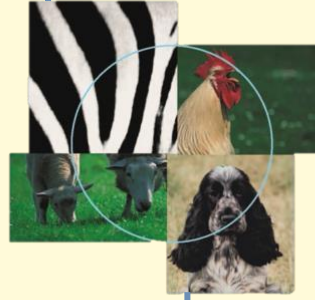


ESVCN
27th Congress



European Society of
Veterinary &
Comparative Nutrition

Congress Proceedings

27th Congress of the
European Society of
Veterinary and
Comparative Nutrition

7th- 9th September 2023

Vila Real | Portugal



utad

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Dear Colleagues and Friends,

It is with great pleasure that I extend a warm welcome to all the participants of the 27th Congress of the European Society of Veterinary and Comparative Nutrition (ESVCN) that gather this year at University of Trás-os-Montes and Alto Douro, in Vila Real. This much-anticipated event arrives after a long delay due to the COVID pandemic, a hurdle we have collectively overcome.



The entire LOC team is thrilled to be able to open the gates of the beautiful UTAD campus to you, so you can enjoy our “house” together with all its surroundings from Porto to the Douro valley. We hope you can fully enjoy some of the historical landmarks and breath-taking landscapes and also our welcoming gastronomic culture, that promises to enrich our meeting.

Thanks to a collective effort it was possible to surmounting obstacles and shape this Congress in what we are sure to be an outstanding program. Over the next three days we will be able to attend engaging sessions, insightful presentations, and participate in stimulating discussions due to an excellent scientific program, created with the many high-quality abstracts submitted. This year, a special additional highlight includes two post-conference talks thanks to the generosity of our esteemed colleague: Professor Josef Kamphues. A special care was also put in the organization of the social program, where we hope you can find a deep trace of the Portuguese culture.

To every author, attendee, and presenter, we owe heartfelt thanks. Your commitment to share your interest and work in veterinary comparative nutrition is key to our common goals. I also wish to express profound gratitude to the ESVCN board for their trust and unwavering support, to UTAD for hosting us, and to our entire LOC team who were always ready to help. A special thanks to Andrea Ellis, Ronald Corbee and Johanna Corbee who have been instrumental in bringing this Congress to live.

Last but certainly not least, a profound thank you to our Sponsors. Without them, being here today would have been extremely difficult, if not impossible.

Thank you all so much for making this Congress a reality. We hope you will enjoy and leave Vila Real with a sense of fulfillment and full of new ideas.

With warm regards,

Ana Lourenço

Chair of the Organizing Committee of the 27th Congress of the ESVCN



Welcome by ESVCN president Aulus Carciofi



Dear friends and colleagues,

The time has finally come, and I would like to welcome everyone to the 27th ESVCN congress! I wish you, and your accompanying family and friends wonderful days in Vila Real!

My first important message is my greatest thanks to the colleagues Ana Luisa Lourenço and Ronald Corbee and their team, who dedicated their time, mind, heart and efforts in building one more great opportunity of interweaving. All their hard work allowed us the possibility of being here, ready to experience three intense days of exchanges, renewed friendships, knowledge, feelings, and ideas. After the challenges of 2021, when we had to adopt the online format at the last minute, Ana, Ronald, and their colleagues from University of Trás-os-Montes and Alto Douro didn't let the baton fall, they remained enthusiastic and willing to work for our Society and dedicated their best so that we could have one more face-to-face event! They had to work twice as hard, as they organized an excellent online congress in 2021, and two years later they carefully prepared a face-to-face event in a welcoming and receptive environment, (even including a boat trip!), so that we could have an experience that flies beyond science and work. The Society is also grateful for the ongoing support from all our sponsors and supporters, enabling the local team to organize the place with comfort and quality to receive us.

So let us have three days of learning, following more than 140 great presentations and discussions about comparative nutrition from different places of the world! The congress program is organized in six main sections of posters and oral presentations, with three main plenary speakers. This time, there will also be a keynote post congress speaker, Prof Kamphues, so don't miss this opportunity. If you are coming for the first time, we suggest that you take the opportunity to meet people who do work similar to yours, but also that you take the chance to interact with researchers from different fields. Ventilating ideas and being comparative can generate surprising results in the long term!

Finally, I can share that the ESVCN annual congress has already incorporated itself into my life in a natural and pleasant way, almost like a special time of the year to see family and friends. For those who have been participating for a while, we all have memories of shared moments in different congresses, so much so that sometimes we are not even sure in which edition of the meeting a certain situation happened, but we know that it was in 'an ESVCN Congress'.

On behalf of the ESVCN Board, I express my gratitude and wishes for a wonderful stay in the 27th ESVCN congress at Vila Real. Welcome!





Local organizing committee

Chair: Ana Lourenço

Ronald Jan Corbee, Filipe Cardoso, Cristiana Rego, Johanna Corbee, Cristina Guedes, Miguel Rodrigues, Luís Mendes, Ana Beleza Silva, Júlia Côte-Real, Tomás Magalhães, João Requicha, and student associations AEMV and NEEZ

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Boards and 2023 awards

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Anne Mößeler (President), Ronald Jan Corbee (Vice-President), Ana Lourenço (Executive Secretary), Veerle Vandendriessche (Treasurer), Annette Liesegang (Past President)

Awards

Helmut Meyer Award: In remembrance of the late Prof. Helmut Meyer, one of the founding fathers of ESVCN, an award is given every year to the best presentation on a general topic at the yearly ESVCN congress. This is a prestigious over-arching ESVCN Award to promote and also to strengthen veterinary and comparative nutrition in research, teaching or consulting. Eligible: All oral presenters who are members of ESVCN Prize: The winner will receive a monetary price and a certificate.

Josef Leibetseder Award: Professor Joseph Leibetseder was a founding member of ESVCN and a founding member of the European College of Veterinary and Comparative Nutrition. He was the Director of the University of Veterinary Medicine in Vienna until 2001. He was an open minded idealistic visionary, who was working in different organisations, in particular for the association “Animals in Therapy” until the end of his life. Eligible: All poster presentations by society members. Prize: The winner will receive a monetary price and a certificate.

Waltham Best Student Award: An award is given to the best oral presentation on a companion animal topic at the yearly ESVCN congress. ESVCN membership is a prerequisite. Eligible: Students, residents, PhD candidates working towards a degree or board certification in the field of companion animal nutrition, and presenting their results at the ESVCN symposium. Prize: The winner will receive a monetary prize plus a travel bursary to enable the winner to travel to and present their research at the American Academy of Veterinary Nutrition annual symposium.

Cat Nutrition Award sponsored by Purina: This award is kindly sponsored by Nestle-Purina in recognition of research in the area of cat nutrition. Eligible: All oral presentations in the field of cat nutrition. Prize: The winner will receive a monetary price and a certificate.

Dog Nutrition Award sponsored by Royal Canin: This award is kindly sponsored by Royal Canin in recognition of research in the area of dog nutrition. Eligible: All oral presentations in the field of dog nutrition. Prize: The winner will receive a monetary price and a certificate.

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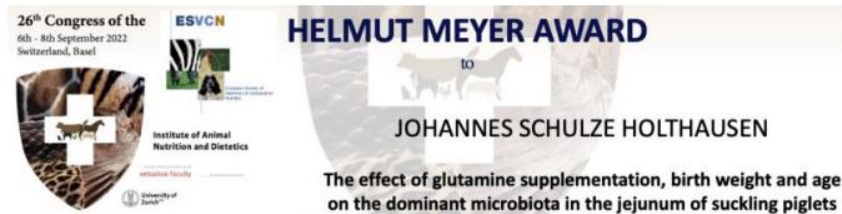


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Award Winners **ESVCN** 2022

The prestigious over-arching ESVCN Helmut Meyer Award was awarded to:



26th Congress of the
6th - 8th September 2022
Switzerland, Basel

ESVCN

HELMUT MEYER AWARD

to

JOHANNES SCHULZE HOLTHAUSEN

The effect of glutamine supplementation, birth weight and age on the dominant microbiota in the jejunum of suckling piglets

Institute of Animal Nutrition and Diagnostics
University of Jyväskylä

Jury: Annette Liesegang (Chair), Britta Dobenecker, Eva Maria Saliu, Angela Gimmel, Jürgen Zentek; Presented by: Annette Liesegang



The prestigious ESVCN Josef Leibetseder Award was awarded to:



26th Congress of the
6th - 8th September 2022
Switzerland, Basel

ESVCN

JOSEF LEIBETSEDER AWARD

to

EVA-MARIA SALIU

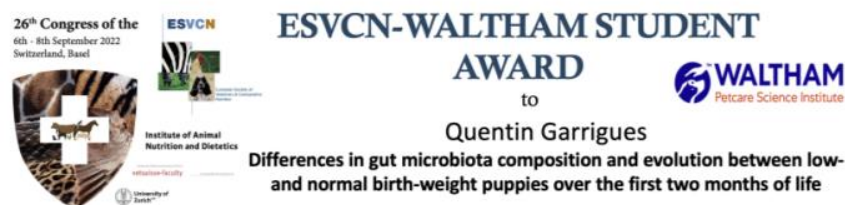
Sows' preference for fibre rich feedstuffs and the impact of fibre particle size on feed intake duration, faecal consistency and short chain fatty acids in faeces

Institute of Animal Nutrition and Diagnostics
University of Jyväskylä

Jury: Stefanie Handl (Chair), Geert Janssens, Anna Lourenço, Esther Plantinga; Presented by: Stefanie Handl



The ESVCN-Waltham Student Award was awarded to:



26th Congress of the
6th - 8th September 2022
Switzerland, Basel

ESVCN

ESVCN-WALTHAM STUDENT AWARD

to

Quentin Garrigues

Differences in gut microbiota composition and evolution between low- and normal birth-weight puppies over the first two months of life

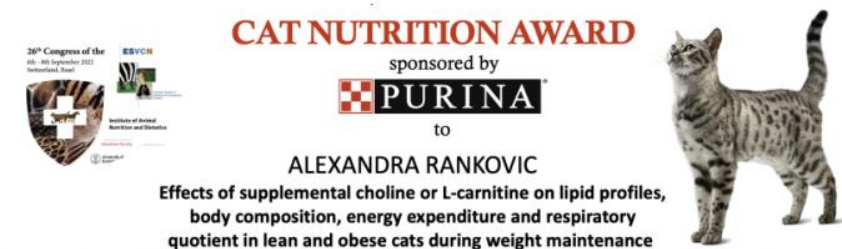
Institute of Animal Nutrition and Diagnostics
University of Jyväskylä

WALTHAM
Petcare Science Institute

Jury: Anne Marie Bakke (Chair), Myriam Hesta, Ronald Corbee, Charlotte Bjornvad, Aulus Carciofi, Richard Butterwick, Jürgen Zentek; Presented by: Richard Butterwick and Anne Marie Bakke



The Cat Nutrition Award was awarded to:



26th Congress of the
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Switzerland, Basel

ESVCN

CAT NUTRITION AWARD

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to

ALEXANDRA RANKOVIC

Effects of supplemental choline or L-carnitine on lipid profiles, body composition, energy expenditure and respiratory quotient in lean and obese cats during weight maintenance

Institute of Animal Nutrition and Diagnostics
University of Jyväskylä



Jury: Brigitta Wichert (Chair), Anna Lourenço, Charlotte Bjornvad, Esther Hagen-Plantinga, Paula Wartelski Pryluk; Presented by: Alessandra Paroli



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Thursday 7th

September

Speaker Stream 1 Aula Magna

Speaker Stream 2 Geosciências Auditorium

8:30-8:55

Welcome session
Chair: Ana Lourenço

8:55-9:40

Tiago Domingos Plenary: The role of ruminant livestock in a sustainable food system: a Portuguese perspective



9:40-9:55

ECVCN to be confirmed ECVCN resident presentation: The winning case report

9:55-10:10

Hannah Godfrey Effect of 12-week feeding frequency treatment on feline physiology, energy metabolism, and physical activity (AAVN-Waltham Student Award winner)



10:15-11:15

1A: Pre and probiotics I
Chair: Annette Liesegang

2A: Animal Nutrition I

Chair: Britta Dobenecker

10:15-10:30

Renjie Yao Effect of feeding grass hay to suckling piglets on their colonic morphology and microbiome at weaning

Blanchard Beneficial response to a very low-carbohydrate diet in dogs with refractory chronic enteropathy: pilot study of 25 cases.

10:30-10:45

Hankel Effect of replacing 10% of the starter diet with fermented rye during the first two days of chick's life on the development of intestinal microbiota

Verbrugghe Dietary fibre increase improves clinical response in cats with chronic enteropathy unresponsive to hydrolyzed protein diets: A case series

10:45-11:00

Mugnier The zebrafish (Danio rerio) model: an accurate model to screen the potential and differential effect of bacterial postbiotics on immune

Magalhaes Nutritional management of cats with chronic kidney disease: a retrospective study in 41 cats

11:00-11:15

Vastolo Amaranthus grains a potential alternative to conventional cereals in monogastric nutrition

Teixeira Evaluation of plasma and urine amino acids levels of cats with chronic kidney disease stages 1 and 2 fed a renal diet

11:15-11:45 Coffee break



11:45-12:45

1B: Pre and probiotics II
Chair: Wendy Wambacq

2B: Clinical cases I

Chair: Brigitta Wichert

11:45-12:00

Pignataro Effect of a nutraceutical treatment on the intestinal microbiota of sled dogs

Pankratz Immediate allergic reactions in the head region in two horses after ingestion of hay – a case report

12:00-12:15

Theodoro What is beyond immunomodulation in yeast or seaweed β -glucans intake in dogs?

Hottat A senior pony with severe dental issues and equine metabolic syndrome: a nutritional challenge ?

12:15-12:30

Souza Repeatability of the effects of dietary supplementation of probiotic on gut fermentative metabolites and microbiota in dogs

Moscoso Case Report: Nutritional Approach to an unbalanced diet and subsequent overcorrection in a female kitten

12:30-12:45

Duangnum-sawang Assessing the Efficacy of Lactobacillus Probiotic Tablets for Improving Canine Oral Health

Pedreira Effects of long-term feeding a diet with moderate protein and low phosphorus in cats with CKD IRIS stage 1 and 2

12:45-13:45 Lunch break

13:45-14:45

1C: Posters: Pre & Probiotics

2C: Posters: Clinical, Equine, Exotics

recorded presentations will loop in the room - please then visit the presenters at their poster

Jeusette A diet supplemented with inulin is able to modify faecal microbiota and its function in cats, leading to an improved vaccine response

Stefanutti Case report: nutritional management of a protein-losing enteropathy in a senior Weimaraner dog

Henriquez In vitro approach for the estimation of the digestibility coefficients and fecal concentration of fermentation products in cats

Stafocche Rickets by hypovitaminosis D in a Pit Bull Puppy fed imbalanced homemade diet

Montserrat Fiber source differentially modulates gut microbiota and host response in dogs

Morrone Nutritional strategy in the management of hyperlipaemia and non-alcoholic fatty liver (NAFL) in a Sardinian donkey

Souza Yeast probiotic *Saccharomyces cerevisiae* effect on fermentative products, immunity and microbiota in dogs undergoing abrupt dietary change

Morrone Comparison of fluctuation in Body Weight, Body Condition and Muscle Condition in Breeding Stallions throughout non-mating period and stud season

Hankel Comparison of fecal microbiota and their fermentation characteristics after the addition of different insoluble fibre sources to complete dog foods

Senes Metabolic shift of nutrition-related parameters in stallions during and after stud season

Penazzi Digestibility, fermentation by-products, and extrusion characteristics of kibble diets for dogs with red lentils pasta by-product

Flaga Effect of dietary linseed oil and vitamin E supplementation on mRNA expression of genes linked to cell survival in white blood cells of racehorses

Lasek Effect of type of detoxifier on fermentation processes determined in-vitro in canine model

Waliczek Effect of dietary linseed oil and vitamin E supplementation on free radicals scavenging enzymes expression in white blood cells of racehorses

Freire Pea flour effects on extrusion traits, digestibility, and fermentation products in faeces of dogs

Sun Body mass, fecal microbiota and fermentation products in zoo felids

Zentek Study on the use of a yeast cell wall preparation on the faecal microbiome in dogs

Janssens Evaluating the effect of bedding type and feeding level on growth performance and metabolic responses in ducks (*Anas platyrhynchos domesticus*)

Melotto The use of metronidazole affects diet digestibility, intestinal permeability, fermentative metabolites, immunity indicators, and microbiome of puppies

Böswald Comparative analysis of pancreatic amylase activity in laboratory animal species

Amaral Mapping of the fecal microbiota of dogs with inflammatory bowel disease after oral intake of yeast cell wall through output of artificial intelligence

Böswald Digestibility of crude nutrients and minerals in C57Bl/6J and CD1 mice fed a standard maintenance diet

Apper A combo of a prebiotic and a postbiotic can modulate the immune system and the gut microbiota of elderly mice in a different way as the prebiotic alone

14:45-16:30

1D: Pre & Probiotics III

Chair: Stefanie Theodoro



2D: Clinical cases II

Chair: Niels Bles

14:45-15:00

Marchi Evaluation of increasing levels of beta-glucan intake on canine digestibility, immunity, and faecal microbiota

Priymenko A case of fatal poisoning with Chinaberry berries in a dog

15:00-15:15

Moravszki Assessment of Labelling and CFU Values of Enterococcus faecium Containing Veterinary Probiotics

Hofmeister Evaluation of two elimination diets using the canine atopic dermatitis extent and severity index (CADESI) in dogs with suspected food allergy – preliminary

15:15-15:30

de Godoy Plasma and fecal metabolomic profile of adult cats fed yeast supplemented diet

de Mik Case report: Hypocobalaminemia and coat discoloration in a dog

15:30-15:45

Vecchiato The effects of a high-protein diet on the fecal microbiota and metabolome of obese and lean cats, before and after caloric restriction

Lucke Nutritional Consultation for a growing Australian Kelpie with hereditary Intestinal Lipid Malabsorption – a Case Report

15:45-16:00

Lasek Effect of species of herbal extracts on fermentation processes determined in-vitro in canine model

Giorgino Case report: Nutritional management of an overweight dog with Myasthenia Gravis

16:00-16:15

Zafalon Markers of satiety, inflammation, and body composition in obese cats fed a diet containing 0.06% beta-glucans

Fusi Dietary intervention in the management of Corpus Callosum dysgenesis in a young English bulldog




16:15-16:30

Miranda Effects of β -glucan derived from different sources in an obese female dogCorbee Case-control study of the prevalence and etiology of ataxia in asiatic lions (*Panthera leo persica*) between 2002 and 2020



