 and 6 µg/g) in ground meat, on the formation of PAHs in grilled beef hamburgers. Raw meat samples presented a total lipid content of 1.36 g/100 g and an endogenous vitamin E content of 1.32 µg/g. Hamburgers of 80 g (two for each experimental groups) were directly cooked in disposable barbecue obtaining well-done level of doneness. An acetonitrile based-extraction and high-performance liquid chromatography with fluorescence detection were used to assay 14 PAHs. The PAHs concentrations were evaluated averaging three measurements for each sample meat. Data were analysed by one-way ANOVA. In relation to the total content of PAHs, no effect ($p > 0.05$) was observed in hamburgers added with 2 µg/g of vitamin E, while a marked reduction ($p \leq 0.0001$) was found in those added with 3 and 4.5 µg/g (59 and 46 ng/g respectively) compared to control samples (76 ng/g). A similar trend was observed for heavy and light PAHs: vitamin E at the lowest concentration did not reduce ($p > 0.05$) their formation, while their content decreased ($p \leq 0.05$) with the other vitamin E levels. Despite low values of PAH4 were observed in control samples, far from the maximum established by the European Union (30 ng/g), the addition of vitamin E reduced PAH4 ($p \leq 0.01$) in all experimental groups, especially ($p \leq 0.0001$) in those added with 3 and 4.5 µg/g. Although grilled hamburgers did not generate high PAHs amount, the effectiveness of vitamin E in inhibiting PAHs formation in grilled beef hamburger depends on the concentration used.

O406

Effect of the inclusion of Opuntia ficus-indica cladodes in the feeding of Cinisara cows on Caciocavallo Palermitano cheese produced in summer

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In Sicily, the cladodes of *Opuntia ficus-indica* are used as fresh supplementation for livestock in summer, season characterized by low fresh pasture availability. The effect of cladodes' integration on characteristics of milk and cheese from Cinisara cows was investigated. In June–July, 16 multiparous cows, that calved in the autumn-spring period, were subdivided into 2 homogeneous groups that, in a 2 × 2 Latin square design, with 2 phases, were fed different diets named control (CN) and cladode (CL). Group fed CN grazed natural pasture (standing hay) supplemented with 4 kg/day per cow of wheat bran, whereas group fed CL received the same diet of group CN supplemented with 15 kg/day per cow of fresh cladodes. In each phase, with bulk milk from each group, 2 cheesemaking was carried out, producing three forms of 'caciotte' for each cheesemaking. After salting, one 'caciotta' cheese was sampled and tasted, while the remaining 2 were vacuum maintained at 4 °C, and sampled and tasted after 15 and 30 days, respectively. Cladodes had low contents of crude protein (6.8% DM) and fiber (34.5%, 25.7%, and 3.0% DM for NDF, ADF, and ADL, respectively), high levels of α-linolenic acid (C18:3n-3, ALA) (19.1% FA) and polyphenols (16.7 mg GAE/kg DM), and an antioxidant capacity (TEAC) equal to 106.1 mmol TE/kg DM. In milk, the cladodes' integration reduced only the urea content (15.5 vs 18.5 mg/100 mL; $p = 0.008$), while in cheeses it influenced more parameters. In particular, products of group CL showed higher polyphenols (6.0 vs 4.7 mg GAE/kg DM; $p = 0.04$) and antioxidant capacity (50.7 vs 49.7 mmol TE/kg DM; $p = 0.04$). In relation to fatty acid (FA) profile, CL cheeses were higher in saturated FA (59.7 vs 57.7% FA; $p = 0.002$), except C18:0, and, among unsaturated FA, in vaccenic acid (C18:1t11) (0.44 vs 0.39% FA; $p < 0.0001$), rumenic acid (C18:2c9t11, CLA) (0.81 vs 0.78% FA; $p = 0.008$), and ALA (0.47 vs 0.33% FA; $p < 0.0001$). At sensory level cheeses from CL diet were more appreciated (5.7 vs 4.9; $p = 0.001$), showing greater color intensity (4.6 vs 4.1; $p = 0.013$), smell (6.0 vs 5.2; $p < 0.0001$) and buttery flavor (5.1 vs 4.5; $p = 0.001$). No difference due to storage time were observed. In summer the cladodes may represent a valid fresh by-product integration, contributing to increase the bioactive molecules in cheese as well as the liking of consumers.

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O26

Application of the Healthy Fatty Index to discriminate the meat nutritional quality of different slow-growing chickens

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Chicken is the first meat consumed worldwide, due to their higher nutritional quality, even if recent studies demonstrated that many differences exist in poultry meat quality due to the dietary strategies, rearing systems and genotypes. At this regard, many indexes, able to estimate and compare its nutritional characteristics, have been designed, mainly referred to lipid composition. The definition of 'healthy' meat regards both the lipid amount and lipid-compounds. The aim of the present research was to apply a novel quali/quantitavive Healthy Fatty Index (HFI) to possibly discriminate the meat nutritional quality of different slow-growing chickens reared outdoor to provide a useful evaluation of the best 'nutritional traits'. One hundred chicks/strain of both sexes were used: Italian crossbreed Robusta Maculata × Sassò (CB), Lohmann Dual (LD), Necked neck (NN) and Red J (RJ). Chickens were reared in pens (2 pens/strain) with an indoor (0.10 m^2/bird) and outdoor (4 m^2/bird) area. The animals fed *ad libitum* the same starter and grower diets. At 81 days of age, 15 chickens/pen were selected and slaughtered. The breast and thigh muscles were excised from the carcasses, sampled, and stored at −20 °C till fatty acids evaluations by GC-FID (mg/100 g). The total lipid content was also quantified by AOAC method. The HFI was determined as follow: $((\text{MUFA} \times 2) + (\text{n-6} \times 4) + (\text{n-3} \times 8) + (\text{n-3})/(\text{n-6}))/(\text{SFA} + (\text{MUFA} \times 0.5) + (\text{n-6} \times 0.25) + (\text{n-3} \times 0.125) + (\text{n-6}/\text{n-3}))$. The results showed big differences in fat content in both meat cuts, in particular RJ had the higher fat content of thigh (17.27% of f.m.) than others, and the lowest breast one (3.23%). LD exhibited the significant higher breast fat content (5.80% of f.m.) than other genotypes. Interesting results were obtained in thigh meat: HFI showed better values in LD chickens than others (10.21 vs 8.55, 8.45 and 8.00, respectively in CB, NN and RJ). In conclusion, the contemporary inclusion of fat content and the lipid profile of meat had a relevant impact in discriminating the nutritional quality of poultry meat from different genetic strains.

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O272

Development of a highly sensitivity analytical method for the determination of PAHs in BABY food (meat puree) by optimized QuEChERS extraction and determination by GC/MS-MS

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Polycyclic Aromatic Hydrocarbons (PAHs) are very important environmental pollutants which can contaminate several types of food. Their presence in specific foodstuffs can also be due to particular cooking practices (i.e. grilling). PAHs are mutagenic and carcinogenic, thus the European Union and the US Environmental Protection Agency (EPA) added these compounds in the priority list of pollutants, so that their presence in food must be steadily monitored. In Europe, the Regulation (EU) No. 835/2011 establishes maximum residue levels (MRLs) for PAHs in food products.
Due to their presence also at very low levels, the analytical determination of PAHs requires high method sensitivity and selectivity. The aim of this study was the optimization and validation of a novel analytical method for the detection and quantification of 4 PAHs (benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[a]pyrene) in baby food, based on GC-MS/MS. Different extraction/purification methods and several detection parameters were compared in order to optimize method sensitivity by means of GC-MS/MS.
The full dataset was evaluated by comparing data using Box Plot which is a very useful tool of graphic representation used for managing quantitative data and to optimize analytical methods. In this regard, the capability of comparing many distributions in the same graph, highlighting the most significant characteristics such as symmetry, range, variance and possible outliers can be considered as the main strength of such tool.
Validation parameters such as specificity, linearity, LODs and LOQs, precision, recovery and ruggedness were determined. From the tests carried out, it was found that the method is very sensitive, reaching concentrations up to 100 ng kg^{-1}. Indeed, the LODs and LOQs were in the range 58–108 ng kg^{-1} for chrysene and benzo[a]pyrene, respectively. The accuracy parameters were satisfactory, in accordance with Regulation N° 183/2011, in terms of both CV% (<6.5%) and recovery percentage (in the range 73–110%).
In conclusion, this method optimization allowed the development of a very sensitive and accurate analytical method for the detection of 4 PAHs in baby food which particularly need the assurance of chemical contaminants absence.

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O485

Effect of aS1 casein level on stretchability of goat curd

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Protein genetic variants of goat milk have been studied for more than 40 years and it is known that α_{s1}-CN presents a remarkable variability that is also connected to different level of synthesis. In fact, dairy goats can present a low, medium or high α_{s1}-CN content connected to more than 10 different genetic variants influencing the expression of this protein fraction. These genetic variants affects both the milk composition and the coagulation properties. In fact, fat globule size and the presence of the polar lipids varies based on α_{s1}-CN content; larger fat globule and low polar lipids are connected to high amount of this protein. Several studies reported a correlation between the milk coagulation properties and α_{s1}-CN content, with shorter coagulation time in the presence of low level but firmer curds in conjunction with high level. Recently, protocols for manufacturing high moisture mozzarella from goat milk have been developed, but no investigation has been performed on the stretching behaviour of goat milk in comparison with cow milk. The present work resumes the outcomes of a study carried out in Apulia and Basilicata Region, aimed at finding goat milks with different profiles of α_{s1}-CN and evaluating the stretching properties of the curd derived therefrom. Electrophoresis was used for casein characterization and Texture Profile Analysis (TPA) for evaluating the stretching properties. More than 30 milk samples were collected and three of them used for the cheesemaking trials al laboratory level. The results of TPA revealed significant differences in the stretching properties in connection with the α_{s1}-CN profile. This study shed light on the stretching properties of goat milk and could be useful for dairy manufacturers for standardizing the protocols for the production of goat pasta filata cheeses.

O548

Essential and toxic mineral content of colostrum and milk in dairy sheep

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The survey was developed to characterize mineral composition in colostrum, milk, and blood of Sarda dairy ewes, and to determine the mineral content of fed and soil. Samples were collected from a representative sample of animals from eight sheep dairy farms in the North-West of Sardinia (Italy) from the parturition to the end of lactation every forty-five days. Fat, proteins, seven among essential and toxic minerals, and total antioxidant capacity were measured in colostrum and milk samples. Minerals were also measured in blood. Data showed that the levels of almost minerals varied among farms. Higher levels of selenium (Se), zinc (Zn) and copper (Cu) were found in colostrum compared to milk. The seasonal evolution evidenced a peak of Se, Zn and Cu at the beginning of lactation and then decrease due to the reduction of supplementation with concentrates associated to the decrease of production level of animals. The content of manganese peaked during spring. The toxic minerals followed two different path, lead (Pb) peaked at the beginning of lactation and then decreased markedly instead cadmium followed the opposite trend; in all lactation phase the values were below the upper limit indicated by the EU in milk products. The nickel content in blood showed no defined trend during lactation. The mineral composition of dietary ingredients determined in different farms is the partial explanation of mineral concentration observed in milk and blood. The level of toxic element Pb is markedly lower than the established for soil Pb hazard level. This study provided new information on the mineral quality of milk in Sarda dairy sheep. Further studies are necessary to create the mineral map of soils in Sardinia, to identify rapid and cheapest methods for determining minerals in milk, and to study the role of dietary supplements in determining mineral concentrations in blood and milk.

O154

The inclusion of hemp (*Cannabis sativa* L.) cake in laying hen diets influences yolk fatty acid profile and sensory quality during the shelf life of eggs

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Consumer interest in healthier and eco-friendly foods is driving towards feeding strategies that can reduce the environmental impact of livestock production while ensuring high-quality products. We assessed the effects of including hemp oil extraction residual cakes in a laying hen diet on eggs' fatty acid (FA) profile and sensory quality over the shelf-life. The trial was performed at a farm raising Lohmann White hens under organic free-range system. One hundred 18-week-old hens (1.550 ± 120 g LW) were assigned to two treatments (2 pens of 25 birds/treatment), consisting of a commercial concentrate diet (Control), and 70% of the same concentrate integrated by 30% of hemp cake (Hemp). After 3 weeks from the start of the trial, the whole production was collected over 10 consecutive days and one-third were immediately analyzed (time 0), while the other two-thirds were stored at 4 °C for 14 and 28 d. For each time, FA profile of yolk (4 eggs/pen), sensory profile (30 eggs/diet), by quantitative descriptive analysis, and consumer liking of hard-boiled eggs (100 eggs/diet) were assessed. Data were analyzed by GLM ANOVA, with diet and storage time as the main effects. At time 0, no effect of diet was found for saturated FAs. Monounsaturated FAs were higher ($p < 0.05$) in Control eggs, particularly oleic FA (38.2 vs. 30.5%, for the Control and Hemp, respectively), while an opposite trend ($p > 0.001$) was observed for polyunsaturated FAs (PUFAs), mainly linolenic (19.5 vs. 24.3%), α-linolenic (0.8 vs. 1.5%) and docosahexaenoic (1.4 vs. 2.2%) FA. However, PUFA in Hemp eggs decreased ($p < 0.05$) at 28 days after laying to a value almost like that of the Control. Sensory quality was little affected by the diet, only pastiness was rated lower in Hemp eggs ($p < 0.01$) possibly related to the higher content of PUFA. For both diet, storage for 28 days reduced opacity ($p < 0.01$) and increased flavor ($p < 0.001$), while odor showed a different ($p < 0.05$) trend in the two diets, increasing in Hemp eggs and decreasing in Control eggs. The consumer panel perceived no difference between the diet except for texture, which tended to be more liked ($p < 0.10$) in Hemp eggs, suggesting that the difference in pastiness identified by the sensory panel was also perceived by consumers. Overall, hemp cake can improve the FA profile of eggs, in terms of PUFA and α-linolenic FA, without adverse effects on the sensory quality of eggs, but this nutritional quality tends to decline over the shelf-life.

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O229

Collagen and extracellular matrix composition of chicken breast meat affected by growth-related abnormalities

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The present study aimed at deepening the knowledge on the implications of the connective tissue components in the development of growth-related abnormalities affecting broilers *Pectoralis major* muscles (PM) with special reference to the wooden breast (WB) and spaghetti meat (SM) conditions. In fact, although with antithetical features, alterations in the connective tissue are among the distinctive traits of these abnormalities which are respectively characterized by an extensive accumulation (up to fibrosis) (WB) and a progressive rarefaction (SM) of the interstitial connective tissue composing the perimysial septa. For this purpose, 3 h *post-mortem* 15 PM were selected from a homogenous batch and grouped according to their phenotype as unaffected (NORM), or severe WB or SM cases (5/group). Total glycosaminoglycans (GAGs) were quantified along with the proportion of soluble and insoluble collagen. Then, collagen type III, selected due to its association with muscle regeneration, was isolated and its amino acid composition assessed by HPLC method. Data were analysed by one-way ANOVA considering the phenotype (NORM, WB, SM) as main effect and, when significant, means were separated by Tukey-HSD test. All statistical differences were considered significant at a level of $p \leq 0.05$. Overall, the findings obtained in this study evidenced that the occurrence of growth-related abnormalities remarkably affects the connective tissue components within the PM. In fact, if compared to NORM and SM, a 3-fold increase ($p < 0.001$) in the concentration of GAGs was found in WB thus suggesting an increased deposition of extracellular matrix. In addition, significant differences ($p < 0.01$) were observed for both insoluble and soluble collagen content among the groups. In detail, the last was found to be significantly higher ($p < 0.01$) in WB in comparison with NORM (0.73 *vs.* 0.58%) whereas SM exhibited intermediate values (0.65%). Remarkable differences were also observed between WB and SM in most of the amino acids composing collagen type III. In detail, among the others, if compared with SM, a significantly ($p < 0.01$) higher amount of glycine and proline was found in WB along with a concurrent increase in the concentration of hydroxyproline (+63%) and hydroxylysine (2-fold) whereas NORM exhibited intermediate values. In conclusion, these findings seem to suggest a key role of the extracellular matrix composition in differentiating WB and SM conditions.

O299

The role of Comisana and Massese breeds on cheese-making efficiency and daily cheese production

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Little is known about the complex cheesemaking process from dairy ewes due to difficulties in milk sampling and in producing high number of model cheeses at individual level. Though, individual information is fundamental to characterize local populations, guarantee breeds conservation and increase farmers' profit. The objectives of this study were to: (i) investigate the variability of cheesemaking traits of sheep milk, (ii) assess the effects of season, stage of lactation and parity, and (iii) compare two breeds (Comisana and Massese) for their cheesemaking ability. The individual milk of 720 ewes from the breeding center of the Italian Sheep and Goat Breeders Association was sampled once between November and April. Milk composition (fat, protein, casein, lactose, total solids) was determined by using an infrared analyzer; cheese yield (%CY) traits and the recovery of nutrients in the curd (%REC) were measured by using the 9-mL milk cheesemaking assessment (9-MilCA) procedure. The following traits were determined for each ewe: three %CY (fresh cheese, cheese solids, and cheese water), and four %REC traits (fat, protein, total solids, and energy); two theoretical %CY traits (*Th*-%CY; fresh cheese and cheese solids) and the related cheesemaking efficiencies; three daily cheese yield (dCY) in fresh cheese, cheese solids, and water retained in the curd. Among the factors affecting the variability of cheesemaking traits, the breed was the most important. Comisana ewes had greater values of %CY traits, recovery of solids and energy, and dCY traits compared to Massese. Days in milk were also significant, showing higher values of almost all the cheesemaking traits towards the end of the lactation, except for the recovery of fat and protein. Parity affected the dCY traits, with the lowest productions for the primiparous vs pluriparous ewes. In contrast, cheesemaking efficiency traits were the worst in the secondiparous. The interaction between breed and parity showed opposite trends among the two breeds for %CY in solids and the recovery of this component in the curd, while season affected only %CY in solids. These results underlined the importance of the animal-related factors in the cheese-making process and that the collection of cheese-making traits at individual animal level is fundamental to characterize local populations, and to provide innovative tools for the conservation of the biodiversity and the sustainability of farming systems in marginal areas.

O292

Effect of a Sulla-based diet on Massese sheeps' milk and cheese

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Sulla (*Hedysarum coronarium*) is a biennial culture belonging to Fabaceae family, which originates from the western Mediterranean area. Sulla is an interesting culture for its adaptability (for example it grows in clay-rich soil, even if it cannot withstand too cold temperatures) and its chemical-nutritional characteristics. In the last period the condensed tannins content of Sulla has aroused great interest for their antioxidant properties. On the other hand, large amount of condensed tannins in the diet of animals can have some anti-nutritional effects. The ability of fresh Sulla in affecting positively the production of milk is well recognized. However, pedo-climatic conditions of the Mediterranean area require studies on its conservation; two possible conservation techniques are hay harvest or dehydration.

This experiment is the first part of a study that compares the use of fresh Sulla and preserved Sulla in the diet (dehydrated or hay). Results of this study concern the comparison between a fresh Sulla based diet and a clover-based diet. The aim of this study was to verify potential differences in animal products, in particular milk and cheese.

The study was carried out in a farm of Pisa Province (Italy) on 12 multiparous Massese ewes. Animals were randomly allotted in six boxes fed two different diets (**S**, Sulla and **C**, clover) in a partial 3 × 2 latin square design. Each experimental period lasted two weeks; one for the adaptation and one for the experimental period.

A part of milk sampled at the end of each experimental period was used to produce cheese. To evaluate the effect of aging, the cheeses obtained were aged two different times, 20 and 40 days. Proximate and fatty acids composition was analyzed on all samples.

Results showed that diet significantly affected fatty acids composition and in particular the content of rumenic acid (C18:2 *c*9*t*11, RA) and alpha-linolenic acid (C18:3 n-3, ALA), being RA higher in **C**, and ALA in **S.**

RA is produced during the bacterial lipid bio-hydrogenation and in tissues thanks to the stearoil CoA desaturase which converts vaccenic acid (C18:1t11, VA) in turn produced in the rumen bio-hydrogenation. Conversely, ALA is one of the most bio-hydrogenated fatty acid which gives VA.

Thus, our results could be attributed to the influence of polyphenols of Sulla on microbial communities of the rumen.

O57

Effect of bactofugation on milk and Pecorino cheese quality

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The presence of clostridial spores in ewe's milk could represent an emerging problem due to several factors related to climate and herd management changes. The proteolytic or lipolytic activity of these bacteria are often responsible for reducing the shelf life of dairy products, so the reduction of milk microbiological contamination is essential. Thus, the use of methods to reduce the number of bacterial endospore-forming agents in raw milk can be used to extend the shelf life of pasteurized milk and reduce the technological problems of dairy products.

Bactofugation (BF) is a method based on the use of a high-speed centrifuge designed to remove bacterial spores from milk at high temperatures. Many studies have evaluated the use of this technology only to reduce bacterial spores and have reported its efficiency in reducing gram-negative bacteria, mainly for cheese production. On the contrary, little is known about the effects of BF on the quality of dairy products, especially in ewe's milk. So, the aim of this work was to verify the effect of BF of raw milk contamination and its quality. Moreover, the effect of this technological application on the cheesemaking properties was verified. A total of 380 raw milk stored in individual bulk tanks was considered from 2018 to 2022. The bactofuge had a centrifugation speed of 10,000 × *g* with a continuous flow, which ranged between 7000 and 12,000 L/h. The efficiency of reducing level of bacterial spores was evaluated in relation to the starting bacterial load level and applied flow rate. About the cheesemaking process, the chemical-nutritional and organoleptic quality of cheeses produced with bactofuged and no-bactofuged milk was also evaluated. The BF treatment reduced the spore level by 80%. This reduction is significantly influenced ($p < 0.01$) by the level of bacterial load. High levels of contamination were associated with higher reduction efficiency. With spore levels below 1500 MPN/L the efficiency is 77%, compared to 83% in milk with more than 4500 MPN/L. Conversely, no significant differences were observed in relation to the extent of treatment. BF did not lead to significant changes in the composition of the milk, except for the reduction in the level of somatic cells (−10 times). In conclusion, BF improved the quality of the milk from a bacteriological point of view, without compromising the qualitative dairy properties during the production process.

O124

Characterization of dry-cured ham microbiome by 16S rRNA profiling during seasoning

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Dry-cured ham is one of the most important Protected Denomination of Origin (PDO) products in Italy and is characterized by a long duration of ripening. There are few studies that describe the microbiome of Italian dry-cured hams, despite the fact that the microbiome of hams undergoes significant changes as they ripen. The current study aimed to examine the microbiome of 40 hams after a year of curing. The hams were produced from pigs that were fed a variety of diets high in crude protein. Near the femoral artery, where microbial changes may mainly take place, samples weighing between 4 and 6 g were taken from each ham. DNA was purified using a magnetic bead based DNA purification kit and an automated platform (Biosprint-96, Qiagen). Thermo Fisher Scientific 16S Ion Metagenomics Kit protocol was used for library preparation. This kit involves the amplification, in two separate PCR reactions, of the V2, V4, V8, V3, V6–7, and V9 regions. Sequencing was carried out in an Ion GeneStudio S5 apparatus using the Ion 520 chip kit (Thermo Fisher Scientific). From the 40 hams, 5502 amplified sequence variants were found, which corresponded to 34 phyla, 90 classes, 185 orders, and 257 families. From the analysis of relative abundances, we identified the three most prevalent phyla in the dataset under study as Firmicutes, Actinobacteria, and Proteobacteria. The Firmicutes phylum, which was particularly represented by the genus *Tetragenococcus*, was found to be the most prevalent. The two halophilic species *Tetragenococcus koreensis* (35%) and *Tetragenococcus halophilus* (8%), which are typically observed in fermented foods like cheese and sausages, were the most frequently seen in our samples. Only 0.5% of all bacteria belonged to the *Burkholderia* genus (Betaproteobacteria) which encompasses also species that could have pathogenic traits for humans and animals. These findings show the possibility to track potential pathogens during maturation as well as the low overall variability of the ham microbiome, which resulted typically composed by salt-resistant species.

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O457

Genetic parameters of growth, feed efficiency and greenhouse gases emissions in Italian Holstein young bulls

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Livestock farming, especially in the dairy industry, accounts for about 50% of GHG of the primary sector. Genetic selection can contribute to reduce its environmental impact. Such selection can be applied directly using breath measurements, but also using indirect indicator traits related to feed efficiency. Purpose of this study is to estimate genetic parameters to verify the feasibility for direct or indirect selection for GHG reduction in Italian Holstein population. The animals involved were 218 young Italian Holstein (genotyped) bulls, candidates to artificial insemination in Italy. Methane and carbon dioxide emissions were collected using the GreenFeed system (C-Lock Inc., Rapid City, SD, USA); feed intake and feed behaviour data were collected using the Roughage Intake Control system (RIC, Hokofarm Group, Marknesse, The Netherlands); biometric measures were collected by qualified and trained technicians. Growth traits showed the largest estimates of heritability, close to 0.40, while RIC derived traits ranged from 0.167 to 0.306 and emission traits ranged from 0.241 to 0.480. Genetic correlations between growth, RIC and GreenFeed traits were moderate to strong, ranging from 0.60 to 0.99. Results suggest that selection indexes can be estimated in order to reduce GHG emissions without compromising growth, body condition, height and feed intake.

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O32

Environmental footprint of heavy pig production system

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This study assessed the environmental footprint of the heavy pig production system in Northern Italy, through a Life Cycle Assessment cradle-to-farm gate model, in order to identify practices along the production chain (breeding, post-weaning and fattening phases) possibly associated with the environmental footprint of herds. Data originated from 18 farms (8 breeding farms – 1052 ± 576 sows, 30.1 ± 3.8 weaned piglets/sow per year – and 10 fattening farms – 3522 ± 1996 places, 170 ± 8 kg body weight (BW) at the sale with 181 ± 13 days/production cycle). For each farm, data on animal and manure management, feedstuff production, and use of energy sources were collected as an annual average. The functional unit was 1 kg BW sold. Global warming (GWP), acidification (AP) and eutrophication (EP) potentials and land occupation (LO) were considered as impact categories. Partition of impact between sows and piglets was based on an economic allocation method. Impact values per production phase were analyzed with linear models to test the effects of farm management (farm size, feed self-sufficiency), ration composition (protein and phosphorus content) and animal responses (weaned piglets/sow/year, replacement rate, average daily gain, feed conversion ratio) variables. The production of 1 kg BW (cradle-to-farm gate) was associated with the emissions of 2.94 ± 0.36 kg CO_2-eq (GWP), 55.6 ± 5.6 g SO_2-eq (AP) 22.3 ± 3.0 g PO_4-eq (EP) and the occupation of 3.49 ± 0.84 m^2/y (LO) on average. Manure management and feed production significantly contributed to all the impact categories and for all the production phases. Farm management traits tenderly affected only the fattening phase: increasing farm size was associated with increasing GWP and increasing self-sufficiency with lower AP and EP. The dietary composition had a very low influence on the impact categories. Increasing productivity (weaned piglet/sow and year) was associated with lower GWP values in the breeding phase. Productivity gains (average daily gain and feed conversion ratio) seemed to mitigate all the impact categories in the post-weaning phase but not in the fattening one. In conclusion, the farm systems investigated showed good performance and low variability in terms of impact values. Differences in management practices among farms were limited and slightly associated with differences in emissions. Therefore, new mitigation practices for lowering the environmental footprint of such production systems need to be explored.

O358

In vitro evaluation of sodium nitrate as a rumen methane reducer

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Nitrate can be used in ruminant rations as an electron (H^+) acceptor to reduce methane (CH_4) production and as a non-protein nitrogen source. However, this additive has a possible toxic effect (e.g. methemoglobinemia) and for this reason, has to be added at minimum safety dosages in diets. *In vitro* tests can be used to safely investigate a wide range of dosages before *in vivo* applications. Therefore, the work aims to study the efficacy of progressive inclusion levels of sodium nitrate ($NaNO_3$) as an anti-methanogenic additive. The *in vitro* fermentation system is composed of eight 500 mL bottles equipped with an infrared gas analyzer for continuous CH_4 assessment. Each bottle was filled with buffered rumen fluid (ratio 1:2) and 3.3 g of a dry substrate and subsequently was hermetically closed and immersed in a water bath at 39 °C for 48 h. Four $NaNO_3$ dosages to supply a final concentration of 2.5, 5.0, 7.5, and 10% of NO_3 on a dry matter basis were tested during four fermentation runs. For each treatment, a corresponding control with comparable levels of sodium and nitrogen was achieved by the addition of urea and sodium chloride.

NO_3 addition has reduced CH_4 yield in a dose-dependent response. Compared to controls, the 10% and 7.5% inclusion levels demonstrated a significant impact up to 48 h of fermentation (149 *vs* 276 mL and 217 *vs* 273 mL, $p < 0.01$). The inclusion level of 5% showed a significant decrement in CH_4 production (195 *vs* 222 mL, $p < 0.05$) until 24 h while the lowest dosage demonstrate a significant effect only in the first 12 h (158 *vs* 173 mL, $p < 0.05$). Considering the total gas, the high inclusion levels (10 and 7.5%) showed a significant reduction to relative control (1118 *vs* 1381 mL and 1264 *vs* 1387 mL respectively, $p < 0.01$) while the lower levels of additive (2.5 and 5%) did not affect the total production after 48 h of incubation. The pH, ammonia, and protozoa counts in the fermentation fluid showed no difference between treatments at the end of fermentation.

A linear regression study between CH_4 production and progressive $NaNO_3$ dosages at different time points allowed to predict a CH_4 decrement of around 5% after 6 h of fermentation for the addition of 1% of NaNO3.

In conclusion, the study demonstrated that NO_3 included at a level considered safe (e.g. around 1% of DM) significantly reduced CH_4 yield without affecting fermentation patterns.

O527

Suckling lamb as net-zero product in Sardinian dairy sheep farms

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The aim of this study was to estimate the net carbon footprint (Net-CFP) of suckling lamb production considering farm's soil carbon (C) sequestration. Six semi-intensive (SI) and six semi-extensive (SE) dairy sheep farms located in Sardinia region were surveyed. Information about flock size, inputs used, purchased feeds, diesel and electricity uses of the last 5 years (from 2016 to 2021) were collected. Data were analyzed by the Tiers 1 and 2 IPCC (2019) equations in a *cradle to farm gate* perspective. The emissions considered were: enteric methane (CH_4), CH_4 and nitrous oxide (N_2O) from manure management, carbon dioxide (CO_2) from diesel and electricity uses, and CO_2 from purchased and on-farm feeds. Greenhouse gas emissions (GHG) were expressed in CO_2 equivalent units to account for global warming potential of each gas, assuming a 100-year time horizon. The GHG emissions related to suckling lamb production were obtained by an economic allocation approach. Net-CFP was obtained as the difference between total GHG emissions and total soil C sequestration potential and dividing them for the functional unit, defined as 1 kilogram of live weight sold (LWS). On average, SI and SE farms had 504 ± 240 (mean ± SD) and 1386 ± 280 heads, respectively. The total area was 50 ± 28 ha in SI and 135 ± 38 ha in SE farms. The number of suckling lambs sold per year were 335 ± 164 and 1129 ± 213 for SI and SE farms, respectively. The CFP was 7.96 and 7.68 kg CO_2eq per kg of LWS in SI and SE farms, respectively. The impact was primarily affected by enteric CH_4, accounting for 53% in SI and 57% in SE farms, respectively. When soil C sequestration potential was considered, a Net-CFP of zero emissions per kg of LWS was observed in all 12 farms. In conclusion, the inclusion of soil C sequestration balanced the emissions achieving the C neutrality. The C credits accumulated over the suckling lamb production could be allocated to the other productions to cover, even partially, the emissions associated to milk and wool.

O234

Effect of THI on milk composition and fatty acid profile of Sarda dairy sheep

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The increase of global temperature is one of the main effects of the environmental impact related to the anthropogenic activities, and it represents an important challenge for the animal productions. THI, an index combining ambient temperature and relative humidity, is used as indicator of heat stress on animals. Animals' health and performance are negatively correlated with the increase of THI, however the mechanisms involved are still unclear. The aim of this study was to evaluate the effect of THI on milk composition and fatty acid profile (FA) of Sarda dairy sheep. For this purpose, 865 individual milk samples from 47 farms were analyzed with Fourier transform mid-infrared spectroscopy for milk components, and with gascromatograph for FA profile. Data were analyzed with a mixed linear model that included days in milk (DIM) classes, parity, lambing month (LM), THI classes and the interaction DIM × THI classes as fixed effects, with farm as random effect. Four THI classes were created according to the quartiles of the distribution of this parameter, with the first quartile representing the lowest values. Almost all the considered parameters were affected by DIM and THI ($p < 0.05$). DIM and THI influenced fat concentration, while protein concentration varied, increasing, only among the DIM. Lactose was influenced by the interaction DIMxTHI: the lowest values of lactose were found at the highest THI values within each DIM class. Long-chain FA were affected by THI and increased with the raise of the THI; short-chain FA were influenced both by DIM and THI, and they decreased with the increase of the THI, according to the reduction of the synthesis of ex novo FA during heat stress. C18:0 was affected only by THI and the highest values were found with high values of THI. Unsaturated FA, such as C18:1 cis9, C18:1 trans11, C18:3 n3 and CLA cis9, trans11, were influenced by the interaction DIMxTHI. Within the same DIM, C18:1 cis9 kept the highest values with the highest THI, whereas C18:1 trans11, C18:3 n3 and CLA cis9,trans11 achieved the highest values with the lowest THI. The results of this study evidenced that milk quality is influenced by interaction between DIM and THI. Some important FAs, whose concentration is associated with high milk quality, are affected by THI and, for this reason, a good management of the animals during the hot season and the selection of resilient animals could contribute to increase the milk quality.

O307

Methane emission pattern in dairy cows evaluated in automatic milking system

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Enteric methane is mainly generated in ruminants during enteric fermentation to remove CO_2 and H_2 from the rumen. With the development and implementation, in the last few years, of new smart sensors for accurate CH_4 emissions prediction in precision

livestock farming, new scenarios are opening up. However, it is well known that these emissions are subject to significant variability, in particular, due to the individual production, the diet, the hour of sampling, and the cows' activity. Aim of this experiment was to study the enteric methane emissions during the automatic milking by a portable laser detector in dairy cows. 40 Italian Holstein dairy cows were enrolled for the trial (lact n° 2.07 ± 1.04, DIM 133 ± 65), they were tied in five groups of eight cows each and completely adapted to the tie stall area (one week). The DMI was monitored and TMR diet was kept constant. Cows were milked a specific time point 6 am (Morning), 2 pm (afternoon) and 10 pm (night). As the milking group was only 8 cows the maximum waiting period before the milking was 50 min. Methane was measured for each cow during the last 3 milkings before group change. The laser was pointed at the cow's muzzle during the whole milking and while the cow was eating the supplementary concentrate. Data was collected in ppm/m and transformed in grams/day. Statistical analysis was performed with a mixed model. Results shown a methane production of 94.21 ± 26.60 ppm/m (44.66 ± 7.32 gr) at 6 am, 110.89 ± 42.77 ppm/m (49.24 ± 11.76 gr) at 2 pm, and 9.82 ± 44.36 ppm/m (48.95 ± 12.20 gr) at 10 pm, ($p = 0.24$). A significant effect of the cow was found ($p = 0.05$). Average methane predicted was 314.92 ppm/m (142.85 gr/day), a lower value than what was expected (over 300 gr/day). This could be related to the time of measurement and the cow's activity (milking and eating the supplementary feed). To our knowledge no data are present in cows during these activities. It is also important to highlight that the inter-cows variability was greater than the inter-milkings/hour variability. We could suppose that cows during the milking time have quite constant methane production, with less interference related to fermentation pattern and/or rumination activity. We can conclude that the milking time could be used as a reference time point to measure methane emissions through portable devices. Future perspectives could evaluate the methane production during the milking in cows exposed to feed additives developed to reduce methane emissions.

O293

Predicting enteric methane emission of dairy sheep using milk fatty acid profiles

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The quantification of methane emissions by livestock plays a pivotal role in setting mitigation strategies in a scenario of climate change. Being the direct measurement of this phenotype expensive and difficult, several equations have been developed for its prediction. Milk fatty acids (FA) profile represents one promising proxy for the prediction of enteric methane (CH_4) in dairy species. To date and according to our best knowledge, no equations are available in literature for dairy sheep. The aim of this work was to estimate and evaluate the methane emission of dairy sheep using prediction equations originally developed for dairy cattle. Data of milk FA profile of 993 Sarda dairy ewes were used. The methane emission was estimated in terms of methane yield (MY, CH_4 g/kg DMI) and methane intensity (MI, CH_4 g/kg FPCM) using 9 equations developed for bovine milk: 6 for MY and 3 for MI. The correlations among the two estimated CH_4 values and milk composition and FA were analyzed. Mean values of MY obtained from the considered equations were very similar among them, ranging from 19.4 to 20.4 g/kg of DMI. Those of MI ranged from 15.1 to 21.0 g/kg of FPCM. Means values of MY and MI, obtained by the means of all considered equations per each animal, were 19.9 and 18.7, respectively, in agreement to previous reports on direct measurements in sheep. The value of MY corresponds to 9–10 kg of methane emitted per ewe per year, in agreement with the IPCC and other previous estimates. The methane emissions were positively correlated with milk fat and negatively with milk yield, and lactose. The methane emission was negatively correlated with short-chain FA with almost all the trans isomers of C18:1 and C18:2 (including isomers of conjugated linoleic acid, CLA), arising from the biohydrogenation of polyunsaturated FA (PUFA), and with several PUFA belonging to the n-3 family. Positive correlations were found with medium and long-chain saturated FA, with several odd and branched-chain FA, monounsaturated (cis isomers) FA, and some PUFAn-6. In conclusion, prediction equations for bovine can be advantageously used also in sheep. The pattern of correlations confirms the metabolic pathways shared by the enteric methane and milk FA and highlights a desired positive correlation between reduction of methane emission and increase of milk quality, in terms of beneficial FA concentration.

O243

Carbon footprint of intensive vs. extensive dairy farming in Gargano area

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Carbon footprint is an indicator of environmental sustainability quantifying the greenhouse gas (GHG) emissions generated by the individual or collective activities.

Two dairy farms located in Foggia province (CaseStudy1, CS1; CaseStudy2, CS2) both raising Mediterranean buffaloes (B) and crossbred Italian Friesian cows (C) with different management systems (intensive vs. extensive) were studied to investigate the impacts of milk production. Data about herd characteristics (live weights, mortality, fertility, production), diets composition, and manure management of dairy buffaloes ($n = 136$ vs. $n = 78$, CS1B and CS2B respectively) and dairy cows ($n = 44$ vs. $n = 31$, CS1C and CS2C respectively) were collected to evaluate the GHG emissions on a year basis according to FAO assessment model (GLEAM-i ver 1.9, https://www.fao.org/gleam/resources/en/).
Considering the global warming potential (GWP) of buffalo farms, the more extensive system in CS2B was found less impactful than the intensive CS1B, showing –33.7% of total GHG emissions (expressed as kg CO_2-eq/year) and –50.8% of total CH_4 (expressed as kg CH_4/year). Both feed intake (kg DM/year) and milk production (kg/year) were lower in CS2B (–48.3% and –52.7 % respectively), so that it resulted more impactful (+42.5%) when milk emission intensity (kg CO_2-eq/kg protein) was considered. The total N_2O emission (kg N_2O/year) was +56.7% in CS2B due to the different manure management.
Regarding the dairy cow farms, CS2C was found less impactful in terms of both total GHG and CH_4 (–21.1% and –37.5% respectively) emissions. Feed intake and milk production were lower in CS2C (–32.4% and –36.9% respectively), resulting the milk emission intensity higher (+25.8%) than in CS1C. Consistently with emissions in buffalo farms, the total N_2O emission was higher (+56.6%) in the extensive system.
Data processing suggested that GHG emissions were closely related to feed production practices, diet composition and manure management in the different farms. On this regard, formulating diets that match the nutritional animal requirements, improving feed digestibility and availability, introducing alternative protein source or by-product, and implementing manure and animal waste processing, could allow a more sustainable use of natural resources, limiting the potential negative environmental impacts while preserving farm remunerability.

O141

GIS model application for the assessment of nitrogen emissions from cattle

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In Tuscany, cattle farming has over 85 thousand heads at the end of 2020 and is part of a context of economic, environmental, and social sustainability. However, it is also involving emissions of climate-altering gases such as methane from enteric fermentation and nitrogen oxides, as well as nitrate leaching, which comes from manure management. Therefore, a Tier 2 method was applied to model nitrogen emissions within the Tuscan territory to estimate the environmental impact of cattle farming. The method used IPCC equations and georeferenced data adopting GIS software. The methodology started with the collection of the provincial data (National Livestock Register database) about the size, category, and age of the Tuscan bovine population. Subsequently, the energy and protein requirements in the ration for both, the dairy and beef category, were estimated by using CNCPS equations. Holstein and Limousine were the reference breeds for dairy and beef models, respectively. Basic assumptions were made in adopting coefficients recommended by the IPCC to estimate nitrogen losses of different manure management systems resulting from: (i) volatilization of ammonia and nitrogen oxides caused by fermentation during manure storage management; (ii) direct emission of nitrous oxide; (iii) nitrogen leaching during the storage of livestock waste. The results obtained, as an annual estimate of nitrogen losses, were georeferenced by province. The basic coefficients or assumptions of the model are estimated through the tables proposed for the Tier 2 method or the bibliographic search of variables for the characterization of a reference animal. The differences between the Tuscan provinces are considerable for all the parameters considered. For instance, N_2O emissions ranged from over 40% in the province of Grosseto to just over 0.1% in the province of Prato. This huge difference is proportional to the number of animals raised, mainly for meat, and to the surface area of the territory covered. The aspect related to nitrogen leaching is considered only as N that leaches into the soil or runs off during manure storage at feedlots or outdoor areas. The part of N leached at ground level as a result of field distribution is not covered by the model but managed by the nitrogen balance at the farm or territorial level. In conclusion, we note important differences within the Tuscan territory, to be evaluated through diversified sustainability indicators based on the variables considered.

O477

Predicted methane emission: a new breeding value for the Italian Holstein

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Livestock farming is indirectly linked to GHG emissions, mainly due to enteric fermentation. The livestock sector indirectly contributes to GHG emissions through activities related to feed production, manure spreading and storage, nitrogenous fertilizers, fossil fuels consumption and deforestation. Methane and carbon dioxide from cattle emissions have been shown to be heritable, providing the basis for applying genetic selection for their reduction. A national breeding program can provide a relevant contribution to reduce greenhouse gas emissions. This study aims to set up a methane emission (CH4) breeding value through an indirect way starting from literature formulas. It is possible to derive traits thanks to the availability of data from the national milk recording system. For this preliminary study individual CH_4 has been derived using literature algorithm expressed as ratio of CH_4 and Energy Corrected Milk. The derived trait on average is equal to 0.76 ± 0.17 MJ/kg/d. A test-day repeatability model was applied. Fixed effects were the herd-test-date (HTD), the interaction parity*stage of lactation and the interaction age at calving*parity. Random effects included cow with records (permanent environment effect), animal and error. Parameter estimation has been estimated on a subset of the Italian Holstein population randomly extracted; procedure was repeated 3 times. Editing resulted in cows with lactation stage between 5 and 365 days in milk, with at least 2 test-day records and belonging to HTD classes with a minimum of 3 contemporary animals. Sires were required to have at least 5 daughters in 3 herds. Final data-set was 632,840 repeated records from 39,574 cows and 1434 sires. Pedigree (76,268 animals) included individuals with records and their ancestors up to 6 generations back. Heritability and repeatability were 0.11 and 0.29, respectively. Genetic parameters were applied to the entire test-day data-set in order to estimate breeding values for the pCH4 index. The new breeding value will be standardized with mean 100 and SD 5, as all functional ANAFIBJ indexes. This work is performed in order to give a tool to encourage farmers to start a selection for better environmental mitigation; the availability of direct phenotypes would be more effective; however, these data collection is not cost and labor free. To overcome these limitations, international effort is exchanging experience in order to build up critical mass GHG data measured in dairy cows.

O209

Implementation of water footprint assessment methodology on buffalo mozzarella cheese

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The Water Footprint (WF) is a multi-dimensional indicator of the amount of direct and indirect freshwater used to make a product available. The WF comprises three water fractions (green, blue, and grey) specified geographically and temporally. This study presents the preliminary findings of a cradle-to-factory gate WF of Italian buffalo mozzarella cheese.

The farm production input data were collected directly from 10 dairy buffalo farms selected to represent the medium to large-size central-southern Italy farms. Data related to buffalo mozzarella cheese production were collected on-site from two cheese factories receiving the raw milk produced by the investigated farms. The accounting methodology was based on a Life Cycle Assessment (LCA) approach in compliance with the principles established by the Water Footprint Network method for water assessment. This study has also involved using a biogeochemical process-based model (DNDC) to account for the green, blue and grey WF components from on-farms soil cultivation. The reference unit involved in the study was 1 kg of buffalo mozzarella cheese evaluated at the cheese factory gate.

The average annual WF estimated, considering the farms and cheese factories input data, resulted in about 7000 L water per kg of buffalo mozzarella cheese with the following distribution among the green, blue and grey fractions: 78%, 8%, and 14%, respectively. The green WF was the most significant contributor, especially for the share attributable to the extra farm feed bought by the farmers, which was the prominent hot spot. Also within the blue fraction, the extra farm feed was the main water-demanding driver, followed by the on-farm crop irrigation, animal drinking water and, to a lesser extent, the cleaning of barns and milking parlours, and the sprayed water used by the cooling system. The grey component of the WF was almost equally shared between the on-farm and extra-farm feed production. The cheese factory had a marginal role in the overall mozzarella WF.

Data regarding WF buffalo milk products are still scarce. However, considering the rise of the buffalo population in Italy, especially in the southern regions, the results from WF studies could support the improvement of environmental policies and support farmers' decision-making about enhancing water efficiency.

O496

Net carbon footprint of Sardinia beef cow-calf system

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In Sardinia, cattle are raised mainly in agroforestry-pasture systems where herbaceous and arboreal species are simultaneously grazed. These systems could capture and store significant quantities of carbon (C) in biomass and soil contributing to decrease the environmental impact of beef production. However, tree and soil C sequestration potentials are not included in the majority of carbon footprint (CFP) studies. Since livestock farms are part of the agriculture sector that emits, but also absorbs CO_2, the overall impact of a livestock supply chain process is given by total balance of emissions and removals made by plants and farm soil. Thus, the aim of this study was to estimate the Net-CFP of a sample of 10 beef farms located in Sardinia. The selected farms adopted the cow-calf grazing system with an open cycle to produce 6–8 months old calves for fattening. Purebred and crossbred Sardo-Bruno, Sardo-Modicana, Angus, Blu-Belga, Charolaise and Limousine were reared mainly on pasture. Data were collected by interviewing the farmer for a complete life cycle inventory (LCI) of cradle-to-gate farm production processes. The LCI included information of the last 5 years, from 2016 to 2021. System boundaries included: enteric CH_4, CH_4 and N_2O from manure, CO_2 from energy and fuel consumptions, CO_2 from purchased and on-farm feeds emissions. Greenhouse gas emissions (GHG) were expressed in CO_2 equivalent units to account for global warming potential of each gas, assuming a 100-year time horizon. Net-CFP was obtained as the difference between total GHG emissions and total tree and soil C sequestration potential and dividing them by the functional unit, defined as 1 kilogram of live weight sold (LW). The 10 farms had on average 38.0 ± 25.1 ha (mean ± SD) of natural pasture, 45.7 ± 41.1 ha of Meriagos (agroforestry pasture system like Dehesa E7.3 biotope), and 28.5 ± 34.5 ha of annual grasses on 113.5 ± 53.0 ha of total area. On average, farms had 41 ± 22 cows with 33 ± 19 calves sold per year at 308 ± 80 kg of LW and 9 months of age. Total emissions were mostly related to the enteric CH_4 and on-farm feed emissions. The inclusion of C sequestration lowered the emission intensity achieving the C neutrality. In conclusion, C sequestration potential compensate emissions of extensive beef production systems. The C credits accumulated in the cow-calf phase could partially or totally cover emissions of the fattening phase, usually carried out in farms where C sequestrations are limited.

O337

Product Environmental Footprint (PEF) as evaluation tool of environmental sustainability in beef cattle farms

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Beef production plays a pivotal role in the Italian livestock industry. Still, it contributes to environmental impacts, along with traffic, domestic heating, industry, and energy production sectors, especially in Northeast Italy, where air pollution is very high because of the region's climate specificity and geographical position. The Product Environmental Footprint is a life cycle assessment (LCA) based method to quantify the environmental impact of products suggested by the European Commission. The LCA provides data from which we can separately review the contribution of all impacts in different production stages, this method is standardized and allows comparations among studies and production systems. This study was financed by the Veneto Region through the Rural Development Program 2014–2020, within LOWeMEAT project, which was aimed to evaluate different environmental impacts of the Italian beef meat production chain. We estimated 16 different environmental impact categories of the current production system starting from data from 8 beef farms used as 'reference farming systems' for the Northeast Italy beef sector. Those farms reared 400–2800 cattle, mostly Charolais and Limousine purebreds imported from France. The functional unit was 1 kg of beef live weight at the farm gate. The crops' production phase, specifically the fertilizers' use, and manure management, significantly contribute to most impact indicators. The fattening phase is mainly affected by diet composition and intake. Moreover, we estimated the contribution of management interventions taken by farmers aiming at improving farms' environmental performance and concluded that interventions such as precise fertilization of crops and the adoption of automatic feeding systems did not have a major contribution to decreasing climate change impact, but they affect mainly the use of fossil and mineral resources. The combination of precise fertilization, robotic feeding, and manure digesters can decrease climate change impact by 20%. Using a biogas digester significantly reduced fossil fuel impact since farms contribute to energy production. PEF is a promising tool for calculating farms' environmental impact and giving us important data to consider the benefits of different interventions, but still needs improvements in the characterization of some impacts e.g. renewable energy sources. The widespread PEF uses will allow us to compare the environmental impacts of EU production systems.

O542

Modelation of the effect of heat stress on milk yield and milk composition in purebred and crossbred cows

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Environmental factors have a direct impact on milk production and other traits, however, there is a lack of knowledge on modeling of the effect of heat stress and the effect of genetics of cow.

Therefore, the objective of this study was to analyze the impact of heat stress (using a temperature-humidity index (THI)) on milk production (kg/d), fat (%) and protein (%), considering the effects of breed, herd, and parity. We used 55,412 test days records of Holstein Friesian, Brown Swiss, Simmental and Crossbreed cows, belonging to 106 herds located in 5 different provinces of the Veneto region in Italy. We fitted linear models to assess the fixed effects. Then, sinusoidal models were fitted to link the production traits with THI using records of temperature (°C) and relative humidity (RH%) of the different provinces. The parameters of the sinusoidal models were estimated using non-linear squares functions with R and they were classified by months and days in milk (DIM). All the fixed effects of the production models were significant as well as the parameters of the sinusoidal model ($p < 0.001$). Milk production presented a phase of increase between the months of January to March and a decrease between June to November with an amplitude of 1.7 kg/d, an average of 35 kg/d, when compared with the THI model. So, the trough of production is delayed respect to the crest of THI. For fat and protein % the delay is much shorter and the amplitude was 0.22% and 0.11% and the mean 3.67% and 3.31%, respectively. In conclusion, the THI and milk yield and milk composition can be modeled and compared through sinusoidal curves. The next step will be to adapt the sinusoidal model to different breeds and herds to study heat stress resistance and resilience.

O79

Alpine herbs affect rumen in vitro degradability and methane emissions

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Grazing ruminants intake variable amounts of medicinal plants when fed on mountain pasture. These plants contain secondary metabolites (tannins, phenols, essential oils, saponins) that have shown an inhibitory effect on rumen methanogenesis. Few studies were conducted to evaluate the effect of Alpine herbs on rumen fermentations, and their potential use as feed to mitigate rumen methane emissions. This study aimed to evaluate the effect of 6 Alpine herbs on rumen fermentations and methane (CH_4) emissions.

Six Alpine herbs (*Alchemilla vulgaris* L., *Sanguisorba officinalis* L., *Tanacetum vulgare* L., *Cicerbita alpina* L. Wallr., *Galium odoratum* L. Scop., *Sisymbrium officinale* L. Scop.) and a grass hay (control) were incubated using an *in vitro* automated system in 3 consecutive runs, in 3 replicates and using as inoculum the rumen fluid of 3 dairy cows, for a total of 189 batch fermentations and 27 blanks. The kinetic of gas production (GP) was measured and, after 24 h, samples of liquid and of gas were collected from each bottle. A mixed model was implemented in R to evaluate the fixed effects of 7 treatments on dry matter degradability (DMd), volatile fatty acid (VFA), GP at 24 h (mL/g DM) and gas composition (H_2, CO_2 and CH_4). CH_4 was expressed as proportion of gas produced (% v/v), per g of DM incubated (mL/g DM) and per g of DMd, and was also computed from VFA profile. Orthogonal contrasts ($p < 0.05$) were built for all the traits investigated, comparing the effect of each Alpine herb to the control test.

The Alpine herbs showed a lower DMd than the grass hay ($p < 0.001$) and produced a lower amount of VFA ($p < 0.001$) and of GP ($p < 0.001$) but, when data were expressed as g of DMd, no differences were observed among herbs and grass hay. All the tested herbs changed the VFA profile increasing the acetic acid proportions and reducing those of propionic and butyric acid ($p < 0.001$), increasing the CH_4 emission computed from VFA. Some herbs slightly reduced ($p < 0.01$) the proportions (% v/v) and the amounts (mL/g DM) of CH_4 emitted compared to grass hay. However, when data were expressed per g of DMd, no differences were observed between substrates.

In conclusion, the tested Alpine herbs showed a lower degradability than the grass hay and it was related to the higher fibre and lignin content. These herbs also showed a lower emission of CH_4 than predicted from the VFA profile suggesting a potential role of plant secondary metabolites in the modulation of fermentations.

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O361

Study of methane emission in the mediterranean buffalo through the use of laser methane detector and its correlation with microbial population from different matrices whitin the agridigit project

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The laser methane detector (LMD) is a tool for methane (CH_4) remote sensing. Different uses are possible and one regards livestock emissions measurement. CH_4 measure is got by pointing the laser beam toward the source. Average CH_4 density between detector and target is displayed in ppm*m.

The aim of this study was to test that technology for qualitative assessment of CH_4 emission in buffalo breath. Former results, on a sample subset, showed high variability among animals. That preliminary analysis also showed some repeatability in terms of peaks' numerosity and intensity, letting suppose this approach as appropriate to identify low emitting animals. Moreover, we aimed to test the correlation between faecal and saliva microbial community and LMD data. Ruminants produce CH_4 in the rumen and a previous study highlighted a correlation between rumen bacteria and CH_4 emission, measured by LMD. One minute laser scans were performed at milking on 40 multiparous lactating buffaloes, (first third of lactation), at nostril, twice per day, 15 days/month during the cold/mild, January to April (20 animals; 82.4 ± 32.59 Days in Milk; parity 3.27 ± 1.15) and hot, June to September (20 animals; 52.46 ± 29.51 Days in Milk; parity 3.21 ± 1.32), season. Besides, once a month, saliva and faeces were sampled and a 5-min continuous laser scan was performed, on animals captured in a crush. Results, based on a wide data set (328,990 measurements) confirmed the high variability of CH_4 emission among animals, with an approximatively normal distribution, that would allow to select for this trait. As for the main environmental factors likely influencing this phenotype, the stronger effects were days in milk, parity, milking entry order, milking session (morning/afternoon) and mixer wagon operator. Surprisingly, neither the season nor the rumination activity showed significant effect on emissions at milking, but season influenced the microbial population. Eventually, there was no correlation between LMD measure and faecal and saliva microbiological analysis. In conclusion, the instrumental measure of CH_4 emission seems viable for selecting low emitter animals. Among the environmental effects it is not clear whether season impacts on emission. Further in deep studies are requested for the identification of suitable proxies of enteric gas production.

O460

Development of a screening method for GHG and NH3 measurements – first results based on a European study

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The effects of cattle farming systems and individual management practices on GHG emissions have been studied by applying a screening method on eight European countries totalizing 60 dairy farms and four seasonal measurements.

This method is based on indoor and outdoor CO_2, CH_4, and N_2O concentration measurements and on a questionnaire developed to estimate the carbon mass balance at the building scale. The results of the questionnaire were also used to characterize the farm diversity.

The dairy population in the barn where the air samplings were done ranged from 8 to 979 cows. The feeding quantity was between 14 and 31 kg DM/animal/day and the milk production between 18 and 50 kg milk/animal/day. Most of them had concrete or slatted floors (40% and 35% respectively) and one fourth were separated urine and feces. Minimum difference to deduce emission estimates were 9 mg m^{-3} for CO_2 and 0 mg m^{-3} for CH_4, NH_3 and N_2O. We also considered a minimum threshold of 5 for the ratio $(C-CO_{2,\ in}-C-CO_{2,\ out}) \cdot (C-CH_{4,\ in} - C-CH_{4,\ out})^{-1}$ because of enteric emissions in dairy houses. Almost 90% of observations were above these thresholds except for N_2O.

This international survey conducted with the same measurement method helped identifying the main factors influencing GHG emissions as well as the existing low-emitting systems: the calculated emissions based on concentration measurements showed that CH_4 emissions from manure could double those from the enteric fermentation of the animals; the detection level of the N_2O emissions was below 1 g N-N_2O/animal/day; in some farms, the emission results showed very few seasonal variations over the year while, for other ones, seasonal variations were well observed; the results showed well detectable GHG emission reductions related to changes in farm practices.

O214

Cradle-to-grave life cycle assessment of buffalo mozzarella cheese

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This study presents the preliminary results of a Life Cycle Assessment (LCA) of buffalo Mozzarella cheese produced in centre Italy in the district of Amaseno (FR). The LCA was performed by a cradle-to-grave approach and the functional unit was 1 kg of buffalo Mozzarella cheese evaluated at consumers' homes. Therefore, the life cycle analysis included processes from farm activities to end-of-life disposal of wasted mozzarella and packaging.

Data on the processes included in the life cycle were collected from: ten buffalo dairy farms for the primary production, two cheese factories producing mozzarella cheese from raw milk of the investigated farms, four mozzarella retails and 150 costumers to characterise mozzarella consumer habits.

The CH_4, N_2O and NH_3 emissions from housing, animals and manure storage were first estimated using a farm-model tool developed by our research group. Regarding soil management, the GHG emissions, ammonia volatilization, nitrate and phosphate leaching were estimated using a biogeochemical process-based model (DNDC). Estimated and collected data were finally modelled by SimaPro 9.1 and the environmental impacts considered were Global Warming Potential (GWP, kg CO_2 eq), Acidification Potential (AC, kg SO_2 eq), and Eutrophication Potential (EU, kg PO_4^{3-}eq). The GWP emissions per kg of mozzarella consumed were 12.5 kg CO_2 eq. The farm activities were the primary environmental GHG hotspot accounting about 90%, mainly driven by enteric fermentation and manure management, instead consumer behaviours (3.5%) and cheese factory activities (3%) had lower contribution. The AC was 0.138 kg SO_2 eq., the 97.5% was related to milk production, the 1.7% was associated to dairy and remain to consumption stage. The EU was 0.041 kg PO_4^{3-}eq., the 98% derived from buffalo milk production, the 1.5% from cheese making processes and the remain from consumer habits.

The LCA provided the environmental burdens of buffalo mozzarella cheese from cradle to grave. The identification of hot spot may support the development of strategies aimed to mitigate environmental impacts and improve sustainability of buffalo Mozzarella cheese.

O197

Reconstruction of functional features of fecal microbiome in dogs fed different diets

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The prediction of functional abundances of microbial taxa based on 16S rRNA bar code was investigated with PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) on 348 fecal samples of medium and large size dogs (86 males, 145 females and 117 castrates of both sexes), aged from 1 to 10 years. The dataset, collected from 8 in-house dietary intervention studies in healthy dogs, was used to assess the effect of sex or 3 diets (D1: 171 dogs fed kibble diet; D2: 87 dogs fed semi moist diet; D3: 90 dogs fed home-made diet or a complement diet with raw meat, BASE). In the study we investigated the variability of enzymatic functions of gut microbiome in relation to diet and sex. We aimed to understand how the bacteria that inhabits the gut of healthy subjects are interconnected from a functional point of view and if enterotypes can be identified in dogs. PICRUSt2 has been employed to impute MetaCyc Enzyme Consortium (EC) pathway abundances from the original microbial abundance data. We performed Principal Component Analysis (PCA) on the pathway abundances, inferring the factors diet and sex. The significantly enriched pathways highlighted that subjects were clustering apart based on these factors. In particular, we observed that dogs fed home-made diet and BASE diets were overlapping and when dogs belonging to these two diets were considered together as a one group (D3), the clustering of the subjects in the 3 dietary groups (D1, D2, F3) was more defined. The same result was obtained analyzing the dogs for the factor sex (male, female and castrated). Looking at the patterns, we recognize the most 25 significative correlated KO terms regarding the diet and sex. About half of the pathways were highly correlated with industrially type of diet, such as D1 (kibble) and D2 (semi moist), whilst the other half were strongly correlated with a D3 (home-made and BASE diets). Our analysis and interpretation of results provides a preliminary evaluation of the effects of diet and sex on the functionality of the gut microbiome reconstructed with PICRUSt2 in healthy dogs. These data are promising but deserve further investigations using a larger dataset of healthy subjects, to consider confounding effects as the breed, age and size.

O11

Blood and clinical parameters of obese and lean cats, and the effects of a caloric restriction

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Obesity is the most common nutritional disease in cats and it is generally managed by caloric restriction (CR) to promote weight loss (WL). The aim of this study was to compare blood parameters of obese (OB $n = 12$; body condition score (BCS) ≥ 7/9) and lean cats (CTRL $n = 12$; BCS =4–5/9) and evaluate the effect on the same parameters of CR in OB cats. OB and CTRL cats were fed an extruded diet (CP 50%, EE 9.4%, CF 5.7% on DM basis) with no CR for 30 days (T1). At T1, CR (40 kcal ME/kg body weight (BW)) based on ideal weight (IW), was applied only to OB cats for 90 days (T2) to promote WL. The BW and BCS were assessed every week, and energy adjustments were made accordingly. Blood samples were collected at T1 in OB and CTRL and at T2 in OB to assess cholesterol (CH), triglycerides (TRG), acute-phase proteins (haptoglobin, HP; serum amyloid A, SAA), insulin and IGF-1 concentrations. To determine differences in BW and blood parameters between groups, and before and after CR in OB, parametric or non-parametric tests were used as appropriate. Differences were considered significant when $p < 0.05$. CR resulted in WL for all OB, however none reached the IW. Median (range) BW and BCS were 6 (4.9–10.3) kg and 8/9 (7/9–9/9) at T1, and 5 (4.7–9.2; $p < 0.01$) kg and 7.5/9 (6/9–9/9) at T2, respectively. Overall, an average WL of 9.2 (4.1–14.3) % of the starting BW was recorded during WL period. TRG were higher in OB vs CTRL, but within the reference range [87 (59–407) vs 57 (29–159) mg/dl, respectively; $p < 0.01$]; insulin tended to be higher in OB vs CTRL [38 (17.2–110) vs 21 (4.2–68) UIU/mL, respectively; $p = 0.060$]. On the contrary, SAA was lower in OB vs CTRL [2.0 (1–5) vs 8.5 (1–15) µg/mL, respectively; $p < 0.05$], while CH, HP and IGF-1 did not differ. The TRG significantly decreased in OB after CR [T1: 87 (59–407) vs T2: 61 (38–164) mg/dl; $p < 0.05$], while insulin, IGF-1, SAA, HP and CH remained unchanged. Dyslipidemia is common in obese cats, but it seems to be reversible after a few months of CR. SAA is known to be positively correlated with obesity in humans; however, in cats, it seems to have a great individual variability and it was previously reported to remain unchanged after a WL period. The present results confirm that obesity can result in higher serum insulin in cats and that a few months of CR is effective in achieving a safe and successful WL. However, metabolic and inflammatory status of obese cats needs to be further investigated.

O12

Effects of oral supplementation with coconut oil on fecal metabolome of healthy dogs

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Based on studies on digestion and absorption of fats, medium-chain triglycerides (MCT; from C6:0 to C12:0) can be rapidly absorbed through enterocytes by passing the lymphatics. Therefore, MCT appear as an interesting energy source to improve the absorption of fats, fat-soluble vitamins, and essential fatty acids, which might be impaired in common canine gastrointestinal disorders, such as chronic enteropathies (CE). Virgin coconut oil (VCO) is the main vegetable fat rich in MCT, and it has shown antimicrobial properties when supplemented to the diet of piglets. To date, effects on intestinal microbiota induced by VCO have not been tested in healthy dogs. This study aimed to evaluate the effects of oral supplementation with VCO on fecal metabolome and fatty acids excretion in healthy dogs. Twelve healthy adult dogs, privately owned, were fed a commercial dry diet (CP 23.5%; EE 17.0%; CF 1.71%; ash 6.18%, as fed) for 30 d and later received, for further 30 d, the same diet supplemented with VCO at 10% of ME. Fecal samples were collected at the end of each treatment and analyzed for moisture, pH, bacterial metabolites, and fatty acids. Data were tested for normality and analyzed through the Wilcoxon matched-pairs signed rank test or the paired *t*-test. Differences were considered statistically significant for $p < 0.05$.

The owners did not report food refusal problems when VCO was added, while other studies reported a worsening in palatability when MCT were added at 22–25% of ME. Supplementation with VCO led to an increase in fecal ammonia (+53%; $p = 0.012$) and C12:0 (+535%; $p = 0.009$) excretion, while C14:0 tended to be higher (+59%; $p = 0.056$). C18:3n6 tended to decrease (−18.3%; $p = 0.052$), while total medium-chain fatty acids (MCFA) were higher (+109%; $p = 0.018$) in dogs receiving VCO. Fecal moisture, fecal fat content, pH, and volatile fatty acids were not affected by VCO. Coconut oil induced changes in fecal fatty acids excretion but poorly modulated the fecal bacterial metabolome. Data from this study showed that MCFA are not completely absorbed by the intestinal mucosa of healthy dogs and are in agreement with our previous study in which MCFA absorption in CE dogs was impaired. Further investigations are needed to understand the impact of oral supplementation with VCO on canine fecal microbiota and metabolome.

O286

Selection signatures in Italian hunting dogs

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Dogs were first domesticated to assist humans in the hunt. As hunting techniques evolved, so did dogs, which became more and more specialized in different activities. Hound dogs (HD) were the first to be selectively bred to pursue the prey and bring it down, working in packs but without any human assistance. Scent hounds track the quarry using their keen sense of smell and come in a variety of sizes, according to the prey they follow and the terrain they have to deal with, whereas sighthounds mainly rely on their eyes and then chase the prey thanks to their impressive speed. Gun dogs (GD), instead, include pointing, flushing, and retrieving dogs, all of which work in partnership with hunters using rifles or shotguns.

The present study aims to investigate the genomic differences between Italian GD and HD. 60 GD (Bracco Italiano, Lagotto Romagnolo, and Spinone Italiano) and 49 HD (Segugio Italiano a pelo forte and raso, and Cirneco dell'Etna) were genotyped with 230K SNPChip. After quality control, exclusion of relatives, and sample size reduction (max 12/breed), a total of 36 GD, 36 HD, and 120836 SNPs were retained. Multidimensional scaling (MDS) and admixture analyses were performed. GD and HD were compared using Wright's fixation index (F_{ST}) and single-SNP cross-population extended haplotype homozygosity (XPEHH).

The MDS plot showed a clear separation between the two groups and the single breeds, with the exception of the overlapping of the two Segugio Italiano. Similar results were found by the admixture analysis.

The SNPs in the top 1% of the distributions of both F_{ST} (>0.33) and XPEHH (>2.79) were selected and mapped on CanFam3.1, leading to the detection of 32 genes. Among these, *RSPO2* and *USH2A* are known to be responsible, respectively, for the presence of furnishing and the ticked and roan coat patterns in dogs, which are traits mainly present in GD breeds included in this study. Other 7 genes are related to dog domestication and behavioural traits, mainly aggressivity. Moreover, we also identified genes associated with hearing, vision, and sense of smell, which might have been differently selected in GD and HD for specific hunting activities.

To the best of our knowledge, this is the first study investigating the genomic differences between Italian HD and GD breeds. The genes we identified are involved in pathways related to morphological and functional phenotypes contributing to making these dogs specialized in the work for which they were selected.

O490

Influence of grain-free and cereal-based diets on postprandial glycaemic response

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Recently, pet-food companies have been marketing several products such as grain-free diet to meet specific nutritional requirement. The study aimed to evaluate the administration effect of two commercial kibble diets (grain free GF vs. cereal-based CB) on postprandial glycemic and insulin response in healthy dogs. Fifteen adult neutered healthy dogs (mean age 5.00 ± 1.30 years; body weight 21.1 ± 5.36 kg; and BCS 4.20 ± 0.86 on 5 points scale) were homogeneously divided into two groups, alternatively fed the two diets for 5 weeks. The diets were formulated with chicken as main protein source and different carbohydrate sources. The metabolizable energy (3990 vs. 3991 kcal/kg a.f.) and protein (31.6 vs. 31.7 % a.f) of GF and CB diets were similar. Blood samples were collected (~10 mL) at recruitment and at the end of each nutritional phase. To measure dogs' post-prandial glycemic and insulinemic responses, blood samples were collected fasting (baseline sample, time 0) and 120, 180, 240, and 360 min after the meal administration.

Regarding the biochemical profile, when the dogs fed GF diet reported higher ($p < 0.05$) level of ALT than CB. On the contrary, CB diet showed higher ($p < 0.01$) level of fructosamine compared to GF. Similarly, the cereal-based diet had the highest ($p < 0.05$) levels of lipase. Concerning the glycemic response, no significant effect of the diet was observed, with the exception of mean glucose concentration, which resulted higher ($p < 0.01$) when dogs fed CB diet compared to GF diet (89.3 vs. 82.8 mg/dl). Otherwise, several differences were reported for insulin response. Indeed, cereal-based diet had higher ($p < 0.01$) level of insulin mean concentration (11.9 vs. 9.44 mIU/l) and area under the curve (3897 vs. 3674). On the contrary, when dogs fed GF diet showed higher ($p < 0.01$) insulin peak (18.1 vs 13.1 mIU/l), which need more than 1 h to be reached compared to CB diet. Additionally, this last resulted in lower ($p < 0.01$) minimum point.

The high presence of protein of animal origin and different starch sources in GF diet can modulate the insulin response. Diets based on different grains (CB) is rapidly absorbed into the intestine. Nevertheless, cereal-based diet is rich in dietary fibre positively affecting intestinal fermentation. This could have implications in populations of performance or working dogs as well as therapeutic impacts on the management of conditions such as diabetes mellitus.

O301

English and American Golden retrievers differentiation: from sperm morphometry to genomic data

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The identification of genomic and semen-related differences among dog breeds or varieties is pivotal for the improvement of the reproductive management of livestock species. Given their peculiar population structure, dogs represent an excellent animal model for this purpose. In particular, the Golden Retriever has to date only one British standard officially recognised by the FCI, but there is also another morphotype, commonly called ‘American', which presents differences in coat colour and body size and structure. Recent evidence on differences in sperm quality parameters in relation to the colour variants of the Arctic fox prompted us to investigate the possible differences in terms of semen morphology and genomics in Canis Familiaris. 20 Golden Retrievers, 10 English aged 5.4 ± 3.5 and 10 American aged between 2.8 ± 2.2 were enrolled for this study. Semen was collected by manual stimulation after 3 days of abstinence, the second fraction of the ejaculate were used for quantitative and qualitative analysis. Sperm counts were performed with the Makler Counting Chamber. 100 spermatozoa, each ejaculate, were observed in bright field under a Nikon Eclipse 80i microscope (100×), captured with a digital camera (Nikon DS-Ri1) and analysed with the software SCA® CASA System and Nis Elements Imaging Software 4.00.02 (Nikon, Tokyo, Japan) for the measurement of head area, perimeter, max and minimum feret. After appropriate quality control, 230K SNPchip data were used to assess the genomic differences between American and English dogs through population structure and selection signature (FST, XP-EHH, and ROH) analyses. Fitting semen morphologic parameters with a mixed model, differences between the varieties were found for minimum feret, perimeter, and roughness, with higher values in English dogs. American and English Golden retrievers were well distinguishable also from a genomic perspective. Eleven genes were identified as the most differentiating both by FST and XP-EHH analyses. Moreover, the two groups presented very different homozygosity scores for 109 genes. Some of these genes play an important role in spermatocyte development and functionality, possibly accounting for the observed differences in sperm morphology. In addition, several of them have been previously related to dog behaviour, size, and coat colour.

O150

Resistance versus susceptibility to Leishmania: genetic differences in a population of English Setter dogs

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Leishmaniosis is a parasitic disease caused by kinetoplastid protozoa belonging to the genus *Leishmania*, which is transmitted to vertebrates via infected female phlebotomine sand flies. The main causative agent of canine leishmaniasis in Europe is *L. infantum*, which is highly diffused in the Mediterranean area and causes a spectrum of clinical signs that varies greatly from asymptomatic/mild to a very severe disease in dogs. The natural susceptibility or resistance to *Leishmania* infection is the result of complex interactions between the parasite and the host immune system. These interactions are due to the differences among individuals, and their genetic asset. Various genetic mutations in different breeds have been suggested as potential genetic markers to be used in the study of *L. infantum* infection resistance/susceptibility. Aim of this study was to evaluate the genotype of 4 SNPs (CBD1 gene), described as associated with resistance (rs852670798 and rs853079810) or susceptibility (rs850814192 and rs851268228) to *L. infantum* infection, in a cohort of 12 English Setter dogs, raised sharing the same habitat (private hunting dogs kept in outdoor kennel) and same repellent treatment for leishmaniasis vector (phlebotomine sand flies), but with different responses to this infection. The dogs in this study were 10 males and 2 females, age ranging between 1 to 13 years (mean age ~6 years). Four age classes (with three dog each) were defined and identified as I to IV: age ≤2 years old, 2 < age ≤4 years old, 4 < age <8 years old, and age ≥8 years old. Of these dogs, sampled during spring 2021, 8 animals were positive to the ELISA test for *L. infantum* infection, while 4 animals were negative. A preliminary analysis of the obtained data showed no correlation between the different haplotypes found in the cohort and the ELISA test result (*p*-value =0.49). Nevertheless, as suspected, a quadratic association between the age of the animal and the ELISA test result was found (R^2 of the model =35.2%, *p*-value =0.05). A follow-up on the cohort will be performed and the animals will be re-evaluated in the following infectious seasons to separate the effect of the age from the genetic one. Furthermore, the samples were genotyped using a high density SNP chip and we are testing different SNP-based approaches to investigate other possible genetic effects on resistance/susceptibility of *L. infantum* infection.

O245

Use of lactic acid bacteria in forage production of Alto Sannio area

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The aim of this study was to validate biotechnological innovations capable of enhancing the relationship between forages used in dairy cattle farming and sustainability and biodiversity of Alto Sannio Area (BN). For this purpose, a microbial culture appropriately isolated and selected from the natural bio-reserves Area was used in hay making. Honeybee foragers (*Apis mellifera ligustica* S.), as indices of environmental quality, were used for the isolation of lactic acid bacteria. The isolated strains were identified through multiple culture-dependent approaches, consisting of Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE), 16S rRNA gene sequencing and Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR), and selected by biochemical assays (APILAB system and API ZYM) for their enzymatic characteristics and their attitude in making hay. *Lactiplanctibacillus plantarum* (LPUM1), selected for the ability to metabolize a wide range of sugars and for its contribution to the enrichment in useful compounds with functional properties, was validated through its direct use (inoculation to reach 10^6 CFU/g) on three hectares of sun-dried meadow biomass composed of *Hedysarum coronarium* L. (40%), *Lolium perenne* L. (30%) and *Trifolium* spp. L. (30%). The biomass was then collected in round bales (350 kg), stored for four months. Hay with no microbiological inoculum was used as control. Samples ($n = 20$), collected from inoculated and no inoculated bales, underwent microbiological and chemical analyses.

The use of *L. plantarum*, characterized by a ready growth in hay, showed charge levels about 3 logarithmic cycles higher than those detected in control hay. Moreover, control hay was characterized by the presence of both fungi and clostridia being significantly higher (by 3 and 4 logarithmic cycles, respectively) than those found in the inoculated hay. Chemical components highlight that the inoculated hay was characterised by dry matter (DM) 82.5 (±0.62, SD) g/100 g, ash 11.3 (±0.51) g/100 g DM, NDF 51.0 (±1.72) g/100 g DM, ADF 40.9 (±0.59) g/100 g DM.

Although the results are not conclusive, the efficacy of strains isolated from honeybee foragers and used in hay making could be considered a valuable strategy for introducing healthy elements from the environment into the dairy chain strengthening the Alto Sannio identity.

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O583

Heavy lamb production as a way to differentiate the dairy sheep livestock system

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The optimization of meat production could be a way to improve the sheep farm's economic performance. The aim of this work, carried out with 'Agnello di Sardegna PGI' and 'Distretto rurale della Barbagia' farmer organizations, was to meet the needs of sheep farmers to have practical information on the production systems of other types of lamb, besides the traditional Sardinian suckling lamb, by exploiting the grazing resources. A study evaluated the costs of production of pasture-fed heavy lambs, weaned at different age. 64 lambs (Ile de France sires X Sarda ewes), born on 03/03/2022 ± 11 days (means ± s.d.) were divided into two groups, homogeneous for sex, based on weaning age: early weaning group, EW; weaned at 39 ± 6 days old and 14.3 ± 2.6 kg body weight (BW), and late weaning group, LW, weaned at 48 ± 10 days old and 15.7 ± 4.1 kg BW. Each group grazed a sown pasture and was supplemented daily with 150 g/head of concentrate and 250 g/head of alfa-alfa hay. Group intake supplement was measured daily by weighing the offer and the orts. During the trial animals were weighted weekly and average daily gains (ADG) were calculated. At 81 ± 8 days of age lambs were slaughtered; pre and post-slaughtered weight were recorded and carcass yields (CY) were calculated. An evaluation of the costs incurred for the two groups was made. The labor, feeding and farm general management costs were considered. Meanwhile the costs of a stall-fed based system were estimated, assuming equal performance. The ADG and CY data were analyzed by lme procedure of R. The ADG and carcass weight did not differ between groups (0.220 ± 0.001 and 0.221 ± 0.001 kg/day and 11.1 ± 0.13 and 11.5 ± 0.11 kg, emmeans ± S.E., for EW and LW group, respectively). The LW group showed higher CY (50.6 ± 0.61 and 52.3 ± 0.51 %, p-value =0.059). The total costs (as group) were 3.44 and 3.45 euro/kg BW for EW and LW group, respectively. The results provide a knowledge base for sheep farmers wishing to undertake these kinds of production. Grazing is important to increase meat quality as well as to reduce cost production, taking into account the total costs in a stall-fed based system were estimated to be 3.67 and 3.63 euro/kg BW, for EW and LW group, respectively. In view of the recently published costs of the traditional Sarda suckling lamb (5.21 euro/kg BW), the production system studied seems to be a good way to differentiate the dairy sheep livestock system.

O433

Effect of pomegranate extract on post-thawed sperm quality

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Sperm cryopreservation is a powerful tool for livestock breeding programs. However, it is well-recognized that cryopreservation causes sublethal damages to sperm cells reducing their functional life span. In buffalo, sperm cells are more susceptible to hazards during freezing and thawing than in cattle. There is strong evidence that antioxidants enhance sperm quality parameters maintaining the motility and the genetic integrity of sperm cells. Pomegranate contains polyphenolic compounds which have

antioxidant properties. Recent findings showed that pomegranate improved sperm quality parameters after freezing in cattle, goat, rats, and rooster. Therefore, the aim of this study was to evaluate whether supplementation of different concentration of pomegranate extract (PE; CRA-ACM) could improve the post-thaw sperm quality in buffalo. Frozen-thawed sperm from 4 different Mediterranean buffalo bulls were thawed at 37 °C for 40 seconds, incubated in Talp medium with 0, 0.1, 1 and 10 µg/mL PE for 2 h at 5% CO_2 in humidified air. After thawing, sperm kinetic characteristics were evaluated with a Sperm Class Analyzer (SCA) system while sperm viability and membrane integrity were assessed respectively by Trypan Blue/Giemsa and HOS test. The incubation with 1 µg/mL PE increased sperm membrane integrity (35.5 ± 1.7, 40.3 ± 3.5, 45.7 ± 2.7 and 34.5 ± 2.1%, respectively with 0, 0.1, 1 and 10 µg/mL PE; $p < 0.05$). However, PE did not affect sperm progressive motility (21.6 ± 0.2, 18.6 ± 0.2, 20.8 ± 0.1 and 20.9 ± 0.5%, respectively with 0, 0.1, 1 and 10 µg/mL PE) and viability (71.3 ± 1.8, 67.8 ± 2.2 64.3 ± 1.4 and 65.3 ± 1%, respectively with 0, 0.5, 1 and 10 µg/mL PE. Interestingly, sperm kinetic characteristics such as VAP, STR and LIN were higher with 1 µg/mL PE (VAP: 24.9 ± 1.9, 27.5 ± 1.5, 31.5 ± 1.1 and 27.9 ± 0.9 µm/s; STR: 34.3 ± 1.5, 41.4 ± 2.8; 47.6 ± 1.9 and 36.5 ± 1.3%; LIN: 53.0 ± 1.7, 59.1 ± 2.3, 61.1 ± 2.9 and 59.3 ± 1.4 % respectively with 0, 0.1, 1 and 10 µg/mL PE; $p < 0.05$). In conclusion, these preliminary results indicated that the incubation with 1 µg/mL PE affected the quality of buffalo frozen-thawed sperm improving sperm membrane integrity and sperm kinetic characteristics. However, further investigations on fertilizing ability are required to draw definite conclusions.

O584

A transgenerational study on the effect of birth month of female ancestors on lactation curves of Italian Simmental cattle

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Heat stress (HS), exerts a direct negative effect on the welfare, the productive and reproductive performances of dairy cattle. The occurrence of HS during pregnancy of a cow could also affect future generations. Previous works carried have highlighted an effect of calving month of granddam and great grand-dam on the EBV for performances traits of their granddaughters (GDA) and great granddaughters (GGDA). This effect has been interpreted as an indication of transgenerational epigenetic inheritance. In the present work, the effects of calving months of three generations of female ancestors on lactation curves of Italian Simmental cattle were investigated. Data were 455,684 test day (TD) records for milk, fat and protein yields, and SCS of 77,903 lactations of Italian Simmental cattle provided by the 'Associazione Nazionale Allevatori Razza Pezzata Rossa Italiana'. For each cow, data from the dam (D), granddam (GD), and great granddam (GGD) were available. Data were analyzed with a mixed linear model. that included the fixed effects of cow, D, and GD birth months, dim class (23 levels of 15 d each starting from parturition), year of production and parity; Herd Test Day and individual lactation were treated as random. Birth months of cows and GD affected significantly milk yield TD records Lowest milk TD LS means both for cow and GD birth months were observed for February, March, and May. These effects were observed also on the whole lactation curves. Birth months can be regarded as indicators of the effect of the pregnancy period. Early embryonic life is a crucial period for the mammalian development and stress factors may negatively affect the pregnant cow and future generations. In particular, if the period of conception of cows is considered, lowest LSmeans for milk TD records have been observed for conceptions that have occurred June, July, and August, i.e. in the hotter months. Results of the present work confirm the negative effect of stress during pregnancy on productive performances on offspring. In particular, the negative effect of the conception month of the producing cow highlights the negative direct effect of HS experienced during the first month of foetal life on future performances of an individual. The negative effect of the GD month of conception support to hypothesize a transgenerational inheritance of epigenetic marks that have been caused by the occurrence of HS during great grand-dam pregnancy in their great grand daughters.

O441

Use of natural antioxidants in poultry diet balanced for antioxidant capacity

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Plant feed additives (PFA) has been widely investigated as natural antioxidants alternatives to vitamin E (VitE) in poultry diets at variable doses, without testing their antioxidant capacity. This led to inconsistent and sometimes misleading results. This study aimed at testing the possibility to balance diets for their antioxidant capacity using PFA as alternative antioxidants to VitE and to evaluate the effect of this procedure on animal antioxidant status and performance. A grape skin extract and green tea mixture (GGMIX) and a complex of polyphenols from wood hydrolysates (PWH) were tested *in vitro* for their antioxidant capacity using the ABTS test, which provide the results as Trolox (hydrosoluble VitE analogue) equivalents (155.56 and 31.83 μM trolox/g respectively). Based on these results, GGMIX, PWH or synthetic VitE (Tocopherol acetate), were added to the basal starter, grower, and finisher diets for broilers to obtain the same antioxidant capacity expressed by 50 mg/kg of VitE. Three groups of 84 broilers were raised for 42 (50% of the animals) and 84 (remaining animals) days and fed the diets containing GGMIX, PWH or VitE. Animals' antioxidant status, health status, growth performances, and lipid metabolism were evaluated. Blood samples were collected at slaughter. No differences were found in animal oxidative status (FRAP: 3.53 vs 3.58 vs 3.67 μmol ascorbic acid/mL, ABTS: 23.0 vs 22.6 vs 22.7 % inhibition, MDA: 0.78 vs 0.91 vs 0.83 μM/mL at 42 d; FRAP: 4.28 vs 4.41 vs 4.32 μmol ascorbic acid/mL, ABTS 22.9 vs 22.3 vs 23.5 % inhibition, MDA: 0.88 vs 0.95 vs 0.84 μM/mL at 84 d for GGMIX, PWH and VitE respectively) and health status, growth performances and lipid metabolism. The preliminary evaluation and the rational inclusion of PFA in broilers diets allows for the satisfaction of their antioxidant requirements, resulting in similar animal's oxidative status and performance. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 774340.

O267

A holistic characterization of pasture area of Centre-South Italy

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Natural pastures are the results of the combined influence of climate, soil properties, vegetation dynamics and grazing animal activities. Aiming to characterise an Apennine pasture area in Montenero Val Cocchiara (MCV) municipalities (Isernia province, Italy) recognized as Sites of Community Importance (SIC) by Natura 2000 network, a multidisciplinary study was carried out. MVC pasture area is localized in an intermontane basin originally located at the bottom of an ancient lake, at about 850 m of altitude. MVC peatland valley (Pantano della Zittola, about 900 hectares) is partially covered by peat layers where Pentro horses and bovines are raised extensively.

During the 2019–2022 grazing period (May–July), 16 top-soil samples (0–20 cm of depth) were studied for the soil organic carbon (SOC) content. Soil profiles from three selected sites were classified according to World Reference Base for Soil Resources, and biomass production evaluation and phytosociological investigation through vegetal transepts ($n = 55$, 0.5 m^2) were investigated. Grazing animal data and farm numbers were collected from official archive for the 2012–2021 period. Animals predated by wildlife were also recorded.

Sapric Histosol (Hypereutric) soils of MVC derived from degraded ancient peatland were characterized by high SOC average values (4.9 ± 1.53%, SD). Herbaceous vegetation, including 15 – 20 species, showed a prevalence of grass followed by legume plants. The annual variability of turf was confirmed by the average height of plants which varied from 5 to 55 cm, and by the turf production which ranged from 0.5 t DM/ha (2019) to 5.8 t DM/ha (2022), with an average value of 3.7 (± 2.31) t DM/ha.

On the entire municipal area, the grazing animal population decreased from 1678 heads in 2012 to 1354 heads in 2021 and a reduction (−37%) of farms was observed during the same period. The annual average number of predated animals accounted for hundred equines.

Observing MVC pasture area from a multidisciplinary point of view, it should be noted that soil characteristics could be influenced by the ground water fluctuation which may affect, in the long term, the role of grassland as sink of carbon. Furthermore, variations of livestock and farm number likely reflect the general trend of abandonment of marginal areas in favour of more productive areas of low-lying lands.

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O509

Cosmetics based on goat milk whey enriched by essential oils from Mediterranean bushes

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Milk whey still represents a burning issue for the very expensive cost of disposing. Notwithstanding the studies to use milk whey as raw material and source of milk by-products, also characterized by healthy properties, the dairy Small-Medium Enterprises face this charge daily. Moreover, in recent decades the demand for healthy and mostly natural cosmetics is increasing. The skin care properties of goat milk are well known since the ancient times, and literature offers many studies concerning the use of goat milk in cosmetics, while few studies deal with the skin care properties of milk whey. From the hydrolysis of whey proteins, bioactive peptides are obtained with important antioxidant, antimicrobial, anti-inflammatory, anticancer, immunostimulant, osteoprotective and dermo-protective properties. The peptides

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obtained from the protein fractions of goat's milk and whey showed important antioxidant properties and scavenging activity of the superoxide anion O_2^-, i.e. capable of transforming oxygen free radicals into non-radical compounds, free of reactivity and therefore of toxicity. Object of this study was to attain six skincare products from goat milk whey: 3 types (hand, body, and firming cream) added with essential oils extracted from 2 bush species, typical of the Mediterranean area, *Lavandula angustifolia* Mill. (lavender) and *Spartium junceum* L. (Spanish broom), rich of antioxidant compounds, chosen in the Cilento area (South Italy). Flowering tops of cultivated lavender and flowers of Spanish broom were handily collected and hydrodistilled to obtain the essential oils. Their volatile compound profiles were obtained by GC and GC-MS. Both oils showed high quality level: the lavender oil contained linalool and linalyl acetate (32 and 41% respectively) as the main components, while in the Spanish broom essential oil, linalool and tetradecanoic acid dominated (27 and 19%, respectively). For the basis of the creams, milk whey was ultra-centrifugated (500 rpm at room temperature) for 15 min, then formulated with natural ingredients, the essential oils and preservatives permitted, so that final products were >99% natural. The shelf-life evaluation showed unaltered properties till 90 days in glass jar closed at room temperature kept in the dark. Further studies will be carried out to evaluate the effect of the prototypes first *in vitro*.

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O312

Antioxidant enzyme activity of rabbits fed dietary bovine colostrum supplementation

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Bovine colostrum (BC) has a high nutritional value due to its content of macronutrients, micronutrients, and bioactive compounds which confer it anti-microbial, anti-inflammatory and antioxidant properties. The use of BC is not confined on humans, but its supplementation as a nutraceutical for both production and companion animals of all ages has been documented.

Although the antioxidant properties of BC are currently known in various animal species, to the best of our knowledge studies on BC effect related to antioxidant status in rabbits have not been performed.

The aim of this study was to investigate the effect of dietary supplementation with two different concentrations (2.5% and 5.0%) of BC on antioxidant status and gene expression of antioxidant enzymes in liver and *Longissimus dorsi* (LD) muscle of rabbits.

New Zealand White rabbits ($n = 39$) were divided into three groups ($n = 13$) and fed until slaughter (91 days of age) with a commercial diet (CON group), CON supplemented with 2.5% (BC-2.5 group) and 5% of BC (BC-5 group). Blood was collected at slaughter from 10 animals/group ($n = 30$) to determine the activity of antioxidant enzymes Catalase (CAT), Glutathione peroxidase (GPx) and Superoxide dismutase (SOD); liver and LD muscle were collected from 10 animals/group ($n = 60$) for RNA extraction and subsequent antioxidant enzymes gene expression analysis through Real Time PCR.

Despite the increase in oxygen radical absorbance capacity (ORAC) values found in complete feed (CON: 113.00 ± 3.8; BC-2.5: 136.3 ± 4.5; BC-5: 150.70 ± 5.8 μmol TE/g), no significant differences in plasma CAT, GPx and SOD concentrations were found. Similarly, there were no changes in gene expression of CAT, GPx and SOD in tissues of rabbits after BC supplementation compared to CON ($p > 0.05$). A significant tissue-related effect has been observed in mRNA level of SOD and GPx, which were significantly higher in LD ($p = 0.022$) and liver ($p = 0.001$), respectively.

We speculated that the lack of alteration in the investigated parameters may reflect the total phenol content (TPC) found in the three experimental diets, which was equal between groups (3.85 ± 0.15, 4.09 ± 0.27 and 3.84 ± 0.08 mg GAE/mg in CON, BC-2.5 and BC-5 groups respectively). Due to limited literature on the topic, further research is needed to evaluate the potential practical application of BC in rabbit rearing.

O447

Livestock biodiversity and endogenous area development: the POD ‘Provolone del Monaco’ model

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Biodiversity is the indispensable element to ensure the adaptation of living organisms to changing environmental conditions. Animal husbandry is the basin from which to draw, improve or even create production chains that satisfy the changing needs of human populations. The creation of certification procedures that combine quality paths, with well-defined standards, with products of animal origin determines the enhancement of the territories involved and, indirectly, the safeguarding of their biodiversity. The main aim of this work is to emphasize the decisive role of the quality certification of *Provolone del Monaco* as a driving force for the development of both the cheese makers/producers of the product and the territory. For this purpose, through the administration of standardized questionnaires, a model of detected preferences has been carried out giving evidence of the importance of quality certification in influencing the consumer's purchasing actions. Considering that *Provolone del Monaco* received POD recognition in 2008, and that its area involves up to thirteen municipalities of the Sorrento peninsula, we could justify in terms of Brand fidelity the growth of the turnover.

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O427

Assessing signatures of selection and climate adaptation in European and African livestock

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Livestock farming is an economically and socially important sector of agriculture and contributes 40% of the value of the agricultural output globally. Currently the global demand for livestock products is increasing with the rapidly growing human population, urbanisation, increase in incomes and a shift in diet. Climate change is likely to affect agricultural systems on many levels such as heat stress in livestock, changes in production and quality of feed crop, water availability, animal growth and milk production, disease, reproduction, and biodiversity. The domestication and dispersal of livestock along with environment-mediated selective pressures have shaped phenotypic variation and left specific signatures in the genomes of locally adapted breeds. Here we studied existing and new genome-wide genotype data to understand livestock adaptation to climatic extremes by using three species (cattle, goat, and sheep). Breeds from challenging climates throughout Europe and northern/central Africa were compared. For each comparison, we performed selection signature analysis through sliding-windows F_{ST} and XP-EHH, and identified the genes intercepted by significant selection sweeps. We applied a consensus by majority approach to select the genes under putative selection within and across species. Around 30% and 21% of all the genes identified across species were previously described in the literature to be associated with fat deposition and overall feed efficiency, and to adaptation to harsh climates, respectively. Similar proportions were recorded in the within-species comparisons. These preliminary results suggest a key role of energy management in the adaptation strategies of ruminants, likely in the shape of increased resilience, rather than resistance to adverse climate.