16:30-17:00 Coffee break

17:00-19:00


ESVCN AGM ESVCN Members only - Annual General Meeting

8:30-9:55	ECVCN AGM	ECVCN Diplomates only - Annual General Meeting Chair: Ronald Corbee		
10:00-10:45	Javier García Alonso	Plenary: Understanding pet rabbit nutrition and feeding		
10:45-11:15 Coffee break				
11:15-12:15	3A: Companion Animals I Chair: Tomás Magalhães		4A: Farm Animals I Chair: Lonneke Noorman	
11:15-11:30	Blees	Bioavailability of collagen hydrolysates in healthy dogs after single and prolonged intake	Blanchard	Association between ruminal microbiota and ruminal lesions in cattle slaughtered in a slaughterhouse in the south of France
11:30-11:45	Flanagan	Impact of dietary supplement combinations on glycemic control in overweight colony beagle dogs	Eppe	Potential association between Negative Energy Balance and thyroid cysts in milking dairy cows: preliminary results
11:45-12:00	Vecchiato	Effects of two diets on the clinical assessment and lipid profile of diabetic dogs on insulin therapy	Rehman	Effects of chitosan loaded manganese nanoparticles on the production performance, serum metabolites, meat quality, and intestinal histomorphometry in broilers
12:00-12:15	Dezorzova	Effect of TEKRO Cardiodiet on electrolytes in cats with HCM	Papadopoulos (recorded)	Effects of dietary supplementation of pomegranate peel in laying hens on egg quality and nutritional parameters
12:15-13:15	 3B Posters: Companion Animals	recorded presentations will loop in the room - please then visit the presenters at their poster	4B Posters: Farm Animals	
	Pagani	Can recyclable retort pouches improve wet pet food shelf life?	Grzeskowiak	Fiber composition in sows' diets influences bile acid profile in colostrum
	Carciofi	Correlation between dog breed size and processing and starch cooking requirements of diets	van Hees	Oat hulls in the diet of newly weaned piglets modified gastric conditions and morphology
	Henriquez	Determination of digestibility and urinary pH in owned cats by an In-home approach	Barc	The action of zearalenone on enzymes of phase I (CYP1A2) and phase II (COMT, UGT) mycotoxin metabolism in the liver of healthy and ketotic cows
	Godfrey	The effect of isoenergetic reduction of dietary macronutrients on physical activity, energy expenditure, and body composition in cats	Vandendriessch	Nutritional emulsifiers are an effective tool to improve intestinal morphology and metabolism of piglets during the post-weaning period under intensive farming
	Pedreira	Validation of the reduction of days of total faeces collection for apparent digestibility protocol in dogs	Wilke	Reducing the protein content in broiler diets – performance based effects on environmental impacts
	Henriquez	Comparison between conventional and marker methods for the fecal collection in the digestibility trials in cats	Tahir	Effects of iron oxide nanoparticles supplementation on the growth performance, viscera development, physiological health markers, and meat quality in broilers
	Danyer	Association of Cat Owners' Nutritional Habits with Cats' Body Condition Score	Kölln	Effect of increasing dietary vitamin D 3 contents on bone breaking strength of young layer pullets of different genetic origin
	Delgado	Preference of Diets Containing Clove, Blueberries or Kelp in Dogs	Henriquez	Evaluation of dog owners' perception as to the physical activity performed by their animals associated with the quantity of food supplied
	Hetényi	Unconventional feeding methods in Norwegian dogs	Principe	Possible nutrient deficiency in cats at 10% of total calories from snacks and treats
	Lucke	Dog feeding practices with regard to exercise routine in Switzerland – a survey	Perini	Does the season influence the food intake of adult cats?
	Ruggiero	Nutritional survey at Naples University VTH	Perini	Determination of nutrients in veterinary diets marketed in Brazil for weight loss in cats
	Vecchiato	Pet rabbits housing and feeding practices: results from a survey in Germany	Menniti	Non-compliances in healthy weight plans at a small animal nutrition clinic: a case series

13:15-14:15 Lunch break

14:15-15:15	3C: Companion Animals II Chair: Han Opsomer		4C: Farm Animals II Chair: Marco de Mik	
14:15-14:30	Baptista da Silva	Assessment of nutrient concentrations and labeling adequacy of commercial insect-based pet food products	Saliu	Performance of sows and piglets receiving diets enriched with coarse or finely ground hay or sugar beet pulp
14:30-14:45	Henríquez	Environmental impacts of extruded dry dog food produced in Brazil, determined by the Life Cycle Assessment (LCA)	Przybylo	Intake of starch in first weeks of life affects lambs behaviour and has a medium-term impact on ruminal fermentation
14:45-15:00	Ribeiro	Effects of choline or L-carnitine supplementation on plasma fatty acids in lean and obese cats during weight maintenance	Saliu	Age dependent short-chain fatty acid absorption in the proximal colon of piglets fed high or low fermentable fibre sources
15:00-15:15	Maturana Delgado	Study of the Potential Antioxidant Effect of a Diet Containing Fresh Blueberries on Exercise-Induced Stress in Dogs	Wilke	Monitoring of environmental impacts of partially substituting soybean meal with rapeseed meal or haemoglobin meal in diets for broilers

15:15-15:45 Coffee break

15:45-16:30	3D: Companion Animals III Chair: Marco Fantinati		4D: Equine Chair: Anne Mößeler	
15:45-16:00	Bosch	Nutritional considerations of the diet of Neolithic dogs: a literature review and scenario analyses	Son	Changes in the nutritional profile, mycotoxins, and phytoestrogens from spring until late summer in selected horse pastures
16:00-16:15	Principe	Untargeted serum metabolomic analysis comparing healthy and diabetic dogs	Pombo	Use of live yeast and thermostable yeast in the blood parameters of maintenance horses
16:15-16:30	Stafoche	Mixed feeding practice reduces weight loss time in obese dogs	Kaya	Perceptions of sustainable horse feeding practices in the Netherlands

17:30-00:00 Gala dinner Menu sponsored by:  Busses will leave promptly at 17:30 outside MIRAGORGO Hotel



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[to Abstract List](#)


**Saturday 9th
September**
Speaker Stream 1 Aula Magna
Speaker Stream 2 Geosciências Auditorium

9:00-9:55

Marshall McCue
 Chair: Jürgen Zentek
 Plenary Using 13C-tracers to characterize nutrient use during feeding and fasting in comparative animal models

10:00-11:15

5A: Protein I

Chair: Esther Hagen-Plantinga


**RAÇÕES
VALOURO
S.A.**

10:00-10:15

Krüsselmann

Influence of the preservation of alfalfa silage on fiber bound protein

10:15-10:30

Ruiz

Effect of dehydrated black soldier fly larvae inclusion in the diet on the welfare status of laying hens at 32 weeks of age

10:30-10:45

Nery

Whole transcriptome analysis in broiler chicken fed mealworm meal

10:45-11:00

Holthausen

Influence of oral glutamine supplementation on microbial gene abundance and associated metabolic pathways in stomach content of neonatal piglets

11:00-11:15

Theodoro

Body composition and protein intake effects on plasma amino acids concentrations in cats

Forero

Metabolic disease prevalence of captive neotropical parrots (Amazona spp.) in São Paulo, Brazil

11:15-11:45 Coffee break

11:45-13:30

5B: Protein II

Chair: Aulus Carciofi

11:45-12:00

Theodoro

Weight loss and methionine, tryptophan, valine and threonine supplementation effects on plasmatic amino acid concentrations in dog

12:00-12:15

Zhang

Effect of extrusion and phytase on the digestibility of plant-based pet foods

12:15-12:30

Souza

Changes in fecal microbiota and functional profile in dogs fed diets with higher or lower protein and fiber concentrations

12:30-12:45

Fredrich

The effects of hydrolyzed chicken liver on digestibility, faecal characteristics, and faecal fermentative end products of adult dogs

12:45-13:00

Brociek

Nutritional and ecological comparison of meat- and plant-based dog feeds

13:00-13:15

Baptista da Silva

Acceptance of insects in pet food products – a survey among Belgian dog and cat owners

13:15-13:30

Santos Neto

Potential to black soldier fly (*Hermetia illucens*) larvae meal based extruded diet to improve canine oral health

13:30- 14:10

LALLEMAND

LALLEMAND ANIMAL NUTRITION

5C: Posters Protein

recorded presentations will loop in the room - please then visit the presenters at their poster

Pagani

Determination of eleven essential amino acids (EAA) by HPLC-DAD/FLD in extruded and retorted grain-free and novel protein diets for dogs

Pagani

Determination of eleven essential amino acids (EAA) by HPLC-DAD/FLD in protein feed materials for extruded pet food intended for dogs

Pignataro

Analysis of the digestibility of amino acids in complete dog food

Kaya

Protein intake in horses and the risk of environmental nitrogen overload

Renna

In vitro rumen fermentation characteristics of defatted insect meals as compared to conventional plant-based meals

Cesar

Comparison of different protein ingredients in digestibility and metabolic parameters in adult dogs

Ruiz

Effect of dietary supplementation with 5% black soldier fly larvae (*Hermetia illucens*) on production parameters of laying hens from 23 to 27 weeks of age

Ruiz

Effect of reducing soybean in laying hens' diets on productive performance

6A: Exotics

Chair: Angela Gimmel

Gudeta

Metabolic responses of Nile tilapia to alternative feed resources

Hsu

The effects of hydrolyzed protein on global metabolomics in adult dogs

Kamphues

Gastric ulcers in pigs – the focus could have been too much on average particle size, missing out the effect of the share of coarse particles in the diets?

Zillinger

Modulation of bone metabolism in young goats fed a phosphorus and/or nitrogen-reduced diet

Muscher-

Modulation of GCN2/eIF2 α /ATF4 Pathway in the Liver and Induction of FGF21 in Young Goats Fed a Protein- and/or Phosphorus-reduced Diet

Banse

Preliminary data on the effects of feeding diets without addition of mineral phosphorus or calcium on performance, serum and bone parameters of growing goats and sheep

Schulz

Effect of a high fat, low carb diet on calcium and phosphorus digestibility in pigs

Schmitz

Effect of a high fat, low carb diet on calcium and phosphorus digestibility in pigs

Processing temperature of full-fat insect meals has limited effects on in vitro rumen fermentation characteristics

Ayala

Goat colostrum as supplement for piglets

Forero

Profile and prevalence of obese captive amazon parrots among other lipid accumulation lessons

Pignataro

Follow-up in 167 homemade diets for maintenance and pathologic conditions in dog

Carciofi

Vegetable protein sources in kibble diets for cats: extrusion, digestibility, and faecal characteristics

Placido

Evaluation of calcium, phosphorus, and sodium concentrations in commercial diets for adult dogs and cats

Carmo

Macronutrients, essential minerals, and toxic metals concentrations in commercial unconventional diets for healthy adult cats

Wichert

A new teaching tool: Peer- facilitated learning to introduce veterinary students in scientific work – using in-vitro techniques

14:10-15:00 Lunch break

15:00-16:00

Awards and Closing

Society Updates and Awards are presented to winning abstracts
Chair: Aulus Carciofi (ESVCN President)**Post Congress Plenary Talks**

Chair: Luis Mendes

16:00-16:45

Josef Kamphues

Plenary: Prevention of gastric ulcers in pigs by changes in the diminution technique of cereals

16:45-17:30

Josef Kamphues

Plenary: Amino acid requirements of dairy cows

Sunday 10th September

Post Congress Tour**14:30-23:00 Post-congress tour: Bus leaves from hotel MiraCorgo - please be there by 14:15 at the latest**

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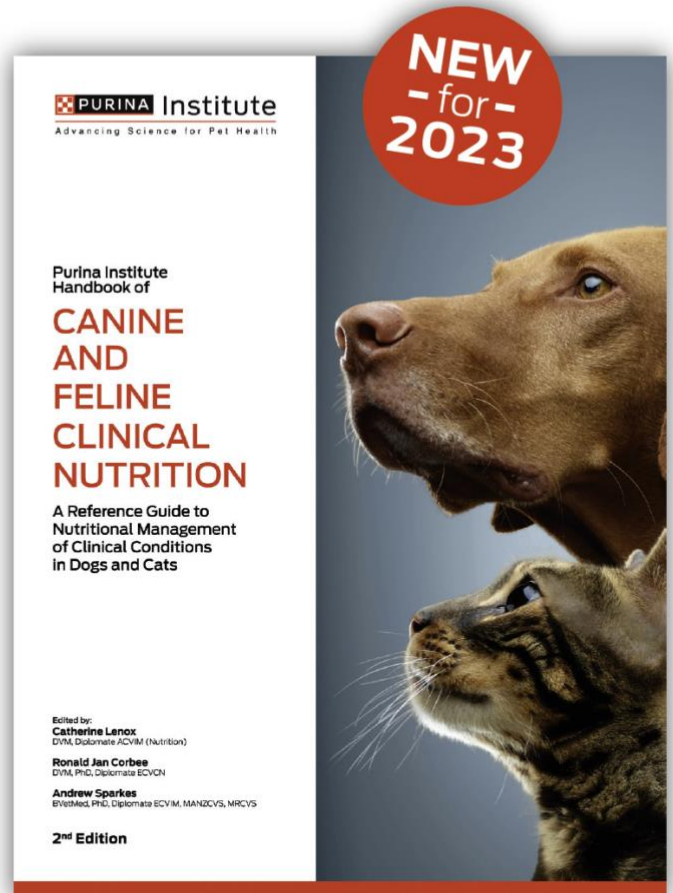
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ABSTRACTS

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Plenary session Thursday

Chair: Ana Lourenço

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Speaker: Tiago Domingos

Tiago Domingos, M.Sc. in Physical Engineering (IST) and Ph.D. in Environmental Engineering (IST), is an associate professor in Environment and Energy at IST, the engineering school of the University of Lisbon. Tiago Domingos teaches or has taught courses such as Thermodynamics, Environmental Modelling, Environmental Economics, Ecological Economics and Sustainable Development and coordinates the M.Sc. in Environmental Engineering at IST. He is coordinator of IST-Ambiente, the IST platform for Environmental Science and Engineering, and president of MARETEC – Marine, Environment and Technology Centre.

Tiago Domingos is a researcher in Ecological Economics and Ecological Modelling, having most notably worked on DEB theory for the metabolism of organisms, the useful exergy approach to energy and economic growth, carbon responsibility indicators, ecosystem services, comprehensive accounting and sustainable agriculture, with more than one hundred papers in international peer reviewed journals.

Tiago Domingos is founder and CEO of Terraprima, an IST Spin-off, winner of the European Commission's "A World You Like with a Climate You Like" competition for the best climate solution in Europe in 2013.

The Role of Ruminant Livestock in a Sustainable Food System: A Portuguese Perspective

Tiago Domingos

MARETEC, LARSyS, Instituto Superior Técnico, Universidade de Lisboa

Conclusion:

- **Ruminant livestock play a key role in sustainable agri-food systems**
- **Grazing on legume rich pastures is key in this, by leveraging land that cannot be used for food production to provide a sustainable nitrogen source for the food system**
- **It seems possible to devise systems that ideally are ideally sustainable, such as**
Sown Biodiverse Permanent Pastures Rich in Legumes +
Montado/Dehesa Agroforestry +
Optimised Intensive Fattening Beef Cattle



Feeding One Versus Four Meals Daily Decreases Postprandial Respiratory Quotient and Increases Rate of Food Consumption in Cats over Twelve Weeks

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Introduction: Similar to humans, feline obesity has become a pandemic. In clinical practice, treatment of obesity involves daily calorie restriction provided in small meals distributed throughout the day [1], based on the assumption that cats eat multiple small prey in the wild [2] and the perception that multiple meals may alleviate begging for food [3]. Our laboratory previously studied the physiological response to feeding frequency in cats over 2 weeks and found that feeding one versus four meals daily resulted in better satiety and may lead to increased fat metabolism [4], but its longer-term effects are not yet known. Therefore, this study investigated the effects of isocaloric provision of one meal versus four meals daily on body composition, physical activity, respiratory quotient (RQ) and energy expenditure (EE), rate of food consumption, and fasted blood glucose and serum insulin concentration in cats over a 12-week treatment period.

Animals, materials and methods: This study involved 16 adult male neutered domestic shorthair cats fed a high-fat high-protein dry kibble to maintain body weight and fed either once daily (FF1, n=8) or four times daily (FF4, n=8). At weeks 0, 2, and 12, dual energy X-ray absorptiometry, actigraphy, indirect calorimetry, blood collection after an overnight fast, and videotaping during the first meal of the day were performed. Blood glucose was measured using a portable glucometer and serum insulin concentration was analyzed using a commercially available species-specific enzyme-linked immunosorbent assay kit. Data were analyzed using the GLIMMIX procedure with treatment as a fixed effect, time as repeated measures, and cat as subject. Significance is reported when $P < 0.05$ and tendency is reported when $0.05 < P < 0.1$.

Results and discussion: Body composition and EE were not different between treatments, but FF1 cats had lower RQ in fed state than FF4 cats ($P_{\text{treatment}} < 0.05$). These observations are similar to our previous study where a three-week treatment period was employed and with wet instead of dry food [4]. The similarity of glucose and insulin concentrations, glucose-to-insulin ratios, and homeostatic model assessment of insulin resistance during the fasted state between the two treatments was also consistent with the previous 2-week study [4]. In contrast to our previous study, total daily physical activity counts did not differ between treatments, but feeding once daily resulted in greater crepuscular activity as FF1 cats tend to be more active during dark hours at weeks 2 and 12 compared to week 0 while FF4 cats experienced no change ($P_{\text{treatment} \times \text{time}} = 0.07$). This may also explain why FF1 cats consumed their first daily meal at a faster rate at week 12 than at week 0 while FF4 cats were slower at weeks 2 and 12 than at week 0 ($P_{\text{treatment} \times \text{time}} < 0.05$).

Conclusion: Overall, limiting feeding frequency to once per day for 12 weeks, without caloric restriction, may increase fat oxidation and nighttime activity, but result in similar body composition and energy expenditure as compared to cats fed four times a day. Longer studies exploring metabolic and satiety markers and those allowing greater food intake are warranted to better understand the biological significance and the role of feeding frequency in feline weight management.

References: [1] German & Martin (2008) Encyclopedia of Feline Clinical Nutrition:4-43; [2] Kane et al. (1981) Nutr. Res. 1(5):499-507; [3] Delgado & Dantas (2020) Vet. Clin. North Am. Small Anim. Pract. 50(5):939-53; [4] Camara et al. (2020) Plos One:0238522

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Session 1A Pre and probiotics I
Chair: Annette Liesegang



1A1. Effect of feeding grass hay to suckling piglets on their gastrointestinal morphology and microbiota at weaning

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Introduction: The addition of insoluble fibre to creep feed affected the digestive tract development of suckling piglets in previous studies [1,2]. In this study, we investigated the effect of grass hay – as a source of structure and insoluble fibre – on the colonic microbiota and its possible link with gastrointestinal development in suckling piglets.

Animals, materials and methods: 15 sows (TN70, parity 1-8) were divided over two groups based on balanced parities. The control piglets (CON; n=7) received commercial creep feed from day (d) 2 of age. The treatment piglets (GH) received the same creep feed but additionally, were provided with grass hay (mean particle length, 6 cm) in a separate feeder until weaning (28d). At weaning, one piglet per litter with the median weight was euthanized. The size and morphometry of digestive tracts and spleens were examined. The content of the mid-colon was collected for microbiota analysis through 16S rRNA amplicon sequencing. The DADA2 R package was used to process the data into ASVs according to the pipeline tutorial [3]. The microbiome data analysis was performed by vegan package in R and phyloseq. The results of morphology were evaluated through GLM. Differences were considered statistically different if $p < 0.05$. This study was approved by Ethics Committee of ILVO with an application number: Aanvraag dierproef 2022/420.

Results and discussion: Morphologically, the weight of the emptied small and large intestine (SI, LI) was higher in GH than CON (223 vs 280 g, 91 vs 77g $p < 0.05$). Grass hay also increased LI length (163 vs 149 cm, $p < 0.05$). The depth of colon crypts decreased in GH (178 vs 156 μm $p < 0.05$). Meanwhile, the villus height in jejunum and villus height to crypt depth ratio in ileum was increased in GH. Notably, the SCFA in caecum were increased in GH but lower in colon than CON. Principal coordinate analysis did not present different clustering of the microbiota community and there was a trend that *Lactobacillus* proportions in GH were greater than in CON, namely 19.6% vs 8% ($p = 0.08$). The growth of GIT was stimulated, and more nutrients might be absorbed at proximal sections with fewer digestive pressure was left to the distal places in GH due to the results of morphology and SCFAs. Meanwhile, like previous studies on other insoluble fibres for humans and rats [4,5], grass hay provision to pre-weaning piglets seemed to stimulate the growth of *Lactobacillus* in the colon. More precise research is needed to evaluate whether the effects of adding grass hay are predominantly attributable to physical or chemical factors, and to determine the robust gut in the suckling period benefits the health and performance of piglets post-weaning.

Conclusion: The grass hay provision to suckling piglets promoted more nutrients produced and absorbed at the front end of intestine, and stimulated the development of GIT. However, it did not alter the colonic microbiota diversity. Therefore, providing grass hay to pre-weaning piglets could be an attractive strategy during weaning transition and for promoting long-term health.

References: [1] van Hees et al., (2019) *J. Anim. Sci. Biotechnol* 10:83; [2] van Hees et al., (2022) *Anim Nutr* 12,284-296.[3] Collahan et al., (2016) *Nat Methods* 13, 581-583 [4] Gibson et al. (1995) *Gastroenterology* 108,975-982; [5] Zhong et al., (2009) *Clin Nutr* 28,575-580;

1A2. Effect of replacing 10% of the starter diet with fermented feed during the first two days of chick's life on the development of intestinal microbiota

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Introduction: The complex process of the intestinal bacterial colonisation is determined by early exposure to environmental bacteria during the first days of chick's life [1]. With the offer of fermented feeds, high numbers of lactic acid bacteria ($> 9 \log_{10}$ cfu/g feed) can enter the gastrointestinal tract of animals, which can have significant effects on intestinal microbial ecology [2]. We hypothesized that replacing 10% of a starter diet with fermented feed only during the first days of life will influence lastingly the development of intestinal microbiota composition of chickens.

Animals, materials and methods: Day-old broiler chicks ($n = 160$; Ross 308) were randomly divided into two groups with 80 birds each, housed in floor pens and fed with conventional complete diets (based on wheat, corn and soybean meal) in three phases. Ten percent of the

Table 1. Characterization of the control and experimental diets offered during the first two days of chick's life (values in g/kg dry matter [DM] unless otherwise stated)

Parameter	Starter diet	Control diet	Exp. diet
Dry matter (g/kg as fed)	885	838	830
Crude ash	54.5	54.3	53.7
Crude protein	241	232	235
Crude fat	53.7	53.3	54.6
Crude fibre	29.2	29.7	30.5
Starch	464	467	469
Sugar	50.1	50.4	43.5
AME (MJ/kg DM)	14.0	13.9	14.0
L-lactate		0.520	1.157
D-lactate		0.064	0.736
pH		5.97	5.77
Lactic acid bacteria (\log_{10} CFU/g diet)		2.91	8.14

Protein and energy requirement recommendation by [3], 23% crude protein and 12.4 MJ ME/kg diet.

starter diets were replaced by either rye mixed with water (control diet) or fermented rye (fermented anaerobically with the help of a starter culture consisting of 1k2079 *Lactobacillus plantarum*, 1k2103 *Pediococcus pentosaceus*, and 1k2082 *Lactococcus lactis* for 24 h at 37 °C, experimental diet). Each diet was offered to 80 birds only during the first two days of the chick's life (Table 1). On days 7, 14, 21, and 42, intestinal contents from the ileum and caeca were processed for the 16S rRNA gene sequencing ($n = 20$). The statistical analysis was done with R (version 4.1.2). The `adonis()` function in the "vegan" package (version 2.5.7) was used to evaluate differences of community structure by permutational multivariate analysis of variance (PERMANOVA). Selected alpha diversity indices (Observed, Shannon) were calculated with the package "phyloseq" (version 1.36.0). Pairwise comparisons of means were conducted with the package "rstatix" (version 0.7.0).

Results and discussion: Pairwise permutational analysis of variance of the factor "Group" (Control vs. Experimental group) indicated the strongest contrasts in β -diversity at both sampling sites until day 14 ($p < 0.001$, $R^2 > 0.40$). Microbiota composition in caecal samples differed significantly between the Control and the Experimental group until day 42. Consistently higher mean values of bacterial richness and Shannon index of Experimental group were observed in the ileum during the whole study period when compared with the Control, though no significance was detected.

Conclusion: The replacement of 10% of the starter diet during the first two days of the chick's life with fermented feed had lasting effects on intestinal microbial ecology until slaughter age of the animals.

References: [1] Stanley & Bajagai (2022) *Animals* 12(20), 2890; [2] Bunte et al. (2020) *Microorganisms* 8(5), 638; [3] Aviagen (2022). Ross Broiler: Nutrition Specifications.

1A3. The zebrafish (*Danio rerio*) model: an accurate model to screen the effect of heat-inactivated *Lactobacillus* strain on immune responses

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Introduction: Due to its high fecundity, genetic tractability, small size, rapid development, gut function, immune gene conservation with mammals, and availability of biomarkers (Brugman, 2016), the zebrafish (*Danio rerio*) has become a widely used vertebrate model to study immune responses and gut barrier function under numerous conditions that matter for human and animal health. It represents a good model to test the modes of action of strain-specific postbiotics which appear to interact with the host cells and have a positive impact on the gut barrier and immune system. We have previously demonstrated that heat-inactivated *Lactobacillus* strains can improve gut barrier characteristics of healthy zebrafish in a bacterial species-dependent way. We wanted to continue our screening by evaluating the impact of those products as immunomodulators for activating antiviral defence. The main goal of this study was to investigate the antiviral activity of heat-inactivated *Lactobacillus* strains using zebrafish challenged with a fish pathogenic viral hemorrhagic septicaemia virus (VHSV). Our hypothesis was that postbiotics were able to modulate the immune response in a strain-dependent way and exhibit immunomodulant and anti-viral properties.

Animals, materials and methods: The protocol received the agreement of the local ethics committee (n°: 202203A-CNU-019). We carried out a 31-day study using wild-type adult zebrafish (24 fish/tank; 3 triplicates/treatment). Treatments consisted of 1] non-supplemented basal diet (**C1**), 2] non-supplemented diet and virus challenge after a 3-week (**C2**), and 3 treatments using the basal diet supplemented with 6×10^6 CFU heat-inactivated bacteria/g feed and a viral challenge after a 3-week supplementation period: 3] *L. paracasei* H108 (**T1**), 4] *L. plantarum* HA-119 (**T2**), or 5] *L. helveticus* HA122 (**T3**). Each heat-inactivated bacterial strain was characterized by electronic and atomic force microscopy. Before the viral challenge (21 d-period), 54 fish/treatment (18/tank) were sacrificed and intestinal and kidney collected to perform histomorphometry and immune response profiling using gene expression (qRT-PCR) and immunoblotting. At 21 d, 30 fish/treatment (10/tank) were challenged with VHSV. Fish survival of fish and viral clearance (qRT-PCR) were monitored for 10 days. Differences between control and experimental groups were estimated by a two-way ANOVA and Bonferroni *post-hoc* test with significance accepted at $p < 0.05$.

Results and discussion: The level (immunoblotting) of TNF- α , IFN- γ , IL-10, and alkaline phosphatase was higher in gut and kidney compared to that of control with T1, T2 and T3. Interestingly antiviral response genes, such as IFN γ , IFN γ 1, Mx, and CD8a were up-regulated in both gut and kidney (except Mx in gut) by the diet supplemented with T2. A better numerical (T3, $P=0.11$) and statistical (T2; $P<0.01$) survival was observed in postbiotic groups compared to the treatment C2, while a trend ($P<0.10$) of a lower number of viral copies was detected in the T1 group. Based on higher cumulative percent survival and a strong immune activation, fish fed with the T2 diet had the strongest antiviral defense against VHSV. The T1 group also displayed considerably higher positive immunomodulatory responses without influencing survival rate of fish.

Conclusion. This study shows that heat-inactivated bacteria can be immunomodulators for activating antiviral defense. Further research to characterize bacterial wall and molecules will be useful to understand the exact immune functional role(s) of the inactivated bacterial strains, and to optimize their use.

References: Brugman, S. (2016). The zebrafish as a model to study intestinal inflammation. *Developmental & Comparative Immunology*, 64, 82-92.

1A4. Amaranthus grains a potential alternative to conventional cereals in monogastric nutrition

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Introduction: *Amaranthus* spp. is considered an under-exploited alternative crop to conventional cereals in the diet of monogastrics and cultivated in the semi-arid Mediterranean area. The inclusion of Amaranth in pig diets could have nutritional and economic benefits. The present research aims to explore the proximate composition, fatty acids content and *in vitro* fermentation parameters of four amaranth grain species (*A. cruentus*, *A. hypochondriacus*, *A. hybridus* and *A. tricolor*).

Animals, materials and methods: Chemical assay of the amaranth grains, moisture, ash, crude protein, crude fiber, ether extract, starch and total dietary fibre, were carried out according to the standard procedures of AOAC [1]. Fatty acids analysis was measurement in gas chromatography after lipids transesterification [2]. The *in vitro* gas production technique was used to evaluate the fermentation parameters using the caecal content as inoculum [3].

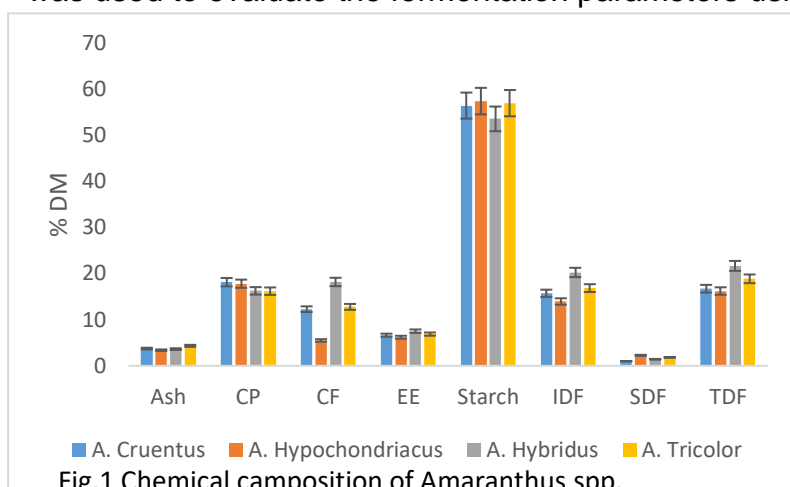


Fig.1 Chemical composition of Amaranthus spp.

Results and discussion: All species were characterised by a starch, crude protein (CP) and total dietary fiber (TDF) contents higher than 50, 16 and 16% on a DM basis, respectively. Among the species some significant differences were observed (Figure 1), *A. cruentus* reported the highest ($p<0.05$) CP. While *A. hybridus* showed the highest ($p<0.01$) amounts of crude fiber (CF) and TDF. In terms of fatty acids profile, *A. hypochondriacus* showed the highest ($p<0.05$) level of α -linoleic acid. *A. hybridus*

and *A. tricolor* resulted in the highest and lowest ($p<0.01$) levels of saturated fatty acids (SFA), respectively. Furthermore, *A. tricolor* showed the highest ($p<0.05$) content of polyunsaturated fatty acids (PUFA). *A. cruentus* and *A. tricolor* reported the highest ($p<0.05$) levels of n3- and n6-PUFA. Regarding the peroxidation index (PI), *A. tricolor* showed the highest ($p<0.01$) value due to the high amount of PUFA. Concerning the *in vitro* parameters, *A. hypochondriacus* and *A. cruentus* showed the highest ($p<0.01$) levels of degraded organic matter (DOM) and gas production (OMCV). Moreover *A. hypochondriacus* reported the highest amount of short chain fatty acids (SCFA). Increased SCFA production might be related to the more soluble fraction of dietary fiber, which is readily fermentable. Whereas *A. cruentus* had the highest ($p<0.05$) level of butyric acid. In this regard, the high proportion of butyrate could be useful for the colonic epithelium as a main energy source for cell growth and differentiation [4].

Conclusion: In conclusion, considering the nutritional characteristics in terms of chemical composition and fatty acids profile and the results obtained from the *in vitro* fermentation, it is possible to state that the four varieties of amaranth grains tested could be used as a promising non-conventional source in monogastric nutrition.

References: [1] AOAC (2019). Official Methods of Analysis, 21st ed.; Association of Official Analytical Chemists International: Gaithersburg, MD, USA; [2] Christie (1993). In *Advances in Lipid Methodology-Two*, ed. by Christie WW. Oily Press, Dundee, Scotland, pp. 69-11; [3] Theodorou (1994). *Anim Feed Sci Technol* 48:185-197. [4] Vastolo (2019). *Animals*, 9, 556.

Session 1B Pre and probiotics II

Chair: Wendy Wambacq

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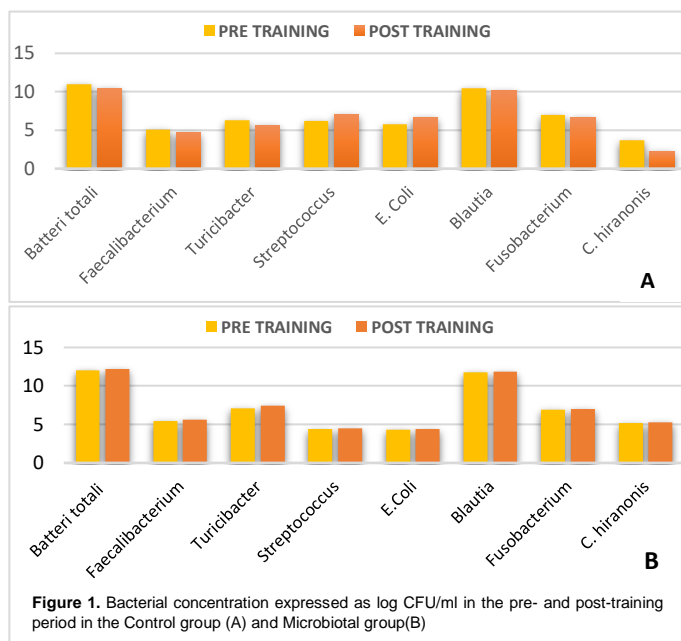
1B1. Effect of a nutraceutical treatment on the intestinal microbiota of sled dogs

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Introduction: Exercise can induce a stress condition in sporting dogs (Keller *et al.*, 2021), promoting an inflammatory response due to muscle injury and oxygen depletion (Tysnes *et al.*, 2020). The use of specific supplements appears very important to counteract the stress condition. The main purpose of this study is to evaluate the effect of a specific nutraceutical product, Microbital, on the intestinal microbiota of adult sled dogs and its ability to improve the dysbiotic condition in animals after a competition/training period.

Animals, materials, and methods: Microbital is made up of prebiotic fiber, FOS, *Lactobacillus reuteri* NBF 1, polyphenols and microencapsulated butyric acid. 20 healthy adult dogs were split into two groups: Microbital group that received the supplement under study and the Control group to which it was not administered. The study lasted 4 months. Faecal samples were collected from each dog at two specific moments: pre and post training period; the workout was an aerobic endurance with gradually increasing run times at different intensities. Through the microbiological analysis, specific bacterial groups were quantified to evaluate the dysbiosis index of each group.



Results and discussion: The results obtained showed an increase in *Streptococcus* spp. and *E. Coli* in the control group after the training period; these bacteria are enteropathogenic whereas beneficial bacteria like *Faecalibacterium*, *Turicibacter*, *Blautia* concentration decreased. The administration of Microbital caused a very little increase in enteropathogens compared to the control group (Fig. 1). The alterations found in the control group are the same that are found during acute and chronic intestinal inflammation, condition not found in the Microbital group (Fig. 2). However, the most important data to observe is how the Microbital

administration reduces the intestinal microbiota alteration.

Conclusion: Microbital manages to counteract the dysbiosis caused by post-exercise stress and could be a valid aid in athletic dogs by limiting the inflammatory damage. In fact, the group of dogs that did not receive the nutraceutical product shows further increase in dysbiotic condition after training.

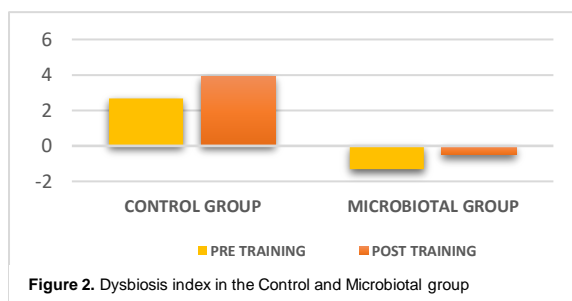


Figure 2. Dysbiosis index in the Control and Microbital group

References: Keller A., et al. "Efficacy of Nx4 to Reduce Plasma Cortisol and Gastrin Levels in Norwegian Sled Dogs During an Exercise Induced Stress Response: A Prospective, Randomized, Double Blinded, Placebo-Controlled Cohort Study". *Front. Vet. Sci.*, 2021, 8. doi:10.3389/fvets.2021.741459.
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1B2. What is beyond immunomodulation in yeast or seaweed β -glucans intake in dogs?

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Introduction; β -glucans intake may modulate gut microbiota, inflammation, immunity, and glucose and lipid metabolism. Differences in structure originate different responses on animals, as the modulation of innate immunity by β -glucan from *Saccharomyces cerevisiae* and the induction of innate humoral response by β -glucan from seaweed (*Euglena gracilis*) [1]. Considering that few studies explored other possible metabolic actions of these two β -glucans, the present study investigated their effects on nutrient digestibility, fermentation products and faecal microbiota composition, postprandial insulin and glucose response, and serum lipid parameters in dogs.