O285

Genomic characterization of the Comune di Sicilia goat, a local Sicilian genetic resource

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Local breed conservation is important to maintain enough genetic variability to face future changes, preserve unique genetic variants of interest, and support human economy and culture. This is particularly true in Southern Italy's marginal areas, where goat farming traditionally relies on autochthonous breeds. Among these, the *Comune di Sicilia* (CS) goat is an ancient breed counting about 500 heads, reared in semi-extensive conditions in western Sicily primarily for milk production. To attribute the breed status of a population, historical, phenotypical, and genomic characterization are needed. Thus, with the BIOSAVE project's support, this study aims to investigate CS's genetic relationship with other Italian goat breeds and identify selection signatures related to specific morphological traits.

79 CS goats, sampled from two farms located in Palermo, were genotyped with Illumina SNP65 bead chip and compared with 437 goats of 14 breeds. After quality control and exclusion of related animals, 487 goats, including 72 CS, were retained. Multidimensional scaling (MDS), admixture, and genetic distance analyses were performed. Runs of homozygosity and the related inbreeding coefficient (F_{ROH}) were calculated. ROH and F_{ST} were used to compare polled/horned and with/without microtia CS individuals.

The results indicated that CS subjects clustered together and were discernable from other breeds, and located near Derivata di Siria, Argentata dell'Etna, Nicastrese, Messinese, and Aspromontana populations. The best-fitting admixture model ($K = 12$) revealed two unique signatures in CS. Another relevant genomic cluster found in CS was in common with other Southern Italian breeds.

CS presented a F_{ROH} similar to other breeds from Southern Italy, half of which derived from ROH >16 Mb implying recent inbreeding events or a reduction in the breed consistency.

The low F_{ST} values found comparing horned and polled goats suggest that this trait is not fixed in the population yet. Some genes detected in goats with microtia are related to the same phenotype in humans or characterize another breed presenting microtia, the LaMancha goat.

These results support that CS can be genomically distinguished from other breeds reared in Italy. Further investigations are needed to identify the genes responsible for peculiar morphological traits found in this population.

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O372

BITE v.2: a user friendly R package for genomic analysis

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Genotyping and high-throughput sequencing have experienced a dramatic cost reduction, forcing geneticists to acquire cutting edge bioinformatic skills for managing large genomic datasets. However, neither all researchers have a proper bioinformatic education nor the learning curve is an easy one in the ‘big data’ domain. Moreover, researchers are often puzzled when it comes to the selection of the most appropriate tool, especially when the integration of multiple analyses is required to obtain meaningful results.

BITE (BioInformatics Tools for Everyone) has successfully served the scientific community providing a set of user-friendly functions for SNP data handling and facilitating visualization, interpretation, and usage of results from third-party software in biodiversity analyses. Besides its capability to interact with the most common genomic data file types, BITE returns publication-grade plots, summary statistics, reports and ready-to-use files in downstream analyses.

The new version of BITE will be focused on replacing outdated and no longer maintained dependencies, guaranteeing long term support and usage (ie. Docker instance), with new functionalities and improved performance.

In this iteration genomic data also be handled in VCF format. Functions for quality control checks and exploratory analyses on genetic diversity and structure are updated with flexible choices. For example, F-statistics and genetic distances will be provided and LD pruning have been implemented. Moreover, MDS or PCA (and Supervised PCA) will be obtained, together with a dynamic visualization tool to better explore population relationships. Finally, an updated function for genomic-informed subsampling is provided to harmonise sample sizes in case of extremely unbalanced sampling efforts. Furthermore, BITE will allow managing third-party output files from software that estimate global membership coefficients (e.g. Admixture). The package will return rectangular or circular barplots, maintaining a consistent colouring of the clusters as the number of ancestral populations increases. A function to identify the best number of ancestral populations will be also included. Besides the possibility to run TreeMix software with or without bootstrapping, it is now possible to plot *f3* and *f4* statistics. Moreover, a specific function to choose the optimal number of migration edges has been added. All graphics have been improved and a report of all analyses can be created by the user.

O481

Copy number variants in 23 Italian local chicken breeds

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Copy number variants (CNV) are structural variations inside the genome that contribute to several adaptive and economic traits in livestock. The present study aimed to investigate and characterize the presence of CNV, copy number variation regions (CNVR) and candidate genes of 23 Italian local chicken breeds: Ancona, Bianca di Saluzzo, Bionda Piemontese, Cornuta di Caltanissetta, Livorno Bianca, Livorno Nera, Mericanel Della Brianza, Modenese, Mugellese, Ermellinata di Rovigo, Millefiori di Lonigo, Padovana Argentata, Polverara Bianca, Padovana Camosciata, Padovana Dorata, Polverara Nera, Pepoi, Robusta Lionata, Robusta Maculata, Romagnola, Siciliana, Valdarnese, and Valplatani. A total of 530 animals (20 to 24 animals per breed, 50% males and 50% females) were genotyped with 600 K Affymetrix® Axiom® HD chip and 580,954 SNPs were available for CNV analysis. The Hidden Markov Model of the PennCNV software was used to identify CNV on 28 chromosomes, and the R package HandyCNV was used to generate and visualise the CNVR, the chromosome location and the gene annotation.

A total of 4266 CNV were identified with the highest number in Ancona (480), Valplatani (408) and Bianca di Saluzzo (348), and the lowest in Polverara Nera (41) and Robusta Lionata (40). To make the interbreed CNVR frequency comparison possible, core CNVR were identified using CNV that had overlapping chromosomal locations in different breeds. This resulted in 292 interbreed CNVR with frequency >15% in at least one breed. Chicken quantitative trait loci datasets and Ensembl gene annotations were used to estimate potential phenotypic effects of CNVR on breed-specific traits. Various CNVR identified in the present study overlapped with genes involved in skeletal muscle development and function, fatty acid deposition, heat stress response and climate change adaptation.

The distribution of the CNV and the comparison of the differences among the CNVR of the 23 local chicken breeds reported in the present study allowed a comprehensive characterization of the breeds. However, further research is needed to confirm if the CNV observed in the breeds are also linked to variations in the specific phenotypic aspects. Further experimental and functional validation of CNVR would help in the conservation of Italian local chicken breeds.

O64

Mapping of heterozygosity-rich regions in Italian and worldwide goat populations

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The heterozygosity-rich regions (HRR) are genomic regions with high variability, which can provide information on populations' diversity and may harbor genes related to functional traits such as immune response, adaptation or fertility. To our knowledge, there are few studies characterizing HRR in goat species. Italy provides a high source of information for caprine genetic resources with its 36 recognized breeds, mainly reared in small farms, in semi-extensive systems, and exploiting marginal areas. Italian ($n = 30$) and Worldwide ($n = 19$) goat populations, for a total of 1287 individuals genotyped with the Illumina GoatSNP52k BeadChip, were investigated for population structure and HRR patterns using both Sliding Windows (SW) and Consecutive Runs (CS) methods (common parameters: minSNP =10, no missing or opposite genotypes and minLength =250kb). The among-breeds relationship analyses clustered the populations in accordance with their geographical distribution, highlighting Asiatic goats as the most distant cluster. The HRR analysis reported overlapping results by both detection methods with high correlation coefficients ($r = 0.996$–1) for the descriptive parameters of number, length and diversity rate. The Alpine populations showed the highest mean number of HRR (N_{HRR}) per individual (12.70) followed by the goats of central-southern Italy (11.59), while African and Asian goats showed relative low values (8.60 and 4.88, respectively). CR and SW reported the same total number of HRR (1130) corresponding to 166 HRR islands. CHI01, CHI11, CHI12 and CHI18 showed 66% of the islands, involving 33, 16, 22 and 30 populations, respectively. In particular, 67% of the populations shared the same hotspot in CHI01, including *STAG1* and *PCCB* reproduction-related genes. Almost all the Italian and Alpine goats had the CHI18 island in common, related to the fiber production from fleece. The hotspot in CHI12 shared by some Alpine and Italian, as well as African and Asian breeds, harbor genes involved in productive and adaptation to climate traits. The results highlighted similar genomic patterns between geographically close breeds probably due to gene flow. Moreover, HRRs across breeds and common HRR hotspots underlined a high level of genetic diversity that could give this species the possibility of facing new challenges linked to the onset of diseases or climate change.

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O374

An ancient DNA perspective on the genomic variation of present day Sardinian cattle

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The Sarda cattle is an autochthonous breed from the island of Sardinia. This population has never been subjected to artificial selection or strong phenotypic standardization and its evolution has been mainly driven by environmental adaptation, leading to the great within-breed heterogeneity we observe today. Sarda cattle are usually raised in isolated herds, with a very limited exchange of animals. Due to geographical and genetic isolation, groups of individuals from specific areas show peculiar morphological traits, as e.g. a markedly reduced size (i.e. 110–115 cm at withers) compared to the breed average. A possible alternative explanation is that these animals still retain ancestral traits at the phenotypic and genomic level. Aim of the present work was to compare genomic information between (i) groups of Sarda animals farmed in different areas and (ii) between present-day Sarda cattle and ancient domestic bovines from 11 archeological sites of Sardinia.

To this end, a total of 64 Sarda animals farmed in nine areas were genotyped with a medium density SNP panel. Only common SNP mapping on autosomes were retained for the subsequent analyses. The ancient DNA (aDNA) analysis targeted 30 samples covering ca. 6000 years (5000 BCE-1300 CE). DNA extraction has been carried out in a dedicated facility following strict laboratory protocols to prevent contamination from modern sources. After extraction, seven samples showing the presence of cattle DNA in a PCR-based test have been purified with a customised target hybridisation capture kit to increase the yield and subsequently used to build genomic libraries prior to sequencing on an Illumina platform. After QC and trimming, the reads have been aligned against the cattle reference genome (ARS-UCD1.2) with recommended aDNA settings. MapDamage 2.0 software confirmed the occurrence of the damage pattern typical of aDNA.

According to the MDS analysis of the modern cattle genotypes, the individuals basically clustered according to the sampling area. Moreover, even if the average minor allele frequencies were quite similar among groups (MAF range of ±0.02), the inbreeding levels largely varied.

As a next step, the variants corresponding to the medium-density panel markers will be extracted from the aDNA genomes and compared to the modern Sarda cattle genotypes.

O371

Genomic profiling of several Lazio Indigenous goat breeds in the Italian context

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Italy is home to many local goat breeds, some of which are still unknown in terms of their genetic makeup while representing an important economic and cultural intake, especially in marginal areas. In this study, the Illumina Goat¬_IGGC_65K_v2 chip was used to genotype 142 samples of the Capestrina, Fulva, Grigia Ciociara, and Bianca Monticellana Italian goat breeds. The genotypes were merged to genome-wide SNP data from 33 Italian breeds to assess the Lazio indigenous goat's genetic profile. After filtering for minor allele frequency, missing genotype call rate, and individual missingness, the final dataset contained 49,115 SNPs. The overall genetic structure was then evaluated using the MDS plot, Admixture analysis and runs of homozygosity (ROH). According to inbreeding coefficients based on ROH (FROH), Bianca Monticellana appears to be highly inbred, whereas the Fulva, Capestrina and Grigia Ciociara exhibit heterozygosity levels comparable to those of other breeds. The admixture outcomes show that the Lazio breeds are not genetically homogeneous. In fact, the Bianca Monticellana differs from other breeds from Lazio and central Italy, most likely because of genetic drift, whereas the Fulva is closer to the Grigia Ciociara and Capestrina. Additionally, each Lazio breed has a structure that could be related to the flock. For example, the Fulva breed seems to be split into two sub-populations, which refer to the two flocks that were sampled. The Neighbor-Joining system classifies the Italian goat breeds into three categories based on their geographic distribution, locating all the Lazio breeds in the same branch. Finally, Treemix results support the above findings additionally indicating no genetic migrations between the indigenous goat breeds of Lazio and the other breeds. These conclusions underline the importance of several local breeds as reservoir of genetic diversity, essential to maintain biodiversity and to improve animal breeding strategies.

O90

Phenotypic characterization of semen production and quality in Italian chicken and turkey breeds

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Native Italian chicken (22) and turkey (8) breeds are an important resources of avian biodiversity. The development of intensive farming systems has reduced the genetic pool of the local avian breeds, putting them at potential risk of extinction. The project 'TuBAvI-2' aims to preserve the Italian poultry genetic resources, by supplying new data and tools for the implementation of a nationwide conservation program. The phenotypic characterization of the breeds is an important task within the project. The purpose of this work was to assess the fresh semen quality of different Italian chicken and turkey breeds, that up till now has never been evaluated before. In total, 38 cockerels: Bionda Piemontese (BP, $n = 7$), Robusta Maculata (RM, $n = 11$), Mericanel della Brianza (MB, $n = 9$), and Siciliana (SI, $n = 11$); and 13 turkeys: Romagnolo (RO, $n = 5$), Bronzato Comune (BC, $n = 5$), and Ermellinato di Rovigo (ER, $n = 3$) were used. All donors were trained for semen collection for a period of 2–6 weeks. Each ejaculate was evaluated considering quantitative (volume, concentration) and qualitative (sperm membrane integrity, motility) sperm traits.

Sperm variables were compared among the breeds using the one-way ANOVA followed by Scheffe's test. In regard to chicken breeds, a higher value of sperm volume was recorded in BP (402 ± 25 µL) ($p < 0.05$), higher sperm concentration was found in the SI breed ($3.4 \pm 0.1 \times 10^9$ sperm/mL), which resulted in a significant difference compared to MB ($2.3 \pm 0.2 \times 10^9$ sperm/mL) and RM ($1.6 \pm 0.1 \times 10^9$ sperm/mL), no significant differences were obtained in terms of total, progressive motility, and kinetic parameters. The MB breed had the higher sperm viability (96.2 ± 0.9%) when compared to the BP breed (89.2 ± 1.6%) ($p < 0.05$). In turkey breeds, no significant differences, neither for quantitative, nor for qualitative traits were recorded, except for sperm viability, which was lower in the ER breed ($p < 0.05$). Overall, ER breed had a lower sperm quality.

For the first time, the results provided a phenotypic characterization of semen production in Italian avian breeds and this knowledge is useful in predicting the fertilizing ability of each donor. Moreover, the quality of fresh semen is an important prerequisite for the implementation of a successful freezing protocol. Further studies are planned to evaluate the main structural components of the sperm membrane, such as the lipid composition and protein profile, to obtain a more comprehensive overview.

O225

An over-time distribution map of Apis mellifera mitotypes in Italy constructed using an environmental DNA approach

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For the role that *Apis mellifera* plays in the agroecological systems, a growing interest across Europe is emerging to preserve the genetic integrity of honey bee populations and subspecies. Honey is an incredible source of environmental DNA that contains traces of all organisms that directly or indirectly were involved in its production, including that of the honey bees that produced it. Specific mitochondrial DNA (mtDNA) lineages that characterize several *Apis mellifera* subspecies can be detected from the same honey sample, providing approximate population genetic information useful to estimate the diffusion and frequency of honey bee mitotypes. In this study, more than 2400 honey samples produced over the last five years (2018–2022) in several Italian regions were collected from different beekeepers. Honey samples were produced in nine regions of the North of Italy, in Sardinia and Sicily. DNA was isolated from all these samples and PCR amplified fragments were separated using a size-based assay to detect A, M and C mtDNA lineages. A second assay based on Sanger sequencing was used to discriminate C1 and C2 mitotypes, which are associated with the *A. m. ligustica* and *A. m. carnica* subspecies, respectively. The results showed that the C lineages were the most frequent mitotypes all over Italy and the C1 mitotype was the most prevalent, as expected. Nevertheless, a similar distribution of honey with different mitotypes was obtained in all other regions of the North of Italy and mtDNA haplotypes associated with non-endemic subspecies (A and M lineages) were present and continuously represented over years. The A lineage was highly represented in Sicily. Metadata about the geographical origin, the altitude of the production site and beekeeper information were collected and merged with the results of mtDNA analysis to produce a distribution map of the main honey bee mitotypes. The obtained results will be useful to design conservation strategies of *A. mellifera* genetic resources in Italy. Moreover, we demonstrated that eDNA from honey can be exploited to design cost-effective non-invasive and simple methods to obtain information on the genetic distribution of honey bee mitotypes in a large geographic area.

Acknowledgements

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O115

Variability in semen freezability within an Italian chicken breed

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In Italy, 53 local chicken breeds were described; the majority (67%) were classified as extinct and 21% at risk of extinction. Therefore, attempts for conservation of Italian avian breeds are urgently required. Sperm cryopreservation is a powerful tool to implement the *ex situ in vitro* conservation technique in bird populations at risk. The aim of this study was to assess the variability in semen freezability between birds within the Italian endangered Bianca di Saluzzo (BS) poultry breed, in order to develop a conservation programme. BS ($n = 18$) roosters were housed at the Poultry Unit, Animal Production Centre, University of Milan (Lodi, Italy). After a semen collection training period, semen donors were selected and semen doses frozen in liquid nitrogen. Each day of collection, quantitative (volume, concentration) and qualitative (viability, total motility (TM), progressive motility (PM), kinetic parameters) sperm parameters were measured. Semen was diluted to 1×10^9 sperm/mL with Lake pre-freezing medium containing 2% N-methylacetamide, loaded into 0.25 mL French straws, frozen for 10 min over a nitrogen bath at 3 cm of height and stored in liquid nitrogen at −196 ° C in cryogenic tank. The straws were thawed at 5 °C for 100 s and sperm parameters were assessed. A total of 10 laying hens per selected male donors were inseminated twice to assess *in vivo* fertility and embryo viability. The mean volume and sperm concentration recorded in fresh ejaculates were 0.327 ± 0.17 mL and $3.303 \pm 1.27 \times 10^9$ sperm/mL. Semen quality of fresh samples was significantly different among birds and only 6 were selected as donors. Furthermore, according to availability of semen doses, 3 birds (BS1, BS2, BS3) were used for artificial insemination trial. Higher values in PM and TM were found in BS2 (PM 25%, TM 98%) and BS3 (PM 33%, TM 98%) compared to BS1 (PM 18%, TM 81%) ejaculates. As expected, a general significant decrease in sperm quality occurred after the freezing-thawing process. Fertility and embryo viability were 30.6% and 95% in BS2, 7.5% and 66.7% in BS1, 8.45 and 57% in BS3. High individual variability was found in semen freezability, not always related to fresh sperm quality. *In vitro* quality of thawed semen was associated to the results obtained in the artificial insemination test. However, furthered analyzes must be performed to confirm the present results and to identify quality sperm markers for the selection of the best semen donors in conservation programs.

O346

About the origin of traditional sheep breeds from South America: a comprehensive investigation

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Documented human migration routes help population geneticists to reconstruct the origin of livestock breeds that have been transported around the world since their domestication. Sheep were brought to South America through different routes, during the Spanish, British and Portuguese conquests of the New World. In the late colonial period new breeds from the European and African continents have been introduced in South America. Previous studies tried to discover the origin of Creole sheep, but this issue is still open. To shed light on their genetic composition we analysed some Creole breeds from Colombia, Uruguay and Brazil, joining genotypes of Italian, Spanish, British and African breeds available from previously published studies and new data generated in the framework of the Smarter project (www.smarterproject.eu). A total of 2789 sheep from 50 breeds were analysed with a final panel of 16,698 SNPs after a QC performed with Plink 1.9. Diversity indices were calculated with Arlequin 3.5.2, whereas the phylogenetic relationship and migration events were inferred using Treemix 1.3. The genetic structure was explored by PCA with SNPrelate 1.3 and with ADMIXTURE 1.3. Taken together, all results showed a high degree of admixture as well as high heterozygosity levels. The lowest Fst value (0.007) was found between Bergamasca and Biellese sheep, and the highest (0.329) between the Border Leicester (UK) and Namaqua Afrikaner (South Africa) sheep. The heterozygosity levels ranged from 0.31 (Uruguayan Creole) and 0.43 (African White Dorper). Both the PCA and the ML tree revealed a weak subdivision in three main clusters (Spain + Italy; Africa; UK). The Creole breeds from Colombia grouped mostly close to all the Caribbean, Brazilian, Mexican, and some African breeds, while the Uruguayan Creole clustered between the British and the Spanish breeds. Moreover, the most probable number of admixture events inferred was 10 of which the most interesting were from the British branch toward the Brazilian and Uruguayan Creole. Finally, the Admixture results partially agreed with previous studies but also gave more clues about the gene flow that occurred in the past. These preliminary results could help in discovering the genetic makeup of the Creole sheep and in preventing uncontrolled cross-breeding in the future.

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O513

Genomic tools for the characterization of the not officially recognized livestock populations: a case study in Mascaruna goat and Pecora Nera sheep from Sicily

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The analysis of genomic data is an important tool for the management of small and endangered populations. Italy holds a large number of small ruminant populations, some of which do not have a recognized genetic structure. The majority of these populations are kept by smallholders under extensive production systems and represent an important economic resource in marginal areas. The conservation of their genetic diversity is compelling in the light of facing future challenges as climate change and emerging diseases. This study investigated the genome-wide structure of two Sicilian animal resources, namely Mascaruna (MAS) goat and Pecora Nera (NER) sheep. These populations are included in the Corial project that aims to study and characterize those animal resources not officially recognized as breeds. A total of 72 and 36 individuals of MAS and NER, respectively, were genotyped using the caprine and ovine 50K BeadChip. The genome-wide data of the two populations were analysed respectively in comparison with others Italian goat and sheep breeds. For both populations, the results displayed moderate levels of genetic variability estimated using observed and expected heterozygosity, effective population size and inbreeding, and they were comparable to that of the other Italian local goat and sheep populations. Multidimensional scaling, model-based clustering and measurement of population differentiation within each species, distinguished MAS and NER from the other breeds, indicating a clear genetic differentiation. The individual data of MAS and NER highlighted genomic admixture with Sicilian goats and sheep, respectively, and showed the lowest genetic differentiation towards Argentata dell'Etna for MAS and Pinzirita for NER, i.e. the breeds that could be considered the ancestral populations of origin. The genomic characterization of these populations represents a fundamental step to plan breeding programs and conservation strategies. The numerous potential uses of genomic information may make routine genotyping desirable for the management of small populations. Our study described an example on two small ruminant populations and confirmed that genome-wide analyses are valuable tools for managing all small and endangered animal genetic resources.

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O342

Looking for the genes involved in local adaptation in sheep

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Throughout their domestication, sheep have adapted to environmental heterogeneity, from the tropics to the highlands. Climate and human selection have shaped the genome and left genetic 'fingerprints' on breeds bred in diverse regions worldwide. The small ruminants have evolved to be highly efficient and provide a valuable model for identifying the genetic pathways and mechanisms underlying adaptation. Landscape genomics can help understand the relationship between genetic architecture and environmental variables and provide information on a species' evolutionary history at different spatial scales. Thus, we aimed to investigate regions of the genome that may be associated with environmental adaptation. The dataset included 501 sheep from creole populations from Uruguay ($n = 100$), Spain ($n = 100$), Africa ($n = 72$) and South Africa ($n = 29$) belonging to the SMARTER project*, and samples from Brazil ($n = 100$) and Colombia ($n = 100$) from a private database and genotyped with the OvineSNP50 or Ovine HD BeadChip (Illumina Inc., USA). A

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total of 23.098 autosomal SNP were included in the analysis after the quality control (SNP call rate: 0.9, MAF: 0.05 and pruned for LD-*indep-pairwise: 50, 5, 0.2*). Climatic variables were downloaded from WorldClim using each population's geographical coordinates. The population structure analysis was performed using MDS (multi-dimensional scaling) and Admixture. The landscape genomic approach (Latent Factor Mixed Model) was used to determine SNPs significantly associated with the geographic and environmental variables. The MDS plot suggested a closer genetic relationship between the Colombian sheep and Spain and African sheep. The Brazilian and Uruguayan Sheep formed an independent group. The Admixture analysis considered 20 potential clusters (K), at K = 4, the Brazilian was distinguished, and the Colombian wool creole and hair-type hair sheep breeds were grouped. The FDR – *q* value was calculated for each locus based on the *p-values* in R. We identified genes related to oxidative stress (*CAT, BLF),* thermotolerance (*FGF2, GNAI3, PLCB1*) and altitude (*PPP1R12A, RELN, PARP2*) that show the adaptation of breeds to specific environmental conditions. Thus, our results could contribute to breeding or conservation plans for locally adapted breeds in the face of climate change.

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O48

The supportive breeding of native Mediterranean brown trout (LIFE Nat.Sal.Mo project, Molise region): the effect of ovarian fluid on frozen sperm motility parameters during artificial fertilization

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The semen cryobank realised within the LIFE Nat.Sal.Mo. project is an important tool to safeguard the genetic integrity of Mediterranean trout (*S. cettii*) at risk of extinction, thanks to its practical use in the supportive breeding. This practice consists in the use of artificial reproduction (AR) to support the natural one. In particular, frozen semen doses in combination with cross-fertilization schemes are used in AR to maximize the genetic variability of offspring to be used in restocking activities. Generally, in AR practices the use of a basic saline solution (D532) is recommended as artificial fertilization medium (AFM) to boost sperm motility. However, in order to maintain a reproductive microenvironment (RM) as similar as possible to the natural one we focused on the use of ovarian fluid (OF) alone as activation medium to sperm motility. Therefore, the aim of the present study was to evaluate the effects of only OF on the post-thaw sperm motility of *S. cettii* compared to D532 and a combination of them at 50% (OF50%).

Sperm samples from 5 males were frozen using a freezing protocol optimised in our laboratory. Each sperm sample was thawed at 40 °C for 5 s and the motility was activated with OF100% and OF 50% of each female ($N = 5$) or D532, then analysed by CASA system. Within each treatment, the data obtained was compared with ONE-WAY-ANOVA, followed by Duncan's test. To assess the fixed effects of male and female ID and their interaction, we used the GLM procedure. OF100% and OF50% enhanced ($p < 0.05$) respectively the total motility (50.4 ± 2%; 51.8 ± 1.9%) and duration of movement (46.1 ± 1.5s; 46.1 ± 1.5s) than D532 (38.5 ± 3.2%; 31.2 ± 1.8 s); higher values ($p < 0.05$) of velocity parameters (VSL, VCL, VAP) were obtained with D532 than other treatments. Sperm traits varied depending on parental ID and interplay between male × female ($p < 0.05$).

Although D532 boosted kinetic parameters more than OF, the authors retain that the sperm velocity could not be a key parameter for a successful fertilization in our cross-fertilization system. In fact, in a controlled RM that already favours the gametes encounter, obtaining a greater number of post-thaw motile sperm/egg that move for a longer time could potentially increase the reproductive efficiency of *S cettii*. Thus, the use of OF alone in the AR could be a simple and effective activation/fertilization media to facilitate the management of riverbank operations, avoiding preparation, transport and use of AFM.

O568

On the road to domestication: a preliminary comparative genomics approach to reconstruct human and livestock correlates

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Since the Neolithic transition, human history has been accompanied by the presence of livestock species whose biodiversity has been molded by repeated human migratory waves. In such a context, complex phylogeographic patterns emerged as a consequence of a combination of natural and artificial selective processes. With the aim to investigate similarities and divergences between the diffusion processes of three livestock species and post-Neolithic human expansion, a comparative framework has been developed including genome-wide SNPs data of goats, sheep, cattle and humans in the Mediterranean region and in the domestication area. Using public repository, we retrieved genomic data from 1.906 goats, 1.924 sheep, 1.962 cattle and 1.363 human individuals. Concerning livestock species, only autochthonous breeds were considered for the analyses. All datasets have been filtered for minor allele frequencies (–maf 0.05) and missing call rate (–geno 0.1) using the software plink and obtaining final datasets consisting of 48.858 SNPs for goats, 33.670 SNPs for sheep, 23.079 for cattle and 421.611 for humans. To explore common pattern of genomic structure, MDS plots were generated for each species, while the software ADMIXTURE was used with K values ranging from 2 to 10 for each dataset separately. The obtained Q-matrices of ancestry coefficients were then been used to construct an interpolation map. Genetic diversity indices were also calculated for each main genomic group representing a specific geographic area in order to assess the presence of pattern of genetic diversity in accordance with stepping stone diffusion processes from the domestication center to peripherical Mediterranean regions. Our results showed that while several common patterns such as a general genomic cline conform with an expansion process from the Fertile Crescent to central Europe can be observed for all species, other local signals seem to be more related to species-specific events reflecting a more complex scenario. For example, while cattle, goats and humans showed similar genetic discontinuities in the Italian Peninsula, sheep seem to be more homogeneous. In cattle this genetic signature could be related to the close relationship between Italian and Balkan Podolian breeds which have been shown to come from the same migration wave. Further analyses and additional samples would be necessary to obtain a more detailed overview of the post-domestication processes of humans and livestock.

O203

Millefiori Piemontese, genetic characterization of an endangered local chicken breed

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Several chicken breeds are recognized in Italy, and the Piedmont region counts three breeds recognized by the MIPAAF: Bionda Piemontese (BP), Bianca di Saluzzo (BS) and Millefiori Piemontese (MP). The MP is an autochthonous breed widely reared in the province of Cuneo until the '60s as double-purpose chicken. Historical sources report that the MP is similar to the Ancona breed but with some specific differences: the MP is heavier than Ancona breed (males up to 4.5 kg, females up to 3 kg), the body is more compact (similar to the BP) and the earlobe colour is red. The MP has a mottled plumage pattern (white and black), yellow skin and shank and the comb is red and erected, with regularly shaped dents, even if less developed compared to other Piedmont breeds.

MP substitution with commercial chicken lines caused its dramatic decline, and almost seemed extinct in the '90s. Some MP individuals have been found in the province of Cuneo (4 breeders preserved a few subjects throughout the years) so a conservation and valorization project of this breed is currently carried out in the TUBAVI project. These subjects ($n = 50$; 16 males and 34 females), phenotypically like the MP, were selected in order to evaluate the genetic variability. Blood samples were collected from each individual for DNA genotyping by a set of 25 microsatellite markers chosen by their high polymorphism. Total number (Na), and effective number (Ne) of alleles, observed (Ho) and expected (He) heterozygosity, and F (Wright's inbreeding coefficient) index were surveyed. In the sample only one locus is not polymorphic; 88 alleles were identified. The average number of alleles per locus was 3.2, and the effective number of alleles was 2.34. The Ne resulted lower due to the presence of low-frequency alleles that can be easily lost by genetic drift, thus reducing the genetic variability of the breeds, and increasing their risk of extinction. Observed and expected heterozygosity were 0.57 and 0.52 respectively with $F = -0.82$ but no significant Hardy-Weinberg Equilibrium deviation was observed. The analysis of the genetical distance from other Italian breeds demonstrated the peculiarity of this genetic group.

Further analyses will be performed in order to define the breed standard, productive and reproductive performances and the breeding programs. As this data and information are unique, it is fundamental to further study and protect this breed, thus preserving and valorizing the Italian avian patrimony.

O507

Genetic diversity and runs of homozygosity in Rendena Cattle

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The dual-purpose (dairy and beef) Rendena cattle breed is native to Val Rendena in the northern Italian region of Trentino-Alto Adige. The breed is of medium build, with short, robust legs and small feet that make it well-adapted to grazing in steep Alpine pastures. The Rendena is also valued by farmers for its longevity, fertility and disease resistance; however, the genomic diversity of this breed is almost unknown. Here, the GGP Bovine 100K SNPchip (Neogen) genotypes and mitogenome sequences from 140 Rendena individuals from 31 farms (collected in 2018) were used together with genotyping data from 40 additional samples (collected in the year 2000) to assess changes in population structure and molecular diversity over the past 20 years. The SNP genotype dataset was used to estimate within-breed diversity and inbreeding, and, with data from 31 local and cosmopolitan cattle breeds, to assess population structure and evolutionary relationships. Principal Component Analysis (PCA), Neighbour-net and Admixture analyses suggested a shared ancestry with the Brown Swiss group. In addition, the distribution of runs of homozygosity (ROHs) appeared homogeneous across chromosomes and was related to chromosome size. Most ROHs were private or common to a few animals. Exceptions were found in three genomic regions on BTA6, BTA10 and BTA16, where ROHs were shared by >25% of the individuals. Noticeably, the ROH on BTA6 consisted in 33 SNPs shared by >50% of the animals over a region harbouring genes relevant for meat (NCAPG, LCORL) and milk production (LAP3). The same region was also found to be under selection in F_{ST}- and XP-EHH-based analyses contrasting alpine *vs* cosmopolitan breeds. The mean inbreeding level recorded for the 2000 and 2018 populations indicated an increase in the average inbreeding from 0.055 to 0.086, and PCA showed partial overlap of the two groups. Most mtDNA variants belonged to the T3 haplogroup which is known to be widespread in Europe; however, 10 animals with T2 haplogroup variants and single occurrences of T5 and Q1 lineages were found.

O151

Evaluation of fatty acids profile of milk produced by mid-lactating Holstein cows fed with enriched olive cake diet

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The use of by-products such as olive cake (OC) for alternative animals feed is widespread to reduce cost associated with animal nutrition (due to the recent increasing costs), to limit environmental impact and to enhance the quality of meat and milk. In fact, it is well known that OC is rich in unsaturated fatty acids (UFA) and polyphenols. The aim of this study was to evaluate the effects of supplementing enriched OC (EOC) to mid-lactating Holstein cows on milk fatty acids (FA) profile. A total of 20 cows were enrolled into 2 homogeneous groups (10 for EOC group and 10 for CTR group) according to BCS (2.43 ± 0.26), lactation period (113 ± 47 d), and milk yield (31.42 ± 3.28 kg/d). The EOC group was fed with a 7% inclusion of enriched destoned OC, whereas control group (CTR) received a conventional diet. Milk samples were collected at d 0 and at d 28, to compare FAs in EOC and CTR group at d 0 and at d 28. FAs were extracted, methylated, and separated with a gas-chromatographer fitting a CP-Sil88 column. Peaks of individual FAs were identified and quantified using a 37 FAME standard. Data were analyzed with the PROC GLIMMIX of SAS. Values are expressed as a percentage of total FAME. The results showed that the inclusion of EOC for 28 days modified the FA profile of milk. In fact, at 28 d, among saturated, short- and medium-chain FAs, C10:0 (2.84 vs. 3.68; $p = 0.08$), C13:0 (0.16 vs. 0.24; $p = 0.08$), and C14:0 (12.73 vs. 14.64; $p = 0.06$) tended to be lower in EOC compared with CTR group. A lower content of C12:0 (3.77 vs. 4.93; $p = 0.05$), C15:0 (1.61 vs. CTR 2.01; $p = 0.03$), C16:0 (33.73 vs. 36.76; $p < 0.001$) was also observed in EOC compared with CTR group. Concerning long-chain fatty acids, EOC group had greater C18:1 cis-9 (20.32 vs. 16.63; $p = 0.02$) compared with CTR. C18:0 tended to be greater in EOC compared with CTR group (6.68 vs. 4.51, respectively; $p = 0.08$), whereas C22:2 (0.05 vs. 0.09; $p = 0.07$) and C24:0 (0.04 vs. 0.08; $p = 0.09$) tended to be lower in EOC compared with CTR. These results point out the beneficial effects of supplementing EOC since it improves milk FAs profile that are positively related to human health and are indicative of a better nutritional and nutraceutical properties of milk. Thereby, using this by-product may be considered as a good alternative to conventional feed, giving moreover an added value to the final product.

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O402

Effect of hemp leaves on milk yield and quality in dairy cows: preliminary results

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Industrial hemp (*Cannabis sativa* L.) leaves are a by-product that usually ends up as a waste in the landfills. As this part of the plant is a good source of protein and fiber, it could be used as a forage for ruminants. Hemp leaves (HL) were supplemented to dairy cows in order to assess its effect on the milk yield and its composition over a short experimental period. Six Italian Simmental (34.6 ± 6.1 months of age) lactating cows were randomly assigned into two treatment groups in an experiment that lasted for 6 weeks (3 periods of 2 weeks). The two experimental groups were fed the same total mixed ration, based on corn-silage, without (CTR) and with a supplementation of fresh hemp leaves (HL). The leaves of hemp (Futura variety) were manually collected at the Center for Cereal and Industrial Crops (CREA-CI), located in Rovigo (Northern Italy), in August 2022. The chemical composition (on DM basis) of the HL was 27.59% of DM, 19.59% of crude protein, 7.12% of ether extract, 13.88% of ash, 30.49% of NDF, 15.81% of ADF, 5.20% of ADL and 0.09% of AIA. The inclusion of HL in the cows' diet was done gradually since there was a first refusal of consumption. During each period of 2 weeks, the cows of HL group were supplemented with 0.5 kg of HL on day 1 and 2, 1 kg of HL on day 3 and 4, 1.5 kg of HL on day 5 and 6 and then without the HL. The milk samples were collected and milk yield was recorded under the effect of day 5 and 6 of each period. HL supplementation did not affect the milk yield but altered the milk quality. On average, milk yield was 25.07 kg/d and milk protein was 3.68%. Parameters such as milk fat and milk total solids decreased with the HL supplementation (4.71 vs. 4.53% and 13.72 vs. 13.43% resp.; $p < 0.05$). For this reason, also the energy content of milk resulted higher for the CTR than the HL group (356 vs. 345 kJ/100 mL; $p < 0.05$). In conclusion, HL can be included as an ingredient for lactating cows, even though the palatability could be low in the beginning of consumption. Further studies might be carried out in order to assess not only the positive transfer of bio-active compounds to the milk and dairy products; but also, to the cannabinoids content of the leaves and its role in the cow nutrition.

O325

Quebracho condensed tannins fed to lactating goats: effect on methane production

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This work studied the effect of different levels of inclusion in the diet (0, 2, 4, 6%) of a commercial purified condensed tannins (71%) extract from quebracho (*Schinopsis balansae*, QE), on methane production in Alpine lactating goats. A repeated Latin square design $(4 \times 4) \times 2$ was applied using eight goats housed in individual respiration chambers and kept in individual metabolic cages for total faeces and urine collection. Each period lasted 28 days, four of which were for sample collection.

The four diets (CTR, Q2, Q4, Q6) were based on meadow hay (on average 53.2%) and compound feed (corn meal, corn flakes, barley meal, and whole extruded soybean) added with the different levels of QE. The CTR had the following chemical composition on DM: 91.9% OM, 15.2% CP, 3.86% EE, 37.1% aNDFom, 23.3% starch. Statistical analysis was performed using the Mixed procedure of SAS, considering the fixed effects of square, period within square, treatment, and the random effect of goat within square.

Treatment did not affect DMI, but reduced OM, aNDFom, and CP digestibility, up to 13%, 24%, and 23%, respectively, as the level of QE increased ($p < 0.001$).

Fat and protein corrected milk was similar between all treatments while Q6 reduced the milk protein yield compared to the CTR (96.3 vs 102 g/d, $p = 0.036$). The inclusion of QE linearly reduced the dairy efficiency (FPCM/DMI; $p = 0.007$).

Methane production was significantly reduced only by Q6 compared to CTR (39.1 vs 43.4 g/d, $p = 0.036$), however, when the methane production is expressed in relation to the DMI, QE linearly decreased the methanogenesis and Q4 and Q6 were significantly lower than the CTR (19.4, 17.8 vs 21.6 g CH_4/kg DMI, $p < 0.001$). Also considering the digestibility, QE linearly reduced methane production per kg of digestible DM ($p = 0.007$) and OM ($p = 0.008$) but linearly increased the methane production per kg of aNDFom ($p = 0.011$). Methane production in percentage of both gross energy intake and digestible energy intake were linearly reduced by QE ($p < 0.001$ and $p = 0.030$, respectively). No effect of QE was observed when methane production was expressed in relation with the milk, milk fat, and milk protein yields.

Given the reduction of diet digestibility induced by QE and the lack of methane reduction per kg of milk, this study shows that including QE in the diets for lactating goats is not an effective tool to reduce the sector's methane intensity emission.

Acknowledgements

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O295

Effect of a blend of essential oil on *in vitro* rumen fermentation for SARA prevention

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Subacute rumen acidosis (SARA) is a nutritional disease when the ruminal pH drops below 5.8 for more than 5–6 h/day. A decrease in ruminal pH causes reductions in rumination and salivation, negatively impacting animal health and causing economic losses for the farmer. One way to address SARA is to add essential oils to the ration, affecting volatile fatty acids (VFA) synthesis pathways, ruminal pH and methane (CH_4) production. This study aimed to evaluate the effects of a blend of essential oils (3 g/head/d) (allyl-sulfide, cinnamaldehyde, eugenol, and limonene) on ruminal pH and fermentation. Four rumen-fistulated cows were fed in a cross-over design 2 different diets in 2 periods: control diet (CONTR) vs essential oil (EO) diet. The individual rumen fluids of these cows (CONTR and EO) were used to carry out *in vitro* incubations for 24 h to evaluate gas production (GP), VFA, and pH; CH_4 production was predicted in function of VFA concentrations. The substrates incubated in the *in vitro* analysis were the control diet (CONTR, 18% starch on DM) and one modified to induce SARA, replacing 35% of CONTR with wheat starch (SARAD, 46% starch on DM). The buffer was prepared with a reduced amount of sodium bicarbonate and disodium phosphate in order to induce SARA *in vitro*. At the end of incubation (24 h), GP and pH values were recorded, and samples were collected to determine VFA. Statistical analysis was conducted using SAS studio (SAS® Studio 3.8), considering the fixed effects of the inoculum, incubated substrate, period, cow, and their interactions. On average, the pH of the EO inoculum after 24 h of incubation was 5.63, while the CONTR was 5.57 ($p < 0.001$). However, for SARAD substrate, EO tended to maintain a higher pH (5.42) than the CONTR inoculum (5.39; $p = 0.074$). The GP (mL/200 mg DM) at 24 h was higher with the EO than the CONTR (75.5 vs 70.4 mL, $p < 0.001$). On the contrary, total VFA (mmol/l) of EO inoculum was lower than CONTR (51.7 vs 54.8; $p = 0.029$). EO decreased the molar proportion of acetate (59.8 vs 61.9%; $p = 0.009$) and butyrate (13.9 vs 15.8%; $p < 0.001$) while increasing the molar proportion of propionate (21.6% vs 17.6%; $p < 0.001$) compared to CONTR. The EO resulted in less methane than CONTR, 13.7 mmol/l vs 16.1 mmol/l ($p < 0.001$), respectively. In conclusion, the tested blend of essential oils seems to be promising in modulate rumen fermentation; further evaluation on microbiome modification is required.

O178

Evaluation of the effect of leaf extracts from deciduous trees (*Castanea sativa*, Fagus sylvatica and Quercus robur) on ruminal fermentation

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It is known that polyphenols can modulate rumen fermentation. In this context, considering the high polyphenol structural variability, three Fagaceae species were selected: beech (*Fagus sylvativa* L. Fs), chestnut (*Castanea sativa* Mill. Cs) and oak (*Quercus robur* L. Qr). Thus, a polyphenol fraction was obtained from each species. Chemical composition analyses highlighted polyphenols identity and relative abundance discriminate the three species. Indeed, hydroxycinnamic acids were mainly abundant in beech, while they were minor constituents in oak and chestnut, which in turn contain an appreciable tannic component. This latter mostly consisted in hydrolysable tannins in chestnut, and condensed ones in oak. Quali-quantitative differences were also related to their flavonoid content. To investigate the impact of the three polyphenol fractions on *in vitro* ruminal fermentation, they were supplemented in the ratio of 0, 5 and 20% to a standard diet and incubated *in vitro* at 39 °C with a ruminal *inoculum* obtained by three young bulls. Polynomial contrast analysis was performed in order to observed dose effect. The chemical diversity has led to different intra-species findings on ruminal fermentation process. Dose-dependent effects were also recorded, in terms of fermentation rate and gas production. All polyphenolic fractions at 5% reduced gas production; chestnut leaf extract was the most active fraction followed by oak extract. The decreasing effect on gas production (gp) occurred during the 1st hour of incubation, while the fraction from beech leaves exerted a slower reducing effect on gp. On the contrary, at 20% a significant increase in gp was found, together with an abrupt slowdown in the fermentation rate. The polyphenol fractions also appeared to modulate and differently affect the production of volatile fatty acids (VFA). All fractions increased total VFA compared to control

diet. At the 20% treatment dose, the beech and chestnut fractions exerted a lower increase in VFA, while the oak doubled VFA production. Furthermore, treatment at 5% for all fractions significantly reduced acetate, valerate, iso-valerate and BCFA, while increasing propionate and butyrate production. In conclusion, although belonging to the same family, the leaves of three species provided extracts with different chemical and nutritional characteristics, resulting in various effects on ruminal fermentations, diversely exploitable.

O112

Relationship between feeding management and milk characteristics in dairy buffalo

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The quality of buffalo milk is strongly influenced by feeding, genetics, season, lactation stage, parity, and management. This study aimed at understanding how feeding management might affect milk quality. The study was carried out in 10 water buffalo farms located in the Amaseno (Italy) for a period of 12 months. On a monthly basis, samples of total mixed ration (TMR), feces, bulk milk, and information of diet and feeding management were collected for each farm. TMR particle size was determined by sieved samples on farm using Penn State Particle Separator (PSPS), and the geometric mean length (GML) was obtained by measuring the particles. TMR and faeces samples were collected and then analyzed at the laboratory for chemical characteristics: crude protein (CP), ash, ether extract (EE), starch, neutral detergent fiber (aNDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). The apparent digestibility of the diet was estimated by an equation using ADL as a marker. The bulk milk sample was analyzed for the determination of fat, protein, and lactose, casein and solid not fat percentages, urea (mg/dL), freezing point (°C), pH, titratable acidity (°SH), somatic cell count (SCC) and cheesemaking attitude (RCT, k_{20}, a_{30}). To find a correlation between the quality of diet and the quality of milk, Pearson correlation was calculated between all the variables. The content of diet DM (%) was negatively related to the content of milk protein ($r = -0.43^{**}$), fat ($r = -0.33^{**}$), casein ($r = -0.37^{**}$) and a_{30} ($r = -0.20^{*}$). Buffaloes fed with diets containing lower DM (46.9 ± 3.5%) showed higher milk yield (MY, +0.53 l/head/d) and lower SCC. Diets with higher DM (57.2 ± 4.2%) had lower crude protein and NDF contents compared with low DM %. The content of dietary proteins (%) was negatively related to the content of milk proteins ($r = -0.31^{**}$), fat ($r = -0.35^{**}$) and casein ($r = -0.29^{**}$), and positively related to milk urea ($r = 0.33^{**}$) and RCT ($r = 0.31^{**}$). Diets with higher proteins (13.4 ± 0.8%) had lower NDF content, lower GML and higher starch content compared with diets containing low (10.6 ± 1.2%) proteins. Moreover, buffaloes fed with diets containing higher proteins content showed higher MY (+0.52 l/head/d). Milk yield was negatively related to SCC ($r = -0.32^{**}$). Furthermore, the season also influenced the quality of the milk; there was a greater content of fat, protein and casein in the winter months and autumn months compared with other months.

O238

Tobacco cv. Solaris seed cake in the diet of growing beef calves

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After cold oil extraction from *Nicotiana tabacum* L. cv. *Solaris* (PCT/IB/2007/053412) seeds, the co-product *Solaris* seed cake (CP 34.7 g/100 g DM) was tested as a possible alternative protein source in beef cattle diet.

Ten male crossbred Friesian growing beef calves raised in a commercial farm located in Benevento province, average weight 157.9 (±24.5, SEM) kg and average age 228.5 (±71.9) days, were divided in two homogeneous groups, Solaris (SOL) and Control (CTR). According to the nutritional needs of the selected animals, two isoenergetic and isonitrogenous diets were formulated. Groups were daily fed 21 kg of legume and grass haylage, *ad libitum* mature grass hay and concentrates mixture made by 40% of corn, 40% of barley, 10% of commercial feed and 10% of *Solaris* seed cake, and by 40% of corn, 40% of barley, 20% of commercial feed, respectively for SOL and CTR group. The trial lasted 66 days, including 16 days of adaptation to the experimental diets. Group feed intake (offered-refusal) was monitored weekly, individual body weight (BW) was recorded at 0- and 66-days, and body condition (BCS) of animals was scored at 0-, 34- and 66 days. Average daily gain (ADG) was calculated. Feeds offered and left over were weekly sampled and chemical composition was analysed according to official methods. The effect of the dietary treatment on individual BW, BCS and ADG was processed by analysis of the variance also considering the covariate effect at 0 days (GLM, ANCOVA; SPSS Inc., Chicago, IL, USA). Significance was declared at $p < 0.05$.

The daily group intake of concentrate was in average 35.4 (±1.21) kg DM and 36.5 (±1.31) kg DM for CTR and SOL group

respectively, indicating the palatability of *Solaris* seed cake. The dietary treatment did not affect ($p > 0.05$) individual BW (207.7 kg vs. 215.9 ± 9.8 kg, respectively for CTR and SOL), ADG (0.86 kg/d vs.1.05 kg/d ±0.15, respectively for CTR and SOL), and BCS (3.31 vs. 3.41 ± 0.16, respectively for CTR and SOL).
Although not conclusive, results suggest that the innovative *Solaris* seed cake represents a promising alternative ingredient in growing calves' diet. This co-product from the innovative energy crop may contribute to the reduction of feeding costs in beef farming and could represent a great opportunity for re-designing the tobacco cultivation, especially in inner Mediterranean areas.

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O202

Changes of rumen microbiota composition in dairy cows fed with different lipid source

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Dietary lipid sources can increase beneficial effects in dairy products, but the degree of saturation of fatty acids could affect rumen microbiota. The aim of this experiment was to evaluate the influence of two different lipid sources, one saturated (Megafat88; FAT), and one unsaturated (whole soybean flaked; SOY) on rumen microbiota composition of high-producing dairy cows fed a hay-based ration. The study involved 8 Italian Friesian dairy cows, and reticular pH, rumination time, and microbiota composition (bacteria and protozoa) were determined. DNA from rumen contents was extracted, purified, and sequenced to quantify bacteria populations. Sequencing was performed on Illumina MiSeq. For protozoa count, liquor samples are mixed with glycerol and the microorganism were counted in Burker-chamber counter. Data were analyzed with JMP pro v 17.1. For the statistical analysis, a linear mixed model was used with a fixed effect: Saturated Fat vs Unsaturated fat. The different lipid sources influenced bacteria community, but the differences were not significant. Prevotellaceae family showed a tendency ($p = 0.1$) and decreased in SOY compared to FAT. Selenomonadaceae family had a higher relative abundance ($p = 0.07$) in SOY, as well as Rikenellaceae. and Veillonellaceae, while Lachnospiraceae did not differ. Data also revealed differences among cows, even if subjected to the same lipid source. Animals were significantly different within the same treatment for Ruminococcaceae ($p \leq 0.05$) in SOY, while Veillonellaceae and Selenomonadaceae families in both SOY and FAT treatment. Rikenellaceae presented a significant difference in FAT ($p \leq 0.05$). Protozoa count revealed a higher concentration in SOY compared to FAT (10.2 vs 8.8 respectively, $p \leq 0.05$). The analyzed genera showed a significant increase in SOY for Epidinium, and Entodinium ($p \leq 0.05$), while Isotricha increased in FAT ($p \leq 0.05$). In conclusion, the dietary lipid source affected rumen microbiota composition, especially the protozoa community. The degree of saturation of lipid sources affected microorganisms in different ways for each cow, underlying the individual characteristics of each animal, and how much the host variability remains one of the major aspects to explain the final animal response.

O215

Could eating time be a useful indicator in dairy farm management?

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The increasing demand for dairy animal products will require maintaining an efficient dairy livestock system. Feed efficiency represents a relevant part of the whole efficiency because feed inputs are the most important factors that affect dairy farm costs. Precision dairy farming has been well-developed, with an increase in the use of advanced technologies such as milking robots, automatic feeding systems, and accelerometers in commercial farms. This study focuses on the data generated by these sensors, which provide increasingly accurate eating and ruminating times for each animal: the aim is to evaluate the links among milk production, feeding management, and behavior in the perspective to associate eating time with feeding efficiency. Data were collected from about 800 lactating cows of two breeds (Holstein Friesian – HF and Italian Simmental – IS) distributed in 14 dairy farms in North-East Italy. Farms were distinguished by the presence of the same sensor (SenseHub^{TM} Dairy, Allflex® Livestock Intellingence™; SCR Engineers Ltd., Netanya, Israel) validated for recording the eating times. Aspects related to the individual traits of the cow, management choices, and ration characteristics were examined. In general, the milk production corrected for fat and protein (FPCM) was foreseeably higher in HF than in IS (33 vs 27 kg/d). Considering feeding behavior, the two breeds were similar in view of eating time, while rumination time was higher in HF by almost 1 h per day (572 vs 517 min/d). Statistical analysis, in particular Principal Component Analysis, was performed considering animals grouped into five eating time classes (≤ 180, 181–220, 221–260, 261–300, and ≥301 min/d). The results showed that cows with longer meal intervals (≥ 301 min/d) had greater values for ruminating time. The production of milk and FPCM followed a similar trend, although there were no differences in milk components (fat and protein). Furthermore, as eating time increased, there was a decrease in milk urea, somatic cells count, and BCS. This study provides useful information by understanding the close association between feeding behavior and animal or management factors. Eating time appears well related to several productive and management traits of lactating cows, and its relationship with feeding efficiency warrants investigations.

O48

Can olive cake by-products be employed to produce silages with enhanced nutritional characteristics?

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The alternative use of agri-food by-products in animal feeding has been already suggested as promising. Olive cake (OC) has good potential to be included in ruminant diets, but studies aiming to increase its shelf-life, besides the drying techniques, are quite scarce. In addition, OC has a high content of polyphenols, which were previously associated with beneficial effects on ruminant products. The present study aimed to investigate the ensiling of OC mixed with different proportions of wheat bran (B) to preserve its shelf-life and polyphenols content. Moreover, hetero- (1) or homo-fermentative (2) inoculants were alternatively supplemented to promote the proper acidification of substrates. A total of 8 different treatments were fermented in 16 laboratory silos for 55 days, namely, OC; B + 1 and B + 2; 5 different mixtures of OC and B, namely HIGH OC + B; HIGH OC + B + 1; HIGH OC + B + 2, with a higher proportion of OC, and LOW OC + B + 1; LOW OC + B + 2, with a lower proportion of OC. Samples collected previous (t0) and after (t55) ensiling were analyzed for gross composition, pH, volatile fatty acid profile (VFA), lactic bacteria counts, and polyphenol profile. Additionally, the *in vitro* true degradability (IVTD) and aerobic stability of silages were evaluated. Data were analyzed using a linear model to compare the treatments listed above. B and OC naturally differed for gross composition, having OC higher content of NDF and lower dry matter (35% DM). Consequently, DM was higher in LOW OC + B (~50%) vs. HIGH OC + B (~40%), thus affecting the required pH values (RpH) as an indicator to prevent pathogen growth. All pH values after 55 days of silages were lower than RpH, particularly in B + 1/2, probably because of higher lactate proportion (~55%, $p < 0.001$). Conversely, OC + B silages had a higher propionate proportion (~60%, $p < 0.001$). However, lactic bacteria count in all treatments was higher than B silages. Regarding polyphenols, most flavonoids increased after fermentation because of silage weight loss. Hydroxytyrosol did not increase, except for HIGH OC + B + 1 (+40%), probably produced from native oleuropein-aglycone (−95%) of OC. Unfortunately, HIGH OC + B silages, in contrast to B lonely, reduced aerobic stabilities and IVTD (65% *vs* 85%; $p < 0.001$). Overall, ensiling a mixture of OC and B, both at high and low proportions, can be a promising solution for OC long time *on-farm* preservation, however, some limitations about IVTD and aerobic stability must be considered for high OC silage.

O407

A dynamic mechanistic model to forecast the oscillatory feeding behaviour of lactating dairy cows

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Daily feeding behavior of lactating dairy cows was reproduced by using the model proposed by Fischer on 1996 and a stock and flow diagram structure was used. In particular, the model included specific stocks for protein, soluble carbohydrates, slowly digestible fiber, and very slowly digestible fiber present in rumen-reticulum compartment at a specific time. The regulation of intake was based on feedback structures for distention, chemostatic, and protein effects. Model inputs were dietary levels of main nutrients as well as their rates of digestion and passage. In this trial, 20 Holstein milking cows were random selected to observe the individual feeding intake in an experimental facility (Romeo and Enrica Invernizzi, San Bonico, Piacenza, Italy) for four consecutively days. The cows had (mean ± SD) 38 ± 7 months and a average milk yield of 33.3 ± 4.36 l/cow/day. The feeding behavior was monitored by using the automatic intake recorder Roughage Intake Control System (RIC; Hokofarm group, Marknesse, The Netherlands). The raw data were reported in an electronic spreadsheet, including number of visits, intake per visit (kg 24 DM), and visit duration (min). The other indices of feeding behaviors were calculated from these primary indexes. In particular, the eating event unit was the single 'meal' (time of the RIC visit or consecutive visits within 20 min). After the number of meals taken (n/day), the ingestion per meal (kg DM/meal), the duration

of the meal (min/meal), and the time lapsed (min) between two successive meals were calculated, as well as the rate of feed ingestion (kg DM/min). Oscillation differences in terms of frequency and amplitude (meals per day and amount eaten per meal), and variability of observed meals (kg/per hour) were discussed regarding the average meal of each animal during the testing period. On average, the animal had 7 ± 2 meals/cow *per* day and 18 ± 9.55 visits/cow *per* day. The average intake per meal was 3.60 ± 1.12 kg. The average DMI was 23.81 ± 2.73 kg/cow *per* day and the average time spend to feeding was 21.0 ± 4.60 min per meal. The oscillatory pattern simulated by Fischer's model was able to describe meal fluctuations due to dietary components, whereas, in this trial, animal components had a substantial impact on the fluctuation. The research group will add new feedback loops in the stock and flow diagram modelling structure to try to accurately simulate the daily oscillatory system of lactating cow behavior, as well as daily DMI.

O386

Oxygen barrier plastic films: an opportunity to improve silage quality and reduce the use of plastic

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Plastic is widely used to cover bunker silos and to preserve the ensiled mass from the aerobic deterioration, thus achieving the best anaerobic conditions and allowing the storage of silage for a long period. In this trial, a comparison between two types of films on sorghum silage fermentative quality was carried out: (i) a conventional polyethylene metallocene (PE-met) film or (ii) an innovative combined oxygen-barrier film (2 in 1, OB), with the same physical and mechanical characteristics of PE-met, but still able to maintain silage quality while reducing the use of plastic at farm level. Furthermore, it has been verified the practical aspect of using this new combined barrier film (i.e. OB) in reducing both plastic use (−35%) and covering time. The bunker was divided into left and right parts (the bunker is 8 m wide, with the 1 m central buffer zone between treatments) and they were assigned to one of two tested films (i.e. PE-met or OB covering systems). High-density polyethylene (HDPE) silo bags with the same fresh chopped sorghum (i.e. 6.7 Department of Animal Science, Food and Nutrition (DIANA), 0.3 kg as fresh) were collocated in different zones of the feed-out face, being central (i.e. 4 for each treatment) or peripheral (i.e, lateral, near the wall, or apical for a total of 6 for each treatment) parts, respectively. After 6 months from harvest and approximately 2 months after bunker opening, silo bags were taken off from the ensiled mass, weighed, and subsampled to analyze fermentative, chemical, and microbiological parameters. The dry matter values were lower ($p < 0.05$) in peripheral samples of PE-met than in central samples (29 % DM vs. 32% DM, respectively). On the contrary, greater ($p < 0.05$) ash (7.85 % DM vs. 7.46% DM), acetic acid (3.71% DM vs. 2.31% DM), 1,2-propanediol (4.65% DM vs. 2.54% DM) and ammonia nitrogen (5.80% TN vs. 4.88% TN) contents were detected in peripheral samples of PE-met than central samples, respectively. The lowest ($p < 0.05$) ammonia nitrogen, acetate and propionate contents were measured in peripheral samples of OB. No differences among treatments were observed for pH, lactic acid, and microbiological counts. The results indicate that combined barrier film is an innovating tool that allows achieving a safe and well-fermented product in comparison with a traditional PE film. Furthermore, this trial confirms that using this new combined barrier film allows for reducing the covering time by 60% improving comfort and security conditions of the farmers.

O554

Novel feedstuffs obtained mixing different by-products: nutritional evaluation

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The use of agro-industrial by-products as animal feed could be an important component of the global strategy to reduce the environmental impact of agro-industrial and livestock production. Indeed, the use of some by-products as animal feed has been explored and could represent socio-economic and ecological benefits (e.g. recycling, cost reduction and feed-to-food competition). The prickly pear by-products, obtained from *Opuntia ficus-indica* (L.) Mill. fruits could be an excellent resource for feeding ruminant. The fruit is mostly eaten fresh after peel removal. This results in a large availability of this by-product which, like other by-products, is subject to seasonality and is difficult to store. In this study, we present the results obtained ensiling prickly pear peels (PPP) with straw or wheat bran in different percentages, with the aim of increasing the PPP storage aptitude. For this purpose, fresh PPP are ensiled alone (PPPA) and with the addition of straw (5% fresh weight: PPPS) or wheat bran (6%, PPPB6 or 12%, PPPB12, fresh weight). Six vacuum-packed micro-silos were prepared for each treatment and, after 40 days of storage, the state of conservation was assessed. Therefore, a total of 24 silage samples were analyzed for chemical analysis (dry matter, protein, fat, ash, and Van Soest fiber fractions). To evaluate the state of conservation, the pH was measured, N-NH3 and organic acids (acetic, propionic, butyric, lactic) were detected. Fermentability was determined by incubating each dry sample inside a bottle containing sheep rumen fluid at 39 °C under anaerobic conditions to evaluate the disappearance of organic matter, final products (volatile fatty acids) and fermentation kinetics. The differences between the micro-silos were evaluated with an ANOVA model. The results showed that the substrate significantly ($p < 0.01$) influenced the storage aptitude, chemical composition, and fermentation characteristics. The addition of straw or wheat bran increased silage dry matter (14.23%, 14.38, and 17.25% for PPPS, PPPB6, and PPPB12, respectively), which was the lowest for PPPA (8.84%). The use of wheat bran with PPP showed significantly higher protein percentages. Overall, the quality of the silages was excellent for the lactic/acetic acid ratio (> 2.3%), for the low pH values (range 3.82–4.01), and for the N-NH3/total N ratio (range 0.44–4.92). In addition, wheat bran silage, although more expensive, produces better quality silage.

O81

High levels of nitrate in fresh meats: does nutrition play a role?

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The 'natural' presence of quantifiable amounts of nitrate in fresh meats has been reported by several authors. The source of such presence has never been identified. The animal diet can play a role, but it has never been demonstrated.

In this work, 160 samples of animal feed were analysed for the detection of nitrite and nitrate. Samples of commercial concentrate feed for cow (23), chicken (23), swine (23) and equine (16), forage (7), alfalfa (13), hay (7), seeds of barley (7), wheat (7), corn (7), soy (7), oats (7), bran (7) and other seeds (6) were analysed. The monitoring was carried out by capillary ion chromatography with conductivity detection, and the most interesting results were 'confirmed' using another consolidated ion chromatography method.

Alfalfa samples showed the highest nitrate concentrations, with mean level of 994.0 mg/kg, and the highest nitrate concentration (1321.9 mg/kg). High nitrate concentrations were also detected analysing legume and meadow hay samples (184.0 mg/kg) and forage (mean level: 401.1 mg/kg) Among processed feeds (simple, compound/complex and complementary), feeds for cow and equine showed the highest concentrations (mean levels equal to 16.5 and 39.6 mg/kg, respectively), followed by feed for swine and for chicken. This last type of feed was characterized by nitrate levels lower than those detected in other raw materials (bran, oats, soy, barley) and slightly higher than wheat and corn samples. Regarding nitrite detection (8 samples in the range 3.7–21.8 mg/kg), the study demonstrated no correlation between nitrite amount and feed type.

The monitoring demonstrated that alfalfa is the feed that mainly contribute to nitrate intake in animals, followed by other types of grass such as forage and hay. Commercial feeds contribute to a lesser extent, and feed for chicken is characterized by very low levels of nitrate, lower than those detected in other raw materials such as oats, bran, etc.

A preliminary trial was also made by comparing meat samples obtained by introducing alfalfa in the diet of 2 livestock units of beef cattle with 2 units not fed with alfalfa. After slaughter, nitrate concentrations equal to 36.1 and 37.1 mg/kg were detected where alfalfa was used with no quantification in other 2 samples. This preliminary result confirmed a possible role of animal nutrition when nitrates are quantified in fresh meats.

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O419

Camelina sativa and Cynara cardunculus cakes supplementation on performance and milk composition in dairy goats during the transition period

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The main aim of the research was to improve the quality of goat milk and derived milk products by exploiting the specific properties of agro-industry co-products such as camelina and cardoon cakes. Thirty-six Alpine multiparous dairy goats (age: 3.04 ± 0.85 years; average daily milk production: 3.93 ± 1.23 L) at ninetieth days of pregnancy were housed in single box and randomly assigned into four isonitrogenous and isoenergetic different diets. The groups were ($n = 9$/group): a control diet (C), without experimental supplementation; a diet supplemented with 5.4% of cardoon cake (CD); a diet supplemented with 7.6% of camelina cake (CAME); a diet supplemented with 6% of a mixture of cardoon and camelina cakes (CACD). Individual feed intake levels and milk yield were recorded daily. Live body weight (LBW) and body condition score (BCS) were recorded weekly; milk samples were collected at day 4, 7, 14, 21, 28; ruminal fluids were collected at day −28, 2, 28 and pH was measured. Collected data were analysed by mixed procedure of SAS for repeated measurements. Significant level was considered for $p < 0.05$ and tendency for

$0.05 < p < 0.1$. No significant treatment effect was observed in milk production, LBW, BCS and ruminal pH ($p > 0.05$). No differences were observed in milk protein content, caseins, urea and lactose ($p > 0.05$). Milk fat content tended to be higher in CACD compared with other treatments at day 4 (CACD 6.95 ± 027, CAME 5.60 ± 0.25, CD 5.91 ± 0.25, C 6.16 ± 0.24 g/100 mL; $0.05 < p < 0.1$) and in C compared with CAME and CD at day 7 (C 5.84 ± 0.24, CAME 5.05 ± 0.25, CD 5.14 ± 0.25 g/100 mL; $0.05 < p < 0.1$). CD linear score of somatic cells count (SCC) values tended to be higher compared with other treatments ($0.05 < p < 0.1$). Moreover, unsaturated fatty acids (UFA) were significantly higher at day 4 in CACD than other groups (CACD 2.15 ± 0.11 vs CAME 1.66 ± 0.10, CD 1.67 ± 0.10, C 1.7 ± 0.10 g/100 g; $p < 0.05$) and at day 7 C group was higher than CD group (C 1.76 ± 0.10 vs CD 1.42 ± 0.10 g/100 g; $p < 0.05$). Camelina and cardoon are valuable sources of unsaturated fatty acids, as PUFA, and their intake can affect their content in milk, implying greater food quality and possible health benefits for consumers. In our trial, supplementation with cardoon and camelina cakes did not change performance of transition dairy goats while was able to modify fatty acid profile of their milk. Overall, these co-products could be excellent protein sources alternatives to soy also because more environmentally sustainable.

O420

Impact of maternal milk from goats fed with *Camelina sativa* and *Cynara cardunculus* cake on goat kids' growth performance and health status

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Cardoon and camelina cakes, are an excellent source of functional components, in particular polyunsaturated fatty acids (PUFA) such as omega-3, branched fatty acids and flavonoids. The aim of this project was to evaluate the effects of maternal milk from goats fed with cardoon and camelina cakes on growth performances and blood metabolic biomarkers in goat kids. Thirty-six Alpine dairy goats were enrolled and divided into four experimental groups ($n = 9$/group) characterized by different diets: a control diet (C), without experimental integration; a diet supplemented with 5.4% of cardoon cake (CD); a diet supplemented with 7.6% of camelina cake (CAME); a diet integrated with 6% of a mix of cardoon and camelina cakes (CACD). The offspring was fed for the entire duration of the trial exclusively with milk from the goats belonging to the mother's experimental group. Goat kids were fed two times a day and housed in a common box. After birth, the quality of colostrum was evaluated and each goat kids were fed its mother's colostrum. Live body weight (LBW) and average daily gain (ADG) were measured and calculated weekly. At the beginning and at the end of the trial, blood samples were collected from jugular vein. At the end of the experimental period, the male goat kids were sent to the slaughterhouse according to farming practices. Carcass weight and carcass yield (carcass weight/slaughter weight) were measured. Collected data were analysed by mixed procedure of SAS for repeated measurements. Significant level was considered for $p < 0.05$ and tendency for $0.05 < p < 0.1$. No significant treatment effects were observed in growth performances ($p > 0.05$). It has been observed a significant difference between sex in LBW and ADG parameters (LBW male: 7.04 ± 0.14, female: 6.48 ± 0.14 Kg; ADG male: 0.20 ± 0.005, female: 0.17 ± 0.005 Kg/day; $p < 0.05$). No significant differences between treatments were found in carcass weight and carcass yield ($p > 0.05$). CACD kids showed lowest values of glucose and highest values of NEFA, at day 28 compared with other treatments. Although camelina and cardoon cakes did not show significant differences in performance and health status among the experimental groups, they were able to provide goats with an adequate nutritional intake during transition period, ensuring a suitable pre- and post-natal development for the goat kids. Consequently, cardoon and camelina cakes can be considered as valuable substitutes of conventional feed protein ingredients.

O462

Effect of fennel seeds as a dietary supplement in grazing goats: preliminary results

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Fennel (*Foeniculum vulgare*) is an annual plant belonging to the family of *Apiaceae*, widely used in Mediterranean areas for its aromatic and medical properties. Fennel is well-known for carminative, digestive and galactagogue properties which makes its seeds particularly used during the lactation stage. In our trial, 20 pluriparous Cilentana goats homogeneous for body weight (BW: 50 ± 2 kg), parity (3rd) and milk yield in the previous lactation (1450 ± 120 g/head/day), immediately after kidding (first week of March 2022) were equally divided into two groups (C: control; F: fennel) fed on a permanent pasture (9:00 am to 4:00 pm). In the pen both groups received 400 g of concentrate mixture composed by barley and corn meals (50/50) (CP: 100,4 g/kg; EE: 31,6 g/kg; UFL/kg: 1,06) and group F diet was supplemented with 15 g/head/day of organic dried fennel seeds (procured by Biokyma© S.r.l) mixed with concentrate mixture at the time of administration. By the beginning of May, milk yield was daily measured while samples of milk and pasture were monthly collected up to September, and analysed, as well as concentrate samples, for chemical composition. Data were analysed using the two-way ANOVA with JMP software (version 11, PROC GLM, SAS 2000). According to previous trials using fennel seeds, milk yield was significantly higher in group F (1809.59 g vs 1418.27 g for group F and C respectively; $p < 0.001$), probably due to the high content of anethole in the fennel seeds. The anethole has estrogenic properties thus able to stimulate the prolactin secretion. In contrast, no differences were observed for fat (C: 4,27% vs F:4,01%), protein (C: 2,93% vs F: 2,88%) and lactose (C: 4,25% vs F: 4,13%) content. However, the investigation on other possible effects that such integration may have on milk quality (i.e. aromatic and fatty acid profile) are in progress. In conclusion, this result is particularly interesting since goat milk in this area is mainly intended for cheese making, therefore a strategy which allows to increase animal performances without the ‘dilution effect’ could be advantageous.

O93

Cashew (*Anacardium occidentale* L.) apple by-products: a promising feed in animal nutrition

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Cashew is a tree from tropical and sub-tropical areas. Native to tropical America, it was introduced in several countries of Asia, Africa, and Central America as an economically important agricultural crop. The nuts are the first interest of cashew production, while the apples are wasted as by-products. One ton of nuts produced is lead to 10–15 tons of apples as by-products. According to this estimation, about 61.13 million tons of apples are generated every year and only 10% of them are transformed into juice, vinegar, jam, chutney, or soft drinks. The valorization of cashew apples in animal nutrition could contain the environmental impact and provide a supplement to the animals, especially during the dry season when feed resources are limited. This study aimed to assess the nutritional characteristics of cashew apple by-products as feed for ruminants. To achieve this aim cashew by-products led the processing technic (whole, up part, down part, middle part, and pulp) were collected regarding the variety (yellow and red) and cultivation areas (Sudanian and Guinea) in Benin Republic. In total of 20 samples (5 parts/variety (red vs yellow) in 2 cultivations areas) were analyzed in terms of chemical composition using AOAC procedures: dry matter (DM), crude protein (CP), ether extract (EE), ash, and Neutral Detergent Fiber (NDF). In addition, the total (TS) and free sugars (FS) content were also quantified in the whole apple and pulp. The *t*-test and Tukey’s HSD test were applied to all data using JMP software with the following model: $Y_{ijk} = U + Z_i + V_j + P_k + (V^*P)_{jk} + {}_{ijk}$. The results showed a significant ($p < 0.01$) difference between the areas for most of chemical parameters except for the sugar content. All by-products resulted low in DM (between 10 and 24% a.f.) highlighting the conservation issue. The highest CP, EE and NDF content was found in the pulp (11.7, 4.40 and 32.9% DM, respectively; $p < 0.001$). The up, middle, and whole parts reported the highest ($p < 0.01$) of non-structural carbohydrates (NSC); in particular, the results of the analysis showed that the whole cashew apples are richer ($p < 0.01$) in TS and FS than the pulp. Considering the interaction varieties and parts of by-products, all parameters were statistically different. The results suggest apple pulp as the best by-product from cashew production to be considered in animal nutrition. Further investigations need to be performed; the study of *in vitro* fermentation characteristics is still in progress.

O133

Automation to support rationing of dairy and beef cattle: comparisons and practical examples of application

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Getting a balanced ration delivered to and consumed by cows is fraught with mechanical and human error risks. Animal feeding requires skill and attention to detail to deliver a high-quality, uniform ration to all cows.

Technology, even more automation, provides valuable support to the farmer in preparing the ration.

This research has analysed three kinds of farms in upper Po valley, with Freisan Holsteins cows, equipped or not with technologies supporting cattle rationing (conventional mixing wagon, an optical sensor for image analysis, automatic feeding systems – AFS) to point out the step forward such technologies provide to animal farmers. Comparisons occurred evaluating the TMR samples before and immediately after the distribution (before animal feed sorting) using the Penn State Particle Separator. In addition, the average length of the fibres retained by the first sieve (>19 mm diameter) was also assessed, together with the effect of the filling level of the mixing wagon. The data collected during the monitoring underwent processing using the general linear model multivariate procedure followed by Tukey's test for post hoc multiple comparisons.

According to the results, using an optical sensor or an AFS resulted in a slight but significant lengthening of the ration fibre compared to conventional mixing wagon technology (5.7 ± 2.7 cm and 5.6 ± 2.2 cm vs 4.8 ± 2.4 cm).

Compared to a conventional mixing wagon, image analysis in supporting TMR preparation results in a ration granulometry that does not differ from that of an expert operator.

The TMR from AFS is coarser than that from a conventional mixing wagon due to the reduced volume of the mixing container. This aspect was confirmed by studying the effect of the level of the mixing of the conventional mixing wagon: the mixing wagon filled fully results in a more refined TMR texture.

In conclusion, it can be stated that technology provides proper support in the preparation of the ration, also providing help to less expert operators, avoiding daily alterations of the unifeed with consequent metabolic and production problems in the cattle.

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O281

Effect of feeding enriched-olive cake on metabolic and milk performance response of mid-lactating Holstein cows

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The use of olive cake (OC) as animal feed is an interesting and sustainable alternative to its disposal, because it may decrease the costs associated with animal feeding, valorizing a by-product and at the same time improving the quality of the products. However, a limited number of studies have evaluated the effect of processed OC on metabolism and performance of dairy cows. Thus, the aim of this study was to assess the effects of adding dried enriched-OC with leaves and olive wastewater in the concentrate of mid-lactating Holstein cows on metabolic profile, rumen fermentations, and milk performance. A total of 20 cows were allocated, according to days in milking, parity, and milk yield, into 1 of 2 dietary isoenergetic and isonitrogenous treatments: control group (CTR) was fed a conventional concentrate, whereas treated group (EOC) was fed a concentrate with 7% of OC integration during 28-d experimental period. Blood and milk samples were collected at 0 and 28 d (end of period) together with rumen liquid samples. Data were analyzed with the proc GLIMMIX of SAS. Compared with CTR, cows of EOC group showed lower milk protein (3.31 vs. 3.14 ± 0.06%; $p = 0.01$), lactose (tendency; 5.0 vs. 4.95 ± 0.03%; $p = 0.06$). Cows in EOC group had greater ($p < 0.05$) blood cholesterol (6.54 vs. 5.91 ± 0.1 mmol/L), P (2.73 vs. 2.47 ± 0.08 mmol/L), Zn (9.96 vs. 8.70 ± 0.5 mmol/L), FRAP (163 vs. 154 ± 5 µmol/L), ROM (13.1 vs. 12.1 ± 0.5 mg of H_2O_2/0.1 dL), and AOPP (32.6 vs. 29.1 ± 1.5 µmol/L), but lower ($p < 0.05$) glucose (3.7 vs. 3.9 ± 0.1 mmol/L), albumin (35.6 vs. 36.2 ± 0.3 g/L), ALP (68.3 vs. 72.9 ± 2.2 U/L), and creatinine (81.3 vs. 84.3 ± 1 µmol/L) compared with CTR cows at 28 d. A tendency for lower rumen butyrate in OC compared with CTR cows was also obtained (11.65 vs. 13.78 ± 0.77 mmol/L; $p = 0.06$). These results suggest that OC integration at 7% in the concentrate does not affect milk yield, but milk protein and lactose due likely to the higher low-degradable fiber content of OC which in turn decrease protein and energy metabolism in the rumen (lower rumen butyrate and blood glucose). However, OC positively modulated the liver metabolism and inflammatory response, but further research is needed to evaluate the response of the oxidative status (greater ROMt and AOPP) which was unexpected since OC brings a high amount of polyphenols content.

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O308

Field study on the effects of pasteurization on colostrum quality of Holstein dairy cows

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Calves are born with almost no antibodies and they strictly depend on colostrum ingestion to acquire maternal passive immunity against infectious diseases. The neonatal calf should receive at least 3 to 4 L of good quality colostrum within 6 h of birth to guarantee the intestinal absorption of a proper amount of maternal immunoglobulins (**Ig**) that will protect it from diseases for the first months of life. Good quality colostrum has more than 50 g/L of Ig and less than 100,000 ufc/mL of bacterial count (**BC**). Bacterial transfer to newborn calves with colostrum is a highly health-risky practice, and neonatal diarrhea is still the first cause of calf death. This field study was carried out in 2021 on a commercial Italian Holstein dairy farm with the aim of analyzing the effect of pasteurization on colostrum quality. Colostrum from 28 cows was sampled at the harvesting and after pasteurization at 60 °C for 60 min. All samples were frozen and subsequently sent to the laboratory for the assessment of Ig content by electrophoresis and BC by optofluorometry. The proportion of colostrum samples with less than 50 g/L of Ig tended to increase with pasteurization, varying from 21% before- to 43% after- heat treatment (Chi-square test, $P = 0.086$). Pasteurization reduced to zero the share of colostrum samples with BC >100,000 ufc/mL, which was 46% at the harvesting (Chi-square test, $p < 0.001$). These field results suggest that, while pasteurization treatment is fully effective in reducing colostrum BC, attention should be paid to its negative effects on colostrum immunity properties. Poorer Ig content leads to lower calf serum Ig concentration and little is known about the effects of the heat treatment on other colostrum components (e.g. growth factors). From the sanitary side, colostrum pasteurization looks optimal solution to face colostrum contamination problems.

O278

Precision feeding: amino acid balance in dairy cows fed two different levels of total crude protein

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Aim of the project was to evaluate the possibility to reduce crude protein (CP) content of dairy cows' diet by balancing the amino acids profile of the ration, through the individual administration of rumen protected (RP) methionine (MET) by the automatic milking system (AMS). The effects of the CP level were evaluated on milk yield (MY) and composition, total tract fibre digestibility (TTDpdNDF) and nitrogen excretion. The trial was performed in a commercial dairy farm equipped with two AMS. Twenty primiparous and 20 multiparous (MULT) cows were enrolled in the trial and fed a silage-based ration (15 CP %, DM), supplemented with RP MET (18 g/h/d) and lysine (LYS, 39 g/h/d). The cows were randomly assigned to 1 of 2 group and supplemented (5 kg/h/d) by AMS, a low (15% CP, LP) or a high protein (20 CP%, HP) pellet, containing both 0.2 % of RP MET, for a total CP content of 15.5 and 16.3% in LP and HP diets, respectively.

The treatments were fed alternatively to each group for 4 periods of 4 weeks each in a cross-over design, at the end of which individual milk, faeces and blood samples were collected to analyse milk composition, fibre digestibility and milk and blood urea content. Daily individual MY was recorded by AMS. Chemical composition and protein digestibility of feeds were analysed monthly. Data were statistically analysed by a mixed model with repeated measure, with treatment, periods, DIM, parity and interactions as fixed effects and cow as random. Data recorded before the beginning of the trial were used as covariate.

Considering all cows, MY and energy corrected milk (ECM) were similar between diets (40.0 vs 40.1 kg/d and 37.9 vs 38.0 kg/d for LP and HP), while MULT cows had a higher ($p < 0.05$) MY and ECM with the HP diet (40.5 vs 41.2 and 39.2 vs 39.5 kg/d).

Milk composition was not influenced ($p > 0.05$) by the CP level of the diet, nor considering fat (3.71 LP vs 3.62 HP, %), protein (3.51 LP vs 3.48 HP, %) or casein (2.77 LP vs 2.74 HP, %). Interestingly, milk urea content was significantly reduced in the LP diet (22.64 vs 24.74 mg/dl), as well as blood urea content (3.92 vs 4.52 mg/dl, for LP and HP, respectively). The TTDpdNDF was not affected, and was on average 76.11 and 76.25 % pdNDF, for LP and HP. Overall, the supplementation of RP MET and LYS permitted to reduce the CP content of the ration with a significant reduction of nitrogen excretion, maintaining MY, milk composition and TTDpdNDF, thus improving nitrogen efficiency.

O263

Impact of dietary lipid source on fecal microbiota composition in dairy cows

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The degree of saturation of the dietary lipid sources could affect the microbiota of the animal digestive tract. Aim of this experiment was to evaluate the influence of two different lipid sources, one saturated (hydrogenated fatty-Megafat 88; FAT), and one unsaturated (soybean full-fat flaked; SOY) on fecal microbiota composition of high-producing dairy cows fed hay – based ration. The study involved 8 Italian Friesian dairy cows and pH, rumination time, rumen and feces microbiota composition (bacteria and protozoa) were determined. DNA from rumen contents was extracted, purified, and sequenced to quantify bacteria populations. Sequencing was performed on Illumina MiSeq. Data were analyzed with JMP pro v 17.1. For the statistical analysis, a linear mixed model was used with a fixed effect: Saturated Fat vs Unsaturated fat. The different lipid sources significantly influenced bacteria community in feces. Ruminococcaceae family showed a tendency ($p = 0.06$) being higher in FAT compared to SOY (27.9% vs. 24.5%), while Peptostreptococcaceae were higher in SOY compared to FAT (3% vs. 1.95%). Rikenellaceae were significantly higher in SOY (21.8% vs 19.7%), while Prevotellaceae did not differ, as well as Fibrobacteriaceae, Lachnospiraceae, Christensellaceae and Veilonellaceae. Moreover, within the same treatment group, the cited families were significantly different among animals for both SOY and FAT, with the exception of Rikenellaceae, which did not differ in SOY treatment. Since this part of the study followed a similar analysis of the rumen microbiota of the same animals, it was possible to observe substantial differences among the two. Surprisingly, Proevotellaceae, the most abundant family in the rumen, showed a decreased abundance (11% on average), while Rikenellaceae, which are not so represented in the rumen, increased their relative abundance in feces. Ruminococcaceae were more abundant in feces compared to the rumen, and even the family composition, in terms of genera, was different. In conclusion, the lipid source affected fecal microbiota, showing differences in families that are not abundant in the rumen, but highly represented in the intestinal tract of different mammalian species, including cows, buffaloes, and humans. At the same time, data underlined the individual response even when animals are fed the same lipid source, and the unicity of the intestinal environment compared to the ruminal one.

O491

Effects of 3-nitrooxypropanol (3-NOP, Bovaer®10) on methane emission and productive performance of mid-lactating dairy cows fed a typical diet for Grana Padano cheese-making

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Dairy farming contributes to GHG emissions due to enteric methane production. A nutritional approach has been proposed to mitigate enteric methane production. The study aimed to investigate the effects of the addition of 3-NOP (Bovaer®10, DSM, Switzerland), an inhibitor of enteric methanogenesis, to the diet of dairy cows on methane emissions, feed dry matter intake, milk yield (MY) and composition (fat, protein, lactose). The study was carried out at the CERZOO (Research Center for Livestock and Environment, s.r.l., Piacenza, Italy). Thirty dairy cows (initial MY 36.8 ± 7.5 kg; days from calving 154 ± 67) were balanced into two groups and fed for nine weeks a typical Grana Padano cheese diet (corn silage 37.3%, alfalfa hay 24%, barley straw 2.2%, concentrate 36.5%, 1.63 Mcal kg net energy for lactation, 15.8% crude protein, on dry matter (DM) basis). The diet was either supplemented (TRT) with 600 mg/kg DM of Bovaer®10, (corresponding to 60 mg/kg of 3-NOP) or not (CTR). Single cow was the experimental unit and was monitored for feed intake (Roughage Intake Control system; Hokofarm Group, The Netherlands), enteric emissions (CH_4, CO_2, H_2; GreenFeed system, C-Lock, Inc., Rapid City, SD), MY and composition (Afilab, Afimilk Israel). A fourteen-day pre-treatment period was observed for covariate adjustment of collected data. A mixed model with repeated measures was used for data analysis (JMP®, Version 17.0.0, SAS Institute Inc., Cary, NC, 1989–2021), with the cow as random and the period as a fixed effect. DMI was numerically ($p = 0.11$) lower in TRT (24.6 kg/d) compared with CTR (26.2 kg/d) group. The milk, fat, protein, and lactose yields were similar between groups. The inclusion of 3-NOP in the diet affected ($p < 0.05$) the enteric emissions per kg of DMI, with a reduction of CH_4 (15.8 vs. 8.4 g/kg DMI), an increase of CO_2 (535.6 vs. 587.5 g/kg DMI) and H_2

(0.08 vs. 0.40 g/kg DMI), respectively for CTR and TRT groups. The feed efficiency (fat and protein corrected milk/kg DMI) was numerically higher in TRT (1.30) compared with CTR (1.26) group. Including 3-NOP in a diet fed to lactating dairy cows significantly reduced (47%) the enteric CH_4 emission without affecting the DMI, MY and its composition, with a numeric improvement of milk efficiency, suggesting it could represent an interesting option to reduce enteric emissions for a more sustainable dairy breeding.

O463

Effects on feed efficiency and resilience in the transition period of Holstein dairy cows fed with different sources of rumen-protected choline

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Transition is the most challenging period in the dairy cow career, mainly for the abrupt modifications in metabolism and sever inflammation occurring at the onset of lactation. One critical aspect of this physiological process is the hepatic oxidation of lipids. Choline is an essential nutrient involved in the transport of triglycerides from the liver, thus, feeding rumen-protected choline (RPC) to cows could be helpful to prevent fatty liver. With the aim to evaluate effects of 2 sources of RPC supplemented from 21 d pre- to 35 d postpartum on metabolism, health and performance, 24 Holstein dairy cows fed a corn-silage based total mixed ratio (TMR) were randomly assigned to 3 groups: TMR unsupplemented (CTR), TMR supplemented with 60 g/d of micronized RPC (P1) or with 25 g/d of coated RPC (P2). Either RPC supplements provided 15 g/d of choline chloride. Individual dry matter intake (DMI), rumination time, milk yield (MY), its composition and BW were recorded daily. Data were analysed under a mixed model (SAS) with cow as random effect, and parity and days in milk (DIM) as fixed effects. All the cows were in a good health status and no clinical signs of ketosis were recorded. No difference was observed for DMI, rumination time and BW. On day 7 before calving, CTR cows had higher levels of biomarkers related to inflammation (e.g. ceruloplasmin 2.9 vs 2.3 vs 2.2 µmol/L, $p < 0.02$, for CTR, P1, P2 respectively), oxidative stress (e.g. higher myeloperoxidase: $p < 0.06$, as well as Reactive Oxygen Metabolites (15.1 vs 11.9 vs 11.2 mg H_2O_2/dL, $p < 0.03$, for CTR, P1 and P2, respectively). On day 3 after calving, P1 vs P2 showed higher concentrations of plasma glucose (NS), and lower NEFA (NS) and BHB ($p < 0.1$), and a higher Liver Functionality Index (3.3 vs 2.7, NS). Treated cows had numerically higher MY, fat and protein output. P1 tended to have higher milk efficiency between 11 to 21 DIM compared to CTR ($p < 0.07$) and P2 ($p < 0.11$). Overall, feeding RPC to dairy cows in the peripartum helps them (but response to P1 seems more pronounced) to afford the inflammometabolic challenges and increases their resilience and performance.

O128

Nutritional profile of hay from tef (Eragrostis teff) genotypes grown in South Italy

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Annual warm-season forages could be used in temperate climate to fill production and nutritional gaps in summer when water is scarce, as well as in arid and semi-arid conditions.

This study evaluated the nutritive value of tef [*Eragrostis teff* (Zucc.) Trotter] forage, an annual grass native to Ethiopia that can potentially provide large quantities of biomass for ruminants in the hottest months of the year.

Fifty genotypes were sown in Foggia (Apulia, Italy) and grown during the spring-summer of 2017. A completely randomized block experimental design with two replicates was used. During the plant growth, parsimonious irrigation was applied using a micro-irrigation system. For each genotype, a first (C_1) and a second (C_2) mowing at the earing stage was performed. After drying, the samples were ground and subjected to nutritional analysis. Statistical analysis was conducted through a full factorial ANOVA and Fisher's LSD post-hoc test for means' comparisons. Interactions with mowing and agronomic yield has been also considered. The significance level adopted was 5% ($p < 0.05$). The mean yield resulted significantly higher ($p < 0.01$) for the first cut (6.0 ± 3.1 t DM/ha) respect to the second one (3.5 ± 3.1 t DM/ha). Overall, the compositional values (min-max, on DM basis) of the genotypes under test were: 12.5–18.6% crude protein, 1.1% crude fat, 55.4–62.0% aNDFom, 36.6–41.6% ADF, 12.0–15.28% ash. The first mowing was higher ($p < 0.01$) than the second as far as the ash (13.8 ± 0.9% *vs* 13.3 ± 0.1% DM), and the ADF (39.4 ± 0.2% *vs* 37.8 ± 0.2% DM) contents.

In contrast, the second cut was significantly higher ($p < 0.01$) in crude proteins (15.3 ± 0.1% *vs* 15.9 ± 0.1% DM), and in aNDFom (57.9 ± 0.2% *vs* 58.9 ± 0.2% DM). No effect of mowing time on

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crude fat (1.1 ± 0.1% DM) was observed. The genotype × cut and genotype × cut × yield interactions were significant for all the chemical parameters studied, except for the crude fat.

These preliminary results demonstrate tef to be a valuable fodder plant in water-scarce environments, such as the areas of Mediterranean basin. However, the choice of the genotype should be further investigated in light to select the most performing ones, especially in the case of multiple mowings.

O39

Rumen lipid biohydrogenation of insect meals: results of an *in vitro* study

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Full-fat insect meals generally contain high ether extract (EE) contents. Therefore, they could be used as potential lipid sources to increase the energy density of diets destined to farmed animals, but also, in some cases, to improve the fatty acid (FA) profile of derived food products. This trial was designed to evaluate eight full-fat insect meals (*Acheta domesticus* – ACD; *Alphitobius diaperinus* – ALD; *Blatta lateralis* – BL; *Gryllus bimaculatus* – GB; *Grylloides sygillatus* – GS; *Hermetia illucens* – HI; *Musca domestica* – MD; and *Tenebrio molitor* – TM) as potential lipid sources in ruminant nutrition. The EE content of the tested meals ranged from 203 g/kg dry matter (DM) in GB to 392 g/kg DM in TM. Unsaturated FA represented more than half of total FA in the tested meals, except for HI (42.3 g/100 g FA) and BL (48.8 g/100 g FA). The tested meals were incubated *in vitro* using the rumen fluid obtained from four cannulated sheep fed a 70:30 forage to concentrate diet, on a DM basis. After 24-h incubation, the FA of rumen digesta were measured. A low branched-chain FA content in the ruminal digesta was found for all the tested insect meals (≤ 7.32 g/100 g FA). The ACD, TM, GB, GS and ALD kept high concentrations of FA with potential human health-promoting effects as unaltered after ruminal biohydrogenation (e.g. C18:2 n-6: from 12.8 to 22.3 g/100 g FA; C18:2 *c*9*t*11: from 0.04 to 0.53 g/100 g FA; C18:1 *t*11 (+*t*9–10): from 1.8 to 4.5 g/100 g FA). Various and complementary factors can explain the obtained results, such as the EE content of the insect meals as well as their FA composition and unsaturation level. First, the high amount of EE in the insect meals could have exerted an inhibitory effect on the ruminal microflora. Moreover, some insect meals (ACD, TM, GS) were rich in polyunsaturated FA (PUFA) which could also have exerted an inhibitory effect on the ruminal microflora responsible for their biohydrogenation. The FA profile of the rumen digesta of ACD, ALD, GB, GS and TM, being rich in n-6 PUFA, could be interesting to improve the quality of ruminant-derived food products. At this regard, HI seems to be much less interesting. Indeed, both HI meal and its rumen digesta were particularly rich in lauric (C12:0) and myristic (C14:0) acids which are transferred to ruminant-derived food products without being further modified, thus increasing the level of undesirable atherogenic compounds in these products.

O440

Relationship between diets characteristics and milk composition in lactating dairy cows fed silage- or hay-based diets

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To study the relationship between diets characteristics and milk productivity and composition in dairy cattle, a total of 21 dairy herds located in northern Italy, 12 fed silage-based (SB) and 9 fed hay-based (HB) total mixed ration diets, were monitored over a period of 8 months. Diets, pooled feces, and bulk milk were sampled on a monthly base in each herd. Productivity was registered. Diet and feces analysis included Dry Matter (DM), ash, Crude Protein (CP), soluble protein (SolP), ether extract (Fat), Neutral Detergent Fiber treated with amylase (aNDFom) and Neutral Detergent Residue treated with amylase (aNDF), Nitrogen bound to NDF (N-NDF), Acid Detergent Fibre (ADF), lignin (ADL) emicellulose (E), cellulose (C), starch (St) and undigested NDF (uNDF). Nutrients digestibility were estimated using uNDF as marker. Milk analysis included fat, protein, lactose, casein (KCnb), somatic cells, urea, and coagulation properties, including RCT, A30, K20 and AMAX.