Animals, materials and methods: One food formulation was used, with the following treatments: CON – control, without β -glucan addition; B-Yest – 0.115% of β -glucan source from yeast cell wall; B-S15 – 0.155% of β -glucan source from seaweed; B-S31 – 0.310% of β -glucan source from seaweed. The products were analysed, and the inclusion established aiming the intake of 15mg (B-Yeast and B-S15) and 30mg (B-S31) of β -glucan/kg/day. Thirty-two beagle dogs were used, 8 dogs per food, distributed in a block design. Blocks lasted 84 days: the first 42 d for washout followed by 42 d receiving the experimental diets (protocol n° 001974/20). Faecal microbiota (16S rRNA gene Illumina sequence) and serum lipid parameters were analysed on 1st and last day of the experimental diet intake. After 15 d of experimental diet intake nutrient digestibility was determined; at day 25 post-prandial insulin and glucose responses were evaluated (dogs were fed and blood collected for 720min), and at day 30 fresh faeces were collected to evaluate fermentation products. Data were subjected to ANOVA and Tukey's test. For postprandial glucose and insulin responses, repeated-measures analysis of variance was used. For faecal microbiota, beta diversity was analysed using the relative abundances of bacterial genera (Aitchison distance) ($P < 0.05$, and $P < 0.1$ as trend).

Results and discussion: Higher apparent digestibility of gross energy, organic matter and dry matter was observed for B-S15 than the B-S31 diet ($P < 0.05$), with the others showing intermediate values. No difference was found in serum lipid parameters ($P > 0.05$). There was a trend towards a lower average glucose concentration in dogs fed the B-Yest diet ($P < 0.1$), and a shorter time to insulin peak response ($P < 0.05$) than observed for the treatment diets. An increase in luminal viscosity with consequent lower digestion and absorption rates of carbohydrates may explain these differences [2]. The predominant phyla in dog faeces were Firmicutes, Fusobacteria and Bacteroides. Diet was responsible for 12.6% of the variation in faecal microbiota, and differences was only observed at the end of the diet intake period ($P < 0.05$). The microbiota of dogs fed B-S31 was different than B-Yest or B-S15, with greater mean relative abundances of *Erysipelotrichaceae*, *Ligilactobacillus*, and *Turicibacter* than the other two experimental diets. This family showed a positive correlation with acetate, propionate and butyrate production [3], and although non-significant ($P > 0.05$) faeces of dogs fed this diet had numerically higher short-chain fatty acid concentration. Some limitations to be considered are the number of animals and the use of only healthy dogs.

Conclusion: The yeast β -glucan tended to reduce mean glycaemia and to shorter insulin secretion time, possibly modulating glucose metabolism deserving future studies. The diet with higher inclusion of seaweed β -glucan induced larger changes in faecal microbiota, with higher proportion of desirable bacteria related to intestinal health.

References: [1] Theodoro et al. (2022) XXI Congresso CBNA Pet. [2] Granfedt Y et al. (2008) Eur. J. Clin. Nutr. [3] Bermingham et al. (2017). Peer J.

Acknowledgments: Kemin Industries to fund the study; BRF Pet Food; BRF Ingredients; ADIMAX Pet Food; CAPES; FAPESP (2020/04120-4).

1B3. Repeatability of the effects of dietary supplementation of probiotic on gut fermentative metabolites and microbiota in dogs

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Introduction: The study aimed to evaluate the repeatability of the effects of yeast probiotic supplementation (0.12 g viable *Saccharomyces cerevisiae*/animal/day) on gut fermentative metabolites and microbiota in dogs.

Animals, materials and methods: The same diet was offered for 28 days to 16 adult beagle dogs, distributed in a completely randomized design, divided into groups of 8 dogs each: control, without probiotic, and probiotic group. The probiotic was individually applied on top of the diet. On day 28, faecal samples were collected for ammonia analysis [1], pH, phenols, indoles, short-chain (SCFA) and branched-chain fatty acids (BCFA), total biogenic amines, and microbiota (Trial 1). After 3 months, the experiment was repeated (Trial 2), under the same conditions. Biogenic amines were analysed by HPLC. Phenols, indoles, SCFA, and BCFA were analysed by gas chromatography. Quantification of bacterial taxa was performed by qPCR and the dysbiosis index (DI) was calculated [2]. Data on the relationship between probiotic supplementation and faecal variables in trials 1 and 2 were analysed by principal component analysis (PCA) using a correlation matrix. Comparisons among groups were performed by similarity analysis (ANOSIM, $P < 0.05$).

Results and discussion: The control group was positively correlated with higher faecal concentrations of ammonia, spermidine, putrescine, and total biogenic amines, higher relative abundance of *Escherichia coli*, pH, and DI in both trials ($P < 0.05$, Figure 1). While higher faecal butyrate concentrations and higher relative abundance of *Faecalibacterium*, *Clostridium hiranonis*, *Turicibacter*, and *Bifidobacterium* were positively correlated with the probiotic group in both trials

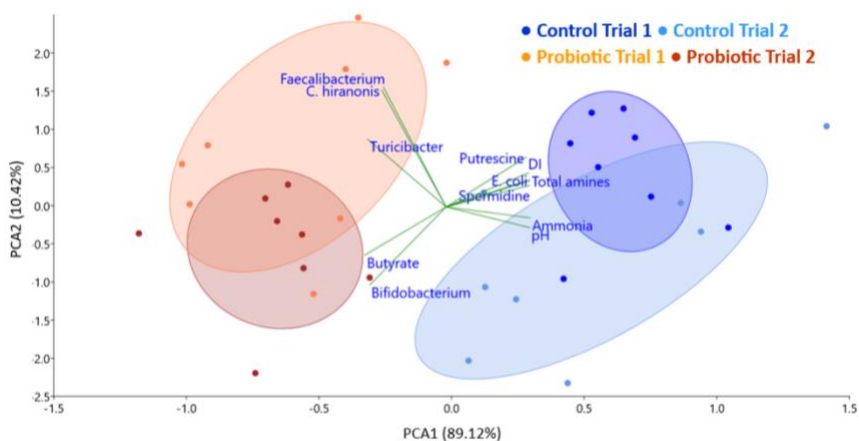


Figure 1. PCA of faecal variables that correlate with the dietary supplementation or not of probiotic in trials 1 and 2.

($P < 0.05$, Figure 1), agreeing with Stercova et al. [3]. Yeast is capable to modulate the intestinal environment through binding sites against potential pathogens and by producing metabolites such as butyrate [4]. ANOSIM indicated a difference between control and probiotic groups ($P < 0.05$, Figure 1). However, there was no difference in the results of the same treatment between trials 1 and 2 ($P > 0.05$, Figure 1), demonstrating that the effect of the probiotic on the analysed variables was consistent between the trials. Despite the complexity of the interactions in the gastrointestinal tract and the non-controllable experimental errors, it was possible to validate the main results of the probiotic with the repetition of the study.

Conclusion: The inclusion of 0.12 g of *S. cerevisiae*/animal/day can modulate the intestinal microbiota and its metabolites, favouring eubiosis. Furthermore, there is repeatability in the response to probiotic consumption after a 3-month in dogs.

References: [1] De Brito et al. (2010) Anim. Feed Sci. Technol. 159:150-155; [2] Alshawaqfeh et al. (2017) FEMS Microbiol. Ecol. 93,11; [3] Stercova et al. (2016) J. Anim. Sci. 94: 2909–2918; [4] Van Den Abbeele et al. (2020) J. Anim. Sci. 98, 6.

1B4. Assessing the Efficacy of *Lactobacillus* Probiotic Tablets for Improving Canine Oral Health

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Introduction: Periodontitis, and gingivitis are common oral diseases in dogs that develop due to the accumulation of microorganisms and calcium, resulting in dental plaques that can lead to infection and inflammation. *Lactobacillus* probiotics have been shown to reduce bacterial infections [1]. This study aimed to evaluate the effects of *Lactobacillus* probiotic tablet on oral health in dogs.

Animals, materials, and methods: The study included 23 dogs (10 males and 13 females) aged 1 to 9 years (mean age 4.3 ± 5.75 years) with consent from their owners. The study protocol was approved by the institutional animal care and use committee at Prince of Songkla University (66/2518), Thailand. Dogs were randomized into two groups: a control (CON) group of 11 dogs and a probiotic (PRO) group of 12 dogs. The PRO group received a mixed *Lactobacillus* probiotic tablet (1 g) containing *Lacticaseibacillus paracasei* SD1, *Lacticaseibacillus rhamnosus* SD11, *Lacticaseibacillus rhamnosus* SD4, and *Limosilactobacillus fermentum* SD7 (10^7 CFU/g), while the CON group received a tablet without probiotics. The owners were instructed to administer one probiotic tablet to the dog once a day for a duration of 4 weeks. At day 1 (T0), week 4 (T4), and 8 (T8), the dogs were examined by trained veterinarians. Gingival index (GI), plaque index (PI), and calculus index (CI) was evaluated at the canine and 4th premolar of the maxilla and the canine and 1st molar of the mandible [2]. No dental polishing was conducted before the study. Oral microbial sample was collected from the buccal surface of the teeth and gum by using sterile cotton swab. The collected oral sample was stored at -20°C until bacterial analysis by real-time PCR. Wilcoxon Signed Rank test was used to test within group changes in GI, PI, and CI before (T0) and after (T4 and T8) the intervention. The differences of these indexes and bacteria levels between the two groups (probiotic and control group) were analyzed by Mann-Whitney *U* test.

Results and discussion: The dogs were fed either commercial dry food or a combination with homemade food by their owners. There was no difference in the GI, PI, and CI as well as bacterial levels between CON and PRO at T0 ($p > 0.05$). The PRO group showed a decrease in GI for both maxillary and mandibular teeth at T8 compared to T0, as well as a decrease in PI at T4 ($p < 0.05$). The CI of maxillary and mandibular teeth in PRO increased between T0 and T8 ($p < 0.05$). When compared to the CON group, maxillary teeth of dogs in the PRO group showed a significant reduction in PI between T0 and T4 ($p < 0.05$), while a tendency of decrease was observed between T0 and T8 ($p = 0.069$). The total bacterial concentration (log CFU/ml) was not different between the PRO and CON groups at any time point. The PRO group showed a 1.2 and 1.0-fold increase in lactobacilli concentration at T4 and T8, respectively, compared to the CON group ($p < 0.05$). In contrast, the concentration of total streptococci, *Aggregatibacter actinomycetemcomitans*, and *Porphyromonas gingivalis* decreased by 0.9, 0.7, and 0.6-fold at T4, and by 0.9, 0.8, and 0.5-fold at T8, respectively, compared to the CON group ($p < 0.05$).

Conclusion: The oral administration of *Lactobacillus* probiotic tablets may modify oral microorganisms by enhancing potential beneficial bacteria and suppressing selected pathogenic bacteria in dogs. This study showed that *Lactobacillus* probiotics may have positive impacts on dental plaque reduction and overall oral health in dogs.

References: [1] Pahumunto et al. (2022) Arch Oral Biol. 142:105513. [2] Harvey et al. (2015) J Vet Dent. 32:16-21.

Session 1C Pre and Probiotics Posters



1C1. Effects of dietary inulin supplementation on the faecal microbiota and its function and on vaccine response in cats

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Introduction: The objective of this study was to determine if a low dose of dietary inulin supplementation in cats is able to induce a prebiotic effect, modifying the faecal microbiota and its function, and to enhance the immune response following a vaccine challenge.

Animals, materials and methods: Neutered male (n=12) and female (n=14) cats, 4-year-old, from the same colony, were fed a complete and balanced diet formulated for adult cat (% as fed, protein 35, fat 13, ashes 7, crude fibre 1.6, moisture 6) (Control) for 1 month (T0) before they were randomly allocated to 1 of 2 groups. The first group remained with the control diet, the second group received the same diet supplemented with 0.7% inulin for 6 weeks (Part 1). Spontaneously voided fresh faecal samples were obtained at T0, and after 3 and 6 weeks. Faecal short chain fatty acids (SCFAs) (butyrate, acetate, propionate) (T0, T3, T6) and the faecal microbiota (T0, T6, massive sequencing of the V3-V4 hypervariable region of the 16S rRNA gene) were analysed. In the second part of the study, after a 6-month washout period with the control diet, the cats were again divided into 2 dietary groups (control vs. 0.7% inulin) (day -42). Six weeks after the change of diet, the cats were primo-vaccinated against the feline leukaemia virus (day 0), with a vaccine booster 21 days after the first dose. Serum specific IgG immune response (anti p45 FeLV antibodies measured by ELISA) was followed before vaccination (day -42, 0), after first vaccine (day 5, 11, 14, 21) and after booster (day 25, 28, 34, 42, 56).¹ Faecal SCFAs and serum igG results (mixed generalised linear model for repeated measurements with diet, time and their interaction as fixed effects, and with cat identity as a random effect, log-transformed variable in case of non-normal distribution) and microbiota taxonomy (linear discriminant analysis effect size (LEfSe)) were statistically analysed. A P -value ≤ 0.05 was considered as significant. A P -value ≤ 0.1 was considered as a trend. Values are presented as mean \pm standard deviation. No multiplicity correction was applied since the study was considered an exploratory pilot experiment and the analysis of each time or group of times aimed to answer different questions.

Results and discussion: Compared to the control group, the inulin group had higher faecal butyrate concentration after 6 weeks of supplementation (137 ± 51 vs. 95 ± 32 $\mu\text{mol/g}$ dry matter, $P = 0.02$). The difference after 3 weeks of supplementation was not yet significant between control and inulin group (106 ± 58 vs. 93 ± 33 $\mu\text{mol/g}$ dry matter, $P = 0.6$). The others SCFA were not significantly different between groups. LEfse analysis of the faecal samples showed higher *Firmicutes* phylum (+14%), one of the main butyrate producers, and lower *Bacteroidetes* phylum (-56%), *Prevotellaceae* family (gram-negative bacteria) (-51%) after 6 weeks of inulin supplementation compared to the control group. Compared to the control group, the inulin group tended to have a higher specific serum IgG response after first vaccine only ($P=0.09$), with significant differences at day 5 (6.9 ± 5.7 vs. $4.0 \pm 3.2\%$, $P = 0.04$) and day 11 (25.7 ± 18.1 vs. $14.4 \pm 9.6\%$, $P = 0.05$) post-vaccination.

Conclusion: Taken together, these results suggest that a six-week dietary supplementation with a low dose of inulin in cats is able to modify the faecal microbiota and its functional metabolites (butyrate), tending to lead to an earlier immune response after vaccination. The biological significance of the observed effects remains unclear.

References: [1] Lehmann R. et al. 1991, J Am Vet Med Assoc 199, 1446–52

Conflict of interest: this study was funded by Affinity Petcare SA

1C2. In vitro approach for the estimation of the digestibility coefficients and fecal concentration of fermentation products in cats

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Introduction: According to 3R's principles (replacement, reduction and refinement), there is a global tendency to reevaluate the use of animals in scientific studies, through the development of alternative methods [1]. The objective of this study was to compare, in cats, the results for fecal concentration of fermentation products through total tract apparent digestibility (TTAD) and *in vitro* methods.

Animals, material, and methods: Nineteen foods and different methodologies (*in vitro* and *in vivo*) were used to determine the digestibility of dry (DM) and organic matter (OM) and the concentration of fermentation products. Two *in vitro* approaches were applied for the comparison of the TTAD: the static method (IVD), simulating the gastric and small intestine phases of the digestion [2], or this same method followed by simulation of fermentation in the large bowel (IVDF) [3]. The concentration of the fermentation end-products in the fecal samples in the *in vivo* methods was also used as a reference for the *in vitro* fermentation. For the TTAD, the DM and OM digestibilities were

Table 1. Digestibility (%) of dry and organic matter determined by *in vivo* and *in vitro* methods.

Variables	TTAD	IVD	IVDF	SD	p-value*
Dry matter	80.42 ^c	84.97 ^b	87.43 ^a	2.21	<0.01
Organic matter	84.96 ^b	84.53 ^b	87.61 ^a	2.03	<0.01

TTAD: total tract apparent digestibility; IVD: static method; IVD+IVF: static method followed by simulation of fermentation in the large bowel; SD: standard deviation. ^{abc} Means followed by common letters on the line do not differ. *p<0.05 = significant.

Table 2. Correlation and prediction equations of parameters significance

Variables	Correlation	Equation
Organic matter	0.79	TTAD (%) = 22.54 + 0.74 * IVD (%)
pH	0.60	In vivo = -16.10 + 3.30 * in vitro
Acetate	0.54	In vivo = -99.16 + 0.21 * in vitro
Butyrate	0.58	In vivo = -77.35 + 0.15 * in vitro

TTAD: total tract apparent digestibility; IVD: *in vitro* digestibility determined by both methods.

Results and discussion: The TTAD of the OM was similar *in vivo* and *in vitro* and a correlation was found between them (Tables 1 and 2). On the other hand, the results determined by the IVDF overestimated the DM and OM digestibility. Although the IVDF simulates the three steps of digestion (the gastric, small, and large intestine phases), the results obtained by the IVD method were closer to the *in vivo* technique, which, in turn, only simulates the first two phases. These results are in line with another study [4], which used the same IVD methodology in cats. In this study, the fermentation products in the fecal samples or in the fecal inoculum media, presented different concentrations, but with significant correlation between them, regarding pH, acetate, and butyrate (Table 2).

Conclusion: The TTAD of OM by IVD showed similarity and high correlation with *in vivo* method, which suggests that it could be used in future trials as a reliable *in-vitro* alternative. The results related to pH, acetate, and butyrate showed that the *in vitro* fermentation method can be used as a trend indicator, but not for the evaluation of concentration itself.

References: [1] Eggel, M et al. (2021) Lab. Anim., 55, 233–243 [2] Hervera, et al. (2007) J. Anim. Physiol. Animal Nutri., 91, 205–209; [3] Williams, et al. (2005) Anim. Feed Sci. Technol. 123-124 Pa, 445-462; [4] Ponciano Neto (2015) XIV CBNA Congress SP Brazil; [7]

1C3. Fiber source differentially modulates gut microbiota and host response in dogs

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Introduction: Dietary fiber mainly comprises carbohydrate polymers which cannot be digested or absorbed by the small intestine of mammals. These carbohydrates polymers can vary a lot among ingredient sources [1] and can come with a wide variety of bioactive compounds [2], therefore each fibrous ingredient can exert different effects on the host response. The aim of this study was to determine the gastrointestinal and health effects of two different fiber sources (from cereals vs. fruits), expecting different outcomes from each treatment.

Animals, materials and methods: Twelve healthy Beagle dogs were enrolled in a latin-square design (3 diets x 3 periods) including a control diet low in fiber (CTR; 6% Total Dietary Fiber (TDF)), a cereal-fiber supplemented diet (BRA; 12% TDF) and a fruit-fiber supplemented diet (FRU; 12% TDF). BRA and FRU diets were formulated to contain equivalent levels of soluble and insoluble fiber (1:10). Every period lasted 6 weeks, with a digestibility trial and faecal and blood collections performed in the last week. Faecal short-chain fatty acids (SCFA) and faecal microbiota taxonomy (16S rRNA) and functionality (Shotgun-NovaSeq 6000) were analysed at the end of each period. Blood biochemistry and complete cell counts were also determined. Statistical analysis was performed by analysis of variance (ANOVA) using the General Linear Mixed Model (GLMM) procedure (R software). For taxonomical and genomic differences between groups, 'limma-voom' R package v.48.3 was used.

Results and discussion: Results showed reductions in nutrient digestibility (DM, OM, GE, CP & fiber) in fiber supplemented diets compared to the CTR diet, whereas no significant impact on faecal consistency was observed among treatments. BRA showed a tendency to increase total SCFA compared to CTR ($P = 0.056$) and FRU tended to increase butyrate ($P = 0.086$). Both BRA and FRU diets decreased faecal branched-chain fatty acids, probably due to the greater access of fermentable fiber, which shifted fermentation in favour of predominantly saccharolytic bacteria. BRA was associated with lower levels of serum triglycerides and FRU showed a tendency to decrease total serum cholesterol ($P = 0.075$). When looking at the microbiota taxonomy and functionality, BRA showed the highest levels of alpha diversity and significantly increased some beneficial genera such as *Lachnospira*, *Bifidobacterium* and *Faecalibacterium* when compared to CTR diet. BRA also showed an overabundance of genes related to carbohydrate and amino acid metabolism. FRU diet, on the other hand, had a different impact on bacterial taxonomy and microbiota functionality and rendered higher levels of total and CD8⁺ lymphocytes in the blood. Differing effects associated with FRU could be attributed to its higher content of total polyphenols.

Conclusion: These results demonstrate that increasing from 6 to 12% TDF by including cereal or fruit fiber sources in the dog diet slightly reduce nutrient digestibility but do not affect faecal consistency. Fiber from cereal sources seems to provide greater beneficial effects on the microbiota compared to fruit, even though fruits sources of fiber could be beneficial modulating the immune system.

References: [1] Marlett (1992) Journal of the American Dietetic Association. 92:2–175; [2] Sagar et al. (2018) Comprehensive Reviews in Food Science and Food Safety. 17:512–531.

1C4. Effects of the dietary inclusion of yeast probiotic *Saccharomyces cerevisiae* on gut fermentative metabolites and fecal microbiota of dogs undergoing an abrupt dietary change

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Introduction: The study aimed to evaluate the effects of the dietary inclusion of yeast probiotic *Saccharomyces cerevisiae* on gut fermentative metabolites and fecal microbiota of dogs undergoing an abrupt dietary change.

Animals, materials and methods: Sixteen adult intact beagle dogs (5 years of age, eight males and eight females) were randomly divided into two groups: The control group (n=8, body weight: 11.64±0.49kg) and the test group (n=8, body weight: 11.51±0.73kg) supplemented with a yeast probiotic *Saccharomyces cerevisiae* (150mg of Actisaf® Sc50, Phileo by Lesaffre, Marcq-en-Barœul, France; 1x10¹⁰ CFU/g). All dogs were fed a commercial adult dog diet with lower protein (20.42% crude protein- CP) and lower-fiber (6.10% total dietary fiber–TDF) concentrations for 21 days. At the 22nd day, dogs were abruptly transitioned to a commercial diet with higher-protein (27.52%-CP) and higher-fiber (27.20%-TDF) concentrations until day 49. On days 0, 21, 35 & 49, fresh feces samples from each dog were collected to analyze pH, ammonia, biogenic amines and microbiome analysis (dysbiosis index according to Alshawaqfeh et al [1] and relative abundance of main bacterial species). The experiment was approved by the Committee of Ethics in Animal Use of the Federal University of Parana (Brazil). Main statistical analyses were performed using Minitab® (version 19.2020.1) software. Each dog was an experimental unit. Yeast probiotic supplementation and sex were treated as fixed effects; the day of measurement was treated as a repeated measure. An analysis of variance (two-way ANOVA) was performed, using the function General Linear Model, to assess the effects of supplementation and interaction with the day of measurement. Fisher LSD with Bonferroni correction was applied as post hoc test. Beta diversity, using Bray–Curtis distances, and its plots were analyzed and generated using Past software 4.03. Analysis of similarity (ANOSIM) was used to evaluate the similarity of the microbiota and KO terms between groups at different time points. Differential abundance of KEGG Modules and KO terms between groups (at day 21 & 49) and diets was analyzed using linear discriminant analysis (LDA) effect size (LEfSe) on MicrobiomeAnalyst [2]. KEG Modules and KO terms with log LDA-score higher than 2 and adjusted P< 0.05 for false discovery rate were considered significant.

Results and discussion: The test group had lower (P<0.05) fecal pH and ammonia concentrations at day 23, lower (P<0.05) total biogenic amines production at day 21 & 49 and higher (P<0.05) fecal concentrations of butyrate regardless of the day, compared to control group. Moreover, the test group showed lower (P<0.05) dysbiosis index than control group regardless of the day and higher (P<0.05) relative abundance of *Bifidobacterium* (at days 35 & 49) compared to control group. Beta diversity demonstrated that the dietary supplementation of the probiotic resulted in modulation of the intestinal microbiota on day 49 (P<0.05) and a trend (P= 0.091) to change the microbiota on day 21 compared to the control group.

Conclusion: These results indicated that the dietary inclusion of yeast probiotic *Saccharomyces cerevisiae* can have beneficial effects on the intestinal microbiota of dogs. Further investigations are needed to evaluate possible benefits in animals sensitive to dysbiosis such as weaning puppies or dogs with gastrointestinal disorders.

References: [1] Alshawaqfeh et al. (2017) FEMS Microbiol. Ecol. [2] Chong et al. (2020).

Conflicts of Interest: Héloïse Legendre, Rodolphe Rabot and Achraf Adib Lesaux work at Phileo by Lesaffre, which sells the yeast probiotic product evaluated. The other authors declare no conflict of interest.

1C5. Comparison of fecal microbiota and their fermentation characteristics after the addition of different insoluble fibre sources to complete dog foods

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Introduction: Fibre in the pet food industry is used to dilute caloric density in weight reduction diets [1]. Common sources of dietary fibres differ in their chemical composition and physiochemical properties, which determine fibre fermentability [1]. Final products of carbohydrate-fermentation in the large intestine are energy containing, making a small, but significant, contribution to the total energy available [2,3]. The aim of the present study was to evaluate whether different insoluble fibre sources supplemented to a canine diet have comparable effects on fecal microbiota and bacterial fermentation.

Animals, materials and methods: Eight female Beagle dogs were fed a commercial basic diet as control (0.452 MJ ME/kg body weight (BW)^{0.75} and day) to which either cellulose processed as powder (PC) and granulate (GC) or lignocellulose (LC) were added at 2 g/kg BW. Contents of total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) are shown in Table 1. The diets were tested in a four-period (14 days), four treatment

Table 1. Dietary fibre content (g/100 g DM) of the basic diet and the supplements

Parameter	Basic diet	Supplements		
		PC	GC	LC
TDF	11.3	98.7	90.9	94.8
IDF	12.6	99.3	90.2	92.8
SDF	1.87	0.00	0.52	1.89

cross-over Latin square design. A pre-collection period of nine days was followed by a total collection period of feces for five days for individual estimation of apparent total tract digestibility (ATTD) of TDF and SDF. A part of fecal samples at days 13/14 were taken for measurements of pH, ammonia and volatile fatty acid (VFA) concentrations and microbiota analysis.

The statistical analysis was done in R (version 4.1.2). Multiple and pairwise comparisons were conducted with the package “rstatix” (version 0.7.0).

Results and discussion: The addition of all three fibre sources to the basic diet altered fecal microbiota slightly, but differently. In comparison to PC and GC, bacterial composition appears to be less affected when LC was added to the basic diet (indicated by Bray-Curtis community dissimilarity). VFAs concentration had a smaller decrease when LC was added to the basic diet, though no significance was detected (Table 2). The measurement of VFA in feces only indirectly provides information on the production rate of fatty acids by intestinal bacteria and no statement can be done on the absorbed amount. Comparing the three products, however, ATTD of SDF was significantly higher when LP was added instead of both cellulose products, ultimately indicating for a higher production rate of VFA.

Conclusion: With regard to microbiota composition, lignocellulose compared to cellulose

Table 2. ATTD of dietary fibre, bacterial fermentation products in feces and fecal pH value of dogs

Parameter	Control	PC	GC	LC	p value
ATTD TDF, %	23.7 ±12.4	15.4 ±5.62	13.9 ±8.50	16.3 ±7.55	0.133
ATTD SDF, %	31.9 ^{a,b} ±7.01	28.6 ^b ±12.8	25.5 ^b ±15.1	41.1 ^a ±7.06	0.044
Ammonia, g/kg	1.17 ±0.35	1.07 ±0.35	1.10 ±0.22	0.98 ±0.24	0.636
Total VFA, mmol/kg	101 ±19.8	91.9 ±15.2	95.7 ±20.2	99.1 ±25.1	0.954
pH	6.88 ±0.212	7.10 ±0.433	7.11 ±0.234	7.06 ±0.152	0.149

^{a,b} Different superscripts within one row indicate significant differences at $p < 0.05$.

seems to be more suitable as an inert fibre source in low calorie canine diets but is also more than a simple diet diluent.

References: [1] De Godoy et al. (2013) *Nutrients* 5, 3099-117; [2] Drochner (1993) *Arch. Tierernaehr.* 43, 95-116; [3] Rechkemmer et al. (1988) *Comp. Biochem. Physiol.* 90, 563-68.

1C6. Digestibility, fermentation by-products, and extrusion characteristics of kibble diets for dogs with red lentils pasta by-product

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Introduction: The increase in food demand is redirecting pet food industries to search for alternative protein and carbohydrate sources, so as to improve their products sustainability.¹ Even though dog foods using lentils are available, little research has been performed on their effects on extrusion, digestibility, and gastrointestinal environment. The substitution of broken rice (BR) by lentil pasta by-product (LP) as the main carbohydrate source was evaluated on extrusion characteristics, digestibility, and microbial fermentation of by-products in the faeces of dogs.

Animals, materials and methods: A diet based on BR was formulated for dog maintenance, and the BR replaced was with 33%, 66%, and 100% LP, when the study ingredient was the sole carbohydrate source. Parameters were adjusted for the BR diet and kept constant, allowing observations of the impact of LP on processing parameters and kibble macrostructure. Thirty-two Beagle dogs were divided into 4 groups (8 for each diet) and fed at maintenance level ($Kcal/d=110 \cdot BW^{0.75}$) to determine apparent total tract digestibility (ATTD) of nutrients and nitrogen balance; after 12 d of adaptation, faeces and urine were quantitatively collected for 5 d. Fresh faeces (< 15 minutes after elimination) were collected for 3 d to measure pH and fermentation of by-products. Data were analysed by ANOVA and means were compared by polynomial contrasts according to LP inclusion amount ($P<0.05$).

Results and discussion: A quadratic increase on mechanical energy transference (kWh/t), temperature and pressure of extrusion was observed with the increase of LP. Even with this, a quadratic reduction in kibble expansion, and an increase in hardness was observed, without affecting starch gelatinization. The ATTD of crude protein, dry matter, and crude energy presented a quadratic reduction, while the dietary fibre ATTD increased quadratically after LP inclusion. According to the polynomial regression, up to 66% of replacement LP did not reduce ATTD, with similar values compared to the BR diet. Nitrogen balance did not change, but a linear increase in faeces production and moisture, and a linear decrease in faeces pH was observed with higher LP, without changing faecal score.² Faecal acetate, propionate, total short-chain fatty acids (SCFA), branched-chain fatty acids, and lactate all increased linearly with LP inclusion, without altering ammonia concentration in faeces. The increase in LP inclusion promoted a linear increment in cadaverine, tyramine, histamine and spermidine; while spermine exhibited higher concentrations in faeces for the LP 33% diet. The effects on both extrusion parameters and kibble formation, as well as in ATTD and fermentation of by-products in faeces can be explained by the higher dietary fibre and lower starch content of LP in comparison with BR. The increase in fibre with lower starch may increase the mass resistance to flow inside the extruder, increasing shear, temperature and pressure, but the reduced starch content ended reducing expansion due to the lower plasticised mass necessary for cell structure formation.³ The higher fibre intake by dogs due to LP inclusion can also explain the reduction in digestibility. The dietary fibre of LP showed to be fermentable by gut microbiota, increasing the concentration of desirable fermentation products including SCFA, and spermidine.⁴

Conclusion: Lentils pasta did not reduce ATTD in up to 66% of inclusion, exhibiting potential benefits to intestinal health as a source of fermentable fibre.

Acknowledgments: BRF Pet Food; BRF Ingredients; ADIMAX Pet Food; Dage! Mangimi Srl

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1C7. Effect of type of detoxifier on fermentation processes determined in-vitro in canine model

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Introduction: Detoxifiers are designed to detach harmful mycotoxins and metabolites from the feed particles and retain them on their surface. After binding to the detoxifier, the harmful substance passes unabsorbed through the digestive tract and is excreted in the faeces. Therefore, the aim of this study was to determine the effect of different types of detoxifier (charcoal, bentonite, yeast cell wall) on in-vitro fermentation parameters (gas production, pH, VFA, lactic acid and ammonia).

Animals, material and method: In-vitro gas production (GP) was estimated using gas test (glass syringes) and the fermentation parameters such as: pH, volatile fatty acids (VFA), short-chain fatty acids (SCFA), branched chain fatty acids (BCFA), lactic acid (LA), ammonia concentration and degradability of organic matter were determined [1]. Chemical composition of detoxifier (charcoal, bentonite, yeast cell wall) was analyzed using standard analytical methods [2]. Faeces of four adult dogs were collected immediately after defecation and transported to laboratories. Afterwards, faeces were mixed with phosphate buffer as inoculum and incubated without (control group) or with different types of detoxifier (0.5 g/20 ml). Fermentation parameters were determined before and after fermentation (that lasted 24 h). Results were analyzed using one-way analysis of variance [3].

Results: The highest pH value after fermentation (6.4) was observed in the bentonit group, then for charcoal (pH=6.2) compared to the lowest for yeast cell wall, (pH=5.3; $p<0.01$). Maximum GP was the lowest in the control group (7.27 ml/g DM of inocula) and then for bentonit (9.8 ml/g DM of inocula) ($p<0.01$). GP max for yeast cell wall was about two times higher than others (23.3 ml/g DM of inocula). VFA (383.1 mmol/g DM of inocula), SCFA (369.4 mmol/g DM of inocula) and butyric acid (50.1 mmol/g DM of inocula) production was the highest for yeast cell wall ($p<0.01$). The lowest production of VFA and SCFA were observed after fermentation of bentonite.

Table 1. Chemical composition of detoxifier components (% of DM).

Items	DM	Ash	CP	EE	CF	NDF	ADF	ADL	I-NSP	S-NSP
charcoal	89,3	89,3	-	-	-	-	-	-	-	-
bentonite	91,7	91,7	-	-	-	-	-	-	-	-
yeast cell wall	94,4	5,2	33,9	0,7		0,7	0,6	0	38,5	2,9

DM – dry matter; CP – crude protein; EE – ether extract; CF – crude fibre; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid detergent lignin; I-NSP – insoluble non starch polysaccharides; S-NSP – soluble non starch polysaccharides;

Conclusion: The type of detoxifier affected the fermentation parameters measured in-vitro in the canine model. Taking into account the fermentation parameters, only yeast cell wall was fermented, charcoal and bentonite were not fermented. Using large amounts of mineral detoxifiers can affect the microbiome and weaken the fermentation processes.

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1C8. Pea flour effects on extrusion traits, digestibility, and fermentation products in faeces of dogs

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Introduction: Studies in humans suggest that pea may have functional effects on intestinal health, by increasing faecal short-chain fatty acids [1], and data in dogs suggest reduced insulin response to diets based this pulse seed [2]. However, although peas have been used in commercial extruded formulations, especially in grain-free products, little research has been conducted for dogs. The effects of dehulled pea flour (PF) as the main carbohydrate source was evaluated on extrusion characteristics, digestibility, and microbial fermentation products in the faeces of dogs.

Animals, materials and methods: A control diet for dogs was formulated using broken rice (BR) as the starch source, and two experimental diets were produced replacing 50% of BR and 100% of BR by PF, keeping constant all other ingredients. Diets was produced in a single screw extruder. Parameters were adjusted for the BR diet and kept constant, allowing to observe the impact of PF on processing parameters and kibble macrostructure. Twenty-four beagle dogs were used to determine apparent total tract digestibility (ATTD) of nutrients (8 per diet); after 10 d of adaptation, faeces was quantitatively collected for 5 d. Fresh faeces (< 15 minutes after elimination) were collected for 3 d to measure pH and fermentation products. Data were analysed by ANOVA and means compared by polynomial contrasts according to PF inclusion amounts (P<0.05).

Results and discussion: A linear decrease on mechanical energy transference (kWh/t), temperature and pressure of extrusion was observed with the increase of PF (P<0.05), justifying the quadratic reduction in kibble expansion, and the increase in density and hardness (P<0.05). No changes were observed in starch gelatinization. The PF inclusion resulted in linear reductions on ATTD of DM (from 85 to 82%), fat (93 to 91%) and gross energy (from 89 to 86%) (P<0.05) but did not alter ATTD of protein (P>0.05). Faecal production and moisture content increased linearly (P<0.05), although the faecal scores remained constant despite PF inclusion. Faecal pH decreased and acetate, propionate, total short-chain fatty acids, and lactate all increased linearly, and isobutyrate, isovalerate, and ammonia decreased linearly with the increase of PF intake (P<0.01). The reduced starch and higher dietary fibre and protein contents of PF in comparison to BR may explain these effects. Less starch reduced the viscosity development and mass resistance to flow inside the extruder, resulting in reduced application of mechanical energy and less cell structure formation. Also, higher fibre intake reduced TTAD of most nutrients, but not of protein. The dietary fibre of PF showed be fermentable by gut microbiota, increasing the concentration of fermentation products, which plays important role in intestinal health, and reducing ammonia concentration [3, 4].

Conclusion: Dehulled pea flour showed be an adequate ingredient to formulas supplemented in fibre and targeting controlled energy digestibility. It also promoted the formation of fermentation products in the colon, and is an interesting fermentable fibre source.

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References: [1] Dahl et al. (2012). *British J of Nutr* 108:S3-S10; [2] Carciofi et al. (2010) *Rev. Bras. de Zootec* 39, 35-41; [3] Maria et. al. (2017). *J of Animal Science* 95:2452-2466 [4] Carciofi et al. (2008) *J Anim Physiol Anim Nutr* 92, 326-336.

1C9. Study on the use of a yeast cell wall preparation on the faecal microbiome in dogs

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Introduction: Yeast cell walls from *Saccharomyces cerevisiae* contain mannan-oligosaccharides and β -glucans. The aim of the study was to investigate dose-dependent effects of dietary yeast cell wall inclusion in dogs, taking into account the effects on the intestinal microbiota based on investigations of faecal samples. For this purpose, feeding trials were carried out on a group of sled dogs, which were not in training or competition.