Milk yield did not differ between dietary typology (33.64 vs 34.31 kg/d in HB and SB diets respectively) and HB diets increased milk fat decreasing K20. Excess CP affected negatively the coagulation properties AMAX ($r = -0.266$; $p < 0.001$), A30 ($r = -0.181$; $p < 0.001$) and RTC ($r = -0.154$; $p < 0.05$), increasing urea ($r = 0.284$; $p < 0.05$) and reducing KCnb ($r = -0.169$; $p < 0.046$); SolP tended to reduce RTC ($r = -0.357$; $p < 0.05$) and K20 (-0.413; $p < 0.05$), depressing KCnb levels ($r = -0.331$; $p < 0.013$). The

RTC and K20 were positively affected by C levels in diet ($r = 0.171$ and 0.173; $p < 0.05$). Dietary Fat was positively related with all lipidic fractions in milk ($p < 0.05$), which increased with aNDFom and ADF estimated digestibility ($p < 0.05$). The latter had a positive impact on the estimated cheese yield ($r = 0.134$; $p < 0.05$ and $r = 0.173$; $p < 0.001$ respectively). Dietary starch levels were positively related with milk yield and composition ($p < 0.001$) while aNDF proportion reduced lactose levels ($r = -0.246$; $p < 0.001$). This project received funding from the European Union's Horizon 2020 research and innovation program, under grant agreement No 777974.

O9

Milk metabolome reveals pyrimidine and its degradation products as the discriminant markers of different corn silage-based nutritional strategies

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In this study, the impact of 6 different feeding systems on the chemical profile of milk was studied. The bulk milk samples were collected by the tank of 36 farms containing both afternoon and successive morning milking. The 36 farms represented a random subsample of a cluster previously identified in a previous work by our research group. These farms raised Holstein-Friesian cows, housed in freestall barns, fed TMR, and with no access to pasture. Overall, 6 main nutritional strategies, based on high use of (1) high-moisture ear corn (HMC) and legume silage (cluster 1), (2) compound feed (cluster 2), (3) corn and soy meals (cluster 3), (4) HMC and soy meal (cluster 4), (5) cornmeal and protein compound feed (cluster 5), and (6) HMC and protein compound feed strategies (cluster 6) were considered. The orthogonal projection to latent structures discriminant analysis combined with variables' importance in projection method and Volcano plot ($p < 0.05$), discriminated milk samples according to the high-moisture ear corn (HMC) included in the diet formulation. The most discriminant compounds, identified as a function of the HMC, belonged to aminoacids, peptides pyrimidines, and pyridines. The discriminant metabolites belonged to metabolic pathways of pyrimidines and vitamin B6. The pathway analyses revealed that the inclusion of HMC in the diet formulation strongly affected the pyrimidine metabolism in milk, determining a significant up-accumulation of pyrimidine degradation products, such as 3-ureidopropionic acid, 3-ureidoisobutyric acid, and 3-aminoisobutyric acid. Pyrimidine intermediates (such as l-aspartic acid, N-carbamoyl-l-aspartic acid, and orotic acid) showed a high discrimination degree. The inclusion of alfalfa silage in the diet formulation was potentially correlated with the vitamin B6 metabolism in milk, being 4-pyridoxic acid the most significant and up-accumulated compound. Therefore, pyrimidine derivatives were outlined as potential biomarkers of HMC-based diets, likely involving a complex metabolism of microbial nitrogen based on total splanchnic fluxes from the rumen to mammary gland in dairy cows. Also, our findings highlight the potential of untargeted metabolomics in both foodomics and feedomics studies involving dairy products.

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O160

In vitro rumen methane production is not affected by the feeding efficiency of donor bulls

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In recent years, much has been said about the role of livestock in the planet environmental problems. In addition, more recently, the difficulty of supplying many feeds is exacerbating the economic crisis in the livestock sector. Improving feed efficiency in ruminant farms can help mitigate both problems by reducing feed consumption. For the same dry matter intake, methane production (MP) in the rumen does not appear to be substantially linked to feed efficiency. However, the scientific literature provides controversial results. The aim of this work is to understand whether and how variations in feed efficiency affects rumen microbial population, fermentation, and MP. Among the young bulls of Italian Simmental breed in performance test and in feed intake control for the determination of Residual Feed Intake (RFI), 15 extremely efficient (RFI = −1.20 kg DM/d) and 15 non-efficient subjects (RFI = +0.88 kg DM/d) were identified. The bulls were slaughtered at the age of about 17 months. The RFI groups are equal in terms of daily gain, body weight, and carcass weight and, dressing out. During slaughter, rumen fluid was collected for microbiological analyses and *in vitro* fermentation. Samples of rumen fluid were stored for protozoa counting and microbiota DNA analysis. A self-assembled batch rumen fermentation system was used to evaluate fermentation parameters and MP. The system consists of eight glass bottles connected to gas counters and an infrared gas analyser that measures the methane concentration. At the end of fermentation, pH, volatile fatty acids (VFA), and ammonia were evaluated. Microbiological analysis on rumen fluid has identified 171 genera. In terms of relative abundance, five were significantly higher, in non-efficient group and three were higher in efficient group (including *Methanosphaera* and a genus belonging to *Veillonellaceae*). However, from the evaluation of the diversity between the genera in the two communities' analogous Shannon diversity index values emerge. The protozoa counting gave values that were not different between the RFI groups for both *Entodinia* and *Holotricha*. The total gas and MP, recorded during *in vitro* fermentations, did not differ between the RFI groups ($p > 0.05$). Moreover, the fermentation liquids were similar for pH and ammonia. However, the VFA composition varied in terms of propionic acid (higher in the non-efficient group) and A:P ratio (higher in the efficient group).

O264

Ensiled tobacco cv. Solaris biomass: chemical, microbiological, phytopathological and nutritional characteristics

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Nicotiana tabacum L., cv. *Solaris* has been developed as no-GMO energy plant (PCT/IB/2007/053412). Due to its strong vegetative capacity, cv. *Solaris* has been suggested as a crop for both biomethane production and animal nutrition, especially recommended for inner areas traditionally devoted to tobacco cultivation.

Aiming to study the characteristics of ensiled biomass to be used as forage, cv. *Solaris* whole plants (19 t), harvested in October and chopped (3 cm length), were ensiled in a bunker-silo and a microbial culture containing viable cells of *Lactiplantibacillus plantarum* (LPUM1) was added at a concentration of about 10^6 CFU/g of biomass. During the feed-out phase, lasted 150 days, samples ($n = 5$) from the feed-out area of the ensiled biomass (SiloSolaris) were collected every 30 days to investigate the chemical and microbiological traits.

On representative samples ($n = 25$) of SiloSolaris, dry matter (DM), crude protein (CP), ash, fibre components, and pH were determined according to official methods. Microbiological analyses were performed, and lactic acid bacteria, clostridia and fungi were quantified. To verify the presence of *L. plantarum* in samples, five bacterial colonies were randomly selected and purified from the MRS agar plates, and characterized using the RAPD-PCR technique. Fungal species were culture-purified on agar media and subjected to taxonomical identification through analysis of the interspace transcribed regions (ITS) and comparison with GenBank.

From chemical and nutritional point of view, SiloSolaris was characterized by DM 18.6 (±1.71) g/100 g, CP 16.9 (±0.32) g/100 g DM, ash 17.5 (±1.21) g/100 g DM, NDF 46.4 (±6.65) g/100 g DM, and ADF 35.9 (±3.57) g/100 g DM and by an average pH value of 5.7 (± 0.65). Microbiological results on the inoculated batches showed a relevant presence of the selected bacterial culture, which maintained charge levels of about 10^7 CFU/g of biomass. In particular lactic acid bacteria, due to their high growth rate, limited the number of fungi and clostridia at about 10^2 CFU/g.

Taken together, our results suggest that ensiled *Nicotiana tabacum* L., cv. *Solaris* biomass, obtained by adding the selected microbial cultures for the control of the fermentation processes, could provide a novel forage for ruminants' diet.

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O536

Effect of milk replacer supplemented with Ascophyllum nodosum on gut health of Holstein calves

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A correct management of the neonatal period of calves can influence the future fertility, age of calving, production, and age at removal from the herd. Particularly, calves have high susceptibility to gastrointestinal diseases and the neonatal calf diarrhea (NCD) is recognized worldwide as one of the biggest challenges for both the beef and dairy industries. Indiscriminative antibiotic treatment is discouraged and according to One Health principle it's necessary a drastic reduction in its use. In this scenario, algae extracts contain many bioactive metabolites of antimicrobial and antioxidant value that can be considered as alternative to antibiotics. The aim of the present study was to investigate the effect of Ascophyllum nodosum on calves feeding milk replacer twice daily until weaning. A total of twelve pre-weaned Holstein Frisian calves, housed in individual pens in the same environmental conditions, were divided in two groups: control group (CTRL, n:6), fed with milk replacer, a bovine's milk powder that respects the nutritional needs of calves; and treatment group receiving milk replacer with 10 g of Ascophyllum nodosum enriched in diet (TRT, n:6) for 42 days. The animals were included in the trial after the assumption of high-quality colostrum. The fecal score was evaluated daily (scale: 0–3) to monitor the incidence of diarrhea. The body weight was evaluated weekly and every two-week feces were collected for microbiological evaluation (total bacteria, Lactobacillus spp. and coliform bacteria) on selective medium. According to our results, the zootechnical performance did not differ between the experimental groups, and the treatment did not affect the palatability of milk. The TRT showed a lower incidence of moderate diarrhea ($p < 0.0113$) confirming a positive effect of the treatment on the fecal consistency. TRT showed a higher content of albumin, calcium, phosphorus, and total cholesterol ($p < 0.05$). In conclusion, the algae supplementation could be considered as potential alternative to antibiotic, to prevent gut diseases, to increase animal health and reduce the antibiotic use in neonatal calves.

O489

Mediterranean agro-industrial by-products as ingredients in a sustainable feeding strategy for dairy sheep: an in *in vitro* trial to evaluate rumen ecosystem

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The agro-industrial production chain contributes to the environmental impact producing wastes that need to be disposed. Most of them are edible source rich in bioactive compounds, such as polyphenols (PP) and PUFAs. Hence, these by-products can be used as ingredient in animal precision feeding with the aim to modulate the rumen fermentation, to improve the quality of the end-products to reduce the effect of the waste disposal, simultaneously satisfying the 3R principles of production sustainability. Three diets formulated including citrus pulp (CTP, 1.7% of DM), grape-marc (GM, 1.7% of DM), cardoon meal (CM, 19.0% of DM) as partial replacers of conventional ingredients, and other three diets formulated adding to the control diet (CON) the pure extracts of the characteristic PP of each by-product, with the same inclusion level of their presence in the raw material, (naringin and hesperidin mix, PCTP =1.02 mg/g of DM; condensed wine anthocyanins, PGM =2.60 mg/g of DM; silymarin, PCM =5.70 mg/g of DM) were tested in an *in vitro* trial with the aim to set up a feeding strategy for dairy sheep. The feeds were isoproteic and isoenergetic and were compared to CON. Feeds were fermented in triplicate and the experiment was replicated the following week with the aim to have two runs. At the end of fermentation time (24 h), samples were collected to be analysed for fatty acid profile, NH_3 concentration, CH_4 potential production (MPP) and NDF degradability. The markers of amylolytic and cellulolytic activities were affected by CM (g/100 g of rumen liquor DM: C14:0iso CON =0.162, CM =0.277, PCM =0.218, $p < 0.001$; C17:0ante CON =0.270, CM =0.433, PCM =0.342, $p < 0.05$) and by PCTP (g/100 g of rumen liquor DM: C13:0iso CON =0.122, CTP =0.110, PCTP =0.168, $p < 0.05$; C14:0iso CON =0.162, CTP =0.161, PCTP =0.238, $p < 0.05$). In contrast, GM affected volatile fatty acid profile (g/100 g of rumen liquor DM: C4:0iso CON =2.520, GM =1.498, PGM =1.49,7 $p < 0.05$; C3:0 CON =0.149, GM =0.091, PGM =0.076, $p < 0.05$; C5:0 CON =0.836, GM =0.500, PGM =0.464, $p < 0.05$). NH_3 concentration varied significantly with CTP feed inclusion (mg/L: CON =1.76, CTP =1.85, PCTP =0.76, $p < 0.001$). NDF degradability was slightly lower in GM (CON =86.58%, CP =86.11%, GM =75.36%, CM =79.66 %, $p < 0.05$). Grape marc and CM showed the lowest MPP (−25% and −40%, respectively). Concluding, polyphenols affected rumen microbial metabolism in a different way, depend on their variety and if used as extract or not.

O60

Effect of increasing concentration of Salicornia on *in vitro* degradability of dietary dry matter and neutral detergent fibre

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The salinization of the Mediterranean basin's areas needs to find some adaptation strategies for the cultivation of plants for animal feeding. Salicornia (Linnaeus 1753), a halophytic plant is characterized by moderate content of crude protein (130 g/kg Dry Matter 'DM') and low content of lignin (19.6 g/kg DM). However, Salicornia contains also high concentrations of NaCl (>150 g/kg of DM). This study aimed to assess the influence of dietary partial replacement of polyphitic hay with pre-dried Salicornia in a diet formulated accordingly to requirements of dairy sheep in an *in vitro* study by using Daisy II Ankom Incubator. Four diets were formulated with

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increasing replacement of polyphitic hay (H) with pre-dried Salicornia (S): 700 g/kg H, 600 g/kg H and 100 g/kg S (10%), 500 g/kg H and 200 g/kg S (20%), 300 g/kg H and 400 S g/kg (40%) on a diet DM basis. All diets contained equal amounts of extruded linseed (20 g/kg DM), alfalfa meal (122 g/kg DM), corn and barley mix (40 g/kg), and fava bean (122 g/kg DM). *In vitro* DM and NDF degradability, pH values, ammonia formation, and volatile fatty acids (VFA) profile were evaluated. Increasing concentrations of Salicornia in the diet were associated with a significantly higher content (+128%) of ashes and a decrease of ether extract (−150%). Moreover, we observed greater DM degradability (+22%) with Salicornia supplementation. These effects were particularly observed in the diets with 40% of Salicornia. On the contrary, no differences were revealed for NDF degradability ($p > 0{,}05$). No differences were observed for the pH values, while ammonia only increase significantly (+3.5-fold) in 40% diet in comparison to treatment containing 0% Salicornia. Regarding the VFA profile, isobutyrate decreased and butyrate significantly increased ($p < 0.05$), with 40% inclusion of Salicornia. The total VFA produced did not significantly vary across the treatments. The acetate/propionate ratio increased significantly ($p < 0.05$) by up 288% with Salicornia inclusion of 10%, 20%, and 40%. In conclusion, the inclusion of Salicornia until 20% did not have significant effects on the dietary DM and NDF degradability, whereas higher inclusion percentages did. However, *in vivo* applications will be necessary for the evaluation of the effects of Salicornia on the degradability of diet ingredients.

O506

A comparison of waxy versus regular maize in diets for post-weaning piglets: effect on growth performance, colon volatile fatty acids concentration, and gut microbiota

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Starch is composed by two fractions, amylose (AA) and amylopectin (aa). The relative amount of two fractions affects the digestibility of different starch types. Usually starch from regular maize is composed by 20% AA and 80% aa, while the waxy maize type is composed almost entirely by aa. The extent of starch digestibility may influence the gut health and development of post-weaning piglets. The present study aims to investigate the effect of a high digestibility maize (waxy type) on growth performance, diarrhoea incidence (DI), and gut microbial composition. A 42-day trial was carried out in a commercial farm. The study has been divided in two parts: the first (**F1**) and the second (**F2**) post-weaning phase, respectively lasted 14 and 28 days. Experimental groups (control, **CON**; waxy, **WAX**) has been composed each by 30 weight homogenous piglets weaned at 28 d of age. Diets were isoenergetic and provided the same amount of starch. All diets components were the same except for the starch source, which was either regular or waxy maize starch. Animals were fed *at libitum*, and the amount of supplied feed was daily weighed. At 0, 14 and 42 days of trial, piglets were weighted and sampled for faeces. Data were analyzed by means of ANOVA, using the JMP software.

Results showed that in comparison to CON, WAX group consumed more feed during the F1 period ($p < 0.01$), however no difference between groups were found in the F2 period. Higher feed intake (FI) contributed to a higher average daily gain (ADG) for WAX-F1 group ($p < 0.001$), but no significant difference has been detected for life body weight (BW) at the end of the F1 period. However, despite no difference for both, ADG and FI, BW of WAX was higher than CON at day 42 of trial ($p = 0.05$). Moreover, the dietary treatment did not affect the DI in both F1 and F2 periods although an increased digestibility of the carbohydrates in the piglets' diet may suggest a worsening in the DI. In addition, preliminary results showed higher concentrations of the total amount of volatile fatty acids (VFAs) in the colon tract for CON-F2 in comparison with WAX-F2 ($p < 0.05$). This outcome was predictable due to the lack of fermentable starch in waxy maize. In conclusion, maize with high digestibility led to the improved growth performance of post-weaning piglets. Besides, the difference in VFAs concentration between groups suggests a different development of the gut microbiota. This hypothesis will be verified by ongoing analyses.

O52

Nutraceuticals: a promising alternative to the use of drugs for intestinal wellness in dogs

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Reactive oxygen species (ROS) in living systems induces an imbalance of redox homeostasis and causes oxidative stress which leads to several pathological conditions in humans and pets, including gastrointestinal disorders. Nutritional supplementation with antioxidant substances could be useful to manage these conditions. In this study, we evaluated the total phenolic content (TPC) and antioxidant activity of three natural substances (bromelain, quercetin, and *Lentinula edodes*) and a formulation containing their combination using TPC, DPPH• (2,2-diphenyl-1-picrylhydrazyl), and ABTS•+ (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic diammonium salt acid)) analyses. We determined TPC and scavenging activity (DPPH• and ABTS•+) by spectrophotometric assays and performed a one-way ANOVA model. The formulation showed the highest ($p < 0.0001$) TPC (4 ± 0.2 mg GAE/g DM (dry matter)) and $ABTS^{\bullet+}$ (125 ± 3 μmol TE/g DM) while, quercitin showed the lowest ($p < 0.0001$) TPC (2 ± 0.2 mg GAE/g DM) and $ABTS^{\bullet+}$ (11 ± 3 μmol TE/g DM). However, quercitin presented the lowest ($p < 0.0001$) $DPPH^{\bullet}$ (EC_{50}: 1 ± 10 μg/mL) followed by the formulation (EC_{50}:138 ± 10 μg/mL), *Lentinula edodes* (EC_{50}: 231 ± 10 μg/mL) and bromelain (EC_{50}: 434 ± 10 μg/mL). These natural products (bromelain, quercetin, and *Lentinula edodes*) may exert their beneficial effects on gastrointestinal health through various mechanisms, including their antioxidant properties, which may help reduce oxidative stress and inflammation in the gut and promote tissue repair. Also, these natural products have been suggested in literature, for their anti-inflammatory and immune-modulating effects that may regulate the immune response and prevent excessive inflammation in the gut. In conclusion, based on the analysis of the antioxidant capacity of the natural substances, it was found that quercetin, bromelain, and *L. edodes* individually demonstrated variable antioxidant capacities. However, the combination of the three ingredients exhibited a promising and strong antioxidant effect. To date, there are no reports of a feed supplement incorporating all three ingredients. Indeed, we have an ongoing *in vivo* trial on healthy dogs to assess the safety and effectiveness of a formulation which includes the three tested ingredients. The current *in vitro* findings suggest that the combination of quercetin, bromelain, and *Lentinula edodes* has the potential to be a valuable addition to current treatments for canine gastrointestinal disorders.

O473

Effect of nutrient self-supply through choice feeding on growth performance, feeding behavior

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This study aimed to compare the growth performance, empty body nutrient deposition efficiency, and feeding behavior of growing pigs fed on a standard two-phase feeding or a choice feeding regime. The experiment was performed with 12 Swiss Large White barrows between 23.2 and 108.0 kg body weight (**BW**). Six pigs assigned to the standard (**ST**) treatment were offered *ad libitum* access to a grower (**ST-G**) and finisher (**ST-F**) diet from 23.2 to 63.4 kg and from 63.4 to 108.0 kg, respectively. The ST-G and ST-F diets were formulated based on the Swiss feeding recommendation for swine for an average BW of 40 and 80 kg, respectively. The other six pigs assigned to the choice (**CH**) treatment had constant *ad libitum* access to both a grower (**CH-G**) and a finisher (**CH-F**) diet formulated for a reference BW of 20 and 100 kg. All diets were isocaloric and differed only in the crude protein and essential amino acid content according to the reference BW used for feed formulation. To determine the empty body nutrient deposition rate, pigs were scanned using dual-energy x-ray absorptiometry at 25.8 and 103.8 kg BW. Individual feed intake and feeding behaviour were monitored with automatic feeders. Changes in BW were determined weekly. Data were analysed with the PROC MIXED (SAS, v9.4) with treatment and litter of origin as main and random effect, respectively. Compared to ST pigs, CH pigs ingested more feed daily (2.49 vs 2.36 kg/d; $p = 0.05$) and grew faster (1.04 vs 0.97 kg/d; $p = 0.02$). Total crude protein consumption tended to be greater (32.87 vs 31.38 kg; $p = 0.08$) in CH than ST pigs due to a numerically greater intake of the protein-rich CH-G diet during the finisher period. A greater crude protein intake in CH than ST pigs was accompanied by a greater (177 vs 159 g/d; $p = 0.04$) daily protein deposition rate but a similar protein efficiency. Regarding feeding behaviour, CH pigs went more often to the feeder, spent less time at the feeder, ate less feed per visit, and had shorter intervals between two meals than ST pigs ($p < 0.01$ for each) in the grower but not in the finisher period. Considering the feeding behaviour traits, the CH pigs with a greater protein deposition potential preferred the protein-rich CH-G over the CH-F diet. In conclusion, these results show that, like the wild pigs, the domesticated modern pigs maintained the ability for an adequate nutrient self-supply according to their nutritional requirements.

O121

Mulberry leaf meal as alternative feed ingredient in rabbit nutrition: preliminary results about productive performance and meat quality

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Mulberry (*Morus alba*) leaf meal (MLM) can be used as an alternative raw material in rabbit feeding, especially in small production systems. However, data about its applicability under commercial conditions are still scarce. This study aimed to evaluate the productive performance and meat quality of rabbits fed diets containing MLM. A total of 140 weaned, 42-days-old, crossbred rabbits (Hycole × Grimaud, initial live weight [LW]: 1335 ± 48 g) were allotted to 2 isonitrogenous and isoenergetic dietary treatments (5 multifunctional cages/diet with 14 animals/cage) in a commercial farm: C (control group, commercial diet without MLM meal) and MLM10 (commercial diet with 10% of MLM inclusion as a partial replacement of alfalfa meal). Two feeding phases were considered: post-weaning (42–63 days of age) and fattening (64–90 days of age), with MLM being included in the fattening phase only (C and MLM10: crude protein of 15.04% and 15.11%; crude fiber of 16.21% and 15.29%). Growth performance and mortality rates were recorded at 63 and 87 days of age, and 3 rabbits/cage were selected to be slaughtered at 90 days of age. At slaughtering, the LW, caecal pH, hot carcass weight (HCW) and chilled carcass weight (CCW) were registered, and dressing percentage and relative organ weights (liver, spleen, kidneys [%CCW] and gut [%HCW]) calculated. After 24 h, the pH and colour of the *Longissimus thoracis et lumborum* (LTL) and thigh were registered. Data were analyzed by Student's *t*-test (IBM SPSS software, $p \leq 0.05$). Growth performance and mortality rates were not affected by dietary MLM inclusion ($p > 0.05$). Relative organ weights were also similar between MLM- and C-fed animals ($p > 0.05$). Differently, the caecal pH was higher in MLM10 than in C rabbits (6.12 ± 0.39 vs 5.82 ± 0.31; $p < 0.05$). The MLM-fed rabbits also displayed lower slaughter LW, HCW and CCW than C animals (3064.1 ± 115.37 vs 3157 ± 81.55 g, 1902 ± 69.28 vs 1973 ± 85.07 g and 1875 ± 54.64 vs 1942 ± 96.19 g, respectively; $p < 0.05$) – potentially attributable to less fat deposits. Furthermore, LTL a* (redness index) of MLM10 rabbits was higher when compared to C group (0.19 ± 0.67 vs −0.42 ± 0.91; $p = 0.05$) – reasonably related to the higher iron content of mulberry leaves than alfalfa. In conclusion, MLM can be used in rabbit diets without negatively affecting growth performance. Further research characterizing the caecal microbiota and fermentative activity are recommended to better contextualize the pH changes.

O357

Different formulations of benzoic acids as strategy to replace therapeutic dose of ZnO on health and performance of weaned pigs

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This study aimed to evaluate the effects of two diets supplemented with an acidifier or with a mix of three acidifiers in comparison with a basal diet supplemented with a pharmacological dose of zinc oxide (ZnO) on health and growth performance of weaned pigs. At weaning (26 ± 3 days, d0), 540 piglets were weighed and divided into three groups (52 litters, 3–4 piglets from each litter/group, six replicates/group): (i. basal diet supplemented with a pharmacological dose of ZnO (2400 ppm) (CO); ii. basal diet supplemented with pure benzoic acid at 5 kg/ton of feed (BAC); iii. Basal diet supplemented with a mix of acidifiers at 4 kg/ton of feed (BAC+). Feed intake (FI) was registered from each replicate weekly. Mortality was daily recorded. Cumulative mortality was calculated and expressed as percentage considering the pigs in the box. At d14 and d28, piglets were individually weighted and lesion measures were assessed on the ears and tail. The lesion index (LSI) was calculated. The statistical analysis was performed in R v4.1.1 using car and lme4 packages. Data on BW and ADG were analyzed using an ANOVA model considering the group (CO vs BAC vs BAC+), box and litter of origin as factors and the piglets as experimental unit. Data on FI, feed to gain (F:G) and LSI were analyzed using an ANOVA model considering the group as factor and the box as experimental unit. During the period d0-d14, the CO had a higher ADG compared with BAC and BAC + groups ($p < 0.001$). Considering the whole experimental period (d0-d52), BAC had a lower ADG compared with CO ($p = 0.05$) and a significantly lower ADG compared with BAC+ ($p = 0.001$), while no difference between CO and BAC + was observed. Piglet's cumulative mortality was higher in BAC and BAC + compared with CO for the periods d0-d14 ($p = 0.03$; $p = 0.01$ respectively) and d14-d28 ($p = 0.02$; $p = 0.01$ respectively). Piglet's mortality was never different between BAC and BAC + groups. At d28, BAC had a higher LSI for the ear compared with BAC+ ($p = 0.05$) and CO ($p = 0.03$), while no difference was observed between BAC + and CO. In addition, LSI for the tail was higher in BAC compared with BAC+ ($p = 0.02$) and CO ($p < 0.0001$), and BAC + had a higher LSI for the tail compared with CO ($p < 0.01$). Overall, the substitution of a pharmacological dose of ZnO with pure benzoic acid during piglets' weaning was not beneficial in terms of growth performance; while if combined with other acidifiers it seems to be able to replace ZnO, maintaining the growth performance of piglets.

O339

Dietary supplementation of *Ascophyllum nodosum* and *Lithothamnium calcareum* as functional feed additives for weaned piglets

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The weaning phase is one of the most critical stages in pig farming. At weaning, piglets should cope with several stressors exposing animals to develop multifactorial gastrointestinal disorders where pathogenic *Escherichia coli* play a pivotal role. Farmers resort to antibiotics treatments to restore the health status of animals. Due to the raising concern on antimicrobial resistance, novel strategies are required to control enteric infections and reduce the use of antibiotics. Functional feed ingredients provide bioactive compounds able to improve animal health and decrease infections. The aim of this study was to evaluate an innovative dietary combination of algae on performance, nutrients digestibility, intestinal health and oxidative status in post-weaning piglets challenged with *E. coli* F4+. Forty-eight weaned piglets (28 ± 2 days) were allotted to two different groups ($n = 24$/group) balanced per weight: control group fed with a commercial diet (CTRL), and algae group fed with commercial diet supplemented with 1.5% of *Ascophyllum nodosum* and 0.5% *Lithothamnium calcareum* for 27 days (ALGA). At day 13, twelve pigs/group were challenged with a single dose of 10^7 UFC of *E. coli* F4+ generating two infected sub-groups (CTRL + and ALGA+). Body weight was recorded weekly for the evaluation of growth performance. Fecal samples were collected weekly and daily from day 13 to 20 for assessing nutrients digestibility by insoluble-ash method and microbiological analysis by plate counting. Serum samples were obtained at day 27 to assess the antioxidant barrier and oxidative status using colorimetric tests. Mixed model was used for the collected data and ANOVA was performed for evaluating the oxidative status. Results showed a significant increase in terms of average daily gain in ALGA + compared to CTRL + group from 17 to 20 days (1119.45 ± 84.55 vs −33.33 ± 88.31 g/day; $p < 0.0001$). Plate counting showed a comparable trend for fecal *E. coli* shedding, total, coliform, and lactic acid bacteria CFU in each group over 27 days. Nutrient digestibility did not show differences among groups for all timepoints. A significantly higher serum antioxidant barrier was registered in piglets fed with algae compared to not-supplemented groups at day 27 (363.26 ± 16.24 vs 230.69 ± 32.08 HClO/mL). In conclusion, the supplementation of *A. nodosum* and *L. calcareum* could be considered a promising dietary strategy to contrast *E. coli* infections thus decreasing the use of antibiotics in swine farming.

O355

Characterization of major algal species and their prebiotic potential

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In animal health, the need for a sustainable system that limits the phenomenon of antibiotic resistance is becoming increasingly evident. In this context, functional foods or ingredients are gaining ground and are also being researched as waste products from the supply chain. Among these, algal biomass is particularly rich in polysaccharides, which could potentially be exploited as prebiotic functional ingredients for both human and animal health applications. To this end, this study aimed to determine the potential prebiotic role of two algae species employed in animal nutrition, *Ascophyllum nodosum* and *Lithothamnium calcareum*. Initially, a metabolomic profile characterization was performed by LC-QTOF-MS system, in order to assess the content of bioactive compounds of the two algal species. Therefore, the effect of *A. nodosum* and *L. calcareum* extracts and their combination was evaluated *in vitro* on the growth of *Lactobacillus plantaurum*. The antioxidant capacity was determined by the ABTS Radical Cation Decolorization Assay, and the total antioxidant capacity was expressed as the percentage of inhibition of radical scavenging (PI%). The antimicrobial effect of *L. plantaurum* cultured with algal extracts against porcine O138 *E. coli* was evaluated following the microdilution bacterial growth method. The metabolomic characterization confirms the functional potential of algal extract, showing a high amount of all the bioactive compounds including polysaccharides. Thanks to the presence of these compounds algal extracts are also able to improve the growth of *Lactobacillus plantaurum in vitro*. In fact, after 8 hrs of incubation both algal extracts improved *L. plantarum* growth, increasing it by about 30%. Furthermore, co-culture of *L. plantarum* with the extract of the algal combination allowed for increased growth as early as 6 hrs of incubation. Moreover, the presence of different bioactive compounds in the algal extracts, allowed an improvement in both antioxidant and antimicrobial capacity of *L. plantaurum*. The results obtained from this study disclose that the extracts of *A. nodosum and L. calcareum* can be considered valid prebiotics in order to

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enhance the functional properties of *L. plantaurum*. Further *in vitro* studies will be conducted to complete the knowledge about the employment of the algal extract as prebiotic, in order to subsequently evaluate their effect *in vivo*.

O537

Functional characterization of hempseeds for nutritional applications

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Protein-energy malnutrition is a global challenge that requires attention, especially with population growth and ongoing food security plans. Therefore, nutritional research is investigating new protein sources for the food/feed sector, that can ensure a high nutritional profile and a key role in environmental protection. Among these, industrial hemp (*Cannabis sativa* L.) can be a promising source of high-quality nutrients. Today, following the re-establishment of the cultivation of hemp plants (<0.2–0.3% of delta-9-tetrahydrocannabinol), there is a growing interest in the production of the seeds due to their relevant nutritional value. While the nutritional profile of hempseeds (HSs) is mostly described in the literature, the functional aspect requires further studies.

The aim of the study was to assess the total phenolic content (TPC) by the Folin-Ciocalteu method and the antioxidant activity by the ABTS assay of HSs, comparing them to linseeds (LSs), matrix widely used in feed sector, following *in vitro* and *ex vivo* digestion protocols. At the same time, the efficacy of the two models was evaluated.

For the *in vitro* protocol, 0.5 g of sample was subjected to oral, gastric (39 °C × 2 h) and intestinal (39 °C × 4 h) digestion using commercial enzymes. Considering *ex vivo* digestion, after collecting gastric and intestinal fluids in piglets (days 40, $n = 24$), they were pooled. Then, 0.5 g of sample was subjected to gastric digestion (39 °C × 2 h) in gastric fluid, and further exposed to intestinal fluid (39 °C × 2 h).

Collected data showed that dry matter digestibility was not statistically significant between *ex vivo* and *in vitro* protocols (48.13 ± 1.10% vs 56.01 ± 0.50%, respectively). The same trend was observed for protein digestibility. Simultaneously, HSs showed an interesting functional profile. More specifically, HSs, after *in vitro* digestion, showed a higher TPC (846.18 ± 23.81 mg Acid Ascorbic Equivalent (AAE)/100 g), although not statistically significant compared to that of LSs (451.44 ± 59.29 mg AAE/100 g). This trend was also confirmed for the antioxidant profile of HSs and LSs (2368.58 ± 117.89 vs 1334.45 ± 43.59 mg Trolox Equivalent/100 g, respectively), showing statistically significant differences ($p < 0.05$).

These results encourage further investigation of the bioefficacy of HSs including both the identification of molecules involved and the setting up of *in vivo* trial, to identify the best level of inclusion of this matrix to ensure high animal performance and welfare.

O353

Effect of different doses of camelina cake as soybean meal substitution of growth performance and gut health of weaned pigs

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The camelina cake (CAM) is a co-product that could represent an alternative protein source to replace part of soybean meal, improving the environmental sustainability of the monogastric production. This study aimed to evaluate the effect of different doses of CAM in substitution of soybean meal on performance and gut health of weaned pigs. At 14 days post-weaning (d0), 64 weaned piglets were assigned to 4 groups balanced for body weight (BW) and litter and fed a standard diet (CO) or a diet with the inclusion of 4% (C4), 8% (C8) or 12%(C12) of CAM. Diets were isoenergetic and isoproteic. Pigs were weighed weekly. Faecal and blood samples were collected at d7 and d28 for microbiota (v3-v4 regions of the 16s rRNA gene) and ROMs analysis. At d28, pigs were slaughtered; pH was recorded on intestinal contents, organs were weighed and jejunal samples were collected for morphological and gene expression analysis. Data were fitted by a mixed model including diet as a fixed factor and batch and litter as random factors. From d0-d7 the CAM inclusion linearly reduced the average daily gain (ADG) ($p \leq 0.01$). No effect was observed in the subsequent weeks, but considering d0-d28, CAM significantly reduced the ADG ($p = 0.01$). From d0-d7, the increase in CAM inclusion reduced linearly the feed intake ($p = 0.04$) and increased linearly the feed to gain ($p = 0.004$). The liver weight was increasing linearly with the CAM dose ($p < 0.0001$). ROMs concentration, intestinal pH and morphology, and gene expression in the jejunum were not affected by the diet, except for the expression of zonulin-1 ($p = 0.07$, quadratic effect). The inclusion of CAM increased the alpha diversity indices at d28 ($p < 0.05$). The C4 diet promoted the abundance of Butyricicoccaceae_UCG-008 and Erysipelatoclostridiaceae_UCG-004, which are usually correlated with resilient gut microbiota. In conclusion, despite the reduction of the ADG of piglets, the inclusion of CAM did not affect gut health and enhanced resilient gut microbiota. CAM could be evaluated as a potential alternative protein source in weaned pigs.

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O343

Effect of the administration of two phytoextracts pre-mixes compared to the pharmacological dose of ZnO on the health, growth performance and faecal microbial profile of weaned pigs

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Weaning is one of the most critical phases in pig's life, often leading to post-weaning diarrhoea (PWD). Zinc oxide (ZnO), at pharmacological dose has been largely used to prevent PWD, however, due to antimicrobial resistant issue, the EU banned its use from June 2022. Phytoextracts and essential oils are possible alternative studied for their antimicrobial, anti-inflammatory and antioxidant abilities. The aim of this study was to evaluate the effect of two feeding additives containing phytoextracts and essential oils compared to ZnO on health, performance and gut health of weaned pig. At weaning (d0), 96 piglets (7058 ± 895 g) were assigned to one of 4 groups balanced for body weight (BW) and litter: CO (control group), ZnO (2400 ppm ZnO from d0 to d14); Blend1 (Cinnemaldehyde, Aiowan and Clove and essential oils, 150 g/100 kg feed); Blend2 (Cinnemaldehyde, Eugenol and Short and Medium Chain fatty acids, 200 g/100 kg feed). Pigs were weighed weekly. Faeces were collected at d13 and d35 for microbiota (v3-v4 regions of the 16s rRNA gene) and *Escherichia coli* count analysis. At d35, pigs were slaughtered; pH was recorded on intestinal contents and jejunal samples were collected for morphological and gene expression analysis. Form d0-d7 the Bland2 had lower average daily gain (ADG) ($p < 0.05$). ZnO and Blend1 never differed in ADG and feed intake. At d14, caecum and colon pH were lower in Blend2 than in ZnO. CO had a higher abundance of haemolytic *E. coli* than Blend1 ($p = 0.01$). At d13, ZnO group had a lower alpha diversity ($p < 0.01$) and a different microbial beta diversity ($p < 0.001$) compared to the other groups. At d13, pigs from Blend2 the ZnO group were characterized by a higher abundance of Prevotellaceae_NK3B31_group (LDA score =4.5, $p = 0.011$), *Parabacteroides* (LDA score =4.5, P adj. = 0.005), CO was characterized by UCG-005 (LDA score =4.3, P adj. = 0.005), NK4A214_group (LDA score = 4.2, P adj. = 0.02), Blend 2 by *Megasphaera* (LDA score =4.1, P adj. = 0.045), and *Ruminococcus* (LDA score =3.9, P adj. = 0.015) and Blend1 by Christensenellaceae_R-7_group (LDA score =4.6, P adj.<0.001) and *Treponema* (LDA score =4.5, P adj.<0.001). Jejunal expression of *NFKB2* ($p = 0.05$) and *IALP* ($p = 0.08$) was higher in the Blend2 group compared with the CO group. In conclusion, Blend1 allowed to obtain the same performance as ZnO through modulation of the gut microbiome, the reduction of haemolytic *E. coli* and the inflammatory status of the gut.

O28

Cardoon meal as an alternative sustainable ingredient in broiler feeding

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Poultry industry represents a suitable production sector for meeting the growing demand for high nutritional value proteins due to the short production chain and low costs. However, this sector weighs on the environment in food vs feed competition for the use of soybean meal (SM). Cardoon meal (CC), resulting from the extraction of oil for energy or bioplastics production, is a matrix rich in proteins and fibre, and a source of bioactive molecules, such as polyphenols. Thus, it could allow the substitution of soybean meal lowering feed costs. This study aimed to evaluate the effect on broiler performances of a diet with CC in replacement of SM at three inclusion levels: control group (C), fed with a basal diet; S85C15 group, fed C diet with the 15% of CC and 85% of SM; S80C20 group, fed C with the 20% of CC and 80% of SM; S70C30 group, fed C with the 30% of CC and 70% of SM. The trial lasted 35 days, no mortality episodes occurred, and the animal growth was similar. Considering the feeding periods, among the parameters evaluated (weight gain, WG; feed intake, FI; feed efficiency, FE), the only differences were registered for FI. In particular, FI was higher in the first two periods (starter and grower $p < 0.0001$; finisher $p = 0.0006$) in the S80C20 groups than the others. The maximum FI in the S80C20 group was registered either in the first and in the second week (0.207 kg, $p < 0.0001$ and 0.606 kg, $p < 0.0001$, respectively), while the highest WG was in the S85C15 group (0.122 kg, $p < 0.0001$) in the first week and CON (0.286 kg, $p = 0.0012$) in the second week. Finally, the FE was higher in the S85C15 group in the first week (1.393,

$p = 0.0466$), in the C group (1.976, $p = 0.0434$) in the second week, and in the other weeks FE resulted not significantly different among groups. Considering the per-week evaluation the CC dietary inclusion slightly affected the birds' performances. However, in the last weeks and considering the whole period, the results of this trial evidenced that no detrimental effect occurred when chickens fed CC at the three inclusion levels tested. FI, WG and FE data of each period or week, were processed as a completely randomized design with repeated measures using the MIXED procedure of SAS with the period (or week) and time as fixed effects. These findings highlighted that the CC dietary inclusion could be a valid alternative in the partial replacement of SM.

O87

Evaluation of essential oils from natural extracts and medium chain fatty acids on piglets growth performance, salivary stress markers and gut health

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This study evaluated the effect of adding an additive (Gastroherb Plus®) composed by essential oils from natural extracts (Oregano) and medium and short chain organic acids (MCFAs) to the diet of weaning piglets on growth performance, salivary stress markers and microbiota composition. At weaning, a total of 210 piglets (26 d, 7.66 ± 1.09 kg) were allocated, according to their body weight, to two experimental treatments. Each treatment was replicated 7 times, with 15 piglets per pen forming the experimental unit. Piglets were assigned randomly into the control group (CTR) fed the basal diet and the treated group (T) fed the basal diet mixed with a dosage of Gastroherb Plus® corresponding to 1.5 kg/ton of complete feed. The body weight and feed consumption were taken at 0, 14 and 35 days. Performances parameters were analysed by means of ANOVA for repeated measures, accounting for the effect of the treatment, time, and their interaction. Cortisol, IgAs and antioxidant capacity were analysed were analysed considering the GLM procedure of SAS v.9.1. Saliva samples were taken at day 14, 21 and 35 of trial for cortisol and IgAs quantification using a competitive and a sandwich ELISA kit while total salivary antioxidant capacity was assessed with FRAP test. At the end of the trial, 7 animals per group were sacrificed and caecal content was sampled in order to perform microbiota evaluations. No significant differences were observed on BW, average daily gain, feed intake, and Feed convertion ratio, while increased fecal consistency was outlined in T compared to C. At day 21 no significant differences were noticed for cortisol in control (0.753 ± 0.455 ng/mL) and treated animal (0.921 ± 0.328 ng/mL). On the other hand, at day 35 d the treated group showed a significant decrease in salivary cortisol (0.672 ± 0.308 ng/mL vs 1.289 ± 0.645 ng/mL, $p < 0.05$). The levels of salivary IgAs and antioxidant capacity were not significative along the trial in control and treated animals. Sequencing the V3-V4 regions of the bacterial 16S rRNA gene produced a total of 2,815,715 reads. After bioinformatics processing, these yielded 741 OTUs with 336,691 total counts across all 14 samples. Firmicutes and Bacteroidetes were the most abundant phyla in both groups and a total of 12 differentially abundant genera (p-value <0.05) were identified in the treated group. In conclusion, the administration of Gastroherb Plus® positively affected salivary stress markers and gut health of weaned piglets.

O107

The use of sugary and salty food industry leftovers as alternative to cereal grains does not affect the growth performance in growing and finishing pigs

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A more sustainable feeding strategy in pig production needs to be achieved in terms of reducing food waste and environmental footprint. One of the potential alternatives is the reintroduction into the feed chain of the food industry leftovers, also known as ex-food or Former Food Products (FFPs). The presence of manufacturing errors, packaging defects, and logistic challenges, makes these products no longer suitable for the human market. Due to the richness in lipids, starch and energy, FFPs-based diet could meet the needs of growing and fattening pigs. In this study FFPs were divided in two main categories (sugary and salty) and they were used in growing-finishing's diets in order to replace conventional ingredients and investigate their effects on growth performance. Thirty-six Swiss Large White male castrated pigs were assigned to the three grower and finisher experimental diets: (1) standard diet (ST-G; ST-F), 0% FFPs; (2) 30% conventional ingredients replaced by sugary FFPs (SU-G, SU-F); (3) 30% conventional ingredients replaced by salty FFPs (SA-G, SA-F). The grower and finisher diets were formulated to be iso-energetic and iso-nitrogenous. The body weight of all animals was monitored weekly starting from birth and right before the slaughter. In addition, the total feed intake (FI), the average daily gain (ADG), the average daily feed intake (ADFI), the feed conversion ratio (FCR) and the gain to feed were calculated for both the growing and finishing phases. These measurements were carried out to evaluate the growth performance of the animals according to the different experimental diets. Data were analyzed with the MIXED procedure of SAS. The model used included the dietary treatments as fixed effect and the pigs as random effects. Least squares means were calculated and considered statistically significant at $p < 0.05$. The results showed that the feed efficiency in terms of FCR was significantly higher ($p < 0.05$) in grower pigs fed ST diet than those fed the SA and SU diets, even though the days on feed, total FI, the ADFI were similar between the groups. However, when considering the overall mean of the entire period (growing-finishing), the use of FFPs did not affect the growth performance of pigs. In particular, BW, ADFI, ADG and FCR were similar between the three groups ($p > 0.05$). In conclusion, no detrimental effects on growth performance are present when sugary and salty FFPs are included in the diets of growing pigs and finishing pigs up to 30%.

O132

Antimicrobial and immunomodulatory evaluation of Olive Mill Wastewater Polyphenols for dietary supplementation in an *in vitro* swine intestinal model

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The olive oil industry produces large amounts of milling waste waters (OMWW) containing a consistent quantity of polyphenols (PP), which could be recovered and exploited for their anti-oxidant and antimicrobial effects. The use of PP as feed additives can have a relevant impact on the agro-food chain with special regard to the improvement of product quality, reduction of drug use, environmental sustainability and circular economy (e.g. use of by-products). The intestinal porcine epithelial cell line IPEC-J2 is considered as reference model to study innate immunity in the intestinal tract and host-pathogen (including *Salmonella* strains) interaction. The present study aimed to investigate the immuno-modulating and anti-microbial effects of OMWW-PP on an *in vitro* model (IPEC-J2) of intestinal *Salmonella typhimurium* (ATCC 12028) infection. To this purpose, a relative normalized gene expression analysis through RT-qPCR and a penetration/adhesion assay were performed. First, two PP extracts (dried and liquid OMWW) at different phenolic concentrations (0.35–0.7–1.4–7–14–70 and 140 µg/mL) were tested for their cytotoxicity through trypan blue and XTT assays, treating IPEC-J2 for 24 h. A significant reduction of IPEC-J2 viability was observed for both extracts only for the highest concentrations (140 and 70 µg/mL). For IPEC-J2 treated with 0.35–7 µg/mL OMWW PP, total RNA was extracted, reverse-transcribed and gene expression levels of *CXCL8, NOS2, TGFB, IL18, MYD88, NFKB1* and *NFKB-P65* were evaluated using *GADPH* as reference gene. Compared to untreated cells, a significant decrease of *MYD88* expression was induced by 0.35 µg/mL of dried OMWW PP and by 0.35–1.4 µg/mL of liquid OMWW PP. *NFKB1* was downregulated by 0.7 and 7 µg/mL of dried OMWW PP. Moreover, liquid OMWW PP induced a significant decrease of *CXCL8* at 0.35, 1.4 and 7 µg/mL, and of *IL18* at 0.35 and 0.7 µg/mL. Liquid OMWW PP treatment significantly increased *TGFB* expression at 0.7 µg/mL and *NOS2* at 0.35 and 0.7 µg/mL. Bacterial invasion was conducted adding 1 mL of 10^8 UFC/mL of *S. typhimurium* suspension (at 37 °C in 5% CO_2 for 1 h) to IPEC-J2 cells pretreated for 24 h with 0.35 µg/mL liquid OMWW PP. The IPEC-J2 PP pretreatment induced a significant decrease of bacterial invasion capacity compared to untreated cells. To summarize, our results suggest a possible immuno-modulating and anti-microbial action of OMWW polyphenols in swine cells.

O478

Effect of keratinase processed feather meal-based diets on the meat quality characteristics of broiler chicken

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The effect of keratinase processed feather meal-based diets on meat quality of broiler chicken was studied. Keratin-rich soil was collected from abattoir, hair, horns and poultry feather dumpsites to identify and isolate keratinolytic organisms for keratinase production. White feathers from slaughterhouse were washed, air dried, treated with keratinase and oven-dried at a temperature of 80° C to a moisture content of 10 %. The untreated and treated feathers were milled into Untreated and Treated Feather meals, respectively to formulate 22 % CP and 3100 kcal/KgME diets used in the study. A total of 1050 Ross 308 day old broilers were weighed and randomly allotted to seven treatments in a completely randomized design, with six replicates per treatment and 25 birds per replicate and managed intensively in a deep litter system throughout the 8 weeks duration, in a single phase regime. Treatment 1 (control with 0 % feather meal), Treatments 2, 3 and 4 contained 8%, 16% and 24% treated feather meal (TFM) while treatments 5, 6 and 7 contained 8%, 16 % and 24% untreated feather meal (UTFM). After slaughtering, meat quality and organoleptic parameters were determined. Data were analyzed using one-way ANOVA (SPSS, 2006) and Duncan test was used to separate significantly different ($p < 0.05$) treatment means. There were significant differences ($p < 0.05$) in the meat quality parameters across the treatments. T1 had the highest cooking yield (71.89 %) while T6 had the least value of 55.28 %. Cooking loss was highest in T6 (44.72) and lowest in T1 (28.11 %). The highest water holding capacity (WHC) was recorded in T6 (1.39 %) while the least was recorded in T7 (0.50 %). pH range from T3 (6.24) to T1 (5.96). Drip loss was highest at T4 (6.51 %) and lowest in T1 (4.08 %). Organoleptic parameters were not significantly different ($p > 0.05$) among the treatments, exception for appearance and flavor that were significantly different ($p < 0.05$). The highest value for appearance (7.56) and flavor (7.64) were recorded in T7 while the least values of 6.76 and 6.88 for appearance and flavor were recorded in T1. The study concluded that enzyme-treated feather meal based diets enhanced broiler meat water holding capacity while untreated feather meal based diets increased the meat's pH. More so, appearance and flavour were enhanced by enzyme-treated feather meal based diets at 24% while meat juiciness was improved at 16% inclusion level of enzyme-treated feather meal in the diet.

O51

Effects of hydrolyzed yeast on growth performance, meat quality, and intestinal immune status of broiler chickens

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Ensuring optimal production performance while sustaining animal health is the goal of the poultry industry. However, due to decades of rigorous selection, the animals' sensitivity to various diseases has increased. Thereby, there is growing interest in the use of feed additives with immunomodulatory properties. This study aimed to determine the effect of hydrolyzed yeast (HY) on growth performance, meat quality, and gut health of broiler chickens. A total of 320 male 1-day-old broiler chickens (ROSS308) were divided into two homogeneous groups, receiving either the basal diet (CTR) or the basal diet supplemented with 500 mg/kg HY (TRT). Each group was composed of 160 chickens, distributed among 8 replicates (20 birds/replicate). Growth performances were measured for each feeding phase (starter, d0–10; grower, d10–21; finisher, d21–42). At the end of the trial (d42), all the animals were transported to the slaughterhouse. Carcass and breast yield and meat quality parameters (pH, colour, and water holding capacity) were determined on three representative animals for each replicate. Jejunum tissue samples were collected from one representative animal per replicate and snap-frozen in liquid nitrogen for gene expression analysis. Gene expression of Adiponectin system (AdipoQ, AdipoR1, AdipoR2), and tight junctions (ZO1, OCLD, CLD3) was determined by qPCR, normalizing for GAPDH, RPL4, and YWHAZ. Growth performances were analysed using a MIXED procedure for repeated measurements (SAS version 9.4), accounting for the effect of treatment, time, and treatment × time interaction. Slaughter yields and gene expression were analysed using one-way analysis of variance, using the GLM procedure. Differences between groups were considered statistically significant at $p < 0.05$. The analysis revealed no significant effects of HY on growth and slaughter performance, as well as on meat quality parameters ($p > 0.05$). Similarly, no significant differences were noted between CTR and TRT groups for gene expression of AdipoQ (2.185 vs 2.741, $p = 0.495$), AdipoR1 (1.876 vs 1.661, $p = 0.446$), ZO1 (1.117 vs 1.125, $p = 0.9661$), OCLD (1.160 vs 0.968, $p = 0.2118$), and CLD3 (0.935 vs 1.070, $p = 0.6418$). Only a trend was observed for AdipoR2 (CTR =0.844, TRT =0.622, $p = 0.095$). The present results suggest that 500 mg/kg HY in the diet of broiler chickens didn't affect production performance nor the expression of genes related to intestinal immunity and integrity.

O302

A new-designed feeding device for sustainable hay feeding management in ponies

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In nature, equines consume frequent, small forage-based meals during the day. The use of slow feeding devices (SFDs) helps to safeguard the equine need to forage when fed restricted amounts by reducing hay intake rate in equines (IR, g/h). In addition, climate change is progressively reducing the availability of resources and SFDs are of growing interest as tools for decreasing hay waste in horse feeding management. The aim of this study was to assess the use of different SFDs – a new-designed slow feeder (HB) and small-holed (3.5 × 3.5 cm holes) hay net (HN) – on the IR of ponies. Nine healthy ponies belonging to two breed-type – 5 Shetland (SH) and 4 Welsh Cob (WC) – mean ± SD:12 ± 4 years, BCS 7.6 ± 0.7/9, BW 222 ± 51 kg were involved. After the adaptation period, a Latin Square design was applied by providing hay through 3 feeding methods: 3 kg of hay fed on the ground (G), 3 kg of hay fed on small holed hay net (HN), and 3 kg of hay fed in a new-designed slow feeder (HB). For each hay feeding method, the IR (g/h) was measured during the morning meal, 3 h/day for 3 repetitions/pony. A mixed model was performed to assess differences in IR (g/h) among the feeding methods, breed-types and their interactions. Significance was set at $p < 0.05$. Considering differences between feeding methods, our results showed that the IR was significantly lower ($p < 0.0001$) in HB (365.44 ± 37.76 g/h, $p < 0.0001$) and HN (363.03 ± 31.53 g/h), compared to G (677.87 ± 31.53 g/h). No difference in IR was found between HB and HN suggesting that these methods are equally useful in reducing IR in ponies. Moreover, our findings revealed that IR was influenced by the breed-type ($p < 0.0001$) – SH ponies showed lower IR for G (563.86 ± 42.03 g/h), HB (274 ± 50.35 g/h) and HN (277.29 ± 42.32 g/h) compared to WC ponies for G (791.89 ± 42.94 g/h), HB (456.88 ± 56.29 g/h) and HN (448.78 ± 46.73 g/h). Body morphology therefore also influences the IR of ponies. In conclusion, HB could be a promising SFD for the feeding management of ponies by exerting a similar IR to HN and by favouring a more natural feeding posture. HB are likely to promote an even lower hay wastage than HN which should be evaluated further as may lead to a more sustainable equine feeding management.

O297

Effect of two feeding managements on the microbiota of different intestinal compartments of the horse digestive tract

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Feeding horses unbalanced diets rich in starch still represent a common mistake in their feeding management, even if it is well-known that an adequate forage:concentrate ratio is crucial for promoting a healthy microbiota. The composition of microbiota changes among the different intestinal segments but the knowledge about such diversity according to the feeding management is currently scanty in horses. The aim of the present study was to evaluate the effects of two different feeding managements – one based on high amounts of starch (HS) vs. one based on high amounts of forage (HF) – on the microbiota composition of different intestinal segments of the horse digestive tract. Nineteen Bardigiano horses, 14.3 ± 0.7 (mean ± sd) months of age, were randomly assigned to two dietary groups – HS ($n = 9$) vs. HF ($n = 10$). At the end of the fattening period horses were slaughtered and digesta content were sampled from: duodenum (DU), jejunum (JEJ), ileum (IL), apex of the caecum (CAE), sternal flexure (SF), pelvic flexure (PF), right dorsal colon (RDC), and rectum (RE). Samples were stored at –80° until the DNA extraction and 16S rRNA amplicon target sequencing. Alpha diversity indices were used to find differences according to the feeding management and to the selected intestinal segments. Shannon index showed higher diversity (Kruskall Wallis test, $p < 0.01$) in CAE, PF and RDC in horses fed HF. β-diversity showed a clear separation of the microbial communities as a function of the diet (PERMANOVA; $p < 0.01$). At the highest taxonomic level, the 88 ASVs fell into two main clusters. One cluster included CAE, PF, RDC and RE of horses belonging to HF characterised by a high abundance of *Peptococcaceae, Paraprevotellaceae, Oscillospira, Bacteroides, Campylobacter* and *Lactobacillus*. The second cluster included DU, JEJ and IL of horses belonging to HS and it was characterised by high abundance of *Lactococcus, Fusobacterium, Ruminococcus, Bacillus and Clostridium.* Moreover, specific differences in microbial composition were shown in each of the selected intestinal segments suggesting that they may be considered as a distinct ecological niche. The feeding management

highly influenced the microbiota composition in horses and HF resulted to be associated with a higher bacterial diversity in CAE, PF and RDC. Further research should describe the relationship between the feeding management, the immune system and the metabolic status of the horse.

O279

In vitro evaluation of biochar from chestnut and vine residues gassification as possible feed additive: antioxidant and antimicrobial activities

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Biochar has been used for different agriculture and environmental applications to minimize waste and increase the circular economy's efficiency. Particularly, the use of biochar as a feed ingredient has the potential to improve animal health, feed efficiency, and livestock housing climate. In the scientific understanding, it is still unclear why biochar, as a feed additive, promotes the observed effects. The variability in physical-chemical-biological properties depends on pyrolysis temperature and waste used. In the present work, two different biochar from (i) chestnut waste (CB) and (ii) vine residues (VB) were characterized for chemical composition and functional properties. The chestnut and vine biochar were extracted into hot water (90 °C for 3 h head-over-heels) and the extracts were analyzed for inorganic/organic components by HPLC-MS. The antioxidant activity was determined by a colorimetric assay (ABTS assay). The antioxidant activity of vine biochar is significantly higher than chestnut biochar (110 TE/gr vs 54 TE/gr; $p \leq .01$). In the present study, the extracts were tested for their effects on *in vitro* growth inhibitory activities against enterotoxigenic *E. coli* species. Overnight-grown *E. coli* were inoculated into tubes containing 20 mL of Luria-Bertani medium (LB) supplemented with 0, 25, 50, and 100 µL/mL of biochar water extracted. Tubes were cultured aerobically at 37 °C and the bacterial growth rate was determined spectrophotometrically by optical density at 600 nm (at 60 min intervals for 6 h). The same concentrations of biochar extract (0, 25, 50, and 100 µL/mL) were tested on growth of *L. plantarum* and *L. reuteri* cultured in MRS broth. The optical density at 600 nm was checked every 2 h for a total of 12 h. Our results demonstrate that both biochar extracts exerted a significative inhibitory activity dose depending ($p \leq .01$) against pathogenic *E.coli* strains; the maximum percentage of inhibition, corresponding to a reduction of 29% of bacterial cells, was observed after 2–3 h of incubation with 100 µL/mL of biochar extracted. On the contrary, the growth of *L. plantarum* and *L. reuteri* was not negatively influenced. Significant differences between groups were performed by one-way ANOVA (GraphPad Prism). These preliminary results suggest that an appropriate dosage of biochar may be interesting to inhibit the growth of pathogenic *E. coli* and consequently a valid alternative for the reduction of the use of antibiotics.

O97

Effect of a combination of three yeasts on growth performance and gut health of weaning piglet

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The present study aimed to investigate the efficacy of a yeast mixture (YM, Levustim B0399, Vetoquinol; *Kluyveromyces marxianus fragilis B0399*, *Pichia guilliermondii*, and *Saccharomyces cerevisiae,* intact inactivated cells) on growth performance and gut health of weaned piglets. Forty-eight male piglets (27 ± 1.7 d, 7.19 ± 0.54 kg) were randomly allocated to two homogeneous experimental groups and involved in a 28-days trial. Both groups received a basal diet with (T) or without (C) inclusion of 0.8% YM during weeks 1 and 2, and 0.6% during weeks 3 and 4. Bodyweight (BW) was evaluated on days 0, 14, and 28 d, and fecal score recorded daily. Fecal samples were collected on 4, 14, 21, and 28 d for microbiota analysis. On day 28, 12 piglets for each group were sacrificed and the ileum tissue was sampled for morphological analysis (villi height (VH) and width, crypts depth (CD), and VH:CD ratio) and for analyzing the overall mucins profile using Alcian-Blue combined Periodic acid-Shiff (PAS) staining. Differences in the means between the experimental groups were determined by analysis of variance (ANOVA). Growth performance and fecal score were analysed by a MIXED procedure of SAS for repeated measures, while morphological parameters were analysed using a GLM procedure. In all the cases, the piglet represented the experimental unit, and significance was declared for $p < 0.05$. The experimental piglets showed similar growing performance, while increased fecal consistency was outlined in T group ($p < 0.01$). Sequencing the V3-V4 regions of the bacterial 16S rRNA gene produced a total of 31.573.670 reads. After quality filtering, 23.794.504 sequences were left for analysis. Firmicutes and Bacteroidetes were the most abundant phyla in both groups at all sampling points (70% and 20% of the total fecal microbiota, respectively). T group resulted in differentially abundant taxa compared to C, among which *Bifidobacterium* ($p = 0.006$), *Coprococcus 2* ($p = 0.015$), and *Clostridium Sensu Stricto 1* ($p = 0.019$). Villus height and width were significantly affected by yeast supplementation ($p < 0.001$; $p = 0.014$, respectively). Mucin profile revealed that mucus in ileum goblet cells was heterogenous, with a higher presence of PAS-positive mucins in the villi of T piglets ($p = 0.037$). Overall, the administration of a yeast mixture to weaning piglets showed positive effects at gut level, decreasing potentially harmful bacteria while improving beneficial genera and intestinal morphology.

O29

Effects of bakery by-products inclusion in the broiler's diet on growth performance, carcass yield and gene expression profiling

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Feed-food competition is one of the major topics to be assessed globally to improve the sustainability of livestock farming. The EU promotes the use of former foodstuffs (FFs) in animal feed as a promising alternative. Among FFs, bakery by-products (BBP) have been shown to be effective in ruminant and swine diets, but few studies have explored their effects on poultry.
The aim of this study was to evaluate the effect of dietary inclusion of BBP in broilers replacing corn and soybean meal on growth performance, carcass and organ yields, blood lipid profile, and expression of liver genes.
200 one-day-old male ROSS 308 chicks were assigned to 4 dietary groups according to their average live weight (LW 38.0 g ± 0.11; 5 replicates, 10 birds/pen). The 4 dietary groups were: Control (CTR: commercial feed), L-BBP (6.25% BBP), M-BBP (12.5% BBP) and H-BBP (25% BBP). Growth performance was evaluated throughout the trial. On day 34, 60 birds (3 birds/pen) were slaughtered and blood parameters, hot and ready-to-cook carcass and organ yields were assessed. 20 liver samples (5 samples/group) were collected in RNAlater and stored at –80 °C until gene expression analysis by NGS sequencing (Miseq Illumina). 11 liver genes (Acox1, FABP1, HSPA2, CASP6, CAT, FADS2, LPL, SOD1, SREBF2, ACTB and GAPDH) were evaluated according to their involvement in lipid and stress metabolism. RNA expression levels were normalized using housekeeping gene. The data showed no differences in LW and average daily gain between groups. The average daily feed intake (g/d) and feed conversion ratio showed a linear decrease ($p < 0.05$) following the increasing levels of dietary BBP inclusion (CTR: 62.5 g/d, 1.57 and H-BBP: 57.5 g/d, 1.39 respectively). Likewise, the results for carcass yields showed no differences between groups, while gizzard yield showed a linear decrease ($p < 0.05$), with the lowest value found in the H-BBP group. Although a linear increase ($p < 0.05$) in blood cholesterol, triglycerides, and serum glutamic-pyruvic transaminase was recorded, no differences in the expression of LPL gene and SREBF2 were found. In particular, the inclusion of BBP did not significantly modify the genes expression at hepatic level. These results may suggest that the dietary inclusion up to 25% of BBP could be used in broilers without impairing performance traits. However, the results recorded in blood lipid profile showed an influence of the fatty acid composition of feed and this is worth of further investigation.

O70

Effects of *Citrus aurantium dulcis* essential oil and *Yucca schidigera* saponins on broiler performance and health

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Avian coccidiosis is an economically relevant infection for the poultry industry, usually prevented by synthetic coccidiostats. The increasing resistance of avian coccidia prompted the search for new prevention methods. *Yucca schidigera*'s (Ys) steroidal saponins reduce the oocyst cycling and improve the chicken's performance. *Citrus* spp. essential oil (EOs) possesses gastroprotective ulcer healing actions, and intense antiparasitic activity, due to the presence of d-limonene. The anticoccidial activity of *Citrus aurantium L.* var. *dulcis* (Ca-EOs) and its potential association with Ys saponins has not been tested yet in chickens. This study aimed to evaluate this combination on chickens naturally exposed to coccidia. One-d-old female broiler chicks ($n = 140$) unvaccinated against coccidiosis were divided into 4 experimental groups ($n = 35$ each), maintained on natural light/dark photoperiod, watered, and fed *ad libitum* with a diet free of coccidiostats. The trial lasted 9 weeks, from 1 (*t0*) to 63-d-old (*t9*). The treatments (Tr) included: control group (Ctrl); Tr_1 (Ca-EOs 5%, 1 mL/L); Tr_2 (Ys 100% pure extract, 0.05 mL/L); Tr_3 (EOs + Ys, 1.05 mL/L) administered in water in bell-shaped tanks. Performances and coccidia oocysts count were evaluated weekly. At *t9* all groups were slaughtered and serum biochemical parameters were evaluated. Data were analyzed using STATA software. Results showed no differences among the groups in feed daily intake, feed conversion ratio, and mortality rate. The body weight showed differences ($p < 0.05$) among all treatments, with Tr_2 and Tr_3 maintaining greater body weight during and to the end of the trial (3186.7 ± 195.8 and 3274.1 ± 346.7 kg respectively) compared to Ctrl and Tr_1 (2626.7 ± 204.7 and 2948.8 ± 397.5 kg respectively). No oocysts were observed in the first 4 weeks in all groups. From 4th to 9th week, Tr_1, $Tr_{2,}$ and Tr_3 showed significantly lower oocysts than Ctrl ($p < 0.05$). The oocyst count was significantly lower in Tr_1 than Tr_2 and Tr_3 ($p < 0.05$), with a proportional decrease from 4th to 9th week in Tr_3. Differences among the treatments were detected in serum AST, cholesterol, total protein, albumin, Ca, and P, with the higher value recorded in Tr_2 and Tr_3 groups ($p < 0.05$), albeit within the physiological range. These preliminary data indicate that *Y. schidigera* confirms its anticoccidial activity, that *Citrus* spp. EOs can reduce coccidia life-cycle with coccidiostat effects, and the effects increase when Ys and EOs are combined.

O356

Efficacy of low protein diets with different amylose/amylopectin ratio on growth performance and health of weaned pigs

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In pigs, the use of low crude protein (CP) diets can reduce the NH_3 excretion and improve gut health. One risk in feeding piglets with low CP diet, is the reduction in performance. This could be due to a possible asynchronism between the blood picks of AAs and glucose, resulting in an low protein synthesis and increased NH_3 excretion. This study aims in maximizing the growth of weaned pigs with a CP diet enriched with amylopectin, which would increase the synchronism between the AAs and glucose picks. At weaning (d0), 90 pigs were divided into 3 groups balanced for body weight (BW) and litter (10 box/diet,3 pig/box): (1) Control diet (CO): standard diet (Phase1: 18%; Phase2: 16.6%; Phase3: 16.7%); (2) Low CP diet (LP) (Phase1: 16%; Phase2: 14.7%; Phase3: 14.5%); (3) As LP but common corn was replaced with Waxy corn characterized by 99% of amylopectin (LPW). The 3 feeding phases were d0-d14; d15-d28; d29-d49. BW and feed intake (FI) were weekly recorded until the end of the trial (d49). The faecal score (FS) and lesion score index (LSI) for the tail and ears were also recorded. Data on BW and average daily gain (ADG) were analysed using an ANOVA model considering the group, box and litter of origin as factors using piglets as the experimental unit. Data on FI, feed conversion ratio (FCR), LSI and faecal index were analysed using an ANOVA model considering the group as factor and the box as experimental unit. The pigs were healthy during the whole trial. The BW at d7 and d14 did not differ between groups. From d21 to d49, the LP had lower BW than CO ($P < 0.01$). BW of LPW did not differ from the CO until d28, then it was reduced ($P < 0.05$). The ADG d0–14 tended to be reduced in the LP ($p = 0.06$) and was not reduced in the LPW. From d15-d28, d29-d49 and the whole period (d0-d49), the CO group had a higher ADG compared with LP and LPW groups ($p \leq 0.01$). The FI never differs between the groups. The FCR did not differ from d0-d14 but it was lower in the CO group during the period d14-d28 ($p \leq 0.01$). The FS and LSI did not differ between the groups. Despite the reduction of the ADG, the reduction of the CP did not affect pigs' FI, health and LSI. The slight modification of the amylose/amylopectin ratio in low CP diet do not reduce the performance of pigs in the Phase1. Further investigations to determine the effect of the LP diet on NH_3 excretion and gut health are ongoing.

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O347

Implication of authorized level of Zn provided from different sources on the performance and health of low and normal birth weight piglets post-weaning

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This study aims to compare the effect of two Zn sources on performance, microbial profile, and gut status in low (L) and normal (N) birth body weight (BBW) piglets after weaning. At farrowing, 64 piglets were selected based on their BBW and divided into normal BBW (NBBW >1 kg; 32 piglets) and low BBW (LBBW <1 kg; 32 piglets). At weaning (25 days of age, d0), piglets were allotted into 4 groups (8 replicates of 2 piglets/group): (1) LBBW piglets fed a standard diet plus 120 ppm of Zn from $ZnSO_4$; (2) NBBW, piglets fed a standard diet plus 120 ppm of Zn from $ZnSO_4$; (3) LBBW fed standard diet plus 120 ppm of Zn from a potentiated source of ZnO; (4) NBBW piglets fed a standard diet plus 120 ppm of Zn from a potentiated source of ZnO. Piglets were weighted weekly until d21 post-weaning (end of the trial). Feed intake (FI) and faecal score were recorded daily. On d9 and d21, one piglet per replicate was slaughtered and colon content was collected for microbiota analysis; the pH from the distal jejunum, cecum, and colon was measured; and jejunum tissue was collected for morphological analyses. Data were analysed using a linear mixed model or a generalized linear mixed model with a Poisson distribution including treatment, class of BBW and their interaction as fixed factors, and the litter as a random factor. There was an interaction between Zn source and BBW for faecal index during d0–14 and d0–21 ($p < 0.01$). Faecal index was lower for pigs fed a potentiated source of ZnO compared to piglets fed $ZnSO_4$ (d0-d9, $p = 0.04$; d0-d14 and d0-d21, $p < 0.001$). Piglets from LBBW group had lower BW and FI throughout the study ($p < 0.01$) compared to NBBW piglets. Pigs fed potentiated ZnO tended to have higher ADG from d0 to d9 ($p = 0.07$) and from d9 to d14 ($p = 0.08$). On d14, piglets fed potentiated ZnO tended to have higher BW ($p = 0.09$) than pigs fed $ZnSO_4$. In the overall period G:F was higher ($p = 0.04$) for piglets fed potentiated ZnO compared to piglets fed ZnSO4. The pH of the jejunum in piglets fed potentiated ZnO was lower than in piglets fed $ZnSO_4$ ($p = 0.02$). An interaction was observed in villus height ($p = 0.053$) and absorptive mucosal surface ($p = 0.02$) on d21, resulting higher in NBW piglets fed $ZnSO_4$. Further data on immunohistochemistry in jejunum mucosa are under evaluation to disentangle the mechanism of action behind the observed positive effect of the potentiate ZnO on the growth performance of LBBW and NBBW piglets.

O530

From tie-stall to loose house with Automatic Milking Robot in dairy cow systems: economic, production and animal welfare implications

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Tie-stall housing is still a widespread system on dairy cattle farms, despite being considered unacceptable due to evidence of poor animal welfare, as stated in a minority opinion included in the scientific opinion published in 2009 by the European Food Safety Authority (EFSA) on the welfare of dairy cows. The transition to more welfare friendly systems could be stressful for the animals due to a huge adaptation effort to a new environment (e.g. housing, social group), but also for farmers from an economic and productive perspective. The objective of FREECOW project is to develop a model for a sustainable transition from tie-stall to loose housing in dairy cattle husbandry evaluating the evolution of some measures six months before and six months after the change of system. A dairy farm, located in Pianura Padana and housing approximatively 35 lactating cows, was monitored for one year and data regarding animal welfare, feeding, milk production and quality, sustainability, and economic were recorded before (tie-stall housing with pipeline milking system) and after the transition to a loose housing system (with Automatic Milking Robot – AMR; GEA Dairyrobot R9500). As expected, the average avoidance distance at the feed rack significantly increased ($p < 0.001$) from tie-stall (5.20 ± 20.0 cm) to loose housing (40.34 ± 47.7 cm). The percentage of very fat cows decreased from 32.1% in tie-stall to 0% in loose housing system ($p = 0.023$), as well as the percentage of dirty udder and teats (from 37.2% to 0%; $p < 0.001$). Cows were fed diets based on corn silage, grass hay, corn meal, and compound feed. Feed selection was higher when cows were in tie-stall, especially for feed with a particle size in the range between 19–8 mm, which were less ingested by cows. The daily average milk production in tie-stall was 28.4 kg/cow. A decrease to 24.4 kg/cow (1.84 milking/cow/day) was found when the AMR was switched on; however, two weeks later it increased to 30.1 kg of milk/cow/day (+19%), with 2.81 milking/cow/day, and finally it stabilised three months later at 30.4 kg of milk/cow/day (>6% than in tie-stall). Incomplete or fail milking decreased from 6.87% to 3.84% (−44%) in two months and the average rejection from the AMR box decreased from 3.43 rejection/cow/day to 0.63 (−82%). Data on milk quality, sustainability and economic still need to be analysed. Preliminary results show an impact of the new husbandry system with probable positive effects for both the animals and the farmer.

O125

Transcriptomic and phenomic data integration to identify markers of subclinical mastitis in Holstein cattle

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In this study, we integrated the RNA-Seq profiling of milk somatic cells upon healthy and naturally affected by subclinical intramammary infection (sIMI) cows with host phenotypic data, in order to gain more insights into the complexity of the molecular mechanisms driving the animals' response to infection, and identify hub variables for early detection and prediction. Bacteriological screening was performed on 188 Holstein cows reared in one herd in order to identify the experimental groups, which were defined as follows: (i) animals with no history of mastitis and negative at the bacteriological examination (NEG; $n = 9$) and, (ii) animals positive at the bacteriological examination for different pathogens (sIMI; $n = 22$). Host-related phenotypic traits included milk yield and composition traits (i.e. protein, casein, fat, urea), udder health traits (lactose, somatic cell count- SCC, pH and conductivity) and immune cells population obtained with flow cytometry. The Data Integration Analysis for Biomarker discovery using Latent Components (DIABLO) approach was used for identifying highly correlated transcriptomic and phenotypic data capable of discriminating between NEG and sIMI animals. Then, interactions among variables with $r \geq 0.90$ were used for the network construction, which was explored using the cytoHubba plug-in within Cytoscape for the hub variables identification. Genes belonging to the selected hub variables were then submitted to a receiver operating characteristic (ROC) in order to test their predictive ability for the discrimination of NEG/sIMI animals. This integration analysis confirmed a strong correlation between the transcriptome and the leucocyte populations ($r^2 = 0.72$), and udder health traits ($r^2 = 0.64$), strengthening the need to systematically include them for the screening of sIMI at herd level. The good predictive performances (Sensitivity >0.89, Specificity >0.81, Accuracy >0.87 and Precision >0.69) obtained with the ROC analysis on the selected hub genes might suggest their inclusion as putative biomarker of sIMI. Among these genes, the major histocompatibility complex (MHC), class II, DO alpha (*BoLA-DOA)* and class II transactivator (*CIITA*) could have a key role as regulators of the animals' response to sIMI and therefore should be further valuated in future studies.

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O499

Preliminary validation of ultra-wide band device as an innovative tool to assess chicken behaviour

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The observer bias and the difficulty to obtain objective data represent the main limitations in behavioural studies.

This research is aimed at developing and validating an Ultra-Wide Band device (UWB) as an innovative tool to detect three broad behavioural categories of hens (i.e. ambulation activity, peaking and spatial location). The agreement between UWB and conventional behavioural observations was defined. 60 Leghorn laying hens (18 weeks of age) were raised in barns and free-range systems (3 replicates of 10 hens each). Each hen was identified by a number ring and equipped with a bib characterized by different colours. Four recording sessions (2 in the morning and 2 in the afternoon) of 2 h were performed for each replicate. During each session, the UWB collected behaviours every 2 s, and the hens were videotaped with a camera. The filming started 10 min after the application of UWB to the bibs to allow the hens to get use to them.

An expert observer analysed the video to record the same behaviours collected by UWB (i.e. activity, peaking, and location) and the same synchronized sample interval of UWB (i.e. 2-s) was chosen for the instantaneous sampling. Cohen's kappa (κ) and McNemar's tests were used to evaluate the agreement between data collected by the observer and UWB at each scan. Sensitivity and accuracy were also reported. Intraclass Correlation Coefficient (ICC) was used to evaluate the agreement on the total occurrence of the behaviours at each session.

There was fair agreement for the locomotor activity recorded by the observer and UWB ($\kappa = 0.257$), and the difference was statistically significant ($p < 0.001$). The sensitivity was 81.6% and 45.8% for resting and ambulation behaviours respectively, while the accuracy was 74.9%. A higher agreement was found for peacking ($\kappa = 0.349$) and the difference was not significant ($p = 0.906$). The accuracy was similar (71.0%) but the sensitivity was higher than 50% for both pecking (56.5%) and no pecking (78.3%) behaviours. The accuracy for the identification of the animal's position in the area was 62.5%. Regarding the total occurrence, the agreement was good for pecking (ICC =0.613) and excellent for ambulation (ICC =0.762) and resting (ICC =0.797).

These preliminary results indicate that UWB could be useful to monitor locomotor activity, pecking, and position of hens. The low concordance of the kinetic activity could be due to the greater accuracy of the UWB than the human eye.

O452

Impact of decreased nutrient density at dry-off on inflammatory conditions in dairy cows

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Dry-off is a stressful event in dairy cows, particularly if milking is stopped abruptly. A safety threshold of 15 kg/d of milk at dry-off has been proposed and several ways are used to achieve this yield and concurrently promote mammary involution. Nutrient restriction effectively reduces yield but can alter metabolism and result in slightly lower production at lactation resumption. This study aimed at assessing whether reducing nutrient density at dry-off would affect inflammatory conditions and liver function from dry-off to early lactation. Twenty-four Holstein cows with yield greater than 15 kg/d were fed either only grass hay ad libitum for 7 days before dry-off (HF, $n = 12$) or continued to receive a standard lactation diet (CTR; $n = 12$). After dry-off, both groups received only grass hay for 7 d. Blood samples were collected at –7, –3, 0, 1, 4, 7, 14 and 28 d from dry-off (DFD), and –14, –3, 3, 7, 14 and 28 d from calving (DFC) to assess blood cell count and inflammatory biomarkers. Data were analyzed with repeated measures mixed models (proc GLIMMIX of SAS). Dry-off significantly altered blood cell count and concentrations of inflammatory biomarkers. No differences between groups were noted in red and white blood cell counts and hematocrit. Compared with CTR, in HF monocytes were lower (0.81 vs 0.64 ± 0.04 K/µL; $p = 0.01$) and eosinophils higher (0.44 vs 0.68 ± 0.11 K/µL; $p = 0.02$) at 0 DFD. The lower haptoglobin (0.08 vs 0.19 ± 0.03 g/L; $p = 0.02$) and ROM (14.4 vs 16.1 ± 0.61 mgH_2O_2/100 mL; $p = 0.04$) concentrations at 4 DFD in HF vs CTR, suggested a reduced inflammatory response in HF after dry-off. Nevertheless, after calving, HF cows had lesser albumin (34.4 vs 36.6 ± 0.54 g/L at 14 DFC; $p < 0.01$) and paraoxonase (85.6 vs 103.5 ± 5.58 U/mL at 14 DFC; $p = 0.03$), higher globulin (40.7 vs 36.2 ± 1.57 g/L at 7 DFC; $p = 0.05$), haptoglobin (0.35 vs 0.11 ± 0.08 g/L at 14 DFC; $p = 0.03$), bilirubin (6.37 vs 4.38 ± 0.51 µmol/L at 7 DFC; $p < 0.01$), GOT (170 vs 109 ± 14.2 U/L at 7 DFC; $p < 0.01$), and myeloperoxidase (483 vs 417 ± 19.2 at 7 DFC; $p = 0.02$), highlighting an exacerbated systemic inflammatory response. These results agreed with the lower milk yield observed in those cows and previously reported, indicating that severe nutrient restriction at dry-off might worsen the adaptation to ensuing lactation. Further research is needed to decipher the mechanism underlying this carryover effect.

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O501

Assessing the motivation of Leghorn hens to access outdoor space and pasture resources

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The choice of suitable genotype, especially in free range poultry production, is crucial because it is strictly linked to the use of outdoor space.

Accordingly, the aim of the present study was to evaluate the motivation of chickens to use outdoor pasture in comparison to feed. The feed, for all animals, represents a primary need ensuring nutrition of the organism; thus, feed could be considered a control test to compare the hen motivation towards different resources.

To this purpose, 9 white Leghorns hens at 38 weeks of age (1.6 kg body weight) were housed in a pen equipped with a shelter (inside 10 hens/m^2, outdoor 10 m^2/hen). To reach the outdoor area, hens had to pay a cost by pushing through a one-way transparent weighted door. The door weight was 150 g and increased 100 g every 2 days. Each animal was identified by a ring provided with a chip. A monitoring system (ChickenGate), consisting in an antenna placed near the pushing door was used to record the animals which reach the resource.

Results showed that for both the resources (pasture and feed) hens reduced the visit number as a consequence of the gradually increase of door weight. In particular, the maximum weight pushed by hens to reach the pasture was 650 g whereas 450 g for feed. However, independently from the resources, animals showed a great individual variability.

Surprisingly, between the feed and grass resources, animals have a tendency to choose the grass. In fact, hens were available to pay a highest cost to access to the outdoor area and use the pasture resource.

However, it is important to consider that hens, at equal weight, have performed higher number of visits to feed resource in comparison to pasture. This difference is probably due to the time of grazing and exploration of the outdoor area, as well as comfort activities such as sand baths, that require more time than the pecking the feed.

This study showed that, despite the biological-physiological need for animals is the achievement of the feed, the motivation of the animals is stronger oriented to the pasture resource.
This trend is probably justified by the fact that the grass resource provides a multitude of activities (feeds, kinetic and comfort behaviours) compared to the feed intake and it is surely affected by the breed used. It is widely known that the interest in outdoor is affected by the genetic strain; accordingly, the assessment of motivation could be useful also for differentiating genetic strain.

O576

Melatonin administration in heat stressed ewes: redox and immunity status of their offspring

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Scientific evidence demonstrates that a stimulus or insult [i.e. heat stress (HS)] applied pre- or post-natal growth results in permanent alterations on animal health and wellbeing of the offspring. The melatonin (MEL) is a small indoleamine with antioxidant, free radical scavenger, and an anti-inflammatory effect. The aim of the present study is the evaluation of the effect of MEL administration on the redox status (total antioxidant capacity-TAC, glutathione-GSH, and lipid peroxidation-TBARS), cytokines' profile (interleukin-(IL)-1β, IL-6, IL-10, and IFN-γ), and Immunonoglobulin (IgG) production in the ewes' blood and colostrum samples, and in the blood of lambs. Pregnant ewes ($n = 31$) were exposed to HS (Temperature Humidity Index =27, severe HS) for the first 100 days of pregnancy and allocated into two groups, the MEL group, subjected to MEL implant 16 days before mating, and the control group (CON, no melatonin implant). A total of 37 newborn lambs were divided according to MEL and CON group of mothers. Blood samples from ewes were collected at lambing (L0), 24 (L1) and 48 (L2) h later, from lambs at birth (L0), 24 (L1) and 48 (L2) h later, and then 5 (L5), 10 (L10) and 40 (L40) days after birth. Colostrum was collected at L0, L1 and L2. The free radical scavenging activity of DPPH was used to determine the TAC, the GSH and TBARS concentration were assayed using commercial kits, and cytokines were determined by ELISA. Results on redox status demonstrated that TAC values were different between the two lambs' groups at L0 and L1. The GSH level was higher in MEL lambs compared to CON at L0. On average, TBARS levels were lower at L0 in MEL lambs compared to CON ($p = 0.049$). In ewes, IFN-γ level was affected by treatment ($p = 0.006$) and time of sampling ($p < 0.0001$), registering on average a lower concentration in MEL than in CON. Moreover, the highest level of IL-6 in MEL ewes on L1 ($p < 0.05$) was registered, concomitantly to an increase of IL-10 level in MEL lambs in comparison to CON lambs on L2 ($p < 0.05$). In colostrum sample, the CON group had higher level of IFN-γ secretion than MEL ($p = 0.013$) with increased levels of IL-10 at L1 in comparison with L0 ($p < 0.05$). On average, CON lambs registered a higher level of IgG than MEL lambs, with an increased level from L0 to L2 ($p = 0.04$). Present study demonstrated that MEL could be used in HS ewes to cope with the crucial first days of their offspring showing antioxidant and immunomodulatory effects.

O165

Substitution of pollen source with commercial protein diet in honey bees nutrition: effects on survival rate and health

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Administration of protein-supplemented artificial diets in period of low food availability is a common practice in beekeeping. However, no data are yet available on protein artificial diet effects on honey bees health. In this study, effects of administration of commercial protein diets on bees survival rate, social (i.e. glucose oxidase activity, GOX) and individual (i.e. phenoloxidase activity, PO) innate immune systems were preliminary investigated. Protein diets effects were tested on two honey bee imago ages to assess whether protein diet could compensate for lack of pollen sources in newly emerged bees and improve the health of foragers. Bees were fed three commercial diets: beet sucrose candy diet without protein (control) and enriched with 1.7% and 7.7% of protein, respectively.
In newly emerged bees, administration of high-protein diet determined a higher mortality compared to bees fed control and low-protein diet. High-protein diet also stimulated GOX production at 10th day of feeding, determining a reduction of PO activity. After 20 days of feeding, GOX activity significantly decreased in all diet group to level like T0 except in the control group. PO activity in newly emerged bees did not show significant differences among diet groups at 10th day of feeding while resulted significantly lower in bees feed high-protein

diet compared to other diet groups at 20th day. Survival rate of forager bees resulted lower than in newly emerged in all diet groups with a higher survival recorded in control group than in experimental ones and no differences were recorded on immune systems.

Therefore, administration of high-protein diet determined a significant reduction of honey bees lifespan, are not able to compensate lack of pollen source in newly emerged bees and negatively affect the health of forager bees, determining an increase of mortality.

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O526

Grey Piedmont's rabbit welfare evaluation in three housing systems

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The Italian Ministry of Health's guidelines for rabbit breeding invite breeders to renovate their cages for fattening rabbits in groups. In this prospective, the production of commercial hybrids is considerably advantaged (fast grow rhythm and slaughter weight at about 70–77 d) compared to local breeds like Grey Piedmont's rabbit (GP; slow growth rhythm and slaughter at 150 d). Thus, to evaluate welfare of GP rabbits in three housing systems, 300 weaned rabbits (30 d) were randomly divided in 3 different housing systems: G (in group with 8 rabbit/pen; <40 kg/m^2), SC (in standard single cage); and M (mixed system, i.e. in group G, from 31 d to 80 d in pen, then in single cage SG, from 81 d to 150 d. The behavioural observations were repeated, in the morning on 10 rabbits/housing systems by two trained operators, at 4 different times: 55 (T1), 70 (T2), 85 (T3) and 100 (T4) days. At the end of behavioural observations tonic immobility test (TI) was performed (10 rabbits/housing system); rabbit's lesions and mortality were also recorded. All traits were analysed with R for Housing system and Time effects, and the significance was set at $p < 0.05$. As for kinetics activities, 'walking' was increased in G and M rabbits at T1 and T2 observations, whereas 'turning on itself' was the highest in SC rabbits ($p < 0.05$). Feeding activities were higher ($p < 0.05$) in SC and M rabbits at T4. The main static category was 'lying down' in SC while 'crouching' in G and M rabbits ($p < 0.05$). Stereotypies were higher ($p < 0.05$) in SC, and M at T4. Positive ('allo-grooming' and 'smelling others') social behaviours were found in G and M rabbits at T1 and T2; whereas in G rabbits at T3 and T4 social encounters became negative ('dominance', 'submissiveness', 'escape', 'biting others'). SC and G rabbits showed the highest TI values ($p < 0.05$), especially at T3 and T4. Lesions and mortality were also the highest in G rabbits at T3 and T4 ($p < 0.05$). Group housing for longer (>80 d) fattening cycle, such as in Grey Piedmont's rabbits, can seriously affect welfare, improving drastically negative behaviours between animals at the onset of puberty (~80 d), with consequent increases of injured rabbits and mortality. The M system could be adopted to guarantee the maintenance of an appropriate welfare standard, even at older ages.

O259

Linking animal welfare and antibiotic use in buffalo farms

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Antimicrobial resistance (AMR) is a major global health emergency; 25,000 people die each year in Europe from infections sustained by resistant bacteria. Inappropriate antimicrobial use (AMU) is reinforcing AMR, threatening public health. The European Medicines Agency (EMA) has classified antimicrobials according to their importance in human medicine. Specifically, the EMA's Category B refers to critical antimicrobials, the use of which should be allowed only when no viable alternatives are available. For this reason, there is growing institutional attention to implementing actions for the optimization of AMU. In particular, the World Health Organization's Global Plan of Action on Antimicrobial Resistance recommends a close intersectoral collaboration between public health and animal health (One Health). The purpose of this study was to report the first data on AMU in Italian dairy buffalo farms and relate them to animal welfare levels. Data from 102 farms were collected over a three-year period (2015–2017); AMU was estimated separately by age group (adults, heifers and calves) using the Defined Daily Dose for Italy (DDDAit). Welfare was assessed using the ClassyFarm protocol for dairy water buffaloes. The farms involved in the study had an average size of 228 adults, 116 heifers and 144 calves. Antimicrobials were used almost exclusively on adults, where overall AMU was relatively low, averaging 1.51 DDDAit/head. No significant correlation between AMU and welfare levels was found. This could be due to the low levels of AMU recorded, which hinder the detection do not allow for evidence of variability between different levels of well-beingof such effects. It remains to investigate the causes of treatment and the molecules used paying particular attention to those classes considered critical for human medicine.

O521

Transition period for Brown Swiss and Holstein dairy cows: feeding behavior and metabolic status

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The most widespread dairy breed in Europe as in the world is the Holstein breed (HO) because of its high milk production. Brown Swiss breed (BS) is also wide managed by dairy breeders especially in country across the Alps for its milk quality. Despite this general information, few studies has been reported regarding the differences between two breeds in feeding behavior, feed efficiency and apparent nutrient digestibility, especially during the transition period. For that reason, we carried out a nutritional trial in which 10 Holstein dairy cows and 9 Brown Swiss dairy cows were monitored during the transition period (i.e. from −21 to 28 days in milk DIM), in both the first and second lactations. Cows were housed at the experimental dairy farm CERZOO (San Bonico, Piacenza) and fed with TMR ad libitum with the roughage intake feeding system (RIC, Hokofarm Group, Netherland) for measuring daily dry matter intake (DMI) and feeding behavior. Further, milk quality was monitored daily though a NIRS instrument in the milking parlour and lying time through a pedometer (Afifarm, Israel). Additionally, milk samples were weekly collected for urea determination. The DMI was similar between the two breeds for the first lactation, but the trend of the intake during the transition seems to be different ($p = 0.005$) around second calving starting from one week before calving until the 4th of lactation. A significant breed effect in both lactations was reported for the parameter DMI on body weight (DMI/BW %), with greater ($p < 0.05$) values for HO compared to BS. Concerning the feeding behavior of the first transition period, there are no differences between HO and BS. Otherwise a different feeding behavior was observed between two breeds ($p < 0.05$) in the second lactation: the daily eating time was higher for HO than BS around calving (from −14 to 7 DIM) and the DMI for each meal as well as the time spent for each meal were higher for HO than BS during the whole transition period. Number of daily meals are equal for both breeds. Lying time, milk yield, ECM and FCM were higher in HO ($p < 0.05$) in lactation, but the feed efficiency did not differ. As reported in previous studies, milk protein and urea contents were higher in BS than HO, whereas fat and lactose contents were similar between breeds. Data from this study suggested the adaptation of each breed to the transition period is different, in particular for traits related to feeding behavior, performance and milk quality.

O488

Associations between the detailed milk mineral profile and total and differential cell count in Holstein Friesian dairy cows

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Udder inflammations are one of the most challenging issues in herds with high-yielding dairy cows. Indeed, after infertility, mastitis is the most economically impactful disease in dairy farms worldwide, due to its detrimental effects on milk yield and quality, and pharmaceuticals and culling costs. Specifically, udder tissue inflammation causes alterations in the concentration of major milk components including mineral elements which have a role in the maintenance of osmotic equilibrium of cells and, especially in the form of salts, in the stability of structure of casein micelle. Somatic cell count is the most known and used indicator of mastitis but recently, a novel measure, namely the differential somatic cell count (DSCC) which represents the percentage of polymorphonuclear neutrophils (PMN) combined with lymphocytes, has been proposed in combination with SCC for achieving more information about the udder health status. This study was aimed at evaluating the relationships between somatic cell score (SCS) and DSCC and the detailed milk mineral profile constituted by 16 macrominerals (Ca, P, Mg, Na, K, S) and 10 microminerals (Al, B, Ba, Cr, Cu, Fe, Mn, Sr, Ti, Zn) in a population of 1013 Holstein Friesian cows belonging to 5 herds located in northern Italy. Milk mineral concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Data were analyzed with a linear mixed model including the fixed effects of cows' days in milk, parity, SCS and DSCC (discretized in quartiles) and the random effect of herd/date. We observed that increasing SCS was associated with a linear increase in milk Fe and Na content ($p < 0.001$) and milk S concentrations ($p < 0.01$). Differently, an increase in DSCC was associated with a linear decrease in milk Na concentrations ($p < 0.001$) and milk Mg concentrations ($p < 0.01$), and with a linear increase in milk K concentrations ($p < 0.01$). Our findings revealed that changes in the concentration of minerals that are sensitive to inflammation and osmotic balance are associated with SCS and DSCC. However, the associations found for DSCC were not necessarily in accordance in terms of direction with those for SCS, which suggest that milk mineral profile might be sensitive to different stages of the disease.