Animals, materials and methods: A total of 21 adult dogs from a private animal husbandry were used. In a randomized cross-over design 4 groups received the diets for at least 3 weeks without washout period without or with different concentrations of the product (Biolex MB 40, Leiber GmbH Bramsche, Germany). Dogs were fed individually, treatments were T1: 0, T2: 4, T3: 8 and T4: 16 g of yeast product/kg dry food (Royal Canin Pro Facility). Measurements in the faeces included dry matter content, pH-value, short-chain fatty acids and microbiome analysis by Illumina sequencing, and the intestinal resistome by detection of resistance genes by qPCR. Data were compared using analysis of variance and Tukey test or by Kruskal-Wallis and Mann-Whitney test. Statistically significant differences were determined with a probability of error of $p < 0.05$.

Results and discussion: The trial showed that the yeast preparation was well tolerated in all doses. All faecal samples were comparable with regard to dry matter content. pH-value and concentrations and relative proportions of short chain fatty acids were very stable. Partially dose-dependent, but especially at the highest dose, significant changes in the dominant faecal bacterial phyla occurred (Table 1).

Table 1: Effect of the yeast product on the faecal bacterial phyla of dogs

Bacterial phyla, %	Added yeast product, g/kg diet				p-value ¹
	0	4	8	16	
Firmicutes		73.4	81.5	74.6	0.127
Bacteroidetes	23.0	20.0	15.3	17.7	0.251
Actinobacteria	2.5 ^a	2.7 ^a	2.5 ^a	4.5 ^b	0.038
Fusobacteria	2.1	1.1	1.2	0.3	0.071
Proteobacteria	0.4	0.2	0.5	0.7	0.149

The highest supplementation of the yeast product increased the abundance of Actinobacteria, caused by the genus *Collinsella* and other subdominant genera. Interestingly, the highest supplementation also led to significantly increased abundance of the lactate-amplifying genus *Megasphaera* and the genus *Catenibacterium*. The shift in the faecal microbiome also led to significantly reduced abundance of other dominant genera such as *Faecalibacterium* and *Fusobacterium*. The genus *Lachnoclostridium* was significantly reduced when the yeast product was added compared to the phases with control feed, while *Erysipelatoclostridium* was detected in higher proportions. The data on the occurrence of antimicrobial resistance genes showed no differences between the treatment groups. Richness, Shannon Index and evenness of the microbiome were not influenced by yeast product supplementation.

Conclusion: The highest dosage of yeast product used also had the most significant effects. These can be characterised in the sense of a prebiotic with effects on the abundance of different genera of the intestinal microbiota.

1C10. The use of metronidazole affects diet digestibility, intestinal permeability, fermentative metabolites, immunity indicators, and microbiome of puppies

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Introduction: We aimed to evaluate the effects of metronidazole on diet apparent digestibility coefficients (ADC), intestinal fermentative metabolites, permeability, immunity, and microbiome in puppies.

Animals, materials and methods: Twelve growing Beagle dogs were divided into a control group (n=6), without, and a test group (n=6), with oral administration of metronidazole (15 mg/kg BW) for 5 days. Dogs were fed the same diet for 80 days. On days 20, 50, and 80, after metronidazole use, faeces and blood were collected for chemical analysis [1] and analysis of short-chain (SCFA) and branched-chain fatty acids (BCFA), IgA, microbiome, and serum concentrations of NF-κB and FITC-dextran. Faecal microbiome was analysed by 16S rRNA. The ADC data were analysed by the t-Student test (P<0.05). Faecal and blood data were analysed by analysis of variance (ANOVA) and means were compared by Tukey's test (P<0.05). Faecal microbiome was analysed by linear discriminant analysis (LDA) of effect size (LEfSe, P<0.05).

Results and discussion: The use of metronidazole reduced (P<0.05) the ADC of dry matter (73.3 to 70.5%), organic matter (77.0 to 74.4%), and crude protein (CP, 78.3 to 73.5%), with no difference for other ADC (P>0.05). In addition, the metronidazole group showed lower faecal concentrations of butyrate (8.35 to 7.81 mM/mol) and higher isobutyrate (5.51 to 5.61 mM/mol), isovalerate (5.50 to 5.66 mM/mol), and total BCFA (P<0.05). These results are consistent with lower CP ADC and higher intestinal permeability in the metronidazole group (P<0.05). The metronidazole group had higher serum NF-κB and lower faecal IgA on days 50 and 80 (P<0.05, Table 1). This indicates an increase in inflammatory processes in the gut and a change in local

Table 1. Means of NF-κB (pg/mL), intestinal permeability (Perm., μg/mL), faecal IgA (μg/mL), and diversity index in dogs receiving (M) or not (C) metronidazole as treatments (T).

immunity.

Item	Day 20		Day 50		Day 80		SEM*	P-value		
	C	M	C	M	C	M		T	Day (D)	TxD
NF-κB	177b	211ab	275a	278a	182b	287a	9.5	0.010	0.002	0.063
Perm.	0.507	0.540	0.501	0.627	0.496	0.493	0.011	0.048	0.092	0.115
IgA	161.0b	432.0a	69.1c	22.6d	59.3c	26.3d	42.90	0.172	0.001	0.033
Chao1	341a	192c	223b	192c	249b	209c	21.85	0.001	0.006	0.004
Shannon	4.1a	3.6b	4.0a	3.7b	3.8b	3.8b	0.08	<0.001	0.342	0.003

*SEM: standard error of the mean.
a,b Means with distinct letters differ (P<0.05).

These results were probably due to changes in the gut microbiome caused by metronidazole, such as a reduction in diversity (P<0.05, Table 1) and abundance of genera considered indicative of eubiosis, such as *Blautia* and *Turicibacter*, and an increase in *Streptococcus* (P<0.05, Figure 1). Similar results were observed by Pilla et al. [2] in adult dogs.

Conclusion: Oral administration of 15 mg metronidazole/kg/BW results in reduced dietary CP digestibility, increased permeability, NF-κB, and reduced butyrate and microbial diversity in puppies.

References: [1] AOAC (1995), Official Methods of Analysis 16th ed.; [2] Pilla, R. et al. (2020) J. Vet. Intern. Med 34:1853-1866.

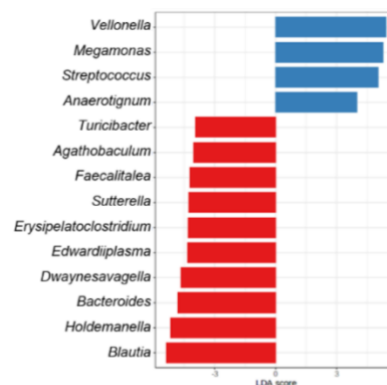


Figure 1. LDA score of bacteria that differ between dogs of the Control (red) or Metronidazole (blue) groups (P<0.05).

1C11. Mapping of the fecal microbiota of dogs with inflammatory bowel disease after oral intake of yeast cell wall through artificial intelligence

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Introduction: Inflammatory bowel disease (IBD) causes dysbiosis [1] and prebiotics can modulate it [2]. Conflicting data in literature compromise research advances in the field and artificial intelligence can provide a more accurate overview of the overall microbiota changes in these in the light of this new and promising methodology.

Animals, material, and methods: Fecal microbiota data of dogs with controlled IBD after oral intake of the yeast cell wall at 15mg/kg (n=6) or placebo (n=6) for 60 days were evaluated through a software of artificial intelligence EzBioCloud®. The dysbiosis scenario, average taxonomic compositions and microbiota network were analyzed and new biomarkers for this disease and treatment outcome were identified. Tukey's test and Linear Discriminant Analysis Effect Size (LEfSe) were used to analyze the results and the p-value was set at 1.0%.

Results and discussion: The results indicated that yeast cell wall favor the growth of symbiotic bacteria instead of pathogenic ones. The Firmicutes/Bacteroidetes ratio, an initial biomarker of dysbiosis [4], improved and these taxonomic families are related to the production of antiinflammatory compounds. The placebo group had a greater abundance of bacterial groups related to the worsening of the disease [5,6] (*Paeniclostridium spp.* and *Collinsella aerofaciens* p<0.01 *Clostridium ramosum* and genera *Lactonifactor* were found as biomarkers for the disease in dogs. The first is associated with inflammation of gut mucosa and the second related to inflammation in chronic diseases such as IBD [7]. *Blautia* genera, specifically specie *Blautia glucerasea*, family *Veillonellaceae* and *Veillonellales* spp. were the biomarkers found for the treatment with yeast cell wall (Figure 1). These bacteria are responsible, respectively, for producing short-chain fatty acids, then upregulating Treg cells and for producing bacteriocins against pathogenic bacteria [8]. It is possible that the yeast cell wall had a better effect due to its composition which includes mannanoligosaccharides. No differences were found for alfa diversity in any of the groups.

Conclusion: The yeast cell wall can modulate the gut microbiota of dogs with IBD and improve the growth of symbiotic bacteria such as *Blautia* genera and *Veillonellaceae* family. Those were the biomarkers for the prebiotic supplementation and positive outcome.

References: [1] Matsuoka et al. (2015). Seminars in Immunopathology, 248: 47-55; [2] Orel et al. (2014). World J of Gastroenterology, 20: 11505-11524; [3] Amaral et al. (2020). Master Thesis, University of São Paulo, 108f.; [4] Stojanov et al. (2004). Microorganisms, 8:1715; [5] Vijay & Valdes (2022). Eur J Clin Nutr, 76:489-501; [6] Milani et al. (2016). Sci Rep, 6: 25945; [7] Jergens et al. (2021). Gastroenterology, 8: 669913; [8] Liu et al. (2021) Gut Microbes 13:1875796.

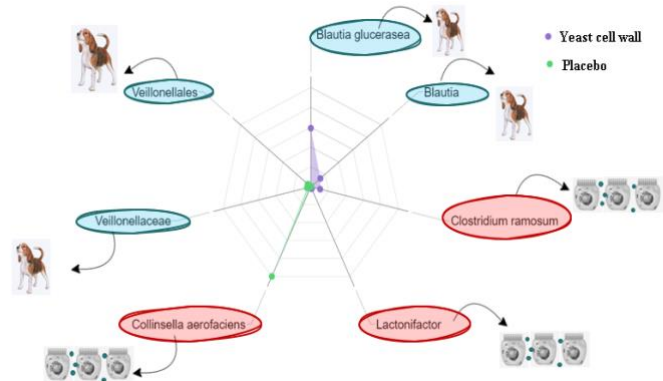


Figure 1. Biomarkers found for IBD dogs and yeast cell wall supplementation outcomes.

Session 1D Pre and probiotics III

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1D1. Evaluation of increasing levels of beta-glucan intake on canine digestibility, immunity, and faecal microbiota

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Introduction: Yeast beta-glucans (BG) modulate the immune system and microbiome of dogs [1,2], but the optimal inclusion dose remains unknown. The aim of the study was to evaluate the effects of 0.0, 0.07, 0.14, and 0.28% inclusion of *Saccharomyces cerevisiae* 1.3/1.6 BG in dry extruded diets on digestibility, immunity, and faecal microbiota of healthy adult dogs.

Animals, materials and methods: Four male Border Collies and four male and female English Cocker Spaniels, all with body condition score (BCS) 5/9 [3], aged 3.5±0.5 years, were randomly distributed into two balanced 4x4 latin squares. For each diet, 28 days were destined to diet acclimation and 7 to sample collection. Variables evaluated were: apparent digestibility coefficients (ADC) of nutrients [4]; faecal concentrations of short chain fatty acids (SCFA) and branched chain fatty acids (BCFA), ammoniacal nitrogen, lactic acid, IgA and pH; lymphocyte immunophenotyping, intensity and percentage of phagocytosis; oxidative burst; and faecal microbiota (by Illumina®). Data were analysed using PROC MIXED and PROC GLIMMIX procedures and Tukey-Kramer test (p<0.05).

Results and discussion: There were no differences in faecal compounds and faecal pH in all treatments with BG. Higher crude protein ADC was observed in the 0.14 and 0.28% BG consumption (p<0.0001), but no differences were found for other nutrients. Phagocytosis, oxidative burst and lymphocyte populations were not modulated by any of the treatments; however, 0.14% BG was able to modulate lymphocyte T CD4+:CD8+ ratio (p=0.0368; Figure 1). No differences in Faith (p=0.1414) and Pielou-evenness (p=0.1151) indexes were observed between treatments, despite positive and negative modulations observed in 5 phyla, 15 families, and 25 genera after consumption of BG diets (Table 1). Beta-diversity was different between intestinal microbiota after 0.0 and 0.14% BG consumption (p=0.047), which demarcates taxonomic variability among them.

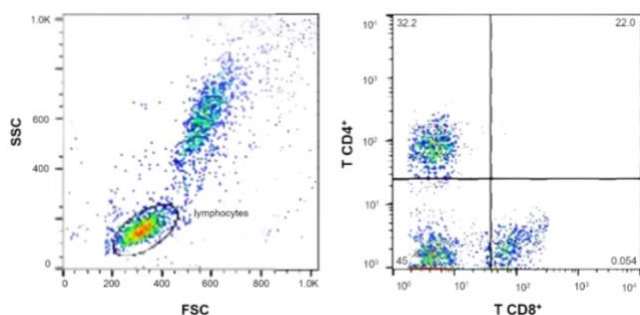


Figure 1. Lymphocyte proliferation activity and T CD4+:CD8+ ratio of the experimental groups

Table 1. Effect of beta-glucan (BG) in dog diets on average relative abundance (%) of main bacterial phyla, family, and genus.

Phyla	Treatments (% BG inclusion)				p
	0.0	0.07	0.14	0.28	
Bacteroidetes	26.38 ^a	19.75 ^c	21.30 ^b	21.73 ^b	<0.0001
Firmicutes	34.18 ^c	46.59 ^b	58.54 ^a	45.54 ^b	<0.0001
Proteobacteria	2.21 ^a	1.72 ^b	1.42 ^c	1.67 ^b	<0.0001
Family					
<i>Bacteroidaceae</i>	16.17 ^a	13.77 ^b	10.99 ^c	11.76 ^c	<0.0001
<i>Erysipelotrichaceae</i>	2.99 ^c	4.56 ^b	9.94 ^a	2.02 ^d	<0.0001
<i>Lachnospiraceae</i>	15.28 ^d	19.77 ^b	16.91 ^c	22.89 ^a	<0.0001
<i>Prevotellaceae</i>	7.74 ^a	4.37 ^b	7.85 ^a	7.99 ^a	<0.0001
Genus					
<i>Blautia</i>	8.91 ^b	10.78 ^a	8.71 ^b	11.03 ^a	<0.0001
<i>Faecalibacterium</i>	4.76 ^c	5.48 ^b	9.01 ^a	6.03 ^b	<0.0001
<i>Fusobacterium</i>	32.33 ^a	23.18 ^b	14.09 ^c	23.94 ^b	<0.0001
<i>Prevotella</i>	4.94 ^b	3.34 ^c	7.32 ^a	7.81 ^a	<0.0001

^{a-b}Averages in the same line followed by different letters differed by 1% in the Tukey-Kramer test adjusted by PROC MIXED.

Conclusion: The 0.14% *Saccharomyces cerevisiae* 1.3/1.6 BG inclusion was the optimal dose in promoting intestinal and immune parameters.

References: [1] De Oliveira et al. (2019) Comp. Immunol. Microbiol. Infect. Dis. 62:1–6; [2] Santos et al. (2022) Fermentation 8:2; [3] Laflamme (1997) Canine Pract. 22: 10-15:7; [4] AAFCO (2019) Washington, DC.

1D2. Assessment of Labelling and CFU Values of *Enterococcus faecium* Containing Veterinary Probiotics

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Introduction: Use of probiotics is becoming more widespread in veterinary medicine, but according to publications the reliability of commercial preparations is questionable [1, 2]. The purpose of our study was to evaluate labelling, ingredients and CFU values of some probiotics marketed for dogs and cats containing *Enterococcus faecium*. **Materials and methods:** 13 commercially available probiotics claimed to contain *E. faecium* were evaluated. The following parameters were measured and compared with the labelling of products: CFU of *E. faecium*, presence of other microorganisms (*Saccharomyces cerevisiae*, *Lactobacillus spp.*), and additives like prebiotics, toxin binders, electrolytes.

Results and discussion: 12 products clearly gave the used *E. faecium* strain, but all the 13 products' labels listed the expected CFU value. 10 contained *E. faecium* DSM 10663 and 2 products contained *E. faecium* DSM 7134. It must be mentioned, the DSM 7134 strain is approved by the European Food Safety Authority (EFSA) as feed additives only for calves, piglets, sows, chickens, minor poultry species, but not for pets [3, 4].

Table 1. Label claims and measured CFU/g values of the probiotics

Sample ID	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
Label claim (CFU/g)	5.5 x10 ⁵	3.5 x10 ⁷	3.5 x10 ⁷	1 x10 ⁹	1 x10 ⁹	5 x10 ⁸	5 x10 ⁸	1.125 x10 ⁸	2 x10 ⁸	2 x10 ⁸	2 x10 ⁸	2 x10 ⁸	1 x10 ¹⁰
Measured (CFU/g)	0	5 x10 ⁸	8.9 x10 ⁸	1.11 x10 ⁹	1.31 x10 ¹⁰	3.41 x10 ¹⁰	7.8 x10 ⁹	2.4 x10 ⁸	1.3 x10 ¹¹	8 x10 ⁸	8 x10 ⁸	5 x10 ⁸	4.47 x10 ¹¹

According to our investigations, in case of the previously mentioned 12 products CFU values even exceeded their label claims. In case of the 13th probiotic where the *E. faecium* strain failed to be declared, there was no bacterial growth (Table1). For 11 products the manufacturer used only *E. faecium*, for 2 products *L. plantarum* was added and other 2 were supplemented with *S. cerevisiae*. Seven products contained prebiotics, 4 toxin binders, 2 electrolytes, and 6 none of them. 4 products used together prebiotics and toxin binders, 2 prebiotics and electrolytes, but none of them had all these three ingredients. All but two products used appropriate dosing recommendations. The 2 exceptions used mocha spoon as a dosing unit, which is not reliable. Contrary to the earlier studies [1, 2] these products had high number of viable bacteria, all but one contained more than 1 x 10⁸ CFU/g, which means higher than 1 x 10¹¹ CFU/kg feed *E. faecium* content. EFSA [5] detected limited improvement in faecal scores of dogs feed with *E. faecium* DSM 10663 at the minimum dose of 1 x 10⁹ and maximum of 3,5 x 10¹⁰ CFU/kg feed. According to EFSA Opinion [6] the dose from 1 x 10⁹ to 1 x 10¹² CFU/kg feed for cats is safe, and small but significant improvement of the faecal scores can be detected. Based on these data our 12 products (92%) contained the appropriate concentration of *E. faecium* DSM 10663.