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O189

NIR spectrophotometric investigation to highlight early signs of ketosis in dairy cows

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Ketosis is a metabolic disorder characterized by excessive production of ketone bodies due to altered metabolism of sugars and fatty acids. Before the appearance of evident symptoms, the subclinical form increases the risk of developing other metabolic and reproductive diseases, such as compromising the welfare of the farmed animal, as well as the qualitative and quantitative characteristics of the produced milk, resulting in serious economic and productive losses for the farm. For this reason, an early diagnosis and, consequently, an equally timely treatment of the disease still in a subclinical form is essential.

Based on these assumptions, the possibility of exploiting NIR spectroscopy combined with multivariate spectral analysis of water absorption patterns as a real-time monitoring tool directly in the milking parlour may represent a sustainable approach for the characterization of the production variables related to the early onset of metabolic disorders in intensive farms. The water molecule, in fact, is responsible for the establishment of a dynamic network of hydrogen bonds with the remaining components present in the solution and any perturbation affecting the system, such as the appearance of ketosis, causes structural changes in the aqueous matrix that can be detected by analysing the spectral pattern of water absorption. In this perspective are included the principles of Aquaphotomics. This modern scientific discipline uses water as a holistic biomarker able to reflect the overall effect of all the components of the system for immediate and non-invasive discrimination of the state of health of an animal, an opportunity for ethical and sustainable animal production that falls within the objectives of precision animal farming.

This study aims to summarize the first results obtained from applying this innovative strategy for the early detection of ketosis in dairy cattle in three farms located in the Lombardy region selected for the execution of the experimental activity.

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O43

The environmental cost of impaired welfare in dairy sheep farming

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Small ruminant farming accounts for only 6.5% of livestock greenhouse gasses (GHG) emissions, which amounts to less than 1% of anthropogenic GHG. However, this sector has a high potential to reduce its emissions by adopting good farming practices. In this sense, one of the challenges should be to find the best win-win mitigation strategies according to the 'One Welfare' perspective, simultaneously improving animal, human and environmental welfare. The present work aims at estimating the variation of GHG emissions due to impaired welfare conditions in Mediterranean dairy sheep farms, and consequently their mitigation potential. Therefore, a dairy sheep farm of the Mediterranean semi-intensive farming system, representative of good practices, was selected as a baseline scenario. From this baseline, six impaired welfare scenarios, covering the most common impaired welfare conditions (i.e. mastitis, lameness, gastrointestinal nematodes infestation – GIN, high stocking density, heat stress and water deprivation) were modelled using case studies published in the scientific literature. Each of the scenarios was characterised by different input values (obtained through modelling), used to assess the carbon footprint with Agrecalc© (a carbon calculator developed by Scotland's Rural College). The variation in emission intensity (EI) between the baseline and the scenarios for producing 1 kg of fat and protein-corrected milk (FPCM) was, then, assessed. All the impaired welfare scenarios presented an increase in EI compared to the baseline (2.38 kg CO_2 eq/kg FPCM). The high stocking density scenario, with animals housed in <1.5 m^2/head, was associated with the highest increase in EI from the baseline (+10.0%, 2.84 kg CO_2 eq/kg FPCM). The mastitis (2.55 kg CO_2 eq/kg FPCM), GIN (2.54 kg CO_2 eq/kg FPCM), and lameness (2.43 kg CO_2 eq/kg FPCM) scenarios caused rises in EI of +6.8%, +6.5% and +2.1%, respectively. The water deprivation (+1.6%) and thermal discomfort (+1.0%) scenarios showed lower increases in EI. The present results suggest that adopting good practices to enhance animal welfare, driving the conditions from impaired to baseline, might proportionally mitigate the farm's environmental impact. Emphasising multidisciplinary, through the collaboration of experts in animal welfare and environmental impact assessment, can help identify effective on-farm strategies contributing to climate change mitigation.

O335

Testing of a pilot system for the automatic monitoring of activity and body temperature of beef cattle

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Advances in the development of precision technologies for monitoring cattle activity at the individual level make it possible to detect health issues or disease outbreaks early. This study aimed at testing a low-cost and versatile set of equipment for the individual monitoring of beef cattle's activity, eating and drinking times, and body temperature. The trial was carried out in a Charolais beef cattle farm located in the Po Valley within the LOWeMEAT project financed by the Rural Development Program 2014–2020 of Veneto Region. The devices used in the study included top- and front-view cameras, a thermal imaging camera, and an RFID-based system. They were installed in a single pen where bulls were housed for the adaptation period after their import from France and allowed to gather data from 10 batches of animals (on average 27 bulls/batch). In order to validate data registered automatically from the devices and ensure the equipment was able to identify some diseases in bulls earlier, a trained veterinarian collected data on bulls' health through direct observation (coughing, nasal discharge, lameness, etc.), and drugs' treatments, and measured the rectal temperature manually. This has enabled the comparison of behavioral activities between healthy and sick bulls in the days immediately preceding the drug treatment. Since bulls' behavior can be affected by different time periods during the day, the effect of treatment/no treatment on behavioral traits was tested within specific timeslots of the day (active phase in the morning; active phase at sunset; rest phase during daylight; evening; night) by a one-way ANOVA (Proc GLM of SAS 9.3; SAS Institute Inc.). The distance travelled by bulls three days before treatment was significantly lower ($p < 0.01$) than that of healthy bulls (~3 meters/10 min) during daylight phases. In addition, bulls tended ($p = 0.09$) to increase their time spent drinking (~2 min/10 min) two days before treatment in the evening. Eating time did not show differences between treated or healthy bulls. The environmental temperature highly influenced the body temperature registered by the thermal imaging camera, often deviating from the rectal temperature measured manually. This pilot thermal and tracking cameras system showed promise in testing, notwithstanding that the recording of behavior needs to be proven on a large scale to help detect accurately 'problem' bulls earlier, and a correction factor should be included for the body temperature.

O134

Ex-hive trials on the efficacy of hop beta acids on the control of the honey bee parasite Varroa destructor

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Recently, interest in α- and β-acids of hop (*Humulus lupulus)* has increased for their acaricidal effects. In fact, some components of hop β-acids have been found to be effective on the ectoparasitic varroa mite (VM) (*Varroa destructor*), with a low impact on its host, the western honey bee (HB) (*Apis mellifera*). The present contribution (PRIMA project 1812/PLANT-B) aimed to assess the HB tolerability of hop β-acids (HBA) and their possible acaricidal effect.

Three consecutive trials under controlled conditions (34.3 ± 1 °C; 72.0 ± 1.5% RH) were performed. Young emerging HB (24 h old), 34.8 ± 1.2 per cage, were submitted to an experimental scheme including: 6% oxalic acid (OA) in water (positive control) and tap water as negative control; the treatments, namely HBA at 0.125%, 0.25%, 0.5%, 1%, 2% were dissolved in acetic acid (MetCOOH) or alternatively in ethanol (EtOH), with relative negative controls. Three replicates for each treatment were set up. After 24 h of acclimatation, a volume of 1.6 µL of treatment/control per bee was spread on the HB. Cages were daily checked and the consumption of water, 66% sugar solution (SS66), VM death (DVM) and HB death (DHB) were recorded.

The treatment that had the highest impact in terms of DVM was EtOH/HBA 2% (31.3 ± 4.7%), comparable to OA (32.7 ± 3.8%; $p > 0.05$), with a dose-dependent increase compared to untreated bees ($p < 0.01$). The EtOH-dissolved treatments showed no statistical differences for DHB except for EtOH/HBA 0.25% ($p < 0.01$) that was the highest. The SS66 and water consumption resulted the highest for the HB exposed to EtOH/HBA 0.125 and 0.25% ($p < 0.01$).

MetCOOH-dissolved treatments showed no effect on DVM but were ineffective compared to OA (32.7 ± 4.6%) ($p < 0.01$). Conversely, the highest dosages MetCOOH/HBA 1% (38.3 ± 5.4%), 2% (34.6 ± 3.8%) and OA (37.0 ± 3.2%) had higher DHB than lower dosages ($p < 0.01$) or controls. The SS66 and water consumption resulted positively affected ($p < 0.01$) at the dosages of MetCOOH/HBA 0.125% and 1% (108.0 ± 22 µL and 101.5 ± 31.5 µL); furthermore, the negative control MetCOOH/W elicited higher consumption of SS66 (83.8 ± 18.1 µL) and water (27.4 ± 7.3 µL) in comparison to the EtOH/W counterparts (46.9 ± 13.4 µL and 13.7 ± 2.0 µL).

The results showed that HBA in EtOH gave DVM and DHB levels comparable to classical treatment with OA; oppositely HBA in MetCOOH seemed to be less effective as acaricide. Further investigations are needed to explain such differences and to test the applicability as hive level.

O523

Management of dairy heifers: can operant conditioning decrease stress and ease animals' monitoring and manipulation?

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The aim of this study was to see if conditioning Holstein heifers aged between 9 and 14 months to come into contact with humans can bring benefits in terms of reducing stress in animals and allow easy manipulation. The trial involved 60 heifers (Young, $n = 24$, 286 ± 45.2 days and Old, $n = 36$, 396 ± 69.5) raised in the same farm and conditions in two adjacent pens according to the age. In the 1st week each animal was tested for its human-animal relationship (HAR) using the Avoidance Distance Test (ADT) and classified as Confident ($n = 20$, ADT ≤0.45 m), Neutral ($n = 21$, 0.45 > ADT ≤1.05 m) or Shy ($n = 19$, ADT >1.05 m). Subsequently, about half of the animals of each HAR class were subjected to an operant conditioning treatment (TR, $n = 29$) for 8 weeks running, twice per week, while the remainders were considered as control group (NTR). TR heifers were subjected to target training for 8 sessions and then were positively reinforced to let be touched on the muzzle, rump and perineum. In the final week the animals were exposed to measurement and manipulation. For each animal, heart rate (HR), Root Mean Square of Successive inter-beat interval Differences (RMSSD) through Polar Equine and fecal cortisol metabolites (FCMs) were measured at the beginning of the trial to evaluate individual baseline levels. The HR and RMSSD were measured a second time, at the end of the trial, during a session of measurement and handling procedures, FCMs was measured 12 h after the procedures and ADT 24 h after them. An ANOVA model with period (first or second measurement), temperament, age, conditioning and their interactions as fixed effects was run. TR heifers showed higher RMSSD (14.2 vs. 16.9, $p = 0.077$), that is a slightly lower stress level. Confident animals showed lower basal FCM value (29.1 vs. 30.3 vs. 38.4 ng/g, $p = 0.039$) followed by neutral and shy heifers, but after manipulation FCM showed a higher percentage increase in confident and neutral animals compared with the shy ones (80.5 vs. 44.3 vs. 10.8%, $p = 0.005$). Between the first and last measurement, the ADT decreased (0.82 vs. 0.17 m, $p < 0.001$) and the RMSSD increased. The ADT decreased more in shy and neutral heifers than in confident heifers ($p < 0.001$). This study demonstrates how conditioning some heifers leads to the reduction of some acute stress indices in the trained heifers, but above all helped to improve relationship with humans in both TR and NTR heifers, especially in those that were neutral and shy.

O369

Behavioural characterization of two local Italian chicken breeds

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Behavioural investigation plays a pivotal role in phenotypic characterization of traditional poultry breeds, in addition, data about birds' reactivity and fear responses could supply effective tools in birds' welfare and coping ability analysis and in rearing systems improvement. The aim of the present work was to characterize behavioural traits in 2 local breeds from Lombardy Region (Italy): Mericanel della Brianza (MBZ) and Milanino (MLN). Five families (1 rooster +5 hens), housed in the same conditions and fed the same diet, were studied. Birds' (1 rooster +2 randomly chosen hens / family) individual reactivity was tested by tonic immobility test (TI): number of induction (N, max 3), TI duration (s, max 180 s), number of vocalization and emergence test (ET, max latency time 180s), head emergence out of the box latency (s), first step out of the box latency (s), bird's complete emergence out of the box latency (s), number of vocalizations and defecations. Data have been analysed by ANOVA using SPSS® General Linear Model procedure, sources of variation were breed, sex and breed*sex ($p \leq 0.05$). Birds' weight (WEI) was included in the analysis as dependent variable. Home-pen based time budget (HPB; 30 min.; 5 min. scan; N of birds performing the behaviour, 7 repetitions; 14 single parameters; 4 cluster parameters; % of the birds performing the recorded behaviour) and Qualitative Behavioural Assessment (QBA; 23 variables rated; mm) were performed on the 10 families. A non-parametric Mann–Whitney *U* test was applied to data analysis considering the breed

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as the main effect ($p \le 0.05$). MBZ and MLN birds showed significant differences in tonic immobility duration (s; LS Means ± s.e.; MBZ 1.00 ± 0.119 vs. MLN 1.45 ± 0.119). High number of inductions and low tonic immobility duration were recorded in MLN hens. HPB analysis revealed significant differences between MBZ and MLN in eating (11.38 ± 5.53 vs. 2.67 ± 3.90), activity (25.69 ± 9.43 vs. 42.28 ± 11.98), perching (16.72 ± 3.42 vs. 8.19 ± 6.37) and standing (15.73 ± 6.74 vs., 28.47 ± 9.70) frequencies. Qualitative evaluation of the families revealed some differences in MBZ vs. MLN descriptions: active (88.00 ± 3.46 vs. 106.00 ± 14.28), bored (32.40 ± 18.65 vs. 0.80 ± 0.84) and nervous (0.00 ± 0.00 vs. 41.00 ± 29.90). Different breed specific reactivity and fear responses have been described: MLN birds showed lower fear responses, the same birds show lower eating and perching activities and are perceived as active and quite nervous.

O524

Effect of yeast supplementation with *Saccharomyces cerevisiae* fermentation products (SCFP) on performance and metabolic profile in suckling Holstein calves

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Calves are born with a physically and metabolically underdeveloped rumen. Stimulating the correct development of the rumen, its papillae, and microbiome is crucial to achieve successful weaning. This study evaluated the effect of supplementing milk replacer and calf starter with Saccharomyces cerevisiae fermentation products (SCFP) during the pre-weaning and for the first 10 d post-weaning. Eighteen Holstein heifer calves received 3 L of colostrum within 4 h after birth and transitional milk for the 2nd and 3rd meal, then milk replacer until weaning (60 d). Calf starter was offered *ad libitum* from 5 to 70 d. Calves were randomly allocated to 1 out of 2 experimental groups: control (CTR; $n = 9$) or supplemented group (SCFP; $n = 9$). The CTR group received no supplementation, whereas the SCFP group received 1 g/d of SmartCare in the MR from 3 to 60 d plus 5 g/d of NutriTek from 5 to 70 d as solid feed (Diamond V). Groups were balanced for birth weight (BW). Feed intake (milk replacer and calf starter) was measured daily. BW and body measurements were taken immediately after calving and then weekly. Several blood samples were collected during the study for metabolic profile assessment. Data were analyzed with repeated measures mixed models (proc GLIMMIX of SAS). Overall, calves showed similar growth performance during the study with an average daily gain from 0 to 60 d of 757 ± 80 g/d. However, the daily gain (calculated weekly) was higher in SCFP compared with CTR at week 4 (681 ± 230 g/d vs 856 ± 145 g/d in CTR and SCFP respectively; $p = 0.05$). Feed intake did not differ between groups. Compared with CTR, SCFP had greater plasma concentration of β-hydroxybutyrate at 60 (0.26 ± 0.08 mmol/L vs 0.32 ± 0.05 mmol/L CTR and SCFP respectively; $p = 0.04$) and 70 d (0.41 ± 0.10 mmol/L vs 0.46 ± 0.08 mmol/L CTR and SCFP respectively; $p = 0.06$), and urea at 70 d (4.32 ± 0.92 mmol/L vs 4.89 ± 0.99 CTR and SCFP respectively; $p = 0.06$), which may indicate greater metabolic activity at the rumen level immediately before and after weaning. These data seem to support the beneficial effect of adding *Saccharomyces cerevisiae* fermentation products to the diet of suckling calves, reducing weaning stress through improved rumen development and absorption efficiency. Further studies are warranted to evaluate potential carry-over effects of SCFP in the post-weaning period.

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O14

Welfare on arrival at the slaughterhouse: handled versus unhandled horses

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Many horses are transported each year to Italian slaughterhouses. In 2021, 29,937 horses (18,386 reared in Italy and 11,551 imported) were slaughtered. Long distance is considered a risk factor for the welfare of the transported horses and journeys must comply with specific requirements of the EC 1/2005. In particular, unbroken horses are not allowed to travel for more than 8 h, while broken horses can travel up to 24 h, but must travel individually, and be watered and fed every 8 h. Hypothesizing that journey conditions would affect the welfare of both handled and unhandled horses, the study aimed to describe their journeys and journey conditions and document their welfare status on arrival at the slaughterhouse using a standardized protocol. The welfare of 1019 horses travelling on 47 different days was evaluated at three slaughterhouses, one in Reggio Emilia and two in Bari province, respectively. Those horses travelled long distances (37 ± 11 h) and were born in Poland (429, 42%), France (316, 31%), and Spain (219, 21.5%), but 39% of them resulted unbroken at the Broken/Unbroken test performed the day of arrival. The broken horses travelled in single bays, perpendicular to the direction of travel, in most of the cases tied and unloaded by handlers who sometimes used rough handling methods. Instead, the unbroken horses travelled loose, in small groups of familiar horses (3 or 4 horses), stopping and resting in control posts or private farms during their journeys, and always self-unloaded. None of the horses arrived dead or severely lame at arrival, however, other health issues were noticed. Unexpectedly, the prevalence of injuries (24% *vs* 1.5%, $p < 0.001$) and nasal discharge (11.6% *vs* 4.3%, $p < 0.001$) was higher in the broken than in the unbroken horses. This may be due to the journey conditions, which limited the ability to balance and lower their heads, causing injuries mainly at the heads and tails, and respiratory disorders. Contrariwise, gastroenteric disorders, mainly diarrhea, were more frequent in the unbroken (6.6%) than in the broken (0.5%) horses. This could be because those horses stopped more times during their journeys changing more times diets. Our data confirmed that the journey conditions are crucial factors for the welfare of the travelling horses.

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O464

Assessment of dairy farm welfare using a benchmarking tool

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Animal welfare has become a critical issue for the livestock sector, due to the awareness of the livestock industry following concerns expressed by the public, scientist and NGOs. This has led to the implementation of new tools designed to meet this objective. The present study aimed to use ten animal and resource-based indicators to create an index and develop a benchmarking tool that can be used to dynamically assess the welfare of cattle on farms with different husbandry systems and provide farmers, veterinarians, extension officers and stakeholders reliable information that can be used as a decision support tool for dairy farm welfare. Therefore, 1891 cows from 204 herds housed in free ($n = 111$) and tie stall ($n = 93$) husbandry systems in South Tyrol (Northern Italy) were evaluated using an individual scoring system for each assessed indicator. The analysis performed using generalized linear mixed model of SAS statistical software. The results showed that 17.6 % of herds (36 farms) had an average score below 60 (out of 100) which means that based on the indicators immediate intervention should be adopted to improve welfare. South Tyrol dairy farms performed well in terms of lameness, skin alterations, avoidance behaviour, number and space of boxes, and dystocia rate scores, while water supply, cow cleanliness status, claw conformations and getting up behaviour offered significant potential for improvement. Substantial differences were observed between housing systems (loose house vs. tie barn), with scores of less than 60 out of 100 points in 9.9 % ($n = 11$) and 26.9%, ($n = 25$) ($p < 0.05$), of the herds kept in free and tie stall, respectively. A slight difference was found when comparing the percentage of herds with high scores, corresponding to 30.6% ($n = 34$) of herds housed in free and 22.6% ($n = 21$) ($p < 0.05$) of herds housed in tie stall housing systems. In addition, it appeared that animals reared on tie stall farms had more frequent problems with body condition, cow cleanliness, and skin alterations. All individual welfare indicators in the top 25% of herds had scores above 80, indicating that this may be a feasible target for free stall and tie stall herds, respectively, to promote good dairy cow welfare. The overall objective must be to adopt measures to improve the scores on all farms closer to this level.

O8

Evaluation of inter-observer reliability of animal-based welfare indicators in the case of trichotomous variables with two or more observers

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This study focuses on the problem of assessing inter-observer reliability (IOR) in the case of trichotomous categorical animal-based welfare indicators and the presence of two or more observers. A modified Animal Welfare Indicators (AWIN) assessment protocol was applied by three observers (A, B and C) on ten dairy goat farms in Piedmont (NW Italy) during the alpine summer grazing season. From these observations and using the body condition score (BCS; scores: -1; 0; 1) as an indicator, we compared the performance of the most popular agreement indexes available in the literature. The IOR was calculated between pairs of observers (A-B; A-C; B-C) using Scott's π, Cohen's K, K_C, K

PABAK, Holley and Guilford's G, Holsti's H, Krippendorff's α, Hubert's Γ, Janson and Vegelius's J, Bangdiwala's B, Andrès and Marzo's Δ, Quatto's S, Quatto's S^* and Gwet's $\gamma(AC_1)$, and among three observers (A-B-C) using Krippendorff's α, Fleiss' K, Hubert's K, Andrès and Marzo's Δ, Quatto's S and Quatto's S^*. For all the indexes, confidence intervals were calculated using the Bootstrap Method in R software; when they were easy to implement, confidence intervals were also calculated using closed formulas of variance estimates. Our results show that some of the calculated indexes, such as Scott's π, Cohen's K, Krippendorff's α, Fleiss' K and Hubert's K were affected by the paradox effect: when the observed concordance rate (P_0) was high, these indexes sometimes gave very low agreement values ($P_{0AB} = 86\%$; $\pi = 0.23$; Cohen's K, $\alpha = 0.24$; $P_{0AC} = 76\%$; π, Cohen's K, $\alpha = 0.07$; $P_{0BC} = 83\%$; π, Cohen's K, $\alpha = 0.33$; $P_{0ABC} = 82\%$; α, Fleiss' $K = 0.21$; $P_{0ABC} = 73\%$; Hubert's $K = 0.19$). Bangdiwala's B, Gwet's $\gamma(AC_1)$ and Quatto's S^* were not affected by this phenomenon, and seemed to be the best indexes for evaluating the agreement between two observers for trichotomous categorical indicators ($P_{0AB} = 86\%$; B, $\gamma(AC_1) = 0.84$; $P_{0AB} = 93\%$; $S^* = 0.84$; $P_{0AC} = 76\%$; B, $\gamma(AC_1) = 0.73$; $P_{0AC} = 88\%$ $S^* = 0.73$; $P_{0BC} = 83\%$; B, $\gamma(AC_1) = 0.81$; $P_{0BC} = 92\%$; $S^* = 0.81$). Quatto's S and Quatto's S^* gave the most reliable agreement values in the case of three observers ($P_{0ABC} = 82\%$; $S = 0.73$; $P_{0ABC} = 91\%$; $S^* = 0.79$). The Bootstrap Method turned out to be simpler compared to the implementation of closed variance formulas and provided effective confidence intervals for all the considered indexes.

O74

Growth rate and hair cortisol variation of beef cattle in pastoral and silvopastoral systems

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Pasture-based livestock systems are threatened by climate change; indeed, their economic viability is undermined by a reduction in their productivity. In the Mediterranean, the increase in drought periods decreases pasture allowance and quality. Moreover, elevated temperatures, intense solar radiation and high relative humidity cause the decrease of animal intake, which in turn cause a reduction in the performance of extensive livestock systems and the decline of animal welfare. Heat stress can induce hormonal changes, including an increase of stress indicators such as blood and hair cortisol. Mitigation and adaptation strategies need to be implemented to increase the resilience of extensive livestock systems to cope with climate change. In this study, we investigated the adoption of agroforestry as mitigation and adaptation strategy because the integration of trees in pastoral systems can mitigate heat stress by reducing animal heat load.

In the present experiment, the average daily gain (ADG, kg/day) and cortisol accumulation (pg/mg) were measured in Maremmana beef cattle to compare animal performances according to two different grazing systems (GSs): pastoral (PA) and silvopastoral (SP). The two-years experiment was carried out in a real farm located in southern Tuscany from March to August 2021 and from March to September 2022. In the experiment, 50 and 40 growing Maremmana heifers and steers with comparable initial weight and age were used in 2021 and 2022, respectively, allotting the animals in two groups: PA and SP. In both years, microclimate parameters were monitored throughout the summer to compare the heat load in PA and SP systems with the black globe humidity index.

A linear mixed-effect model was used to determine the effects of GS on ADG and cortisol. In 2021, the highest ADG was recorded in spring for PA, when pasture allowance was greater. In 2022, the overall effect of GS was not significant ($p = 0.46$). ADG was lower in summer respect to spring in 2021 and 2022 with a greater reduction in PA (significant interaction between GS and time, $p < 0.01$). Regarding cortisol, statistical analysis shows a significant difference ($p < 0.05$) between GSs in 2021, and in tendency also for 2022 ($p > 0.05$).

This study shows that agroforestry can mitigate the heat load on grazing animals during Mediterranean summers. However, novel feeding strategies in agrosilvopastoral systems should be further studied to improve the resilience of extensive livestock farms.

O403

Evaluation of fecal and urinary excretion and behavior of Italian Simmental dairy cows during the access to exercise pasture for 2 and 4 hours per day

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Prolonged indoor confinement of dairy cows can have negative consequences on animal health, behavioural expression, welfare and lifespan. Many studies have reported positive effects of pasture and grazing but few experiments are available on other outdoor conditions (i.e. exercise pasture). The objective of this experiment was to evaluate the environmental impact of faecal and urine output and behaviour pattern of the cows during the paddock access time (PAT) for 2 and 4 h per day. Six Italian Simmental cows were divided into 3 pairs balanced by DIM, parity and milk yield. Following a 3 × 3 Latin square design, the cows were assigned to 3 experimental groups: CTR, without outdoor access; U2, exit once a day (11.30–13.30); U4, exit twice a day (divided into U4am: 9.00–11.00 and U4pm: 14.00–16.00). The trial start at the end of October 2023 and each of the 3 periods lasted 2 weeks. The grazing possibility of fenced area (total surface of 600 m2) was very limited. Water was available ad libitum. Manual collection of total faecal and urine output and behavioural observations of the animals during the outdoor access were carried out during 4 days per period. Animals were fed a corn silage-based TMR and milking was performed by an automatic system. A statistical analysis of the results was carried out considering only data of the outdoor activity, using a simplified model with two factors: PAT (3 levels: U2, U4am, U4pm), animals ($n = 6$), and interaction PAT × animals. The total distance covered by the animals in the external fenced area was on average 782 ± 458 meters in 2 h without significant differences among PAT ($p = 0.95$). On the contrary, the animal effect on this parameter was very high ($p < 0.01$). No correlation was found between the external environmental temperature (ranged from 4 to 23 °C during the trial) and the outdoors locomotion activity of the animals ($r^2 = 0.06$). The amount of faeces produced by the animals during the 2 h spent outdoor was significantly influenced ($p < 0.001$) by PAT (2.74, 3.82 and 2.17 kg of fresh faeces respectively for U2, U4am, U4pm). Instead, the amount of urine produced was influenced by the animal ($p < 0.01$) with mean values per cow ranging between 1.55 and 4.77 kg. In conclusion, the urinary excretion of cows during the paddock access is characterized by a high individual variability while the faecal production appears to be higher in the central hours of the day (from 11.30 to 13.30) than in the morning and afternoon.

O453

Space use by laying hens in a cage-free system: effect of genotype and enrichment with perches

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To evaluate the effect of the genotype (Hyline Brown vs. Lohmann White) and the enrichment with additional perches on a wall in front on the aviary (enriched or not) on space use in an aviary system, 1800 pullets (17 weeks of age) were allocated in 8 pens of a three-tiers aviary at the Experimental Farm of the University of Padova (two pens per experimental group). For the purposes of the present study, the following data were recorded from 26 to 35 weeks of age: the weekly oviposition rate, the distribution of hens and eggs in the aviary. Data were analysed by ANOVA using a mixed model with genotype and perch enrichment as main effect, week of age as a random effect, and pen as a repeated measure.

Oviposition rate was higher in white compared with brown hens (95.5% vs. 91.6% present hen) which was also associated to a higher deposition rate of dirty eggs (13.5% vs. 3.20%) and a lower deposition rate of broken eggs (0.79% vs. 2.08%) ($p < 0.001$). The distribution of the hens in the aviary during the morning showed a higher presence of white hens in the nests (6.21% vs. 3.48% of observed hens) and on perches of the enriched walls (7.13% vs. 2.52%) compared with brown hens ($p > 0.001$). On the other hand, a lower rate of eggs laid in the nests (88.2% vs. 92.2% total eggs) and a higher rate of eggs laid on the floor (11.4% vs. 2.48%) was recorded in white compared with brown hens, while the rate of eggs laid out of the nests on the tiers showed an opposite trend, being higher in brown compared with white hens ($p < 0.001$).

The presence of additional perches decreased the rate of hens on the floor (33.2% vs. 37.2%; $p < 0.05$) while 4.82% of hens were found on the additional perches when these were available. The perch enrichment decreased the eggs laid in the nests (88.3% vs. 92.1% total eggs) and increased the eggs laid on the tiers out of the nests (3.41% vs. 2.29%) and, especially, the eggs laid on the floor (8.25% vs. 5.60%) ($p < 0.001$). These results corresponded to a higher oviposition rate of dirty eggs (10.5% vs. 6.67% present hen; $p < 0.001$) and a lower oviposition of broken eggs (1.01% vs. 1.79%; $p < 0.01$) in pens enriched with additional perches compared with not enriched pens.

Since significant interactions were observed between genotype and enrichment with additional perches, it can be concluded that the design of the aviary requires genotype-specific adaptation to optimize space use by laying hens for optimizing their welfare and productive performance.

O69

Relationships between on-farm animal welfare assessment, milk yield and quality in dairy cows

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The demands of customers have raised pressure on dairy farmers to enhance the welfare of their animals. Dairy farmers may now choose from different on-farm animal welfare assessments that are either optional or part of mandatory programs. In Italy a checklist called 'Classyfarm' has been proposed to evaluate animal welfare based on European legislative references, EFSA suggestion and literature data. The aim of the present study was to evaluate if the animal welfare score, obtained using the Classyfarm checklist, was related to the milk yield and quality in a cohort of Tuscan dairy farms (DF). A total of 34 DF were included in this study. Four different area presented in the check list were scored (structure (S), management (M), animal-based measures (ABM), and total welfare (TW)). S area was composed by 32 different items, M area by 29 items, ABM area by 19 items and TW area consisted of 105 items. The score of each area were classified as 'medium' if it was under 70, 'high' between 70 and 80, and 'very high' if greater than 80. Data about milk yield and quality were collected from each DF. Milk quality was obtained by the mean of 5 different milk pool analysis; the parameters evaluated were the somatic cell count (SCC), the total bacteria count, % of fat, % of protein and % of lactose. A non-parametric test (Kruskal–Wallis) was used to evaluate the effect of the 4 areas on milk parameters. Results showed that DF with very high M and S scores had greater milk yield compared to those with medium M score ($p < 0.03$). The difference in milk yield was significant only for the month of check-list application. SCC tended to be lower in DF with very high ABM score compared to those with medium score ($p = 0.08$). Significant differences were found for the other milk quality parameters, according to the Classyfarm scores. The study showed that very high M and S scores could have a relation with productive parameters which may represent useful indicators of animal welfare. The tendency of lower SCC with very high ABM may be related to a direct effect of ABM (i.e. the incidence of mastitis is smaller for cleaner cows) or to an indirect effect related to better environmental situation that in turn led to better immune system response with a reduced illness susceptibility. In conclusion, the application of Classyfarm checklist may enhance farmers' understanding of the animal welfare, leading to an improvement of animal health and productivity.

O123

Mass spectrometry-based characterization of the bovine milk peptidome upon Streptococcus agalactiae and *Prototheca* spp. infection

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During mastitis there is a substantial change in the proteins in milk. A variety of proteinase are released from both microrganisms and host immune cells, which results in the increase of proteolitic activity and consequently, the production of hundres of endogenous peptide fragments. Hence, this study aims to investigate the differences in the milk endogenouse peptidome upon *Streptococcus agalactiae* (*Strep. agalactiae*) and *Prototheca* spp. subclinical intrammary infection (IMI) in Holstein cattle. Endogenous peptides were isolated from the milk of animals negative at the bacteriological examination (Neg, $n = 14$), animals positive for *Strep.agalactiae* (Sa+, $n = 10$) and animals positive for *Prototheca* spp. (P+, $n = 11$) and analysed by mass spectrometry (MS)-based approches. Untargeted analyses performed with Matrix Assisted Laser Desorption/Ionisation Time-of-Flight (MALDI-TOF) MS and nano-High Performance Liquid Chromatography (nanoHPLC)-Orbitrap MS/MS combined with discriminant analyses allowed the identification of 40 peptides with the higher capacity to discriminate the different groups: Neg, Sa+, P + and Positive (Pos, Sa + plus P+). These peptides were validated by a targeted Ultra High Performance Liquid Chromatography (UHPLC)-triple quadrupole MS/MS analysis. Final data were Log transformed and significant differences among groups were detected by appling a linear model including the bacteriological status (Neg, Sa+, and P+) as fixed effect. Overall, the results showed a larger abundance of peptides in positive samples. A panel of 32 peptides belonging to β-casein, αs1-casein, αs2-casein, k-casein, β-lactoglobulin and butyrophilin (subfamily 1, member A1) was significantly different between Pos and Neg. In particular two peptides derived from k-casein and αs1-casein were the most promising (Fold Change, FC >2 and p-value <0.001). No peptide was able to discriminate between Sa + and P+. Further analyses in a larger number of animals are definitely needed in order to propose these peptides as biomarkers of IMI which could help for the detection of subclinical pathological conditions and the appliciation of targeted therapies.

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O247

Linking animal welfare and biosecurity, a case study in buffalo farms

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ClassyFarm is an integrated system for the categorization of farms according to the risk assessment (RA) methodology. It is an Italian innovation aimed at facilitating and improving the synergy between breeders and competent authorities, in order to ultimately improve the safety and quality of food of animal origin. ClassyFarm gathers and processes data referred to the following areas: biosecurity, animal welfare, health and antimicrobial usage. It can be applied to several livestock species, included water buffaloes. Upon request of the Italian Ministry of Health (IMH), the National Reference Centre on Water Buffalo Farming and Productions Hygiene and Technologies (CreNbuf), in collaboration with the Italian Reference Centre for Animal Wefare (CreNBA), developed a RA-based checklist (CL) for the on-farm assessment of buffalo welfare and farm biosecurity level, included in the ClassyFarm system. The multiple-choice CL consists of 80 items. Each item is scored according to 3 categories: 'insufficient', 'acceptable' and 'excellent'. The assessment system for animal welfare includes non-animal based (N-ABMs) and animal-based measures (ABMs). N-ABMs are divided into 2 macro-areas: Area A (32 items) 'Management factors' and Area B (31 items) 'Housing factors'. ABMs are assessed in Area C (17 items). Biosecurity was assessed using 15 indicators. The CL has been tested in 100 farms, with an average size of 412 heads (min 81, max 2240). The overall welfare value was on average 61.55% (on a scale from 0 to 100%) and the average biosecurity score was 43.31%. The statistical analysis was performed by Spearman Rank correlation coefficient using GraphPad Prism 8.0.1. (GraphPad Software, San Diego, CA, USA). The two variables were found to be positively correlated (Spearman's Rho =0.501; $p < 0.001$). The average welfare values of the specific areas were: A, 61.76%; B, 42.17%; C, 70.43%. At least one potential legislative non-compliance was recorded in 39.80% of the farms. These CLs represent a functional and effective tool to assign animal welfare and biosecurity indexes to farms, allowing to improve farm management and housing conditions, giving answers to consumers and adding value to farmers' good practices. The IMH is promoting the application of this system at European and international levels.

O54

Walking distance and maintenance energy requirements of sheep during mountain pasturing (transhumance)

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Sheep pasturing has become an important means for landscape management and conservation in marginal areas of mountain regions by reducing succession with dwarf shrubs and bushes, thus creating space for valuable forage grasses. Furthermore, mountain sheep pasturing for meat production also contributes to local food security. However, little is known about the energetic expenditures of sheep during mountain pasturing, which is important information for optimizing the productivity and economic efficiency, environmental aspects (e.g. biodiversity) as well as animal welfare. Therefore, the aim of the following study was to estimate the maintenance energy requirements of ewes over the whole mountain pasturing period (transhumance) considering movement patterns assessed by using satellite-based Global Positioning System (GPS) tracking devices. Energy requirements for walking increased rapidly at the beginning of transhumance (May–June) (4.14–4.17 MJ/d), which could be explained by the longer walked distance and by overcoming variable altitude during that phase. Walking speed (2–8 m/min) was slower compared to previous findings due to the difficult terrain of mountain pastures on which sheep moved. Energy demand for walking was correlated with walking distance (0.45, $p < 0.001$) and walking speed (0.26, $p < 0.001$). Results out of this study contribute in promoting the efficiency and consequently the rentability of alpine sheep pasturing systems. The latter ensures the production of local food and further preserves the ecosystem services linked to this low-input production system.

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O294

Assessment of the allostatic load in horses at the slaughterhouse

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From an ethological and ethic perspective, it is becoming more and more crucial to assess animal welfare at the time of slaughtering. The aim of this study was to evaluate horses' allostatic load at the slaughter with a non-invasive method through the sampling of faeces and hair to measure 11-oxoetiocholanolone (11-oxo) and cortisol (HCC), respectively.

The concentration of 11,17-dioxoandrostanes in faeces is one of the several faecal indicators that are used to evaluate the HPA axis activity retrospectively over the medium term (1–2 days) being a metabolite of cortisol. Hair cortisol has been used to evaluate the HPA axis activity also retrospectively but over the long term and with a lag time of about 14 days.

This study involved a total of 42 Trotter horses (11 females and 31 males, aged 1 to 20 years old) that were sampled at the slaughterhouse.

Following the animal's evisceration, faeces were removed from the large intestine and immediately frozen for lab extraction. Methanol was used in the first phase of the extraction, and diethyl ether in the second. The dried-down ether phase was then solubilized in buffer and kept at −20 °C until analysis. The levels of 11-oxo in faeces have been measured using a competitive ELISA kit that is commercially available (Cayman Chemical, No. 501420 Ann Arbor, MI, USA).

Hair samples were obtained by using an electric clipper to trim the hair close to the skin. Samples were washed with isopropanol and extracted with methanol for 16 h at 37° C. The leftover residue was then evaporated and dissolved in ELISA buffer. An in-house ELISA technique was used to measure the HCC.

The faecal 11-oxo concentrations obtained in this study were generally lower than those in control samples (mares at parturition characterized by high HPA axis activation) while the HCC were in agreement with those from studies using the same biological sample in animals at the equestrian centres with moderate sport activities.

Thus, sampling hair and faeces at the slaughterhouse looks promising to offer an easily collecting and standardizable sample to monitor animals' stress before and close to the slaughtering.

O156

Can forage legumes play a role in sustainable dairy farming? Preliminary evidence on buffalo and cow herds in Campania, Southern Italy

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Climate change and associated weather variability, together with factors such as the need to maintain soil fertility, competition for arable land, and rising protein feed costs, are driving changes in forage systems. The aim of this study was to investigate whether the re-introduction of legumes in farm forage plans can play a role in improving economic and environmental sustainability of forage systems in livestock areas of the Campania Region. This note provides preliminary results related to the forage yield. The study sites were a 154-hectare dairy buffalo farm located in an irrigated, flat area with a maritime Mediterranean climate, and a 60-hectare dairy cattle farm located in a rainfed Apennine foothill area with a subcontinental Mediterranean climate. At both sites, two grass forages (ryegrass, *Lolium multiflorum*, and wheat, *Triticum aestivum* cv. Ludwig) and one clover forage (a 50/50 mixture of *Trifolium alexandrinum* and *Trifolium squarrosum*) were tested in pure or intercropped cultivation. A total of 9 theses were compared: 4 intercrops with legume/grass ratios of 50/50 and 75/25, and 5 controls, i.e. the pure clover crop and the pure ryegrass and wheat crops fertilized or not with nitrogen. For both sites, the sowing and harvest dates were November 2020 and May 2021, respectively. The trial was arranged into three blocks, each of which was divided into 9 plots (5 × 4 m), one for each thesis. The physicochemical characteristics of active layer of soil of each plot was determined. When scheduled, nitrogen fertilization was carried out at the beginning of the grass tillering stage. At harvest, biomass production and floristic composition were estimated in a 1 m^2 area of each plot and forage samples were collected. The overall results can be summarized as follows: (a) the role of legumes in maintaining soil fertility is less pronounced under frequent manuring; (b) the rapid growth of ryegrass tends to reduce the incidence of clovers; (c) the slow germination of clover promotes weed growth; (d) ryegrass needs well-prepared soils, whereas wheat is more adaptable (larger seeds) and gives better yields than ryegrass; (e) the 50/50 intercropping of wheat and clover gives the highest and most stable yields providing a protein yields of 0.5 t/ha in marginal areas (poorly fertile soils, inland hills, low temperatures) and 1.0 t/ha in fertile environments (well-prepared soils, plains, temperate climate, frequent manuring).

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O522

Discriminating between limiting and reducing factors in dairy farms through yield gap analysis

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Yield gap analyses, at farm level, are used to estimate the difference between the potential and actual production of farms and describe the causes of this difference. This study aimed to find some indicators which, on a local scale, could help farmers calculate their yield gap and understand whether their constraints were mainly due to limiting (feeding-related) or reducing (management and health) factors. Twentynine farms located in the Po Valley were checked on average 4.89 times over a two-year period. At each visit, milk yield (MY) and dry matter intake (DMI) were recorded, TMR homogeneity index (HI) was measured and samples of TMR and faeces were collected to determine TMR composition and digestibility. Income over feed cost (IOFC), summer-winter ratio (SWR) in MY and digested dry matter (DDM) were calculated. Farms, on the basis of their average daily milk yield per cow, were divided into three milk production classes: Low (L), Medium (M) and High (H), characterised by ≤31.1, 31.1–36.7 and ≥36.7 kg/head/day MY, respectively. Data were analysed through an ANOVA mixed model (SAS release 9.4) using the production class as fixed effect and the farm nested in the production class as random effect. A stepwise regression was run to assess the relationship between the feeding-related variables and MY. The achievable milk yield (AMY) of a farm (no reducing factors) and AMY with no feeding-limited factors (NFLMY) were calculated on the basis of the stepwise model. Achievable (AIOFC) and not feeding-limited IOFC (NFLIOFC) were calculated as well. H farms showed higher IOFC ($p < 0.001$), DMI ($P = 0.006$), DDM ($p < 0.001$), digested crude protein (DCP, $P = 0.019$), CP intake ($P = 0.084$), HI ($P = 0.09$), SWR ($P = 0.041$) and lower HI coefficient of variation ($P = 0.04$). The conversion of DDM into milk was higher ($p < 0.05$) in H and M compared with L farms. The explicatory variables of stepwise regression were DDM and crude protein (CP) with a R^2 of 0.716 ($p < 0.05$). Replacing MY with AMY led to the same MY between L and M farms, whereas replacing MY with NFLMY reduced the difference between M and H. The same trend was found for IOFC. The M farms had a yield gap mainly due to feeding-limited factors, whereas for L the yield gap was also due to reducing factors. Further research with a deeper insight on reducing factors is warranted.

O540

Effect of omitting dry period in Saanen and Alpine goats on metabolic profile and performance in the subsequent lactation

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The dry-off is a challenging practice in high-yielding goats due to their lactation persistence, forcing dairy farmers to restrict nutrient supply. Dietary limitation potentially induces detrimental effects on late gestation dairy goats. Omitting dry period has been proposed as a strategy to cope with this limitation, but its metabolic effects have never been tested. Twenty-eight dairy goats (20 Saanen and 8 Alpine) were either abruptly dried off 60 d before the expected calving (DP; 10 Saanen and 4 Alpine) or milked continuously till kidding (CL; 10 Saanen and 8 alpine). Blood samples were collected at –2, –1, 1, 2 and 4 weeks from kidding (WFK) and analysed for plasma metabolic profile. Milk yield was recorded, and milk samples were analysed for fat, protein, lactose, and somatic cell contents during monthly dairy herd improvement checks at –8, 1, 3, 10 and 11 WFK. Data were submitted to ANOVA considering time as a repeated measure and group (DP or CL), its interactions with breed and time, and the 3-way interaction as the fixed effects. No differences were noted in milk yield after kidding. Compared to DP, CL had higher protein, lower lactose and higher SCC in milk ($p < 0.05$). In plasma, DP had higher β–hydroxybutyrate, urea and lower creatinine at –2 and –1 WFK ($p < 0.05$) pointing a higher ketogenesis and higher amino acid catabolism due to the lower nutrient content in dry diet. In comparison with DP, Saanen–CL had higher glucose at –2 and –1 WFK and lower NEFA at –1 WFK ($p < 0.05$), while Alpine–CL had higher NEFA at –2 WFK ($p < 0.01$), suggesting that CL improved prepartum energy availability in Saanen, but not in Alpine goats. CL had higher γ–glutamyl transferase at –2, –1 and 1 WFK ($p < 0.05$), reflecting greater liver activity compared with DP goats. CL had higher cholesterol, albumin, and lower haptoglobin at –2 WFK ($p < 0.05$), reflecting lower prepartum inflammation compared with DP. Despite that, Saanen–CL had higher ceruloplasmin ($p < 0.05$), suggesting that the beneficial effect of CL on inflammation was less marked in Saanen

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than Alpine goats. CL had lower myeloperoxidase at 1 WFK ($p < 0.01$), reflecting a milder immune system activation at the onset of lactation, but higher reactive oxygen metabolites at 2 WFK ($p < 0.05$), suggesting greater oxidant species release. These outcomes suggest that omitting dry period is a potential strategy to cope with the issues hindering dry off in high-yielding goats without negatively affecting metabolism and milk composition.

O126

Trend in management scenarios of smallholder livestock farming systems affects ecosystem services: a landscape perspective from Campo di Segni (Lazio, Central Italy)

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The spatial transformation of cultivated and pasture lands may threaten the provision of some ecosystem services and limit human well-being. Because of the abandonment of traditional agronomic and pastoral practices, in agricultural systems characterized by mountainous and smallholder farming, common landscape transformations are reafforestation and loss of historic and traditional buildings or rural structures.

Through a space-time reconstruction of the main environmental and agricultural characteristics, we quantified the modifications of the agro-pastoral landscape of the Campo di Segni plateau (3566 ha, Lepini mountains, Central Italy) that has occurred in the last 60 years.

Our sources have been historical and recent bibliographic elements, direct interviews with local farmers, cartographic and diachronic (1954 *vs* 2016) geostatistical analysis of land use from aerial photograms.

The pastures in the municipality of Segni were assigned in the 1950s to 62 farmers, rearing 334 cows, 230 horses, 39 pigs, 210 sheep and 120 goats, corresponding to 645 Animal Units (AU); in 2016 the pasture were assigned to 28 farmers, rearing 200 cows, 180 horses and 330 sheep, or 513 AU). The negative trend of the livestock load was 20.5%.

Accordingly, the temporal analysis has shown a clear process of re-naturalization of the lands used for grazing or cultivation in the past. In fact, we found 706.4 ha of new forest or encroached land uses (+19.81% respect to 1954) and only 142.4 ha of regression of natural areas (+5.55% respect to 1954); the 74.64% of the study area has not undergone changes respect to the land use of 1954. In some cases (99.2 ha, 2.78%), the loss of grazing areas has been caused by urbanization processes.

Several traditional rural buildings (i.e. farm huts, lèstre – in the local dialect) are in a state of abandonment and are in danger of falling down, as well as the other traditional facilities serving the breeding systems (drywalls, macere, fountains, wells and cisterns, volubri, etc.)

This analysis suggests that the study area still retains many valuable elements to justify and support an extensive livestock farming system, but urgent interventions and enhancements are needed so that this environmental and cultural heritage is not definitively lost, as already happened in other Apennine contexts.

O269

Evaluate, drive and communicate the sustainability of complex systems: a proposal

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There is a growing need to measure the sustainability (or unsustainability) of a farm among researchers, policy makers and farmers. The evaluation is useful to know the sustainability level of a farm, to drive or to monitor and evaluate the CAP application with its cross-compliance, greening and rural development measures but also to communicate to the supply chain and to the end-consumer this property. A farm with livestock is a complex production system with interaction between the productive activities carried out by the farmer and the natural, social and economic environment. These interactions develop several functions measured with specific indexes and unit of measurement that make impossible express them in a single value. Aim of this work is to propose a method to express the sustainability of a complex system in an all-inclusive way transforming the expression of the sustainability functions into a dimensionless scale that allows adding these evaluations into a single final value. To this purpose has been identified three domains or components (environmental, territorial and social-economic) characterized by several functions (clusters). To evaluate with a dimensionless unit each cluster was applied the principle of the theory of the risk (UNI EN ISO 12100:2010). A matrix evaluation based on four grades for the farm functions (lacking, rare, probable, highly probable) and the environmental, territorial and social-economic vulnerability of the farm settlement area (light, medium, heavy, very heavy) respectively, give sixteen values classified into four classes: negligible (1); medium (2–3); high (4–8); very high (9–16). Careful attention should be given to the selection of clusters and indicators that measure them, since the functions considered will influence the outcome. The evaluation of each cluster could be eventually multiplied by a coefficient proportioned to the importance of the function expressed or to the function to be boost. The sum of the clusters evaluation gives the Sustainability Index (SI) of the farm expressed on four classes (A, B, C, D) and three sub-classes for each class (+, 0, −), representable graphically and interpretable in a simple way. In conclusion the method lends itself to be used as (i) sustainability assessment tool (to measure farm sustainability); (ii) farm development tool (to increase sustainability); (iii) policy makers tool (to reach or improve the CAP objectives); (iv) communication tool (as commercial label).

O306

Assessment of individual dairy cows *in vitro* rumen methane emissions

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Research in the last years has resulted in a better understanding of factors influencing methane (CH_4) emissions, the main greenhouse gas produced by ruminants. A large proportion of the variation in enteric CH_4 emissions from animals can be explained by diet composition and feed intake. Nevertheless, differences in CH_4 release might be ascribed to each individual cow. Aim of the study was to screen the CH_4 production in individual dairy cows using the Gas Endeavour® *in vitro* system (GE, Bioprocess Control, Svezia). For this purpose, 58 Italian Friesian dairy cows from the University of Bologna dairy research farm were enrolled. Animals had, on average, 3.5 years of age (±1.36), 176.5 DIM (±110.52), 40 l/day milk yield (±11.79), 3.92% fat (±0.43), 3.45% protein (±0.23). Diet fed to all animals was mainly composed by alfalfa hay (35.1%DM), wheat hay (11.16%DM), and cereal mix (48%DM). Rumen fluid was sampled with an esophageal probe at 9.0 am after feeding. After collection, the rumen fluid was filtered with a sieve (1 mm diameter pores) to eliminate gross material, and then measured for its pH using a pH meter (PH20er, VWR). The rumen fluid was thus used as inoculum for *in vitro* TMR fermentation, according to the Tilley and Terry (1963) modified technique. CH_4 production was measured *in vitro* using the GE. CH_4 production was evaluated at 3 different time points: 8 (T1), 12 (T2) and 24 (T3) hours of incubation. Data distributions were different per time point, as expected (170 mL in T1, 204.4 mL in T2, 228.8 mL in T3, on average). In addition, we observed a significant correlation between CH_4 production at T2 and T3 ($r^2 = 0.99$, $p < 0.01$). Rumen fluid pH was 6.13 (±0.38) on average, and positively correlated with CH_4 production ($r^2 = 0.3$, $p = 0.28$). On the opposite, age ($r^2 = -0.33$, $p = 0.83$) and milk yield ($r^2 = -0.12$, $p = 0.79$) were negatively correlated. The result showed that cows with the same diet had different CH_4 productions also *in vitro*. Differences between the groups remained constant for all fermentation time points evaluated. Interestingly, a high correlation of CH_4 production was achieved between T2 and T3. In conclusion, these results indicated that 12 h of fermentation are sufficient to have reliable data on CH4 production *in vitro*.

O479

Modelling GWP of milk production using technical efficiency indicators

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The livestock sector is a major source of greenhouse gases (GHG), contributing to climate change. Currently, a range of mitigation strategies has been identified to improve the sustainability of animal production. In particular, among the most important drivers that can help decrease the impact per kg of milk are the increase of individual milk production and feed efficiency, as well as the increase of the on-farm production of highly digestible forages. However, other aspects of farming, and in particular, some indicators of technical efficiency, can play an important role in the mitigation of GHG and, therefore, in the modelling of global warming potential (GWP) related to milk production. In order to assess the relative weight of some technical variables in the estimation of GWP of milk production, data from 50 farms of northern Italy was used. Herd fertility and management data were provided by Associazione Nazionale Allevatori della Razza Frisona, Bruna e Jersey Italiana (ANAFIBJ). The simplified equation for the evaluation of GWP started from the results of Life Cycle Assessment, performed with EF 3.0 method characterization factors, as suggested by PCR Formaggio Grana Padano DOP; differences between biogenic and fossil methane were taken into account. The Functional Unit was 1 kg of Fat and Protein Corrected Milk (FPCM). The average duration of lactation was 316.3 ± 21.7 d, the average calving interval was 425.2 ± 22.0 d and the average lactation number per cow was 2.33 ± 0.26. The average GWP was 2.02 ± 0.29 kg CO_2 eq/kg FPCM. The MIXED procedure was carried out for assessing the relationship between GWP and farm indicators; farms were evaluated as random effect. PROC REG was performed in order to evaluate the predictive capacity of the simplified GWP equation (R^2 0.74 and RMSE of 0.13). The results obtained highlighted that duration of lactation, calving interval and number of lactations per cow were significantly related to GWP. In addition, two genetic indices were related to the GWP: the Economic Health (IES) and the predicted Feed Efficiency (pFE) indices. The simplified equation is a method easily applicable on a farm scale and uses already existing information including herd female fertility and management data. This could represent a useful tool for estimating the impact of milk production of the individual farm, and also for having an overall vision of the impact at a national level, following its evolution over time.

O559

Meat quality assessment of raw meat from two Mediterranean autochthonous pig breeds reared in sustainable conditions

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The sustainability of livestock farming in relation to global anxiety about climate change and the quality of agro-ecosystem services has become a fundamental issue for the world and the scientific community. Many aspects of meat quality are related to genotype, sex, and age of the animals as well as the production system. In Mediterranean countries, farmers in marginal areas engage in environmentally friendly production systems using native genotypes that are well adapted to the environment and are able to exploit the feed resources available on the territory. The black pig breeds are autochthonous in many countries and reared in the interior of the Mediterranean region. Although in Italy, the black pig breeds have been studied and analysed for years, and have been divided in different populations, in Greece, there is a lack of information about the productive performance of black pigs. The current study, funded by the GREEN FUND GREECE no.003141, aimed to evaluate the effect of genotype on the quality parameters, the chemical composition and fatty acid profile on the *Longissisum lumborum* muscle in pig slaughtered at 9 and 12 months of age. For this purpose, 20 piglets were selected from 2 pig farms (1 Greek and 1 Italian) and divided into 4 experimental groups (5 animals per group): E9-Greek breed, slaughtered at the age of 9 months, E12-Greek breed, slaughtered at 12 months of age, I9 Italian breed, slaughtered at 9 months of age, and I12 Italian breed, slaughtered at 12 months of age. Pigs were slaughtered at a licensed abattoir in their country of birth and meat quality analyses were carried out at the University of Bari. The preliminary results show that the E12 meat is less dark with better values of tenderness than I12. The genotype did not influence the chemical composition of 9 months pigs, but on the other hand the I12 have lower protein content and intramuscular fat than E12. As far as the meat fatty acid composition is concerned, the results show a higher percentage of SFA but a lower of MUFA in Italian groups. The concentration of n-3 and n-6 was higher in E9 and E12 than the Italian groups. The meat health indexes were not influenced by genotype but were influenced by age of slaughter. This trial could be a first step to evaluate meat characteristics of Greek black pigs and, therefore, a tool to exploit the diversity of production systems and to raise awareness of the relevance and value of this animal genotype.

O143

Estimating enteric methane emission in dairy cows exploiting longitudinal data measured on single animal and at farm level to refine IPCC equations

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The dairy sector accounts for approximately 30% of GHG emissions of the global livestock sector and for 4% of total anthropogenic GHG emissions. Around 80% of the CH4 emission of the dairy sector originates from enteric methane, a significant source of anthropogenic greenhouse gas production. To achieve the new global methane pledge to tackle climate change and reduce the emission of 30% by 2030, it is necessary to improve the monitoring tools and foster the adoption of mitigation strategies in farm. This study aimed to refine the estimation of the enteric methane emission of dairy farms in Italy by exploiting the longitudinal Dairy Herd Improvement (DHI) and Livestock Environmental Opendata (LEO) project data collected on single animals or at the farm level.

Data on single animals included monthly milk yields and fat and protein composition, while data at the farm level comprised culling and replacement, herds composition by categories, feeding stuff, and diet administered per animal categories and seasons.

Data were collected by the Italian Breeders Association (AIA) in about 9500 Holstein dairy farms throughout Italy, rearing over one million animals. The effect of implementing IPCC/

ISPRA equations for the Net Energy for Lactation, the Net Energy for Maintenance, and for the Ratio of Net energy available in diet for maintenance to digestible energy was estimated using DHI/LEO data as an alternative to the adoption of the default Tier 2 values suggested by ISPRA. Enteric emissions were calculated per herd, categories, animals, and kg of milk. Then, 1549 dairy farms were sampled and divided into 3 classes of average size per cow/year (156 farms up to 100 lactating cows; 640 up to 200; 753 with >200). Preliminary results indicated that emissions per kg of milk from the largest size classes appear to be equal to or slightly lower than those in the class with the lower heads number. The different level of farm management in relation to farm size could influence the level of emissions. The results will contribute to a more precise quantification and monitoring of enteric emission at the farm level, to identify differences among herd size, regions, and seasons, and to deploy targeted large-scale mitigation actions.

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O553

Agroforestry to support carbon-neutral dairy productions in Tuscany

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Agroforestry is considered an appropriate strategy to cope with climate change through both adaptation and mitigation solutions. Agroforestry is defined as 'the practice of deliberately integrating woody vegetation (trees or shrubs) with crop and/or animal systems to benefit from the resulting ecological and economic interactions'. Agroforestry combines on the same land surface food, feed, and timber production, stocking carbon in the biomass of trees, reducing soil erosion and flooding risks, and improving nutrient cycling and animal welfare. Among agricultural sectors, livestock is the most impacting in terms of greenhouse gas (GHG) emissions. At the same time, livestock productions maintain socio-economic sustainability in both marginal and intensive agricultural areas. Carbon insetting refers to any activity undertakes to reduce GHG emission or to sink carbon from the atmosphere within a production system. The development of integrated crop-livestock-tree systems can help the transition to a low-carbon agriculture through stocking carbon in tree biomass.

A preliminary scenario analysis was conducted to assess the carbon sequestration potential of a poplar-based agroforestry systems in eight dairy cattle farms (Mugello area, northern Tuscany) and in six dairy sheep farms (Manciano area, southern Tuscany). Firstly, a LCA analysis was conducted by collecting primary data in both cattle and sheep farms. The functional unit was 1 kg of fat (4%) and protein (3.3%) corrected cow milk or sheep milk (6.5%, 5.8%, for fat and protein, respectively). Secondly, the Eco Yield-Safe model (a dynamic model for predicting resource capture, growth, and production in agroforestry systems) was used to estimate carbon sequestration of a poplar agroforestry system in both study areas.

Global worming potential was 1.14 CO_2-eq kg^{-1} for dairy milk and 4.11 CO_2-eq kg^{-1} for sheep milk. Total emissions per ha of utilized agricultural area varied from 4.13 to 30.78 Mg CO_2 ha^{-1} y^{-1} in dairy cattle farms, and from 3.85 to 7.53 Mg CO_2 ha^{-1} y^{-1} in dairy sheep farms. The Eco Yield-Safe model predicted a total above-ground biomass after ten years of 21.59 Mg ha^{-1} of timber in Mugello area and 12.02 Mg ha^{-1} in Manciano area. The potential carbon compensation of the agroforestry systems was 3.69 and 2.03 Mg CO_2 ha^{-1} y^{-1}, respectively. The average potential carbon compensation ranges from 12 to 89% (average 33%) and from 26 to 52% (average 43%) in Mugello and Manciano farms respectively.

O30

A sustainable alternative grain legume for livestock: agronomic and nutritional traits of three lupin (*Lupinus* spp.) species

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With the aim of evaluating lupin grain as a sustainable source of nutrient and bioactive compounds for animal feeding, the main agronomic traits, nutrients and antioxidant phenols of *Lupinus albus* L. (Luxor), *Lupinus luteus* L. (Dukat) and *Lupinus angustifolius* L. (Wonga), grown side-by-side in the Mediterranean area, were studied.

Proximate composition was determined using the official methods of analyses, fatty acid profile by gas chromatography, total phenolic content (TPC) and the scavenging activity (DPPH• and ABTS•+) by spectrophotometric assays. A one-way ANOVA was used to assess the significance of accessions effect and means were separated by the Tukey HSD test ($p \le 0.05$).

Seed yield resulted significantly different among lupin species: *L. albus* proved to be the most productive species (2.27 Mg/ha) compared to *L. luteus* and *L. angustifolius* (1.49 and 1.77 Mg/ha, respectively) that were not significantly different.

Regarding nutritional traits, *L. luteus* showed the significantly highest crude protein content (396 g/kg, as fed) and *L. albus* the highest oil content (93 g/kg, as fed) and the lowest crude fiber content (120 g/kg, as fed).

The FAs resulted of nutritional interest for *L. albus* and *L. luteus*; the former showed the significantly highest content of the oleic acid (51%), the latter, the significantly highest content of linoleic acid (50%) and alfa-linolenic acid (8%). *Lupinus luteus* showed the significantly highest polyunsaturated FAs (58%), in particular for those of n6 (50%) and n3 series (8%), while, *L. albus* the highest monounsaturated FAs (58%) and the lowest saturated FAs (16%).

Antioxidant properties, namely total phenolic content and scavenging activity (DPPH• and ABTS•+), were the highest in *L. luteus* and *L. albus,* respectively. The highest content of Apigenin 1 derivatives was observed in *L. luteus*, while the highest level of Apigenin 2 ones was determined in *L. albus*.

Among studied species, *L. albus* resulted largely the most productive species showing an interesting protein, oil and unsaturated fatty acid contents, and antioxidant acitivity; *L. lutes* showed lower yields but emerged for polyunsaturated FAs content, and *L. angustifolius* did not show valuable traits compared to the other lupin species. The present data suggest that *L. albus* can be considered a valuable crop and feed resource for livestock.

O62

The value of sheep transhumance for rural landscape: a GPS tracking approach

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In the Mediterranean and Alpine regions, transhumance is a form of pastoralism based on the seasonal driving of livestock along established drovers' roads. In 2019, it was inscribed on the Representative List of the Intangible Cultural Heritage of Humanity due to its contribution to conserving marginal areas' natural and cultural heritage. In particular, the grazing patterns of transhumant flocks can contribute to maintaining High Nature Value Farmland (HNVF), defined as those areas in Europe where agriculture supports, or is associated with, either a high species and habitat diversity and/or the presence of species of European conservation concern. This research investigates the potential role of sheep transhumance in the conservation and sustainable use of marginal areas in the Eastern Alps. We monitored two flocks of sheep for 16 months (from November 2020 to March 2022) using GPS (Global Positioning Systems) GSM collars. Two sheep per flock were equipped with GPS collars collecting the animals' position every hour, except for one day/week, when positions were collected every 15 min. We obtained digital maps of protected areas (Natura 2000 network) from the European Environment Agency and land use from CORINE Land Cover 2018 with a resolution of 10 m. We defined as HNVF the pixels classified as '243-agricultural land with significant natural vegetation', '231-pastures', and '321-natural grasslands'. The two flocks moved for 1587 km and 1118 km, with a daily movement of 3.23 ± 2.92 km and 2.83 ± 2.19 km, respectively. The altitudinal gradient ranged from 0 to 2340 m a.s.l. The two flocks intersected protected areas (Natura 2000 network) for 18% and 8% of total GPS positions and HNVF areas for 35% and 28%, respectively. The results confirmed the potential role of transhumant sheep flocks in conserving rural landscapes and HNVF and suggest that further information should be acquired on the effects of grazing practices on these habitats. This knowledge could inform specific policies and the promotion of knowledge of the multifunctionality of transhumance to support this traditional livestock system.

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O505

An agronomic-food strategy to improve the environmental impact in terms of water saving and food efficiency

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Producing fodder, using less water. Often a forced choice. This requires new fodder routes.. We know that 1/3 of Europe's available water resources are used for agriculture. In Southern Europe it is as high as 80%. An agronomic revision is needed in the production of fodder with high quantity and quality, but less need for water. Excellent dairy farming must be considered, even without maize. The working strategy goes in a different direction: make the most of the autumn-winter rains for fodder production by using autumn sowing and spring mowing grasses that favour leaf and stem production over grain production. These are crops that require little fertilisation, taking advantage mainly of slurry spreading. Following the logic of water saving, maize will be replaced with a similar crop in terms of energy input, but with lower water requirements. Think of fodder sorghum, which does not produce grain, but allows a huge fodder mass, which can be used to feed dairy cows. Unlike maize, sorghum has exceptional drought tolerance and can withstand periods of low water, without becoming stressed. It does not require the use of pesticides (less disease) and is less content with nitrogen fertilisation than maize. Forage sorghum has a high height and a highly developed leaf apparatus, which guarantees high yields. It can be cut either once at physiological maturity or twice at an earlier degree of maturity (pre-wilted). It has high digestibility of fibrous fractions (BMR variety) with low lignin content. Many researches confirm that sorghum-silo can be used in dairy cows as a total replacement for silage, with no undesirable effects on performance, but positive effects on milk content (fat and protein). Personally, we tend to combine two varieties of BMR fodder sorghum, with a traditional (non-BMR) variety, which acts as a carrier, given the low lignin, with fibre that is very good from a nutritional point of view, but which inevitably tends to be fatty. Rationing can improve the carbon footprint. Here, too, sorghum holds the cards. Less water and less fertilisation allow it to be a less energy-intensive plant. Highly digestible fibres allow less emissions from the animal. The discussion concerns the forage scaffolding shifted to the maximum utilisation of winter cereals. Since they, too do not require irrigation water, fertilisation, as mentioned, is often linked to spreading

O231

Multi-scale integrated accounting of buffalo farms' metabolism

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The analytical approaches currently in use to evaluate the sustainability of livestock systems are based observing the end products, these approaches do not allow to enter into the details of the production process by integrating the social, economic and environmental domains. The following contribution illustrates a relational model applied to evaluate the relative sustainability of buffalo farms in Campania Region (Italy). We applied the MuSIASEM approach (MultiScale Integrated Accounting of Societal and Ecosystem Metabolism), a relational multicriteria framework allowing the characterization and evaluation of different metabolic features of the system. The accounting method evaluate the metabolic performance, in an upward and downward causality, by means of viability (techno-economic constraints), feasibility (bio-physical constraints), desirability (societal norms) and externalization (system opening). All analyzed farms showed a high metabolism, in terms of use of resources and production of different forms of waste products. The dependence on external systems (externalization for the import of feed) appeared to be extremely evident, with repercussions on the effectiveness of the production systems in attracting the local workforce. Evident critical issues also emerged in farms with biodigesters.

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O418

Effects of different automated feeding management on milk traits and rumination time of dairy cows during hot season

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During summer, dairy cows reduce dry matter intake and change their feeding behaviour pattern. The automated feeding management (AFM) systems allow to set TMR preparation and distribution according to the leftover level at the manger and by scheduling lag times in their work. The aim of this study was to evaluate the effect of a different AFM setting in the summer months on daily rumination time (DRT), milk yield (MY) and its quality. The TMR was distributed for six weeks with (A) leftover <70 mm, without machine-stop, or (B) leftover <90 mm, with a machine-stop from 9:00 AM to 2:00 PM, alternatively on week basis. The temperature humidity index was monitored on hourly basis. Rumination time was recorded by an ear tag accelerometer to allow *a posteriori* stratification of cows according to their starting level of DRT as low (L1, 532 ± 36.7 min/d, $n = 12$), medium (L2, 572 ± 39.8 min/d, $n = 13$), and high (L3, 603 ± 29.7 min/d, $n = 13$). Individual milk yield was automatically recorded at each milking. Milk samples from 16 Italian Friesian cows (8 primiparous and 8 pluriparous) were weekly tested for composition and cheese-making properties. Data were analysed according to a general linear model where each dependent variable was tested for the fixed effects of DRT (L1, L2, and L3), AFM settings (A and B), and periods within them.

The AFM setting did not affect individual DRT nor MY. Milk pH, clotting time, curd firming rate, and final curd firmness on the 16 sampled cows did not differ between A and B settings, with only a trend ($p = 0.068$) for a lower fat content for B. The cheese-making features were also unaffected by DRT level; however, MY was higher ($p = 0.032$) in L2 than L1 cows. Throughout the study, DRT was still higher in L2 and L3 than L1 cows ($p < 0.05$); its level affected milk fat content (lower in L2 and L3 than L1, $p < 0.05$) and fat yield (lower in L3 than L1; $p = 0.027$). These results confirm that a good rumen activity at the beginning of a stressful period can help to cope with it; on the contrary, the tested AFM settings did not allow to reduce the negative effect of heat stress on DRT and MY. Further study will be necessary to really exploit the opportunities offered by the different AFM systems in managing critical summer situation in dairy herds.

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O458

Animal breeding sustainability: the Italian Holstein experience

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To increase the environmental sustainability of the dairy sector a holistic approach is needed. Methane and carbon dioxide emissions have been shown to be heritable in cattle, providing the basis to apply genetic selection for their reduction. For this reason, the Italian Association of Holstein, Brown and Jersey breeders (ANAFIBJ) is working on data collection of innovative phenotypes and, in the future, to set-up routine recording in commercial dairy farms. Since 2018 ANAFIBJ, has started to record GHG data on young genotyped Italian Holstein bulls passing into the Genetic Center. For this purpose, the GreenFeed system (C-Lock Inc., Rapid City, SD) has been installed and used. In three years, a dataset of more than 11,200 phenotypic records collected on more than 200 young bulls has been set-up. Preliminary analyses showed that animals emit 223,6 g of CH4/d with a heritability (h2) of 0.396. Thanks to this experience ANAFIBJ has the intention to contribute further and set up a routine recording system for these phenotypes implementing experimental protocols to apply in commercial farms. For this purpose, Laser Methane Detector Mini (LMD, Crowcon, Abingdon, UK) is currently being tested at ANAFIBJ Genetic Center and a data collection protocol is under investigation. Once a standard protocol will be defined, individual CH4 emissions will be collected in 3000 genotyped Italian Holstein dairy cows (some of them daughters of the young bulls recorded at the Genetic Center) distributed in 100 commercial farms throughout the country. At the Genetic Center, in addition, several phenotypes will be collected in order to better define the GHG data emission. Main biometric measures will be recorded and samples of ruminal fluid and faeces will be collected. Biological samples will be frozen and stored at −80 °C, until instrumental and bioinformatic analysis. Activities in commercial farms and in experimental stations will allow to study the interaction between host and environmental microbiome, and to evaluate the reliability of faeces as a proxy of rumen sample. Furthermore, it will be possible to estimate the genetic parameters and to develop models for genetic and genomic evaluations of methane emissions.

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O424

Expectation from precision livestock farming (PLF) in reducing environmental impact of dairy farms

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Livestock productions must be profitable and environmentally sustainable, and it must assure the optimal animal's health and welfare.

Precision livestock farming (PLF) offers the opportunity to improve the efficiency in resource use. It allows to monitor in real-time a wide series of parameters regarding animals and farm, like feed intake, animal welfare, rumination rate, behaviour, oestrus, energy consumption, environment conditions and management. It allows to detect early onset of health problems, verify diet accuracy and distribution, simplify reproductive management and monitor farm energy consumption. This can help improve production, the efficient use of resources and consequently the farm's environmental impact.

The aim of this study is to demonstrate that the digital technologies with which the animals can be monitored continuously have positive effects on the farm efficiency and the environment by improving the performances and the welfare of dairy cattle.

The effectiveness of PLF technologies in reducing the environmental impact was evaluated in three innovative dairy farms located the Lombardy region. Three LCA analyses of the farms were carried out before (year 2018), during (year 2019) and after (year 2020) the introduction of some PLF technologies. Primary data were collected by giving the farmers a questionnaire, while secondary data were obtained from databases and literature sources.

The functional unit was 1 kg of FPCM, the system boundaries were from cradle-to-farm gate and the environmental categories were: global warming, acidification, eutrophication, energy consumption. All the data were processed with CAP'2ER® (Niveau 2) developed at the French Institut de l'Elevage.

In 2018 the estimate carbon footprints (CFPs) of these farms were 0.78, 0.74 and 0.90 kgCO_2 eq/kg FPCM respectively; in 2019 CFP's were 0.80 for two farms and 0.93 kgCO_2 eq/kg FPCM, while in 2020 it was 0.83, 0.81 and 0.83 kgCO_2 eq/kg FPCM.

The results of this study showed that a continuous monitoring of animals may improve the early recognition of problems due to health, fertility and productivity, allowing immediate actions by the farmer. A proper utilization of production inputs can improve the farm efficiency and environmental performances, but the different situation at farm level required further studies to explain the different extent of the effect in each farm.

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O313

Does age affect learning capacity and grazing activities of Holstein cows managed with Virtual Fencing collars?

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Virtual Fencing (VF) can be a helpful technology in managing herds in pasture-based systems. VF system can replace physical fences with virtual ones defined in a GIS system: when an animal crosses the virtual border, the VF collar emits a sound of increasing pitch, followed by a weak electrical impulse (0.1 J). If the animal continues to walk forward, this deterrent stimuli sequence is repeated up to three times, until it returns to the defined paddock. It is well known that animals can learn the systems easily, but there is a lack of information, if learning capacity decreases depending on the animal age. Therefore, the study aimed to investigated whether animals of different ages differ in their ability to learn VF, and whether this has an impact on their behavior as well. The experiment took place in the summer 2022 in the Swiss lowlands on four strip-grazing paddocks, comparable in terms of forage biomass and botanical composition. Four groups of five lactating Holstein-Friesian cows each, equipped with VF collars (Nofence®), were allocated in separate paddocks after the daily milking. These groups differed in terms of age: two old groups (O1 = average lactation of 5.2; O2 = 4.8) and two young groups (Y3 and Y4 = first lactation). After a seven-day training period (T), each paddock was gradually increased by VF during five consecutive grazing periods, based on the forage biomass availability. Furthermore, to collect detailed information on animal movements, each cow was equipped with a leg pedometer (IceQubes, Peacock Technology Ltd) that recorded the daily step count (DS). To assess differences among groups, the total number of daily sounds (S) and electrical pulses (EP) emitted by the Nofence collars were elaborated. All data were analyzed using the GLIMMIX procedure of SAS Software. The results showed that age had no impact on animals' response to the VF. Thus, during the training, only O1 received significantly less EP than the other three groups. This difference was not observed after the training. Moreover, considering the entire trial duration, O2 and Y4 differ from O1 and Y3 in terms of S emitted by the collars, with Y3 and O1 that registered the highest and the lowest number of S respectively. Finally, there were no significant differences in DS among groups. In conclusion, results suggest that age does not affect animals' learning capacity to learn and interact with the VF, and their grazing activities as well.

O274

Assessment and validation of individual water intake of dairy cows from reticular boluses

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The whole dairy cattle community, scientists and farmers in particular, are putting efforts to improve environmental sustainability of the sector. Future generations of dairy cows must optimize natural resources use without impairing quantity and quality of their output. Water use in cattle, for example, can be more sustainable and efficient. Sensor data can often be good predictors of complex traits, like health status, fertility and stress. However, a large quantity of reference data is needed to develop robust predictive models and decision-support systems. In this study we explored the possibility to predict cow's water intake (WI) by mean of longitudinal temperature recorded by specific reticular boluses (smaXtec animal care GmbH, Austria) inserted into 8 cows undergoing a feeding trial at the experimental farm of the University of Bologna (October to December 2020). Cows, 4 primiparous and 4 pluriparous, were farmed in tie-stall with an individual drinker available. The WI was registered every day for 28 d, i.e. 7 consecutive d per each diet ($n = 4$); thus, data referred to adaptation phases were excluded. To quantify the number of times the reticular temperature dropped and duration of such drops, a daily 'drop area' was obtained for each cow by subtracting the area under the reticular temperature curve from the area under the body temperature curve. The 'drop area' was subsequently linked to the respective daily WI, body weight (BW), and milk yield (MY). Before boluses validation, the reference WI (140 ± 34 L/d) was adjusted for systematic effects (cow, parity, diet, and days in milk) using the GLM procedure of SAS v. 9.4. Then, the GLMSELECT procedure selected the most important predictors of WI among all imputed traits: mean, SD and minimum of both body and reticular temperature, MY and BW. Different models were tested by combining the predictors offered using a 5-fold cross-validation (75% training, 25% validation). Based on the mean square error in cross-validation, the best model was the one with all predictors available ($R^2_{CV} = 0.90$). When MY and BW were masked, the accuracy decreased ($R^2_{CV} = 0.65$). In both models, the 'drop area' was selected as one of the most important predictors and, when used alone, the R^2_{CV} was 0.61. Results are promising and suggest that there is potential to explore dairy cattle water efficiency: WI could be in fact estimated from sensor data in free stall barn/commercial contexts where recording individual intakes is not feasible.

O394

Modeling heat flows in heat-stressed dairy cows using System Dynamics techniques

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Heat stress (HS) condition is due to an excess of heat load (HL) in the cow's body, which can be expressed by increased body temperature. As a systems science methodology used in modeling complex environmental, ecological, and biological systems, System Dynamics (SD) can be used to understand the heat flows and HL accumulations in dairy cows. The aim of this work was (i) to analyze the differences in the HS response of dairy cows exposed to cooled and uncooled environments in terms of body temperature and milk production, (ii) to use these data to test a simple SD model built based on THI variation and on the HS response of dairy cows. Data on barn weather and milk yield of nine dairy cows were collected during August 2022 from a dairy cattle farm. Cows were selected based on their milk yield (MY) (49.1 ± 4.2 kg/d), days in milk (DIM) (97.6 ± 14.7 d), the number of lactations (NL) (3.4 ± 1.4) and average daily milking (ADM) (2.9 ± 0.5). The cows were divided into two groups housed in the same barn, but one in a cooled open environment (C, 5 cows) and the other in an uncooled open environment (UC, 4 cows), at the beginning of the HW period based on the weather forecast. The body temperature (BT) of each cow was recorded daily from August 8 to August 18 2022 with vaginal dataloggers. Animal and weather data from August were then used to feed the dynamic heat stress model and to calibrate it. Then, the milk yield predictions of the SD model were evaluated. As main results, during the HW period, the average MY was 50.1 ± 6.2 kg/d and 43.9 ± 5.3 kg/d, and BT was 39.5 ± 0.3 °C and 40.0 ± 0.3 °C, for C and UC groups, respectively ($p < 0.05$). Those results imply that both groups were affected by the HW, but C group adaptation was greater due to the cooled environment. The SD model can capture the HS response of the cows in the uncooled environment (with $R^2 = 82\%$, CCC =0.89) more accurately than the cows in the cooled environment (with $R^2 = 66\%$, CCC =0.80). Additionally, the model reproduced the dynamic pattern of the MY better for the uncooled environment according to the visual inspection of the results. These findings demonstrate the use of the SD model for predicting the MY of dairy cows under HS conditions and point out the potential improvements in the model structure, which includes considering additional variables related to animal response (e.g. body temperature variations, cooling conditions) to describe the HS variations over time more accurately.

O431

Milking parameters related to mammary gland health and milk characteristics in Italian Mediterranean buffalo

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The aim of this study was to verify the influence of different milking machine setting parameters on milk yield and quality in Italian Mediterranean buffalo and to define those that are the most appropriate in this species. Individual data from official recording system (following ICAR international standard) collected by Italian Breeders Association were considered for each farm. Milking machine and equipment settings data were acquired within the Milking Control Service activities (SCM) following the ISO regulation 5707 and 6690. A total of 260,000 milk Test-Day (TD) samples, collected from 57,000 buffaloes from 213 buffalo farms in Campania region, between December 2018 and February 2022, were utilized. Information on ID, age, parity and days in milk were available for each animal. Data for each TD consist of milk yield (MY), fat percentage (FP), protein percentage (PP), lactose percentage (LP), and somatic cell count (SCC–cells/mL) that was log-transformed to calculate the Linear Score (LS). Milking System Height (MSH), Vacuum Level (VL), Pulsation Rate (PR), Pulsation Ratio, Automated Cluster removers system (AD), Diameter Length ratio of pipeline (DL), Effective Reserve status (EUR) were collected from milking machine. After editing, data were analyzed through a mixed linear model using JMP Pro 16 software (SAS). A logistic regression model was used to evaluate the relationship between the levels of vacuum and the presence or absence of EUR. An improper setting of the milking system (insufficient EUR) has been observed in 37% of farms. An incorrect setting of the milking in these farms (29.3% of the total TD) was responsible for a higher LS and a lower LP, compared to those with normal setting although no differences were recorded for MY and PP. Except for PP, the MSH significantly ($p < 0.001$) influenced all milk parameters. Similarly, VL affected all milking characteristics ($p < 0.001$) while PR was significant on LS and FP only ($p < 0.001$). The presence of the AD showed a significant effect ($p < 0.001$) on MFP and MY. DLR and EUS were significant on MLP only ($p < 0.001$). These results suggested that buffalo have a specific requirement for milking parameters and that, as already recognised in dairy cattle, an incorrect setting of the milking was responsible for either an increase of LS and a reduction of lactose. Thus, an appropriate milking setting is necessary to ensure milking efficiency and to preserve mammary gland health.

O300

Contrasting climate change effects on dairy cattle through Machine Learning approach

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Climate change's adverse effects include, among others, rising average temperature (e.g. in the next few years is expected 1.5 °C warming compared to the 1850–1900 baseline), shifting precipitation patterns, more frequent extreme weather events, longer cold periods, and increased levels of solar radiation, among others. On livestock, climate changes will impact the amount and quality of products, health, welfare, and fertility, causing significant economic losses. Therefore, modelling the impact of climate change using short (i.e. days or weeks) and long periods (i.e. decades) forecasting are crucial to react on time and preventing risks.

The first step to achieve this objective is the statistical elaboration of climatic and animal production historical series to develop an accurate prediction algorithm. Longitudinal data on milk production (yields and components) and somatic cell count, as a proxy for udder health status, were provided by 'Pezzata Rossa Italiana' Breeder Association and LEO project (www.leo-italy.eu). Historical climatic series were provided by CMCC, in the Highlander project frame. We applied a Machine Learning approach to identify the climatic variables with the most significant impact on animal production and welfare. The best-fitting models for each parameter were then applied to climate projections until 2050, dynamically downscaled under RCP8.5 scenario at 2.2 km over Italy. The outputs were compared with historical climatic data (from 1989 to 2021), obtained with the same procedure, and plotted on risk maps identifying areas across Italy with higher probability of a drop in dairy production and animal welfare. Such maps, together with IoT technologies and real-time data, will contribute to developing a decisional support system (DSS) and setting up tailored actions to prevent the global warming impact of animal production.

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O15

Application of infrared thermography for detection of udder health disorders in dairy cattle

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Udder health (UH) disorders are one the most common economic and welfare concern in dairy cattle. UH disorders may be difficult to detect and tend to increase during the transition to automatic milking systems (AMS).

This study aimed to document whether infrared thermography (IRT) could be a possible tool for the on-farm detection of UH disorders in dairy cattle. The study was conducted on an organic dairy farm with about 140 fresh AMS-milked Holstein Friesian cows. Multiparous fresh cows ($n = 34$) were monitored from January to early March (36 < Temperature Humidity Index <54) during their first use of AMS. Milk somatic cell counts (SCC), milk conductivity, and udder clinical exam (presence of skin lesions, redness, firmness, pain, and warmness) were gathered during the first three days of AMS use and then weekly. Concurrently, 3 images (left, right and back views) of each cow's udder were taken at a 1-m distance using a portable IRT camera. For each of them, average (avg) and maximum (max) udder surface skin temperatures (USST) were calculated and included as outcomes in regression models with somatic cell score (SCS, obtained from SCC) as a predictor. Cows were also scored as healthy or problematic based on clinical inspection and milk characteristics (i.e. problematic if at least one of the following features was present: udder nodules and pustules; redness, pain, or warmness in at least one quarter; SCC >200,000 cells/mL; conductivity >80 mS). This score was used to evaluate whether cows with UH disorders had statistically different USST, and to evaluate the diagnostic merit of IRT.

Left avg USST resulted to be the skin temperature mostly associated with SCS, increasing proportionally as SCS increased (Left avg USST $=33.62 + 0.23 \times \text{SCS}$; $p = 0.0002$). Left avg USST was different between healthy and problematic cows (31.7 ± 0.2 °C (95%Confidence Interval (CI) = 31.3–32.1) *vs* 32.5 ± 0.2 °C (95%CI =32.1–32.8); $p = 0.007$) and a USST of 32.2 °C was calculated as the threshold. IRT sensitivity and specificity were 81.48% (95%CI for sensitivity =71.30–89.25%) and 46.67% (95%CI for specificity =35.05–58.55%), respectively, considering as true positives the cows that were scored as problematic and had an avg USST ≥32.2 °C. Our results suggest that IRT can capture increased SCS, and is sensitive but not specific in detecting UH disorders. This could be due to other factors (e.g. udder cleanliness) which may interfere with the on-farm use of IRT for the detection of UH disorders.

O113

Results from Italian National Breeders Association (AIA) nationwide collection of setting and efficiency parameters in milking systems of Italian dairy farms

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The performance of some milking machine, regardless the herd size, fully relies on the appropriate configuration and functionality of the milking equipment. Among the settable technical parameters of milking devices, pulsation fine-tuning, and vacuum stability are keys for their direct consequence on efficiency and udder health.

Here we present the results of the largest by far and national-wise assessment of the milking equipment exploiting data, collected during the 2022 Milking device and routine inspection (Milking Control Service – SCM) campaign associated with DHI recording. This study, in fact, covers 1060 herds spanning different size and breeds, 92,743 lactating cows, 12,949 pulsator tests, and 210 vacuum drop tests. The dataset includes eight pulsation parameters (ratio, rate, 4 pulsation phases percentage, limping, dipping) and three vacuum stability parameters (undershoot, overshoot, vacuum drop).

Milking machine parameters have been collected by trained personnel equipped with VaDia®, the last generation milking sensor with an accuracy of ±0.2 kPa and a measuring frequency of 200 Hz. Values above the ISO standard thresholds have been considered anomalies and correlated at the farm level with the incidence of mastitis diagnosed by SCC (threshold above 200,000) and other reproductive and productive data collected routinely via DHI and LEO Project (PSRN 16.2 Livestock Environment Opendata). The expected outcomes will provide the first large-scale overview of the performances of milking machines in Italy, assess the correlation between milking equipment settings and udder health statistically, and, finally, reckon the impact of milking device anomalies on dairy farm profitability.

O324

Identification of the most impacting environmental variables on dairy cow's milk yield using Machine Learning methods

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The effects of climate change have been tangible in different aspects of life on earth: from human and animal health to ecosystemic levels. The food production sector has also been affected since climate change can cause economic losses and decrease food quality. The phenomenon is well-documented in the livestock sector, in particular in the case of milk production. Dairy cows are highly sensitive to environmental changes, which cause a reduction in milk yield and quality (i.e. fat and protein content). These changes can also affect the cow's health, shown by an increase of somatic cell counts during heat-stress conditions. Many studies have been done in this regard, however, it is not well known the individual impact and how quickly these climatic conditions can affect milk production. To understand these effects, dairy cows' records from 1990 to 2020 were analysed. The production data of each animal was paired with the climatic variables (i.e. temperature, relative humidity, precipitation, etc.) evaluated for up to 22 days before the functional control date. The single environmental variables were evaluated, to avoid index bias. Given the high amount of data and the high dimensionality of the issue, we applied a Machine Learning (ML) approach to identify the best climatic variables affecting milk production. ML techniques, indeed, identify unexpected and non-linear relationships across the data. We developed a pipeline that can: (1) remove collinear climatic variables to maintain the most informative ones before data analysis; (2) identify the best algorithm family to use climatic variables as milk production predictors; (3) optimise the parameters of the best algorithm to increase its accuracy; (4) evaluate the importance of all variables to subset the most important ones; (5) explain how each variable can affect the milk production. The ML pipeline identified temperature and humidity as the most influencing variables for predicting milk quality variation. Moreover, the long-term effects (i.e. weeks) are more impactful on milk production than the short one (i.e. days). This study shows how ML algorithms can be used to assess the impact of climate change on animal productivity. This ML application can help farmers make efficient decisions and avoid economic losses, by identifying how environmental conditions can affect milk production. Furthermore, the algorithms used can be easily applied to different production data in the livestock sector.

O451

Comparison of different methods to estimate dairy cows' production potential

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Milk yield dynamics and production performance reflect how dairy cows cope with the surrounding environment. To optimize farm management, these parameters have been recently studied in the context of precision livestock farming. Starting from milk yield measurements recorded by on-farm sensor systems, many mathematical models have been implemented to transform raw data into useful information for the farmer. This study aimed at comparing three methods that allow to estimate the individual milk production potential, using daily data recorded by the milking robots of 14 dairy farms (7 Holstein, 7 Italian Simmental). Perturbed lactation model (PLM), iterative Wood model (IW), and quantile regression (QR) were compared in terms of estimated total theoretical production and total milk loss. The results from the three methods were in line with each other in terms of estimated total theoretical production (correlation coefficients around 0.99), while they were moderately correlated in terms of estimated milk loss (correlation coefficients between 0.56 and 0.65). The median total theoretical production was equal ($p > 0.05$; pairwise Wilcoxon test) when estimated by IW and PLM (9642 kg and 9704 kg on 305 days of lactation, respectively), while it was higher when estimated by QR (9830 kg on 305 days of lactation). In case of the total milk loss, the distribution of the estimated values was different for the 3 methods, and especially for QR compared with the other 2 techniques: QR produced less variable estimates, with a higher median milk loss of 5% (compared to 4% of IW and PLM). Since QR overestimates the quantities of interest, IW and PLM should be preferable to estimate the individual milk production potential. For both IW and PLM, the estimated total theoretical production increased in multiparous compared to primiparous cows (Wilcoxon test); a similar pattern was observed for the percentage of milk loss. As expected, the total theoretical production estimated by the 2 methods resulted to be higher for Holstein cows than for Italian Simmental ones (mean of 10,577 kg and 8576 kg on 305 days of lactation, respectively); the total milk loss, instead, resulted to be lower for Holstein breed than for Italian Simmental (mean of 4.3% and 4.9%, respectively). Analyses were carried out with RStudio software (R version 4.1.2).

O183

Impact of the digital and technological transformation of Italian livestock farms in the last decade

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The National Institute of Statistics (ISTAT) has just concluded the latest decennial General Census of Agriculture, covering the entire universe of farms in Italy. The census survey collected data in relation to all aspects characterizing the Italian agricultural and livestock sector, including a number of specific questions aimed at analyzing both the degree of computerization and the presence of innovation, detected for the first time in census surveys.

The data collected confirm the presence of an ongoing modernization process in the Italian agricultural world, particularly in the livestock sector. Although the incidence of digitized farms has increased by about four times since 2010 and 10% have made at least one innovative investment, very large territorial and typological differences remain.

According the 2020 Census, in Italy there are 213,984 farms with breaded animals. The bigger part of the livestock farms are located in the South 41.4%, in the North area are located 39.7% and the Center is represented by 19.2%. In the Northern Italy 67.3% of the farms declared that they are computerizing their activities.

Analyzing the data by dimension, results that the average UAA (utilized agricultural area) of the digitized respondents is equal to 44.6 hectares. The incidence start from 2.1% of units with UAA between 0 and 1 hectare to 70.9% of those with more than 10 hectares.

In terms of LU (Adult Livestock Unit), 68.2% of computerized farms have more than 10 heads.

Furthermore, it is the largest and youngest-managed livestock farms that show a major propensity for digitalization. If the farm is conducted by people up to 44 years of age, the digitization rate reaches 34.6%; where the farmers have more than 65, it is only 15.8%. In terms of ULA (Annual Work Units) the average value recorded is equal to 2.5 units.

The objective of this paper is to investigate the IT and innovation level of livestock farms in relation to their structural and territorial characteristics. The most modernizing farms in terms of both digitization and innovation will be analyzed, identifying the characteristics, location and features that favor the process of technological advancement in Italian agriculture through some digitalization index. In addition, a focus was carried out to analyze the point of view of the farmers regarding the opportunity or the obstacles offered by the digital transformation and the adoption of some PLF.

O528

Precision livestock farming to control changes in behavior and production in dairy cows based on variations in environmental THI

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The trial evaluated the effect of Temperature Humidity Index (THI) on behavior of 417 Friesian dairy cows in two farms through a year from October to September. Data recording was carried out by neck collars containing sensors (Cowscout – GEA Farm Technologies). The following parameters were continuously monitored (24/7): rumination, feeding, inactivity. The monitoring of the climatic parameters was carried out at 3 different heights inside the stall (low: near the ground; medium: at the height of the animal's head; high: under the roof) to evaluate the potential incidence of the THI recording sensors location.

Data were analysed using SAS statistical software. The climatic parameters were significative affected by the sensors position ($p < 0.001$), but also by the farm's area (62 vs 67 for THI average). Interaction between sensors position and farms was statistically significant, highlighting how farm management affects the THI detected above all in low and medium positions (60 vs 71 and 62 vs 69 respectively as THI average). Model for the evaluation of the behavioural parameters included farm as fixed effect and THI as a covariate, as well as the interaction between the two effects. The effect of THI was tested both as diurnal and nocturnal THI and as the number of continuous hours above two thresholds: 72 and 78. The results showed that the covariates tested always had a significant influence on the behaviors analysed. It should be noted that as the THI increased, the animals' behaviour in the two farms changed reporting different estimates but similar trends. This was probably linked to the response capacities of the animals to climatic stress in the two farms due to the different habits of the climatic-environmental conditions found on average in the two farms. As regard the milk production, the effect of the THI was evident with a general decrease for each point of increased THI, which was greater when analysing the consecutive hours above the threshold 78. The study emphasized the importance of efficient tools for both environmental and individual animal control, which contribute to animal health and welfare and avoid production losses.