Conclusion: Results show that this strain of *E. faecium* remains viable and stabile during processing and storage condition, and combination with other functional ingredients (toxin binders, electrolytes, prebiotics) have no negative effects on it. In contrast to the negative experiences of previous years [1, 2], the fact that more than 90% of the investigated preparation listed correctly the strains, and the measured CFU values which even exceeded the claimed numbers, proves that the tested probiotic preparations are more reliable and have better quality, which is presumably also due to the strict European Union regulations.

References: [1] Weese et al. (2011) Can Vet J 52:43-46; [2] Lata et al. (2006) Acta Vet. Brno 75:139-144; [3] Hanchi et al. (2018) Front. Microbiol 9:1791; [4] EU Register of Feed Additives; [5] EFSA Journal 2014;12(6):3727; [6] EFSA Journal 2014;12(3):3602

1D3. Plasma and fecal metabolomic profile of adult cats fed yeast supplemented diet

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Introduction: Functional ingredients are ingredients that are intended to produce positive health outcomes through their physiological activity in the body [1]. Yeast cell wall products have shown antioxidant properties [2], modulation of the inflammatory response [3], and short-chain fatty acid production in the intestine [4]. An important compound to induce these responses is beta-glucan, a polysaccharide that can act in enhancing the scavenging of reactive oxygen species capacity and induce immune modulation [2]. Thus this study aimed to investigate the effects of yeast (*Saccharomyces cerevisiae*) supplementation on the metabolomic profile of adult cats. It was hypothesized that yeast supplementation could cause changes in the metabolomic profile of adult cats.

Animals, materials, and methods: The study was a complete randomized design with 20 cats divided into 2 treatments (n=10). Animals were fed complete and balanced extruded diets formulated for maintenance. The control diet (CMLA), mainly composed of chicken meal low-ash and rice, and a test diet of similar ingredient composition with 1600ppm of beta-glucan (CMLA Yeast) added at the expense of rice. The study had a 42-day period, with a 7-day washout period prior to treatment supplementation. Fecal and plasma samples were collected on days 0 and 42. Global metabolomics was analyzed by ultra-performance liquid chromatography and high-resolution mass spectrometry with a heated electrospray ionization source. Statistical analysis of the log-transformed data was carried out using Array Studio in Jupyter Notebook, with a paired t-test used to compare the treatment groups. The statistical significance was set at $P < 0.05$ and $q < 0.05$.

Results and discussion: The main changes were observed in lipids, fatty acids, amino acids, energy metabolism, and xenobiotic metabolites. Data indicate that metabolites from lipids, fatty acid, and amino acid metabolism increased fold change of cats fed the yeast compared with cats fed the control. In the plasma dataset, monoacylglycerols (e.g. 1-oleoylglycerol (18:1)), were significantly lower ($P < 0.05$) in the CMLA Yeast group in relation to CMLA. Although 3-hydroxybutyrate (BHBA) was significantly higher, a ketone body that plays an important role in beta-oxidation. Data also show a decrease ($P < 0.05$) of the fold change of plasma and fecal metabolites for energy metabolism of cats fed the yeast compared with cats fed the control diet.

Conclusion: In conclusion, the current research has emphasized several potential advantages of incorporating yeast into the diet of cats. Furthermore, the study has demonstrated that supplementing with yeast could contribute to regulating the metabolism of cats in terms of lipids, amino acids, and oxidative stress metabolites.

References: [1] Day et al. (2009). Trends Food Sci. Technol. 20:388-395. [2] Nakashima et al. (2018). J. Nutr. Sci. Vitaminol. 64(1):8-17; [3] Bi et al. (2022). Poultry Sci. 101(4):101712; [4] Xue et al. (2017). Anim. Nutr. 3(4):399-405.

1D4. The effects of a high-protein diet on the fecal microbiota and metabolome of obese and lean cats, before and after caloric restriction

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Introduction: Cat obesity is effectively managed with high-protein (HP) diets and caloric restriction (CR) [1]. In cats, fecal microbiota (FM) is influenced by HP diets [2-3], while the effect of CR on FM is poorly understood [4]. This study aimed to assess the difference in FM of obese (OB) and lean (LN) cats fed a HP diet with no CR, and then the effect of CR on FM of OB.

Animals, materials and methods: At T0, 13 healthy LN and 12 OB cats were fed a high-protein (HP) mix feeding regimen (mixF, on dry matter basis: dry food=DF: crude protein (CP) 50%, ether extract (EE) 9%, crude fiber (CF) 6%, starch: 14%, ash 7.6%; wet food=WF (42g/cat/day): CP 63%, EE 23%, CF: 0.2%, ash 10%) according to their current maintenance energy requirements (ER) for 30 days (T1). Then, a CR (40kcal/kg ideal body weight (BW)/day [5]) was applied to OB for 90 days (T2), reducing the DF while keeping the daily WF fixed for nutritional adequacy. ER adjustments were made weekly to promote weight loss. Fecal samples were collected in OB and LN at T0 and T1, and in OB at T2, to evaluate FM and metabolome, selected bacteria, and the feline dysbiosis index (fDI [6]) by qPCR. To determine differences in FM, 2-way ANOVA analysis was used with “mixF” and “OB vs. LN” as main effects with FDR correction (q-value), while pairwise tests were used to assess the effect of CR in OB. Statistical significance was set at p and q<0.05. Data are expressed as mean ± SEM or median (range).

Results and discussion: At T1 vs. T0, an increase of fecal iso-butyric acid was observed both in OB and LN (+31% and +46% q<0.05, respectively), while an increase of iso-valeric acid was observed in LN (+44%, q<0.01). Iso-acids are indicative of bacterial protein catabolism and could be stimulated by the HP content of mixF. In OB, at T1 vs. T0, a decrease of *Faecalibacterium* spp. (5.2 ±0.3 vs. 6.2 ±0.3 Log DNA, q<0.05) and *Bacteroides* sp. (5.0±0.3 vs. 6.0±0.2 Log DNA, q<0.01) was observed. In both groups, at T1 vs. T0, a decrease of *Streptococcus* spp. (OB: 2.7 ±0.2 vs. 3.1 ±0.2 Log DNA, LN: 2.9 ±0.1 vs. 3.5 ±0.3 Log DNA, q<0.05) and *Bifidobacterium* spp. (OB: 2.5±0.3 vs. 4.1±0.4 Log DNA, q<0.001, LN: 3.0 ±0.2 vs. 3.8 ±0.4 Log DNA, q<0.05) was observed. According to previous studies [2-4], the decrease of short-chain fatty acids producers *Bacteroides* sp., *Faecalibacterium* spp. and *Bifidobacterium* spp., might occur due to HP content of mixF. OB had a significantly lower abundance of *C. hiranonis* compared to LN (T0: 4.7 ±0.5 vs. 5.5 ±0.3 Log DNA, q<0.05; T1: 4.0 ±0.6 vs. 5.2 ±0.3 Log DNA, q<0.01). *C. hiranonis* has a key role in gut dysbiosis, as it is involved in bile acids metabolism [6]. However, most of OB had *C. hiranonis* concentration within the reference range, and the fDI between OB and LN did not differ (p>0.05). At T2 vs. T1, the WL was 9.2% (4.1-14.3), and an increase in fecal pH (7.1 ±0.2 vs. 6.7 ±0.04, p<0.05) and a decrease of n-butyric and n-valeric acid (-24% and -29% p<0.01, respectively) were observed, probably due to CR that led to a remarkable reduction of CF intake (-49 ±6% DM). CR did not affect FM.

Conclusion: The HP diet modulated cat FM toward proteolytic metabolism, while HP+CR decreased the amount of fiber fermented by FM. However, it is unknown how protein fermentation could affect the gut health in a strict carnivore animal, like the cat.

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1D5. Effect of herbal extracts on fermentation processes determined in an in-vitro canine model

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Introduction: Herbal extracts contain various active ingredients, including essential oils with antibacterial properties which can affect the fermentation process. Due to high concentration of active ingredients, small amounts are effective. Therefore, the aim of the effect of different of herbal extracts on in-vitro fermentation parameters.

Animals, material and methods: Faeces of four laboratory dogs (adult Beagle, aged 3-5 years), fed the same commercial diet, were collected immediately after defecation and transported to laboratories. Subsequently, faeces were mixed with phosphate buffer as inoculum and incubated without (control group) or with addition of samples of herbal extracts (0.5 g/20 ml). In-vitro gas production (GP) was estimated using gas test (glass syringes) and the fermentation parameters such as: pH, volatile fatty acids (VFA), short-chain fatty acids (SCFA), branched chain fatty acids (BCFA), lactic acid (LA) and ammonia concentration, and degradability of organic matter were determined in a canine model [1]. Chemical composition of herbal extracts (*Yucca schidigera* - YS, *Curcuma longa* - CL, *Rosmarinus officinalis* - RO and *Echinacea purpurea* - EP) was analyzed by standard analytical methods [2]. Fermentation parameters were determined before and after incubation (that lasted 24 h). Results were analyzed using one-way analysis of variance [3].

Results:

Table 1. Chemical composition of herbal extracts used in the study (% of DM).

Extract	DM	Ash	CP	EE	CF	NDF	ADF	ADL	I-NSP	S-NSP
<i>Yucca schidigera</i>	95.6	69.6	0.9	0.5	0	25.7	35.2	0.8	0.9	0.9
<i>Curcuma longa</i>	95.2	17.1	5.1	5.7	0	0.4	0.1	0	1.0	0.8
<i>Rosmarinus officinalis</i>	94.5	2.3	1.2	9.5	0	0.5	0.7	0	0.4	0.7
<i>Echinacea purpurea</i>	88.5	62	3.9	11.8	0	0.9	0.3	0	0.8	0.7

DM – dry matter; CP – crude protein; EE – ether extract; CF – crude fibre; NDF – neutral detergent fibre. ADF – acid detergent fibre; ADL – acid detergent lignin; I-NSP – insoluble non starch polysaccharides; S-NSP – soluble non starch polysaccharides;

Table 2. Fermentation parameters of herbal extracts used in the study (unit of DM).

Extract	GP max ml	ph24	SCFA mmol	BCFA mmol	VFA mmol	N-NH3 mg%
<i>Yucca schidigera</i>	49,6 ^A	5,3 ^{BC}	317,7	10,0 ^A	327,7	72,6 ^{ab}
<i>Curcuma longa</i>	50,8 ^A	4,5 ^{AB}	284,6	9,5 ^A	294,1	56,1 ^a
<i>Rosmarinus officinalis</i>	50,2 ^A	4,6 ^{AB}	314,4	8,9 ^A	323,3	58,0 ^a
<i>Echinacea purpurea</i>	49,3 ^A	4,4 ^A	307,6	9,7 ^A	317,4	56,9 ^a
Inoculum	25,0 ^B	6,1 ^C	294,6	22,1 ^B	316,7	116,2 ^b
<i>P value</i>	<0,01	<0,01	0,7	<0,01	0,7	0,02

Conclusion: The oils contained in herbal extracts could inhibit the growth of bacteria, which significantly influenced the in vitro fermentation process. Polyphenols (tannins) contained in the extracts can protect part of the dietary protein against degradation, which causes the process of inhibiting the formation of ammonia. The highest fiber content in YS may have contributed to higher VFA levels during fermentation.

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1D6. Markers of satiety, inflammation, and body composition in obese cats fed a diet containing 0.06% beta-glucans

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Introduction: Feline obesity is linked to several negative health effects. There is evidence that beta-glucans can mitigate the negative effects associated with feline obesity [1], as well as promoting satiety in other species [2, 3]. Due to the lack of studies with obese cats, the present study aimed to evaluate the effects of an inclusion of 0.06% yeast beta-glucans in the diet of obese cats on markers of satiety, inflammation, and body composition.

Animals, materials and methods: Nine client-owned obese cats [obese group (OG); body condition score (BCS) 8-9/9] and nine client-owned lean cats [control group (CG); BCS 5/9] were fed for 15 days with a control diet (0.0% beta-glucans) and then were fed for 90 days with a test diet (0.06% beta-glucan), so that the daily food amount was calculated for body weight maintenance. Blood samples were collected before (T0) and after 90 days (T90) of the test diet intake in order to evaluate gene expression of amylin, interleukin 10 (IL-10), ghrelin, and glucagon-like peptide-1 (GLP-1) by RT-PCR. The body composition was also evaluated at T0 and T90 by the deuterium isotope dilution technique. In order to compare groups, a repeated measure ANOVA test was performed. When differences between the averages occurred, the Tukey test was run ($p \leq 0.05$).

Results and discussion: At T90 there was a lean body mass (LBM) gain (in kg) in both groups without any change in body fat (Table 1). There were no variations in expression of amylin, GLP-1, ghrelin, and IL-10 after beta-glucans intake. Although the beta-glucans intake did not change body fat, there was an increase in LBM. In small animals, the relationship between

Table 1 - Mean of lean body mass and body fat values in obese cats and ideal body condition score cats, before and after supplementation with 0.06% beta-glucans.

Variables	BCS ¹		SEM ⁴	p value		
	CG ²	OG ³		BCS ¹	Beta-glucans	BCS ¹ *Beta-glucans
LBM ⁵ (Kg)	3.10	4.18	0.197	0.0010	0.0320	0.7415
LBM ⁵ (%)	82.58	72.40	2.294	0.0072	0.0547	0.3729
BF ⁶ (Kg)	0.69	1.61	0.173	0.0018	0.1960	0.5850
BF ⁶ (%)	17.41	27.59	2.356	0.0072	0.0547	0.3729

¹BCS: Body condition score; ²CG: Control group, with BCS 5; ³OG: Obese group, with BCS 8 and 9; ⁴MSE: standard error mean; ⁵LBM: Lean body mass; ⁶BF: Body fat.

beta-glucans and possible increase in LBM is not known. In human athletes, beta-glucan supplementation provided increased muscle strength and upper limb muscle mass [4]. In rats, there is evidence that beta-glucans have positive effects on muscle function, such as improved muscle performance during resistance exercise [5], improved myocyte oxidative metabolism [6], and reduced fatigue recovery time [7]. Regarding satiety markers, unlike what have been shown in humans [8] and dogs [2], there was no increase in their concentrations after beta-glucans intake. It is noteworthy that the method used in the present study was to evaluate the relative gene expression of these markers, while in the studies mentioned above, their plasma/serum concentrations were measured, which may explain the different results. It would be important to analyse gene expression in adipose tissue in future studies. Regarding IL-10, there was no variation after beta-glucan intake. This cytokine has an anti-inflammatory effect, and it may be co-elevated in obese animals in response to increased pro-inflammatory cytokines [1].

Conclusion: The beta-glucan addition in the diet at the concentration of 0.06% was able to increase the muscle mass of obese cats, but it was not sufficient to provide an increase in satiety markers and a decrease in IL-10.

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1D7. Effects of beta-glucan derived from different sources in an obese female dog

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Introduction: Obesity results in a chronic low-grade inflammatory state [1]. Therefore, the immunomodulatory effects of beta-glucans (BG) may be beneficial for obese dogs [2]. However, the possible adverse reactions of this nutraceutical are little known. This report shows a skin reaction after ingestion of BG from two different sources [*Saccharomyces cerevisiae* (SC) or *Euglena gracilis* (EG)] in an obese dog.

Animals, materials and methods: One obese dog was referred to the Pet Nutrology Research Center to be included in a clinical trial. A complete anamnesis, physical and clinical examination were carried out. The patient was a 6-year-old, female spayed mixed breed,

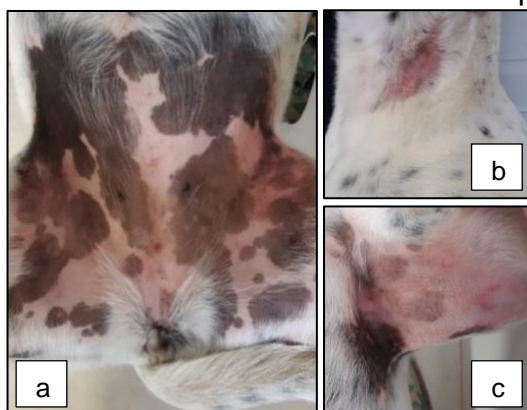


Figure 1. Patient erythematous lesions in abdomen (a), neck (b) and axilla (c)

with a body condition score (BCS) 8/9 [3] and muscle mass score (MMS) 3/0-3 [4]. Complete blood count, kidney and liver function, cholesterol, triglycerides and blood glucose levels were within the canine reference range. A control diet without the inclusion of BG (26.00% crude protein, 16.50% ether extract, 1.77% crude fibre and 5.43% ash in dry matter basis) was consumed for 30 days and there were no clinical manifestations. Then, an isonutrient diet with 0.01% inclusion of SC 1.3/1.6 BG was introduced. After 30 days, the owner attended the research center to reporting intense pruritus, alopecia and erythema on the dog's abdomen, neck and armpit (Figure 1).

Seven days after removing the treatment, all skin lesions and allergy signs disappeared. To better understand the allergic condition, the owner accepted a new challenge with an isonutrient diet with 0.01% EG 1.3/1.6 BG. In 15 days, the same skin manifestations returned, and it disappeared after seven days of control diet consumption.

Results and discussion: As the dog came from a clinical study and ingested only the isonutrient food, we can confirm that the BG were related to the allergic skin reaction. There are few reports of allergy to BG in animals with comorbidities in the literature, with obesity being a possible cause, as adipose tissue stimulates inflammatory and immunological metabolic reactions. So far, only one study has reported cases of allergic reaction to BG in dogs [5], in which a skin reaction to SC 1.3/1.6 BG was observed in two animals with pre-existing inflammatory bowel disease, which was related to allergic reaction. Yeast-derived BG are extracted from the cell wall of the yeast SC. This means that the immune system can properly recognize its molecular pattern to trigger an immune response as expected. Furthermore, it is known that some yeast cell wall proteins are known to trigger adverse skin reactions in people with food hypersensitivity [6]. It is possible that the gp200 glycoprotein is associated with the allergic reaction to yeast-derived BG. About EG 1.3/1.6 BG, the possible mechanism involved is unknown. Once consumed isonutrient diets, the adverse reaction can involve interactions between BG and other specific ingredients.

Conclusion: Obese patients may have an allergic reaction to BG, regardless of their origin (SC or EG). The mechanisms involved remain little understood.