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O532

Effects of particle size distribution of TMR on peNDF, starch intake and milk production in Calabria dairy herds

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Finding an optimal balance between the amount of physically effective fibre (peNDF) and rumen fermentable carbohydrates in the diets of high-producing dairy cows is necessary to prevent sub-acute ruminal acidosis (SARA), optimize digestion, nutrient utilization, and improve dairy production. The physical effectiveness of a diet depends on several factors, of which fiber content, forage particle size and starch fermentability are the most important. In a recent study on 13 dairy farms located in Calabria, we evaluated the particle size distribution of the TMR by Penn State Particle Separator (PSPS), reporting an excess of the long fraction (46% of the farm), of the very short fraction (15%), or of both these fractions (38%). Furthermore, a selective consumption of the very short fraction was observed in the 85% of the farms. In this work we show the effect of particle size distribution on the peNDF content of the diet. Additionally, the starch content of the diet and the quality of the milk were evaluated. In the 13 herds, only information related to the high production group of cows was considered in the study. Samples of TMR were collected in three points along the face of the feed both at the feed delivery (t0) and after feeding, just before the new fresh feed delivery (tf). Weights of feed offered and of refusals were also determined. All samples were analysed for particle size using the PSPS and its DM, NDF and starch content were determined. Furthermore, the nutritional composition of the milk was analysed by FT-IR. The peNDF content was estimated for each sample as the sum of the % of the three sieves (19 mm, 8 mm, 4 mm) multiplied by the NDF content of the sample. All diets analysed met the NDF values recommended by Penn State University (>28%) except farm 1 (26,8%). In farm 1, 2 and 11 (2 TMR-S and 1 TMR-H) peNDF >8 values were less than 15% and starch values were greater than 33%. Several authors indicate that peNDF >8 values less than 15% in diets rich in highly fermentable carbohydrates would increase the risk of SARA. In addition, the refusal of the long fraction and a selective consumption of the fine particles found in these 3 TMRs can be considered as a further element of concern. Regarding milk production, no abnormal parameters were found, probably because it was bulk milk and not individual milk. It therefore seems desirable, even in TMRs with good amounts of NDF, to verify the granulometry of the TMR.

O241

How much is the environmental benefit of using precision livestock farming in Italian dairy farms?

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Despite the significant improvement in efficiency of livestock sector since the 1950s and the consequent reduction of greenhouse gas (GHG) emissions per unit of product, further efforts are needed to improve the sustainability of animal productions. The aim of the study was to quantify the environmental impact of milk production and evaluate the role of Precision Livestock Farming (PLF) among the mitigation strategies of GHG emissions. Using Life Cycle Assessment (LCA) in five dairy cattle farms in Lombardy, the Global Warming Potential (GWP) of milk production was evaluated. The assessment was repeated creating scenarios with or without the use of PLF solutions for monitoring production, fertility and udder health. Environmental benefit of introducing the Automatic Milking System (AMS) was evaluated in two farms: 8 scenarios were created assuming increases in Milk Yield (MY), by 5% and 15%, changes in milk fat and protein content and Somatic Cell Count (SCC), increases in Purchased Feed (PF) and in energy consumption. Secondly, the reduction in GHG emissions due to the adoption of automatic heat detection systems was estimated into two farms that currently use automatic oestrus detectors by simulating a scenario without the use of sensors; days in milk, MY, dry matter intake, PF, number of lactating, dead and sold cows, age at first calving were modified. To evaluate the environmental advantage of the udder health PLF monitoring systems, a mastitis detection capacity 5% lower than the actual estimated detection capacity was considered in two farms that currently possess precision systems for monitoring udder health. An increase of average SCC was assumed as well as changes in MY, discarded milk, PF and number of heifers. The AMS adoption resulted in a reduction of kg CO_2 eq./kg of Fat and Protein Correct Milk (FPCM) from 1.13% to 5.80% compared to conventional milking, with MY increase as main driver. Using PLF systems to detect heats decreased the GWP of 1 kg of FPCM by almost 10% compared to visual heat detection. Regarding the udder health scenario, every 5% more cows with mastitis detected led to a reduction of GWP by an average of 0.5%. All LCA scenario analyses involving the use of PLF systems resulted in reduced GWP. The application of PLF seems to reduce the environmental impact of milk production at farm level, especially when technology enhances the herd efficiency.

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O484

Influence of kisspeptin on buffalo superovulation

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The low number of embryos recovered per donor is undoubtedly the major constraint of multiple ovulation and embryo transfer (MOET) in buffaloes. Recent studies suggested to modify the MOET treatment in order to avoid steroid profile alterations and to improve the expansion of cumulus cells during maturation, as well as the oviduct vascularization and motility. Aim of the present study was to evaluate the use of kisspeptin, a hypothalamic neuropeptide, that has been shown to stimulate the GnRH release by activating the LH pulse necessary for oocyte maturation and ovulation. Italian Mediterranean Buffalo cows were superovulated with a conventional FSH protocol ($n = 10$; CTRL group) or with the same FSH protocol and the use of kisspeptin ($n = 10$; KISS group) Kisspeptin was administered 3 times IM on days 11–13 and in slow release with the aid of ialuronic acid on day 13 of the MOET cycle and animals were sacrificed 18 h after last GnRH. Antral follicular count, recovery rate and oocyte quality were recorded, and plasma and follicular fluid were collected for steroid profile determination. Granulosa cells (GCs) were collected to analyse the mRNA expression of gonadotropin receptors (LHR and FSHR) and genes involved in steroid synthesis, as the cytochrome P450 family 19 (CYP19A1) and the steroidogenic acute regulatory protein (STAR). Oviducts were also collected to evaluate the mRNA expression of estrogen receptor 1 (ER1), progesterone receptor (PGR), vascular endothelial growth factor (VEGF) and VEGF receptor (FLK1). Interestingly, steroids plasma concentration increased in KISS group (40 ± 2.0 *vs* 15.4 ± 0.9 pg/mL of E2 and 1.1 ± 0.2 *vs* 0.4 ± 0.04 ng/mL of P4, respectively in KISS and CTRL groups; $p < 0.01$) while no differences were recorded in intrafollicular steroid concentrations. Even if the recovery rate was lower in KISS group (44% *vs* 56.6% of CTRL group; $p < 0.05$), the percentage of oocytes exhibiting proper cumulus expansion was higher (72.7% *vs* 28.1%, respectively in KISS and CTRL groups; $p < 0.01$) compared to CTRL group. In addition, the expression of CYP19A1 decreased while the expression of STAR in GCs increased in KISS group compared to CTRL ($p < 0.05$). Finally, an increased expression of PGR, ER1, VEGF in the fimbria was observed ($p < 0.05$) in KISS group compared to CTRL. The results suggest that the exogenous kisspeptin treatment enhance steroidogenesis, improving both oviductal and ovarian activity, and may help the ovum capture in superovulated buffaloes.

O195

Impact of heat stress on growth of Italian Limousine

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Quantifying heat stress (HS) is one of the major challenges in animal breeding programs. Environmental conditions can dramatically change over space and time, especially with outdoor rearing, affecting consequently growth and health status of the animals. The objective of this study was to assess the impact of heat stress on beef cattle growth, using a population of Limousine cattle kept outdoors. Data for average daily gain of Italian Limousine, raised between 1990 and 2022, were combined with air and surface temperatures, relative humidity, and wind speed obtained from the official site of National Aero-Space Agency (USA). Temperature-humidity index (THI) was also calculated, using the formula: $(1.8 \times AT) - (1.0 - RH) * (AT - 14.3) + 32$.
As a measure of growth, we considered average daily gain (ADG) and environmental measures for a time period defined as in between two body weight measures of the same animal. For the same time interval, we calculated average (aTHI) and maximum (mTHI) THI. Overall, we used 38211 weight records of Italian Limousine from 118 herds, which showed at least 5 animals for the entire period. Correlation between ADG and mTHI was −0.14 ($p < 0.01$), suggesting that HS negatively impacts animal growth. Five classes of aTHI and mTHI using the first four quartiles as breaking values were also created. Effects of aTHI and mTHI and the 5 classes of aTHI and mTHI on ADG, also including the effects of sex and heard-year (fixed), and animal and test-date (random) were evaluated. Considering aTHI or mTHI as a linear covariate the model showed no significance of the effect. Instead, when aTHI and mTHI where considered in classes, they showed significant effects. These results confirm that growth is negatively affected by the increase of THI, with the consequence of lower animal welfare in addition to economic loss for the beef cattle industry. This study suggest that mTHI can be considered a more appropriate indicator of heat stress as compared to aTHI, explaining more variability for the considered trait. This study should be expanded, including a possible genetic determination of heat tolerance in beef cattle.

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O573

Study on the intergenerational and transgenerational epigenetic effect of in ovo chicken embryo stimulation

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The term epigenetic modifications refers to phenotypic modifications in an individual caused by mechanisms that are not related to changes in the DNA sequence. In vertebrates, crucial events of epigenetic reprogramming occur during the stages of early embryogenesis and germ cell development. We hypothesized that when administered in ovo by a single injection, synbiotic and choline, both of which are bioactive substances, can stimulate embryonic development and produce a life-long impact on the phenotype of chicken (such as gut health, immune system, performance, mortality, and physiological traits). We hypothesized also that epigenetic changes that were acquired in one generation, as a result of the in ovo impact on embryonic and long-term postembryonic development, can be permanently inherited and propagated in future generations. The first aim of this project is to analyse whether epigenetic changes that were acquired in one generation, as a result of the prenatal in ovo impact on embryonic and long-term postembryonic development, can be stably inherited and propagated in future generations. The second aim is to investigate the physiological relevance of intergenerational and transgenerational inheritance. The final aim is to identify the molecular mechanisms behind this type of information transfer by using a high-informative multi-omics approach. The proposed project is realized on the chicken model (Gallus domesticus), Green-legged Partridgelike chicken, a dual-purpose Polish breed. Compared to mammals, birds have several advantages, especially for studying transgenerational epigenetic inheritance. The major advantage is that a bird embryo develops outside the mother, and the maternal influence is reduced only to the egg composition. The idea and the first results of the project will be presented.
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O586

Dry vs wet aging in the enhancement of culled goat meat quality

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Goat meat has great potential in meat market, due to the high nutritional value and the predominant content of polyunsaturated fatty acids. These features make goat meat a healthful meat for human consumption. Within the overall production, meat from culled goat represents a substantial share, which is usually addressed to petfood or rendering, since it is less appreciated by consumers. Aging can enhance the quality of meat from culled goats for human consumption, increasing its market value. This study aims to evaluate the effects of aging time and aging methods on the quality of meat from culled goat. Thirty goats were involved in the trial. The hind limbs were divided in the two experimental groups: the right ones were subjected to dry-aging and the left ones to the wet-aging, for a period of 5 weeks. At each aging time (W0, W1, W2, W3, W4, W5) samples were taken from *rectus femoris* for the analysis of rheological, colorimetric, and oxidative parameters, as well as volatile profile. Samples for sensory test were collected from *vastus medialis*. The dataset was subjected to an analysis of variance (ANOVA) and to a Tukey test for repeated measures. Tenderness, evaluated through Warner Bratzler shear force and panel test, increased in dry-aged meat and decreased in wet-aged meat during aging time. ($p < 0.01$). Yellowness decreased with aging time and showed higher values in dry-aged meat ($p < 0.01$). Dry-aged meat showed higher concentration of oxidation products compared to the wet-aged one ($p < 0.01$). Aldehydes were the most represented compounds in both aging methods ($p < 0.01$). Overall liking recorded the highest scores in dry-aged meat ($p < 0.01$). Dry-aging has improved the quality of meat from culled goat, more than wet-aging. However, reducing dry-aging time is recommended in order to avoid the qualitative decay of meat due to oxidation.

O587

Effects of nucleotides and yeast supplementation on gut microbial population of calves

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Feed additives improves gut health of the animals, increasing digestion rate and enhance growth performance. Yeast cells and nucleotides have been approved as feed additives and their effect on animal health and productivity are still under investigation. Yeast cells supplementation contribute to the intake of different vitamins and enzymes. Therefore, it may stimulate digestion and balance microbial equilibrium in the gut of weanling animals. Changes in the microflora, due to both yeast cell wall components and direct effect of live yeast, could improve animal health and growth performance by reducing pathogenic bacteria and toxic metabolites. Nucleotides are involved in several biological processes including regulation of biosynthetic pathways and promotion of cell growth and division. In new-borns their requires is high due to several growth and defence efforts. Nucleotide requirements are particularly high in stress conditions, such as nutrient deficiencies or starvation, or during a rapid growth phase. Although organisms are able to synthetize nucleotides, the diet represents an important daily intake. An effect of nucleotides on intestinal health was reported in several monogastric species by modulating the local immune response, increasing the replication rates of enterocytes and promoting mucosal remodeling. However, the effect on gut microbiota of nucleotides and yeast cells oral administration, even in combination, needs to be elucidated.

The aim of the present study is to assess the effect of four supplementation diets on the modulation of gut microbial population of calves.

A total of 40 male calves were selected at birth and randomly divided into four groups based on supplementation diets: (i) 5 g of nucleotides; (ii) 5 g of yeast; (iii) 2.5 g nucleotide and 2.5 g yeast; (iv) no supplementation. The supplementation diets were administered per head directly in the mouth using a large syringe twice a day until slaughter on 25th day. Intestinal samples of each calf were collected, avoiding the intestinal content emptying, and frozen at −80 °C until analysis. The microbiota was investigated by metabarcoding analysis and related to each supplementation diets. Results indicate an effect of nucleotides and yeast supplementation on gut microbiota, even in combination, respect to the control diet. In particular the promoting effect of supplementation diets of beneficial bacteria, as well as the suppression of enterotoxigenic bacteria, was evaluated.

O588

Illumina Ampliseq approach for multi-pathogen detection

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In veterinary field it is essential to analyze large number of samples and simultaneously test different pathogens. For this purpose, a multi-pathogen detection system joined with the MiSeq Instrument (Illumina), was validated for the screening of important diseases, in the framework of LEO project. MiSeq represents a highly throughput NGS platform combining the highest reads quality production (within Illumina instruments) and the possibility to use a wide range of read size.

Sequencing of 250–300 bp paired end (PE) reads is possible only using MiSeq Instrument enabling to analyse target genes (amplicon-based), and to perform microbiota analysis by 16S metagenomics.

Since 2021, three different AmpliSeq panels were validated for:

- 14 **Abortigenic agents** of cattle and sheep-goats: *Salmonella enterica*, *Brucella* spp., *Campylobacter jejuni*, *Leptospira interrogans*, *Chlamydophila abortus*, *Coxiella burnetii*, *Listeria* spp., *Toxoplasma gondii*, *Neospora caninum*, Schmallenberg virus (SBV), bovine viral diarrhea (BVDV), bovine herpesvirus types 1 and 4, bluetongue virus (BTV) and goat herpesvirus 1.
    - **Results for cattle**:
        - 62 out of 65 samples results match with DNA microarray (Nanostring) results. The 3 discordant samples were co-infection not detected by the DNA microarray;
        - Sensitivity was 100% (l.c.l 95.56% – u.c.l. 100%);
        - Non-specific results not recorded;
    - **Results for sheep-goat**:
        - 67 out of 67 samples results match with qPCR results;
        - Se was 100% (l.c.l 95.69% – u.c.l. 100%);
        - Non-specific results not recorded.
- 19 **Respiratory pathogens** of bovine: *Bibersteinia trehalosi*, Bovine alphaherpesvirus 1, Bovine coronavirus, Bovine herpesvirus 5, Bovine mastadenovirs, Bovine orthopneumovirus, Bovine respiravirus, *Chlamydia psittaci*, *Histophilus somni*, Influenza D, *Mannheimia haemolytica*, *Moraxella bovis*, *Mycoplasma bovis*, *Pasteurella multocida*, Pestivirus B, Pestivirus D, Pestivirus H, Pestivirus A, *Trueperella pyogenes*.
    - **Results**:
        - all 28 positive samples match with qPCR results;
        - Se 100% (l.c.l 90.19% – u.c.l. 100%).
        - Non-specific results not recorded;

Results on isolates, reference material and field samples, in comparison to well established methods (qPCR and Nanostring), remarked:

- high potential of NGS for diagnostic purposes (the panels are also open to new pathogens of interest)
- possibility to test up to 384 samples in the same sequencing run
- reduction of hands-on laboratory sessions and costs.

O589

LEO livestock national bio-repository: a tool for the conservation of Italian livestock genetic diversity

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Any advance in livestock molecular genetics has only been achieved upon the availability of biological specimens. Specimen resources enable research, service, and education and represent the knowledge reserves for understanding species and breeds.

In Italy, in the last decades, several farm animal collections have been organized as a by-product of research projects or through specific initiatives of research centers, universities, breeder associations, etc. However, none covers the Italian biodiversity nationwide, and few are still under regular maintenance.

To fill the gap, LEO project (PSRN 16.2 Livestock Environment Open-data) in 2019 broadened its objective to include the creation of the largest and complete *ex-situ* biorepository of Italian of livestock genetic diversity. The key objective of LEO gene banks is to guarantee the safe and long-term storage of genetic material and associated data.

The LEO farm animal bio-bank has been created following the recommendation deployed in the FAO Guideline 33: 'Innovations in cryoconservation of animal genetic resources.'

The new infrastructure, located at ConsDABi biodiversity research center, permits the conservation of biological material at room temperature, refrigerated (4–6 C°), frozen (–20 C°), and cryopreserved (liquid nitrogen). Protocols regulate the Leo biorepository workflow for sample acquisition and storage and legal rules for data and sample access and usage.

Up to now, LEO Biorepository contains over 630,000 biological samples belonging to 79 bovines breeds, 2 buffaloes breeds, 84 sheep breeds, 60 goat breeds, 69 horse and 11 donkey breeds, 25 swine breeds and 46 rabbit breeds. In detail 315,535 hair bulbs, 51,985 tissues from bioptic ear tags, 119,978 nasal and salivary swabs stored at room temperature. Frozen samples include 68,132 DNA, 57,341 blood, and 16,067 tissues of various sources (muscles, cartilage, etc.). Finally, are cryopreserved 5452 straws.

Chronologically, the samples in the gene bank span up to thirty years. The biobank repository increases at the rate of thirty-thousand new samples per years and will be regularly maintained and updated.

In this framework, LEO Gene banking genetic resources fit within Strategic Priority 9 of the FAO Global Plan of Action for Animal Genetic Resources 'Establish or strengthen *ex situ* conservation programs'.

O590

Stress assessment in juvenile sea bass (Dicentrarchus labrax) during transport density simulation

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Transport of live fish is an important process in aquaculture. During transport, fish are subjected to different stressful factors like fasting, handling, vibration, overcrowding and water deterioration. Plasma cortisol is considered the most reliable acute stress index. The aim of this preliminary study was to simulate different transport densities and evaluate the effects on stress by measuring plasma cortisol levels. Before creating groups, blood samples were kept from 25 animals to determine the initial cortisol (IC) levels. Two juveniles' European bass (*Dicentrarchus labrax*) groups were kept for 24 h at two different travel densities: the control group at 25 kg/m^3, while the experimental one at 55 kg/m^3. The juvenile fish involved in the trial came from a single stock and their weight was 41 ± 2 g (mean ± SD). Three tanks of 2 m^3 were used for each group (triplicate), with a total of six tanks. The transport lasted 24 h. Blood samples were taken for both groups before the travel to determine basal cortisol (BC), and at the end of the travel to determine post stress cortisol (PC). All animals were drawn from the tank and immersed in an anesthetic bath. Plasma was sampled from the caudal vein with heparinized syringes (Pic solution, 1 mL). Subsequently the blood was transferred to heparinized tubes and centrifuged. The plasma obtained was transferred to microcentrifuge tube and stored at –20 ° C until processing. The ELISA kit (DRG® Cortisol ELISA) was used to assess cortisol levels in all samples.

Analysis of variance (ANOVA) was performed to detect significant differences in the cortisol values found. The IC values showed lower values than the values found after the creation of travelling groups. In both densities groups the PC values were greater than BC ($p < 0.01$), with higher values pf PC in high densities animals compared to low densities ones ($p < 0.01$). the lowest cortisol values were observed at IC moment (60.94 ng/mL) and the highest were the PC in the high-density animals (337.73 ng/mL).

The differences between initial cortisol and basal cortisol underline that even handling alone is a stressor for the fish. Moreover, differences in cortisol found in BC and PC indicate that transport is stressful and increasing density is a factor able to enhance stress, with economic losses for farms.

O591

Effect of a diatomaceous-based dietary additive on milk production, antioxidant status and immune response during peripartum in buffaloes

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The physiological stress that ruminants undergo during peripartum induces hormonal and metabolic changes as well as lowering of immune response. In order to improve animal welfare and productions, the use of products rich in antioxidants such as algae is receiving increasing attention. The aim of this work was to study the effects of diatom dietary inclusion on the health and milk production of buffaloes during peripartum and early lactation. Hence, a commercial additive (Decosel®) consisting mainly of diatomaceous earth was used for the trial. Eighty pregnant Italian Mediterranean buffaloes, balanced for parity, were divided into a control group (CG; $n = 40$) and a diatom group (DG; $n = 40$). Both groups were fed with the same diet during entire trial; additionally, only DG received daily 60 g/head of additive. The trial lasted 120 days. Blood was draw from jugular vein through sterile Vacutainer tubes and the antioxidant activity, inflammatory markers, proliferation and PBMCs and monocytes viability were analysed. Milk was daily collected to determine physico-chemical parameters. Data were analyzed using a two-way ANOVA, where dietary treatment and time were set as independent variables. Diatom inclusion did not affect chemical composition of milk, but it favored a decrease of the somatic cell score in multiparous buffaloes of DG during early lactation ($p < 0.05$). An increase of enzyme activity (SOD, Catalase and GSPx) and antioxidant capacity (measured by FRAP and ABTS) during whole trial were found in DG ($p < 0.01$), but no difference in oxidative products of plasma was detected between groups. Results of inflammatory markers revealed only a tendency to decrease after the first 30 days of lactation in all animals. However, if treated with H_2O_2 or stimulated with ConA, PBMCs of DG produced a better response in viability and proliferation than CG ($p < 0.01$). Results indicate that dietary supplementation with diatoms in buffaloes during peripartum enhances *in vitro* immune response during calving and early lactation. However, buffaloes are characterized by a low metabolic and catabolic activity which does not allow to observe the potential effects of diatoms in milk production, quality, and oxidative status. These outcomes encourage further studies aiming to deepen knowledge on this field, as a strategy to reduce clinical and metabolic pathologies after calving and the use of antimicrobial substances, as well as improving milk yield of buffaloes.

O593

A promising mix of protein sources obtained from biorefinery as partial replacement of dietary ingredients in aquafeed for gilthead sea bream (Sparus aurata) broodstock

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There is an increased research activity focusing on new protein and oil sources able to secure the sustainability of aquaculture productions. The factors that mainly limit the replacement of fishmeal and fish oil with alternative ingredients are their lack of essential amino acids and in long chain-polyunsaturated fatty acids (LC-PUFA). In particular, a balanced diet is necessary for broodstock, to guarantee the adequate development of brain and larval tissues and reproductive performance.

The aim of this study is the evaluation of the reproductive performance of gilthead seabream broodstock (*Sparus aurata*) fed on experimental diets based on innovative and valuable ingredients produced utilizing aquaculture wastewater and fish processing wastes. To date, no data are available on the potential inclusion of such ingredients coming from biorefinery as fish and plant-based ingredients substitution in broodstock aquafeed.

A control diet (CONTR) was prepared using a blend of conventional protein sources while an experimental diet (H1) was prepared using a mix of alternative and valuable ingredients (microalgae, *Nannochloropsis sp*; duckweed, *Lemna minor*; insects, *Hermetia illucens* and macroalgae, *Alaria esculenta*) as a partial replacement (10%) of fishmeal and vegetable protein sources.

Gilthead seabream broodstock (male weight average 0.512 ± 0.087 kg and female weight average 2.522 ± 0.34 kg) were randomly allocated in circle-shaped 5000-L-fiberglass tanks, (22 fish/tank, 15 males and 7 females) in a RAS, under controlled rearing conditions, and nutritionally challenged for 6 months, 2 months before pre- and 4 months post-spawning. Being the alternative ingredients produced using aquaculture waste, diets were evaluated at nutritional, chemical and microbiological levels No contaminants were found in the diets, which resulted similar in their proximate composition and fatty acids and amino-acids profile, except for the Docosahexaenoic acid (DHA), for which H1 diets resulted more enriched respect to the control (CONTR). Spawning quality parameters were significantly different between H1 and CONTR broodstock ($p < 0.05$). In particular, the mix inclusion seems positively affect the hatching parameter and larval survival at 6 days post-hatch ($p < 0.05$). No alterations in terms of skeletogenesis, gut, and muscle development were found in larvae sampled from H1 with respect to CONTR group. Further studies are underway to verify the health parameters of the broodstock.

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O585

Suitability of Life Cycle Assessment (LCA) for the estimation of donkey milk environmental impact: a case study

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Nowadays donkeys are interested by two innovative aspects: onotherapy and milk production. Donkey milk, similar to human one, characterised as high added value food, is also adopted as raw material in cosmetics. Growing public interest suggests a pilot study on the sustainability of donkey milk, according to LCA criterion Two different models were described: Real Scenario Model (RSM) and Increased Milk Production Model (IMPM), representing the farm with declared milk yield (1 kg of raw milk as functional unit, FU) and the same, hypothesising a larger size of FU, respectively. Allocation involved RSM and IMPM scenario both, thus different values of impact categories, i.e. GWP (kg CO_2

equivalents), Acidification Potential (ACP, g SO_2 equivalents) and Eutrophication Potential (EUP, g PO_4^{3-}) were recorded. GWP, improved by mass allocation, showed the lowest amount of equivalents in IMPM, compared to economic and no allocation criterion ($p < 0.05$). In the RSM allocation modes affected differently GWP, the small size of FU determined the largest amount of CO_2 equivalents ($p < 0.05$) without allocation, whereas in the mass mode were found lower ones than economic allocation ($p < 0.05$). ACP and EUP followed the same trend. No differences were found in IMPM (no, mass and economic allocation) results, moreover mass allocation values recorded in the RSM framework, were no significantly different compared to IMPM ones. ACP and EUP of RSM affected by economic allocation were less sustainable ($p < 0.05$) than all IMPM values, were also less sustainable than RSM equivalents improved by mass allocation ($p < 0.05$), even though lower than no allocation ones ($p < 0.05$) in RSM scenario. As expected, large milk yield improved almost all impact categories, while the increase was proposed as a model. RSM and IMPM both were affected by allocation criterion, largely adopted to attribute pollutants to byproducts. Economic and mass allocation depicted a representative framework where (in RSM) the role of donkey meat contributed to subtracting equivalents from milk, the main product. Donkey farms, often characterized by ecosystem services (onotherapy or landscape), can express their added value by means of allocation criterion or the travel cost method. This research is aimed at proposing a pilot study as a touchstone, also given the growing appeal of donkey milk.

O24

A genome wide-association study for morphological and hunting performance traits in Braque Français Type Pyrénées dogs

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High-throughput genotyping technologies offer a great potential to increase our understanding of the genomic basis of canid variation. Braque Français Type Pyrénées (BRA) are smart, agile, and friendly dogs originally developed for tracking, hunting, and retrieving feathered game. On a population of 48 unrelated BRA dogs (27 males, 21 females), single nucleotide polymorphism (SNP) genotype data from the CanineHD Whole-Genome Genotyping BeadChip and evaluation scores for 12 traits related to morphology and hunting performance are available. The assembly CanFam3.1 was used as the reference genome. After quality filtering (MAF >5%, call-rate >95%), 96,816 SNPs on the 38 dog autosomes (CFA) and 44 dogs were retained; residual missing SNP genotypes were imputed using the LHCI method implemented in Beagle v.4.0. Phenotypic scores were expressed on a semi-quantitative scale from 1 (worst) to 6 (best), and were mostly poorly to moderately correlated except for some morphological traits (e.g. $r = 0.81$ between the conformation of the head and that of the eye). A genome-wide association study (GWAS) model that included the effect of sex and the genetic kinship matrix based on SNP genotypes was used to analyse the association between SNP loci and phenotypes. The strongest signal of association was found on CFA05 (at position 33,872,506 bp) for the shape and position of the eye (*p*-value =1.346e-05), located within the *STX8* gene. For the hunting performance, the strongest association was detected on CFA32 (at position 10,959,734 bp) for the trait ‘connection to the hunter conductor’ (*p*-value =3.639e-05); this SNP is located ± 100 kb off of four know genes (*NUDT9*, *DSPP*, *SPARCL1*, *DMP1*). Among these, *SPARCL1* has been associated with morphological traits in dogs. A larger number of significant associations (*p*-value <0.001) was found for morphological traits than for traits related to the hunting performance (211 v 178). These results advance our understanding of the genetic basis for morphology and hunting behavior in dogs and identify new variants which are potential targets for further research.

O15 AQUACULTURE

The evolution of aquaculture in the Balkan region in the last 70 years

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This study represents the analysis of aquaculture history in the Balkan region (Albania, Bosnia and Herzegovina, Croatia, Greece, Montenegro, North Macedonia, Romania, Serbia, Slovenia and Turkey) from 1950 to 2019. In this study are investigated farmed species, their diffusion in the different countries and the functional relation between aquaculture and capture fishery. Diversity of farmed species is also investigated, differences between marine and freshwater aquaculture, together with the differences between fish and mollusc aquaculture.

In the Balkan countries, aquaculture started in the 50-ies. In the beginning, fisheries production was about 27 times higher than aquaculture production. In 2019 aquaculture volumes were comparable with fisheries; with a total increase of 92 times since its beginning. Total aquaculture production in 2019 was more than 571,000 tons for a corresponding economic value of about 2200 million US$.

In 2019, marine aquaculture represented 70% of total aquaculture production. Fish production represents 98% of the annual economic value in the region. Main farmed species was European sea bass with 186000 tons of production. Over a total of 64 species farmed, only 4 made 92% of total production. Turkey was the main producer country (in 2019) with more than 371,000 tons produced; the first 3 countries made 91% of total aquaculture production in the region. Calculated models indicate that total aquaculture production in 2030 will oscillate between 697,000 and 760,000 tons, thus showing an expansive growth. Instead, fisheries production shows a decreasing trend with expected production of about 260,000 tons in 2030. Actually, this study represents the most extensive study of aquaculture in the region and it can be considered a valuable reference point for future common aquaculture strategies in the area.

O19

Genetics of udder volume and maternal ability in Italian beef cattle

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Selection in beef cattle has been focused mainly on productive traits; however, reproductive, and functional traits have gained interest in recent years. Calving ease, calf's protection and milk yield have been shown to be an important trait for calf survival and its subsequent growth, particularly in beef cattle reared in an open pasture and loose system. Traits related to the udder and milk production can influence calf growth and weaning weight being milk the main source of calves' feeding, playing therefore a great influence on farmer's income in beef cattle contests.

This study aimed to estimate genetic parameters for udder volume (UV), maternal ability (MP) and a combined trait obtained by summing the two traits (UV + MP) in Marchigiana (M), Chianina (C), Romagnola (R), Maremmana (MM) and Podolica (P) beef cattle.

Traits were evaluated on cows through linear type score of UV, evaluated as the distance of the bottom part of the udder and the hocks, and by interview to farmers on MP produced. Both traits were evaluated on a 1–5 scale system, i.e., from very small to abundant volume for UV, and from scarce to abundant in interviews on farmers for MP. Records were obtained within the I-BEEF project financed by the Ministry of Agriculture through the EU rural development funds from 2017 and 2021 and involved 13,738 cows, including 4585 M, 4336 C, 964 R, 947 MM, and 2951 P.

A GLM of SAS allowed the identification of 3 main non-genetic factors affecting the scores. These factors were identified in the herd-year classifier within breed, the calving distance (i.e., interval from calving to type trait evaluation), and the age at calving by parity (classified in 1° parity, 2°, 3° and other) at animal evaluation classes. These factors were all accounted in the subsequent across-breed genetic analysis carried out through single and bi-trait animal model-REML, accounting for 79,487 animals in the pedigree file. Heritability estimates were 0.09 for UV, 0.06 for MP, and 0.12 UV + MP, which agreed or was lightly lower than estimates obtained in other beef and dual-purpose cattle breeds reported in the literature. The genetic correlation between UV e MP traits was 0.88. The investigated traits were chosen to be included in new estimated breeding values for Italian beef cattle breeds.

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P596

Ensiling of agro-industrial by-products mixtures with Lactiplantibacillus plantarum: chemical and microbiological characteristics

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In last years, the climate challenge and the need of an ecological transition has attracting the interest toward the use of agro-industrial by-products for livestock feeding, in line with the green and circular economy. The olive oil, winery, and dairy industries result in large amounts of by-products, which are usually underused or not valorised, constituting an added cost for their disposal as waste. The inclusion of these by-products along with wheat straw in animal feeding, beyond as nutrients source, may have beneficial effects on animal health and quality of products, due to their content in bioactive compounds such as polyphenols. Ensiling is regarded as a suitable method to preserve many types of fodder and by-products, overcoming seasonal availability. In this study, we evaluated the possibility of ensiling mixtures of olive oil industry by-products (olive leaves, olive mill waste water and olive pomace), wheat straw, grape pomace, and cheese whey. Five different mixtures composed of 97% of by-products and 3% molasses were inoculated with a commercial *Lactiplantibacillus plantarum* strain, and vacuum packed into plastic bags at lab-scale. The chemical, microbiological characteristics, total phenol contents and antioxidant activity (DPPH) were evaluated at the ensiling time (D0) and after 4, 7, 15, 30 and 60 days. The results showed a good aptitude of the by-products to be ensiled in mixtures, with a pleasant smell and good visual features. The different mixtures of by-products significantly ($p < 0.05$) affected Fleig's score, total phenols content, antioxidant activity and microbial cell densities of total mesophilic aerobic bacteria, lactic acid bacteria, enterobacteria, yeasts, and moulds, with a low number, or absence, of undesirable microorganisms. Acidification differed among the silages, resulting in the highest rate (pH <4.5; D60) in the silage containing olive and grape pomaces, straw, and cheese whey.

It can be concluded that the ensiling of agro-industrial by-products in the mixture gives satisfactory results in terms of microbiological and antioxidant characteristics for potential use in animal feeding and environmental and economic savings.

P598

A 33-year retrospective analysis of economic value of Mediterranean aquaculture as a suitable base for future management of aquatic resources in the region

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This study is based on the aquaculture economic value data in the Mediterranean region from 1984 to 2017 and on aquaculture production data from 1950 to 2017. Both functional and geographical expansion of aquaculture is investigated, considering two main complementary aspects of aquaculture: farmed species and farming countries. The calculated models on the Mediterranean global value of aquaculture foresee that it is in a stationary phase and that in the next future, it will keep constant about 4.8 US$ billion. Aquaculture's economic value is based on a few successful species and countries, as shown by the application of Pareto principle. In the future 80% of economic value of aquaculture will be provided by 4 or 5 species and the 99% of value by 20–22 species. The most relevant species of aquaculture economic value are Nile tilapia, gilthead seabream and European seabass. Even if the first Mediterranean country is Egypt, which aquaculture is based on extensively farmed tilapia, the analysis of relative aquaculture growth indicates that the leading countries are Israel, Greece and Cyprus. These countries should be taken as possible models for country aquaculture development. The situation described here shows economic and productive aspects that can be used as a powerful reference point for the strategic management of aquaculture in the Mediterranean.

P22

Identification of candidate gene variants for the Alpaca Suri phenotype by whole genome sequencing analysis

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Alpaca is a South American camelids bred for meat and fibre production. Two different phenotypes are described for this species: the short and crimped hair phenotype, known as Huacaya, and the long, straight and luster hair phenotype, known as Suri. To date, the genetic background behind the two phenotypes is still unknown, however, segregation analysis suggests the Suri phenotype as dominant trait. In this research, whole-genome sequencing (WGS) analysis was used to uncover the genetics variant behind the Suri phenotype. Sample consisted of 19 WGS from Huacaya alpacas retrieved from public available repository (NCBI-SRA), 3 new WGS of Huacaya alpacas and 4 new WGS of Suri alpacas. Single reads were aligned to the most updated alpaca reference genome (VicPac3.1) and the genomic joint variant calling was performed. 37,421,914 variant were called, classified as single nucleotide polymorphism (30,749,986), insertions (3,547,803) and deletions (3,124,125). The called variants were then annotated in order to predict the phenotypic effects. 81,859,906 phenotypic effects were predicted, of which 61,094 were of 'high impact', 312,519 were of 'moderate impact', 545,541 were of 'low impact' and lastly, 80,940,752 were classified as 'modifier impact' influencing genomic untranslated regions. Finally, a case-control filtering was performed assuming the Suri phenotype as dominant mutation. 258 out of 35,371,788 variants remains. Five variants located on four loci were missense mutations while the remaining 253 were annotated to untranslated genomic regions. The five missense variants identified are promising candidate for the alpaca Suri phenotype.

P49

Integration of multi-tissues multi-omics data. An example from bovine embryos

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The increasing availability of large multi-tissue data sets which contain gene expression measurements across different tissues and individuals provided unprecedented opportunities to investigate transcriptome variation across tissues and individuals and may reveal interactions between genes and tissues. The corresponding data set is a three-dimensional array: genes, individuals and tissues (or recording times). We present here the so-called 'Partial Triadic Analysis', (PTA), a well-suited statistical tool to get a clear representation of a spatial series of matrices, one for each tissue. PTA is an extension of PCA and allows one to find a structure common to every matrix and to study its stability across tissues. PTA consists in three steps: (i) the interstructure step, where are compared and analyzed the relationships between the different datasets, (ii) the compromise step, where all datasets are integrated into an optimum weighted average, the compromise (or consensus) table, and (iii) the intrastructure step, where the single-transcriptome are compared to the compromise in order to analyze commonalities and discrepancies.

PTA was applied to transcriptomic data from the ANR (Agence Nationale de la Recherche) funded BoSexDim project, consisting of 19 embryo transcriptomes recorded at D40 (40 days after fertilization) and structured by sex (Male / Female) and type (*in vivo/in vitro*). These transcriptomes were recorded for four tissues (brain, liver, gonad and placenta). PTA shows a compromise structured by sex (first axis) and type (second axis). The same set of genes contribute the most to the sex structuration whatever the tissue. However, the differentiation of *in vivo* vs *in vitro* embryos was not made by the same genes according to tissues. Some genes showed an inconsistent, even contradictory behaviour, with an overexpression in one tissue and an underexpression in another one.

This example highlights the power of the Partial Triadic Analysis for depicting the variability of the transcriptome structure across various tissues.

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P95

Factors affecting twinning rate in Italian Holstein cows

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Multiple births are acceptable in some animal species/categories because they allow for the production of more offspring from genetically superior parents. In Italian Holstein (IH), twinning and its association with traditional traits have not been studied yet. However, the perception of farmers is that this phenomenon has been increasing in IH. Through this study, we have evaluated the factors affecting twinning in IH cattle by using an edited dataset of 1,280,346 milk test-day records (TD) collected on 86,089 cows (parity 1–3) in 772 commercial herds distributed across 3 representative Italian provinces. Herds performing synchronization were excluded from the study. The effect of twinning (TW, yes vs. no) on daily milk yield (MY) was investigated through analysis of variance. Fixed effects were parity, classes of days in milk, TW, calving month and test year, and random effects were herd-test-date, cow, and residual. The sex of the twins weas not accounted for and calving events with 3 or more twins were excluded. Twin births accounted for 2.09% of the total TD and 1.32% of the total cows. Cows giving birth to twins did not produce less or more milk compared to cows giving birth to a single calf. For genetic analysis, 50% of the total TD were extrapolated to calculate average and maximum MY in each lactation (55,071 cows and 94,662 lactations). Heritability and genetic and phenotypic correlations of TW with average MY and maximum MY were estimated. Fixed effects were parity, age at calving within parity (6 classes), and season of service, and random effects were herd-year-season of calving, additive genetic animal, and residual. TW was lowly heritable, with additive genetic variance not different from zero as well as the genetic correlations with average and maximum MY. These preliminary findings suggest that there is apparently a low genetic link between productivity and TW and confirm TW to be a complex trait whose variability cannot be easily manipulated through genetics. Since some bulls and dams were connected to several twins in the pedigree, we need to further explore nature and genetic architecture of TW in IH.

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P108

Analysis of mRNAs transcribed from the Ragusana Donkey CSN1S2 I and II genes and variability detection

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In recent, years donkey's milk (DM) has attracted an increasing interest in human nutrition, since it may represent the best natural substitute of cow's milk (CM) for children affected by CM protein allergy. The reason lies in a low casein content and a casein to whey protein ratio closer to human milk than to ruminant milk. Recently, the presence of all four casein fractions αs1, β, αs2 and κ-CN was demonstrated in DM. In particular, concerning the αs2-CN encoding gene (*CSN1S2*), two different donkey cDNAs (*CSN1S2* I and *CSN1S2* II) have been identified. The first (GeneBank FM946022.1) is constituted by 19 exons encoding for a protein (αs2-I) of 236 amino acids (aa); the second (GeneBank FN298386.2), made of 16 exons, encodes for a peptide (αs2-II) of 160 aa. Aim of this study was to analyse the transcript profile of donkey *CSN1S2* I and *CSN1S2* II genes. Milk samples were collected from 8 unrelated donkeys of Ragusana breed. Total RNA was isolated from milk somatic cells. Retrotranscription (RT) was performed using an oligonucleotide dT18, whereas the PCR reaction was accomplished using primers designed using published donkey nucleotide sequences. PCR products were cloned. According to the sequence of randomly chosen clones, the *CSN1S2* I gene, beyond a correctly spliced mRNA, transcribes other three minor mRNAs (a) lacking the exon 11, (b) missing the last 35 nucleotides of exon 17, (c) showing the insertion of an extra exon 12 (named 12', intronic insertions of 105 bp) that introduces a premature stop codon. Analysis of RT-PCR products of *CSN1S2* II gene evidenced a more remarkable variabilty of the splicing events, mainly regarding the alternative insertion of an extra exon 7 (named 7'), or the alternative skipping of exons 9, 10, 11, 12 or 15. The remaining products are characterized by the combination of deletions described above: e.g. contemporary skipping of 11 and 12, 9 and 15 or 9, 10 and 15. Interestingly, the duplication of the tripeptide KQL at exon 13 previously reported (GeneBank FN298386.2) was not detected in all *CSN1S2* II mRNAs sequenced. Therefore, likely it should be considered as an artifact. For *CSN1S2* I, seven SNPs were observed, 5 of them responsible for aa changes: p.T73 > A, p.I109 > V, p.I130 > V, p.I146 > T and p.D217 > Y. Similarly, 8 SNPs were observed at *CSN1S2* II *locus*, 6 of which non-synonymous: p.L63 > F, p.H70 > Q, p.D90 > N, p.129A > T, p.H131 > Y and p.F157 > S. The investigation at DNA level will likely clarify the variability found at mRNA level.

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Heritability of milk conductivity measured by automatic milking system in Italian Holstein cows

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Mastitis is one of the most important diseases in dairy farm, reducing milk production and causing heavy economic losses.

One effective approach to reduce the impact of mastitis is to select disease-resistant animals, performing this selection both on the male and the female lines. This selection could be possible by identifying the progeny of the most resistant cows. However, it is still necessary to find parameters that are both good mastitis indicators and that retain a large additive genetic variance. Automatic Milking System (AMS) contributes to a growing amount of big data measured to monitor animal health, and this data can be used for animal selection. Among the different parameters measured by AMS in milk, Electrical Conductivity (EC) can be a good and cheap candidate to be used as mastitis predictor. Thus, aim of this study was to calculate the heritability of EC in two Italian Holstein dairy cow farms. Two farms were selected based on four features: same AMS model, same cow breed, and similar farm and animal management. Data were measured from June to September 2021, for a total of 16 measurements for each cow. Along with EC, the value of Somatic Cell Count (SCC) was recorded for every milking. Differently from EC, that is measured on milk from each quarter of the udder, however, SCC was measured from the pooled milk of the four quarters. After data editing and pruning on the original dataset (i.e. removing records with missing values for the dependent variable and animals without half-sibs in the dataset), the final one was composed by 3362 observations recorded on 234 cows. Pedigree data were collected and included a total of 51 sires. Sire mixed models were fitted to calculate the additive and phenotypic variances, including the following fixed effects: SCC, contemporary group based on the month of the sampling, days in milk, parity, and farm effect. Random effects included sire and animal effect. Heritability was estimated and varied largely depending on the model used, ranging from 0.03 to 0.35, similar to what already reported in scientific literature. Further observations will be recorded, as well other farms will be involved, to improve the obtained estimates.

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Different optimal contribution selection strategies to manage inbreeding in limited size cattle breeds

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The aim of this study is to investigate the use of Optimal Contribution Selection (OCS) in two local beef cattle breeds to implement conservation schemes aimed at minimizing inbreeding (F). Calvana (CA) and Pontremolese (PO) are Tuscany cattle breeds; the former has a deeper and more complete pedigree (3194 records; Pedigree completeness index, PCI =73%), while the latter shows a more shallow and incomplete pedigree (429 animals; PCI =59%). In these breeds the most common breeding practice is natural insemination, even if doses of semen are conserved for conservation purposes. Mating plans were developed using EVA software v3.0 and for each breed three approaches were tested: (i) the use of only natural insemination, limiting to 2 the number of maximum matings for each bull, (ii) the use of both natural and artificial insemination, limiting to 5 and (iii) the previous approach using until 20 semen doses for each bull. Genetic contributions were optimized using default algorithm parameters with constraint option (dFconstraint =0.01 and mating strategy = mai). Results showed that it is possible to avoid a large increase in F using OCS; the proposed matings obtained offsprings with an F coefficient lower than 1% in CA breed and lower than 3% in PO breed. The use of semen doses can further reduce offspring average F, which passed from 0.007 (1st approach) to 0.006 (3rd approach) and from 0.025 to 0.019 in CA and PO respectively. The large use of semen (20 doses per bull) led as expected, to excessive use of those bulls; percentage of offsprings born by artificial insemination was 10% in CA and 45% in PO deriving from only 6 bulls for each breed. Utilization of animals and especially semen with unknown parents and underestimated average relatedness could be risky. To minimize this risk it might be desirable to genotype breeding animals creating a genomic relationship matrix able to better assess offspring inbreeding coefficients.

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Genes expressed in the udder of Sudanese camels belonging to different ecotypes (GENEROUS)

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Dromedary milk plays a central role as a protein source for humans across the world, especially for peoples living in arid lands for whom other protein sources are scarce. Concurrently, market demand for diversified dromedary milk products has been growing, and this milk is receiving more global recognition as a healthy food.

The possibility of analyzing the expression profiles and metabolic pathways of the dromedary udder offers an opportunity to elucidate the mechanisms underlying the complex traits of camel milk production. Rapid development and decreasing costs of Next Generation Sequencing (NGS) have made high-throughput RNA sequencing (RNA-seq) a fundamental tool for transcriptome analysis aimed at identifying differentially expressed genes (DEGs), new or alternative transcripts, non-coding RNAs, etc.

These discussions lay out a clear path for our work: (1) make clear the genetic mechanisms underlying the expression of genes controlling the quali-quantitative production of milk in camels; (2) establish the basis for the genetic improvement of the species genetics by the discovered markers in the coding regions; (3) unlock the potential of camel milk for economic and human benefit to communities.

Project goals

The following are the four primary goals of the project:

a. analyse and compare the transcriptional profile of genes expressed in the udder of Sudanese lactating dromedary she-camels with extreme phenotypes for milk yield and belonging to the two main Sudanese ecotypes (Darfouri and Kenani camels);
b. analyse the regulatory regions (promoters and 3'UTR) of the genes identified as differentially expressed in the two groups of she-camels in order to identify their polymorphisms and investigate their distribution in a representative sample of the Sudanese camel population;
c. define the genetic variability found at the loci involved in the milk production for dromedary camel populations belonging to other ecotypes (for instance, Arbi Butana, Anafi, Rashaydi camels, etc.);
d. transfer Project-gained knowledge to the scientific and Sudanese camel milk production communities through publications and dissemination activities on how best to implement optimal strategies for more profitable breeding.

The Project is subdivided into four phases and is organized into seven work packages (WPs), each with a specific goal.

Duration: 3 years.

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Characterization of an Iranian buffalo population at CSN1S1 and CSN3 loci

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Water buffalo is globally recognized as an important species for agriculture and local economies in many regions of the world. River buffaloes are found from eastern Asia to Europe. In Iran, there are three main buffalo breeds including Azeri, Khuzestani and Mazandarani. However, local sub-populations (or breeds) exist, mainly identified on geographical origin with little or no documented phenotypic or genetic differences. Buffalo milk quality plays a key role in dairy processing, being the curd formation dependent on total protein and fat content. In this respect, casein variants are known to influence the yield according to the specific genotype at *CSN3* and *CSN1S1* loci. The aim of this study was to characterise a local Iranian buffalo population of the Pareh Sar area (Gilan province, IRAN) for the SNP AJ005430:c.578C > T at *CSN1S1* and HQ677596:c.536C > T at *CSN3* and compare it with the literature data in the Italian Mediterranean buffaloes (IMB). DNA was isolated from 89 buffalo individual blood samples. A duplex artificially created restriction site (ACRS) PCR was carried out using at the same time four primers for generating two amplicons, 86 bp for the *CSN1S1* and 160 bp for the *CSN3*. Enzymatic digestion was performed by *MboI* and *HinfI* endonucleases. The electrophoresis run on a 3% agarose gel in TBE 0.5× at 90V for 45 min allowed the simultaneous identification of the genotypes for both *loci*. Population statistics were run on collected data.

The population analysis showed that minor allele frequencies (MAF) were 0.318 for the CSN1S1 T (allele A) and 0.275 for the *CSN3* T (allele X2). No departure from HW equilibrium was detected. MAF of *CSN1S1* was different ($Z = 2.48$, $p = 0.013$) from published data on IMB (0.425), but this was not observed for *CSN3*. No linkage disequilibrium (LD) was detected between the markers ($r^2 = 0$) and all four possible haplotypes were found. The higher frequency (0.496) was discovered for the *CSN1S1* B-*CSN3* X1 and a similar result was also found in the IMB (BX1, 0.553). The most favourable haplotype for dairy purposes (*CSN1S1* A-*CSN3* X2) showed the lower frequency (0.094) in the Iranian population. Conversely, the AX2 haplotype had a frequency of 0.279 in the IMB. This result leaves genetic improvement opportunities in both populations for this trait, considered as one of the most relevant for its influence on protein content and cheesemaking properties.

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Genomic regions in association with stay-ability in Italian Limousine cattle

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The objective of this study is to identify genomic regions, which variability is associated with cow survival in the Italian Limousine beef cattle populations. Parity records were available for 37,518 cows. Survival was expressed as stay-ability (binary, i.e. 0/1) at a given parity x, i.e. the ability of a cow to survive from parity x to parity $x + 1$, with the parity being from 1 to 6 (e.g. STAY1 for surviving from parity 1 to parity 2).

Threshold models were used. Genomic information (about 14,000 SNP markers) was included for 3039 individuals in the Single-Step GBLUP model. These included 292 bulls and 2747 cows, with some of the cows being phenotyped (2329). Variance components were estimated using pedigree information only, breeding values were estimated including genomic information. Allele substitution effects were obtained using back-solving of the mixed-model equation, variance was obtained as the product of the marker effect by the genotypes. Overlapping windows of 10 contiguous SNP markers were constructed and their impact on the traits was expressed as the proportion of genetic variance explained. Only the top 1% for variance explained were considered hereinafter.

Heritability estimates showed a decline from STAY1 (0.187) to STAY5 (0.096). Some degree of shared genetic architecture among the traits was found. Chromosomes 5 and 6 showed regions affecting all traits, chromosomes 1, 2, 3, 9, 11 and 19 showed regions affecting 5 out of 6 traits. The strongest concordance was found among STAY4 and STAY6, with 60 genomic windows affecting both traits (supported by a 0.31 rank correlation of breeding values for genotyped individuals between the two traits), followed by STAY3 and STAY6, with 28 genomic windows in common (showing rank correlation of 0.07). All other combinations of traits showed 0 to 20 windows in common, with rank correlations (of breeding values) between −0.10 and 0.24.

Stay-ability showed similar genetic architecture over the studied parities, although the overlapping was moderate. Further studies should investigate the degree of discordance, rather than concordance, among the traits. Results should also be validated with some measures of empirical significance (i.e. bootstrapping).

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P190

Effect of CAST and DGAT1 genetic variants on the qualitative and quantitative characteristics of Italian Mediterranean buffalo meat

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In recent years, the interest towards buffalo meat has been increasing due to its nutritional quality: reduced fat and cholesterol, and high iron content.

This study aimed to characterize the chemical-physical composition of Italian Mediterranean buffalo (IMB) meat and to assess the effects of *CAST* and *DGAT1* gene polymorphisms.

Calpastatin is involved in the tenderness process, while the *DGAT1* is a key gene for fat synthesis, although studies have linked it to meat tenderness.

DNA of 56 IMB belonging to one farm was isolated. *CAST* gene was partially sequenced in 10 IMB randomly chosen. SNP discovery showed the transition $A > G$ at the intron 12 (nt 338) that represents the first marker reported at *CAST* in the IMB. Regarding the *DGAT1,* we considered the SNP $C > T$ at the exon 13 (nt 69). The sample was genotyped by PCR-RFLP for both SNP. Twenty out of 56 IMB were slaughtered and a portion of the *L. thoracis et lumborum* muscle was analysed for chemical (centesimal composition), physical and technological aspects (pH; colour: L*, a*, b*, Hue and Sat; texture 20% and 80%; tenderness; cooking losses; water holding capacity) at 7 and 14 days to assess the ripening effects.

Analyses confirmed the values reported in literature at day-7, while data on tenderness were generally rather high. Instead, analyses at day-14 proved the positive effect of the ripening.

Genotype distribution was not homogeneous. Hence, we excluded animals with rare genotypes (5 AA for *CAST* and 8 TT for *DGAT1*). Regarding *CAST*, the interaction between the *meat shade* (Hue) and the day was significant ($p = 0.038$). Such result was partially unexpected because not directly linked to the meat tenderness, despite this association has been investigated in literature. No genotype effects on meat tenderness was found. This might be due to the different genetic structure of the IMB compared to cattle or, simply, to a random allele association between the examined SNP and other causative markers on *CAST*.

The SNP $C > T$ at *DGAT1* was associated with a different level of Texture Profile Analysis (TPA 80%). The CC genotype showed greater compression force than the CT ($p = 0.035$). Although the TPA 80% links to the stroma more than to the myofibril component, we can consider it as a good indication for the improvement of meat tenderness as already reported in literature. In fact, the adipose tissue is defined as a specialised connective tissue-Therefore, in this respect; the role of the *DGAT1* should be reconsidered for affecting, directly or indirectly, the meat texture. Conversely, the lack of *DGAT1* association with fat traits needs further investigation.

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Allelic frequencies of Warmblood Fragile Foal Syndrome (WFFS) gene mutation in the Italian horse population

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When present at moderate to high frequency in a population, harmful recessive alleles can be expression of the heterozygote advantage. This is probably the case of the recessive lethal missense point mutation in the procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 gene (PLOD1, c.2032G > A) that occurs in horses, responsible for the warmblood fragile foal syndrome (WFFS), a congenital pathology characterized by a high rate of miscarriages during the late gestation and severe skin fragility. Live homozygous foals show open lesions and joint hyperelasticity; they have poor prognosis and early euthanasia is advised. Despite this detrimental effect, the frequency of WFFS carriers span between 11 and 30% in Warmblood breeds, probably exacerbated by the selection of traits related to athletic performance in dressage and show jumping, two of the main disciplines where these horses are mostly used and where joint mobility and extreme athleticism are pivotal characteristics. Gene-assisted selection is a powerful tool to identify carriers for recessive traits in order to plan mating programs aiming to reduce allele frequency in a population, avoiding homozygous individuals thus preserving, together with the legitimate objectives of selection and genetic improvement, animal health and welfare. The aim of this study was the evaluation of the WFFS allele frequency in 10 Italian and non-Italian populations bred in Italy (Bardigiano, Tolfetano, Sarcidano, Cavallino della Giara, Murgese, Anglo-Arab, Cavallino di Monterufoli, Sella Italiano, Thoroughbred and Maremmano). A total of 641 subjects were genotyped through an custom RFLP-PCR test and/or the GGP Equine Arrays from Illumina. Most of the tested breeds resulted in not being carriers of the mutation, with warmblood as Tolfetano, Anglo-Arab and Sella Italiano characterized by low carrier frequency (less than 5%) as expected for their admixture with Thoroughbred and Arab. The only exception was Maremmano which showed over 12% carrying subjects. Due to this high allele frequency, an in-depth analysis of the relatives was carried out in order to identify the bloodline(s) responsible for the introgression of the deleterious allele into the Italian breed. Indeed, the average percentage of Thoroughbreds in the Maremmano horse sum up around 15–20% as calculated by genealogical data and molecular-based admixture analysis, allowing a strong hypothesis of responsibility in the introgression.

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Genomewide association study for carcass and meat quality traits in Italian Simmental

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Genetic improvement of meat traits in cattle is usually carried out on growth performances and muscularity score of young bulls in performance test stations. In Italian Simmental (IS), genetic and genomic estimated breeding values for meat and milk traits are combined in a dual-purpose selection index. However, carcass and nutritional quality traits are excluded because expensive to measure and recorded after slaughtering. Our objective was to search for genomic variants associated with technological and nutritional quality of meat. To this aim, 119 IS young bulls were genotyped using GGP-HD v.2–150K that simultaneously interrogate about 139K single nucleotide polymorphisms (SNP). After data editing, 117 calves and 120,188 SNPs on 29 chromosomes (chr) were analysed. The animals were unselected young bulls at performance test station at Fiume Veneto (Italy) slaughtered between 16 and 18 month of age. After measuring 7 carcass traits, *longissimus thoracis m.* was sampled and analysed for assessing meat quality (12 traits) and fatty acid composition (31 Traits). The traits examined were live weight at slaughterhouse (LW), Carcass Yield (CY, %) and centesimal composition, SEUROP, pH (48 h), Colour (48h, L*, a*, b*, c and h), cooking loss (CL7) and Warner-Bratzler share force 7 d post-slaughtering (WB7), the profile of 26 fatty acid (%) and their relevant sum and ratio (5 traits). Genome-wide association study (GWAS) were conducted using GenABEL package implemented in R software (v. 3.6.1) and candidate genes in QTL regions ± 250kb interval were retrieved from Genome Browser (https://genome.ucsc.edu/). LW and CY were 664.8 ± 42.6 kg and 56 ± 2.2%. GWAS allows to detect QTL regions in 24/50 analysed traits and to suggest 238 candidate genes on 21 chr(s). The SNPs associated to pH, colour (b*, c) and fat% allows to target 34 genes. The fatty acid composition traits harboured the largest number of QTL. In summary, 196 genes in QTL region were retrieved for saturated, mono/poly-unsaturated fatty acids and relevant sum/ratios. SNP genotypes close to the FANS gene (chr19) were associated C14:0 and 5 genes located on BTA5 were related to 4 poly-unsaturated fatty acid (C20:2w6, C20:3w6, C20:4w6 and C22:4w6). In conclusion, GWAS allowed to identify a number of QTL region harbouring gene possibly affecting meat nutritional quality.

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Multiplex Digital Expression Gene Analysis (MuDEGA) of 11 liver poultry genes whit NGS approach

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Next-generation sequencing (NGS) represents a powerful tool for gene expression profiling but the whole RNAseq technique is very expensive, limiting the number of samples analysed in a study. However, a specific laboratory protocol for target gene NGS quantification in chicken is not available. The present study aimed to develop a protocol for the quantification of a selected set of genes involved in stress and lipid metabolism. The study was carried out on 24 male local and broiler chicken liver samples stored in RNAlater at −80 °C until RNA extraction. Eleven liver genes, involved in lipid and stress metabolism, were selected: ACOX1, FABP1, HSPA2, CASP6, CAT, FADS2, LPL, SOD1, SREBF2. ACTB and GAPDH were used as housekeeping. Total RNA was extracted with FastGene® RNA Premium Kit and evaluated for its quantity (Qbit®, RNA Broad-Range Assay Kit) and integrity (RIN, Agilent 2100 Bioanalyzer). The mean ± standard deviation (SD) concentration of the extracted RNA and RIN were respectively 7.28 µg/µL ± 3.05 µg/µL and 8.09 ± 0.55. Then, all RNA was reverse-transcribed with First Strand cDNA Syntesis Kit and cDNA was quantified with Qubit®. The literature primer was modified with a specific adapter sequence in 5' and each gene was tested in single PCR amplification: the amplicon ranged from 120 to 300 bp. A multiplex PCR protocol for all 11 genes was optimized. To identify the adequate concentration of cDNA, the following dilution was used for all 24 samples: undiluted cDNA, 1:25, 1:50, and 1:100 for a total of 96 reactions. The library for NGS sequencing was realized. Briefly, the multiplex PCR products were purified (ExoSAP-IT® Express) and the Index PCR was performed. The products of the Index PCR reaction were quantified by Qubit® and size analysed using Bioanalyzer. Each library was diluted to 4 nM and pooled for NGS sequencing on MiSeq Illumina platform. R software (version 4.2.2) was used for statistics by applying R packages to perform reads count (FeatureCounts). Differential expression gene analysis of cDNA dilution was performed using the DESeq2 R package, p-adjusted value <0.05 was used as threshold. The statistical analysis showed significant difference ($p < 0.05$) between undiluted cDNA and the other groups but no significant difference among 1:25, 1:50, and 1:100 dilutions. The differential gene expression was confirmed in real time PCR. The protocol described is fast, cheap and simple new tool useful for digital gene expression analysis in chicken.

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Dissecting the genetic variability of major genes for pig production traits using whole genome sequencing data

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Whole genome sequencing (WGS) datasets produced from next generation sequencing technologies are making it possible to obtain a comprehensive evaluation of the level of genetic variability within and across breeds and populations in many livestock species and to infer their genetic history and relevant population genetic information useful to manage these animal genetic resources. In this study, we mined WGS datasets from about 500 individual pigs or groups of pigs (obtained from DNA-pools) belonging to 30 European and 14 Asian breeds, European and Asian wild boars and other species of the *Sus* genus (*Sus barbatus*, *S. cebifrons*, *S. celebensis* and *S. verrucosus*) closely related to *Sus scrofa*. Two thirds of the datasets were retrieved from the European Nucleotide Archive (ENA) or from previous projects and one-third was newly produced for this study and derived from Italian Large White, Italian Landrace and Italian Duroc pigs. Datasets retrieved from ENA were pre-filtered according to a minimum averaged depth of sequencing of 10× whereas the average depth of sequencing of the newly produced WGS datasets was about 23×. A total of 150 genes were selected according to their already established role in affecting relevant production traits (e.g. growth rate, lean meat and fat deposition, reproduction performances, taste preference, meat quality, including boar taint). Short reads from these genomes were first aligned using bowtie to a customized reference sequence generated from the reference pig genome, including sequence of the selected genes. Variant calling was performed with samtools software. The Ensembl Variant Effect Predictor tool was used to inspect the consequences of the identified variants and potentially deleterious mutations were detected with SIFT. About 2.3% of the detected variants were in coding regions and included a total of 340 missense mutations and other variants with predicted functional effects (e.g. stop codon gains/losses, frameshift mutations). Substantial differences in allele frequencies and allele distributions were observed for these putative relevant variants between European and Asian pig breeds and across the other *Sus* species. This study provided a landscape genome picture of variants that might explain part of the genetic variability of important production traits in pigs.

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Genetic approach for white marking knowledge in Maremmano horse

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The Maremmano is an autochthonous Italian horse breed, fixed in type despite a long history of cross-breeding, probably descendent from the native horses of the Etruscans. The wild and difficult environment of Maremma shaped this animal which became the most important Italian native saddle horse having a good appearance and excellent conformation. The coat is bay or black, the chestnut coat is admitted in females; furthermore, an excessive extension of white markings on the face and limbs it's not desired. Particularly, the excessive white marking on the head and legs is badly valued by breeders. In horse base coat colour is primarily determined by polymorphisms at the Melanocortin-1 Receptor (*MC1R*) and Agouti Signaling Protein (*ASIP*) loci, creating a black, bay or chestnut coat. Likewise, MC1R influences the extent of white marks more than other mutations at different loci. The different arrangements for the 2 most common alleles at the MC1R (E/e) and ASIP (A/a) loci are interesting for the extent of white marks. At this purpose, 1032 (799 bay, 211 black and 22 chestnut) horses, enrolled in the Studbook of National Horse Breeders Association of Maremmano Breed (ANAM), were evaluated for the extension of the white marks (limited/widespread) and genotyped at the two loci to reduce the presence of horses with white markings.

The Hardy-Weinberg equilibrium at loci ASIP and MC1R, tested by 'chi-squared', showed that they are not in equilibrium ($p \leq 0.001$) and this was probably due to the fact that ANAM applied a phenotypic selection against chestnut coat. A pedigree file, including 17,478 horses (10,222 females and 7256 males) registered in the Studbook, was run to set up a single trait BLUP Animal Model to estimate variance components for the trait *white marks extension* considering as fixed effects the only four statistically significative ($p \leq 0.05$) combinations of the two alleles at the two loci (BLUPF90).

The statistical significance of the fixed effects (sex, coat colours, genotype at locus A, genotype at locus E, the nine possible combinations of the two alleles at the two loci and their interactions) was estimated with a general linear model performed by R Software (2022). The *white marks extension* heritability was high and equal to 0.53 ± 0.07. These results encourage to take into consideration the actual genotyping at the ASIP and MC1R loci, currently used only against chestnut coat, also to limit the extension of the white marks badly valued by breeders.

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A first insight into miRNA expression of colostrum in Piedmontese cattle breed

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Colostrum is the first secreted product of the mammary gland immediately after delivery. In addition to having a nutritional role, it is essential to protect the calf in the first weeks of life. Immunoglobulins mainly perform this defensive function, but colostrum is also rich in microRNA (miRNA), in particular in miRNA that act mainly on genes involved in the regulation of the immune system and in the intestinal development of the calf. Therefore, miRNAs from colostrum and milk are thought to be messengers, produced by the mother for the new-borns, that play important functions in regulating the calves' development. To date, studies have mainly focused on milk miRNA of dairy cattle breeds and their effect on humans, while their role in calf is little studied. The present project (still under development) aims to analyse miRNA expression in colostrum of the Piedmontese cattle breed in the first days after calving. A deeper knowledge of miRNA expression patterns in colostrum would allow for a better understanding of the origin, regulation and function of miRNAs in mother-calf communication and to verify whether it is possible to promote the health of calves and improve their neonatal development by optimizing the uptake of miRNAs. All the Piedmontese brood cows of the teaching farm of the Department of Veterinary Science of the University of Turin giving birth for at least their second calf are involved in the project. Colostrum is collected at about 7 different time points depending on the delivery time (soon after delivery, and after 6, 12, 18, 24, 30, 36, 48 and/or 72 h). At this stage of the project, miRNAs were extracted from the colostrum collected at four time points of the first three cows involved in the project. The Maxwell® RSC Instrument using the Maxwell® RSC miRNA Blood Kit was used. The quantity and quality of miRNA extractions were tested with the Small RNA kit of the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) for the following NGS analysis. Library were prepared using the TruSeq® Small RNA Library Prep Kit and were run on the MiSeq instrument (Illumina, San Diego, CA, USA). Sequencing data were uploaded to the Galaxy web platform (usegalaxy.org) and bioinformatics analysis are undergoing using the MiRDeep2 tools for the identification of the miRNAs expressed in the colostrum at the different time points. Differences between time points will also be checked and statistically analysed.

P249

A genome wide association study for diarrhea resistance in pre-weaned rabbits identified markers useful to breed for increased animal welfare

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Animal welfare and reduction of antimicrobial agents are gaining more and more importance in defining sustainable livestock production systems. Adopting novel breeding and selection strategies that directly or indirectly address these aspects can also improve efficiency and farmers' profitability. In commercial rabbitries, diarrhea of newborn rabbits represents one of the major sources of economic losses. Genetic resistance against diarrhea can be dissected using genomic approaches. In this study, we carried out a case-control genome-wide association study for identifying genomic regions affecting the sensitivity to pre-weaning diarrhea in a commercial rabbit population. Animals included in the study were from litters produced by crossing 7 bucks and 45 does. A total of 331 newborn rabbits (149 healthy and 182 with diarrhetic) were selected from litters presenting at least one case and one control animal. Genotyping of the selected rabbits was carried out with the Affymetrix Axiom OrcunSNP Array, which analyse a total of 199,692 single nucleotide polymorphisms (SNPs). PLINK v.1.9 was used for quality checks and data filtering whereas association was carried out with GEMMA v.0.98 via linear mixed models. A main peak of association was identified on rabbit chromosome 12. Results were further validated by genotyping the associated DNA markers in additional cases and controls from another cohort of the same rabbit population. Fine mapping of the region based on whole genome resequencing data obtained from a few cases and controls identified a few candidate causative mutations in genes involved in basic immunological functions. The genomic information here obtained will be useful for implementing a marker assisted selection program aimed at improving resistance against pre-weaning diarrhea and improving animal welfare and the sustainability of the rabbit production system.

P282

Genomic inbreeding distribution in Italian dairy goat farms

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Managing the value of inbreeding within dairy goat farms is becoming a crucial point in light of the increased use of artificial insemination especially in northern Italy. In particular, thanks to the increasing amount of genomic data made available by the Italian Sheep and Goat Breeders Association through its projects, the calculation of the genomic inbreeding could represent the most effective monitoring tool at our disposal.

The purpose of our work is to provide an overview of the current situation of the average level of genomic inbreeding in Camosciata delle Alpi (CAM) and Saanen (SAA) goat farms all along the Italian peninsula.

We analyzed the genomic inbreeding coefficient (F_{ROH}) of 1949 individuals of CAM and 668 of SAA belonging to 70 different farms genotyped with the 50k SNPchip within the CHEESR project. We found an average F_{ROH} value of 0.056 (±0.03) for CAM individuals and 0.063 (±0.03) for SAA individuals considering all the Peninsula. Data at our disposal came from 13 different Italian regions, mostly from two regions in the north of the country, Lombardy (mean F_{ROH}: 0.05) and Piedmont (mean F_{ROH}: 0.05). The highest average inbreeding values were observed Calabria (0.08 ± 0.03) and Valle d'Aosta (0.08 ± 0.06) for CAM and Calabria (0.07 ± 0.03) and Veneto (0.07 ± 0.04) for SAA. At the farm level, we observed an average F_{ROH} value of 0.056 (± 0.01) for the CAM and 0.06 (± 0.01) for the SAA considering only farms with at least 10 genotyped individuals. Using a hierarchical Anova model (Region, Farm|Region) we observed significant differences among farms within the region ($p < 0.001$) for both the breeds and a difference between the values of the single regions only for CAM ($p < 0.001$) with maximum values of 0.10 and 0.11 for CAM and SAA respectively.

Our results underline a highly significant difference between farms' values as well as the low average inbreeding levels of the two populations. This suggests that, as the genomic data increases, it will be possible to use the F_{ROH} as useful information for breeders to monitor the trend of inbreeding within their herds. We hope that with the new projects currently underway we could have a greater amount of genomic data to outline a more precise situation of the inbreeding distribution per farm.

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P303

Toward a new version of medium-density Buffalo SNPchip array for Mediterranean breed

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The water buffalo (*Bubalus bubalis*) is a large bovid used, since ancient times, for human needs. Today it is bred in many countries across the globe. There are two distinct species of water buffalo: river (from western Asia to Europe) and swamp (more common in eastern Asia). In Italy, river buffalo have been present since the Roman period and today Italian Mediterranean is bred for milk production, used to produce 'mozzarella di Bufala campana'.

To study buffalo genomic variability, a first SNP array (Axiom Buffalo Genotyping – ThermoFisher Scientific) containing 90k SNP was developed by the International Buffalo Genome Consortium, covering the water buffalo (river and swamp) genome-wide diversity. However, to improve milk quantity and quality in the Italian Mediterranean breed, also through Genomic Selection, a new and breed-specific version of the Axiom Buffalo array was developed.

The first step was to validate the already existing SNPs Axiom Buffalo array in the Mediterranean breed. All the probes were mapped to the most recent Mediterranean buffalo reference sequence, removing the ones with low mapping quality or without mapping. After that, a quality control of the probes was performed using around 700 Mediterranean animals. Probes with low call rate, monomorphic or with not optimal clustering were removed. The remaining probes were evaluated: SNPs previously identified as important (e.g. present in IMAGE array, already associated with milk characteristics, etc.) were prioritised. In almost all the cases only one probe was kept. Finally, around 85k probes (and 76k SNPs) over 123k available sports were retained.

The second step consisted in filling the new array gaps using WGS Mediterranean data. The sequences were aligned and variant called, using state-of-the-art bioinformatic pipelines. SNPs associated with functional genes were added (for example, casein gene cluster), together with SNPs in the Y chromosome (which was not previously included), and in the regions with lower density, compared with the expected. The final array density reached around 35 SNPs/Mbp. In conclusion, the new version of the buffalo array has 25% more SNPs than the first released, with a very high usage rate in the Mediterranean breed.

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P349

Using udder traits in a selection index for udder health in Italian Mediterranean Buffaloes

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Since 2018, after the introduction of IBMI, the breeding objectives of the Italian Mediterranean Buffalo population have changed, focusing on milk production, mozzarella yield and functionality. Udder conformation (UC) traits play an essential role in both mastitis resistance and milking ability but they are not included in the current selection index despite their importance for health and longevity. We, therefore, propose an aggregate selection index (ASI) considering both the economic importance and the genetic parameters of milk production traits, $MSCS_{150}$ (geometric mean of somatic cell score at 150 days in milk) and the UC traits (fore udder attachment– FUA; rear udder width– RUW; rear legs height– RLH; teat direction – TD; teat length – TL and teat position – TP). The ASI was developed to decrease $MSCS_{150}$ while maintaining the milk production level, hence considering SCS_{150} and MY as breeding objectives and UC traits as additional selection criteria. We first estimated the genetic (co) variances between the UC, MY, and $MSCS_{150}$ traits, then estimated the relative weight for each UC trait. We used a multi-trait animal model with phenotypic records from 15,275 females and a pedigree with 43,395 animals. The CG (herd-year-calving season), the month of calving and the parity were considered fixed effects, and the animal was a random effect. Heritability for MY and $MSCS_{150}$ were 0.391 and 0.134, respectively, and varied between 0.11 to 0.23 for UC traits. Low negative genetic correlations were observed between MY and RLH (−0.119), while the correlations between $MSCS_{150}$ and RLH (−0.350) and $MSCS_{150}$ and FUA (−0.220) were moderately negative. A positive correlation was founded between FUA and MY (0.4272) and as well MY and RUW (0.645). The relative importance of each selection criteria depends on the genetic links between this and the breeding objective. The RUW, RLH, and TD are the most important UC traits to select for reduced $MSCS_{150}$ while maintaining milk production. Using ASI, the expected genetic gain per year for $MSCS_{150}$ and MY is −0.03 and 0.26 kg, respectively; it is also possible to reduce $MSCS_{150}$ by −0.32 and increase MY by 2.57 kg in ten years of selection.

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P19

Genetics of udder volume and maternal ability in Italian beef cattle

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Selection in beef cattle has been focused mainly on productive traits; however, reproductive, and functional traits have gained interest in recent years. Calving ease, calf's protection and milk yield have been shown to be an important trait for calf survival and its subsequent growth, particularly in beef cattle reared in open pasture and loose system. Traits related to the udder and milk production can influence calf growth and weaning weight being milk the main source of calves' feeding, playing therefore a great influence on farmer's income in beef cattle contest.

This study aimed to estimate genetic parameters for udder volume (UV), maternal ability (MP) and a combined trait obtained by summing the two traits (UV + MP) in Marchigiana (M), Chianina (C), Romagnola (R), Maremmana (MM) and Podolica (P) beef cattle. Traits were evaluated on cows through linear type score of UV, evaluated as distance of the bottom part of the udder and the hocks, and by interview to farmers on MP produced. Both traits were evaluated on a 1–5 scale system, i.e. from very small to abundant volume for UV, and from scarce to abundant in interviews on farmers for MP. Records were obtained within the I-BEEF project financed by the Ministry of Agriculture through the EU rural development funds from 2017 and 2021, and involved 13,738 cows, including 4585 M, 4336 C, 964 R, 947 MM, and 2951 P.

A GLM of SAS allowed the identification of 3 main non-genetic factors affecting the scores. These factors were identified in the herd-year classifier within breed, the calving distance (i.e. interval from calving to type trait evaluation), and the age at calving by parity (classified in 1° parity, 2°, 3° and other) at animal evaluation classes. These factors were all accounted in the subsequent across-breed genetic analysis carried out through single and bi- trait animal model-REML, accounting for 79,487 animals in the pedigree file. Heritability estimates were 0.09 for UV, 0.06 for MP, and 0.12 UV + MP, that agreed or lightly lower with estimates obtained in other beef and dual-purpose cattle breeds reported in literature. The genetic correlation between UV e MP traits was 0.88. The investigated traits were chosen to be included in new estimated breeding values for Italian beef cattle breeds.

P360

The muzzle measurement: a new phenotype in three Italian beef cattle breeds

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Nowadays, efficient breeding and genetic improvement of livestock, looking at sustainable farming, are required for facing the increasing worldwide request of meat. The use of new biometric markers to assess food conversion efficiency and suitability to extensive animal husbandry systems can be useful to achieve progressive development on this matter. Specifically, the present work takes place in synergy with the National Association of Italian Beef Cattle Breeders (ANABIC) within the I-BEEF 2 project. At this purpose, the muzzle circumference and width were checked as biometric predictor for productive and efficiency traits in young bulls of three Italian beef cattle breeds (Chianina, CHI; Marchigiana, MAR; and Romagnola, ROM). During performance test, 105 CHI, 103 MAR and 96 ROM were measured twice, at the beginning (7 months of age) and at the end (12 months of age) of the test. The muzzle circumference was taken by a metric tape and the width through an orchidometer. The four measures: initial muzzle circumference (IMC), initial muzzle width (IMW), final muzzle circumference (FMC), final muzzle width (FMW) were correlated (Pearson) with: muscularity (MSC), average daily gain (ADG), weight at the beginning of performance test (IW) and 365 days (FW). As expected, IMC showed high correlations with IMW and FMC (0.69 and 0.73, $p < 0.0001$, respectively) therefore, only one measurement, either in trait and time, could be sufficient to describe the trait. About the three different breeds, in CHI and MAR were observed not significative correlations between IMW, FW and ADG; moreover, in CHI was also observed a correlation between FMW and ADG (0.37, $p < 0.0001$) and in MAR a correlation between FMC, MSC and FW (0.37 and 0.39, $p < 0.0001$). Rather interesting results were finally obtained in ROM breed, where IMC and IMW showed a correlation of 0.47 and 0.41, respectively, with FW ($p < 0.0001$); a correlation between IMC and ADG (0.40, $p < 0.0001$) was also observed. These preliminary results suggest the possibility to currently consider, mainly for the Romagnola breed, that the value of the IMC and/or IMW are an effective morphometric predictors of the future weight and therefore can be used as new phenotypic traits for selective breeding strategies.

P373

Frequencies of CD4 and CD5 flow cytometric phenotypes in Podolica and Maremmana breeds

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One of the most important leukocyte subpopulations is T helper/inducer lymphocytes, characterized by the surface expression of CD4, which is involved in response to peptides presented by class II MHC. Another surface protein CD5 is expressed on T and B1 lymphocytes and has important functions in TCR- and BCR-mediated signal transduction, respectively. The monoclonal antibodies (mAbs) CC26 and CC17 recognize polymorphic epitopes on bovine CD4 and CD5 antigens, respectively. This study aimed to evaluate the frequency of reactivities with CC26 and CC17 in the Podolica and Maremmana breeds of cattle. Blood samples from a total of 86 Podolica (47 bulls and 39 cows) and 67 Maremmana (25 bulls and 42 cows) animals were examined using an indirect two-colour flow cytometric assay. Our results showed that CD4 T cells from 4.7% (6.3% bulls and 2.3% cows) and 34.3% (44.0% bulls and 28.6 cows) of Podolica and Maremmana cattle, respectively, had the CC26-negative phenotype. This phenotype has been found in both Friesian and Bos indicus cattle, and our results also show its existence in these two Italian beef breeds. In addition, 4.6% of Podolica cattle had the CC17-negative (CD5) phenotype. To date, this phenotype has never been found in Bos taurus cattle, whereas only limited in Bos indicus cattle of the Boran breed. Interestingly, the four CC17-negative animals were females from two neighboring herds in the Crotone province (Calabria region, southern Italy), with a high inbreeding rate. Our results open up interesting new possibilities for studies using these two Italian breeds. Future molecular analyses will identify SNPs that alter the epitopes recognized by these two mAbs. *In vitro* stimulation studies will help to highlight the possible effects of these modifications on T lymphocyte effector responses. In addition, the correlation with these animals' genetic indices and/or clinical data will need to be evaluated. Finally, our study demonstrates how flow cytometry can be validly employed for characterizing protein polymorphism, for which monoclonal antibodies are available.

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P378

Sustainable animal breeding in a local cattle breed: a genomic strategy to redefine Reggiana Herd Book standards and breeding goals

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Reggiana is an Italian autochthonous cattle breed, mainly reared in the Emilia Romagna region. At present, a total of about 2800 Reggiana cows, distributed in about 100 farms, are registered to its breed Herd Book. Nowadays, almost all the milk produced by the Reggiana breed is processed into mono-breed Protected Designation of Origin (PDO) Parmigiano-Reggiano cheese that is labelled with the brand name '*Vacche Rosse*' (according to the typical red coat colour – *fromentino* – of the breed). This cheese is marketed at a higher price than those of undifferentiated origin. Phenotypic selection in the Reggiana breed has been applied over the last decades to maintain the breed standard defined in the Herd Book (e.g. solid *fromentino* coat colour, pink or pale muzzle, medium-tall stature, dual-purpose conformation, absence of morphological defects), but a few Reggiana animals do not completely match the breed standard phenotypes. In this study, we defined a strategy to design a sustainable breeding and conservation programme of the Reggiana population, by considering both phenotypic and genetic data. About 70% of the whole Reggiana breed population was genotyped with the GeneSeek GGP Bovine 150k SNP chip. The allele and genotype frequencies and distribution of single nucleotide polymorphism (SNP) markers involved or associated with phenotypic exterior (coat colour and muzzle), morphological traits (e.g. stature and morphological defects), and the presence of deleterious alleles derived from other cattle breeds were analysed. Based on obtained results, information on the genotype at the *Extension locus/melanocortin 1 receptor* (*MC1R*) gene has been included in the Reggiana Herd Book as part of the breed standard. Other DNA markers could be included or could be used to cull some animals carrying unwanted alleles/defects or to design appropriate strategies for their effective eradication from the population. These strategies might be integrated in optimum contribution selection plans that would also carefully evaluate the potential loss of genetic variability and effective population size of the breed. This strategy can be useful to design further refine a genomic-driven sustainable breeding and conservation program of Reggiana cattle that is also linked to the genetic authentication of its mono-breed Parmigiano Reggiano cheese.

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P385

The genetic background of lactation persistency in the Italian Holstein breed

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Improving lactation persistency (LP) might be an effective alternative to maintain the efficiency of milk production without negatively impacting the energy balance, fertility and other health issues, especially in the first period of lactation of dairy cows. Nevertheless, little is still known on LP, mainly due to the standard breeding system for dairy cows that relies on 305-day lactation not allowing them to express their persistence to the fullest. Thus, the purpose of this study was to model lactation curve over time from 1035 Italian Holstein dairy cattle reared in two different herds and to perform Genome-wide Association Studies (GWAS) for unraveling the genetic background behind peak yield (PY), peak time (PT) and persistency (PER).

Test day records ($N = 12,993$) were collected in a two-year period which allowed to model the lactation curve of 1035 cows following the Wood equation producing PY, PT and PER traits. All the dairy cows were genotyped with several different genotype panels and further imputed to ~84k preselected SNPs. The GWAS was performed using the StatGenGWAS in R where a covariance matrix was computed following the EMMA algorithm, including parity, season of calving and farm factors as well as the genomic relationship matrix. Then a Generalized Least Squares method was used to estimate marker effects and the corresponding p-values [suggestive threshold of $-\log_{10}(p\text{-value})$ equals to 4]. The ARS-UCD1.2 genomic coordinates of the region containing suggestive significant SNPs (±50 Mbp) were used to retrieve candidate gene lists and annotations from Biomart in Ensembl release 94.

Several genomic regions and candidate genes were identified for PER on BTA3, BTA9, BTA14, and BTA15.

For PT, four genomic regions were identified, distributed across BTA6, BTA7, BTA17 and BTA18. Finally, for PY, four regions on BTA1, BTA4, BTA13 and BTA15 were found to be significantly associated. A total of 111 genes were located within those significant regions, highlighting the polygenic nature of those traits. Our preliminary findings could help to further characterize the molecular mechanisms behind the LP traits and might additionally corroborate the importance of introducing that information into novel genomic indexes or dedicated SNP-chips customized for persistence genotyping.

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RNA-seq study on Longissimus thoracis muscle of Italian Large White pigs fed extruded linseed with or without antioxidants and polyphenol

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The addition of omega-3 polyunsaturated fatty acids (PUFAs) in the pig diet increases their content in muscle cells, and the additional supplementation of antioxidants promotes their oxidative stability. However, to date, the functionality of these components within muscle tissue is not well understood. Consequently, our aim was to find the differences in gene expression between the different conditions and investigate how potentially differentially expressed genes (DEGs) were implicated in the cellular composition and metabolism of muscle tissue of 48 Italian Large White pigs under different dietary conditions. Here, we make use of RNA-seq data from a publicly available study in which all the involved pigs were slaughtered at the same live weight of approximately 150 kg. The pigs were divided into four groups of 12 subjects each. The diets supplied to the animals during the growing-finishing period were a basal control diet (D1), the same diet enriched with extruded linseed as the source of omega-3 polyunsaturated fatty acids (D2), a diet equal to D2 but supplemented with selenium and vitamin E (D3), and, finally a diet equal to D2 but supplemented with natural polyphenols obtained from grape skin and oregano extracts (D4). To analyze the data, we constructed a DEseq2-centered pipeline and we obtained (all with $p$adj <0.1) 22 DEGs for D3 vs. D1. Functional enrichment analysis with Cytoscape revealed that diet D3 compared to D1 promoted a more rapid and massive immune system response, likely because of a possible improvement in the overall muscle tissue functionality. For D4 vs. D1, we obtained 12 DEGs, of which some we associate with increased anti-inflammatory potential of the muscular tissue, possibly promoting the oxidative stability of omega-3 PUFAs. The obtained results are consistent with the use of selenium and vitamin E or natural polyphenols in diets enriched with omega-3 PUFAs to increase both the content and oxidative stability of omega-3 fatty acids. This type of PUFA possibly provides the cells with greater membrane fluidity and anti-inflammatory potential, important requirements for maintaining cellular physiology. We conclude that supplementing pig diets with omega-3 and antioxidants holds the potential to improve pork nutritional quality, promoting consumers' health.

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P396

Chromosomal instability in bovine induced by environmental pollution

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During the last five years, a special project concerning the monitoring of environmental pollution by using cytogenetic investigation in Romanian bovine has been developed. In this context, our study has had two targets: (1) the identification and quantification of mutagenic agents present in the environment and, especially, in animal feed, in relation to the maximum permitted limits of Romanian and European law in force; (2) the assessment of the pollutants effects on the genetic material integrity of farm animals like cattle and buffaloes.

A cytogenetic investigation was carried out for 300 heads (237 cattle and 63 buffaloes) reared in different farms from all over the country. Our study revealed chromosomal instability for 40 animals (29 cattle and 11 buffaloes) represented by a large number of mono-and bi-chromatidic breakages on autosomes and heterosomes, loss of chromosome fragments and gaps. Our investigation continued through SCEs-test, which is a specific test for identifying the effects of toxic agents on the genetic material integrity. For animals with many chromosomal breakages the number of sister chromatid exchanges (SCEs) was very high for cattle (8–16 SCEs/cell) and buffaloes (10–17 SCEs/cell) compared to the normal animals. Chemical analyses were performed on feed samples used for cattle and buffaloes. Also, the assessment of different metabolic profile parameters has been done.

KEYWORDS: Cattle; buffaloes; chromosomal instability; environmental pollution

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Breeding for fitness and health in mountains and plains: functional traits in local dual purpose Alpine cattle

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The worth of local breeds (LB) has recently started to be highlighted after that a selection only focused on increasing production has caused a severe decline in functionality and robustness in specialized cosmopolitan breeds. The lower productive ability of LB has been strongly balanced by great environmental adaptability, longevity, fertility, disease resistance and lower management costs. LB selection indexes are usually based on productive traits only because accounting for functionality has not been necessary so far. However, genetic progress requires attention to this aspect also in LB. This study aimed to estimate genetic parameters for functional traits in Italian cattle breeds native of the Alps or the close plain: Aosta Red Pied, Aosta Black Pied-Chestnut, Rendena, Alpine Grey, Reggiana. The traits analysed were somatic cells (somatic cell score, SCS), fertility (FERT: parity-conception interval; calving interval), and longevity (LONG: herd life, functional longevity, number of parities), in addition to milk yield. SCS heritability (h^2) varied from 0.07 to 0.13, whereas

h^2 for FERT varied from 0.01 to 0.05. LONG showed an average h^2 of 0.09. Various genetic correlations (r) were found among trait pairs in different breeds. For SCS-FERT r varied from zero to 0.1, for SCS-LONG from -0.4 to 0.10, and it was low-negative for FERT-LONG. SCS-milk and LONG-milk r largely varied from negative to positive values, whereas r for FERT-milk was positive (0.3 on average), suggesting a worsening of fertility following the increase of milk yield. This variability in results could be explained by the selective pressures occurred for the different production aptitudes over the years in the studied breeds (milk; dual-purpose, with different importance for milk and meat; triple-purpose including fighting ability for Aosta Black Pied-Chestnut). Genetic trends also greatly varied among breeds: SCS showed a variation from close to null to positive, FERT from negative to positive, whereas LONG generally showed a positive trend. It should be noticed that positive variations for SCS and FERT mean a trait detriment. Results highlight the importance of considering functional traits also in LB, due to the complex framework of genetic correlation among them including also antagonistic relationships with production. Genetic trends also suggest that selection indexes should account for functionality to preserve the valuable characteristics of LB effectively.

P408

Genome-wide association study between single nucleotide polymorphisms of swine genome with carcass and processed ham quality traits: a preliminary study

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Genome-wide association studies (GWAS) are widely used as a tool to find candidate regions associated with traits of interest for animal breeding and production. Traits such as meat color formation, thigh weight, lean meat content, and adsorbed salt are scarcely investigated because are expensive and difficult to measure. In this research, a preliminary study was performed to identify regions potentially related to carcass and ham quality traits. Genomic information was obtained from 238 commercial hybrid pigs utilizing the GeneSeek® Genomic Profiler genome-wide porcine genotyping array. Carcasses were tested for their hot weight (HCW), backfat and loin thickness, and lean meat percentage (LMPC). The corresponding fresh hams were assayed for weight and ultimate pH, and for the activities of Cathepsin B and Ferrochelatase (FECHA) in the *Semimembranosus* muscle. The lean meat percentage of fresh ham (LMPH), salt absorbed after first (SALT1) and overall salting stages (SALT) were estimated online by the Ham Inspector™ apparatus. Hams were processed in compliance with the procedures established for Protected Designation of Origin Parma ham, and processing weight losses were measured at the main processing stages. HCW showed a significant negative correlation with LMPC and LMPH, while LMPH was correlated positively with LMPC, SALT1, SALT, and weight losses. Twelve SNPs were significantly associated with FECHA. In particular, marker ASGA0004152 (rs81216562) was located in an exon of Ferrochelatase gene (*FECH*), expressing the enzyme Ferrochelatase which is one of the key factors involved in the development of meat color. The results obtained in this preliminary study were achieved by combining innovative and non-destructive technologies for screening hams under processing, measures of enzymatic muscle properties relevant to dry-cured ham quality, and genomic information obtained through a GWAS. Additional studies carried out in a larger number of pigs have been planned to investigate the effect of *FECH* variants on the quality of the dry-cured ham with the main reference to color development and to confirm the GWAS results obtained in this preliminary study.

Acknowledgements

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P412

Use of longitudinal data in genomic management of dairy cattle herds

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The precision livestock farming tools based on sensors, measuring a wide number of phenotypes, make available an impressive amount of longitudinal data not yet fully exploited in selection and mating plans in dairy cattle. The Automatic Milking Systems (AMS) delivers daily a large number of information on each cow's milk production, coupled with the milk somatic cell count (MSCC), rumination and cow activity. The possibility to associate this large number of data with the genomic information that can be obtained at low cost on all females of a herd, opens a wide plethora of opportunities for a genomic precision management approach to dairy cattle herds. The GENORIP project collected longitudinal data from AMS and genotyped all females of 7 herds for more than 5000 individuals with the GGP 100K Bovine SNP chip. Genotypes, milk yields and environmental factors were analysed by an AI machine learning model to estimate the lactation curve in a phenomics approach on more than 500 cows from one herd with AMS. A sensitivity analysis including various environmental predictors showed the value of the approach used and the usefulness to fine tuning the prediction including environmental descriptors. The Pearson correlation between estimated and true lactation was, in the best prediction, 0.85.

The results obtained in predicting the lactation curves allow estimating the future production of calves, managing the herd reproduction according to the expected revenues.

Discussion with farmers provided insights to further develop the exploitation of the approach in managing large herds.

The longitudinal data from AMS coupled with genomic information on all females of a herd represents a new frontier in exploiting added values from SNP chip data in addition to the GEBV estimation.

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P443

Genetic evaluation for profitability in Italian Mediterranea Buffalo

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Italian Mediterranean Buffaloes, in Italy, are selected for milk, fat, protein, mozzarella yield, limb conformation, and mammary apparatus. Buffaloes could be registered in one of the official herdbooks, RIS Bufala and ANASB, both focused on selection and genetic improvement of Mediterranean Buffaloes. Ris Bufala introduced the IPday scoring system, which quantifies the profitability of a buffalo per day of life by estimation of incoming money and outgoing expenses, considering average milk market price, feeding costs, and age at first calving, with penalties for intercalving exceeding 450 days and for age at first calving exceeding 37 months. Collaboration between RIS bufala and SYNERGY has led to changes in data collection and management which paves the way for an update of data and for corrections of any inaccuracies at any time. IPday has been developed using a dataset containing 21,873 lactations recorded from 41,687 buffaloes, 611,653 pedigree records, and 40,162 results of parentage verification. A pipeline has been created in R, has been used in order to identify data, that were incomplete or incompatible with the statistical model. BLUP Animal model method was used to estimate genetic breeding values of animals. Genetic additive components and breeding value estimation are performed by Blupf90 software package, using, in the statistical model, a random animal additive effect, a fixed herd effect and a random error component. A total number of 663,228 breeding values have been estimated using a heritability of 0.27. We performed three consecutive runs of genetic evaluation to assess the stability of the system, truncating the data at the end of 2019, 2020, and 2021. Finally, trend comparison and correlation analysis were conducted between different runs. The results of stability analyses demonstrate a correlation of 95% between consecutive yearly runs. The study results show the potential of using IPday for genetic evaluation for profitability in Italian Mediterranea Buffalo.

P518

Differentially expressed miRNAs in the stool of Bos indicus divergent for feed efficiency

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The fecal microbiota is an emerging field of research in cattle, being an essential component of the gut microbiota and host metabolism. Recent evidence indicates that small RNAs, like microRNAs (miRNAs), may be isolated from feces and can be involved in host-microbial interactions. In this context, transcriptomic analysis of the stool has the potential to reveal the linkages between host-miRNAs and microbiome gene expression, which in turn is expected to influence production traits. For this study, stool samples were collected from the rectal ampulla of 16 Nelore bulls divergent for Residual Feed Intake (RFI) phenotype. Total RNA extraction was performed using Trizol reagent, and sequencing of miRNA was performed using the Illumina Hiseq 2500 platform, with a throughput of 8,000,000 paired-end reads per sample. Reads were trimmed and low-quality sequences were removed. Host reads were mapped to the *Bos taurus* genome (ARS-UCD1.2) with STAR software. Differential expression (DE) analyses were performed using DESeq2 software and analyzed to predict potential regulatory target genes with Targetscan and DIANA miRPath v3.021 softwares. Functional enrichment of target genes was performed with WEB-based Gene seT AnaLysis Toolkit. In total, 163 expressed miRNAs were identified, being seven miRNAs more expressed in the efficient group; bta-miR-126 ($p$adj =0.0019), bta-miR-30a ($p$adj =0.0042), bta-miR-196a ($p$adj =0.0164), bta-miR-205 ($p$adj =0.0401), bta-miR-27b ($p$adj =0.0517), bta-miR-143 ($p$adj =0.0965), and bta-miR-155 ($p$adj =0.0942). Target genes from bta-miR-143 were enriched for PI3K-Akt signaling pathway and target genes from bta-mir-27b were enriched for Type II diabetes mellitus, Insulin resistance, TNF, and Insulin signaling pathway. Previous studies also identified these pathways related to RFI in a Nelore population. Altogether, these results point to miRNAs identified from the stool as potential regulators of feed efficiency, which may provide the knowledge to develop future strategies to manipulate the microbiome.

P519

Microbial diversity in the stool of Bos indicus divergent for feed efficiency

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Microbiome research is gaining attention in livestock species, as it assists in understanding host biological processes under the prism of symbiotic microorganisms. Feed efficiency is a livestock production trait with economic and environmental impacts, and there is increasing evidence that the gut microbiota plays a vital role in its regulation, suggesting that the modulation of an animal's microbiota composition can promote more sustainable and efficient livestock production. Next generation sequencing studies have used 16S rRNA sequencing to describe the microbiota composition, stating that metabarcoding can offer new opportunities to use microbial composition to assess feed efficiency. For this study, stool samples were collected from the rectal ampulla of 16 Nelore bulls divergent for Residual Feed Intake (RFI) value. DNA extraction was performed using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (ZYMO Research Corp), using 150 mg of stool. PCR target amplification for bacterial 16S rRNA was performed using designed primers and amplicons were sequenced in an Illumina Hiseq platform (2 × 250 bp) using the Illumina V3 sequencing kit. After sequencing, raw reads were filtered for quality (>Q25) and trimmed at positions 220 (F) and 175 (R) using QIIME 2 version 2018.8. The filtered data was submitted to the DADA2 package to generate amplicon sequence variants (ASVs) and bacterial sequences were annotated using the SILVA database version 132. The resulting ASV table was used to determine alpha diversity (number of ASVs and the Shannon–Wiener index) with QIIME2. We identified a total of 5006 bacterial ASVs in the Nelore bulls' microbiomes. The most prominent bacterial phyla identified in the both groups were Firmicutes and Proteobacteria. Comparison of samples from different groups using alpha diversity metrics (Chao I index and Shannon index) revealed no significant difference ($p > 0.10$) in the richness of bacteria populations between efficient and inefficient groups. Nonetheless, Pearson correlation analysis between the Shannon index and RFI showed significant association ($p < 0.10$), indicating a relationship between feed efficiency and microbial diversity.

P534

RNA-seq profiling of milk somatic cells in four cattle breeds reared in different management systems

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The lactation cycle is a continuous process where several developmental and physiological changes occur in the mammary gland in particular changes in its functionality, milk yield and milk composition, and mammary epithelial cells (MECs). MECs are involved in the synthesis and secretion of milk, and in the immune response. The principal aim of the present study was to profile the transcriptome of bovine milk somatic cells (BMSCs) in four cattle breeds, some of which not previously investigated: Holstein (HO), Simmental (SM), and Simmental × Holstein (SM × HO) reared in Monterotondo (Italy) in an intensive production system, under the same management and feeding conditions; Podolica (POD), a local breed, reared on a farm located in South of Italy in an extensive management system based on grazing. Total RNA was extracted from BMSCs at days 60 and 120 after calving with the kit TripleXtractor direct RNA (GRiSP Research Solutions, Portugal); RNA quality and quantity were checked with spectrophotometric and microfluidic inspections (RNA 6000 Nano chip, 2100 Bioanalyzer). A total of 60 samples with RIN >7 were sequenced by an external service using the NovaSeq platform (150 bp paired-end). Reads and bases with low quality were removed using Trimmomatic software v 0.36. Cleaned reads were aligned to the bovine reference ARS-UCD v1.2, using STAR software v2.6.1d, and assembled with StringTie, to obtain the count matrix. Normalization of the count matrix and identification of differentially expressed genes (DEGs) of pairwise contrast between lactation stages and breeds were obtained using the R package DESeq2. Results showed that genes encoding for the most abundant protein synthesized during lactation (*CSN1S1, CSN2, CSN1S2, PAEP, CSN3, LALBA*) and implicated in different steps of fatty acids biosynthesis (*FASN, SCD, XHD* and *PLIN2*) were among the top 25 highly expressed genes in all samples. The milk protein and fatty acids biosynthesis genes were mostly expressed at 120 than at 60 days and were expressed at lower level in POD than in the other breeds. Moreover, some differences among the top 25 highly expressed genes were also found between the three breeds reared in the intensive production system. Preliminary results showed that the highest number of DEG between 60 and 120 days was found in SM × HO (677) and at 60 days in the comparison of HO with POD (285).

P592

Genome-wide association studies for serological response to three infectious diseases in Merino of EA GROUP and Fleischschaf

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The presence of diseases in extensive sheep herds implies a decrease in the profitability of the farms, since it reduces the productive levels of sheep and lambs. Furthermore, it generates important expenses in medication and veterinarians that, in short, will reduce the already reduced economic benefit of farmers. For this reason, the selection of animals with genes related to resistance to diseases becomes a fundamental tool to clean up farms from not only a health, but also an economic point of view. The objective of this study was to identify markers associated with resistance to three infectious diseases of both health and economic interest such as those caused by Brucella spp, Chlamidya abortus and Coxiella burnetii. Blood samples from Merino of EA GROUP (1580) and Fleischschaf (552) sheep breeds reared in 16 extensive farms in the South of Spain were genotyped using the GGP Ovine 50K chip (GeneSeek Genomic Profiler) in Neogen (Lansing, MI, USA). SNP genotype quality control was performed using PLINK v 1.9. Markers with a call rate of less than 0.9, minor allele frequency (MAF) lower than 0.05, mapping to sex chromosomes or yielding significantly deviated from HWE genotypes ($p < 0.001$) were filtered out. Individuals with a call rate below 95% were also excluded. 49604 SNPs and 2132 animals were retained to perform subsequent analyses. The phenotypes used in the GWAS were the presence or absence of antibodies for the aforementioned three pathogens. The association analyses were performed with the Genome-wide Efficient Mixed-Model Association (GEMMA) v 0.98.1 package. The P-values obtained for each association were corrected for multiple testing by using the False Discovery Rate (FDR) method. Associations with a q-value below 0.05 were considered as significant. Manhattan plots were built with the ‘qqman’ R package. Nine SNP showed significant genome-wide associations with Coxiella burnetii and seven SNP with Chlamidia abortus in Merino of EA GROUP, while no SNP were significantly associated with Brucella spp. Contrary, 12 SNP resulted significantly associated with Brucella spp in Fleischschaf sheep. Close to these SNP, several genes implicated in the fetal-embryonic development are located, for example IFT52 or GTSF1. Study financed by the CDTI project ‘Selección asistida por marcadores genéticos de resistencia a enfermedades para el ovino extensivo, GENXLAMB’.

P594

A first overview on genome-wide diversity of Italian donkey populations

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Donkeys are widely reared animals, with more than 150 breeds worldwide. In spite of their vital economic importance, especially in developing countries, donkeys have received less attention than other livestock species. Analysis of genomic data is increasingly becoming part of the livestock sector and is an invaluable tool for the effective management of breeding programs in small populations, providing background information concerning genome structure in domestic animals. A scientific working group has recently been established to join efforts and resources for the genomic characterization of local Italian donkey populations. The main objective is to investigate the genomic structure of these important animal genetic resources, in order to provide information on their current conservation status which in turn will foster the management of the genetic variability. Biological samples from about 200 animals (20–30 per population) belonging to nine Italian donkey populations (Amiata, Asinara, Grigio Siciliano, Martina Franca, Pantesco, Ragusano, Romagnolo, Sardo and Viterbese) were collected for the analyses. Considered the absence of a specific beadchip array, we used double-digest restriction site associated DNA (ddRAD) sequencing to obtain approximately 50,000 single nucleotide polymorphisms (SNPs), and to overcome the limitations of previous studies based on microsatellite markers. The dataset will be analyzed to study several aspects of genetic diversity, such as relationships and gene flow, admixture and level of genomic inbreeding. In addition, a comparative analysis of conserved haplotypes will be conducted to identify selection signatures related to physiological processes of adaptation, resistance and milk production. The analysis of the data will pinpoint the genetic distinctiveness among Italian donkey populations. Moreover, the obtained results will contribute to a better characterization of their history and genetic structure.

P595

Preliminary assessment of the wool quality parameters in the Gentile di Puglia merino sheep breed

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A wool quality assessment was conducted in the Gentile di Puglia sheep breed after a period of over three decades. The study took place in Apulia, Italy, on three farms that had registered herds. Wool samples were collected from individual animals, with a total of 100 samples obtained from each farm. The weight of the entire fleece was measured (referred to as TW), and the diameter (FD) of both greasy and scoured wool was assessed using the Fibrelux instrument. The results indicated that the TW varied between 1.54 ± 0.53 kg and 3.44 ± 0.79 kg for animals aged 2 and 3 years, respectively. The average diameter of the wool was concentrated around 20 microns, ranging from 11.3 to 28.1 microns (with a standard deviation of 3.35). In greasy wool, the diameter ranged from 14.82 to 31.48 microns (with a standard deviation of 3.18). The average FD for greasy wool was 23.99 ± 3.184 microns, compared to 20.78 ± 3.37 microns for washed wool. The average washing weight yield was 0.56%, which is relatively low when considering a range between 63% and 67%. This suggests that there is significant room for improvement in animal management practices. The average FD for greasy wool varied among animals aged 2, 3, and 4 years, with values of 22.38 ± 1.71, 23.35 ± 2.75, and 23.18 ± 2.61 microns, respectively. The variation coefficient for these measurements was 5.87 ± 6.79, 8.69 ± 9.88, and 8.383 ± 9.03. These findings will be instrumental in initiating a selection plan for wool improvement in the Gentile di Puglia sheep breed. The study was partially funded by the European Union Next-Generation EU (PNRR) – MISSIONE 4–2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022, with support from the Agritech National Research Center. It is important to note that the manuscript solely reflects the views and opinions of the authors, and the European Union cannot be held responsible for them and partially funded by WOLLY regional research project.

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Tenebrio molitor as a valuable tool for valorisation of agro-industrial by-products in the circular bioeconomy perspective

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The continuous human population growth causes an ever-increasing protein requirement (both for food and feed). To fill this ‘protein gap’, humankind needs to produce new foods and feeds from new sources, as well as valorise agro-industrial waste and by-products (FLW). These strategies can have some help from the use of insects as new bio-farms. Among insects, *Tenebrio molitor* (TM) is a good solution, because it is one of the edible insects that can represent an alternative source of proteins, showing a nutritional profile that compares favourably with meat. Furthermore, TM was the first insect approved by the European Commission for human consumption in 2021. The valorisation of FLW by using this insect as a bio-convertor to produce proteins and other useful nutrients and compounds make TM perfectly positioned for the circular bioeconomy. At the ENEA Trisaia Research Centre, Bioproducts and Bioprocesses Laboratory, we are carrying on the rearing of TM and we have tuned a protocol, based on similar ones available in literature, for obtaining flour to use in food and feed. Specifically, in our experimental trials TM larvae (TMLs) were reared on a standard bran-based diet and then fasted for 2 days, washed, blanched (to increase the shelf-life of the resulting flour), and dried in a ventilated oven at 60 °C for 24 h. Subsequently, the TMLs were milled with a beater. The resulting powder was analysed by Kjeldhal to determine protein content and by Aqualab instrument to determine water activity at 24.6 °C. Flours we produced in the tests showed a protein content of 40% in the powder obtained by TML fed 95% bran and 5% zootechnical yeast, which is in line with values reported in the literature. The water activity in the powder, being 0.374, makes it suitable for storage for a long time without getting mouldy or losing organoleptic characteristics. We are applying this protocol to larvae reared on different diets to test how the composition of the diet influences the protein content of the resulting flour.

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Hemolymph oxidative stress profile of black soldier fly larvae reared on four different substrates

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This study aimed to provide novel information about the hydroperoxide levels and the antioxidant capacity present in the hemolymph of *Hermetia illucens* (black soldier fly, BSF) larvae fed on different diets. Five-days-old *Hermetia illucens* larvae were raised on: (1) broiler feed (CON, control diet), (2) cauliflower waste diet (CAU), (3) apple waste diet (APP), and (4) carrot waste diet (CARR). The larvae were placed in plastic containers (60 x 40 x 15 cm) with a feeding rate of 0.1 g/d of substrate per each larva, considering a growing period of 20 days. The substrates were placed in plastic containers one day before the deposition of the larvae; each container was a replicate and each substrate had 9 replicates. Hemolymph samples were collected from twenty larvae per replicate. Data were analyzed by a one-way ANOVA, using the GLM procedure of SAS (2002) and considering the substrate as main effect. The experimental unit was the replicate. The antioxidant barrier was significantly different among the groups, in fact the concentration of hydroperoxides was higher in the control than in the carrot group (175.1 vs. 131.6 U CARR, respectively, $p < 0.01$) thus demonstrating that the use of carrot as growing substrate, led to a significant reduction in reactive oxygen species (ROS) production in BSF larvae. Concerning the biological antioxidant potential (BAP), it showed differences among the groups, in particular the highest ($p < 0.01$) value was in the cauliflower (4795.5 µmol/L) and the lowest in the control group (4145.2 µmol/L), thus suggesting that the cauliflower-based diet also increased the antioxidant barrier. In general, oxidative stress can be defined as a disturbance in the balance between the production of reactive oxygen species and antioxidant defenses. In insects, ROS are involved in the regulation of various mechanisms and intercellular signaling and act as bactericidal agents. They can also induce cellular senescence, apoptosis, and cell growth regulatory pathways and are involved in immunity; also, in response to nutrient stress, cells enter autophagy, which can lead to adaptation or death. Further studies are needed to explore the mechanisms by which vegetable waste can act as a ROS limiting factor in *Hermetia illucens* larvae, this could be very interesting to determine for how long the larvae can be fed with a specific diet.

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Growth performances of Black Soldier Fly larvae fed on food chain by-products

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The circular economy approach is increasingly relevant for companies, and insects farming match with this business model as the larvae grow on agro-food wastes. In 2021, the European Commission issued Regulation 2021/1372, allowing the use of insect protein meal as feed for some farmed animals. The most common insect currently reared for these purposes is *Hermetia illucens* (Black Soldier Fly), which when processed in its larval stage (BSFL) offers a protein-rich meal.

In the frame of the Italian Ministry of Health 'Ricerca Corrente 2019' programme (Project IZSLT 11/19), this study aimed at investigating the suitability of wine and oil industry by-product as growing media for BSFL and on their crude protein (CP) and fat (EE) content.

Six-days old BSFL were grown for 21 days on olive leaves (OL), olive pomace (OP) or grape pomace (GP). All the substrates were dried and milled at 1 mm before use. The larvae (93) were seeded at 1.5 BSFL/cm^2 in plastic larvaria (in triple), and the whole experiment was replicated twice. The growing substrate was provided twice a week at a feeding rate of 12 mg DM/larvae. The dry substrates were hydrated just before use at the respective water retention capacity.

The BSFL survivorship was not affected by the growing media (94.5–98.0%), but the daily weight gain was higher ($p < 0.01$) in BSFL reared on GP (40 mg/day) in comparison with OP (13 mg/day) or OL (3 mg/day). Therefore, the estimated feed conversion ratio was higher in OL (265.3) fed BSFL ($p < 0.05$) in comparison with OP and GP (41.9 and 15.7, respectively). The CP content on DM was higher ($p < 0.01$) in OL and GP reared BSFL (47.4% and 49.3%) if compared to OP (40.3%) ones. On the contrary, the EE content of BSFL was higher by using OP as growing media (31.3% on DM) in comparison to GP (18.6% DM) ($p < 0.01$), that in turn was higher than in BSFL fed on OL (11.1% DM) ($p < 0.05$). These preliminary results suggest that OL is the worst growing media for BSFL, but OP and GP can exhibit promising growing performance, also in the light of modulating the CP/EE balance in BSFL according to the rearing purposes.

P375

Chemical composition and nutritive value of black soldier fly larvae reared on municipal bio-waste

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Different alternative feed ingredients, such as insect's meal, are considered potential substitutes for partial substitution of soybean meal in a scenario of continuous growth of animal husbandry. Among different insect species, *Hermetia illucens* (black soldier fly, BSF) has received particular attention since it can also be produced on bio-waste such as the organic fraction of municipal solid waste (OFMSW) which, in a circular economy perspective, allows a reduction of the costs of production and allows sustainable rearing. The aim of the present experiment was to evaluate the growth performance, the chemical composition and the nutritive value of BSF grown on OFMSW.

Larval growth was performed in a climatic chamber at constant conditions (25 ± 0.5 °C, 60 ± 0.5% RU, 12:12 h L/D) on samples of OFMSW divided in two parts: untreated (Control) and pulped (Pulp) obtained after a pulping process. Substrate and larvae samples were analysed for chemical composition; *in vitro* digestibility trials by an enzymatic method (pancreatic-pepsin digestion) were conducted to determine energy and protein digestibility of larvae grown on the two substrates. The average chemical composition of untreated and pulped OFMSW was (% DM): EE 14.1 vs 12.7, CP 14.9 vs 14.6, Ash 13.3 vs 17.6.

No statistical differences were observed between pulped and untreated substrates in terms of survival, developmental time and larval biomass production.

The BSF larvae grown on control had higher lipid content than those grown on pulp (34.4 *vs*. 28.5%). In comparison with other studies, larvae grown on OFMSW showed a lower content of protein and higher level of ash, probably due to rearing substrate chemical composition (e.g. high ash content). The values of *in vitro* protein digestibility coefficients were similar between the treatments (68.8 *vs*. 73.3%, respectively for Control and Pulp; $p = 0.073$), while the control group showed significantly higher levels of energy digestibility than the pulp group (82.1 *vs*. 61.9%; $p < 0.001$).

In conclusion, BSF meal obtained by larvae grown on OFMSW in a circular economy approach could be a potential ingredient of feed in poultry nutrition in partial substitution of soybean meal, even if it is necessary to standardize the substrate composition, the nutritional value and to evaluate the microbiological contamination of larvae produced. Moreover, a revision of European legislation should be performed since to date insect rearing on OFMSW is not allowed.

P517

Is under-vacuum packaging a suitable method for Hermetia illucens and Tenebrio molitor larvae storage?

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The use of insects in food and feed needs a robust knowledge of their nutritional properties, but also of the many aspects related to their preservation for maintaining, as long as possible, these features. This study aimed to evaluate the effect of non- or under-vacuum storage on the quality preservation of *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) dried mature larvae. These insects were reared on vegetable wastes (HI) and wheat bran (TM) in a lab-scale facility. Three samples (15 g each) of HI and TM dried larvae were taken to be analyzed, from non-vacuum and under-vacuum bags stored for 1, 3, and 6 months from drying (T1, T3, T6, respectively). Proximate composition, total lipids, fatty acid profile, primary (conjugated dienes, CD), and secondary (thiobarbituric acid reactive substances, TBARS) products of lipid oxidation were analyzed. In addition, biogenic amines analysis was carried out. Two different one-way ANOVAs were performed with R Core Team (2022) for each species to estimate the effect of the treatment and the effect of the storage time. The most interesting results, expressed on 100 g of dried sample, involved lipid oxidation metabolites and biogenic amine formation. In the first case, from a time perspective, HI prepupae from T6 had a significantly higher content of CD (1.98 mmol Hp/100 g) than T1 and T3 groups, whilst TM samples showed major content of TBARS in the T1 group (1.59 mg MDA/100 g). On the other hand, biogenic amine content was higher in HI under-vacuum than in the non-vacuum samples, especially cadaverine, histamine, and spermidine (119.0, 14.8, and 13.8 mg/100 g, respectively). Moreover, tyramine production has been detected notably in T3 HI prepupae (179.35 mg/100 g). About treatment, no significant difference was found in TM, neither in proximate composition nor in amine formation; however, time-wise, cadaverine was detected most in the T6 group (6.13 mg/100 g), while putrescine, tyramine, and spermidine were mostly present in the T3 samples (16.02, 20.5, and 10.68 mg/100 g, respectively). Hence, vacuum treatment did not seem to preserve the product quality of HI, nor significant advantages were identified for TM. Thus, further investigations are required to deepen insect storage to provide a clear picture of their safe use as food and feed ingredients.

P531

Effect of diet composition on growth and temperature development in black soldier fly (Hermetia illucens) larvae rearing

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Black soldier fly (BSF) larvae can use food by-products or residues as a substrate for growth, and these larvae can be used as a protein source for farmed animals. Therefore, their use in animal feeding is considered a strategic solution to improve the sustainability and efficiency of animal production. Insects must be farmed under strict environmental conditions: temperature, humidity, light, and aeration are important factors affecting larvae welfare and the efficiency of their bioconversion. Diets (i.e. substrates) should be formulated carefully since they have a direct effect on microclimatic conditions and larval development.

The goal of this trial was to test four different diets: control (poultry feed), vegetable (carrots, potatoes, and brewer's spent grain: 1:1:1), carnivorous (ground epiglottis and cod waste; 1:1), omnivorous (vegetable: carnivorous; 1:1) and assess their effect on larval growth performance, chemical composition, and substrate temperature. BSF Larvae (6-day-old) were randomly chosen and allocated to the 4 diets (4 plastic containers, i.e. replicates, per substrate, each measuring 32 x 23 x 12 cm, containing 2000 larvae and approximately 2 kg of substrate). The environmental conditions were kept at 27 ± 1 °C and 65 ± 5% RH, with photoperiod of 12L:12D. The temperature of each container was measured twice daily (morning and afternoon) for the entire experimental period (7 days) using a thermal imaging camera. Each container was aerated daily to ensure that the substrate was thoroughly moisturized and rotated.

The preliminary results showed that the carnivorous diet resulted in significantly lower growth performances (–30% larval biomass at the end of the trial compared to the average of the other three groups; $p < 0.01$). The chemical analysis revealed that these larvae had a significantly higher dry matter content (DM; about 44%) than the other groups ($p < 0.01$), implying a high larvae meal yield. The vegetarian diet had the lowest DM (about 22%); which was significantly lower than the other diets ($p < 0.01$).

In terms of substrate temperatures, the control diet showed considerably lower average temperatures ($p < 0.01$) compared to the omnivorous and vegetable diets. However, this did not seem to affect larvae growth in this substrate.

These preliminary findings showed an effect of the diets on substrate temperature and larvae growth; however, the chemical analyses currently in progress will provide further insights on larvae composition.

P38

Qualitative characteristics of mule's milk during lactation

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Despite their inability to reproduce naturally, mules can host embryos, gestate normally and can be surrogate dams and produce milk for the foal. To the best of our knowledge, there are no studies on the nutritional composition of mule milk. The aim of this preliminary investigation is to increase the knowledge on maternal attitude of the mules focusing on nutritional composition of mule's milk and its variations during the whole lactation period. Milk was obtained from a mule dam that had foaled after receiving a mule embryo transplant. The samplings were performed from 6 h after foaling to the 172 days in milk. For each sample, gross, mineral and fatty acids composition were evaluated. Lactation phase was grouped in three periods: early lactation (from 6 h to 15 DIM), mid lactation (from 16 DIM to 110 DIM) and late lactation (from111 to 172 DIM). Data were analysed by ANOVA considering the three periods of lactation as fixed effect. The results showed the following average mule milk composition: dry matter $10.84 \pm 1.591\,\mathrm{g}\ 100\,\mathrm{g}^{-1}$, protein $1.98 \pm 0.554\,\mathrm{g}\ 100\,\mathrm{g}^{-1}$, fat $0.90 \pm 0{,}850\,\mathrm{g}\ 100\,\mathrm{g}^{-1}$, ash $0.39 \pm 0.128\,\mathrm{g}\ 100\,\mathrm{g}^{-1}$, saturated fatty acids $50.00 \pm 4.526\,\mathrm{g}\ 100\,\mathrm{g}^{-1}$of fat, monounsaturated fatty acids $31.80 \pm 3.451\,\mathrm{g}\ 100\,\mathrm{g}^{-1}$of fat and polyunsaturated fatty acids $18.2 \pm 1.849\,\mathrm{g}\ 100\,\mathrm{g}^{-1}$of fat. Dry matter, protein and ash decreased significantly from early to late lactation while the fat content was significantly higher in the early lactation. No difference were highlighted for fatty acids composition that remained stable during the whole lactation. In conclusion, the results show that mule milk composition is similar to horse and donkey milk.

P98

Capillary electrophoresis milk protein profile as effect of CSN1S1 polymorphism and diet energy level in Derivata di Siria goats

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Twenty-seven Derivata di Siria lactating goats, nine homozygous for strong alleles (AA), nine heterozygous (AF) and nine homozygous for weak alleles (FF) at the *CSN1S1* locus, were used to evaluate the effect of genotype, diet and genotype × diet interaction on goat milk caseins. Goats were used in a 3 × 3 factorial arrangement of treatments, with three genotypes (AA, AF, FF) and three different energy levels. High, medium or low energy level (H, M, L) was achieved by supplying a complete pelleted feed containing 65% of alfalfa hay, respectively ad libitum, and at 100% and 70% of the total energy requirements according to INRA guidelines. The experiment consisted of three simultaneous 3 × 3 Latin squares for the three genotypes, with one square for each energy level. Each experimental period consisted of 15 d for adaptation and 8 d for data and sample collection. Milk yield was affected by genotype and diet ($P \leq 0.05$). The lower level was found in FF goats than in AA and AF genotypes (673.7 *vs* 934.5 and 879.8 d/g, respectively; $p < 0.005$) and in goats fed L diet energy level (651.5 *vs* 1042 and 852.8 g/d for H and M diet, respectively). Among caseins, only α_{s2}-casein concentration was lower ($p = 0.023$) in milk from goats fed L diet as compared to H and M diet (2.5 *vs* 2.7 and 2.8 g/kg, respectively). Genotype affected total and individual caseins concentrations ($p < 0.02$) except for κ-casein. Specifically, total casein and α_{s1}-casein concentration was higher in AA than in AF and FF goat milk (24.9 *vs* 20.3 and 19.8 g/kg; 7.2 *vs* 3.7 and 0.7 g/kg, respectively), while FF genotype showed higher values for α_{s2}-casein and β-casein concentrations compared to homozygous AA and heterozygous AF goats (3.1 *vs* 2.3 and 2.5 g/kg of AA and AF goats; 13.6 *vs* 12.4 and 12 g/kg of AA and AF). An interactive genotype × diet effect occurred for α_{s2}-casein concentration, greater in milk of FF goats fed H and M diet energy levels (3.2 and 3.5 *vs* 2.7 g/kg; $p = 0.019$), and for α_{s1}-casein yield

($p = 0.027$), which was higher in AA and AF goats, compared to FF goats, only when fed H diet energy level (8.6 and 4.4 *vs* 0.4 g/d, respectively). The reported results demonstrated that high energy input, as well as strong alleles at *CSN1S1* locus, improve milk production and casein concentration. Moreover, our data confirmed the existence of an interaction between genetic a_{s1}-casein polymorphism and diet that may impact on dairy goats performance.

P322

Effect of environmental factors on content and distribution of Ca, P and Mg in bovine milk

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In Italy, milk produced in the summer season is characterised by a decrease of the contents of lactose, proteins, casein and fat, and by worse physico-chemical properties. Nevertheless, less is known about the impact of seasons on milk minerals content and their distribution between the colloidal and the soluble phase. The aim of the research was to evaluate the influence of seasons (winter, W; spring Sp; summer, Su; autumn, A) on the mineral contents of milk, on their distribution between the colloidal and soluble phases, and their repercussions on the rennet coagulation properties of milk. Within the MIPAAF project INNOVALAT and AGER project FARM INN, a total of 48 bulk milk samples from 4 Italian Friesian cattle herds were collected throughout one year (1 sample per herd per month). Total contents and soluble contents of Ca, P and Mg were assessed in milk and in ultrafiltered whey, respectively. The colloidal contents of were calculated by the difference between total and soluble fractions. Furthermore, the rennet coagulation properties (RCP: r, k_{20}, a_{30}) were measured.

The milk produced in Su was characterised by the lowest content of total Ca (Su 115.28, W 119.02, Sp 119.28, A 118.80 mg/100g; $p \leq 0.05$), total P (Su 92.99, W 96.32, Sp 96.45, A 95.98 mg/100g; $p \leq 0.01$), total Mg (Su 9.85, W 10.22, Sp 10.22, A 10.20 mg/100g; $p \leq 0.05$) and colloidal P (Su 50.14, W 52.17, Sp 50.72, A 52.46 mg/100g; $p \leq 0.05$). The ultrafiltered whey of summer milk showed lowest concentration of soluble Ca (Su 26.35, W 32.53, Sp 31.82, A 30.42 mg/100g; $p \leq 0.001$) and soluble P (Su 40.82, W 44.99, Sp 44.79, A 43.51 mg/100g; $p \leq 0.001$). Also Colloidal P (Su 50.14, W 52.17, Sp 50.72, A 52.46 mg/100g; $p \leq 0.05$). The highest rennet coagulation time (Su 20.47, W 17.19, Sp 20.30, A 18.88 min.; $p \leq 0.001$) and curd firming time (Su 3.79, W 2.50, Sp 2.90, A 2.75 min.; $p \leq 0.05$), as well as the lowest curd firmness (Su 32.43, W 40.199, Sp 42.31, A 36.26 mm; $p \leq 0.05$) were recorded in summer milk. Finally, soluble Ca and P were positively correlated ($p \leq 0.01$) with rennet coagulation time ($r = 0.474$ and $r = 0.381$, respectively). Colloidal Ca was positively correlated with curd firmness ($r = 0.323$; $p \leq 0.05$). Changes in mineral content and distribution of milk were recorded throughout the seasons with repercussion on its RCP. In particular, the increase of soluble Ca and P that take place in summer milk contribute to the worsening of its rennet coagulation time.

P376

Quality comparison of Teramana goat milk with the Saanen breed

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In recent years, the livestock sector has increasingly focused on the selection and breeding of high-production animals, resulting in the progressive abandonment of certain native breeds in almost all of Italy. However, the last decade has seen a reversal of course aimed at recovering the genetic heritage of certain endangered breeds. This research aims at the recovery and enhancement of the endemic goat breed of the province of Teramo (Abruzzo), the goat ‘Teramana’, through the characterization of milk quality parameters, compared to a very widespread and highly productive species, the Saanen goat. Electrophoretic techniques (SDS-PAGE) were used for protein analysis, and the results were validated through immunoblotting (WB). For the analysis of fatty acids, after extraction, the profile was quantified through the use of a GC-FID. The study was conducted between February and June 2022. Forty-one individual samples of milk Teramana goat were collected from different companies in the Abruzzo region, a few kilometers from each other. Similarly, forty samples of Saanen goats were collected from the same farms or in the same area as the Teramana goats. Individual milk samples were collected and used to evaluate milk's chemical composition, fatty acid profiles and protein composition. The findings obtained showed the presence in ‘Teramana’ goat's milk of significative a higher content of β-casein and κ-casein and the same content of α-casein as in Saanen goat's milk. As a result of the higher content of β-casein and κ-casein, dairy trials have shown an increase in dairy yield compared to Saanen milk. The analysis of the acid profile showed significative a higher content of linoleic acid conjugates (CLA). In addition, Teramana's milk is characterized by a greater amount of monounsaturated fatty acids (MUFA) than Saanen, which has a higher content of saturated fatty acids (SFA). The results show that the different protein and fatty acid profiles of the milk of Teramana goats have higher quality characteristics than Saanen goats, increasing the quality and yield of the cheeses. These reasons would contribute to the recovery and the diffusion of these animals, obtaining superior productions from a qualitative point of view, with a consequent increase in the cost/benefit ratio.

P390

Effects of heat stress on Sarda dairy sheep performances in mid lactation

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Heat stress influences physiological, alimentary and productive responses. With high environmental temperatures, feed intake of sheep is reduced as well as digestibility and utilization efficiency. An increase of intake produces additional endogenous heat that would tend to increase exponentially with critical environmental conditions. For these reasons, the animal decreases the dry matter intake, reducing automatically the level of productivity and adopting a set of physiological responses in order to dissipate internal heat. Two of the most important markers of heat stress are the respiratory rate (RR) and the rectal temperature (RT) that show a strong variability with the worsening of environmental conditions. The aim of this work is to study the effects of heat stress on Sarda dairy sheep performances. Twenty-one Sarda dairy ewes (5th month of lactation) in the experimental barn of University of Sassari (Ottava, Italy) cooled with ventilation of 1 m/s, were divided into three groups balanced for dry matter intake (DMI =2.22 ± 0.05 kg/d), milk yield (MY =2.00 ± 0.03 kg/d), body weight (BW =53.9 ± 2.04 kg) and BCS (2.98 ± 0.08). From 29 May to 19 July 2022). One group (AVE) received chopped oat hay ad libitum (CP =7.3% and NDF =63.9%; DM basis); the second (MLQ) and third (MHQ) received chopped alfalfa hay of low quality (CP =19.7% and NDF =43.5%; DM basis) and high quality (CP =23.5% and NDF =39.0%; DM basis) ad libitum. DMI and MY has been monitored daily for 21 days and the two physiological parameters were recorded 3 times/d at 7.00, 13.00, 19.00 h. RR was measured with manual counts within videos of 1 min, whereas ocular globe (PT) and RT using infrared and digital thermometers, respectively. Statistical analyses were made with the software SAS (9.0) testing a mixed model for repeated measurements (fixed effects of THI and group and random effects of day and animal). DMI was not affected ($p > 0.05$) by THI (Kliber, 1964). MY showed a strong decrease with THI >76 (−11.2% compared to THI =71–72; $p < 0.05$) w/out differences among groups. RR and RT were statistically influenced by THI ($p < 0.0001$). RT can be estimated from PT (non-invasive) as RT (°C) = 0.698*PT (°C) + 11.909 ($R^2 = 0.68$). The study underlines the relevance of heat stress on depressing milk yield even without effects on animal intake and in cooled barns. Additional focus need to be oriented on the wind speed effect on animal performance in hot environment.

P445

Effect of the feeding system on the growth performance of Holstein-Friesian calves in the pre-weaning period

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Twenty-four months is the ideal age for the first calving of a heifer since it has been proved to be the most advantageous age for the lifetime productivity of the animal and the farm economical profit. To achieve this goal, calves must follow adequate diets. Aim of this study was to assess whether different types of milk administration (manual or automatic) can influence the growth performance of Holstein-Friesian female calves from birth to weaning.

The study was carried out in two farms located in Turin province (NW Italy) and involved a total of 54 calves: 27 calves were fed the milk *ad libitum* with an automatic feeder (farm A) and 27 calves were fed the milk manually with a teat bucket in two daily meals (farm B). The vaccination program, milk replacer and feeding plan were the same for both farms. At birth, each calf was given 4L of colostrum (Brix score >22%). From the third day of life, starter feed and fresh and clean water were provided *ad libitum*. The calves were individually weighed at birth and then every 10 days up to 70 days of life (age of weaning). An independent samples Student's t test was used to compare the growth performance of the calves between farms. A Functional Data Analysis was used to fit the growth curves of each calf and the average growth curve of the calves for each farm.

The calves reared in farm B had a significantly higher average body weight at birth than those reared in farm A (37.85 *vs* 33.33 kg; $p < 0.01$), most probably as the consequence of the utilization of a close-up dry period feeding regime (administration of a diet with a higher energy content) during the last 20 days of pregnancy in farm B, not performed in farm A. However, from the tenth day of life, the calves reared in farm A showed statistically higher body weight when compared to the calves reared in farm B ($p < 0.01$). The average daily gain of the calves from birth to weaning was significantly higher in farm A when compared to farm B (0.87 *vs* 0.67 kg/head/d; $p < 0.01$). This could be the consequence of the automatic milk administration, as it is known that small quantities of milk provided in many meals daily promote daily weight gain and animal health. Different staff involved in calf management, farm environment, type of calf housing and basic herd genetics may also have played a role and further studies are therefore necessary to confirm the results obtained in this trial.

P539

Biomolecular investigation for Mycobacterium avium subspecies paratuberculosis in raw and pasteurized milk

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Recent research has shown how some bulk tanks contain raw milk contaminated with Mycobacterium avium subsp. Paratuberculosis (MAP).

Considered the correlation between MAP and Crohn's disease, this would represent a human health problem, for this reason control plans have already been activated in several States (precautionary principle).

The aim of this work is to verify the survival of MAP to the milk pasteurization process.

To verify the survival of MAP in raw and pasteurized milk, investigation was carried out using q-PCR in both matrices that provides information on the presence of the pathogen's DNA, but not on its survival in the thermal process.

To test the viability of the MAP, cultures were set up and were periodically subjected to reading and to q-PCR.

Most of the milk consumed and transformed undergoes heat treatments aimed at the total or partial elimination of any pathogens present.

Raw milk represents a food with unique nutritional characteristics, for this reason its consumption is increase in last years; however, milk that does not undergo heat treatments can be a source of zoonosis.

MAP is excreted in milk produced by infected cows; its high resistance to heat treatments characterizes this microorganism and the purpose of this work is to demonstrate its survival to the pasteurization process.

At the end of this work, in parallel with microbiological-cultural tests, we can be deduced that MAP can be survive a pasteurization process carried out with the same modalities developed in the experimentation. Survival does not influenced by the initial concentration of the inoculated pathogen since the presence is confirmed in all the prepared dilutions.

P210

Nutritional Profile of Donkey meat as affected by muscle and aging time

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Donkey represent a ‘sustainable’ source of high-quality meat because of its high nutritional value, mainly due to the large use of pasture in rearing system. The aim of the present study was to evaluate the effect of muscle and aging time (1, 6, and 14 days) on fatty acids profile, amino acids composition and cholesterol content of donkey meat. *Longissimus Dorsi* (LD), *Semimebranosus* (SM), and *Rectus Femoris* (RF), were sampled from each left carcass of 12 donkeys, each muscle was divided into three sections, vacuum packaged, and stored at 2 °C for different aging time. LD muscle showed higher percentage of monounsaturated fatty acids (MUFA; $p < 0.001$) and lower content of polyunsaturated fatty acids (PUFA; $p < 0.05$) compared to RF and SM with the highest value of oleic acid ($p < 0.01$) and the lowest value of n6 ($p < 0.05$). As consequence LD muscle showed lower polyunsaturated/saturated fatty acids (PUFA/SFA; $p < 0.05$) compared to the other muscles. A significant effect of aging time was found, from 1 to 14 days. During aging time donkey meat showed an increase of SFA ($p < 0.01$), due to an increase of stearic acid (C18:0, $p < 0.01$) and a decrease of PUFA decreased ($p < 0.05$), with the lowest values of n6 ($p < 0.05$) and of n3 ($p < 0.05$) polyunsaturated fatty acids at 14 days of aging. No significant differences on cholesterol content as affected by muscle and aging was found. Refers to aminoacids composition, *Longissimus dorsi* muscle showed higher values of aspartate, ($p < 0.05$), methionine ($p < 0.01$), isoleucine ($p < 0.05$), lysine ($p < 0.05$) essential amino acids (EAA; $p < 0.05$), non-essential amino acids ($p < 0.05$) and total amino acids ($p < 0.05$) compared to RF and SM muscles. The results highlight that, regardless from muscles, donkey meat, being particularly rich in PUFA and EAA, could represent a healthy alternative to traditionally consumed red meat. A different aging method could be used in donkey meat to preserve the high PUFA content.

P330

Effects of pig dietary omega6/omega3 ratio on fatty acid profiles and nutritional indices of lipid depots

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Pork products show an unfavourable n-6/n-3 polyunsaturated fatty acid (PUFA) ratio. Also due to their high level in saturated fatty acids (SFA), pork consumption has been associated with an increased risk of chronic diseases. Enrichment of pig diets with n-3 PUFA is seen as a sound strategy to increase their intake in human diets.

Thus, this study investigated the effects of gender and dietary n-6/n-3 PUFA ratio (PR) on the fatty acid (FA) composition and nutritional indices of lipids in subcutaneous (SF) and perirenal (PF) adipose tissues (ATs) and in the *longissimus dorsi* muscle (LD) of pigs. To this aim 24 pigs, balanced for gender and weight, were assigned either to the control (C) or low PR (LPR) group. The C pigs received a barley/soybean-basal diet, while in the LPR group 5% of barley was replaced with extruded linseed, as a source rich in n-3 PUFA. Dietary PR was 9.7 and 1.4 for C and LPR group respectively. The diets were administered for 104 days, from an initial starting live body weight (LBW) of 80 kg and till slaughter at 151 kg LBW. After slaughter, LD samples were analyzed before and after cooking for malondialdehyde content. Further, the FA composition and nutritional indices such as unsaturation (UI), peroxidizability (PI), hypercholesterolemic (HI), hypocholesterolemic (hI), health-promoting (HPI), nutritive value (NVI), atherogenic (AI) and thrombogenic (TI) indices were determined in the LD and both ATs.

LPR group showed overall higher values in PUFA content, in particular n-3 PUFA level, due mainly to α-linolenic acid (ALA) and a lower n-6/n-3 PUFA ratio in LT and in both ATs. However, the higher level of unsaturation did not influence the oxidative stability of both raw and cooked meat.

In the LD, LPR group led to a decrease of TI without affecting the other nutritional indices, whereas the gender exerted no effect. Instead, in both ATs these indices were greatly modified in the LPR group and, PI apart, were all improved. Gender exerted a moderately significant effect, showing better values in PF of barrows. It is concluded that feeding linseed to pigs at 5% level in the diet to reduce PR did not have an adverse effect on the oxidative stability of meat while it contributed to the increase of n-3 fatty acids levels in all tissues examined, improving most nutritional indices, especially in ATs, and this could bring about a positive effect on human health.

P502

Use of microalgae oil in lamb nutrition and its implications for meat quality

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Microalgae are photoautotroph unicellular or multicellular microorganisms and can be used as feed source for ruminants. To our knowledge the applications are still limited and few studies are available on the effects of microalgae on meat quality. After weaning period (30 days of age), 20 Gentile di Puglia male lambs were randomly divided into two groups: C (control diet) and T (test-diet including one microalgae oil capsule of 560 mg/day). The proximate composition of diets was determined. Growth and slaughter performance were evaluated until slaughter (72 days of age). After an overnight fast, lambs were electrically stunned, exsanguinated and processed at a local slaughterhouse. Hot and cold carcass weights were recorded and dressing percentages calculated after dressing and chilling at 2–4 °C for 24 h. Then, carcasses were dissected into commercial cuts. The rib cuts were broken down into fractions tissue. A sample of the *Longissimus dorsi* (LD) muscle was subjected to physico-chemical analysis (pH, colour, texture and fatty acids profile). Statistical analysis was performed using PROC MIXED model in SAS, and significance set at $p < 0.05$. Final live weight was higher in T group compared to C diet (18.91 vs. 16.99 kg; $p < 0.01$). Carcass traits, percentage of the main cuts and proportion of different fractions tissue were not affected by diet ($p > 0.05$), except for perineal fat, which was higher in T lambs than in C group (1.48 vs. 1.17; $p < 0.05$). Diet affected the yellowness (b*) index of the LD muscle that was higher ($p < 0.01$) in meat from T group compared to C; while, the dietary treatment did not affect ($p > 0.05$) pH, tenderness and cooking loss. As for meat fatty acid composition, only linolenic (C18:3n-3) and docosahexaenoic (DHA, C22:6n-3) acids were found higher ($p < 0.05$ and $p < 0.01$, respectively) in T lambs compared to C diet. In conclusion, the results of this feeding trial show that microalgae oil may be successfully included in lamb diet because of it supported growth traits of animals with a favourable fatty acid profile, with a positive effect on nutritional properties of lamb meat.

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P25

The Prima Omega Rabbit project: preliminary results on the impact of dietary linseed on phytoestrogens metabolism in rabbit bucks

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The Omega Rabbit project aims to develop a new quality food product 'Ωrabbit meat' by assessing the impact of different n-3 fatty acids source (flax or algae) on rabbit metabolism and meat quality. Flaxseed is a rich source of phytoestrogens, whose metabolites (enterodiol-END and enterolactone-ENL) can affect estrogenic functions. Thirty New Zealand White rabbit bucks were equally divided into two experimental groups and fed different diets for 110 d (50 d adaptation +60 d experimental periods): control (CNT), standard diet; FLAX, standard diet +10% flaxseed. Semen was (0–60 d) collected weekly, whereas blood serum every 15 d. Phytoestrogen (lignans, isoflavones and their metabolites) of sperm and blood serum, and the distribution of α and β-estrogen receptors (ER) on sperm were analysed by GC-MS and immune fluorescence techniques, respectively. A *t*-test was applied for the comparison of GC data.

Results demonstrated that isoflavones (i.e. daidzein and glycitein) in sperm were higher in FLAX than CNT group until 14 d of experimental period (15-fold) and decreased up to 60 d; whereas lignans (i.e. matairesinol and laricilresinol) showed higher values during the whole trial (2-fold, $p < 0.05$). Phytoestrogen metabolites (equol-EQ, END, ENL) showed an inverse trend respect to the precursors, and they did not exhibit significant differences between dietary groups. On the contrary, serum END and ENL showed higher levels in FLAX group (2-fold, $p < 0.05$), while EQ value was lower (1.5–2-fold, $p < 0.05$) than CNT. No significant differences were recorded for both isoflavones and lignans on serum. Sperm of FLAX group showed higher expression of β-ER than CNT but not for the α-ER; furthermore, a different distribution on sperm surface was also recorded: β-ER were mainly located in the head, while α-ER in the midpiece. In conclusion, flaxseed administration widely affected the phytoestrogen metabolism of rabbit bucks, mainly due to the modulation of precursors/metabolites. Furthermore, the distribution and activation of β-ER of FLAX group sperm denoted a strictly connection between the different phytoestrogens provided and their metabolic activity.

Acknowledgements

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P461

Characterization, virulence, and antibiotic sensitivity pattern of Escherichia coli isolated from chicks in Egypt

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This work was carried out on 200 broiler chicks collected from different Egyptian governorates to characterize *Escherichia coli* (*E. coli*) isolates and detect the virulence and the antibiotic sensitivity pattern of the isolated strains. Examined chicks were suffered from general signs, diarrhea, high mortalities, septicemic picture, and unabsorbed yolk sacs. Samples were collected from liver, heart, and yolk sac from each bird and subjected for bacteriological examination. Colonial morphology and microscopic, biochemical, and serological identifications were done for characterization of *E. coli* isolates. The virulence of the *E. coli* strains was tested using haemolytic activity and Congo red binding assay method. Moreover, *E. coli* strains were tested against different antibiotics using disc diffusion method. The results showed isolation of *E. coli* with an incidence rated of 42.5% (85/200). The colonial morphology and biochemical identification showed typical characters of *E. coli*. Serological identification revealed detection of 85 sero-groups of *E. coli* that represented 60 identified O sero-groups and 25 un-typable ones. The results of blood haemolysis indicated that out of 60 strains of *E. coli*, 35 (58.3%) showed α haemolysis, 10 (16.7%) showed β haemolysis, and 15 (25%) were non haemolytic. Congo red assay indicated that all tested strains of *E. coli* were positive. The antibiotic sensitivity pattern show that *E. coli* strains showed sensitivity (100%) to erythromycin, oxytetracycline, and colistin, but resistance (100%) to ampicillin, chloramphenicol, and flumequine.

P545

Does an insect-based feed formulation in laying hens affect the antioxidant potential and fatty acid profile of the eggs?

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Since eggs are one of the main dietary lipid sources, lipid composition of eggs has become an area of current scientific interest. It has been shown that hens are able to deposit dietary lipids in to eggs yolk, so that is possible to modify the fatty acid composition of an eggs by modifing diet.

The aim of this study was to investigate the effect of dietary substitution of soybean meal with partially defatted meal of *H. illucens* on yolk fatty acid composition and oxidative status of eggs production.

The trial lasted 56 days and involved 108 laying hens (Lohmann Brown) 18-week-old. They were divided into 27 groups (4 hens each) and randomly assigned (nine replicates of four hens each) to one of the three experimental diets isoenergetic, iso-proteic and balanced for amino acids: the fraction of soybean meal was replaced by *Hermetia illucens* meal at 50% (T50%) or 100% (T100%) and at 0% in the Control group.

Eggs were sampled at week 1 (W1), 4 (W4), 8 (W8) and evaluated for fatty acid composition and antioxidant capacity.

Fatty acid analysis was carried out on yolk with a gas-chromatograph on FAME following an extraction with chloroform/methanol and the fatty acid methyl esterification.

Antioxidant capacity was measured on yolk and albumen ethanol extracts spectrophotometrically. Radical probes ABTS and DPPH were employed along with the FRAP method. Results were expressed as mmol of Trolox equivalent.

The antioxidant capacity of eggs showed no differences between treated and control groups

Kruskal–Wallis non-parametric test was applied to test for differences between controls and treatments. Significance is confirmed with *p*-value <0.05. The test was performed after the verification of homoskedasticity (variance equality between groups). A post hoc test with Dunnett's correction was then applied to highlight the differences in detail

The differences in the following 7 fatty acids were significant: C12:0 ($p = 0.005$); C14:0- ($p = 0.002$); C14:1 ($p = 0.002$); C15:0 ($p = 0.005$); C17:0 ($p = 0.005$); C17:1 ($p = 0.026$); C18:4n-3 ($p = 0.004$).

For the following parameters: C12:0; C17:0; C17:1; C18:4n-3 the differences were significant only between T100% and Control but not between T50% and Control. For parameters C14:0; C14:1 and C15:0 there were significant differences both between T100% and Control and between T50% and Control.

The integration of insect meal in the diet did not negatively impact on fatty acid composition.

P311

Evaluation of fiber digestibility in mares during the peripartum

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Horses are herbivorous and hindgut fermenters adapted to eat a fiber-based diet. The adequate digestion of fiber in the hindgut is crucial to satisfy the energy requirements of the horse as well as to reduce particle retention in the intestine – which represents a risk of large colon impaction. Therefore, the aim of this study was to evaluate the fiber digestion (expressed as undigestible neutral detergent fiber content of feces, uNDF) in mares during the *peripartum* period. Eleven healthy Standardbred mares (565 ± 40 kg) were enrolled in the study, and they were monitored from the admission at the Veterinary Teaching Hospital of the University of Bologna (15 ± 6 d before parturition) until discharge (6 ± 2 d after parturition). The daily ration (3 meals) was based on meadow hay (2% of BW) and concentrates (1.6 kg *prepartum*, 2.6 kg *postpartum*). Feed intake (forage and concentrate) was daily recorded and feces were collected 3 d before parturition (T-3), just after parturition (T0), 3 d *postpartum* (T3), 6 d *postpartum* (T6). Feeds and feces were analyzed for chemical composition. Statistical analysis was assessed using a mixed model procedure (fix effect the time, random effect each mare). Results showed variation in fiber fractions in the *peripartum*. The uNDF content (%DM) was at T-3: 47.59 ± 2.4^{AB}; at T0: 46.93 ± 3.08^{B}; at T3: 49.35 ± 2.01^{A}; at T6: 49.22 ± 1.89^{A}, $p < 0.05$. The lowest digestibility was recorded on the day of parturition and increased in the following days (+3%). This result could be related to an increase in gut motility due to hormonal changes and parturition, characterized by uterine and abdominal contractions and increase in catecholamines. After parturition, an increase in fiber digestibility was recorded, probably due to an enhancement of fermentation processes carried by the increase in requirements due to lactation. Moreover, the increase in concentrates in the *postpartum* did not have negative effects on fiber digestibility, confirming that the quantity of starch was adequate (<1 g/meal/day) and the feed quality was high. Indeed, an adequate proportion between rapid fermentable carbohydrates and fiber is well known and associated with an adequate rate of degradation. In conclusion, it could be speculated that fiber digestion in mares is not only related to the quality of the delivered feeds but is also driven by physiological status. Further studies must be done to elucidate the mechanisms that lead to this phase.

P319

Evaluation of objective measurement of coat colors in horses

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During domestication, thanks also to human selection, horses have developed different coat colors. Horse coat color are classified from dark to light as black, dark bay, bay, chestnut, gray and white. This phenotypic trait is often used for breed differentiation indeed horse studbook normally allowed specific coat colors only. Traditionally, evaluation of coat color is performed by trained judges, but this method is sensitive to human error. Nowadays, new technologies such as colorimeter are available to characterize horses coat color. Thanks to those tools we can detect accurately all the shades and the differences between colors. The aim of this study is to evaluate coat color detected with an objective measurement, in three different Italian horse breeds, Bardigiano, Murgese and Tolfetano. In Bardigiano and Tolfetano, all the colors from black to bay are allowed in the studbook, instead for Murgese, only black or roan horses can be included. A total of 272 horses were analyzed with a colorimeter (Minolta Chromameter Reflectance CR-300). Four anatomic areas have been tested with this device; neck, shoulder, stifle to detect the coat color, and lip to identify skin color. Outcomes were in L* a* and b* format, where L* describes lightness, a* describes colour saturation from red to green, and b* describes colour saturation from yellow to blue. From those values, hue [arctan(b*/a*)] that describes real colour, and chroma $(a^{*2}+b^{*2})^{0.5}$ that describes saturation, were obtained. A total of 6 different coat colours were detected by colorimeter in Bardigiano horse (4 shades of bay and 2 of black), 2 for Tolfetano (bay and black), and 2 for Murgese (black and roan). A high correlation between neck and shoulder ($r = 0.70$) and between stifle and shoulder ($r = 0.66$) was found. Principal component analysis was carried on to detect whether breeds differ to each other, based on colorimeter data. From the PCA, the differentiation between Murgese and the other two breeds was clearly measurable, whereas the split between Bardigiano and Tolfetano was not clear. Innovative tools to determine accurately the coat color in horses is therefore essential, especially in cases where there are specific constraints for the registration of animals to the studbook.

P567

Evolution of colostrum characteristics in murgese mares during the first 48 hours after foaling

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The conservation and reintroduction of autochthonous livestock breeds has been increasingly recommended as an effective biodiversity conservation strategy. Over the centuries, they have contributed greatly to the shaping of most traditional Mediterranean landscapes, such as Murgese, the oldest surviving Italian horse breed. More recently, horse breeding has also gained importance for colostrum, with its characteristic properties and the possibilities of its use on a pharmaceutical, but also on a veterinary level. The colostrum of mares is characterised by a unique chemical composition: high percentage of dry matter (30%), a large amount of proteins (10% on average) and immunoglobulin. The foal should ingest the colostrum within the first hours after birth, as the absorption of macromolecules and immunoglobulins is highest in the first hours of its life. Therefore, the administration of colostrum to the foal is essential for its survival in all cases where the mother is unable to do so. In this study, the physicochemical composition and antioxidant activity of colostrum from Murgese mares were analyzed. Colostrum samples from 25 lactating mares were collected in the first 48 h after foaling in six different farms specialized in breeding Murgese horses in the Apulia region, Italy. The collections took place between February/2021 and April/2021. Colostrum samples were collected by manual milking after cleaning the udder and stored in previously sterilized plastic bottles (6–12–24–48 h after birth). The immunoglobulin content was measured with a refractometer at each sampling. The results showed that the values of most chemical parameters in colostrum were highest 6 h after foaling, especially fat, protein and brix content. In contrast, an increase in pH and lactose content was observed 48 h after birth. The colostrum in the first h was characterized by a high content of dry matter, protein and lysozyme and a high antioxidant activity. Its intake in the first 48 h of life guarantees the survival of the foal. In contrast, the foal's life is endangered if it does not absorb colostrum in the required quantity or quality, e.g. if it is abandoned by its mother or orphaned. For these reasons, the study was carried out in collaboration with the Territorial Biodiversity Office, which manages the Eastern Murge Biogenetic Nature Reserve, which has become a ‘public colostrum bank’ for national farms and all horse breeders.

P258

Whole genome sequencing data provide a landscape picture of genetic variability in sea cucumber species

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Holothuria or sea cucumber is a genus of marine invertebrates, belonging to the group Echinodermata, commonly called sea cucumbers. Holothuria species are detritivores, raking sand into their mouth with oral tentacles, extracting and digesting the bacteria, animal and vegetal particles, and voiding the sand through the anus. In this way they churn up significant areas of the seabed, playing a central role in the habitat these species live. Holothuria species have been seen as a promising candidate for aquaculture, as they are resistant to handling and adapt easily to captivity conditions. The interest in Holothuria for aquaculture began in Asia and reached the European market, making it a potentially valuable species for European aquaculture. The genus counts more than 160 species, many of which are uncharacterised at the genome level. However, very recently, few genome drafts became available, allowing to conduct genomic analyses on these species.

Here, we provide a first comparative genome analysis of two species of sea cucumber that are common along Italian shores, *Holoturia polii* (HP) and *Holoturia tubulosa* (HT) utilizing the reference genome of *Holothuria glaberrima* (HG) in the comparison, the only reference genome so far available. The reference genome is composed of 89,105 scaffolds, most of them (54,676) with scaffolds below 10 kbp in size. Scaffolds over 10 kbp represent 1.067 Gb of the predicted 1.1 Gb. We produced whole genome sequencing datasets using Illumina paired-end read for a few HP and HT samples. About 300 million reads per sample were filtered and then mapped to the HG reference genome with standard options. Variant calling was performed considering all samples together. The genome coverage from the datasets of HP and HT was 56% and 58%, respectively. This relatively low coverage is probably linked to the drafted genome assembly for HG, which is highly fragmented. Variant calling produced 72,217,418 single nucleotide polymorphisms (SNPs), mostly on the longer scaffolds that will constitute the core of the analyses. This information has been used to estimate the genetic relationship among the three species and detect genomic regions of high genetic divergence that might be useful to define specific genome regions that characterize different Holothuria species.

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P429

Use of biopromotor to improving depleted coastal and semi-closed marine areas

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The progressive pauperisation of the marine fauna is also testified by the results of fishing obtained along the Ionian coast that indicate a progressive decrease in the quantity and marine species fished. The local economy is heavily penalized by the low yield of fishing caused by problems related to environmental pollution and water heating. Use of bioactivator, mixtures of selected microorganisms, enzymes, plant extracts and mineral catalysts that, once released along the coastal areas, can activate and improve the quality of marine ecosystem.

For the trial in an area close to the mouth of the river Cavone (Pisticci, MT) 2 sites of equal surface area and comparable as by kilograms of fish caught as by number of species fished were identified (C – Control and T – Treated). On site T were weekly released 2 bioactivator (MICROPAN OXYLESS and MICROPAN AQUA PE). During the treatment, in each site, were performed a caught monthly (T0; T1; T2 and T3); on the boat the specimen fished was identified and numerated, individually weighed and delivered to the laboratory in refrigerated conditions (4 °C).

Hence, the following measurements were made: total, fork and head length and maximum height. From linear and weight measurements, morphometric indexes, as relative profile, cranial index and condition factor were calculated. Rheological properties of the raw fish fillets were assessed using an Instron 5544 Universal Testing Machine. Texture Profile Analysis (TPA) was performed using a flat steel probe of 25 mm diameter, through a double compression test elaborated by the incorporated software. AOAC procedures were used to assess the moisture, ether extract, raw protein and the ash.

The results of comparison between T0 and T1 do not indicate significant differences in the parameters considered; at T2 the results indicate an amount of 8,1 times greater in site T then the site C and relative to the number of species have been counted 11 more in area T compared to the C. In T1 and T2, samples analysed have significative differences in total length, relative profile and condition factor for grey mullet (*mugil cephalus*), and striped seabream (*Lithognathus mormyrus*). No significative differences are notable in colour and textural parameters in fishes analysed.

Our study indicates that the bioactivators were effective in the increasing of number of species caught and the quantity fished for each.

P515

Tetrodotoxin presence in mussels: rapid toxin detection based on a Point of Care immunoassay

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With the ongoing climate changes and increasing globalization, alien species have colonized new ecosystems. Recently, in the Mediterranean Sea an increased number of cases of tetrodotoxin (TTX)-contaminated seafood have been reported, resulting in a serious case of food poisoning. Due to this increase in cases, EFSA (European Food Safety Authority) has set a limit to TTX concentration in seafood, which can't be higher than 44 ug/kg. The available methods for TTX detection in seafood require a specialised laboratory, expensive instrumentation, trained staff to conduct the analysis and require a couple of days to obtain the results. Thus, having a suitable method for TTX detection for the infield controls would be highly desirable. To develop a Point of Care (POC) system, we first optimized an Antigen-Antibody enzymatic immunoassay (ELISA) aiming to verify both specificity and antibodies' performance selected on the target toxin. With the purpose of optimisation, TTX detection was performed on laboratory spiked samples of mussels and the extraction approach, and the mussel matrix effect were both investigated. Subsequently, the optimised immunoassay protocol was transposed to an Organic Light Emitting Diode (OLED)-based immunosensor to allow the POC analysis directly in the infield. The developed immunosensor has been tested with the spiked samples obtained experimentally in the laboratory, as previously with the ELISA assay. In the POC protocol no enzymatic amplification step was performed like in ELISA, but it was detected the fluorescence emission produced from the marked secondary antibodies.

The data obtained from the fluorescence intensity allowed good discrimination of the positive and negative samples in relation to the limit set up by the EFSA. The results obtained by ELISA and the POC immunosensor have then been compared and a strong correlation was found between the two methods.

Although further investigations should be performed with samples exposed to natural TTX environment contamination, the present data showed promising feasibility in using POC as a faster detection method for discrimination among contaminated and uncontaminated samples, even when using TTX dosage lower than the EFSA limits for this specific food contamination.

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P549

Effects of diets containing Hermetia illucens and poultry by-products meals on Dicentrarchus labrax intestinal enzymatic activities. Results of a commercial production trial

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Aquaculture is facing the challenge of being developed following the circular economy concept; in this regard, the choice of sustainable, nutritious, and non-conventional aquafeed ingredients should answer this need. The SUstainable fiSH feeds INnovative ingredients–SUSHIN project has recently proposed a combination of *Hermetia illucens* meal (HIM, 8.1 g/100 g as fed) and poultry by-product meal (PBM, 20.6 g/100 g as fed) to substitute up to 40% of vegetable proteins in diets containing low quantity of marine proteins (5.5 g/100 g as fed) for marine species of interest for Mediterranean aquaculture, as European seabass (*Dicentrarchus labrax)*. Thus, the aim of the present trial was to compare the effects of the experimental feed (SSH diet), under commercial conditions (Ittica Caldoli Foggia, Italy). Three thousands mixed-sex E. seabass were randomly distributed into three tanks, 1000 fish each and fed a commercial diet. When an average weight of 300 g was reached, fish were fed for 63 days with two different isoproteic (45%) and grossly isolipidic (20%) diets. A control group (CTR) continued being fed the same commercial diet as during the adaptation phase and the two remaining groups were fed with the experimental diet (SSH). At the end of the growing trial, marketable indexes were recorded, then fish intestine and muscle ($n = 9$/group) were sampled. The intestine activity of glutathione reductase (GR), catalase (CAT), glutathione peroxidase (GPX) and lipid peroxidation was measured. In muscle, conjugated dienes and thiobarbituric acid reactive substances were measured as lipid oxidation markers. At the end of the trial, the total length and body weight of fish fed the SSH diet (472.5g) were higher than the CTR group fish (433.9g). The incidence of the intestine on total weight was lower ($p = 0.05$) in SSH than in CTR fish, being 2.18% and 2.38%, respectively. Intestine lipid peroxidation was lower in fish fed the SSH diet than in the CTR ones. Moreover, lower CAT activity and higher GR activity were also registered in the SSH group than in the CTR group, even though not statistically significant. Muscle lipid oxidative status was not affected by the diets. In conclusion, the SSH diet, which in previous trials showed promising results at experimental level, demonstrated to be a suitable alternative to the commercial feed for E. seabass feeding even when administrated in fish farming plant.

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P56

Meat quality of roe deer hunted in Tuscany: preliminary results

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In the last decades in Tuscany the number of wild animals has drammatically increased and especially roe deer which, due to its small body size, easily adapts to many environments. Therefore, the consumption of meat resulting from hunting is also constantly increasing and is rapidly approaching that of wild boar and red deer. So, it seems interesting to study fat content and fatty acid composition of the meat deriving from this ungulate. At the San Miniato slaughterhouse, 33 samples of muscle *l. dorsi* were collected and, to do a comparison based on sex (male *vs* female) and age (young *vs* adult), were subdivided into: 24 males and 9 females, 10 adults and 23 young. Sampling is still in progress in order to obtain at least 20 samples for each class. The meat samples were frozen and stored at -20 °C until the time of analysis; since the samples were very small, it was not possible to carry out organoleptic analyses, which in the wild are less interesting for the final consumer, but only analysis of the fatty acid profile. A Kruskal-Wallis statistical analysis was performed since some fatty acids did not have a normal distribution and only differences equal to $p \leq 0.05$ were considered significant. Regarding the results, expressed in mg/100g, the only significant difference emerged in the quantity of C18:3n3 both for age (2.91 young *vs* 3.72 adults) and for sex (3.07 males *vs* 3.37 females) with $p < 0.05$. This could be linked to the intramuscular fat content which, although generally very low, is variable with average values of 1.41 g and 1.78 g in adult and young males and 1.07 g and 1.63 g in adult and young females respectively. Other fatty acids are also at the limit of significance. Generally, the acidic composition is similar to that of other ruminants, thus making venison suitable for the human diet. Further investigation will be possible increasing sample numbers, since, at the moment, there are very few scientific works that take into consideration this aspect of meat quality, preferring instead everything related to the management of roe deer in the area.

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P102

Comparison of sialic acids content in cow and buffalo milk in different season

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There are many derivates of sialic acids but the most common in mammalian are Neu5Ac (5-Acetyl-Neuraminic acid) and Neu5Gc (5-Glycolyl-Nuraminic acid), a particular family of monosaccharide of neuraminic acid. They are present as terminal of glycans of the cells and they are involved to cell-cell interaction in the immune system. The humans are unable to synthetize Neu5Gc due to a deletion in CMAH enzyme responsible for the hydroxylation of Neu5Ac in Neu5Gc. The presence of Neu5Gc in human cells comes from the consumption of animal products, and it in humans could result as proinflammatory, associated with an increased risk of cardiovascular disease and cancer; while Neu5Ac has an important role as precursor of neural brain glycoprotein in neonatal phase. For this reason, it is advisable to consume milk and meat rich in Neu5Ac but poor in Neu5Gc. High variability of sialic acids in animal products depends, mainly, by genetic factors and response to stressors, such as metabolic stress due to high production in dairy cows or high ambient temperatures as reported, hypothesizing that the glycolyl form could use as biomarker index of environmental/heat stress response in dairy cows. Hence the aim of the study was to evaluate the sialic acids, Neu5Gc and Neu5Ac, content in two dairy species: cattle and buffalo during different seasons, as stressor marker. Trial was performed on 160 bulk milk samples collected in the morning, one half from Italian Holstein Friesian (Ho), the others from Mediterranean Italian Buffalo (Buf). Sialic acids were extracted from whole milk by BMD derivatization and HPLC analysis. Buffalo milk showed a lower amount of Neu5Gc (2.02 vs 3.10 µg/mL) and a higher ($p < 0.001$) amount of Neu5Ac (146.03 vs 102.16 µg/mL) compared with Ho milk. The seasonal effect was markedly visible ($p < 0.001$) in the glycolytic fraction of Ho milk (high in summer 3.84 ± 0.69 µg/mL low in winter 2.06 ± 0.58). Season did not affect both sialic acids in Buf milk. However, for both species the Neu5Gc was correlated with the THI (Temperature Humidity Index) detected on the day of milk sampling ($r = 0.31$ to $p < 0.004$). In conclusion, buffalo milk had less Neu5Gc and therefore the consumption of its dairy products could contribute less to trigger inflammatory processes. Furthermore, buffalo milk shows a limited temporal variability of sialic acids responding differently to environmental stress than cattle.

P104

CHEESEMINE: ‘from forage to consumer’ valorization project of cheese ripening in mines

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Numerous researches can be found concerning the aging of cheese in caves, an environment that can be assimilated to that of a mine in terms of thermo-hygrometric characteristics. Some studies focused on microorganism in caves, or on relationship between aging environments and chemical characteristics of cheese, as lipids and volatile matter. Instead the CHEESEMINE project focused on every part of the cheesemaking processes, from the grass and the hay production to cheese aging in mine. In 5 dairy farms, in 2 consecutive years, located in Dossena (BG), at 800–1400 m asl and produced cheese in farm, were monitored about grass yield and quality (part for hay and part for pasture), milk yield and quality, protocols of cheesemaking, chemical analysis of milk, microbiological and fungi analysis of milk and cheese (before and after 2 different age of ripening – 90 or 180 d).

The grasses produced 2.5 cuts on average and for more than 50% were used as pasture, for cows and heifers. *Poaceae* were predominant only in the first part of the seasons, *Fabaceae* were less than 15% in all part of the seasons, and so the Pastural Value was low, particularly in august. The farms were small, with 8–45 dairy cows and had low Dairy Efficiency (0,75–1,32), but they showed high quality of milk (fat 4.05 ± 0,61, protein 3.43 ± 0.31, casein 2,71 ± 0.26, despite of Log10 SCC 4.97 ± 0.45 and Log10 TPC 4.31 ± 0.67).

The monitored cheesemaking processes led to produce a disciplinary for this new cheese, called ‘*Ol Minadur*’ and concerned the use of raw milk, the possibility of using a natural dairy starter and the boiler operations for a correct syneresis (43–46 °C for at least 20 min). In 2 years, 5 cheesemakings were monitored for each dairy farms and a total of 250 wheels were produced. In each cheesemaking at least 2 wheels were produced, one sent to mine, and one rippened in farm cellars.

No lactose was found at maturation of 90 d. In 12 more important fungi species found, *Penicilium biforme* was always found, both in farm cellar (74%) and in mine (61%), both at 90 d (80%) and at 180 d (60%).

The main difference between the wheels aged with the same aging time, was represented by the higher level of humidity of the wheels in the mine (38.2 ± 1.5%) than the wheels aged in the farm cellars (32.9 ± 1.9%). Some small differences in lipids content and lipolysis process.

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P106

Effect of different feeding management on the fatty acid profile of buffalo milk

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Buffalo milk is characterized by high levels of protein and fat, and particular attention is paid to its composition and to fatty acids profile. Many genetic and environmental factors affect milk fatty acids composition. Among environmental factors feeding plays a key role. The aim of the present study was to discriminate the variation of Mediterranean buffalo milk fatty acid profile related to different fed diets. The trial was carried out in 10 dairy buffalo farms located in central Italy (Amaseno valley, Lazio). The ten farms were representative of the production area. The diet was fed ad libitum as total mixed ration. Bulk milk samples were collected monthly from December 2021 to November 2022. Milk samples were analyzed for the determination of fatty acid profile by gas chromatography. Fatty acids were classified according to degree of unsaturation by dividing fatty acids into saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA). In total, 90 individual fatty acids were determined: 5 low SFA, 9 odd SFA, 6 other SFA, 12 branch SFA having iso and anteiso configuration, 9 isomers of conjugated linoleic acid (CLA), 33 MUFA subdivided into 21 cis and 12 trans, 16 PUFA subdivided into 6 PUFA n6, 5 trans isomers n6, 4 PUFA n3 and finally 1 Trans isomers n3. Results clearly showed the effect of diet on that fatty acid composition. In particular, concentrated/forage ratio was one of the most important factors. A low amount of branch SFA and a high amount of PUFA n6 were related to high concentrates/forages ratio. Conversely, farms that used large quantities of hay, providing to animals a diet with a low forage concentrate ratio, showed a high presence in SFA branches and MUFA trans, probably related to greater ruminal activity. Within forages is important to differentiate silage portion respect to hay or cut grass. The CLA amount observed, depended on amount of green forage and hay in diet, but it was also influenced by other components of diet. Phytanic acid (Branch SFA) in milk was greater in diet contained grass. This could be used as a marker for ‘grass feed’ diets. Among concentrates, the linseed addition to the diet increased the PUFA n3 concentration. The presence of linseed in diet seems to have inhibited the CLA in milk of grass-fed animals. These results contributed to increase knowledge of how diets, based on quantity of concentrates and composition of lipids present, were able to influence fatty acid profile of buffalo milk.

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Effects of the use of spirulina (Arthrospira platensis) in buffalo feeding on sensory quality of mozzarella cheese

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There is a growing interest to use Spirulina (*Arthrospira platensis*) in human and animal nutrition as an important source of bioactive compounds. In this study, aimed at improving the sensorial and nutraceutical properties of mozzarella, it was utilized as feed supplement. Two groups of 12 dairy buffaloes were enrolled. The Control group (C) received the TMR farm diet (17 kg DM; 130 g CP, 400 g NDF, 0.85 UFL/kg DM), whereas the experimental group (S) was fed with the same diet supplemented with powdered Spirulina (50 g/head/d one month before calving and 100 g/head/d three months after calving). Three mozzarella cheese making were carried out during the 2nd and 3rd month of lactation. A 10-member panel was trained using a specific frame of references and used a specific vocabulary to assess cheese sensory properties through quantitative descriptive analysis, whereas 68 consumers were recruited to express their liking for mozzarella. In addition, a Vickery auction and a Computer Vision System (CVS) colorimetric analysis were carried out.

Significant differences ($p < 0.01$) between S and C mozzarella were observed for several sensory attributes. Spirulina mozzarella resulted in a brighter external appearance (also in accordance with the results of the CVS analysis), with a higher intensities of butter odour and whey flavour than Control. Similar results were observed for sweetness, bitterness, juiciness, tenderness, oiliness (probably due to the increase in MUFA), and greater release of buttermilk. As regards to consumer testing, in blind condition (tasting without information about the product) both products were appreciated with an average score above the neutral score (5); under expected condition (information about product without tasting), consumers tended to express a greater liking for the C mozzarella ($p < 0.10$); in actual condition (tasting with information on product), consumers tended to express a greater appreciation for the S mozzarella ($p < 0.10$).

The growing interest for nutraceutical properties of mozzarella prompted the consumers to offer a higher price for the Spirulina mozzarella (16.20 €/kg) than the Control product (14.08 €/kg) during the Vickery auction ($p < 0.01$). We conclude that information about the products, if given to the consumers, can be the major determinant of consumer willingness to pay for animal-based food products. However, information about nutraceutical properties should be paired with products presenting a good eating quality.

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P159

Does the use of ensiled forage affect the sensory properties of fresh cheese?

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The present work aimed to evaluate the effects of the forage preservation method (silage vs. hay) on the chemical and sensory characteristics of two artisanal, fresh cheeses: ricotta and 'primo sale'. A 6-ha dryland field (760 m above sea level) was sown with a legume-grass mixture (*Lolium multiflorum, Triticum aestivum cv. Ludwig, Trifolium alexandrinum, Trifolium squarrosum* spp.) at a 40–60 ratio. At harvest (early flowering of legumes), half of the forage (3 ha) was chopped at a theoretical length of 1.1 cm and ensiled in plastic film, whereas the other half was air-dried to hay and then stored in 1.2-m-diameter round bales. Three months after the forage harvesting, thirty-two lactating Holstein cows were randomly allocated into 2 groups, silage (S) and hay (H), balanced for DIM (141 ± 76 vs. 141 ± 69 d, respectively, for the S and H group), milk yield (29.6 ± 10.0 vs. 29.0 ± 8.9 kg/ head per d), and milk quality (fat 3.8 ± 0.74 vs. 3.7 ± 0.78%; protein 3.2 ± 0.4 vs. 3.2 ± 0.4%). Group H was fed a TMR containing as the sole forage the experimental hay which for group S was replaced by the experimental silage. A limited amount of the experimental hay (5.0 kg as fed) was included in the silage based TMR to provide a basic level of physically effective fiber. After 10 d of adaptation to the diets, during 3 consecutive morning milking, bulk milk of each group was separately collected to manufacture 'primo sale' and ricotta cheeses. Milk and cheeses from each batch of production were collected to be analyzed for macro components, fatty acids (FA) profile, and sensory properties (Quantitative Descriptive Analysis). Data were analyzed with a one-way ANOVA to assess the effects of diet (S and H). The chemical and FA compositions of ricotta and 'primo sale' were not affected by the preservation method. Regarding the sensory properties, the 'primo sale' cheese produced on the silage diet was more acidic and saltier ($p < 0.05$) than that produced on the hay diet. Similarly, S-ricotta was saltier ($p < 0.001$), but it also scored lower in brightness ($p < 0.001$) and color intensity ($p < 0.05$). The consumer panel did not perceive any differences between ricotta and primo sale produced on S and H diets. The results indicate that although experienced panelists may detect little difference, the forage preservation has no effect on consumers' liking of fresh cheese.

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P161

The effect of nitrite treatment of tuna on the biogenic amines level and total microbial count during storage: a preliminary study

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A recent concern in food safety is the illegal treatment of tuna with high amounts of nitrite to obtain a significant improvement in appearance. During storage, high levels of amines (particularly biogenic amines) can be produced, so, this treatment could mask a significant problem of food safety. The microbial count and possible presence of pathogens is another concern to take into account.

This study describes the preliminary results obtained by simulating the treatment of red tuna with nitrite solutions, then determining the following chemical parameters: histamine, volatile basic nitrogen (TVBN), nitrite/nitrate, ascorbic acid and sulphites, both before and after 5 days of storage. Regarding microbiological aspects, the following determinations were carried out: Total microbial count at 30 °C, enumeration of *Enterobacteriaceae*, *Salmonella* spp, coagulase-positive staphylococci, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Vibrio vulnificus*, beta-glucuronidase-positive *Escherichia coli* and anaerobic sulfite-reducing bacteria.

The analytical determination of histamine was carried out by using HPLC/FLD after sample derivatization, while TVBN was then determined by steam distillation and final titration. Nitrite/nitrate and sulphites were detected by ion chromatography, while ascorbic acid by HPLC/UV-DAD.

Regarding TVBN determinations, no significant differences were observed between treated and untreated samples, while treatment seems to halve the formation of histamine. Sulphites were not detected, while ascorbic acid was quantified in both samples at a mean concentration of 245.5 mg/kg.

As it regards microbiological determinations, most pathogens were not found in all samples. The total microbial count and enumeration of *Enterobacteriaceae* were in 1 order of magnitude higher (10^5 CFU/g vs 10^4 CFU/g) after 5 days of storage in a not treated sample if compared to the same treated with nitrite solution. No other significant change was verified.

In conclusion, this preliminary study confirmed that no other food safety concern is highlighted, apart from nitrite amount, in samples treated with nitrite solution, if the overall quality (especially microbial) of the starting product is good. Further studies are needed in order to clarify the effect of such treatment on samples with higher initial microbial count and/or in presence of pathogens.

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P230

Comparison of meat quality traits among chickens'genotypes with different growth-rates

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Starting from 2018, the European Chicken Commitment (ECC) aims to set standards for improved broiler welfare and supply chain sustainability to be pursued by 2026. One of the topics is the development of medium growing genotypes to be bred in less intensive indoor and outdoor systems. Thus, the present study aimed at comparing the quality traits of meat belonging to the main fast-growing hybrid used for meat production in Italy (namely Ross 308, FG) with some medium-growing (MG) genotypes among ECC recognized ones, which can be used as alternative. A total of 3512 broiler chickens were reared under experimental conditions in an environmentally controlled poultry facility. Birds were divided according to their genotype and gender into four experimental groups ($n = 439$/group). In detail, 4 genotypes were considered of which one FG and 3 MG slaughtered at 42 and 50 d of age, with an average weight of 2.5–2.6 and 2.9–3.1 kg for females and males, respectively. Then at 3h *post-mortem*, 12 Pectoralis major muscles (PM) for each experimental group ($n = 8$) were weighed and used to assess the main meat quality traits (ultimate pH, colour, drip and cooking losses and shear force). Data were analysed by factorial ANOVA considering the main effects of the genotype and gender along with their interaction. Since the interactive term was not significant for none of the parameters, only the main effects were included in the statistical model and when significant means were separated by Tukey-HSD test ($p < 0.05$). As expected, a significant ($p < 0.01$) effect of gender was observed for PM weight with male broilers exhibiting higher weights (+35%) if compared with females. In addition, when considered on their whole, data obtained for the genotype evidenced that it strongly affects the main quality traits. In fact, if compared with FG, MG birds had lower pHu ($p < 0.001$) which were associated with a higher water holding ability as depicted by lower drip ($p < 0.05$) and cooking losses ($p < 0.01$). Overall, PM belonging to MG genotypes had similar features with the only exception being MG2 that showed a higher development of the pectoral muscles (+23% if compared to MG1 e MG3) and increased cooking losses, suggesting a higher similarity with FG. Based on the available information and considering the importance of this issue, the results obtained in this study stresses the importance of including alternative genotypes to be used for broiler production considering ECC goals.

P235

Qualitative characterization of chicken meat according to the main Italian commercial categories

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The present study aims at evaluating quality traits of chicken meat belonging to some of the main commercial categories marketed in Italy: free-range light (L) chickens (medium-growing genotype, slaughtered at 56 d and 1.8–2.0 kg) and conventional medium (M) and heavy (H) broilers (fast-growing genotype farmed under conventional intensive systems, processed at 35 and 40–42 d and 2.5–2.8 kg and 3.3–3.8 kg, respectively). Thus, a total of 15 flocks were considered, and 50 breast fillets and 20 thighs per each flock were randomly collected and grouped according to the selected commercial categories (L = 5, M = 5 and H = 5). The incidence and severity of the growth-related abnormalities White Striping (WS), Wooden Breast (WB) and Spaghetti Meat (SM) were assessed on each breast fillets, while pHu, colour, drip and cooking losses, and shear force were determined on 25 breast fillets and 20 thighs per each flock. The remaining breast and thigh samples, were minced, respectively, and used for the analyses of protein (carbonyls) and lipid (TBARS) oxidation level (5 replicates/ flocks). Data on the incidence of growth-related abnormalities were analysed through Chi-square test, while meat quality was evaluated by One-way ANOVA considering the commercial category as the main effect (L, M, H). Means were separated using Tukey-HSD test. The overall incidence and severity of WS and WB were significantly ($p < 0.05$) lower in L if compared with M and H chickens, while no significant differences were observed for SM abnormality. As for breast meat quality traits, if compared with both M and H, L exhibited lower pHu (5.53 vs. 5.80 and 5.82; $p < 0.001$), drip (1.11 vs. 1.67

and 1.94%; $p < 0.001$) and cooking (12.9 vs. 19.1 and 19.4%; $p < 0.001$) losses. No differences on colour, shear force, lipids and protein oxidation were found. On the other hand, thigh meat quality traits did not vary among the experimental groups with the only exceptions being yellowness, showing the highest values in L (b*; 11.0 vs. 5.08 and 4.06; $p < 0.001$) and TBARS which was higher in L and M compared to H (0.485 and 0.345 vs. 0.174 mg MDA/kg; $p < 0.001$). In conclusion, despite the differences of genetic type, rearing system and age at slaughter characterising the 3 commercial categories analysed, quality traits of breast and thigh meat were quite similar. As expected, breast meat from light free-range chickens presented a lower incidence of growth-related abnormalities and better technological meat characteristics.

P256

Antioxidant activity of cheese obtained from dairy cow fed tannins

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The study aimed to assess the effect of dietary supplementation of tannins to grazing dairy cows in two seasons characterized by a good quality pasture (spring) or a poor-quality pasture (summer) on the antioxidant properties of cheese and cheese *in vitro* digestates. Fourteen lactating dairy cows were divided into a control group (CON) and an experimental group (TAN) receiving 150 g/head per day of tannins supplementation. Animals were allowed to graze on spontaneous pasture or on dry stubble, during spring and summer experiment respectively. The experimental trials lasted 23 d. Cheeses were produced the day before the beginning and at the end of each seasonal trial, using individual raw whole milk and ripened for 25 d. Briefly, the cheese was produced using a batch of 7 kg of milk heated at 38 °C, coagulation with commercial liquid veal rennet, cooking at 75 °C in hot water, and brine salting. On cheese samples, the *in vitro* digestion was performed and cheese antioxidant activities before and after simulated digestation were evaluated based on their free radical scavenging ability by 1,1-diphenyl-2-picrylhy-drazyl (DPPH) radical scavenging assay. Cheese made from TAN milk showed higher ($p < 0.05$) antioxidant activity in both spring and summer season, with 50 and 70% of DPPH inhibition, respectively, compared with the control cheese showing about 30 and 50% in spring and summer, respectively probably due to different levels of tannins-derived bioactive compounds released. The gastric and intestinal digestates showed higher ($p < 0.05$) percentage of DPPH inhibition in TAN treatment, only for cheese produced in the summer trial. On the contrary, the comparable antioxidant activity found in the spring season, was ascribed to the high quality of the basal diet, composed by green pasture which likely provided high levels of antioxidants. This study provided evidence that antioxidant molecules can be transferred from the diet to milk and dairy products, thus enhancing the nutritional quality of dairy products.

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Instrumental measurements of juiciness of meat and plant-based burgers

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Plant-based burgers (PBB) are food products that are designed to have meat-like characteristics, but do not contain any animal-derived ingredients. The sensorial experience of PBB complains about their lack of juiciness of cooked products compared to the meat-based burgers (MBB). As many analytical techniques, established for meat juiciness evaluation, would not seem to be sufficient to characterize PBB the objective of this study is to quantify PBB's juiciness by novel techniques in the burger from the original frozen/fresh *status* to what the consumer could perceives in his/her mouth.

The study was performed on 1 MBB, 1 commercial PBB and 6 self-made prototypes of PBB, for a total of 80 burgers analysed both raw and cooked. The traditional methods employed to quantify meat instrumental juiciness were modified to make them suitable for PBB juiciness evaluation. Physical analyses were performed by two new measuring instruments: Meat Cooking Shrinkage and Water Holding Capacity Trend instruments. These semi-automatic instruments, which measure different parameters on the same sample, provide accurate and fast measurements. Data were analysed by ANOVA GLM procedure (Tukey test, $p < 0.05$). The results were expressed as percentage on the wet basis to provide a correct comparison of the obtained values.

The following parameters were evaluated: total moisture content (TMC), meat cooking shrinkage (MCS), fluid to the mouth (FTM) and cooked specific fluid content per volume (CSFC).

The meat-based burger had a TMC equal to 71%, a significantly higher value (+9.6%) than that from the PBB average. Both MBB and PBB had similar FTM average (48%), but MCS was significantly higher (+11%) in MBB (24%) compared to PBB average. It can be concluded that MBB retained more water than PBB with a CSFC of 0.84 mg/mm^3, a value significantly higher (+0.222 mg/mm^3) than that from PBB average. All the means were significantly different at $p < 0.05$ level. Plant-based burger showed a lower water retention than MBB because plant-based proteins cannot bind together to simulate the interstitial spaces between meat muscle myofilaments. These myofilaments trap and retain water, which is released during chewing to create the juicy sensation.

Further innovations must be explored to reproduce the type of capillary systems of muscle that are crucial to the juiciness of cooked PBB.

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P575

Effect of intramuscular vitamin E injection on Polycyclic aromatic hydrocarbons formation in cooked meat of broiler chickens reared under tropical climatic conditions

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Vitamin E as a potent natural antioxidant, is a well-established micro-nutrient used in animal nutrition to sustain animal health and production, and to reduce the oxidative processes in meat. Several studies have reported the inhibitory effects of vitamin E on formation of carcinogenic compounds during meat processing. The aim of the study was to assess the effect of intramuscular vitamin E injection on Polycyclic aromatic hydrocarbons (PAHs) formation in cooked meat. The study was carried out in a small chicken farm in Uganda. Thirty 28 d old Cobb 500 chicks were randomly assigned to 3 treatment groups (5 replications/group), which were intramuscularly injected on left pectoral muscle with 0.25 mL (12.5 IU) (T1) and 0.5 mL (25 IU) (T2) of *dl* α-tocopheryl acetate (50 mg/mL), and 0.5 mL of physiological saline (C). At 42d, birds were slaughtered and the pectoral muscle was removed for the quantification of carcinogenic compounds after grilling. PAHs were detected using a GC-MS analysis. Data were analyzed by one-way ANOVA. Data for concentration of PAHs that did not meet the assumptions for ANOVA were analyzed using the Kruskal Wallis H test. No statistically significant differences between the three groups were found for all the PAHs detected. The mean concentrations (µg/kg) of PAHs formed in grilled meat were in the range: 0.30–1.55 for Benzo(a) pyrene (BaP), 0.37–2.54 for Benzo(b) fluoranthene, 0.80–1.19 for Benzo(g,h,i) perylene, 0.89–1.97 for Benzo(k) fluoranthene, 0.94 for Dibenzo(a, h) anthracene and 1.18–1.57 for Indeno (1,2,3-cd) pyrene. In particular, the mean contents of BaP (the marker for the carcinogenic potency of PAHs) found in breast muscle were 0.3 µg/kg for group T1 and 1.55 µg/kg for the control group, with no detection for group T2. The mean concentration of BaP was within the tolerable limit set up by EU regulation (5 µg /kg). Even if not significant, an apparent trend of vitamin E treatment towards inhibition of the formation of these carcinogenic compounds appeared to have emerged. In fact, more of the PAHs were detected in the control as opposed to the vitamin E treated groups. The results from the Toxic Equivalent computation also revealed that the concentrations of the compounds detected were within the tolerable limit set by EU Regulation.

P17

Effect of cooling condition on animal and environmental performance in Saanen goats’

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The aim of this study was to evaluate the effect of the use of cooling on animal performance and physiological response in lactating Saanen goats. The study was conducted in a commercial dairy goat farm located in the northwest of Sardinia (Italy), during the summer season. A total of 20 Saanen dairy goats at 120 days in milk (DIM) were allocated to two groups and simultaneously exposed to cooling (COO; $n = 10$ goats; temperature humidity index: THI <80) or to heat stress (HS; $n = 10$ goats; THI >80) conditions for 1 month. Animals were selected from a larger group of 50 animals to be homogeneous for milk production (3.49 ± 0.55 kg/d; mean ± SD), milk composition (fat: 3.05 ± 0.38 %; protein: 3.25 ± 0.22; lactose: 4.50 ± 0.15%), and body weight (BW) (66.8 ± 12.5 kg). Goats were fed a total mixed ration (51.4% DM) composed by 20.8% CP and 38.5% NDF (% on DM basis). Data on environmental temperature and humidity were recorded throughout the experiment by two data loggers. Individual milk production and BW were recorded once a week. Individual milk samples were collected weekly and analysed for chemical components and for milk coagulation parameters (rennet clotting time, RCT; curd-firming time, K20, curd firmness at 30 min, A30). Heart rate, oxygen saturation (SpO_2), and rectal temperature were measured 4 times per week. Water and feed intake were recorded daily. In addition, blood samples were collected on d 0, and on days 10 and 28 of experimental trial, and analysed for haematological, liver and kidney parameters. Results evidenced that DMI did not differ between groups ($p > 0.05$). Water intake was numerically lower in the COO group. Milk production and composition and milk coagulation parameters were not affected by the treatment ($p > 0.05$). Rectal temperature and hearth rate did not differ between groups ($p > 0.05$). Compared to HS, COO goats had higher blood MCH values (6.19 *vs.* 5.88 ± 0.15 pg; $p = 0.049$; COO *vs.* HS group, respectively) and higher SpO2 (74.22 *vs.* 71.55 ± 1.29 %; $p = 0.038$; COO *vs.* HS group, respectively), evidencing a better physiological response to a cool environment. The other blood haematological parameters did not differ between groups. In conclusion, a short period of cooling did not modify milk production traits but slightly alleviated heat stress improving physiological response. However, a longer treatment period may be useful to evaluate the effect on milk production.

P219

Cradle-to-grave carbon footprint of dairy products of local supply chain

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The study was aimed at assessing the Carbon Footprint (CF) of dairy products by an approach of Life Cycle Assessment (LCA) from *cradle-to-grave*. The dairy supply chain included: the *upstream* stage corresponded to the raw milk produced from an extensive dairy farm breeding Pezzata Rossa cattle; the *core* phase referred to the manufacturing and packaging operations of dairy products like: mozzarella, ricotta, primo sale, yogurt, caciotta stagionata and fresh raw milk; the *downstream* stage included the direct selling through a supermarket, a local market and a delivery system based on electric bicycles service, this stage has also included the consumption and waste disposal habits. The functional unit (FU) was a kg of dairy product representing the six selected products reported above. However, the CF of each product was also assessed. Data referred to the year 2021 and the greenhouse gasses investigated (CO_2, CH_4, N_2O and HFC) expressed as kg of CO_2 equivalents (CO_{2eq}) were associated with the following emissive sources: enteric methane, manure management, energy consumption, transports, cooling gas leakage and packaging production and disposal. The CF of one kg of representative dairy product was 7.46 kg CO_{2eq}. The upstream, core, and downstream stages shared 76%, 6% and 18% of CF, respectively. The enteric methane of the upstream phase and the refrigerant gas leakage of the cooling processes during the downstream were the main hot spots accounting 58% and 14.3% of total CF. Conservative crops practices and rational pasture contributed to a soil carbon sink of 1.32 kg CO_{2eq}/FU. The operations of selling through the delivery service highlighted a reduction of about 0.14 kg CO_{2eq}/FU compared to the direct one. Finally, considering the single dairy products, the caciotta stagionata pointed out the highest CF corresponding to 20.7 kg CO_{2eq}.

The identification of the major sources of GHG emissions associated with local dairy products may stimulate debate on environmental issues local network and direct them toward processes, choices and habits that reduce carbon pollution.

P287

Use of brewery spent grain in ruminant nutrition to reduce environmental impact of feed: a metanalysis

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The recent increase of primary commodity prices and the problems related to the climate change suggest a reconsideration of using some agro-industrial by products (AIBP) as animal feeds. Indeed, a large part of AIBP originated from food chain production has not usually considered because of some problems related to their use (high humidity, huge variability in chemical composition and in the availability across different years). Among these AIBP, the residue arising from beer industries, brewery spent grain (BSG), is very interesting due to the high amount of annually produced material and its nutrient composition. In this work, the literature focusing on the chemical composition of this AIBP and its effect on the ruminant performances was analyzed, to provide useful information about the best level of inclusion in ruminant diet and to estimate the contribution to reduce the cost and the environmental impact related to the animal feeds. The review of the literature highlighted a very interesting average nutrient composition of BSG: 22.4% of protein, 46.5% of neutral detergent fiber (NDF), 20.6 acid detergent fiber (ADF) and 8.9% of lipid content, on DM basis. The inclusion of BSG in the diet of dairy cows had positive or not significant effect on dry matter intake and on milk production till a level of inclusion of about 30% of the diet. We estimate an annual amount of BSP produced in Europe of more than 8 million tons, being the beer production in Europe more than 42 million tons with an average % of by-product of 19. This means an annual amount of about 0.4 million tons of proteins and 0.8 million tons of NDF, that (considering a reasonable replacement of 1/5 of the daily amount of dietary protein of concentrates and an average recovery of material of about 50% from the food industries) can be used to integrate the diet of about 56 million dairy sheep or 36.6 million dairy goats or 6 million beef cattle or 1.6 million dairy cows. Moreover, considering to replace part of soybean (650 €/ton; 2.79 kg CO2eq/kg) in the diets of ruminants, a cost save of about 225 million euro and a reduction of 1.15 million tons of CO2 eq per year were estimated. In conclusion, the use of AIBP (i.e. BSG) in ruminant nutrition can give a very positive contribute in ruminant livestock in term of reduction of feed cost and of environmental impact.

P511

Applying the Functional Unit Approach to estimate the environment fooprint of Italian fresh milk

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In the last two decades, significant scientific progress in assessing agriculture and food production over the life cycle showed the high burden of direct emission of GHG on the field.

However, interpreting the outputs is frequently debatable because the approaches employed were based on even commonly accepted principles and methods not well established. In addition, the datasets analyzed were sometimes scarce or local, and the nutritional quality of food not usually included in assessments or comparisons between items. To feel the gap, the Food and Agriculture Organization (FAO, https://www.fao.org/home/en/) recently published methodology guidelines (https://www.fao.org/documents/card/en/c/cb8054en/) for the integration of nutrition and health in life cycle assessment of food items.

The FAO guidelines represent a leap forward in LCAs of food items, bringing new perspectives, expanding the geographic dimension to the global scale, and including different applications of LCAs of food items, ranging from research to commissioned assessments performed by consultants. According to the guidelines, when reporting the results of LCAs, food items' nutritional quality must be converted into a functional unit in place of standard mass units. Nutritional indices are, in fact, an up-and-coming method, especially when several nutrients are to be considered at the same time. Here we attempt to tailor the nLCA approach to our national agri-food sector, using fresh milk production as a case study.

A set of data ranging from DHI longitudinal productive and reproductive data measured on single animal, to *ad hoc* questionnaire administered to farmers were available and used to refine the estimation of the greenhouse gas emissions (kg CO_2e) of whole fresh milk based on NRF indices or across a selection of functional units as kg CO_2 e/100 g of product, kg CO_2eq/EU serving size, kg CO_2 e/100 g dry weight, kg CO_2 e/100 kcal, kg CO_2e/100 g protein and kg CO_2 e/100 mg calcium.

P560

Effects of an essential oil and polyphenol blend for ruminants diets on enteric methane emissions

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One of the crucial challenges to face a global warming is represented by the reduction of enteric emissions from ruminants. Many additives have been tested to reduce methane production in the rumen, using a wide range of different compounds. The most important method to transfer the efficacy of feed additives or premixes is the use of *in vivo* experiments. The aim of this work was to test the capacity of a dietary premix to reduce methane emissions from sheep. The experiment was carried out in the experimental farm of the University of Sassari in Italy. During summer, Sixteen pluriparous Sarda dairy sheep (6th month of lactation) were allocated to two homogenous groups based on BW (53.0 ± 8.2 kg). Both were fed with dehydrated chopped alfalfa hay, a supplementation of whole corn grain (345 g/d, DM basis) and one of them (Treatment group, TRT) was supplemented with 1 g/day per head of premix (Anavrin produced by Vetos Europe s.r.l.). The premix included essential oils (EO) from cloves (*Syzygium aromaticum*), coriander seed (*Coriandrum sativum*), geranium (*Pelargonium cucullatum*); tannins (CT) from chestnuts (*Castanea sativa*); bioflavonoids (BF) from olives (*Olea europea*). The concentrations of these were EO:CT:BF =1:2.5:0.1. Animals followed a 28-day diet adaptation period and 8 week trail. Methane emissions were measured using metabolic cages and ventilated hood system (ISBN 9783031197291), equipped with a digital gas analyzer (GMS810 SICK S.p.A., Vimodrone, MI, Italy), over 24 h (about 400 records/d per ewe once per week during the trial). Dry matter intake (DMI), digestibility and milk yield (MY) were measured during 4 consecutive days each week. The respiratory rate, as a physiological marker of heat stress, was measured 5 times/d (7:00, 10:00, 13:00, 16:00, 19:00h) with manual counts within videos of 1 min. Results shows that DMI, MY and digestibility were not significantly affected by the treatment. On the contrary methane emission (g/kg DMI) was reduced with the use of premix by 16% in respect to control ($p < 0.05$). Methane emissions (g/d) were also positively affected by the THI variation

($p < 0.0001$). Otherwise, RR increased with THI (RR, acts/min =4.3051*THI – 263.45; $R^2 = 0.40$) whereas MY was negatively influenced by THI, recording a strong decrease with a THI >77 (−17.2%). In conclusion the use of essential oils and tannin blends reduced the methane emissions without significantly modify the animal performance.

P40

House soiling: is it a common problem in Italian cats?

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House soiling is a behavioural problem and one of the most common reasons for abandoned cats. In Italy, the population of domestic cats is estimated to have reached 7.3 million, but information on how cats are managed and whether they suffer from house soiling is scant.

This study aimed to describe cats' and cat owners' profiles, how cats are caretaken and whether house soiling is common in Italy.

A cross-sectional online survey digited using Qualtrics was conducted from March to May 2022. It consisted of 18 closed and 3 open-ended questions asking for respondents' housing details (i.e. housing type, housing size), respondents' family details (i.e. number of adults, number of children under 7 years old, number of children between 7 and 12 years old), respondents' pet details (i.e. number and species of owned animals, other than cats), cat's respondent details (i.e. relationship with the cat, number of cats, cat gender, cat breed, cat age, cat medical problem), litter details (i.e. number of litters, type of litter box, type of litter, litter scooping frequency, litter full cleaning frequency), and whether the cat showed inappropriate elimination (together with elimination type, spots, and posture). The link of the survey was spread using different social media (Facebook, Instagram) and also emailed to the members of two cat breeders associations (AFI, AFeF).

More than 2800 responses were received for a total of 3154 cats since many respondents answered for more cats. The majority of respondents were from the North of Italy (49.6%), living in an apartment (69.1%) without a garden (68.7%). Most of the households were made of two adults (47.1%) without children (90%) and other animals (58.1%). The majority of the respondents were amateurs (91.9%) and possessed two or three cats (44.93%). The cats were mainly female or male neutered (44%, 39.9%), of European type (51%). There was usually only one litter box per household (46.6%), covered (50.6%), and located in the bathroom (50.8%). The most common litter was the agglomerate type (51.7%), which was scooped at least once a day (79.3%) and fully cleaned at least once a week (58.2%). The prevalence of house soiling was 21.3%. These cats eliminated urine (39.9%), poo (18.6%) or both (34.2%) outside the litter, but usually in the same spot (56.8%) assuming a squatting posture (35.1%). It seems that house soiling is a common problem in Italy and further studies to investigate possible risk factors are needed.

P77

Effects of extrusion processing on pomegranate peel polyphenols in dry pet food

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Pomegranate (*Punica granatum* L.) peel represents a biowaste from juice extraction, which constitutes approximately 43% w/w of the fruit. It is a source of bioactive compounds, including flavonoids, complex polysaccharides, and hydrolysable tannins, such as punicalagin (PUN), ellagic acid and derivatives (EAD), and gallic acid, with a high nutraceutical value and used as an additive or ingredient by the petfood industry, even if the biological activity of its active compounds can be influenced by the extrusion production parameters which potentially reduce the beneficial effects of the pomegranate. Aim of this study was to evaluate the effect of the extrusion process on the polyphenols (PPs) of pomegranate peel extract (PPE) in a dog food.

A control formula (CO diet) was developed. PPE (Total Phenol Content/TPC: 23 g/100g; EAD: 13 g/100g; PUN: 10 g/100g) was added to the CO diet at an inclusion level of 1% (PPE diet). Foods were extruded in a single screw extruder using the same production parameters (temperature, pressure, steam). Four samples were collected for each phase at 10-min intervals. The 1st sampling step was post-mixing, the 2nd post-grinding, the 3rd post-steam conditioning, the 4th post-expansion, the 5th post-drying, and the last step on the finished product. A total of 48 extractions, in duplicate, were analyzed for TPC using Folin-Ciocalteau assay by spectrophotometry, and for PPs by liquid chromatography. A one-way ANOVA model was performed to determine the differences between extrusion phases.

At the 1st sampling step of the extrusion process, the TPC in the CO diet was 0.09 g/100 g with 0.08 g/100 g of EAD and 0.01 g/100 g of PUN whereas, the TPC in the PPE diet was 0.17 g/100 g, with 0.08 g/100 g of EAD and 0.08 g/100 g of PUN.

At the 3rd sampling step of the extrusion process, the greatest loss of PPs was observed in the PPE diet when the TPC was 0.08 g/100 g (−53%; $p < 0.05$), with 0.07 g/100 g of EAD (−12.5%) and 0.01 g/100 g of PUN (−87.5%; $p < 0.05$) while, in the CO diet, a slight loss ($p \geq 0.05$) was observed for TPC 0.07 g/100 g (−22%), with 0.06 g/100 g of EAD (−25%) and 0.01 g/100 g of PUN. In the following steps of the extrusion process, TPC, EAD and PUN remained constant in both diets.

Data show that steam conditioning represents the main critical point for PPs loss. Future research should aim at improving the stability of pomegranate PPs during the production processes to ensure added value in the utilization of this organic waste.

P153

Artificial senses for evaluation of feces protein catabolites in guide dogs fed different diets during training

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A nutritional plan suitable for the animals trained as guides for blind people represents a trivial fraction of the monetary investment. The suitability of Electronic senses technology for rapidly assess the petfood digestibility in guide dogs during specialized training programs was evaluated. During the training work, 8 neutered adult dogs belonging to the Labrador retriever breed clinically healthy and housed at the Regional Centre Helen Keller of the Italian Blind and Guide Dog School Union, in Messina (Italy) were divided into two homogeneous groups for sex (2 males and 2 females), age (17 months ±1), initial body weight (26.3 kg ±1), and BCS (4.5 ± 0.11), and fed with two commercial diets: a performance diet characterized by low- carbohydrate/high-protein and fat diet (29:39:19% as-fed) and a maintenance diet characterized by high-carbohydrate/low-protein and fat diet (50:24:12 % as-fed). Monthly, for 84 days, on freshly voided fecal samples from each dog, the protein catabolites were analyzed by E-nose (FOX 4000, Alpha M.O.S.) equipped with 18 MOS gas sensors and E-tongue (α-Astree, Alpha M.O.S.) equipped with a set of 7 chemical sensors. The measurement dataset was submitted to statistical analysis by using a Discriminant Function Analysis (DFA) to evaluate the variable time (Days 0, 28, 56, 84) for each diet and the variable diet (GF vs. CTR) independently by the time. In addition, a data fusion was performed to validate the instrumental sensory platform using the quantitative identification of catabolites obtained by traditional techniques. The DFA plot obtained from E-tongue sensors signal showed a clearer separation between the GF and CTR groups than that obtained using the E-nose evaluation, being the protein catabolites water-soluble, reaching the maximum validation score (100) on Day0, Day56 and Day84 and the score 95 on Day28. These results were confirmed by those obtained using spectrophotometry. Furthermore, the discrimination performance increases with sensor signals fusion of E-tongue and E-nose. The results testify that the E-senses technology is a solid and rapid alternative to the traditional methods. Its application, providing rapid information on intestinal fermentation processes, is crucial to verify the adopted dietary strategies on gastrointestinal tract health.

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P157

The gut microbiota differs between working shepherd dogs bred in pastoral condition and companion dogs

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Italy is characterized by ancient pastoral tradition in which the presence of sheepdog breeds is fundamental. These breeds are reared to work with livestock and to protect the herd from the aggression of predator animals. One of the peculiar feature of these breeds lies in their old and typical pastoral diet based on bread, dairy by-product, food waste and what they find accompanying the animals to the pasture. Moreover, they do not undergo any drug treatment and have an excellent state of health. In this context, the aim of this study is to explore, for the first time, the microbiota of dogs bred in pastoral conditions (Sicilian Mastiff) compared to companion dogs (Labrador Retriever) fed with a commercial diet. Genomic DNA has been extracted from faecal samples of 8 Labrador Retriever and 8 Sicilian Mastiff. The 16S gene has been sequenced with Illumina Miseq and raw reads have been processed using QIIME2. The high-quality reads have been classified at phyla, family and genera level with an identity threshold of 75%, 87% and 95%, respectively. A total of 8 phyla, 52 families and 103 bacterial genera have shown a relative abundance greater than 0.1%. The most abundant phylum has been *Firmicutes* (54%) followed by *Bacteroidota* (33%), *Fusobacteriota* and *Proteobacteria* (5%). It is interesting to note that Sicilian Mastiff has been a higher prevalence of *Firmicutes* and almost absence of *Fusobacteriota* and this reflects the ingestion of vegetable that occurs during the accompaniment of animals to pasture. *Fusobacterium*, *Turicibacter* and *Bacteroides* have been very prevalent in Labrador and this reflects a complete diet. *Clostridium_sensu_stricto_1* genus has been most prevalent in Sicilian Mastiff, suggesting the co-evolution between this genera and dogs as reported for wolf, and is able to digest protein. *Catenibacterium* and *Prevotella* have been prevalent in Sicilian Mastiff and they are able to digest the carbohydrate producing short chain fatty acid that are associated with beneficial effects for gut. The beta diversity analysis, based on Bray-Curtis distances, has shown how different are the two diets (PERMANOVA $p > 0.001$). In conclusion, further studies will have to be done but we could hypothesize that the composition of Sicilian Mastiff's microbiome reflects not only the diet but also the conditions in which it has evolved that determine a healthy microbiota and useful for a hypothetical transplantation in selected breeds affected by many metabolic diseases.

P196

Comparison of second and third generation sequence technologies for microbiome studies in dogs

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The characterisation of the bacterial population in different body regions was enabled by the development of high-throughput DNA sequencing techniques. Microbiome composition is commonly determined by sequence analyses of one or two of the variable regions present in the 16S rRNA gene. However, this current 16S profiling method usually fails to provide a deep taxonomic annotation up to the species level. Experiments sequencing the full length of the 16S gene has only recently become a realistic approach. In the study, we applied the second and third generation sequencing technologies to analyze the DNA extracted from blood and feces. A first aim was to compare the taxonomic phylogenetic tree obtained from the two sequencing techniques and the second aim was to evaluate which technique provides a more reliable assessment of the similarity between blood and fecal microbial community. For this study, blood and fecal samples were collected from 18 healthy and 19 dogs with gastrointestinal disease, and DNA was extracted through commercial kits. Two primary data sets were produced. The first was a 16S rRNA V3–V4 regions data set generated by Illumina NovaSeq 6000 platform, on a paired 2 × 300 bp mode. The second was a full-length 16S rRNA data set generated by Sequel IIe PacBio platform. The sequences were analyzed through qiime2 for the taxonomic annotation and statistical analysis. Analyses of the 16S rRNA V3–V4 regions data indicated that alpha and beta diversities of fecal and blood microbiome were significantly different between the two groups of dogs. Principal Coordinate Analysis (PCoA) revealed that healthy and sick subjects were significantly clustered, for both blood and fecal microbiome samples. However, comparison of the full-length 16S rRNA data with the 16S rRNA V3–V4 regions data revealed differences in relative bacterial abundances, together with a higher accuracy of the species call. We conclude that the sequence length of 16S rRNA gene could lead to a better microbial population resolution, thus affecting the final composition results.

P298

Siberian Husky canine breed: a preliminary study on breed's morphology evolution

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Siberian Huskies (SH) are a spitz breed originated in Siberia and selected for their ability as sled dog in USA, official documents and recognitions underline the importance of this breed and its specific ability to cope with Nordic countries adverse climatic and weather conditions SH average number of puppies entered per year in the Italian studbook – ENCI in the period 2012–2021 is 1026.7 (N). The aim of this preliminary research was to assess the closeness of the sampled dogs to the breed standard and to track the evolution of body measurements and indexes with two measurements in 2004 and 2019 in conformation type SH. A Total of 54 adult (age ≥12 months, unrelated in second generation, Italian origins) dogs (sex ratio 1/1; 2004: $N = 8$, 2015: $N = 46$) were measured for Height at withers (HWT, cm), Head total length (HTL, cm), Skull length (SKL, cm), Muzzle length (MUL, cm), Skull width (SKW, cm), Chest girth (CHG, cm); Elbow height (ELH, cm), Chest Height (CHH, cm), Body length (BOL, cm), Cephalic Index (CEI), Muzzle/Skull ratio (MSR), Total head length/Height at withers ratio (HWR), Elbow height/Height at withers ratio, Corporal index (CRI). Data analysis was performed by ANOVA using General Linear Model procedure of SPSS. In our model dependent variables were body measurements and indexes, source of variation were sex and samples' collection year and their interaction, the post hoc Bonferroni test was used to investigate the significant differences ($p \leq 0.05$). Our results show an evident closeness to the breed standard; a clear sexual dimorphism, a slight increase in population HWT (2004 vs. 2019; 54.29 ± 0.45 vs 55.24 ± 0,19), BOL (2004 vs. 2019; 60.77 ± 0.68 vs 62.48 ± 0.28) and CRI (2004 vs. 2019; 94.72 ± 1.60 vs 100.88 ± 0.67), and a slight decrease in SKL (2004 vs. 2019; 54.29 ± 0.45 vs

55.24 ± 0,19), CHH (2004 vs. 2019; 24.57 ± 0.61 vs 22.42 ± 0.25) and CEI (2004 vs. 2019; 55.90 ± 0.88 vs 50.00 ± 0.37). In conclusion, very marginal changes in morphological traits have been found. Further morphological analyses are needed, including in the dataset working bloodlines, to supply objective descriptions of the breed phenotype aimed to preserve SH uniqueness.

P428

Preliminary result of argos-based satellite telemetry on migration of 4 song thrushes (Turdus philomelos) captured in Apulia Region

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The European Birds Directive (2009/147/EC) states that migratory game birds are not hunted ‘during their return to their rearing grounds’, therefore, for each huntable species, Member States shall assess the 10-day period (TDP) in which the pre-nuptial migration starts. For birds wintering in Europe, the onset of northward movements cannot be easily defined. In Italy, the song thrush (*Turdus philomelos*) is among the most representative game bird species hunted during December and January. The Italian Institute for Environmental Protection and Research (ISPRA) declares that thrush migration begins in the first decade of January, while the hunting associations report that the thrushes do not leave before February.

The present work focuses on the first year of study of the Argos-based satellite telemetry system applied on Song thrushes (*Turdus philomelos*) carried out in Puglia in order to obtain more information on the pre-reproductive migration period, on the migratory routes and on the areas of origin of the specimens wintering in Southern Italy. During the wintering period (mid-January 2022) 4 Song Thrushes were captured with nets in a large protected area near Laterza (TA) and promptly each of them was equipped with satellite transmitters. Among these, a non-functioning device was identified from the very first hours, therefore this subject was excluded from the trial. The other 3 transmitters worked regularly until the end of the observation period, set for mid-June. The three reported birds recorded their last presence in Italy on 23rd and 25th March and on 5th April, respectively. Two thrushes seem to have stopped to nest in Hungary (near the border with Ukraine, about 1100 km from Laterza) and in Romania (near the border with Moldova, about 950 km from Laterza), with movements of about 1 km, while the third one moved about 4000 km, also according to the North-East direction since arrive near Ural Mountains.

These preliminary results also provide indications of the efficacy and safety of satellite devices used for the study of song thrush migration. Furthermore, one of the devices is still in operation 11 months after its application, thus proving to be a promising device that will be under investigation during the whole winter season 2023.

Acknowledgements

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P430

Life Cycle Assessment (LCA) of an Italian Mediterranean Buffalo Farm: comparison between three feeding strategies

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The aim of this study was to identify the different environmental impacts that can be observed with three feed management strategies in the organic milk production of Italian Mediterranean buffaloes through Life Cycle Assessment. Data from an organic buffalo farm located in Caserta province were collected and three diet strategies with different proportions of self-produced and purchased feedstuffs and with or without the utilization of the ‘zero grazing’ technique were compared: standard diet based on maize silage and concentrate (Diet A); standard diet for 8 months and zero grazing utilization for 4 months (Diet B); standard diet for 4 months, ‘zero grazing’ for 4 months and haylage for 4 months (Diet C). An attributional approach and a cradle-to-gate perspective were considered, using 1 kg of Energy Corrected Milk as functional unit. The foreground system includes four activities: agricultural process (on-farm feed production), purchasing raw materials, transportation of all material to the farm and procedures in buffalo farm. Data for the background system is based on commercial databases, including EcoInvent (cutoff, v3.6) and Gabi professional, and the Environmental Footprint (EF) 3.0 method was used to quantify environmental impacts. Diet 1 yields the largest impacts in most categories, instead from acidification, ecotoxicity-freshwater, terrestrial eutrophication and particulate matter. On the contrary, the diet 3 outperformed the other scenarios in the remaining categories and it always showed smaller impacts with respect to diet 2. The shifting from diet 1 (Diet A) to diet 3 the farm can reduce the impact of milk production of about 40% in human toxicity-cancer, 14% in the eutrophication of fresh water. The hotspot analysis revealed no significant changes among the three diets: feed production is responsible for the majority of impacts in almost all the categories, while the transportation step becomes relevant regarding climate change, ionizing radiation, ozone depletion and resource use – fossils respectively for 11, 21, 30 and 20%. In conclusion, the application of feeding strategies based on the utilization of self-produced feedstuffs, in particular when the 'zero grazing' technique is applied, reduces the impact for most impact categories.

P437

Preliminary investigation of microbiome in Italian Honeybees (Apis mellifera)

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Honeybees *(Apis mellifera),* unlike other insects, show a highly conserved and specialized gut microbiome, primarily composed of host-adapted bacteria, facultative anaerobes, and microaerophiles that can be transmitted between individuals through social interaction. In honeybees the gut microbiota contributes to food digestion and fermentation of plant components. Furthermore, the bee gut microbiota has been shown to regulate the host immune system and to protect against pathogenic diseases. The aim of this study is to investigate the composition of the intestinal microbiota in a honeybee breeding population selected for 5 years for honey yield, hygienic behavior (HB) and gentleness (GN). To this end 45 colonies, belonging to the honeybee breeding population, were sampled 3 times from June 2021 to October 2021. In total 135 samples were analyzed. For each colony the gut of 10 worker bees were pooled, DNA was extracted and the microbiome was analyzed by sequencing the V3–V4 region in 16S rRNA gene using Illumina technology. Relative abundance analysis showed that *Firmicutes, Protobacteria* and *Actinobacteria* were the most prevalent Phyla, while the most frequent bacterial families were *Lactobacillaceae,* followed by *Orbaceae, Neisseriaceae, Bifidobacteriaceae, Rhizobiaceae* and *Enterobacteriaceae*. Future work will focus on the effect of microbiome composition on honey production and health related traits.

Acknowledgments

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P495

Understanding Medieval livestock diversity in Viterbo's Tuscia using aDNA data

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Osteological samples were selected from three sealed contexts in Tuscia, dated to the 14th century by stratigraphic position and archaeological materials: two are waste pits in urban (Cencelle) and rural (Celleno) areas, while the third (S. Valentino) is a bovine carcass burial from a former monastic settlement, later reused for agricultural purposes.

Different bones were selected from these archaeological sites to understand the Medieval livestock diversity in Viterbo's Tuscia. Most bones are deeply fragmented due to butchery techniques, so complete metapodials were selected to avoid contamination. The analyses were carried out in the ancient DNA (aDNA) Laboratory of DIBAF (Tuscia University) following stringent precautions, commonly used for this analysis.

DNA was extracted and quantified, genomic libraries were prepared and sequenced at a coverage of 1.5 Gb per sample (10Mreads PE da 150bp). A metagenomic analysis was carried out for an early identification of the species: *reads* were aligned with many livestock species reference sequences and the obtained results confirmed the morphologic indications of archaeozoologists through biometric indications. After that, each sample was aligned to the specific reference sequences. Further analyses were carried out to evaluate the damage pattern typical for aDNA, and thus exclude contamination of exogenous DNA, for example during the excavations or laboratory practices.

Subsequent analyses were aimed at determining the sex of the animals to better understand the exploitation mode of the period. Mitochondrial DNA (mtDNA) is usually less difficult to amplify than nuclear DNA, so variants on mtDNA and on Cytochrome B (CytB) were investigated in order to establish the haplogroup and thus the origin of the samples.

Taxonomic determinations confirmed the validity of morphometric criteria used to distinguish between sheep and goats. The predominance of females reverses the proportion displayed by the horncore morphology (M 67.9%; F 32.1%). The prevalence of males in cattle suggested a different type of exploitation: the use for meat of adult oxen possibly belonging to a breed not selected for agricultural work. *Sus scrofa* findings were inconclusive because they were degraded by the practice of double boiling, providing indirect confirmation of the species. It was possible to establish the haplogroup of only one sample of *Ovis aries*, which was found to belong to the HB haplogroup, the most common in Europe.

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Survey of swordfish (Xiphias gladius) in the Mediterranean Sea off Bagnara Calabra: focus on catch sizes

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The swordfish *Xiphias gladius* (Linnaeus, 1758) is found in the open waters of tropical, subtropical, and temperate oceans of the world, including the Mediterranean Sea.

Swordfish off Bagnara Calabra have been caught with harpoon since 200BC, when, in the summer, these animals come to the surface often in male-female pairs. In this work, we present the data of fishing season, from May to July, of the years 2020, 2021 and 2022 and related to swordfish caught by 2 feluccas and 17 longlines. The area of the studyfalls into the Division 37.1.3 of FAO Major Area 37 and included the Tyrrhenian Sea of the Strait of Messina, between the north-eastern coast of Sicily and the western coast of Calabria up to Bagnara Calabra. All the caught swordfish were subjected to measurement oftotal length (TL) and body weight (BW). A total of 1212 swordfish were caught, of which 908 males and 304 females, with a male/female ratio of 3–1.

Females had a mean TL of 238 ± 15 cm with a mean BWof 68 ± 18 kg, males had a mean TL of 200 ± 14 cm and a mean weight of 30 ± 11 kg, falling within the observed ranges for swordfish caught in these areas. The minimum BW recorded was 12 kg, respecting the minimum catch size of 11.4 kg indicated by ICCAT. In our study, the sex ratio was stable in the 3 years, and showed a value similar to that observed in other studies on Mediterranean swordfish, while differed from the ratios observed in waters around Taiwan and in the Atlantic Ocean (the sex ratio fluctuated from 0.4 to 0.7). Female swordfish are known to reach sexual maturity above 20 kg, so all the females caught in this study were sexually mature. This aspect is due to the characteristics of these fishing waters, defined as 'spawning areas' and these data are also confirmed by the high male/female ratio. In all years the fishing peak occurred in June (36% of the annual catch weight), while the lowest quantity was observed in May (about 29% of the annual total). Although the annual trend of fishing remained stable in the number of fish caught (401,408 and 403 for 2020, 2021 and 2022, respectively), in the 2021 catch there was a decrease of more than 1 t compared to the other two years (16.4, 15.2 and 16.5 t for 2020, 2021 and 2022). In 2021 the female BW was significantly lower ($p < 0.0001$) than in the other 2 year while the weight of the males did not show significant variations. More targeted investigations are needed to protect and valorise this natural resource in the Bagnara Calabra area.

P582

Vermicompost production by slaughterhouse waste

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Due to the great importance of the circular economy in agriculture, the objective of this project was to evaluate the possibility of using cattle slaughterhouse waste (SW) to produce vermicompost (VC). Blood (B), bone-meat (BM), obtained grinding heads and cannons, and ruminal content (RC) were used as substrates. Fresh SW have been mixed together (BM 11.5%, B 23.1% and CR 65.4%) and treated with a dryer machine (ENVICE), for 5 h (maximum temperature reached 120 °C). This treatment allowed to eradicate pathogenic microorganisms and to reduce the fresh weight by 60%. After the heat treatment, the SW were mixed with other substrates and treated with a daily inoculation of photosynthetic bacteria (Rhodopsudomonas capsulate) for 1 week, to stabilize the final matrix, before its administration to earthworms. Different combination between dried SW and other substrates were obtained: (A) 20% SW and 80% of mix of charcoal powder, sawdust, and manure (SW20); (B) 25% SW and 75% manure (SW25); (C) 50% SW and 50% manure (SW50). These mixes were separately administered to red earthworms (*Eisenia fetida and Eisenia andrei*) once a week for 4 months. Red earthworms were placed in manure beds inside plastic bins, then different VC from each bin were obtained. The different experimental VC have been compared to each other and with the control, which was obtained from bio-transformation of only bovine manure by red earthworms.

All VC were produced according to the law for chemical composition of 'composted mixed improver' (D.Lgs 75/2010). As the percentage of SW increased in the earthworms food, total organic carbon (from 21% to 28%), total nitrogen (from 1.9% to 3.01%), organic nitrogen, as a percentage of the total nitrogen (from 84% to 93%) and pH (from 7.1 to 8.2) increased. Moreover, humic and fulvic acids contents were higher in VC from SW25 and SW50 (14% and 15% respectively) compared to the VC from SW20 (10%) and control VC (13%). Mineral content was variable, but always within the limits allowed by the law.

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Genome-wide analyses elucidate the population structure of Noticiana sheep breed in the Mediterranean context

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Among livestock species, the sheep genetic resources have played an early major role in the Mediterranean area. Italy has a long history of sheep breeding and, despite a dramatic contraction in numbers, still raise several local sheep populations that may represent a unique source of genetic diversity. The Noticiana is a population of the southeast part of Sicily appreciated both for its dairy products and for its resistance to harsh environment. In this study, the high-density Illumina Ovine SNP600K BeadChip array was used for the first genome-wide characterization of 48 individuals of Noticiana sheep with the aim of investigating its diversity, the genome structure and relationship within the worldwide and Italian sheep breeds context. Moreover, the runs of homozygosity (ROH) pattern and the pairwise F_{ST}-outliers were examined in order to search for potential signatures of selection. Noticiana reported moderate heterozygoties (H_O = 0.336, H_E = 0.324), low levels of molecular inbreeding (F_{IS} = -0.035, F_{ROH} = 0.083), and a low value of contemporary effective population size (*Ne* = 26.2). The high percentage of short and medium ROH segments (93% under 4 Mb) is indicative of a within breed relatedness dating back to ancient times despite the absence of management for the mating plans and the reduced population size. Multidimensional scaling, model-based clustering, the measurement of population differentiation and the neighbor-net showed the clear separation of Noticiana from the rest of the Italian sheep, and highlighted its shared ancestral components with Comisana breed. ROH islands and F_{ST}-outliers approaches in Noticiana identified genes and QTLs involved in milk and meat production, as well as related to the environment-adaptation capacity, and therefore are consistent with the phenotypic traits of the studied breed. Although a wider sampling could be useful to deeper the genomic survey on Noticiana, these results represent a crucial starting point for the characterization of an important local genetic resource. Moreover, they will help designing and implementing conservation strategies in order to recover the Noticiana breed and exploit its products.

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The first semen cryobank in Europe for the protection of Mediterranean brown trout wild populations: the results over four years within the LIFE Nat.Sal.Mo project (Molise region. South Italy)

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The implementation of the first European semen cryobank of Mediterranean brown trout (*S. cettii*) was made possible by the LIFE Nat.Sal.Mo project, which aims to protect and restore the genetic integrity of the native populations of this priceless species that is on the verge of extinction. The interaction of different climate and human threats have severely harmed its biodiversity over the decades, however the introduction of alien trout represented one of the most serious threats to the native populations' intraspecific diversity. The main usefulness of the present cryobank is represented by its practical application to support the artificial reproduction activities aimed to maximize the genetic variability of the offspring and reduce the genetic introgression rate in the native populations of the project area.

From a practical point of view, developing an effective semen freezing procedure was the first step in setting up the cryobank. The fine-tuned cryopreservation protocol allowed for the storage within the cryobank of 1683 semen doses, from 150 native breeders. During the four years of the project, about 30% of these semen doses were used for artificial fertilization purposes. The present activities were accomplished directly on the riverbank by dividing the eggs from each native female into equal aliquots and fertilizing each of them with frozen semen from different males, following a cross-fertilization matrix scheme in order to increase the genetic variability of the offspring. Subsequently, the fertilized eggs were incubated at the hatchery until they reached the eyed stage. In order to elude the domestication of wild stock, the restocking of the suitable areas was carried out using eyed eggs with the 'artificial nesting' method. This technique assured a successful approach, therefore avoiding the exposure of larvae and early life stages to an artificial environment. This innovative technique resulted in excellent fertilization rates *in vivo*, which ranged from 64% to 81%, confirming also the efficiency of the sperm freezing protocol used and increase in the percentage of native specimens in the project's areas.

The remaining genetic resources of the cryobank represent supportive material both to ensure the sustainability of the project in the future, and to play a powerful multiplying effect which will affect other river basins at national and European levels where *S. cettii* populations are at risk of extinction.

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Italian semen cryobank of autochthonous chicken breeds: the case study of Siciliana breed

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The Siciliana is an ancient chicken breed of Southern Italy, originating from Sicily. It is distinctive for its typical double or rose comb. Due to the widespread use of high-performing commercial hybrids, the Italian poultry industry has experienced significant losses in terms of animal genetic resources over the past few decades. This erosion has affected many native genotypes, including the Siciliana. The populations of this native breed are raised in very small numbers, and they suffer inbreeding and a loss of genetic diversity. It is extremely worrying that only 186 individuals were found during a recent census. In fact, the Siciliana breed is listed as 'threatened preserved', and recently, actions have been implemented to preserve this breed within the project 'Conservation of biodiversity in Italian poultry breeds: TuBAvI-2'. Overall, the present project aimed to promote and support the conservation of the Italian poultry genetic resources through the combined application of *in situ* and *ex situ* strategies. The aim of this work was to describe the activities developed and the outcomes achieved within the creation of the first Italian Semen Cryobank of Autochthonous Chicken and Turkey Breeds, about the Siciliana breed.

Sixteen cockerels were raised in outdoor pens on a private breeding farm (Molise region), and after a training period ranging from 3 to 6 weeks, semen was successfully collected from eleven donors with the abdominal massage technique. The quality of each fresh ejaculate was assessed shortly after collection, considering sperm volume, concentration (photometric approach), sperm membrane integrity (SMI, flow-cytometry), and motility parameters (CASA system). Samples deemed suitable were processed and frozen; briefly, semen was diluted with freezing medium to reach a final sperm concentration of 1.0×10^9 sperm/mL and 2% of N-Methylacetamide and packaged into straws (0.25 mL).

The cryopreservation process negatively impacts the post-thaw sperm quality, in fact, we found a significant decrease in SMI (91.7 ± 0.5 *vs* 37.7 ± 1.5), total (91.2 ± 0.5 *vs* 26.3 ± 1.3) and progressive motility (26.7 ± 0.9 *vs* 3.3 ± 0.2). Throughout the project timeframe, 444 sperm doses, from 11 donors were stored in the cryobank. The implementation of semen cryobank for this breed, as well as for other autochthonous chicken breeds, provides an important connection with *in situ* strategies, to counteract genetic problems and prevent the extinction of breeds.

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Growth performances and carcass characteristics of Nero di Lomellina and commercial hybrid pigs: preliminary data

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In the 2020, the *Nero di Lomellina* pig, an autochthonous pig from the area of Pavia, has been recognized as reconstructed Italian pig breed by the National Pig Breeders Association (ANAS). No data on *Nero di Lomellina* growth performances and carcass characteristics are available. The aim of the study was to investigate the effects of the pig genetic type (*Large White vs Nero di Lomellina*) on growth performance and carcass characteristics. Ten *Large White* pigs and ten *Nero di Lomellina* pigs born in winter season were selected and were monitored till slaughter. The animals were weighted at different timepoints and the average daily gain (ADG) was calculated. At slaughter, seven animals per genetic type were selected and hot carcass weight, weight of green ham and loin were recorded. The live weight at birth, weaning and growing phase resulted higher ($p < 0.05$) in *Large White* than *Nero di Lomellina* pigs. At slaughter, the live weight was comparable ($p > 0.05$) in both genetic types. The carcass yield was about 83% and the ham weight was about 17 kg, with no difference between genotypes. The present preliminary data suggest that in the first phase of breeding there are some differences in growth performances between commercial hybrid pigs and *Nero di Lomellina* pigs. However, no difference in carcass weight and carcass characteristics were observed. Further data on *Nero di Lomellina* pigs are needed to confirm the present results and to evaluate meat quality parameters.

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Egg production traits during ageing in the chicken Italian breed Mericanel della Brianza

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Local chicken breeds are a vital source of gene reserves and their conservation has a technical role for the future advancement of the productive system, as well as a social cultural role. The aim of this study was to record the oviposition performance during ageing in the Mericanel della Brianza (MB) bantam breed population conserved at the Poultry Centre for the Conservation of Local Genetic Resources, University of Milan (Lodi, Italy). A total of 61 chicken breeders were selected and organised into 7 families (A-G) kept in floor pens in a controlled environment. Birds received a photoperiod of 15L:9D and were fed *ad libitum* a commercial breeder diet. Egg production (EP) and egg weight (EW) were recorded daily per family constantly from sexual maturity for two consecutive years. Weekly EP (%) and total egg production/hen/year (TEP/H) were calculated. Analysis of variance was performed on EW data considering laying cycle (LC), family lines (FL), month (Mo) and the interaction LC*Mo as sources of variation. Sexual maturity occurred at 24 weeks of age. In the first year (1Y) of age, the oviposition cycle showed a standard trend, while in the second year (2Y) of age, different laying peaks occurred. However, EP was limited and 36% was the peak reached in 1Y and 25% in 2Y. TEP/H was 72 eggs in 1Y and 50 eggs in 2Y. Analysis of variance showed that EW was significantly influenced by all the various factors considered: LC ($p < 0.0001$), Mo ($p < 0.0001$), FL ($p < 0.0001$) and interaction LC*Mo ($p < 0.0001$). The average EW was 34.5 g in 1Y and it significantly increased to 37 g in 2Y. EW increased progressively during the laying cycle in 1Y, while it was constant in 2Y. Mean EW values showed significant differences between FL. The lowest mean EW (33.5 g) was measured in FL-F and the highest (36.9 g) in FL-G. For the first time, the age at sexual maturity was accurately recorded and the oviposition rate studied for two consecutive years in MB hens housed in a controlled environment. The results are useful to improve the breeding management of MB laying hens in order to identify the optimal combination between age at the onset of sexual maturity and egg production.

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High-resolution genomic analysis of Sicilian chicken populations

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Italy counts a high number of local chicken populations, some of which do not have a recognized genetic structure. Two Sicilian populations named Val Platani (VLP) and Cornuta (COS) represent noteworthy local genetic resources. In this study, the genotyping data of 128 animals (50 COS and 78 VPL), obtained with the Affymetrix Axiom600KChicken Genotyping Array, were used with the aim to characterize the genetic diversity, the runs of homozygosity (ROH) pattern, as well as the population structure and relationship within the framework of other local Italian and commercial chickens. Analysis of within-breed diversity showed the two populations to have moderate variability in agreement with the estimated values reported for Italian chickens. The homozygosity hotspots, inferred from ROHs, harbored genes related to immune response and local adaptation traits. The results of among-breeds relationship and genomic structure reported a clear clustering of the populations according to their geographic origin, and showed all individuals grouped by population. The COS formed a non-overlapping genomic cluster and clearly separated from the other populations, but showed an evident proximity to the Sicilian breed (SIC) and its genetic influence. The VLP highlighted intermediate relationships between the COS-SIC group and the rest of the sample, but closer to the other Italian local chickens. Moreover, it showed a complex genomic structure, due to the presence of two sub-populations that match with the different origin of the samples. These results underlined the hypothesis of a defined genetic structure for Cornuta, which could be also the consequence of reproductive isolation, reduced gene flow and inbreeding. The neighbor-joining tree based on genome-wide allele sharing confirmed the differentiation among Sicilian populations, and highlighted the presence of sub-clustering linked to the different farms in which the two populations have been sampled. Although they are not conclusive, our current results represent a starting point for planning monitoring and conservation programmes.

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The artificial insemination for the conservation of the Sicilian honey bee (Apis mellifera siciliana)

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Apis mellifera siciliana Dalla Torre (AMS), the endemic honey bee subspecies of Sicily, is endangered by hybridization with other honey bee subspecies that are frequently imported in the island for productive purposes (i.e. *A. m. ligustica* Spin.). Its unique adaptation to the local climate makes the AMS deserving of preservation; however, the high polyandry of honey bee queens, which mate in flight with several drones from different genetic sources, makes the conservation challenging.

Artificial insemination (AI) is an essential tool that provides complete mating control of honey bees for research and breeding purposes. This technique can be used in conservation practices of native subspecies because it provides the capability to create targeted crosses in order to maintain the genetic integrity of populations.

In spring 2022, as part of the PLANT-B (PRIMA) EU Project, 18 different colonies located in Sicily were analysed, through a morphometry characterization, to the identification of strains with the highest belonging to the AMS subspecies, explained in terms of membership degree of each colony to the AMS subspecific traits. To investigate the validity of the AI as a technique to maintain the pure subspecies characteristics, 20 crossings between virgin queens and drones from the selected colonies were performed in July. Only three queens, belonging to two of the 18 colonies (43.8% and 50.0% of average membership) studied, were successfully fertilized. Missed insemination was due to unborn or dead queens within the nucleus before and after AI, probably because of the adverse environmental conditions that occurred in spring-summer 2022. The morphometric characterization carried out on F1 workers born by AI queens, showed in two cases a membership equal to those of the parental queen rather than the drones, while the value dropped for a colony (31.3%) with respect to the membership degree of the mother queen (43.8%). These preliminary outcomes suggest that worker morphometry is mainly influenced by the parental genotype of the queen compared to drones, but a more extensive study is essential to confirm it. Even if the success of the AI is strongly influenced by climate conditions and the positive result of breeding of virgin queens and quality drones, anyway, the advantages of this technique make it promising for the conservation of the subspecies. The search for high AMS membership mother queen is a prerequisite for further conservation actions.

P140

Phenotypic characterization of an endangered indigenous goat: Comune di Sicilia ecotype

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Characterization of indigenous livestock species is the key to the development of proper strategies for long-term maintenance and use of genetic variation and for guidance in decisions about future utilization and conservation strategies. Comune di Sicilia goat, counts about 500 adults, is bred in western Sicily under low-input management systems and contributes significantly to the livelihoods of resource-poor farmers as a source of protein and income, thanks to its typical dairy products. It is known for its unique adaptive traits, such as heat and drought tolerance, and low disease susceptibility, which make it ideal for production under stressful environments. However, it has received little attention, and, in fact, it is not currently recognized as a breed but only as a local ecotype. The aim of this study was to phenotypically characterize the Comune di Sicilia goat so as to collect all data needed for drafting a breed standard, which, together with genotyping, would allow its official recognition. Phenotypic traits for 78 adult goats (9 males, 69 females) included body weight (BW); withers (WH), croup (CH), and chest height (CHH); chest (CL), length (TL), and croup length (CHL); chest width (CW), hip breadth (HB), and coxofemoral width (CXW); and chest (CC) and shin circumference (SC). Qualitative traits included coat color pattern and type, presence or absence of horns, ears and wattles. Age was estimated based on dentition. Analysis of the morphological descriptors showed some sexual dimorphism (BW: males 54 ± 5 kg, females 43 ± 8 kg; WH: males 75 ± 2 cm, females 71 ± 3 cm; TL: males 81 ± 5 cm, females 73 ± 6 cm; CC: males 102 ± 4 cm, females 82 ± 6 cm), but overall, all parameters showed very little variability. As regards qualitative traits, badger-face was the prevalent coat colour, and there were subjects without horns (50%), ears (5%), and wattles (55%). A relative homogeneity was observed in Comune di Sicilia goats, as well as peculiar phenotypical features, such as diluted pheomelanin and the presence of horns, which might represent environmental adaptative traits. This comes together with an effective population size big enough to be exploited for future breed improvement.

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P142

External egg quality from Siciliana, a native chicken breed of the Sicilian Region (Italy) reared under free range conditions

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Animal biodiversity is an important component of all sustainable agri-food systems. The valorisation of typical products also represents a strategy for the preservation of local breeds. Aim of this study, taking into account the visual criteria of consumer choice, was to evaluate the external characteristics of eggs from the endangered Siciliana (SI) chicken breed compared to Livorno (LI) pure breed and Lohmann white (LW) commercial hybrid ones. No. 63 eggs (21 for each group) were sampled from 3 flocks of 2 local Italian breeds (SI and LI) and 1 commercial strain of LW, all reared in different farm under organic conditions. The following external quality traits were evaluated: egg's weight, length, width, circumference, shape index, shell percentage, and shell index. Eggshell color was determined using a colorimeter (Minolta CM 500) and results were expressed using the CIE L*a*b* system color profile. Furthermore, the image of each eggshell was acquired with a computer vision system (E-eye, Iris visual analyzer 400, Alpha MOS) and results were expressed using the RGB system color profile. E-Eye data were subjected to PCA analysis. As expected, the average weight of the 2 autochthonous breeds eggs was similar (SI =56.80 g; LI =61.23 g) and significantly ($p < 0.0001$) lower than LW eggs (70.33 g). Moreover, the width and circumference of SI group eggs (41.33 and 14.40 mm, respectively) were significantly lower than LI (43.39 mm; 15.04 mm) and LW (45.27 mm; 15.55 mm) groups; therefore, the shape index of SI group eggs (69.21%) was significantly ($p < 0.0001$) different from LW and LI (74.17% and 75.92%, respectively) groups. No significant differences were found for shell percentage and shell index. Regarding shell color, all parameters of SI group (L = 91.88; a = −0.26; b = 10.29) significantly differed ($p < 0.0001$) from LI (L = 94.36, a = −0.87, b = 6.83;) and LW (L = 94.45, a = −0.61, b = 6.07) groups. Results from E-eye showed 5 color codes (2184, 2439, 2455, 2456, 2712) founded only in eggshell of SI and the PCA plot has clearly separated the groups, showing SI at right side of the first component and LW and LI on the left one. Overall, the results, showing how the shape and color of the shell may characterise a breed directing consumers in their choices, can be also taken into account for the conservation and characterisation of a native Sicilian breed.

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P162

Morphological description of Montanina cattle, an old Sicilian (Italy) indigenous ecotype

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Sicily is rich in animal biodiversity and boasts a great variety of indigenous cattle: Modicana and Cinisara, officially recognized, and Montanina, a local ecotype not officially recognized.
The aim of this research was to study the phenotype in a nucleus of 32 *Montanina* adult cows from 3 local farms, located in Nebrodi areas, to identify the peculiar phenotypic traits to create the breed standard. The morphology characterization was made with estimation of weight and with 10 body measurements (expressed in cm), such as Withers Height (WH), Body length (BL), Chest Height (CH), Chest Width (CW), Chest Length (CL), Chest Girth (CG), Rump Length (RL), Rump width Ileum (RW_IL), Rump width Ischium (RW_IS), Hock Girth (HG). The descriptive statistics were calculated for morphological variables and the morphological tips was established through Body Index (BI: CW/CH) and Chest Index (CI: BL/CG). Phenotypically the cows have a red overcoat with an overall black hair coat and a reddish stripe on the back. The horns are white with black tip and have an average length of 27.48 cm (medium – long size). The average values of body weight is 494.55 kg and the average of body measures are 129.3 (WH), 158.4 (BL), 71.2 (CH), 46 (CW), 81.9 (CL), 182.4 (CG), 50.8 (RL), 54.2 (RW_IL), 39.2 (RW_IS) show a variability and this is confirmed by BI index in which some cows are mesomorphs (50%) while other are dolicomorphs (50%). Despite variability, the animals have good skeletal and muscular development. According to CI index, the cows are all mesomorphs indicating a good conformation well adapted to mountain environment. In conclusion, our results show that the Montanina cow has adapted to the difficult local environment and will also be able to adapt to future climate changes. The phenotype, like the coat colour and horns, the shape and the body size makes it unique and different from the other breed present in the territory, but further studies will be conducted to investigate the genetic structure of *Montanina* breed.

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P176

Management of Bagnolese sheep in Campania Region: animal and pasture characteristics

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The Bagnolese sheep, called Malvizza, is a Campania region native breed, with dual aptitudes. It stands out for its productions: lamb, prized for the tenderness of its meat, and milk, with a distinct dairy aptitude, transformed into the eponymous Pecorino cheese. The breed, the result of crossbreeding in which the somatic traits of the Barbaresca can be recognized, has about 7465 individuals and is mainly distributed in the Campania region in the Picentini mountains, Alburni, Vallo di Diano, and Sele plain, and marginally in the hills of Caserta and Benevento. The predominant type of breeding is semi-extensive pasture and feed supplementation is used during energy deficit periods (hot summer and winter); feeding of lambs, destined for replacement, consists exclusively of milk until they are 35–40 days old. The Project SAVEPEB has helped to collect updated information related to the management of this breed. It characterized the entire production cycle of pecorino Bagnolese to improve its competitiveness by implementing a specific quality workflow. Blood samples were collected for DNA bank and cytogenetic analysis from 97 female Bagnolese sheep. Every 15 days, milk samples were collected for the qualitative characterization. The average milk yield was 60.7 ± 17.1 liters/head calculated over 180 days of lactation, with an average fat content of 9.04 ± 1.2 % and protein content of 6.7 ± 0.5 %. Regarding pasture evaluation, representative forages were randomly collected in the identified grazed areas (No. 7 areas) during one year of collecting (No. 6 sampling from October to July). The botanical evaluation was carried out before drying and the samples were analyzed for chemical composition. Energy content was also estimated. Most of the grazing areas consist of legume grasslands or mixed natural pastures, where Leguminosae and Graminacea families are equally distributed. Other species were also identified in variable quantity. Protein and energy content reach relatively high value in some forages, whereas fiber level appear slightly higher compared to other investigation on natural pasture in Mediterranean area. Protein and lignin resulted the most variable parameters, maybe due to the different phenological stage of the plants at the

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sampling time or to the botanical families present. The seasonal variability must also be considered.

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P179

Mitochondrial control-region variation in European local sheep

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According to archaeological and molecular evidence, after domestication in the Fertile Crescent 10,000 years ago, sheep spread to Europe following the early farmers during two main migration events: the first and oldest migration involved sheep populations mainly raised for meat production, and whose current descendants are the tropical hair sheep and the Scottish Soay breed. The second migration from the Middle East to Southeast Asia, Africa, and Europe, instead, spread sheep stocks that had already started to be selected for wool production. To better understand the current distribution of diversity and the phylogeographic differentiation of European sheep populations, we investigated the genetic variation of the mitochondrial DNA (mtDNA) in 53 native breeds from nine European countries (Estonia, France, Germany, Italy, the Netherlands, Portugal, Spain, Sweden, and the United Kingdom). To this end, we examined 770 base pairs (bp) of the mtDNA control region in 625 samples collected in the framework of the EU-funded IMAGE H2020 project. Overall, 467 different haplotypes were identified. Molecular diversity indices showed high haplotype diversity ($h = 0.99$) but low nucleotide diversity ($n = 0.014$). Starting from the same dataset, a phylogenetic network was constructed after including reference sequences of the Asian (*O. orientalis*) and European mouflon (*O. orientalis musimon*), and of the known sheep mtDNA haplogroups (A, B, C, D and E) as a reference. The resulting graph showed that the European sheep haplotypes clustered partly with lineage A (ca. 7%) and mainly with lineage B (ca. 93%). The Analisys of MOlecular VAriance (AMOVA) and the calculation of between-breed Fst indices highlighted that most of the genetic diversity occured within breeds (91.83%) rather than between breeds (5.32%) or between countries (2.85%). Overall, our results suggest the occurrence of high levels of mtDNA variation and high gene flow rates among European native sheep and confirm the expected affiliation of most haplotypes with lineage B, the mitochondrial haplogroup most frequently found in Europe. Furthermore, despite the wide distribution range of the investigated breeds, i.e. from the Mediterranean area to Scandinavia, geography seems to contribute only moderately to the overall partitioning of diversity.

P233

Genotyping-by-sequencing of honey derived environmental DNA can retrieve information on the Apis mellifera subspecies

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Honey contains environmental DNA (eDNA) traces derived from all organisms that directly or indirectly contributed to its production or that have been part of the production niche and environment from which this matrix is obtained. We recently demonstrated that honey constitutes an easily accessible source of *Apis mellifera* DNA useful to retrieve population genetic information. We also recently demonstrated that honey bee mitochondrial DNA (mtDNA) specific lineages detected in the honey can be used to authenticate the entomological origin of the honey. In this study we analysed honey DNA and integrated honey bee mtDNA information with nuclear genome polymorphisms to set up an improved tool that can detect the honey bee subspecies using these two genome levels. To this aim, we designed and tested a genotyping by sequencing (GBS) assay to analyse 121 single nucleotide polymorphisms (SNPs) of *A. mellifera* nuclear genome using eDNA extracted from honey. Results were integrated with information derived from previous studies and whole genome resequencing datasets. Genomic analyses were obtained for 61 specimens (honey samples and honey bees) collected in a few Italian regions (Emilia-Romagna, Liguria and Sicily) and that included: (i) individual honey bees of the subspecies *A. m. ligustica, A. m. mellifera* and *A. m. siciliana*; (ii) groups of pooled DNA samples from more than 30 *A. m. ligustica* workers belonging to the same colonies from which honey samples (see below) have been collected; (iii) honey samples obtained from 32 single hives; (iv) undifferentiated honey samples produced from *A. m. ligustica* and *A. m. siciliana*. The GBS runs produced more than 53 million reads that were used to obtain genotype information of the selected bi-allelic SNPs. Allele frequency estimation combined with several multidimensional scaling approaches were able to correctly assign the honey to the honey bee subspecies that produced it with high correlations between samples and runs. Overall, results obtained from GBS demonstrated the possibility to use *A. mellifera* nuclear genome variability to authenticate the entomological origin of the honey by detecting the honey bee subspecies.

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P246

Comparative analysis of heterozygosity-enriched regions in Reggiana and Modenese genomes provides information on local cattle breed specific variability

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Reggiana and Modenese are dual-purpose cattle breeds mainly reared in the North of Italy and linked to the production of mono-breed branded Parmigiano-Reggiano cheese, which provides the economic income to the farmers that is needed for the sustainable conservation of these autochthonous breeds. The population size of these breeds experienced a drastic reduction in the 1980' and a subsequent slow recovery. Inbreeding is an important parameter that should be monitored to define appropriate conservation programs of local genetic resources. We therefore already evaluated inbreeding in these breeds using pedigree and genomic information based on runs of homozygosity (ROH). Hotspot regions of heterozygosity may be useful to define other relevant population genomic information. Runs of Heterozygosity (ROHet) are regions of continuous single nucleotide polymorphisms (SNPs) with heterozygous genotype. In this study, we obtained a genomic landscape picture of ROHet in Reggiana and Modenese cattle breeds and identified ROHet islands. A total of 2730 Reggiana cows and 564 Modenese cattle (almost two thirds of the actual population for both breeds) were genotyped with the GGP Bovine 150K SNPchip. Quality filters were applied with PLINK1.9. ROHet were identified with detectRuns R package. In total, 38942 and 7289 ROHet were identified in Reggiana and Modenese cattle populations, respectively. The average number of ROHet per animal in Reggiana was 14.24 ± 3.8, with a minimum of 1 ROHet and a maximum of 30. In Modenese breed, the average number of ROHet per animal was 12.91 ± 3.3, with a minimum of 1 and a maximum of 23. For Reggiana, the longest ROHet was on chromosome BTA10, with a length of 1029 kb, while for Modenese the longest ROHet was on BTA21 and measured 1228 kb. The total average size of the genome covered by ROHet for each animal was 2532.38 ± 858 and 2291.26 ± 781 kb in Reggiana and Modenese, respectively. These regions included many genes involved in fecundity, survival, and fitness-related traits that might be involved in defining breed genetic features.

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P253

The Agouti locus and coat colour in cattle: evaluating ASIP gene variability in local and cosmopolitan cattle breeds

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Coat colour in mammals depends on the relative amount of two types of pigments, eumelanin (black/brown) and phaeomelanin (yellow/red), which are in turn controlled by the *Extension* (*E*) and *Agouti* (*A*) loci. While *E* locus encodes the melanocortin-1 receptor (*MC1R*) gene which is involved in both melanins production, *A* locus encodes the agouti signaling protein (*ASIP*) gene that downregulates *MC1R* activity, resulting in phaeomelanin synthesis. According to the classical epistatic interaction between the *E* and *A* loci, wild type alleles at the *MC1R* gene would allow to express mutated alleles at the *A* locus. Cattle breeds have a large variability in coat colour. In this study we focused on two autochthonous breeds (Reggiana and Modenese) and a cosmopolitan breed (Holstein) with different coat colour: Reggiana has a classical red coat colour derived by the recessive mutated allele at the *MC1R* gene; Modenese is characterized by a white-pale grey solid coat colour; and Holstein usually has a spotted white and black coat colour with black derived by a dominant mutated *MC1R* allele. In this study we characterized the cattle *ASIP* gene region variability starting from whole genome sequencing (WGS) data obtained from Reggiana (n.50), Modenese (n. 10) and Holstein breeds (n. 50). We identified four insertion/deletions (indels) which might putatively affect *ASIP* gene regulatory regions. The indels have been also genotyped by end-point PCR and Sanger sequencing in a larger population of the same three breeds and other 10 breeds. The results revealed that the Reggiana and the Italian Holstein breeds were almost completely fixed for alleles that were almost absent in Modenese breed. Variability in the *ASIP* gene in this breed might be involved in determining its white coat colour. These results provide some first evidence on the elusive role of the *ASIP* gene in affecting coat colour also in cattle, similarly to what already reported in sheep and goat, where variants in this gene are associated with white coat colours.

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P291

Giant Grey rabbit breed: a preliminary study on reproductive traits

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Population size reduction and genetic erosion are aspects of concern in rabbit heritage breed conservation. An objective analysis of reproductive parameters of heritage breeds could provide effective tools for the definition of population management strategy. In addition, the inclusion of reproductive efficiency in selection plans could be very helpful in targeting conservation parameters on productive data. The aim of this study was to investigate reproductive traits in the Giant Grey rabbit (GGR) breed. Nineteen litters from three different rabbitries have been analyzed: origin of the breeders (BRO; 3 levels: 0 = homebred buck and doe, 1 = external buck or doe, 2 = external buck and doe), gestation length (GSL; dd), litter size (LIS; N; born alive), percentage of weaned kits (PWK; %; number of weaned kits/number of born alive kits), percentage of show prospect kits (PSK; %; number of selected kits for conformation show/number of weaned kits) were included in the dataset. Descriptive statistics, correlation (Pearson's) and ANOVA analyses were carried out using SPSS® (vers. 28) software. The GLM model included GSL, LIS, PWK and PSK as dependent variables and BRO as a source of variation. Post hoc Bonferroni test was used to investigate significant differences ($p \leq 0.05$) between means. The major part (57.9%) of the litter was produced by mating a homebred with an external breeder. Variable mean values (± std. dev.) are GSL (dd) 32.23 ± 0.832, LIS (N) 6.26 ± 2.05, PWK (%) 88.31 ± 13.29, PSK (%) 83.00 ± 25.22. BRO showed significant effects on PSK and kits out of external parent recorded higher values. Pearson's correlation analysis confirmed the most favourable choice of kits out of both external breeders for conformation competitions. Furthermore, a significant positive correlation between GSL and LIS was found. In conclusion, reproductive traits have been described in the Giant Grey rabbit breed for the first time and further monitoring may play a pivotal role for the implementation of conservation program and the improvement of production.

P309

Different exposure to heat waves in Italian small ruminant populations

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Last decades have seen a dramatic increase in the number and frequency of heatwaves (HWs), and according to climatic projections, the situation is going to worsen. HWs have a severely detrimental impact on livestock leading to reduced performance, impaired feed intake and immunity, pest outbreaks, and increased mortality. For this reason, we aimed to assess the HW exposure of Italian small ruminant populations.

Geographic data about 2022 goat and 4475 sheep farms, related to 47 and 66 breeds, were provided by the Italian Sheep and Goat Breeders Association (Asso.Na.Pa.). The hourly Temperature Humidity Index (THI) from 1950 to 2022 was calculated using ERA5-land. A HW was defined as a period of at least 3 days, between May and September, with a maximum daily THI exceeding the 99th percentile of the reference period (1950–1979). For each year, the number of HW days (nDays) and the average THI measured during the HWs were calculated. HW parameters were associated with all the farms using ArcGis Pro and then fitted in a model including year, breed, and their interaction as fixed factors.

The locations of the enrolled farms saw a significant increase in nDays (from 0.9 ± 2.0 in 1950s to 5.4 ± 5.7 after 2010) and THI (from 69.8 ± 5.3 to 71.8 ± 5.5), especially in Friuli-Venezia Giulia and Liguria regions, respectively. In particular, the mean increase was of 0.08 and 0.07 nDays and 0.02 and 0.01 THI points per year in goat and sheep farms, respectively. All farms showed a notable rise in nDays, with Lariana goat and Istriana-Carsolina sheep farms exhibiting the greatest average value and increase (+0.10/year and +0.12/year). No significant difference was observed among goat breeds in terms of yearly THI increase, though Grigia dei Monti Picentini farms had the highest mean value. Conversely, the year x sheep breed interaction was significant, with Noticiana farms having the highest average THI and Garessina farms the greatest increase (+0.04/year).

Many of the breeds exposed to increasing HW are small and reared in localized areas, and thus even more sensitive to changes in their environment. Knowledge of the climatic risks for local livestock populations is useful to identify both breeds to be carefully monitored or showing a good response to HW (e.g. long-selected in regions subjected to HW): indeed, studying them might allow to understand the mechanism underlying their adaptation and thus the most efficient measures to mitigate the negative effects of climate change.

P340

The Apennine Rock partridge (Alectoris graeca graeca) a taxon to conserve. But what genes and how to keep them ex situ?

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The Rock partridge (RP, *Alectoris graeca*) is a wild Galliform endemic to Europe. The species is of conservation concern (SPEC 1) and reported in the Annex 1 of 2009/147/EC Directive. Conservation efforts are needed, and appropriate *ex situ* farming protocols should be established. Such types of protocols should include: (i) a preliminary genetic characterization to select native genes, (ii) a breeding technique avoiding morphological and behavioural limits. At these scopes, samples of faeces and feathers from 69 wild specimens of Apennine RP (*A. g. graeca*) were collected. The DNA extraction and PCR amplifications were performed following validated protocols focusing on the D-loop control region. The samples matched to four haplotypes registered in GenBank (H3, H8, H24 and H10), and mutations in the interval between 431 and 896 bp of our improved sequences allowed for the identification of 7 longer haplotypes.

Aiming to assess the effect of the intensive rearing on some morpho-functional traits, live weight, tarsus length and depth, tarsus and head width, head, wing, and beak length, were recorded fortnightly from 14 days after hatching (DAH) up to 98 DAH on offspring reared with intensive (G1, 16 chicks) and wild-like (G2, 14 chicks) approaches. Since morphological changes normally occur in multigenerational captive birds, first generation chicks of a wild breeding pair showing H10 haplotype were involved. This was a forced choice given the unavailability of other wild birds.

Comparisons between groups, at different stage of growth, were performed by an unbalanced full factorial ANOVA and significant differences were declared for $p \leq 0.01$.

The G1 birds were constantly heavier than G2 ones, although the statistical significance was achieved only at 14 DAH (55.66 ± 6.65 mm vs. 49.16 ± 4.25 mm). The first signal of captive morphological changes has been recorded at 84 DAH, when G1 birds showed shorter heads (35.43 ± 1.24 mm) and thinner tarsi (4.45 ± 0.31mm) than G2 birds (36.84 ± 1.34 and 4.73 ± 0.29 respectively), as a possible result of limited physical and cognitive exercise in an artificial environment. Our results provide a list of Apennine RP haplotypes and suggest implementing semi-natural breeding protocols to avoid the occurrence of captive morphological changes and the unintended selection of this characters.

Further studies should be focused on other mitochondrial lineages and on how the breeding system affect behavioural attitudes of Apennine RP.

P449

SCALA-MEDI: investigation of genetic and epigenetic adaptation mechanisms in North African livestock

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The SCALA-MEDI project is characterising the genetic and phenotypic diversity of Mediterranean local breeds of sheep and chicken, to understand and exploit their ability to adapt to climate changes, harsh environments and diverse farming systems. The project is using new technologies for remote phenotyping of adaptation traits and genotyping. Moreover, techniques for exploring the genome methylation of animals reared in different environments are being used to study the role of epigenetics on adaptation ability. Biological samples, environmental, production and socio-economic data are being collected on local breeds from Tunisia, Algeria and Morocco, along sheep and chicken value chains. Dedicated questionnaires are being used to characterize farming systems and production chains. Genomic data will be analysed and compared to data produced in Italy and France to identify markers associated with adaptation and product identity. Breeding programmes will be developed to maximise the value of local breeds. Decision-making tools will be proposed to improve management strategies for Mediterranean livestock systems to face future climate change scenarios. Tools to manage breeding and crossbreeding strategies will be designed to improve sustainable production by maintaining diversity and exploiting adaptation abilities, and hence increase the value of local populations. This will promote on farm conservation and use of local genetic resources.

P475

Influence of age on testicular size and libido in creole rams from west of formosa (Argentina)

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Traditionally, sheep production in the semi-arid region of Formosa was linked to traditional and artisanal production systems, to a subsistence economy with low use of inputs. Creole sheep present important adaptive characteristics to the tropical climate, such as tolerance to heat, to ectoparasites, among others. Reproduction is one of the determining pillars in the organization of small ruminant production systems. In males, there may be variations in libido throughout the year, as well as in testicular size and seminal characteristics. Although there is information available on some reproductive variables of various sheep breeds, very little is known about their seasonal variation at different ages in Creole sheep from west of Formosa. For this reason, the objective is to determine the effect of the age of the rams (in young and adults) on the scrotal circumference and service capacity during autumn and winter. The study group consisted of 20 Creole rams from west of Formosa of different ages, 30 ($n = 10$) and 18 ($n = 10$) months of average age, belonging to the *ex situ* conservation nucleus of the provincial hut of the Validation Center of Agricultural Technologies (CEDEVA) of Laguna Yema, Formosa, Argentina. The variables analyzed were scrotal circumference (SC) and service capacity (PCS) during autumn and winter by means of descriptive statistics, and statistical inference by means of ANOVA and Tuckey's test ($p = 0.05$), using the age of the ram and season of the sheep as effects. The results obtained for SC were 27.75 ± 0.34 cm for 18-month-old males, while for 30-month-old males it was 29.9 ± 0.39 cm, with statistical significance ($p = < 0.001$). When considering the season, no differences were found in either of the two ages. In the analysis of the PCS, no effects of age or of the seasons studied were observed. Finally, it can be said that Creole sheep males have good libido and testicular size, both in the autumn and winter seasons of the CEDEVA localized in the semi-arid area of Formosa under natural photoperiod conditions, evidencing that, also at 18 months, they present optimal reproductive behavior. Despite being preliminary data and the desire to extend the sampling to the four seasons of the year, the present study contributes to the reproductive characterization of Creole male sheep from west of Formosa.

P476

Morphologycal description of Angora Chaqueño goats

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In the Chaco region (Argentina), there are goat herds that play a social, economic and cultural role, and which have never been registered in the FAO database (DAD-IS). This local genotype is referred to, by producers, as ‘Angora Chaqueño’ due to the existence of shaggy coats, like the Asian Angora breed. The objective was to contribute to the phenotypic characterization of Angora Chaqueño goats as a preliminary study to recognize that population as a local breed. 104 adult animals (does and bucks) belonging to 14 farms in the department of 25 de Mayo (Chaco province) were sampled, before the spring shed. Information of morphological characters of racial interest were collected following the recommendations of the FAO. With data obtained, descriptive analysis was carried out by frequency tables. The main coat color was white (96.9%), the rest was cream. Almost 96% individuals had long fibers on the thorax, back and hindquarters, the rest had long fibers on the hindquarters only. The fibers were 49.5% curls and wavy 50.5%. The animals have no long fibers in the low legs. The frontonasal profile was straight in 97.9% of the individuals, 86.9% had lateral ears, and 96.1% with intermediate-sized ears (>14 cm). The mucous membranes of the mouth and nostril were dark spotted in 81.9% of the animals, while in 17% were pink, and only 1.1% completely dark pigmented. Around 77% of the hooves had spotted color pattern, 8.8% white, and 4% completely black. Other characteristics of the head, included fringes in 42.6% of the animals and 83.6% had beard. The eyes color was 92.8% brown and 7.2% light blue. Horns were present in 94.1% of the animals, which were classified by their morphology in spiral shape (38.6%), arched shape (19.8%), and straight (35.6%). 93% of the horns had rings marks, and in some cases, they had longitudinal eumelanic lines. Wattles were observed in 15.8% of the individuals. The morphologic characteristics describe in this sample of the Angora Chaqueño population, are reliable enough to lead the future complete study of these animal to avoid the recognition as a local breed of the region where they are rise and to project appropriated conservation programs.

P508

Genetic diversity and population structure in creole cattle

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Unraveling the genetic diversity and population structure of Creole cattle is essential for its genetic improvement, conservation, and to better understand the mechanisms of adaptation to a variety of environmental stressors. Several cattle breeds were imported in South America from the Iberian Peninsula following the discovery of the New Word and later from other European Countries. More recently, indicine breeds were also imported from India and Pakistan. Imported cattle spread all over the continent and adapted, during centuries, to a wide diversity of climatic and farming conditions. To investigate the molecular diversity and demographic history of South American cattle, we collected 918 criollo samples from 17 different populations from eight South American Countries: Brazil ($n=97$), Panamá ($n=69$), Peru ($n=200$), Ecuador ($n=20$), Argentina ($n=184$), Colombia ($n=89$), Uruguay ($n=193$), and Venezuela ($n=53$). Data were genotyped with medium-density SNP chips, except Brazilian samples that were genotyped with the BovineHD BeadChip. Only markers shared by the two SNP chips were kept and, after quality control, 24,831 SNPs were included in the working dataset. Genetic diversity and population structure were assessed using multiple approaches. Observed heterozygosity ranged from a minimum of 0.3 (±0.21) in Criollo Curralerio from Brazil to a maximum of 0.392 (±0.16) in the Ecuadorenian population. Results from Principal Component Analysis and Neighbour-Net analysis mirrored the geographical distribution of the populations sampled across the continent. Admixture analysis was performed including in the dataset also Holstein, Nellore and N'Dama samples, as reference for European *B. taurus*, *B. indicus* and African cattle, respectively. A genetic contribution from African and indicine cattle was recorded in all South American populations, highlighting the admixed characteristic of these cattle. Preliminary findings suggest that South American cattle experienced recursive events of gene flow and admixture from different source populations during centuries. Further analyses will be performed to identify marker variants associated to adaptation to harsh environments.

P544

COVARAP project, valorization and conservation of Piedmont local chicken breeds. Egg production performance in different farms

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In recent years, consumers have become increasingly aware of the dramatic loss of genetic diversity. The replacement of local poultry breeds with commercial strains, selected to maximize their production, have drastically reduced the number of breeds. This is the reason why the poultry sector is increasingly interested in issues of biodiversity conservation and climate change. In Italy, several autochthonous poultry breeds are currently investigated in conservation projects focused on the preservation of their genetic and historic heritage. However, few studies have investigated the opportunities linked to the quality of their productions. Recently, consumers have shown growing interest in food biodiversity, defined as 'food identified at the taxonomic level below the species level, and underutilized or wild species'. Genetic conservation and food biodiversity valorisation are the main drivers of the COVARAP ('Conservation and valorisation of the autochthonous breeds of Piedmont') project. The aim of the project is to improve and enhance the livestock production of two local poultry breeds in the Piedmont (NW Italy) region, Bianca di Saluzzo (BS) and Bionda Piemontese (BP).

In 2023 all the data will be collected from three farms involved in the project. Productive performances (number of eggs layed /day and egg average weight) will be recorded for each farm during a whole laying cycle.

For egg quality parameter analysis, 10 eggs/breed/farm will be collected at 3 different times during BS and BP ovodeposition periods (T1: at the beginning, T2: at the middle and T3: at the end). After the collection, quality parameters (egg weight, width, length, yolk and albumen weight and percentage) will be assessed. Moreover, along with the traditional yolk colour evaluation, through the colorimeter, innovative devices will be used for the evaluation of the DSM YolkFan colour scale (The Digital YolkFan™, DSM). Results will be useful to create an innovative model, able to characterize and evaluate BS and BP egg quality and production performances in different environments. These data will be useful for the identification of the best strategies for the quality enhancement of BS and BP derived products.

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P152

Circular bioeconomy for olive oil waste and by-product valorization: effect of dietary enriched olive cake inclusion in Modicana cows feeding management on cheese quality

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The circular economy and bioeconomy can contribute to transitioning towards more sustainable production and consumption in the olive oil sector. During olive harvesting and subsequent oil production, large quantities of by-products are accumulated in a short time. Several studies have tested olive cake in animal feed to produce derived foods with improved nutritional profiles. Aim of study was to evaluate the effects of olive cake supplementation in the diet of Modicana dairy cows in terms of the quality of the Provola cheese obtained. A total of 24 multiparous Modicana dairy cows were randomly allocated, according to milk yield (MY), days in milking (DIM), and parity, to 1 of 2 dietary groups: the control group (CTR; MY: 12.2 ± 3 kg/d; DIM: 127 ± 17 d; parity: 3.3 ± 1.5) was fed with a conventional diet, without any integration, whereas the treated group (OC; MY: 13.4 ± 4 kg/d; DIM: 137 ± 25 d; parity: 2.8 ± 1.3) was fed with a conventional diet integrated 7% of dried and pitted olive cake. The chemical composition, fatty acid profile and polyphenol content was evaluated in Provola cheeses from CTR and OC groups. The total lipid content was 23% in CTR and 24% in OC samples. Compared with CTR, the fatty acid profile of OC Provola was characterized by lower content of C16:0 (31.07% for OC vs. 32.00% for CTR) and C14:0 (11.30% for OC vs. 12.10% for CTR) and greater content of C18:1 n-9 (22.40% for OC vs. 20.60% for CTR). In general, an increase in PUFAs as well as a decrease in SFAs was observed in OC Provola. These effects on the fatty acids profile of cheese are consistent with the findings of several authors regarding cheese produced by dairy ruminants fed with olive by-products. In addition, the content of total polyphenols in OC Provola was significantly higher than CTR (335 ± 10.65 vs. 170 ± 6.99 mg/kg, respectively).

To conclude, a Provola with functional characteristics would be gladly welcome and well appreciated by consumers. Therefore, the reuse of waste biomass could contribute to ensuring the wellness of people and the environment.

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Mapping the spatial distribution of agro-industrial co-products and sheep livestock farming in Italy

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The production of waste and agro-industrial residues has increased considerably throughout Europe. A huge portion of waste biomass has interesting compounds with nutritional properties and bioactive compounds and can be used as co-products. Sheep may be one of the main targets for the use of agro-industrial residues in animal nutrition. This species can valorise feedstuffs of low nutritional value by supplying various products (e.g. milk and meat). The aim of this study is to map the spatial distribution in Italy of several co-products deriving by tomato, carob, hazelnuts, citrus, olive, grape and pomegranate processing, in relation to sheep population to quantify their potential availability as diet ingredients. Database of National Veterinary Information System and the Italian National Institute of Statistics (ISTAT) have been consulted to obtain data on sheep distribution and agro-industrial waste, respectively, related to the period 2018–2021. The sheep population in Italy is 1054599 heads. Sardinia is the region with the highest sheep percentage (37%) followed by Basilicata, Sicily, Abruzzo, and Lazio (29%). Concerning the agro-industrial residues, Italy shows a heterogeneous distribution across the territory. Grape waste is the most represented with 1,557,572 tonnes, followed by citrus (1,339,523), olives (845,287), tomatoes (253,980), hazelnuts (57,057), carob (25,806), and pomegranates (10,730). Veneto and Puglia reported the highest amount of grape residues (40%), while Emilia-Romagna (12%) and Sicily (9%) the lowest. Olive cake was mainly produced in central and southern regions (74%), especially in Apulia, Sicily, and Calabria. Similarly, citrus by-products are mainly produced in Sicily and Calabria (60% and 24%, respectively). Otherwise, carob waste is produced only in Sicily, Lazio, and Apulia. Hazelnut waste is distributed throughout the country, Piemonte reporting 31% of hazelnuts residues, followed by Lazio and Campania. Pomegranate by-products seems to be evenly distributed in all regions, except Liguria, Piemonte, Valle d'Aosta, and Molise. Tomato pomace is also present in all regions, with Campania, Puglia, Emilia-Romagna, and Lombardy reporting 81% of the total tomato residues. In conclusion, the use of by-products in sheep feeding plan can be assumed given their wide distribution in Italy.

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P237

The concentration of water-soluble carbohydrates of the pasture grazed by lactating ewes might be a main cause of milk fat depression

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The aim of this research was to investigate the causes of milk fat depression in sheep milk, often observed in late winter early spring after the lactation peak. For this reason, 5 farms from the central region of Goceano of Sardinia (Italy) were selected. In these farms tank milk and pasture samples were taken weekly for 5 weeks (12 April 2021–7 May 2021). The supplements used to complete the diet were also recorded. Milk samples were and analyzed for fat, protein, urea and somatic cell count. Milk fatty acids were also analyzed by gas-chromatography. Pasture samples were subjected to compositional analysis to measure their CP, ash, fat and fiber. In addition, water soluble carbohydrates (WSC) were also measured.

The (%DM) chemical analysis highlighted high variability in the composition of the pasture (CP: 6.4–22.1%; NDF: 36.6–60.4%; WSC: 3.5–26.9%). Milk had also a very variable composition, based on the farm considered and week of sampling (Milk fat: 4.40–6.61%; Milk protein 5.13–5.81%; fat to protein ratio: 0.80–1.16%).

The data were analyzed to assess possible statistical associations between pasture and milk components.

Milk fat concentration resulted significantly and inversely associated to the content of WSC of the pasture (Milk fat, % milk =6.536–0.069 WSC, % DM; $r^2 = 0.47$; $p < 0.01$). However, no relationship was found between milk fat composition and the content of NDF, or of any other component, including ether extract, in the pasture. No association was also found between milk fat content and its concentration of trans fatty acids.

Based on these results, a retrospective study on pasture samples dominated by grass species collected during autumn and winter in sheep farms of Sardinia by our research group was carried out. The results showed that the average content of WSC was higher in winter samples than in autumn samples (21.5 vs. 3.5 % DM), with the maximum value of 36.2 and 6.4 % DM, respectively. The average content of CP and the fraction of soluble protein were higher in autumn than in winter samples (27.7 vs. 15.8 and 14.2 vs. 8.3 % DM, respectively), suggesting an inverse relationship

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between pasture WSC and CP content. In contrast, NDF content varied very little (35.8 vs. 37.4 % DM in winter and in autumn samples, respectively).
In conclusion, these results suggest that excess of dietary WSC more than the lack of NDF can be the cause of the milk fat depression often observed in dairy ewes during late-winter, early spring.

P275

Influence of olive cake supplementation on the gut microbial communities in dairy cows

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The use of agro-industrial by-products as feed supplements in animal nutrition represents a strategy to reduce feeding costs and satisfy the nutritional needs of livestock. Particularly, the use of olive by-products, such as olive cake (OC), became widespread as a feed supplement since they are rich in nutraceutical molecules with antioxidant and antimicrobial features, including polyphenols. In this context, the aim of the study was to investigate the effect of the dietary olive cake supplementation, on both composition and dynamics of the faecal microbiota of dairy cow. A total of 18 lactating cows were allocated, according to days in milking, parity, and milk yield, into 1 of 2 dietary isoenergetic and isonitrogenous treatments: control group (CTR) was fed a conventional concentrate, whereas treated group (OC) was fed a concentrate with 8% of OC integration during 28-d experimental period. Concentrates were isonitrogenous (20% of CP/kg DM) and isoenergetic (1.05 UFL/kg DM). Genomic DNA has been extracted from faecal samples and the 16S bacterial gene has been sequenced with Illumina Miseq and raw reads have been processed using QIIME2 pipeline. 10 phyla, 51 families and 78 genera were detected in the control samples whereas 10 phyla, 44 families and 78 genera were detected in treatment samples. Results showed that *Bacteroidota* and *Firmicutes* were identified as the dominant phyla, accounting for over 90% of the total bacterial population. The *Desulfobacterota* phylum, able to reduce sulphur compounds, was detected only in faecal samples of OC cows, whereas the *Elusimicrobiota* phylum, a common endosymbiont or ectosymbiont of various flagellated protists, was detected only in CTR cows. In addition, both *Oscillospiraceae* and *Ruminococcaceae* families were found only in the OC group and their growth can be associated with the presence of the polyphenols content in the diet. These two families are able to ferment complex plant carbohydrates and to produce short chain fatty acids. In conclusion, the predominance of *Firmicutes* and *Bacteroidota*, associated positively to promote the health, and the exclusive presence of *Oscillospiraceae* and *Ruminococcaceae* in the experimental group, confirm that olive cake is a valuable feed supplement for cow's nutrition.

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P284

Characterization of polyphenols and antioxidant activity of olive by-products using for animal feed

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The interest on formulating diets of ruminant with bioactive compounds has increased over the last years with the aim to add health benefits. Olive oil industry generates relevant amounts of olive pomace that represent a by-product rich in high-value compounds (e.g. dietary fiber, unsaturated fatty acids, polyphenols) that could meet this purpose. In the present study, the bioactivity of three different batches of olive pomace (OP1, 2 and 3) was evaluated. The samples were extracted with Soxhlet for 5 h to fall, using 200 mL of pure methanol. The obtained solutions were then evaporated with a Rotavapor (Buchi B-490) and finally the extracts were characterized for their profile in phenolic compounds (hydroxytyrosol, tyrosol, oleuropein, ligstroside, carnosol, myricetin, luteolin and apigenin) through HPLC-DAD. The antioxidant capacity of the extracts was evaluated according to 2,2-diphenyl-1-picrylhydrazyl, D9132 (DPPH assay), Ferric Ion Reducing Antioxidant Power (FRAP assay) and 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS diammonium salt assay) methods; total phenol content and reducing activity was evaluated by Folin-Ciocalteau assay, using in all tests a multi detection plate reader (FLUOstar Omega, BMG Labtech). Tyrosol (222.9 ± 1.8^{a} *vs* 46.7 ± 2.5^{b} *vs* 0.2 ± 0.5^{c}), hydroxytyrosol (224.6 ± 0.4^{a} *vs* 36.9 ± 0.5^{b} *vs* 4.9 ± 0.3^{c}) and luteolin (559.9 ± 2.7^{a} *vs* 474.1 ± 1.7^{b} *vs* 354.7 ± 3.2^{c}), expressed as μg of compound/g of extract, resulted the most represented phenols in OP1, OP2 and OP3, respectively. Phenolic profile showed significant differences among the three samples, even if most compounds were still present, with a different distribution between them, depending on single molecules. Accordingly, the three OP samples showed different antioxidant activities and the highest was recorded in OP1 (124.6 ± 4.2^a *vs* 55.6 (4.1^b *vs* 19.5 ± 1.2^c, 44.5 ± 2.5^a *vs* 19.6 ± 2.4^b *vs* 9.22 ± 0.7^c, and 74.6 ± 3.5^a *vs* 31.5 ± 1.5^b *vs* 6.3 ± 0.07^c for ABTS, DPPH and FRAP, expressed in Trolox Equivalent ± SD, respectively), likely due its higher tyrosol content. The olive pomace application in the animal food industry could be a suitable strategy to obtain safe multifunctional ingredients such as dietary fiber and polyphenols with higher health-promoting effects.

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P333

Nutritional characteristics of innovative feed containing by-products from high-amylose wheat

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New feed ingredients from vegetable co- or by-products can help increasing the sustainability of the feed industry and of the entire livestock sector. The aim of this study was to investigate the nutritional characteristics of pelleted feed containing by-products from an innovative high-amylose wheat genotype to be used for ruminants' nutrition.

Bran and middlings from a bread wheat line (Cadenza HA), characterised by a high content of amylose (>78%) obtained by silencing the *SBEIIa* genes, were mixed to replace, in the same quantities, the conventional counterparts to obtain pellets for dairy ruminants. Individual by-products and pelleted feed were analysed for chemical composition and evaluated *in vitro* using the gas production technique with buffaloes' ruminal *inoculum*.

Both by-products and pelleted feed showed interesting nutritional characteristics, such as crude protein content (13.8%, 12.8% and 17.5% on a dry matter basis for bran, middlings and pelleted feed, respectively), that were similar ($p > 0.05$) to that of non-mutant wheat (15.8%, 18.2% and 19.5% on a dry matter basis for bran, middlings and pelleted feed, respectively).

The *in vitro* gas production parameters at 48 h of incubation showed a high fermentability and potential gas production (OMD equal to 73.59%, 85.57% and 82.09% and OMCV equal to 278.5 mL/g, 350.5 mL/g and 281.0 mL/g for bran, middlings and pelleted feed, respectively).

The fermentation kinetics clearly indicate that the tested by-products undergo a delayed fermentation compared to their conventional counterparts. These results could be due to elevated content of fibre (resistant starch and arabinoxylans) in the high amylose bran and middlings.

According to these preliminary results, it is possible to hypothesize that the tested co-products could be used to replace traditional ones and can express a greater nutritional potential. Further studies are necessary to evaluate the best inclusion level into the diets for ruminants.

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Effect of two different forage sources (oat hay vs. alfalfa hay) fed during pregnancy on the performances of Sarda ewes during early lactation

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Late pregnancy and early lactation are very critical stages for ewes, due to their high requirements and low voluntary dry matter intake (DMI), which might lead to metabolic disorders during pregnancy and subsequent low milk production. The utilization of high-quality forages might limit these risks. Thus, the objective of this study was to evaluate the importance of the quality of forages fed during the pregnancy on the performances of the ewes during early lactation. Twenty-eight pregnant Sarda dairy ewes were divided into two groups, balanced for body weight, days in pregnancy, and expected litter number (mean ± SD; BW, 54 ± 8.7 kg; DIP 115 ± 2; 1.24 litter number). The experiment started 30 days before the expected pregnancy and ended at 30 days of lactation. During pregnancy, one group received ad libitum dehydrated chopped alfalfa hay (AAH; 14.8% CP, 53.3% NDF, 8% ADL, DM basis) and a fixed supplementation of 440 g/d of whole corn grains, while the other group received ad libitum oat hay (OH) (9.6% CP, 65.6% NDF, 4.9% ADL, DM basis) and a fixed supplementation of 350 g/d of whole corn grains and 140 g/d of soybean meal (used to balance the CP of the diet), DM basis. After lambing, all the ewes were fed the same diet, made by the pregnancy alfalfa dehydrated hay fed ad libitum, plus a fixed concentrate supplementation (440 g/d of whole corn grain +135 g/d of soybean meal, DM basis). A factorial design with feeding treatment, time and their interactions as fixed factors and animals as random factor was applied.

The diet fed during pregnancy affected the voluntary DMI of hay (OHpreg =0.702 vs. AAHpreg =1.023 kg/d, $p < 0.01$) and diet (OHpreg =1.161 vs. AAHpreg =1.431 kg/d, $p < 0.01$) during this stage. It also effected the voluntary intake of hay (OHlact =1.336 vs. AAHlact =1.438 kg/d, $p < 0.01$) and diet (OHlact =1.873 vs. AAHpreg =1.992 kg/d, $p < 0.01$) during early lactation, despite in this stage all ewes received the same alfalfa hay. Milk yield (OHlact =2.345 vs. AAHlact =2.477 kg/d, $p < 0.05$) and milk fat content (OHlact =5.08 vs. AAHlact =5.47 kg/d, $p < 0.05$), but not milk protein content, were also affected by the diets fed during pregnancy. Regarding the energy balance, the diet had an effect during pregnancy (OHpreg = –0.520 vs. AAHpreg = –0.120 Mcal/d, $p < 0.001$) but not during lactation (OHlact = –0.205 vs. AAHlact = –0.260 Mcal/d).

This study highlighted the importance of feeding high quality forage during the pregnancy of dairy ewes on their subsequent milk production.

P384

Effects of three forages of different quality on voluntary intake and milk production of Sarda ewes during mid lactation

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The importance of forage quality on the intake and milk production of dairy cattle has been deeply explored in dairy cattle, while quantitative information is lacking in lactating dairy ewes. Previous studies have shown that the lactation persistency of dairy ewes in mid-lactation is negatively affected by the utilization of medium-high doses of sugars and starch, while is positively affected by the utilization of highly degradable fiber sources. For this reason, the choice of the appropriate forage sources is particularly important.

Thus, this study evaluated the productive effects of three different forage sources fed ad libitum. Twenty-one Sarda dairy ewes in the 5th month of lactation were divided into three groups balanced for DMI, milk yield (MY), body weight (BW) and BCS, mean ± SD; DMI 2.22 ± 0.05 kg/d MY 2.00 ± 0.03 kg/d; BW 53.9 ± 2.04 kg; BCS 2.98 ± 0.08. One group received dehydrated chopped oat hay (OAT; 7.3% CP, 63.9% NDF, 38.5% ADF, 4.78% ADL, DM basis) ad libitum and a supplementation of 176 g/d of whole corn grains and 360 g/d of soybean meal, DM basis. The second and third group received dehydrated chopped alfalfa hay of medium quality (AMQ; 19.7% CP, 43.5% NDF, 43.8% ADF, 6.48% ADL, DM basis) and high quality (AHQ) (23.5% CP, 39.0% NDF, 43.3% ADF, 5.8% ADL, DM basis) ad libitum, respectively, with a supplementation for both cases of 528 g of DM/d of whole corn grains. The experimental period lasted 21 d. A factorial design with feeding treatment, time and their interactions as fixed factors, animals as random effect was applied.

The diets affected the voluntary intake of hay (OAT =0.702 vs. AMQ =1.551 vs. AHQ =2.199 kg/d, $p < 0.001$) and voluntary DMI (OAT =1.289 vs. AMQ =2.085 vs. AHQ =2.733 kg/d, $p < 0.001$), NDF intake per day and as % of BW (OAT =0.587 vs. AMQ =0.738 vs. AHQ =0.903 kg/d, $p < 0.001$) and (OAT =1.14 vs. AMQ =1.33 vs. AHQ =1.68 NDFI%BW, $p < 0.001$), respectively. Milk yield was also affected by the diets (OAT =1.491 vs. AMQ =1.755 vs. AHQ =2.028 kg/d, $p < 0.001$). Milk quality was affected by the diets for milk fat percentage (OAT =7.24 vs. AMQ =6.93 vs. AHQ =6.44 %, $p < 0.01$), milk fat yield (OAT =105 vs. AMQ =116 vs. AHQ =125 g, $p < 0.05$), milk protein yield (OAT =83.2 vs. AMQ =96.2 vs. AHQ =110.8 g, $p < 0.01$), and urea (OAT =32.4 vs. AMQ =35.9 vs. AHQ =41.3 mg/dL, $p < 0.001$). This study highlighted the importance of feeding high quality forage during the mid-lactation phase in order to maintain the persistency of lactation and improve milk quality parameters.

P444

The effects of copper nano oxide and prebiotic on growth performance and antioxidant activity in Holstein suckling calves

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The aim of this study was to investigate the effects of copper Nano oxide and Celmanax prebiotic on growth performance and some blood parameters in Holstein suckling calves. 28 Holstein calves with an average age of 1 to 8 days and an average weight of 39.1 kg were used in a factorial experiment in a completely randomized design with 4 treatments and 7 replications. Experimental treatments included: (1) Basic diet without additives, (2) Basic diet +4 gr of prebiotic (PBC) per day, (3) Basic diet +15 ppm nanoparticles of copper (NCU) per day, (4) Basic diet +4 gr of PBC and 15 ppm NCU per day. The Calves fed twice a day: in the morning (8:00 am) and evening (18:00 pm). Blood samples were collected from jugular veins on d 30 and 65, approximately 3 h after the morning feeding and transported to the laboratory. Results showed that the daily weight gain and final body weight were higher in prebiotic supplemented calves than control group ($p < 0.05$). The plasma Cu concentration was greater ($p = 0.05$) for calves fed with NCU (138.4 µg/dl), than for the control group (109.8 µg/dl) on day 65 of the experiment. Total antioxidant activity and glutathione peroxidase were not affected by dietary supplementation of NCU and PBC ($p > 0.05$) but inclusion of NCU in the ration improved ($p < 0.05$) superoxide dismutase concentration on d 30 and 60 of the experiment.

KEYWORDS: Antioxidant; celmanax prebiotic; copper nano oxide; suckling calves

P456

Influence of supplementing bakery by-products and cocoa bean shells to a grass-based diet on feed intake, milk production and ruminal fermentation of early-lactating dairy cows

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Food leftovers from bakery industries (BBP) and cocoa bean shells (CBS) represent losses of food that are normally wasted despite their high energy and polyphenol content. Their use in ruminant nutrition could enhance the re-use of organic losses and convert them into valuable feed ingredients. This study aimed at evaluating if BBP and BBP containing CBS can replace most of a cereal-based concentrate in a herbage-based diet fed to dairy cows in early lactation without negative consequences on the cows' ruminal fermentation and production.

We used 17 early lactating Holstein and Red Holstein cows for 6 weeks, including a baseline measurement (day before start), a 2-wk adaptation (P1) and a 4-wk sampling period (P2). The cows were fed ad libitum freshly harvested herbage and were assigned to one of three concentrate types: (i) a control concentrate (CON), (ii) a concentrate including 55% BBP (BP-) and (iii) a concentrate including 55% BBP and 1% CBS (BP+). The concentrates were similar in protein and net energy contents and were offered according to a fixed allocation scheme from 5 kg in P1 to 6 kg in P2. Feed intake and milk yield were recorded daily, and milk composition twice weekly. Reticular pH was measured continuously (SmaXtec, Graz, Austria). Linear mixed models included the random effect cow and the fixed effects period, concentrate type, their interaction and – for feed intake – baseline herbage intake (R, package lme4).

In P2, CON and BP- cows ingested more concentrate than BP + cows ($p < 0.05$). Across periods, CON cows ingested less water-soluble carbohydrates and more starch ($p < 0.05$) than BP- and BP + cows, which was more pronounced in P2 than P1 ($p < 0.05$). Milk yield decreased from P1 to P2, which was less pronounced feeding CON and BP- than BP+ ($p < 0.05$). In P2, milk lactose percentages were greater in CON than in BP- and BP + cows. Across all periods, the range of the reticular pH of CON cows was smaller compared to BP- and BP + cows ($p < 0.05$). The mean, min and max pH increased from P1 to P2, which was less pronounced feeding CON and BP + than BP- ($p < 0.05$).

Feeding BBP increased water-soluble carbohydrate and decreased starch intake. The relationship between the BBP-related increases of reticular pH and pH range and rumen health needs to be further investigated. The lower concentrate intake and milk yield of cows receiving CBS need further investigation regarding possible anti-nutritional effects of CBS-derived tannins.

P492

Microplastics contamination in dairy cows' total mixed ration

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The demand for milk and meat is continuously increasing with the growth of world population, that is expected to reach about 9.7 billion by 2050. Ruminants are one of the main sources of these animal products, thus boosting their intensive livestock. However, ruminants are exposed to a huge number of contaminants, with potential risks for humans. Among contaminants, plastic materials are probably the most common. Low-density polyethylene (LDPE) and polyamide copolymer (PA-LDPE) films are widely used to cover horizontal silos and to wrap and tie bales; veterinary medicine containers are made of high-density polyethylene (HDPE). In addition, plastics from atmospheric deposition should be accounted in feed contamination studies. Plastics undergo to a systematic fragmentation driven mainly by ultraviolet radiation (UV) and mechanical abrasion, thus generating small plastic fragments (<5 mm), called microplastics (MPs). Recent studies have shown that MPs can potentially contaminate animal feeds, as confirmed by their presence in cow blood and sheep feces.

Aim of this work was to verify the presence of MPs in a total mixed ration (TMR) for dairy cows using a protocol specifically optimized to extract 5 polymers: LDPE, HDPE, PA, polyethylene terephthalate (PET) and polystyrene (PS).

The TMR was sampled in 3 different points of the feed lane of a dairy cattle farm located near Torino (N-W Italy). No plastic disposables or materials were used for sampling or analysis. Samples were dried at 60 °C overnight and grounded with a mill (5 mm sieve). The MPs extraction was done with an environmental

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control blank using a basic digestion followed by Fenton reactions. MPs were finally recovered and quantified using a stereomicroscope.

An average of 17 MPs/g was found in the TMR. Most MPs were fibers of different colors (colorless, black, red and blue). The length ranged from 0.201 mm to 3.734 mm.

In this trial we extracted for the first time MPs from dairy cows' TMR and we verified the MPs presence in the animals diet. The incidence of MPs contaminations of TMRs and feeds (corn silage, hay and concentrate) is under investigation in different farms, as part of a larger project for the optimization of MPs extraction protocol of feeds and animal diets.

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P498

Effects of an electrolyte, antioxidant and osmolyte blend supplement on milk yield and cheesemaking properties in Holstein lactating cows during warm season

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The negative impact of heat stress (HS) on performances can be reduced by selecting for HS resistance and/or, for more immediate effects, by adopting feeding strategies. The study aimed at evaluating the effectiveness of additive supplement on milk yield and cheesemaking properties in Holstein dairy cows during hot season. The experimental trial was carried out under field conditions at the facilities of a dairy farm. The farm consisted of 600 Holstein lactating dairy cows. Cows were milked 3 times daily (05:30, 13:30, 21:30 h). The study lasted 98 d (June–September 2022): 14 d were used as adaptation period and the last 84 d as experimental period. Eighty-two healthy lactating Holstein cows, in the early lactation phase, were selected according to lactation number, days in milk, milk yield (MY) and body condition score. The animals were split into 4 balanced groups of 20 (groups 2 and 4) and of 21 cows (group 1 and 3). The four groups were then randomly allocated to 2 different experimental groups: (1) cows fed only with total mixed ration (TMR), control groups (C; groups 2 and 3); (2) cows fed with TMR + Bovine BlueLite® Pellets Max (BBL) additive (TechMix Europe SL, Spain), treated groups (T; groups 1 and 4). The dosage of BBL additive was 150 g/head/d. The additive was added directly in the mixer wagon and homogeneously distributed over the entire feeding lane. Temperature and relative humidity of the inside barn were recorded daily during the study period using electronic probes connected to a data logger. Feed intake and TMR samples were collected every 2 weeks for each of the four groups. Daily MY was recorded electronically at each milking and summed daily. Individual milk samples were taken every two weeks from all the three milking and packaged in 50 mL plastic tubes containing Bronopol®. Milk characteristics and milk clotting properties were determined. Rumination time, eating time, heavy breathing time and activity pattern were monitored continuously in all cows using wearable sensors (SenseHub® Dairy). Inclusion of BBL improved MY (44.7 vs 44.1 l/head/d, $p < 0.01$) reduced rennet clotting time (20.6 vs 22.6 min, $p < 0.01$) and increased titratable acidity (6.4 vs 6.1°SH/100 mL). Treated cows spent less time in overheat and eating and have shown a propensity for a minimum motor activity associated with a longer low activity time compared with C cows. Those changes clearly show a mitigating effect of treatment in heat stressed lactating dairy cows.

P503

Effects of early gestation maternal energy restriction in dairy heifers on the growth performance, starter intake, apparent total tract digestibility and gastrointestinal weight of dairy calves

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We tested the hypothesis that early gestation maternal energy restriction in heifers will reduce growth performance, feed intake, apparent total tract digestibility and gastrointestinal organ weights of dairy calves. Holstein Friesian heifers ($n = 28$; BW ± SD; 371.57 ± 42.14, age ± SD; 15.7 ± 1.08 months) were divided into three groups based on body weight and age and, from 11 days before artificial insemination were individually fed as following: (i) 0.6 M of their maintenance energy requirements

(M) until day 80 of gestation (nutrient restricted NR80, $n = 11$), (ii) 0.6 M until day 120 of gestation (NR120, $n = 11$), and (iii) ad libitum (1.8 M) until day 120 of gestation (Control, $n = 6$). Heifers were group fed once the individual feeding period ended, with ad libitum access to feed until calving. Twenty-two single female calves were retained after calving. All calves received the same management and diet until slaughter (BW ± SD; 117 ± 13). Daily individual intake was recorded, and body weight and other body measurements were monitored every fourth night until weaning. Calves were housed in a single pen post weaning and received the same management and diet until slaughter. Calves were euthanised at d 135 ± 3 to obtain organs weight. Data was analysed in R with One-way ANOVA and mean contrast separated with Tukey post-hoc test. At birth, calves born to control fed heifers were heavier than NR80 calves ($p = 0.01$) and tended to be heavier than NR120 calves ($p = 0.11$). Similarly, daughters from control fed heifers had larger thoracic circumference than their counterparts ($p = 0.049$). However, these differences disappeared as calves aged. Despite the low birth weight, NR80 calves had the highest ($p = 0.03$) starter intake compared to control and NR120 calves, respectively. However, apparent total tract digestibility remained similar among all calves throughout the pre and post weaning phases. Although, nutritional management during early gestation had no effect on calves ADG, slaughter weight, DMI prior to slaughter, lower gut and foregut weights and total GIT weight, duodenal weight tended to be heavier in control calves than their peers ($p = 0.07$). Results suggest that altering maternal energy in heifers during early gestation reduces birth weight in progenies, however, these animals show capacity to undergo catch up growth. Continued research is necessary to investigate if the no difference in organ weights persists in a long-term.

P138

Replacement effect of dietary vitamin E with different source of polyphenols on growth performance and vitamin E status in post-weaning piglets

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Vitamin E has a vital role as an antioxidant and immunomodulator in animal species. Dietary supplementation with high levels of vitamin E is usually recommended in post-weaning piglets, when they show a decline in serum vitamin E concentration, reduced growth rate and susceptibility to stress. However, due to its synthetic nature and uneven distribution in tissues, the researchers continuously search for cost effective natural alternatives, and polyphenols, due to their antioxidant properties, could be such an alternative.

Therefore, the present study aims to investigate the partial replacement effect of vitamin E as suggested from additive company and literature in broiler with different sources of polyphenols on growth performance and serum vitamin E concentration in piglets.

Piglets ($n = 350$, body weight (BW) 7.20 kg), balanced for sex and BW were randomly assigned to seven experimental dietary groups: CON$^-$ (401 mg/kg vit E); CON$^+$(175.8 mg/kg vit E); groups C, D, E, F and G containing each one 87.9 mg/kg vitamin E + products C (citrus and tannins) D (grape pip soluble), E (dry grape extract and carrier), F (freeze-dried melon juice and flesh, palm oil and microcrystalline cellulose), G (onion soluble and grape soluble) vitamin E equivalent, respectively. The trial lasted 35 days. Individual piglet weights at day 0 (d0) and day 35 (d35) and pen feed consumption were recorded for calculation of average daily gain (ADG) and feed conversion ratio (FCR). Blood samples were collected on d0 and d35 to determine serum vitamin E concentration.

Results showed that after 35 days of differentiated feeding, C group consumed less feed probably due to a taste not appreciated by piglets, exhibiting a lower ($p < 0.05$) final body weight (15.04 kg) with a lower ($p < 0.05$) ADG (0.223 kg) and worse FCR (2.1 kg/kg) compared to all other groups showing overlapping growth performances. No mortality was registered throughout the entire period. No effect of sex was found. Serum vitamin E concentration showed a general increase in all groups at d35 ($p < 0.001$) and no effect of diet was detected; no clinical sign of vitamin E deficiency was noted in any piglet. In conclusion, the partial replacement of vitamin E with polyphenols results in unchanged growth performance among groups, excepting C group; considering vitamin E serum concentration, all integrations seem to be potentially reliable. Anyway, further research is needed to determine the effects also on other physiological indicators.

P364

Top-dressed arginine supplementation to lactating sows influences piglet's performance and gut bacterial and viral profile

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Early colonization of the gut microbiome plays a key role in the development of the gut physiology, immunity and growth of the host. The early development of the gut microbiome is affected by several factors including the mother microbiome and dietary intervention. This study aims to investigate if dietary supplementation of arginine (Arg) to lactating sows can influence the performance, colostrum and milk composition and gut microbiome of sows and their litters. A total of 16 sows were divided into two groups balanced for parity and body weight: control (CO) (fed corn-based diet) and ARG (CO +22.5 g/d/sow of on-top Arg). Diets were fed from 4 days before farrowing (d-4) to weaning (d27). Piglets were weighed at d0, d7, d14, d27, d34 and d41. Colostrum and milk were sampled at farrowing, d10 and d20 to analyse immunoglobulins. Faecal and cecal samples were collected at d27 from all sows and piglets (8 piglets/group) respectively. Library preparation and amplicon sequencing of the bacterial V3–V4 regions of the 16S rRNA gene was performed using the Illumina MiSeq technology. Virus particles were purified and extracted using AllPrep Power Viral Kit. Illumina libraries of viral DNA and cDNA were prepared using Nextera XT DNA and sequenced on the MiSeq platform. Data were fitted using an ANOVA linear model with diet as a factor. ARG did not affect the sow's performance during suckling but increased the average daily gain from d27-d41 ($p < 0.001$) and from d0-d41 ($p = 0.05$). ARG sows had a higher concentration of fat ($p = 0.03$) and lower lactose ($p = 0.06$) in milk at d20 than CO. The gut microbiome of sows and piglets clearly differ in terms of the bacterial and viral community (alpha and beta diversity, $p < 0.05$). Interestingly, bacteriophage viral species changed drastically between sows and piglets. Piglets had a higher frequency of Caudovirales ($p < 0.01$), and sows of Petitvirales ($p < 0.01$). Sow's faecal microbiome, both virus and bacteria, was not affected by the diet. Piglet's cecum viral profile was affected by the sows' diet (higher alpha diversity in the ARG group, $p = 0.01$). Piglets in the ARG group had a higher abundance of *Collinsella* (LDA score >2; $p > 0.05$), Cremevirales ($p = 0.09$) and a lower abundance of Piccovirales ($p = 0.07$).
In conclusion, Arg supplementation to lactating sows can improve the performance and modulate the gut microbiome of their litters.

P468

A new formulation with fermentative S-acetyl-glutathione and Sylibin for dogs with liver disease

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Liver disease is often characterized by oxidative stress. Glutathione (GSH), an important intracellular antioxidant, is primary sintetized in the liver and its concentration could decrease in case of liver damage. In veterinary medicine, there is an increasing interest and use of feed supplements with antioxidant properties. We performed a case-control study, testing a new formulation containing fermentative S-acetyl-glutathione (SAG), Sylibin and other antioxidants (Orange bioflavonoid, vitamin B2, vitamin B12, vitamin E) in dogs with a diagnosis of liver disease. The study duration was 35 days. A total of 24 dogs were recruited and randomly assigned to a treatment group (TRT,male $n = 5$, female $n = 7$, mean age 6.8 yr) and to a control group (CTR, male $n = 6$, female $n = 6$, mean age 6.7 yr). The effects of the supplement on blood parameters for testing liver function and antioxidant activity were investigated using a regression model (generalized linear mixed model (GLMM)- with Gaussian likelihood) using the R language (2022). Most of the liver key parameters were significantly reduced in the TRT group but not in the CTR group (ALT, AST, ALP, GGT and BIL) and no or limited effects were seen on other biochemical values (TP, ALB, GLU, TRI, and PCR). In addition, we reported a significant increase in the level of erythrocyte GSH in the TRT group nearly reaching the minimum physological level (300 Ug/Hb) at the end of the treatment. The hepatoprotective activity and the erythrocyte GSH increase, following the administration of our tested supplement, could be the result of the synergic effect of the included ingredients. This complementary feed may represent an effective help in managing liver disease in dogs.

P564

Effect of oral administration of Bacillus subtilis C-3102 and Pichia guilliermondii to nursery piglets on some inflammatory biomarkers and growth performance at weaning

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This study aimed to evaluate the effect of the oral administration of the probiotic *Bacillus Subtilis* C-3102 (BS), the prebiotic *Pichia*

Guilliermondii (PG) and the combination of both and the base gestation diet on some inflammatory biomarkers and growth performance of nursery piglets from birth until 30 days after weaning. During the last week of gestation a total 20 sows (Large White × Large White) homogeneous for parity, BCS and farrowing date were randomly assigned to one of the four dietary treatments: CTRL, basal gestation diet without any supplement; PRO, basal gestation diet supplemented with 90 mg of BS; PRE, basal gestation diet supplemented with 6 mg of PG and PRE/PRO, basal gestation diet supplemented with 90 mg of BS and 6 mg of PG. Starting from farrowing until weaning the piglets received the powder of different treatment diluted with water the same supplement of their dams by means of an oral-syringe in the follow amount CTRL water; PRO, 9 mg of BS; PRE, 300 mg PG; 9 mg of BS +300 mg of PG for group PRE/PRO. During lactation piglets' health status were monitored every day while body weights were recorded at birth and on +7, +14, +28 days after. At weaning, the piglets were blood sampled to investigate the serum levels of Interleukin 1B (IL-1B), Interleukin 6 (IL-6), Interleukin 10 (IL-10) and interferon gamma (IFN-γ). The piglets of PRE-PRO and PRO showed higher body weights at 21 and 28 days after birth compared to the other two groups. Serum levels of IL-1B and IL-6 were lower ($p < 0.01$) in group PRE/PRO compared to the other three groups. IL-10 and IFN-γ were higher ($p < 0.01$) in groups CTRL and PRE compared to PRO and PRE/PRO. Our findings evidenced that the group of piglets that received probiotics alone or in combination with prebiotics (PRO or PRE/PRO) in the diet decrease significantly pro-inflammatory serum cytokines levels. Moreover, higher anti-inflammatory IL-10 levels may be the result of a more evident inflammatory status of the CTRL and PRE compared to PRO or PRE/PRO-groups.

P13

Horse transportation by air: routes, practices and welfare implications

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Horse transportation by air is common, but information regarding its routes, practices, and welfare implications is scant. Our study aimed to describe the routes, management, and welfare status of horses travelling by air. Data were collected from 77/402 horses travelling on 23 journeys. Information was gained from before to 5 days after the journey. The horses were moved for sale (67%), equestrian competitions (17%), racing (9%), breeding (6%), or relocation (1%). Belgium was the most frequent departure country (15/23 flights, 65%; 38/77 horses, 49%), followed by The Netherlands (4/23 flights, 17%; 20/77 horses 26%), Germany (2/23 flights, 9%; 7/77 horses, 9%), UK (1/23 flights, 4%; 6/77 horses, 8%) and the USA (1/23 flights, 4%; 6/77 horses, 8%). The USA was the most frequent destination (10/23 flights, 43%; 19/77 horses, 25%), followed by Japan (7/23 flights, 30%; 30/77 horses, 39%), South Africa (3/23 flights, 13%; 14/77 horses, 18%), Australia (2/23 flights, 9%; 8/77 horses, 10%) and New Zealand (1/23 flights, 4%; 6/77 horses, 8%). Before departure, horses stayed in quarantine (15 ± 15 days), and on the last day, were considered fit for transport. At the departure airport, 17% of horses showed nasal discharge and two horses had minor injuries. The horses were loaded into 2-horse (17% of horses) or 3-horse type (83% of horses) jet stalls, located before (38%), in between (31%) and after the wings (31%). Inside the stalls, the horses were untied (23% of horses), long (possibility to low head to knee height; 52% of horses) or short tied (25% of horses) and shaving was the most common bedding. Grass hay (89% of horses) or haylage (11% of horses) was offered *ad libitum*, while water was given *ad libitum* (10% of horses), every two (31% of horses) or every 3 h (59% of horses); almost all horses ate (12 ± 7 kg/horse) and drank (17 ± 13 L/horse) in transit. At the check performed before landing, one horse was found with a swelling on the chest and 30% of horses showed nasal discharges. At the arrival quarantine, four horses showed health problems, namely two cases of superficial lesions (cut on the back limb and skin rubbed raw at head level), one case of diarrhoea, and one case of fever (40.7 °C). All horses recovered after 5 days. Overall, this preliminary study has increased our knowledge of transportation by air and its implication for horse health and welfare.

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Effects of milk exosomes from heat-stressed dairy cows on BME-UV1 cells

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Heat stress (HS) represents one of the main problems in the livestock sector and in dairy cows, affecting productivity, health, and general well-being. Recent studies have observed and increased release of membranous extracellular vesicles, exosomes (EXO) in particular, in subjects exposed to HS conditions.

EXO are membrane microvesicles of 30–100nm in diameter that contain a load of substances deriving from the cells including proteins, lipids, miRNA and DNA able to transmit signals to other cells. The present study aimed at verifying the effects of EXO, isolated from milk of 4 Holstein Friesian (FR) and 4 Brown (BR) lactating cows, reared in commercial farms, under thermo-neutral (TN) or heat stress (HS) conditions, on cellular responses in bovine mammary epithelial cell line (BME-UV1).

Preliminary step for removing cell debris and fat globules was performed before the separation of EXO from milk. Milk samples were then centrifuged at 10,000*g* for 30 min at 4 °C. The supernatant was collected and diluted in sterile PBS and ultracentrifuged at 100,000*g* for 1h at 4 °C to obtain the EXO. The EXO pellet was collected and further purified through size exclusion chromatography. After isolation and characterization EXO were used for *in-vitro* test. The BME-UV1 were grown in flasks of 75 cm^3 at 37 °C until they reached confluence. Subsequently, 2.5×10^4 cells were plated in 0.3 cm^3 wells using FBS medium without EXO and incubated at 37 °C. After 24 h, the medium was replaced with medium enriched with EXO FR-TN, FR-HS, BR-TN, BR-HS, and without EXO (control). Before the addition of EXO to cells, EXO were purified using the Toxin Eraser Endotoxin Removal Kit. Cell viability rate was assessed by XTT test, cellular apoptosis susceptibility by using the Apo-ONE® Homogeneous Caspase-3/7 Assay kit and mRNA expression of genes linked to apoptosis by PCR-Real Time.

The results showed a reduction of cell viability in FR-HS compared to FR-TN and an increase of apoptosis (21.2%) in FR-HS compared with FR-TN. These results were also confirmed by an over-expression of pro-apoptotic Casp-3 gene in FR-HS compared to FR-TN. In contrast, no statistical differences were observed between BR-TN and BR-HS. These findings provide insight into the ability of EXO isolated from HS animals to modulate the cellular response and gene expression of BME-UV1 *in vitro* and highlighted that FR-HS and BR-HS EXO were able to induce a breed-specific response in BME-UV1 cells.

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Effect of hemp cake-based diet on laying hen behaviour

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We aimed to assess the effect of a hemp cake-based diet on the behaviour of organically reared Lohmann White hens. We used four sub-groups of 25 animals each. Two sub-groups received a standard diet based on corn flour and soya cakes (50 animals; Group C), two others received the same diet, integrated with 30% hemp cake (50 animals; Group H). The following behavioral categories were identified during two preliminary *ad libitum* observation sessions: feeding, drinking, locomotion, inactivity, laying, exploration, self-grooming, dust bathing. Eight observation sessions were conducted at 1-week intervals from 9.00 to 13.00 using the instantaneous scan sampling technique (3 min sampling intervals). The location (nest, indoor, outdoor) and posture (lying, standing) were also registered. The day of observation was used as experimental unit. Data were subjected to ANOVA using diet, hour of observation and their interaction as factors. Egg production (number and weight of eggs per 25-hen sub-group) was recorded over a period of 10 weeks and subjected to ANOVA using diet as factor. Egg production was higher in Group H than in Group C both in terms of number (17.29 ± 1.29 vs. 13.25 ± 0.58; $p < 0.05$) and total weight (1119.47 ± 68.54 vs. 811.61 ± 44.2 g; $p < 0.05$). Hens from Group H tended to be located more often in the nest (0.26 ± 0.02 vs. 0.20 ± 0.02; $p < 0.10$) and tended to be observed more often inactive (0.21 ± 0.02 vs. 0.18 ± 0.03; $p < 0.10$) and less often feeding (0.17 ± 0.02 vs. 0.22 ± 0.02; $p < 0.10$). In addition, animals from Group H were observed more often laying (0.17 ± 0.01 vs. 0.12 ± 0.01; $p < 0.05$). The results concerning production, location and behaviour all converged towards the same implications as the hens fed a hemp integrated diet, possibly due to a higher level of satiety, ate less, were less active, had a higher production level and as a consequence were more often located in the nest. We conclude that the inclusion of hemp cake at 30% in the hen diet may promote egg production with a reduction of the activity expressed by laying hens.

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Surgical castration: does a non-pharmacological approach improve piglet welfare?

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Surgical castration of male piglets is a welfare issue, as it is a painful and stressful procedure. Among alternative approaches to pain control, the administration of sugar (e.g. saccharose) has effectively reduced procedural pain in neonates, due to the release of endogenous opioids. The aim of this study was to evaluate whether a non-pharmacological approach (administration of glucose) influences physiological and behavioural responses of piglets undergoing surgical castration.

Procedures were approved by Italian Ministry of Health (863/2020-PR). A total of 70 commercial-hybrid male piglets were included in the study, weighted, and assigned to groups balanced for body weight (1.97 ± 0.32 kg). 34 piglets were assigned to one of two pre-operative oral treatments: Group CW ($n = 17$) received 5mL of water, while Group CG ($n = 17$) received 5 mL of glucose solution at 10% and underwent surgical castration under inhalation anaesthesia at 4 days of age. 36 animals were enrolled as controls and underwent manipulation of the scrotum while awake, in the same portable device for anaesthesia; they were also previously treated with water (MW; $n = 18$) or glucose solution (MG; $n = 18$). Glycemia was measured before treatments administration and after surgical castration. Piglets' behaviour was recorded when reintroduced in the farrowing crate and evaluated with continuous focal sampling by a trained observer. The latency until suckling was measured and pain-related behaviours (prostration, tail wagging and abnormal gait) were evaluated for subjects with latency until suckling of 3 min.

Glycemia significantly increased after castration for piglets castrated or manipulated and treated with glucose solution (Wilcoxon $p < 0.05$), whereas the values were physiological both before and after procedures. Latency until suckling did not significantly change among groups (Kruskal–Wallis; $p > 0.05$), despite the higher mean value of MG piglets. Manipulated piglets showed less pain-related behaviour if compared to castrated piglets, whether the oral treatment (Kruskall–Wallis; $p < 0.05$). Although not significant, MG showed less pain-related behaviour than MW. These results suggest that glucose solution provide some positive effects on stress, rather than a control of pain. It might be a low-cost method to reduce manipulation-induced stress in piglet management.

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Identification of potential welfare indicators for high sustainability standard in heavy pig production

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An expert consultation was organized to select animal welfare requirements to be included in a sustainable standard for Parma Ham.

The consultation involved a group of experts to establish a consensus opinion on the most appropriate measures for the welfare of pigs using a Delphi technique. A total of thirty-three experts in the areas of pig welfare were identified through literature searching. General information on the aim, methods and use of data were emailed to participants who were asked to respond within 14 days giving informed consent to participate in the study. Feedback was obtained by nineteen experts.

The study was conducted online using Google Module software and each participant was provided with a link and instructions. They were asked to consider a list of 88 measures that are directed towards the animal (44 animal-based measures) and their environment (44 resource-based measures).

Criteria were identified from the revision of 14 private and public voluntary animal welfare standards for pig production, according to productive category (dry and pregnant sows, lactating sows and piglets, weaner pigs and fattening pigs).

Two rounds of consultation were designed to achieve a degree of consensus among experts: in the first round, participants were asked to indicate how appropriate the selected measures would be to assess the welfare state of the animal, using a six-point scale (0 = lowest score, 5 = highest score). The responses from the first round of consultation were analysed and the results were returned anonymously to the experts. During the second round, participants were asked to indicate the importance of the welfare measures identified in the first-round. In this way, each respondent gave a view on all measures identified within the group. A five-point scale was used to allow the experts to indicate how much importance should be placed on the level of each measure (1 = lowest score, 5 = highest score) in order to reach final consensus.

In this way, a consensus of opinion was reached and a total of 28 animal-based and 19 resource-based measures were selected. In particular, according to production categories 11 requirements for dry and pregnant sows, 12 for lactating sows and piglets, 13 for weaner pigs and 11 for fattening pigs were included in a prototype protocol for sustainable pig production, which has been now under refinement and tested on farm.

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Monitoring the behavior of purebred and crossbreed beef calves in a confined management system to improve their welfare

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The goal of research in animal welfare is to find positive indicators of welfare through the study of species-specific behavior. A behavioral response can be described as the animal's answer to a challenge in its external and internal environment. The aim of this work was to monitor the behavior of purebred and crossbreed beef calves reared in the same optimal environmental conditions according to Classyfarm®.

Thirty beef calves were divided in 10 Limousine (LMS), 10 Bruno-Sarda (SRB) and 10 SRBxLMS crossbreed and by sex (5 males and 5 females for each genetic group) and confined in 6 pens of 45m^2 each. The general activities (resting, standing, position, walking, eating, drinking, and ruminating) and social behavior (displacement for food, water, and space, play fighting, self and allogrooming and stereotypes) in each pen were recorded by two observers for four months. Eating behavior was also observed by videocameras h24 addressed for each feeder. 'Concentrate' and 'Hay consumption', 'Competition for food', 'Resting', and 'Ruminating' were recorded as events, time of duration and frequency for each pen. The data were analyzed using procedure of SAS with repeated measurements by a general linear model with fixed effect of breeds, sex and time.

General activity records showed significant differences between genetic types: SRB calves showed lower percentage of animals for 'Sternal resting' ($p < 0.05$) and higher percentage of animals for 'Standing'($p < 0.01$) than other groups. Females showed higher percentage of animals for 'Ruminating', 'Central position' and 'Standing' than males ($p < 0.05$, $p < 0.01$ and $p < 0.05$). 'Self-grooming' and 'Allogrooming' were significantly higher in females than males ($p < 0.05$) and 'Play fighting' was higher in males than females ($p < 0.01$), because of normal behavior. No significant differences were observed between the genetic types for the parameters of feeding behavior. All the calves showed a higher frequency at the feeder in August than in the other months, linked to the increase in concentrate feeding events ($p < 0.01$). 'Competition for food' events at the feeder also increased in August ($p < 0.01$).

In conclusion, the optimal rearing conditions (Classyfarm®) have allowed the animals to adapt to the environment and to exhibit their own species-specific behavioral repertoire.

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Welfare assessment of horses on pasture: Catria breed as case study

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Welfare assessment involves the evaluation of a panel of welfare measures – resource-, management- and animal-based. Nowadays, there are no validated on-pasture welfare protocols for horses and – accordingly – there is the need to fill this gap as well as to support the official controls of veterinarians. The aim of the present study was to develop and test a welfare protocol for horses kept on pasture. The protocol was developed by a team of veterinarian expert in equine welfare (focus group) following the experience of the Italian National Reference Centre for Animal Welfare (CReNBA). The focus group selected 82 measures which were divided into 6 macro-areas named training, feeding, facilities, ABMs, biosecurity, and health management. Each measure had to be judged with a 3-point score – -1, inadequate; 0, adequate but improvable; +1, optimal. The welfare protocol was tested by one trained operator from June 2021 to August 2022 on 484 horses belonging to Catria breed – an Italian autochthonous breed – which were kept on 32 pastures of Monte Catria (central Apennines). A descriptive statistic was performed on each macro-area to evaluate the frequency (%) of scores for the selected welfare measures.

Here, the measures judged as inadequate to satisfy the welfare standard for horses are described. Considering the training macro-area, the worst scores were given to the daily number of inspections of the animals (55%) and the feeding management according to group composition (88%). This latter aspect was in line with the results obtained within the feeding macro-area in which it was shown an inadequate quality of the diet supplied (empirical for the 79%) in terms of both hay (70%) and feedstuffs (97%). Accordingly, the 52% of the body condition score (ABMs macro-area) resulted inadequate. The worst scores for facilities macro-area was related to the management and cleaning of the bedding of the partum area (91%). Moreover, the biosecurity measures resulted inadequate for the control of pests/wild animals (76%) and foreign visitors (82%). Major concerns for the health management macro-area resulted in vaccination programs (73%) and in the care of the dental board (61%).

The present study shows the usefulness of a specific welfare protocol to identify measures that need to be improved in field condition. Further researches are needed to validate the welfare protocol for horses kept on pasture as a tool to safeguard horse welfare.

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Effect of the season on oxidative response in the Mediterranean Buffalo

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Temperature is one of the most important ecological factors that directly affect livestock production. The Reactive Oxygen Metabolites derived compounds (d-ROMs) and the biological antioxidant potential (BAP) are simple and reliable tests for the measurement of reactive oxygen species (ROS) and the plasma biological antioxidant potential. These tests are considered suitable biomarkers for evaluating oxidative stress (OS). Bovine studies showed that elevated temperatures result in accelerated generation of ROS, causing an imbalance between ROS production and the ability of scavenging systems to detoxify and remove the reactive intermediates. Since such studies remain still limited in water buffalo, the effect of temperature on serum d- ROMs and BAP parameters was planned to assess the oxidative status in an Italian water buffalo population within the **Agridigit-PLF4Milk project**. In this study, only preliminary results on d-ROMs are presented. Blood samples (jugular) were collected 12 times in two years (2021–2022) from 40 buffaloes. One sample per month was taken from each head during hot season (July-September) and during cold/mild season (February-April). The amount of free oxygen radicals in serum samples was determined using d-ROMs test (Diacron, Italy) modified for a microplate procedure. The results are expressed in arbitrary Carratelli Units (UCARR), where 1U CARR is equivalent to the oxidizing power of 0.08 mg H_2O_2/dL. Model included year, season, Temperature Humidity Index (THI), Body Condition Score (BCS) and individual rectal temperature. The minimum and maximum THI values recorded during the experimental phase were 29.6–74.5 and 54–85 for cold and hot season respectively in 2021 and 31.6–66.3 and 47.9–86.2 in 2022. The d-ROMs values ranged between 36.3 and 377 UCARR with a normal distribution (mean =138.39 ± 44.43 U CARR). Statistical analysis showed that levels of d-ROMs were meaningfully affected by sampling year (159.67 *Vs*.118.45 UCARR, $p < 0.001$) and season (133.022 *Vs*. 145,09 in hot and cold/mild season respectively, $p < 0.05$). In our study, buffaloes showed high levels of d-ROMs in cold season of year 2021 when the maximum values of THI, indicator of a heat load index, were recorded, compared to the same season of 2022. A more accurate assessment of OS will be obtained after the determination of BAP levels. Overall, this preliminary results provide, for the first time, insight into the ROS modulation in Italian water buffalo in field conditions.

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Influence of body lesion severity on oxidative status and gut microbiota of weaned pigs

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The objective of the study was to assess the physiological effects of body lesions caused by social stress in weaned pigs. On 45 tail-docked pigs, lesion score (LS) was assessed at 1 week (28 days of age, T1) and 7 weeks post-weaning, T2. Lesions were measured on pig skin, tail, ear, neck, middle trunk, and hindquarters, and scored on a scale of 0 to 2 in accordance with the guidelines provided by the Welfare Quality® (2009). At T1, pigs were categorized as High LS (H, $n = 15$) or Low LS (L, $n = 28$) when the LS was >1 in at least 2 of the locations considered. At T2, based on the previous observation, pigs were separated into 4 groups: High to Low LS (H-L, $n = 10$), High to High LS (H-H, $n = 5$), Low to Low LS (L-L, $n = 20$), and Low to High LS (L-H, $n = 8$). At T1 and T2, blood and stool samples were collected to quantify Biological Antioxidant Potential (BAP), Reactive Oxygen Metabolites (d-ROM), IgA, IgM and IgG and to characterize the faecal microbial profile. BAP was estimated using a FRAP assay and d-ROM where quantified using a d-ROMs test. IgA, IgM and IgG where quantified using an ELISA assay. When compared to the L group at T1, pigs of the H group exhibited a lower BAP ($p = 0.02$). At T2, the L-L group had a lower concentration of IgA compared to the H-H and L-H groups ($p = 0.02$ and $p = 0.04$, respectively), while the L-H group had a lower concentration of d-ROM than the H-H group ($p = 0.03$). At T1, microbial profile of H and L pigs was significantly different ($R^2 = 0.04, p < 0.01$) and L pigs were characterized by a higher abundance of Firmicutes,

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Blautia, *Eubacterium coprostanoligenes*, *Faecalibacterium*, *Megasphaera*, *Subdoligranulum* (*p*adj <0.05). Pigs of the H group were characterized by higher abundance of Bacteroidota, Rikenellaceae RC9, Prevotellaceae UCG-003, uncultured-Lachnospiraceae and uncultured-Oscillospiraceae (*p*adj <0.05). At T2, the H-H group were characterized by UCG-010, H-L by *Agatobachter* and L-L by *Alloprevotella* (*p*adj <0.05). One-week post-weaning pigs with a high LS had an altered oxidative status and a different microbial profile, characterized by a reduction of Firmicutes and an increase of Bacteroidota compared with pigs with low LS. Importantly, these modifications had a long-lasting impact, modulating the immunological response up until 7 weeks after weaning.

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Pig welfare in intensive and organic farms: an assessment by the Classyfarm system and stress biomarkers

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The modern sensibility to welfare has questioned whether intensive pig farming can be 'cruel' on 'unethical', while organic farms seem more respectful of pigs' needs. The study aims to compare stress biomarkers between categories in intensive pigs, correlate them to the official welfare Classyfarm scores, and compare welfare in the fattening sector of organic and intensive farms. The study enrolled 21 sows and 20 post-weaning (PW) pigs from intensive farms and fattening pigs (FP) raised in intensive (20) and organic (23) farms. Haemogram and serum stress markers (complement and bactericidal-SBA- activity, lysozyme, haptoglobin and major acute pig protein-MAPP) were assayed. The Classyfarm checklist assessed in-field welfare on each farm. Depending on data distribution (by Shapiro–Wilk normality test), group differences were tested by One-way ANOVA or Kruskall-Wallis with Tukey posthoc test and significant *p*-value <0.5. No flogosis, stress leukogram or anaemia was evident. Leucocytosis (25×10^3/µl), neutrophilia (10.2×10^3/µl) and lymphocytosis (13×10^3/µl) were evident in PW pigs. These animals showed higher haptoglobin (27.7µg/mL) than FP (16.6µg/mL, $p = 0.02$) and higher MAPP (360.7ng/mL) than sows (188.8ng/mL, $p = 0.03$), which can relate to stressful husbandry practices of this production phase (mi × ing groups, change of diet, etc.). The Classyfarm system scored optimally in all the PW and FP. As for the sows, 2 farms scored suboptimally, with higher haptoglobin (14.2 vs 23µg/mL, $p = 0.019$) and lower leucocytes (12.9 vs 17.6×10^3/µl, $p = 0.009$), neutrophils (5.3 vs 8×10^3/µl, $p = 0.03$) and lymphocytes (6.1 vs 8×10^3/µl, $p = 0.009$). Lower SBA (104% vs 236%, $p = 0.017$) was detected. Classyfarm scored optimally in FP both in organic and intensive farms. Yet, organic pigs showed lower MAPP (173.8 vs 250ng/mL, $p = 0.005$) and higher lysozyme (3.9 vs 3.005µg/mL, $p = < 0.001$). The results suggest that weaning impacts intensive pigs' welfare. Also, suboptimal Classyfarm scores were linked to higher stress markers and worse immunocompetence in intensive sows. Conversely, Classifarm did not detect any welfare lack in FP, which, if reared in intensive farms, showed higher acute phase proteins and lower serum lysozyme, compatible with stress. In conclusion, Classyfarm might be a valuable tool for farm welfare assessment, yet, the combination with serum biomarkers is advised for a complete insight into swine stress

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Serum Oxytocin and social behaviour: a comparison between cows and calves in the Impossible Task Paradigm

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Ruminants are capable of establishing a social bond with humans and a growing interest in circulating hormones, in particular to the oxytocin (OXT), due to their a central role in regulating behaviour and social relationship. In this study we explored the differences between calves and cows in levels of oxytocin, cortisol, and different social behaviours towards humans in the impossible task paradigm. Twenty-six Italian Red Pied calves and 29 adult cows raised in a farm located in Roccabascerana (Avellino, Italy) were included in the trial. In brief, a specific experimental setup was built to induce the violation of the expectation and it consisted of a transparent plastic food container whose inverted lid was fixed on a wooden platform with screws. The daily care of the animals had been ensured by a caregiver considered familiar for the animals. For the test, beside the caregiver, two persons were involved: one experimenter played the role of the stranger; another one manipulated the apparatus and the feed during the trial. During the solvable tests, the container was positioned freely upside down on the lid; while during the unsolvable trials, it was blocked.

Behavioural analysis was performed using video recordings analyzed with the Solomon Coder® software (ELTE TTK, Budapest, Hungary) by a qualified researcher. After the test, blood was collected to determine the serum concentration of oxytocin and cortisol. Statistical tests were performed with IBM SPSS statistical software 26 (IBM Corp., Armonk, NY, USA).

Serum oxytocin levels in cows correlated positively with duration ($p < 0.001$) and frequency ($p < 0.001$) and negatively with the latency of interactions with the caregiver ($p = 0.03$), but this did not occur in calves where cortisol correlated positively with the latency of behaviours directed at the caregiver ($p = 0.01$), and the duration of behaviours directed at the apparatus correlated negatively with people ($p = 0.05$). This highlights a different behavioural strategy between calves and adult cows when placed in front of an impossible task.

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Evaluation of differential somatic cell count by conventional flow cytometry and Fossomatic technology

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Recent studies investigated the relationship between somatic cell count (SCC) and milk composition in water buffalo but there is no information on the differential cell count in buffalo milk. Conventional flow cytometry is a tool to detect changes in cell populations. Differential somatic cell count (DSCC) has recently been described and validated in bovine. The aim of this study was to evaluate the differential cell count by two flow cytometric tools and their correlation with SCC in buffalo milk. Sixty-four bulk tank milk samples were analysed. The percentage of polymorphonuclear cells (PMN), lymphocytes and macrophages were identified using an antibodies multicolor panel direct to CD14, CD16 and CD18 markers, by a conventional bench analyzer CytoFlex (Beckman Coulter). The DSCC parameter was obtained by high-throughput milk analyzer Fossomatic7DC (Foss Analytical). Milk samples were categorized into group 1 (SCC <100,000 cells/mL), group 2 (100,000 ≤ SCC ≤300,000 cells/mL) and group 3 (SCC >300,000 cells/mL). Differences in percentage of PMN, lymphocytes, macrophages, and DSCC parameter among the SCC groups were analyzed by GLM procedure in SAS analytics software. Results showed that group 1 had a significantly lower percentage of PMN compared to group 2 and group 3 ($p < 0.05$). A significantly lower value of DSCC parameter was found in group 1 compared to group 2 and 3 ($p < 0.009$; $p < 0.0001$) and a lower value in group 2 compared to 3 ($p < 0.0003$). The relationship among SCC, percentage of PMN, lymphocytes and macrophages, and DSCC parameter was analysed by Pearson correlation. Results showed that the correlations between SCC and PMN, lymphocytes and macrophages were $r = 0.55$, $r = -0.45$ and $r = -0.53$ respectively ($p < 0.001$). A strong relationship between SCC and DSCC parameter was found ($r = 0.80$; $p < 0.0001$). Finally, comparing the DSCC to leukocytes subset, the correlation was $r = 0.68$ for PMN, $r = -0.42$ for lymphocytes and $r = -0.72$ for macrophages ($p < 0.003 \div 0.0001$). This is the first study that characterizes the differential cell count by flow cytometry in buffalo milk and evaluates the relationship between the two analytical tools in this species. Results show that the differential and somatic cell count could be useful parameters to monitor farm management.

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Cellular immune system variations at different ages from two water buffalo farms

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In recent years there has been an increased interest in understanding the immune system of water buffalo (*Bubalus bubalis*) due to the growing economic impact of this species. Flow cytometry is a powerful methodology for cell analysis that achieve simultaneous measurement of multiple surfaces and intracellular antigens allowing the identification of specific cell subsets. In this study, we assess three multicolor antibody panels to characterize and monitor the leukocyte subsets by flow cytometry. Panel 1, with anti-CD3, CD4, CD8α and TCR-δ antibodies, was assessed to evaluate total T lymphocytes, T helper, T cytotoxic and $\gamma\delta$ cells. Panel 2 with anti-CD79a and CD21 antibodies was designed to evaluate the total and $CD21^+$ B lymphocytes subset. Panel 3 with anti-CD335, CD172a, CD14 and CD16 antibodies, was assessed to evaluate the NK cells and monocytes. Fifty-four buffaloes from two farms were included in the study. The animals were divided into three groups by age: 80–100 days old calves ($N = 18$); 16–18 months old heifers ($N = 18$) and multiparous dry buffaloes ($N = 18$). Heparinized blood samples were incubated with each antibody panel and collected on CytoFLEX flow cytometer (Beckman Coulter, USA). Significant differences among age groups were found in the percentage of $\gamma\delta$ cells ($p = 0.0001$), T cytotoxic ($p = 0.0003$), NK cells ($p = 0.0001$), monocytes ($p = 0.009$) and $CD21^+$ B lymphocytes ($p = 0.0002$) by Kruskal-Wallis test. The Mann-Whitney test was used to compare the parameter distributions between the two farms in each animal group. Results showed that in dry buffaloes total T lymphocytes (76.9 *vs* 69.4; $p = 0.002$) and $\gamma\delta$ cells (17.4 *vs* 10.0; $p = 0.009$) were higher and total B lymphocytes were lower (14.1 *vs* 16.8; $p = 0.054$), in farm 1 compared to farm 2. In the heifers $CD21^+$ B lymphocytes subset was lower in farm 1 compared to farm 2 (83.7 *vs* 87.0; $p = 0.054$). Finally, in calves, the monocytes were found significantly different between the two farms (3.0 *vs* 5.1; $p = 0.008$). Although there are no substantial management differences between the two farms, these results could indicate a different adaptation to the two different geographical areas in which the farm are located. Finally, the cellular immune response at different ages could be a useful approach to monitor health status at animal and farm level.

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An overview of calf rearing conditions in Italian dairy farms

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ClassyFarm is the new IT system of the Italian Ministry of Health for the collection, management and exchange of data on livestock production in Italy. The strength of the system is its interoperability, which allows easy interchange of data among public veterinary services, farmers, farm veterinarians and accredited certifying bodies (e.g. for labelling). Animal welfare is one of the focus area of the ClassyFarm system. In ClassyFarm, on-farm animal welfare is assessed using species-specific protocols set up by the Italian Reference Centre for Animal Welfare. The ClassyFarm protocol for dairy cows in loose housing system includes indicators (AWI) for both lactating and non-lactating animals, including 9 AWIs referred to calves (0–180 days old).

The goal of the present study was to provide an overview of the calf rearing conditions in Italian dairy farms by analyzing the AWIs present in the ClassyFarm protocol and their trend over the 3-year period 2020–2022. The 9 AWIs referred to calves were daily inspections, colostrum management, feeding management, cleanliness of the pens, bedding material for new-born calves, space availability for calves in single pen, possibility for calves in single pens to see and touch each other, space availability for calves in group pen and calf annual mortality rate. Using the ClassyFarm system, 1.062 dairy farm were assessed in 2020, 4.233 in 2021 and 4.027 in 2022, considering the whole country.

Around 58.5% of the assessed farms showed an optimal condition for the AWIs 'colostrum management', 'cleanliness of the pens' and 'space availability for calves in group pen'. For these AWIs, around 41% of the farms showed a good condition and 0.5% a poor rearing condition. The 46% of the farms showed an optimal level of the AWI 'space availability for calves in single pen', while the 53.7% was found good and the 0.3% was found poor. Concerning 'feeding management' and 'number of daily inspections', the 72.7% of the farms showed a good level, the 27% an excellent level and the 0.3% a poor level. The 99.5% of the farms provided adequate bedding material for new born calves and the 98.7% permitted contact among calves in single pens.

The annual mortality rate was under 4% in the 47.0% of the farms, between 4% and 10% in the 37.5% of the farms and over 10% in the 15.5% of the farms.

No differences or trends in calf rearing conditions were seen among the considered 3-year period.

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Maternal inflammatory indicators during transition period and their relationship with growth performance of newborn buffalo calves

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The transition period from late pregnancy to early lactation is extremely critical for producing animals as it is typically accompanied by metabolic stress and an overt inflammatory condition that can affect offspring health, immune responses and production variables. In Buffalo cows no information exists on how maternal inflammation can affect calves' health and growth performance. Therefore, the aim of this study was to determine in Buffalo dams the serum levels of the pro-inflammatory cytokines Interleukin 6 (IL-6), Interleukin (IL-1B) and tumor necrosis factor alpha (TNF-α) during transition period and to evaluate their relationship with colostrum IgG content, calves' immune response and body weights at birth, +7, +14, and +21 days after. Twenty-two ($n = 22$) pregnant heifers were blood sampled at T-30 days before expected calving date, at parturition (0) and weekly until 21 days after. The dams were allotted in two groups ($n = 11$ each) (Low Inflammation = LI or High inflammation = HI) based on the pro-inflammatory cytokine levels detected at T-30. Statistical analysis was performed with SPSS 26.0 software (IBM Company Headquarters, USA). General linear model (GLM) analysis for repeated measures was performed to compare interleukin mean values among different dam's inflammatory status (HI/LI). One-way ANOVA was performed to analyze the effects of inflammatory groups on colostrum and calves' serum IgG content, calves' body weights, and the cytokines levels for each sampling week. Pearson's correlation coefficients between dam's pro-inflammatory cytokines levels and their offspring body weights were calculated. HI-buffaloes had a significant lower colostrum IgG content (60.8 ± 27.67 mg/mL *vs* 73.6 ± 24.61 mg/mL; $p < 0.05$) and lower calves' weights at birth (39.96 ± 3.03 kg *vs* 41.98 ± 3.46 kg respectively; $p < 0.05$), at +7, +14, +21 days after ($p < 0.05$). Significant negative correlations were found in LI-buffaloes among IL-6, IL-1B, and TNFα levels at T0 and calves' weight at birth, at +7 and +14 days after ($p < 0.05$). Significant positive correlations were found in HI buffaloes at T-30 among IL-6, IL-1B, TNF-α levels and calves' weight at birth ($p < 0.05$); moreover, IL-1B at T0 were highly correlated with calves' weights at +7 ($p < 0.01$), +14 ($p < 0.001$) and +21 days ($p < 0.01$). Hence, reducing inflammation in late gestation could potentially enhance buffalo health and production efficiency that can also advantage calf health and growth.

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Effects of grazing two mixtures of forage species on ewes milk production under climate change condition

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In Sicilian forage systems, it is still necessary to identify species that allow to maintain over time meadows for grazing. Native grassland species such as Sulla (*Sulla coronaria L.*) represent a resource with well-known potential in Mediterranean environments. The intercropping combination of sulla with other self-seeding species (e.g. chicory or other cosmopolite species such as clover) would represent a useful strategy to implement grazing resources in Sicilian forage systems. This research aimed to evaluate the effects of continuous grazing of different intercropping forage legume species on live weight (LW), BCS and milk yield and composition in ewes. The forage species were (i) mixture of *Sulla*, *Medicago polymorpha* L., and *Cichorium intybus* L. (SMC); (ii) mixture of *Medicago truncatula* L., *Medicago polymorpha* L., and *Medicago scutellata* (MMM). Twenty lactating ewes of Valle del Belice breed were homogeneously divided into four groups of five ewes on the basis of lactation phase, milk yield, BCS and LW; each group was assigned to one of four grazing sectors of 1500 m^2, consisting of two replicates of SMC and MMM. LW and BCS of ewes were recorded at start and end of trial. Ewes' milk yield were recorded daily, while individual milk were sampled 3 times, every 8 days, and analysed for its components and clotting ability. During the trial forages were sampled to determine chemical composition. During the experiment, lasting 25 days, there was a gradual decrease in milk yield which resulted less pronounced for SMC group than for the group grazing on the other forage mixture. Therefore, the ewes fed on SMC mixture showed a significantly higher milk yield in comparison with the ewes grazing on the mixture MMM (1334 vs 1153 g/d; $p < 0.05$). Furthermore, the milk produced by SMC ewes was characterized by higher protein (5.17 vs 4.85 %, $p < 0.001$) and casein percentages (4.02 vs 3.73 %, $p < 0.001$), lower urea content (37.70 vs 45.38 mg/dl $p < 0.001$) and better clotting parameters measured with Formagraph (a_{30} and a_{2r}, $p < 0.001$). Finally, at the end of grazing period, ewes of SMC group showed a less reduction in LW compared to MMM ewes (–2.05 vs -3.55 kg, respectively), although the difference did not reach a significant level. The results obtained seem to highlight the promising role of the SMC intercrop, leading to a potential quantitative-qualitative improvement of pasture resources and productive performance of grazing ewes in a semi-arid Mediterranean environment.

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Influence of pre-milking teat preparation procedures and bedding type on the bovine milk microbiome: results of a pilot study

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Milk microbiota represents a key point in raw milk cheese production and contributes to the development of typical flavor and texture of cheese. Some studies have recently shown that the microbial composition of milk was related to the microbiota of bedding. Pre-milking teat disinfection and cleaning is essential to reduce risk of exposing the open teat end to environmental pathogens and is an important step in the production of high-quality milk. However, the chlorine products employed in these practices could have an impact both on the milk microbial population and on the cheese making properties.

The aim of the present study was to evaluate (1) the influence of the bedding type on bovine milk microbiota and (2) the influence of chlorine products usage for pre-milking cleaning using both microbiological and metagenomic analyses.

For the bedding trial, the influence of two different types of bedding (straw and mats) on the microbiological quality of bulk milk collected in 24 different farms was evaluated. No significant differences were observed between the two groups of samples with regard to total bacterial count (ranging from 10^2 to 10^3 cfu/mL) and lactic acid bacteria (from 10^2 to 10^3 cfu/mL).

For the second trial, in 10 different farms, pre-milking teat cleaning was performed using lactic acid based detergents for 2 weeks and bulk milk samples were collected on day 8, 11 and 14 of treatment. Subsequently, chlorine-based detergents were used for 2 weeks and bulk milk samples were collected on day 8, 11 and 14 of treatment. Considering all farms as a whole, there were no significant differences in the alpha and beta-diversity metrics, summarizing the richness and the distribution of abundances of the bacterial groups, between the two treatments. Interestingly, significant differences were found in the milk microbiome of each farm compared to the others, regardless of the type of cleaning used, and, within each herd, comparing the two treatments. From the taxonomic analysis, eight to fourteen genera in each herd resulted significantly different (p-value <0.05) with and without chlorine detergents. Among these, *Acinetobacter* was the most common one, but with a different trend depending on the farm.

In conclusion, each farm had its own milk microbiota which was influenced not only by the detergent used for the pre-milking teat cleaning but also by environmental conditions and farm management.

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EU-CIRCLES project: microbial and health evolution of pigs reared in high and low sanitary condition

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The EU CIRCLES project's objectives are to examine the development of the pig's microbiome from the of early colonization to slaughter and to identify microbial features connected to piglet health status in both low and high sanitary conditions in order to inform microbial targeted intervention. In the first step, 96 piglets from 22 litters were divided into two groups at the age of 21 days (d) and assigned to two different weaning and fattening farms selected for their history for good (H group) or poor (L group) scores in terms of productivity (e.g. feed conversion rate), prescription of antibiotics and occurrence of infection where they were monitored until slaughter. Pigs were fed the same diets throughout each phase, regardless of the farm. Samples of faeces and blood were collected at d21 of age (farrowing unit, T1); 42 and 80 d of age (weaning unit, T2 and T3); 98 and 278 of age (fattening unit, T4 and T5). Microbial profile (V3-V4 regions of 16S rRNA), blood formula and immunoglobulins (Igs) were analysed. The alpha and beta diversity indices ($p = 0.001$) significantly highlighted the evolution of the faecal microbiota from piglets to adult pigs. The faecal microbiome at T2, T3, and T4 were significantly impacted by the farm's sanitary condition. At T2, H pigs had lower alpha diversity ($p = 0.002$) and were more abundant in *Prevotella*-related bacteria than L pigs, who had an higher abundance of *Lactobacillus* and *Collinsella*. At T3, L pigs were more abundant in *Terrisporobacter*, Veillonellaceae, and *Catenibacterium*, while H pigs had a higher alpha index ($p = 0.001$) and were richer in Bacteriodales and *Treponema*. An interaction between the sanitary condition and time was observed in the weaning phase for several blood components. At T2, H pigs had lower red blood cells, haematocrit and lymphocytes counts, and higher haemoglobin basophils and neutrophils counts compared to L pigs ($p < 0.001$). At T3, H pigs had higher haemoglobin and lymphocytes counts, and lower basophils and neutrophils counts compared to L pigs ($p < 0.001$). Blood formula and Igs clearly clustered according to time (3 clusters: T1, T2-T4 and T5). Overall, the project shows a notable impact of the sanitary condition and animal maturation on the gut microbiota of pigs.

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Perceived resilience of beef farmers importing young bulls and heifers from France

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Veneto's beef production is strongly characterized by the presence of specialized fattening units relying on imported animals. The purpose of this study was to analyse how farm resilience is perceived by Veneto breeders in a period of climate, social and economic challenges. We submitted a questionnaire to about sixty farmers, obtaining 25 replies. The questionnaire was composed of a first part asking the structural and managerial characteristics of the farm, and a second part related to the importance attributed by the breeder to the functions of his own farm (e.g. food production or valorisation of natural resources), to the risk management strategies applied until now, the main challenges that the farm will have to face in the near future, and the perception that the farmer has about the resilience of his farm in terms of robustness, adaptability and transformability. Farms were classified according to their size, in terms of average heads raised per year, into small (≤500), medium (501–1800) and large (>1800). Data on function and resilience capacities were analysed through ANOVA, whereas the frequency of management strategies used and the challenges perceived by farmers were analysed by using Fisher exact test. The main function for all farm sizes was to 'provide a sufficient agricultural income' (28.6% of importance assigned). Large farms gave more importance to providing good working conditions to employees ($p < 0.05$), whereas for small farms it was more important to care about rural areas attractiveness ($p < 0.05$). Risk management strategies applied in the last 5 years deeply differed among farm different sizes ($p < 0.01$), whereas no difference in resilience, that was on average 3.76 in a 1 to 7 scale, was perceived by farmers. Main challenges also significantly differed between small, medium and large farms ($p < 0.001$), but on the whole economic challenges were considered the most important (45.3%), followed by social (20.3%), institutional (18.7%) and environmental (10.9%) ones. The low resilience perceived can be explained by the high specialization of farms. More care on the adoption of suitable risk management strategies in different farms must be warranted.

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Estimation of the rearing cost for female replacement lambs from birth to first lactation

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One of the most important phases in the management of dairy sheep is the breeding of the replacement, i.e. the young animals that will replace the culled ewes. In Sardinia about 500,000 replacement female lambs are raised every year. From a technical point of view the feeding and growth before weaning, after weaning and mating are fundamental. In previous studies of our group, different mathematical models have been used in Sarda dairy sheep to study the replacement lamb growth and to predict the live body weight at different ages and physiological stages. The aim of this study was to calculate the rearing cost of replacement lambs using growth curves from experimental recorded data, from birth to first lambing. The study was conducted at a commercial farm in North-West Sardinia where a total of 86 sheep born in the same month were weighted at birth and every 7 days. Animals had different ages at the sampling points according to their date of birth. Body weight values were analyzed with a Brody growth model to estimate the average weights reached at different physiological stages. The real cost of feeding observed per each period in the animal group was considered. Suckled milk was considered equal to 5.376 liters per kg of body gain (at price of 1.30 €/liter). Feed costs for starter, hay and commercial mix after weaning were equal to 0.55 €/kg, 0.14 €/kg and 0.45 €/kg, respectively. Body weight and age were highly correlated and the model precisely predicted the growth curve (R^2 of 0.93; $p < 0.001$). Target weight resulted equal to: 3.43 ± 0.54 at birth, 13.35 ± 2.60 at weaning, 32.35 ± 3.20 at puberty, 36.42 ± 3.25 at conception, and 45.87 ± 5.06 at first lambing. The milk and starter intake resulted equal to 1.03 liters/d and 0.083 kg/d per head from birth until weaning. In the post-weaning phase the dry matter intake was 0.873 kg per day. The total cost, to raise one replacement lamb resulted equal: 63.32€ (birth to weaning), 108.33€ (birth to puberty), 132.31€ (birth to conception), and 247.71€ (birth to first lambing). Improvement of replacement practices should be oriented to reduce age at first lambing reaching the target weights at early age (before puberty) and to reduce the overall rearing cost.

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Characterization of cattle slaughterhouse waste and their bioconversion into valuable protein sources and biofertilizer by black soldiers' flies' larvae

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This study aimed to assess the waste products in slaughterhouses in Mbam et Inoubou division and how they can be valorized through the production of black soldier fly larvae. Therefore, the first step was a survey conducted throughout the division to characterize the waste management and waste stream in these slaughterhouses. The surveys were administrated in the slaughterhouses in the target subdivisions (Ombessa, Bafia, Ndikinimeki and Makénéné). Thereafter, organics wastes were collected in these slaughterhouses for the production of black soldier fly larvae. Different waste-based diets (ruminal content and blood) and a control diet (starter chick feed) were formulated and randomly allocated in form of quaplicates to black soldier fly larvae and tested their effects on growth parameters (average weight, weight gain, feed intake and daily growth) and the proximate nutritional composition of larvae. The data obtained were processed and then the results analyzed. It emerged from this survey that 76 animals are slaughtered per week and that these animals generate an average of 0.95 tonnes of blood and 3.69 tonnes of ruminal content deposited in the environment each week. For the bioconversion trial, the results showed variations in physicochemical parameters depending on the type of diet. the control diet exhibited temperatures above that of the others. Growth performance generally varied significantly with diets ($p < 0.05$); the control substrate presented a higher final weight over a short larval phase compared to the other substrates. The feed conversion ratio varied between 1.3 ± 0.05 to 3.4 ± 0.24 with the control recording the highest value. On the nutritional quality aspect, the larvae from the diets made up of slaughterhouse waste presented high values compared to the larvae of the control diet, such as the protein contents which was around (23.67 ± 0.19–46.30 ± 0.15%). The smallest value was that of larvae fed with the control diet. Besides that, the mineral content of larvae fed with slaughterhouse waste was also superior to that of the control.

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Rationing automation in cattle farming: a further path towards sustainability

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Agriculture accounts for 7% of the CO_2 equivalents emitted into the atmosphere, of which 75% is attributable to livestock production. Currently, barn automation technologies may help reduce the pollution potential of animal farming. The AUTOFEED project (https://autofeed.crea.gov.it) aims to deepen the knowledge of total mixed ration (TMR) and automatic feeding systems (AFS) for cattle, which are promising solutions to drive animal husbandry towards increasing sustainability.

In such project, a survey collected the impressions, advantages, and disadvantages of using an AFS.

The interview (both online and directly carried out) provided insights into the end-user feedback about this technology. Despite the limited diffusion of such systems on the national territory (about one hundred robots), 29 answers were collected from farmers with AFS installed and 19 responses from farmers willing or planning to adopt them. The AFS technology, compared to a conventional distribution system with a mixer wagon, has shown a net energy saving higher than 50%, all in favour of environmental sustainability. The interviews also highlighted a net saving in labour greater than 40% that allows for a change in the methods of managing the farming workload. The result is less strict workload management so that the operators previously involved in preparing the ration (executive, repetitive and very rigid task) can be involved in increasingly managerial tasks (i.e. placing more attention to animals), improving the social sustainability of the livestock sector. Moreover, the interviewees reported a +2.92 kg/d increase in feed ingestion and a +3.31 kg/d increase in milk production following AFS adoption.

This causes the economic and environmental sustainability of farming to rise following the spread of the impacts over increased production.
This study found greater sustainability, in all its forms, of AFS compared to conventional unifeed distribution systems.

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Influence of the use of a product based on bacteriocins from Lactococcus lactis susbp. cremoris in pre- and post-milking teat treatment on bovine milk microbiota

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Pre- and post-milking teat disinfection with commercial solutions containing iodine, hydrogen peroxide, or chlorine are procedures routinely used in the herds to reduce the incidence of new intra-mammary infection. These chemical products may contribute to modify the milk microbial population. Therefore, in this study, we investigated the effect of applying a product containing a bacteriocin during the pre- and post-dipping teat disinfection as evaluable alternative of disinfectants. One strain of *Lactococcus lactis* subsp. *cremoris*, producing Nisin A showing *in vitro* a high bactericidal power towards the main mastitis bacteria was used to set up pre- and post- milking products for teat disinfection.
From the same herd, twenty multiparous dairy cows with low somatic cell counts were divided into two groups of ten lactating cows each: for the pre- and post-milking disinfection, the control group (CTR) was treated with a commercial iodine disinfectant, while the treated group (TR) with the product containing the above-mentioned bacteriocin. Milk samples were collected before (T1) and after one (T2), two (T3) and three months (T4) of treatment from two out of the four healthy quarters, selected on negative bacteriological results and low SCC (<100,000 SCC/mL). For the sequencing, the V3–V4 hypervariable regions of the 16S rRNA gene were sequenced by a MiSeq (Illumina) run with 2×250-base paired-end reads.
The bacterial community was prevalently composed of Firmicutes (~54%) and Bacteroidota (~20%). From T1 to T4 a significant increase was detected for Actinobacteria (~14%) and Proteobacteria (~16%) phyla, regardless of the treatment. Seventy-eight genera differed significantly (p-value <0.05) between the CTR and TR groups. The alpha-diversity metric, summarizing the richness and the distribution of abundances of the bacterial groups, increased significantly (p-value <0.0048) in the TR group at T2, T3 and T4, highlighting higher biodiversity. The milk microbiome showed a statistically significant separation both among treatments, timepoints and combining treatments × timepoints (p-value =0.00464).
In conclusion, the use of the bacteriocin-based products in milking routine of dairy cows could enrich the milk microbiome biodiversity with valuable effect on raw milk cheese-making as well as in preventing the emergence of antibiotic resistance.

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Multi-scale integrated accounting of buffalo farms' metabolism

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The analytical approaches currently in use to evaluate the sustainability of livestock systems are based observing the end products, these approaches do not allow to enter into the details of the production process by integrating the social, economic and environmental domains. The following contribution illustrates a relational model applied to evaluate the relative sustainability of buffalo farms in Campania Region (Italy). We applied the MuSIASEM approach (MultiScale Integrated Accounting of Societal and Ecosystem Metabolism), a relational multicriteria framework allowing the characterization and evaluation of different metabolic features of the system. The accounting method evaluate the metabolic performance, in an upward and downward causality, by means of viability (techno-economic constraints), feasibility (bio-physical constraints), desirability (societal norms) and externalization (system opening). All analyzed farms showed a high metabolism, in terms of use of resources and production of different forms of waste products. The dependence on external systems (externalization for the import of feed) appeared to be extremely evident, with repercussions on the effectiveness of the production systems in attracting the local workforce. Evident critical issues also emerged in farms with biodigesters.

Acknowledgements

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P277

Validation of genomic breeding values for feed efficiency using field data: experience from UNIBO experimental herd

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A strategy to maximize genetic gain in dairy farming is to identify genetically elite females through genomic testing. Indeed, direct genomic values (DGV) are early accessible at a cost-effective also in young stock and are subsequently blended to estimated breeding values (GEBV) once prediction of genetic merit is undertaken when phenotypes are available. These criteria can be used by farmers to rank females and service the best ones with (sex-sorted) semen of elite bulls to achieve greater gain while simultaneously reducing the number of non-productive animals. Feed efficiency is nowadays the most popular issue for animal scientists working with ruminants. The purpose of the current research was to validate feed efficiency breeding values using real dry matter intake (DMI) data. For this purpose, we quantified the association between DGV/GEBV of predicted feed efficiency (PFE) and on-field feed intake data. Up to date, the total number of genotyped subjects at the UNIBO experimental herd is 279. Cows are routinely enrolled into experimental nutritional trials in which individual DMI (kg/d) information are collected on a daily basis. DGV/GEBV for PFE are calculated by ANAFIBJ within the national genetic and genomic evaluation; proofs are standardized to a mean of 100 and standard deviation of 5. Data were analysed with a linear mixed model, separately for primiparae and pluriparae, by adjusting the dependant variable DMI for the fixed effects of daily milk yield (linear covariate), PFE DGV/GEBV (two classes: <100 or ≥100), and days-post-calving (<120 or ≥120); random terms were cow nested within experimental trial and contemporary group (experimental trial-test date). Mean PFE was 99.50 (±1.48) while daily individual DMI averaged 23.48 (±3.44) and 26.91 (±3.14) kg/d for primiparae and pluriparae, respectively. The Pearson's correlation coefficient between PFE and feed intake was −0.14, suggesting that cows with higher genetic merit have lower DMI. Differences between least squares means of the two PFE levels were −0.23 (±1.10; $p > 0.05$) and −0.26 (±0.75; $p > 0.05$) kg DMI/d in first- and later-parity cows, respectively. Although not significantly different, estimates indicated that, irrespective of systematic effects, cows with higher genetic merit for PFE have a lower individual daily intake compared to those with a lower genetic merit. Future efforts should be pursued to augment the sample size in order to increase the robustness of these estimates.

P304

First evaluation of economic performances on farms adopting environmentally friendly practices. The case of PLANT-B protocol in Italy

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Adequate pollen nutrition is one of the necessary conditions for successful honeybee management. Therefore, it is key to provide adequate pollen and to enlarge the period during which the bees can find it by increasing the number of pollen sources. This analysis assesses the potential economic results of implementing a strategy to increase honeybee pollen availability by introducing aromatic plants in mixed beekeeping-citrus farming systems (PLANT-B protocol). This is crucial for ensuring the economic sustainability of the strategy and generating the related agronomic, cultural, and environmental benefits. The analysis is based on a sample of ten farms (five beekeepers and five farmers) implementing the approach. Data are gathered through a structured questionnaire designed to estimate economic and technical viability. Farms are subdivided into treated (that adopt PLANT-B protocol with aromatic plants) and no-treated subjects. The survey describes the production techniques and related economic coefficients. This data is used to assess the technical and economic results. We have assessed: (i) the yield of citrus and honey production, (ii) costs associated with citrus fruits and honeybees, (iii) farm gross margins. In particular, we compare (1) the baseline with the first and second years of the PLANT-B protocol application and (2) treated and no-treated farmers with the PLANT-B protocol. The results are encouraging. PLANT-B application shows a significant increase in mean production but with a high variation among farms, while the price level does not change. This generates an increase in the level of revenue but also in its variability. Clearly, also costs increase due to the PLANT-B application only due to the growth in the input prices because, conversely, the input quantity decreases. Specifically, the costs of fertilisers, crop protection, and water have risen sharply: farmers spend more after adverse events. At the end, farmers that adopted or not adopt PLANT-B do have very similar economic results. This preliminary result demonstrated that the introduction of aromatic plants seems economically sustainable. This suggests that farmers could implement this strategy, which can yield not negligible environmental and agronomic benefits.

P425

Development and implementation of a result-based funding mechanism for carbon farming in EU mixed crop livestock systems

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Agriculture is responsible for the emission of about 10% of the generates greenhouse gas (GHG). In the same time it contributes to regulate the CO_2 uptake via carbon sequestration in soils. With the aim to contribute to tackle climate change, the main objective of the 'LIFE CARBON FARMING' (*Development and implementation of a result-based funding mechanism for carbon farming in EU mixed crop livestock systems)* project is to reduce by 15% (633.841 tons CO_2 eq) the carbon footprint of agricultural products (beef and dairy) in 6 years using result-based funding. Carbon finance is viewed as a key to incentive and develop low carbon actions. The project also aims to build up carbon finance mechanisms in 6 European countries.

The project, coordinated by the French Institut de l'Elevage (IDELE), has 31 beneficiaries and involves about 700 mixed crop livestock farms in France, Ireland, Belgium Germany, Spain and Italy, where 18 carbon action plains called 'Carbon farming projects' will be applied. The effects of these plans will be evaluated on GHG emissions and carbon sequestration. These projects will also contribute to improve animal welfare, increase renewable energy, reduce synthetic fertilizers and increase beef and milk production.

The functional unit is 1 kg of live weight gain (LWG) and 1 kg of fat protein corrected milk (FPCM) for beef and dairy systems, respectively. The environmental categories are global warming, eutrophication, acidification, energy consumption, water consumption, carbon sequestration, and the contribution of beef and dairy farms to the rural landscape. These categories will be evaluated in 61 farms (36 beef farms located in Piemonte and in Veneto and 25 dairy farms distributed throughout the national territory).

To evaluate the environmental impact of these farms, 'Calcul des AUTOMATISE Performances Environnementales en Elevage de Ruminants' (Niveau 2) developed at the IDELE was used.

In all farms the effect of the mitigation strategies for carbon footprint reductions and their economic feasibility will be evaluated. The activity will be carried out in collaboration with ASPROCARNE, UNIVARVE, AIA and CRPA of Reggio Emilia.

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P448

LEO: big data zootecnici per la consulenza aziendale e la ricerca

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The Livestock Environment Open data project (LEO, URL: www.leo-italy.eu) is creating a linked open data platform for animal husbandry, merging existing and novel information on Italian livestock populations. Up to now, the LEO semantic graph database contains ten Ontologies, ten controlled vocabularies and handles more than 11 billion semantic triples.

Data are available either as a direct download of preconfigured datasets in Json and csv format or by querying the source information using a unique query language (SPARQL). A steady refresh of the Ontologies grants the updated description of the data domain of interest and the complete navigability of the environment.

Data are collected routinely on 18,194 livestock farms and about 4 million extant animals belonging to 109 breeds, of which 103 are local and autochthonous.

Five data categories are available: laboratory data that include about 40 parameters on milk quality, reproductive and productive field data collected routinely on a single individual, data from Precision Livestock devices (e.g. ruminometers, activometers, milking robots, etc.), health and welfare data and, climate data. The climate data includes two large datasets: the Atmospheric global coverage model ECMWF with a spatial resolution of 0.1 × 0.1 degree (9 square km) and the forecasting weather model COSMO-IT with a spatial resolution of 0.025 × 0.025 (2.5 square km).

The applicative cooperation of several running national databases like BDN (National livestock database) and Si@alleva (DHI National database) enriches the available open data in LEO. The project is coordinated by the Italian Breeding Association and supported by a multidisciplinary partnership from Universities, Veterinary Departments, and computer engineers.

P558

Carbon footprint of dairy milk and milk protein production: case study from Po Valley (Italy)

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One of the biggest challenges for dairy milk production is match the need to increase production to meet a growing increase in food demand while reducing environmental impacts from primary production. Focusing on Carbon Footprint (CF), standardized milk (in kg or liter) is the widest functional unit used to estimate the impact of milk production. Fewer studies investigated the impact in terms of nutritional values of milk, particularly protein, casein, or urea content. Casein content is a key issue in cheese-making yield and the milk urea content is a nitrogen efficiency indicator. Knowledge on the relationship between CF and these paraments could be useful to support the definition of dairy farm managerial and nutritional strategies to improve milk production efficiency and reduce enteric methane emissions and nitrogen losses in manure.

Ten dairy farms located in the Po Valley (Italy), producing milk destinated to Grana Padano PDO production, were investigated through primary data collection. Farm CFs were estimated according to Life Cycle Assessment (LCA) approach. A cradle-to-farm gate system was considered including all the processes necessary to milk production and indirect emissions due to enteric fermentation, manure fermentation and nitrous fertilizers application. A comparison between different functional units were performed: 1 kg of Fat and Protein Corrected Milk (FPCM), 1 kg of Milk Protein (MP) and 1 kg of Casein (MC). Biophysical allocation was used between milk and meat production. Based on FPCM-CF values, the most impactful group of farms had a mean CF of 1.59 kg CO_{2eq}/kg FPCM, 45.79 kg CO_{2eq}/kg MP and 58.34 kg CO_{2eq}/kg MC, meanwhile the least impactful one had a mean CF of 1.23 kg CO_{2eq}/kg FPCM, 35.46 kg CO_{2eq}/kg MP and 47.97 kg CO_{2eq}/kg MC. The assessment of CF according to FPCM, MP and CM leads to the same ranking of farms. The most impactful group is characterized by high value of average days in milk and inter-part period, % milk protein and casein and lower average daily milk production and milk urea content.

These preliminary results highlight the need to better investigate milk urea and protein content as functional unit to investigate milk production environmental impact.

P94

Integration of a health monitoring system using wearable sensors in the management protocol of female calves

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Management of the calves is critical to optimize the weaned calf. Fifty seven percent of mortality is seen in the first 24 h and 75% within 7 days of birth. Dairy heifer calves' management is the second-largest annual expense for dairy farms. The best management can allow to repay the investment in rearing costs with higher milk production and a longer productive life. The possibility to monitoring the calves' health status using wearable sensors could make it possible to improve the management of the calves by intervening in advance. This study aimed at verifying the possibility of using ear tag sensors from birth to weaning (80 d of age) in Holstein calves. The trial was carried out in a dairy farm consisting of 600 lactating cows from 19th July to 28th October 2022. Seventeen female calves were monitored, and behavioral or physiological parameters were registered. Ear tag sensor was applied at birth. The parameters recorded from wearable sensors were suckling, rumination, low activity, and high activity times and health index. The health index is based on the assessment of the calf's vitality which is estimated as a function of the activity parameter. During experimental period live weight at birth and at weaning, the daily quantity and quality of colostrum administered were registered. Consumption of milk and solid feeds were registered 5 days per week. At same time calf health status was monitored using the Wisconsin calf health scoring chart considering rectal temperature, cough score, nasal discharge, eye score, ear score and fecal score. From birth to weaning some behavioral parameters such as low activity and high activity time significantly changed. The greatest low activity time was spent within the first 10 days of life (44.2 ± 13.9 min/d) then decreased until weaning (14.9 ± 15.6 min/d). Rumination time was considered after the 30th days of life. Rumination time increased from 3.6 ± 7.2 to 8.2 ± 11.8 min/d until weaning. Suckling time increased linearly ($R^2 = 0.61, p < 0.01$) from birth to weaning. Some interesting associations between health status and behavioral parameters have been found. Rumination time was positively associated with feed intake and average daily gain ($r = 0.62$ and $0.52, p < 0.01$) and negatively related with fecal score ($r = -0.52, p < 0.01$) and rectal temperature (-0.30, $p < 0.05$). Moreover, the health index alarm anticipated the appearance of the health problem and the increase in body temperature in 90% of cases.

P111

Use of NIRS to predict diet characteristics and digestibility in dairy cows

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To date, Near Infrared Spectroscopy (NIRS) seems to be a valuable aid for feeding management. Portable NIRS tools are recently used for on farm analysis of raw materials, ration and digestibility assessment. This study aimed to control of the diet and the apparent digestibility of dairy cows using a portable NIRS (PoliSPEC^{NIR}, Itphotonics, Italy) with on-farm modality. The study was carried out in 4 dairy cow farms (A, B, C, D), visited 6 times each, for a total period of 12 months. The surveys conducted at each inspection were (i) chemical-physical analysis of ration; (ii) quality control of the preparation of the diet (homogeneity index (HI) and sorting index (SI)); (iii) chemical analysis of feces taken directly from the rectal ampoule; (vi) estimation of the apparent digestibility using lignin (ADL) as a marker. In total, 24 unifeed samples and 24 feces samples were analyzed only with PoliSPEC^{NIR}. The results showed the use of PoliSPEC^{NIR} directly on farm allowed to have a daily control over the quality of diet. Moreover, it can identify, in a short time, the critical points of feeding management. The results about HI (expressed as a pure number, PE) showed that two farms distributed homogeneous unifeed (HI means ± sd was 68.47 ± 6.50 and 66.59 ± 11.04 respectively for farm A and D) and the other two inhomogeneous unifeed (50.28 ± 21.57 and 49.17 ± 9.40 respectively for farm B and C). Inhomogeneous unifeeds were due to the lack of attention in preparing the mixer wagon (MW) or to an excessive presence of wet raw materials or excessive filling level in MW. As regards the SI (expressed as a pure number, PE) measured at 1.5h after distribution of the ration with NIRS, two farms reported a 'modest selection' (36.90 ± 18.15 and 37.93 ± 6.98 respectively for B and C); one farm 'marked selection' (42.32 ± 15.98 for A) and the last farm 'accentuated selection' (73.70 ± 9.04 for D). We have seen that SI is greatly influenced by the type of raw materials used, by the type of feed bunk and feeding management. The use of NIRS for the analysis of feces has allowed to estimate the apparent digestibility of the diet. The apparent digestibility was slightly overestimated by NIRS, especially the digestibility of starch. In conclusion, NIRS instrumentation is extremely useful for analyzing all the critical points in the feeding management of dairy cow, making possible to improve the well-being, production, reproduction, as well as the eco-sustainability of the farm also in economic terms.

P182

Virtual fencing for the management of limousine cattle at pasture: effects on animals' activity budgets as compared to traditional grazing management

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Virtual fencing (VF) is an innovative technology to manage cattle at pasture. VF is based on GPS collars which can contain animals within GPS coordinates. Herd containment relies on the learning capacity of the animal to associate an acoustic cue to a forbidden pathway. When animals cross the VF, the collar activates, and an audio tune is played. If the animal turns back, the audio tune stops; otherwise, at the end of the audio tune, the collar delivers a slight electrical pulse (0.1 J). The combination between the audio cue and the electrical pulse can repeat at most three times, then, if the animals does not return inside the VF, a notification of escape is delivered, and only GPS positioning is maintained active. The aim of the study was to assess whether VF affected the activity budgets of a herd of Limousine cows during seasonal grazing in Tuscany (IT). Thirty Limousine cows were divided into two groups of fifteen. The control group (C) was handled according to the traditional grazing management, letting the animal graze in 15 ha for 6 weeks. The second group (VF) was handled using VF collars (Nofence®, Norway) on incremental surfaces according to biomass availability and consumption rate. For the VF group, five virtual fencing changes were performed, starting from a grazing surface of 4.5 ha, and reaching 15 ha after 6 weeks. Each animal of both groups was provided with smart ear-tags (Allflex®, USA) which registered activities as time spent ruminating, lying, grazing, and moving. Data were analyzed with a linear mixed model. Group, date, and hour were set as fixed effects, and animal within group as random. VF did not affect animal's activity budgets, indeed, time spent ruminating ($p > 0.05$), resting ($p > 0.05$), and grazing ($p > 0.05$) were similar between C and VF cows. Daily hours deeply affected animal's activity budgets ($p < 0.001$) with resting and ruminating activities being predominant during late evening and night, whereas during early morning and afternoon the leading activity was grazing. In conclusion, results suggest that VF does not affect animal normal behaviors during seasonal grazing. Further studies should be carried out on other stress-related parameters to assess whether VF can be an animal-friendly management technique to enhance pasture utilization in extensive grazing systems while reducing farmers' labor.

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P207

Precision beekeeping systems as swarm control tools: a case study

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Precision beekeeping (PB) is raising interest by beekeepers and researcher involved in apidology, giving great opportunities to timely measure any significant changes in the health and production status of honey bees (*Apis mellifera* L.) (HB). One of the main focus is the remote identification of colony swarming as a critical factor on the honey production profitability. This contribution aims to identify some PB parameters that can allow to detect swarms' events remotely in real time and intervene in field.

In spring 2022, a beehive, located PLANT-B (PRIMA project n.1812) apiary at the Experimental-Educational Farm 'Nello Lupori' (Tuscia University, Viterbo, IT) was monitored by a PB system provided from Melixa S.r.l. (Trento, IT). The PB system included a scale to measure hive weight (HW), in-hive temperature (IHT) and flight activity (FA) sensors. On 19 April 2022, during a hive inspection intervention, the occurrence of several queen cells was recorder and just three of them were left; starting from this day the hive was remote monitored in order to evaluate swarm events.

From the April 30th to the May 6th three swarms were recorded by the PB system and two of them were then pursued and cached. The primary swarm left the hive the April 30th between 11:02 and 11:53 when it was recorded a significant weight loss (1.34 kg, $p < 0.01$) compared to the previous day; in the same time-window 12,731 flights were recorded. The swarm was found and captured about thirty meters away from the apiary and the presence of a not marked queen was noted. The secondary swarm happened on May 3th and was recorded from 13:02 to 13:53; a significant ($p < 0.01$) weight loss of 2.12 kg was recorded compared to the previous day and the FA in the swarm period was about 15,073 flights. It was found and caught, with a not marked queen, a hundred meters away from the apiary. These two swarms were found in south-east direction from the original beehive. The last swarm was recorded between 13:23 and 14:14 on May 6th with a FA of 12,450 flights. A significant ($p < 0.01$) decrease of weight of 1.13 kg, compared to the previous day, was recorded. For all the events, the ANOVA did not show significant changes in temperature compared to the previous days. Results show that swarms events could be remote identified with the loss of weight, future developments of PB systems could be the integration of in-app notification in real time of the event or a fine detection of IHT to identify the pre-swarm phase.

P341

Validation of the body condition score measured by a specific app for image analysis in lactating Mediterranean buffalo

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Remote sensing techniques in livestock farming are important to increase efficiency and improve animal and humans' welfare. An application for Android smartphone/tablet (®West Systems) was used for indirect determination of Body Condition Score (BCS) of Mediterranean buffalo. The equation ($BCS = -9.6426 + 0.1139\ AIT$, where AIT = angle between the apex of the rump and the two tangents to the iliac tuberosities) for a Visual Image Analysis (VIA) study on Mediterranean buffalo, was used for the app development. That equation gave good results, making the median obtained by experienced evaluators and VIA comparable ($r = 0.88$, $p < 0.01$).

In this further implementation, we aimed to validate the algorithm, developed in 2008, for its use in Android environment, on different set of animals and against different evaluators (at the time neither technical and specific App nor suitable mobiles were still available). The App works by measuring an angular parameter (ATI) and returns a BCS value. 250 observations from two operators were compared to the values obtained by the App. Agreement among evaluations was assessed by computing correlations of classification within operators, and between operators, and App. Linear regression on official classification (means of two trained operators) and classification obtained by the App, was carried out to assess the accuracy and precision of the method. The difference between App and the mean of evaluators' assessment kept in the same standard BCS (1–9 scale) class for over the 96.85% of cases and within 1 class in the remaining 3.15%. The concordance between App and official method ($r = 0.934$ $p < 0.0001$) was higher than the between operator agreement ($r = 0.863$ $p < 0.0001$). Regression ($y = 0.944x + 0.425$, $R^2 = 0.872$, $p < 0.0001$) showed a slope coefficient very close to one, and a low dispersion, with an average error of a fifth of BCS official scale point (RMSE =0.213). The high consistency between classifications obtained by expert operators and those provided by this method, demonstrated that VIA approach can replace the traditional human based methodology. The next step will be to install one or more measurement spot over the farm's facilities, where animals are used to pass or stay. That way it could be possible to get data automatically and continuously. In conclusion the use of such a technology may help to safely manage the herds and to improve buffalo farming efficiency.

P450

SEBASTIEN: a decision support system for the livestock sector built on environmental, sectoral and geospatial data

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Climate change has a strong impact on livestock systems, in particular on animal production, health and reproduction, on diseases and their spread, and on feed availability. Furthermore, greenhouse gasses (GHG) emissions, where the livestock sector has a significant impact, are influenced by the changes in climate trends. For all these reasons, there is an increasing need to formulate management options that can help the scheduling of farming practices that can be successful under the future climate scenarios. To address these needs the SEBASTIEN (Smarter livEstock Breeding through Advanced Services Tailoring Innovative and multi-sourcE data to users' Needs; GA INEA/CEF/ICT/A20202373580, action n. 2020-IT-IA0234. Cup: J35F21003610004) project was designed. SEBASTIEN aims to deliver a Decision Support System (DSS) for a more efficient and sustainable management of the livestock sector in Italy, with a focus on extensive and intensive cattle, sheep, and goats' farming system. To achieve these goals, SEBASTIEN will generate and re-use large datasets (e.g. data sourced by the LEO and HIGHLANDER national projects), such as environmental and meteorological data, production and reproduction livestock data, and the available sensor data on animal behavior and physiology, and stable inner temperature and humidity. The models derived from investigating these data collections through machine learning algorithms will be implemented in online tools available to the entire farmer community. The online tools will forecast heat stress, determine pasture usage to guide the farmer towards the best grazing areas, and anticipate the increased risk of infection from some target pathogens and diseases (currently: visna-maedi and bluetongue). The tools, available also as mobile apps, will display the target information as risk/availability colour gradients in a browsable, user-friendly maps. The development of a DSS and Services' Platform, developed considering stakeholder's advice, will contribute to make the livestock sector more environmentally and socio-economically sustainable. Finally, leveraging on the ever-increasing data availability the SEBASTIEN platform will be easily extended to provide supporto on additional species, pathologies, and other parameters of interests for the livestock sector.

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An overview of the rabbit spermatozoa proteome

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Despite the importance of sperm and its membrane proteins for fertilization, the rabbit sperm proteome is still poorly explored. Therefore, an LC-MS/MS analysis (EPIC-XS project 186, Horizon 2020) of rabbit sperm proteins extracted with the Pierce™ Cell Surface Protein Biotinylation and Isolation Kit was performed. UniProt database and eggNOG-mapper v2.1.9 were used for annotation and obtaining protein/gene names and tissue specificity information. An overrepresentation test (GO-Slim and Complete) was performed using PANTHER v17.0 to identify the most frequent gene ontology (GO) terms for biological process (BP), molecular function (MF), and cellular component (CC) as well as their fold enrichment (FE). Only results for $p < 0.05$ were considered. A total of 1377 proteins were identified and 859 were reliably quantified after removing contaminants and duplicates. Of these, 770/724 had a protein/gene name assigned and 175 proteins were associated with the cell surface/plasma membrane. Only 3.5% had information on tissue specificity and only 0.7% were already associated with the male reproductive system, according to UniProt. Of the 724 that had a gene name, 707 had an equivalent human protein. Their corresponding IDs were used to search in PANTHER database against Homo sapiens to maximise hits, as no data from *Oryctolagus cuniculus* were available. Of the 697 mapped, the term with the highest fold enrichment for BP was 'tricarboxylic acid cycle' (FE =17.38), for MF it was ' unfolded protein binding' (FE =8.13), and for CC it was 'chaperonin-containing T-complex' (FE =22.15). The terms 'ATP generation from ADP', 'glycolytic process', 'single fertilization', 'fertilization', 'ATP binding', and 'acrosomal vesicle' were also significantly enriched (7.93 < FE <16.11). The TCA cycle (FE =18.80), ATP synthesis (FE =16.88), and pyruvate metabolism (FE =14.77) were the 3 most enriched pathways. An overrepresentation test was also performed for the plasma membrane proteins. Compared to the total proteome, a higher FE was observed for processes related to reproduction, such as sperm-egg recognition, binding of sperm to zona pellucida, and (single) fertilization. These were also among the ten most enriched biological processes. These results contribute to a better understanding of the rabbit sperm (membrane) proteome and function, increasing the possibility of discovering biological targets for various biotech applications.

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An assessment of the deamidation and glycosylation sites of rabbit spermatozoa proteins

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Deamidation of asparaginyl (Asn) and glutaminyl (Gln) residues can lead to altered protein function or even degradation due to changes in the protein structure. Hence, through this process, Asn and Gln may play an important role in certain biological processes. Glycosylation is also an important process that happens from spermatogenesis to fertilization. Rabbit remains one of the most used species in reproduction studies, yet its sperm proteome is poorly explored. In this work a proteomic analysis of rabbit sperm was performed resorting to deglycosylation of surface proteins to help get complementary information (EPIC-XS project 186, Horizon 2020). Sperm proteins were biotinylated and isolated with the Pierce™ Cell Surface Protein Biotinylation and Isolation Kit (A44390, Thermo ScientificTM), according to slightly modified manufacturer's instructions. Peptides were dissolved in triethylammonium bicarbonate and Peptide-N-Glycosidase F was added for deglycosylation. After LC-MS/MS analysis, data were analyzed with MaxQuant v2.0.3.0 (FDR 1%). Enzyme specificity was set as C-terminal to arginine and lysine, also allowing cleavage at proline bonds with a maximum of two missed cleavages. Variable modifications were set to oxidation of methionine residues, acetylation of protein N-termini and deamidation of asparagine and glutamine residues. A total of 1377 proteins were identified and 881 reliably quantified. Regarding deamidated peptides, 4405 were identified, corresponding to 960 deamidation sites. Of these, 810 (localization probabilities >0.75), deriving from 1089 different deamidated peptides, were identified with high confidence. A single deamidation site can be quantified multiple times when it is identified alone as well as in combination with other sites on the same peptide. This is the case for many deamidation sites, leading to redundancy in lists of quantified deamidation sites. Overall, 661 deamidated peptides were reliably quantified (at least 3 valid intensity values in one of the experimental conditions), corresponding to 640 unique deamidation sites on 321 proteins. Also, 279 possible N-linked glycosylation sites were identified (NxS/T motifs). Deamidation and glycosylation sites of (rabbit) sperm proteins are poorly studied despite their importance. This work extends the knowledge in this regard and contributes to a better characterization of the rabbit sperm proteome profile.

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Dietary hemp seed cake in slow-growing broilers: effects on productive traits, antioxidant status and intestinal histomorphology

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The competitiveness of local livestock systems depends on the identification of alternative feeds to high-cost imported ingredients. Among the identification of local alternative feeds, hemp (*Cannabis sativa* L.) shows interesting potential. Considering the growing popularity of hemp cultivation, a large number of by-products are currently available, of which hemp seed cake (HSC), obtained by cold pressing methods, may be investigated as a feed ingredient due to its nutritional properties. Thus, HSC could partially replace conventional feeds in poultry diet being a protein (>30%) and fatty acid source (PUFAs, especially C18:2 n-6). Therefore, to evaluate the effect of dietary HSC on growth, meat traits and oxidation, and intestinal morphometry, a total of 180 male slow-growing broiler chickens (Hubbard) were divided into three groups and fed three grower-finisher isonitrogenous (19.7% crude protein DM) and isoenergetic (ME: 14.5 MJ/kg DM) diets from 14 days of age until slaughter (49 days). Diets varied according to HSC inclusion level: a control diet without HSC (HSC0) and two test diets containing 5% and 10% of HSC (HSC5 and HSC10, respectively). There were a total of 18 floor pens, six replicate pens (2.5 × 1.5 m) per treatment with ten broiler chickens in each pen with stocking density according to EU legislation. Morphometric indices of duodenum were measured at the end of the feeding trial (49 days). Dietary HSC had no adverse effect on growth performance, resulting similar (final BW ~2200 kg and FCR 2.25; $p > 0.05$) and meat traits (carcass and breast yield: 74 and 20%, respectively) among groups. Feeding HSC at both inclusion levels influenced positively ($p < 0.05$)breast and thigh meat muscles fatty acid composition, in terms of n-3 PUFAs series. Moreover, meat from HSC-fed broilers resulted less ($p < 0.05$) susceptible to lipid oxidation compared to control diet. Dietary HSC inclusion at 10% resulted in higher ($p < 0.05$) duodenal villus height, crypt depth and villus height to crypt depth ratio as well as villus surface area. Based on the obtained findings, dietary HSC positively supported productive traits of slow-growing broilers preserving meat from oxidation and also improving intestinal morphometry. Thus, the study assessed that hemp seed cake can be successfully included in the broiler diet, resulting in a valuable ingredient as replacement for conventional feed sources.

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