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Session 2A Animal Nutrition I

Chair: Britta Dobenecker



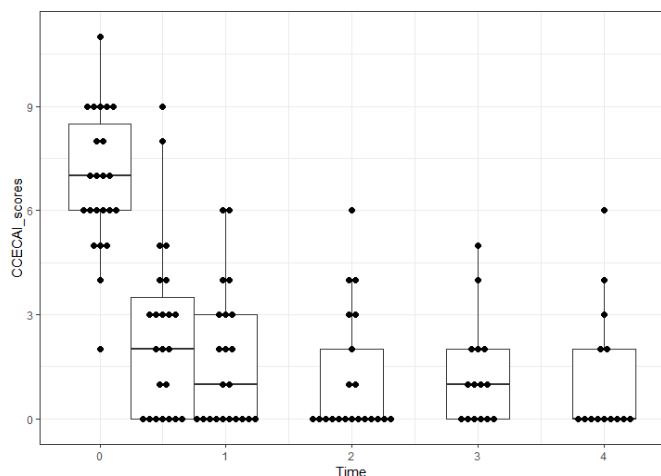
2A1. Beneficial response to a very low-carbohydrate diet in dogs with refractory chronic enteropathy: pilot study of 25 cases

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Introduction : Chronic enteropathies in dogs are quite common. Although the cause can vary, there is an overlap of clinical signs and chronic inflammation of the intestinal mucosa [1]. When reasoned medical management (prescription diets, antibiotic therapy (AB), immunosuppressive therapy (IST)) does not stabilize the symptoms, these enteropathies will be qualified as refractory chronic enteropathies (RCE). Such dogs may remain with various medical treatment for years. Low fat or novel protein home-cooked diet has been proposed [1,2]. But to our knowledge, never has been a very low-carbohydrate home-cooked diet (VLCHD), although dogs can digest and tolerate starch with various genetic and epigenetic capacities [3,4,5,6,7]. We formulate the hypothesis that a lack of tolerance to starch activates various mechanisms and generates a chronic inflammatory state, and that a VLCHD may improve clinical signs of RCT.

Animals, materials and methods: Inclusion criteria: dogs coming to the specialist consultation at Onlyvet vet hospital, and diagnosed with RCE for at least 3 months. They are prescribed, for at least 2 months, a VLCHD, formulated to cover all nutrient requirements [7]. The VLCHD included meat, raw canola oil, vegetables, Vit'i5™ as a mineral, trace elements and vitamins supplement, with apple and cheese as acceptable treats. Medical treatments



were supposed to be stopped after a month time of this diet. After 2 months, the diet could be changed, with the introduction of up to 20% of energy as starch (20CHD). All clinical and biological and gastro-intestinal parameters were followed for 4 months. Wilcoxon test was used for CCECAI and body condition scores.

Results and discussion: A total of 25 dogs were included. The decrease and normalisation of the CCEICA score (figure 1) was significant within 2 weeks, and stayed low thereafter. Clinical signs and body condition score improved significantly with time. After 2 months of diet, most owners

(15/25) refused the reintroduction of starch. In the 10 dogs with 20CHD, the CCEICA score increased again in 3/10 dogs (starch was stopped again in 2/10 and CCECAI got back to 0). Medical treatments were still maintained in the last month of follow up in 9/25 dogs (5 with AB, 2 with IST, 2 with both), sometimes for unclear reasons.

Conclusion: A very low carbohydrate home cooked diet appears as a clinically beneficial option for dogs with RCE. Further studies are needed to understand the underlying mechanism, especially the effect on the microbiota of such a diet.

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2A2. Dietary fibre increase improves clinical response in cats with chronic enteropathy unresponsive to hydrolyzed protein diets: A case series

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Introduction: Dietary interventions are often a key component of therapy for chronic enteropathy (CE) [1]; for which both hydrolyzed protein diets (HyPD) and fiber-enhanced diets are recommended, however response to these interventions can be variable [1,2]. Here, we present a case series of three cats diagnosed with CE that failed to respond to HyPD but presented significant improvement to an increase in dietary fiber.

Animals, materials and methods: Between August 2021 and December 2022, three cats presented to the Ontario Veterinary College's Clinical Nutrition Service for dietary management of CE. All three cats showed clinical signs related to both small and large intestinal problems (see table 1). Blood biochemistry showed no significant findings. One cat presented increased serum folate and was receiving Vitamin B-12 supplementation. Faecal tests were negative. Abdominal ultrasound and intestinal biopsies were consistent with lymphoplasmacytic inflammation in both the small and large intestine. A comprehensive diet history was obtained for the three cats.

Table 1. Patient signalment, and clinical summary of the three cats with chronic enteropathy, when presented for dietary management of medical conditions that responded to fiber addition when hydrolyzed diets failed.

Patient	Body Weight (kg) [Body condition score]	Medication	Clinical signs	Dietary treatment	TDF when clinical signs resolved (g/100 kcal)	Time until clinical improvement (weeks)
12yr FS Norwegian Forest	5.5 [6/9]	Prednisolone	Hyporexia Lethargy Constipation	MFD + psyllium husk	2.9 (MDF: 2.1; psyllium: 0.8)	2 - 3
6yr MN Ragdoll	6.5 [7/9]	Prednisolone Cisapride	Constipation Vomiting	HFD	2.7	1 - 2
12yr MN DSH	6 [6/9]	Prednisolone Sulfasalazine Vitamin B-12	Defecating outside the litter box Vomiting Diarrhea	HFD	2.7	1 - 2

Results and discussion: Table 1 summarizes the clinical assessment of the three cats with CE. In all cats, dietary management included HyPD. Two cats were previously fed HyPD for at least for 2 weeks, unsuccessfully; for the third cat, HyPD were recommended by the Clinical Nutrition Service and worsened clinical signs of CE, this was seen over the first week after transition. HyPD was discontinued because of failure to achieve clinical remission or improvement. HyPD are usually highly digestible and low in fibre content (TDF \leq 2 g/100 kcal) [1]. An increase in total dietary fibre (TDF) was implemented, which resulted in resolution of clinical signs and allowed reduction of pharmacological therapy. Two cats were fed a HFD {TDF > 2.5 g/100kcal [2]} and one was fed a moderate fibre diet (MFD) {TDF 1 - 2.5 g/100kcal [2]} with added psyllium husk supplement. The addition of dietary fibre has been found to provide benefits for cats with chronic diarrhea, colitis and constipation [2,3,4], however there is limited information on the clinical effect when small intestine inflammation is present [2].

Conclusion: These cases highlight the importance of considering fibre supplementation in the management of cats with CE, particularly in cases where HyPD fail to provide adequate control of clinical signs. Further studies are needed to evaluate the efficacy of this dietary approach in a larger population of cats with CE.

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2A3. Nutritional management of cats with chronic kidney disease: a retrospective study in 41 cats

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Introduction: Chronic kidney disease (CKD) is a prevalent metabolic condition in middle-aged to senior cats and dietary intervention is essential to delay its progression and improve prognosis [1]. This study aimed to describe nutritional management in a group of cats diagnosed with CKD and to investigate its association with survival.

Animals, materials, and methods: A retrospective study was performed at 2 veterinary centres with cats, using the following inclusion criteria: confirmed diagnosis of CKD; absence of comorbidities; available information about nutritional management; a complete follow-up from diagnosis to death; and a minimum survival time (ST) of 2 months. All animals were staged and treated according to the IRIS CKD guidelines [2]. Descriptive statistics was performed and the ST was calculated by the Kaplan-Meier method. Associations between ST and the different variables were investigated using the log-rank test and were considered statistically significant when $p < 0.05$.

Results and discussion: Forty-one cats (23 females and 18 males) were included with a median age of 12 years (range: 2 – 19 years) and a median weight of 3.3 kg (range: 1.6 – 6.4 kg) at diagnosis. The most reported clinical signs at presentation were weight loss ($n=27$; 65.9%), anorexia ($n=22$; 53.7%) and/or vomiting ($n=14$; 34.1%), and three cats were in IRIS CKD stage 1, 19 in stage 2, 15 in stage 3 and 4 in stage 4. The transition to one of two commercial renal diets (crude protein: 23.0 – 24.5%; phosphorus: 0.39 – 0.41%; EPA/DHA: 0.41 – 1.5%) was recommended in all cases, regardless of the IRIS CKD stage, but only 56.1% ($n=23$) accepted this food and continued to be fed with it as their main nutritional support. In over half of the included animals ($n=23$; 56.1%), the use of mirtazapine as an appetite stimulant was required. Twenty-six cats received nutraceuticals during their treatment, mostly phosphate binders ($n=23$, 56.1%), but also multivitamin concentrates ($n=4$; 9.8%), iron and B vitamins ($n=2$; 4.9%) and flavonoid-based products ($n=1$; 2.4%). Median overall ST was 272 days (range: 61 – 2442 days), which is lower than previously described in a similar study (771 days) [3]. Only IRIS CKD stage showed a significant association with it ($p < 0.001$), with stage 4 cats having a significantly worse prognosis (Median ST=77 days). Other factors may have limited the prognosis, such as the high rate of animals that did not accept the transition to the renal diet. Thus, although the median ST of the animals that consumed the renal diet was not statistically superior to the others (272 days vs 257 days; $p=0.949$), the patient with the significantly longer survival time was exclusively on renal food from diagnosis to death.

Conclusion: A short median ST was detected in this small group of cats, as a result of a higher prevalence of more advanced IRIS CKD stages, associated with more debilitating clinical signs. Furthermore, the high percentage of cats that did not accept the renal diet suggests that clinicians still need to improve their nutritional approach to these patients by recommending a more gradual and timely transition and increasing owner awareness and compliance. Therefore, an earlier diagnosis and adequate dietary intervention should be reinforced for a better prognosis in cats diagnosed with CKD.

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2A4. Evaluation of plasma and urine amino acids levels of cats with chronic kidney disease stages 1 and 2 fed a renal diet

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Introduction: Chronic kidney disease (CKD) can alter the plasma amino acids (AAs) profile because the supply of AAs to the kidneys can decrease by the reduced glomerular filtration rate [1]. This study aimed to compare plasma and urine AA profile of healthy and CKD cats, and evaluate the effects of a renal diet on plasma and urine AAs concentrations in cats with CKD stages 1 (CKD1) and 2 (CKD2).

Animals, material and methods:

Procedures were approved by the Ethics Committee on the Use of Animals. Six domestic cats with CKD1 and nine CKD2 were included in the renal groups; control group was composed of 10 healthy cats. All animals (both groups) were enrolled on a senior diet (103g protein/1000kcal and 2.9g phosphorus/1000kcal) for 30 days before samples started to be collected. Subsequently, every group received renal diet (87g protein/1000kcal and 1.2g phosphorus/1000kcal) for 60 days. Both diets had different vegetal and animal protein sources. Sample collections were conducted in two periods, T0 (after 30 days of senior diet) and T60 (60 days after T0). The plasmatic level of 38 AAs was analyzed by high-performance liquid chromatography [2]. A repeated measure ANOVA test was performed. When differences were observed, the Tukey test was used ($p \leq 0.05$).

Table 1 – Plasma amino acids concentration observed in the experimental groups

Amino acid	Time	Treatment			p		
		Healthy	CKD 1	CKD 2	Treatment	Time	Interaction
Arginine	T0	16.45	14.68	18.24	0.8595	<0.0001	0.3063
	T60	33.26	40.57	32.28			
Histidine	T0	10.07	7.07	8.64	0.9007	0.0005	0.2840
	T60	14.24	18.31	14.62			
Isoleucine	T0	49.29	38.37	76.97	0.6874	<0.0001	0.5069
	T60	628.58	447.22	494.57			
Leucine	T0	15.10	15.63	15.00	0.1459	<0.0001	0.1502
	T60	39.85	46.84	34.56			
Lysine	T0	11.27	10.69	12.37	0.5451	<0.0001	0.1944
	T60	27.40	26.99	20.08			
Methionine	T0	119.98	112.06	116.56	0.3794	0.0004	0.3420
	T60	395.59	219.46	300.52			
Phenylalanine	T0	3.67	3.54	4.57	0.5800	<0.0001	0.1934
	T60	18.30	20.45	15.76			
Taurine	T0	14.57	13.55	20.81	0.2103	<0.0001	0.8227
	T60	35.40	39.36	45.58			
Threonine	T0	9.08 ^{Aa}	7.87 ^{Ba}	9.89 ^{Aa}	0.2075	0.0075	0.0381
	T60	11.17 ^{Ab}	19.57 ^{Aa}	10.61 ^{Ab}			
Valine	T0	23.38	48.62	29.36	0.1292	0.0383	0.1495
	T60	107.10	121.02	18.36			

^{Aa} Means followed by different uppercase letters in lines differ by 5% in Tukey test

^{ab} Means followed by different lowercase letters in columns differ by 5% in the Tukey test

Results and discussion: Plasmatic concentration of 26 AAs [9 essential amino acids (EAAs) (table 1)] increased at T60 for all groups, except valine in CKD2, which decreased. It was observed a time x treatment interaction for plasma threonine and tyrosine and for urine threonine and alanine. Urine levels of 28 AAs (10 EAA) increased at T60. Despite moderate protein levels, plasma concentration of 9 EAAs increased after renal diet, in both renal and control groups. It demonstrates the importance of adequate dietetic AAs profile independently of total dietetic protein content in the renal diet formulations. Although there was no difference between healthy and cats with CKD, in early CKD stages there was still no renal function impairment sufficient to cause changes in AAs profile. These results are relevant because early studies showed that renal patients had lower EAAs concentrations than healthy individuals [3], and this can cause negative consequences, such as impaired protein synthesis, decreased albumin levels, compromised immune function and loss of lean body mass [4].

Conclusion: Healthy cats and cats with CKD1 and CKD2 had no difference on plasma and urine AA profile. After eating a renal diet, these cats had increased plasmatic and urine concentrations of non-essential AAs and EAAs.

References : [1] Brunetto et al. (2021) *Metabolites* 11(12): 844; [2] White et al. (1986) *J. Autom. Chem.* 8:170-177; [3] Suliman et al. (2005) *Am J Clin Nutr.* 82:342-349; [4] Parker et al. (2019). *J. Vet. Intern. Med.* 33(2):680-685.

Session 2B Clinical cases I
Chair: Brigitta Wichert



2B1. Allergic reactions in the head region in two horses after ingestion of hay – a case report

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Introduction: In February 2022, we were contacted regarding two horses (Bavarian Warmblood, gelding, 22 years old and Westphalian Warmblood, mare, 18 years old) within a small herd of nine animals, who had suffered an allergic reaction, especially in their head region (oedema of eyelids, lips, and mouth), after the ingestion of hay. Initially signs of mild colic and watery diarrhoea were also seen in both horses. Several hours later, urticaria had affected their entire bodies. The first-responding veterinarian administered glucocorticoids and the clinical signs then subsided quickly and completely. Because similar incidents have been reported in the attending veterinarian's area of practice, she suspected that the two horses might have had contact with caterpillars of the oak processionary moth (OPM). The owner of the horses therefore submitted a sample of the hay they had been consuming to verify this suspicion. Oak trees were not present in the vicinity of the horses. As the horse owner purchased hay from many different suppliers, no information could be provided on the tree population around the hayfields.

Animals, materials and methods: Since the sensory examination of the hay did not reveal any specific findings, we decided to use DNA metabarcoding (AIM - Advanced Identification Methods GmbH; Leipzig, Germany) for further examination. A sample of the hay was subjected to Next Generation Sequencing. The sequences were then compared to different databases (BOLD database [1], NCBI GenBank [2]) and assigned to different species.

Results and discussion: All species detected (about 50 % Ascomycota, 20 % Arthropoda, 30 % other Eucaryota) were screened and tested for their potential to cause contact dermatitis. DNA of OPM could not be detected, but another representative of Lepidoptera, *Lymantria dispar* (gipsy moth, GM) was found.

Like OPM, stinging hairs of GM caterpillars can also cause various ailments. In humans, itchy urticaria, eye irritation and respiratory distress have been described [3,4,5]. Although there are no case reports for animals, a comparable symptomatology can be assumed in horses. The fact that only two horses within a herd of nine animals developed clinical signs might be explained by nest distribution in the hay and an individual sensitivity to environmental- and feed-related allergens.

Conclusion: We presume a causal connection between the clinical signs observed in the horses and the GM DNA detected in the hay sample. DNA-Meatbarcoding of hay samples appears to be a suitable method to screen for caterpillar species when feed-related adverse reactions are suspected in equine patients.

References: [1] <https://boldsystems.org/index.php/databases>; [2] <https://www.ncbi.nlm.nih.gov/genbank/>; [3] Tuthill et al. (1984) Am J Public Health 74(8):799-803; [4] Gooderham et al. (2021) J Cutan Med Surg 25:555-6; [5] Croitoru et al. (2022) CMAJ April 4, 194:E500.

2B2. A senior pony with severe dental issues and equine metabolic syndrome: a nutritional challenge?

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Introduction: Equine metabolic syndrome (EMS) is a collection of risk factors for endocrinopathic laminitis characterized by insulin dysregulation (ID) present in middle aged and older horses [1], [2]. Geriatric horses are also prone to develop dental abnormalities and pituitary pars intermedia dysfunction (PPID) [2], [3].

Case history: A 28-year-old gelding Shetland pony, 221 kg, body condition score (BCS) 5/9, with a history of being overweight (BCS: 7/9 – 4 years ago) was referred to the nutritional service due to an acute phase of chronic laminitis, PPID, high basal insulin concentrations (858pmol/L) and hypertriglyceridemia (1.11mmol/L). Furthermore, severe failing dentition (diastema's and 5 missing teeth) was diagnosed. As initial treatment, the pony was receiving: pergolide (2.27mg/kg SID), suxibuzone (6.8mg/kg BID), pentoxifylline (7.2 mg/kg BID) and metformine (25mg/kg BID). Apart from continuous pasture access, hay and a soup-type diet were fed. Willow branches were also offered to keep the pony busy during the day.

Nutritional advice & discussion: EMS with hyperinsulinemia and hypertriglyceridemia, PPID and dental issues were all taken into account when designing the nutritional advice. The prescribed diet consisted of grass pellets, fibre-rich mash, beet pulp, and a roughage balancer. All the pelleted ingredients were mixed and soaked in warm water for a period of 20min. This "soup" type of diet was used to ease swallowing and to facilitate enzymatic and bacterial digestion due to the small particle length [4]. A salt lick stone without flavour, and water were always available. The pony was kept inside during the acute phase of laminitis. This prescribed diet contained the same amount of energy (2.2 EWP_a) but was 7% higher in crude protein compared to the previous diet. The amount of sugars and starch was reduced from 1g/kg BW/meal (previous ration) to 0.45g/kg BW/meal in the prescribed diet. The fat intake was increased from 2.2g/kg DM to 6.8g/kg DM mainly coming from the flaxseed in the fibre-rich mash. Despite being mainly a "soup" diet, dry matter (DM) intake represented 1.17%/kg BW/day and 27.6% crude fibre/kg DM was fed (previous diet: 33% crude fibre/kg DM). Furthermore, all vitamins and minerals requirements were met by adding a roughage balancer. Short grass fibres was offered in between meals to occupy the pony and the willow branches (*Salix spp.*) were kept to chew. Willow bark contains salicin, polyphenols and catechol and therefore has analgesic and anti-inflammatory properties [5].

Follow up & conclusion: Due to the development of hyporexia from unknown origin, the main challenge was to provide a highly palatable diet and to ensure the pony's welfare. The owner was asked to check which feed was less palatable by offering the soaked ingredients separately. Therefore the fibre-mash and the beet pulp were replaced by another fibre-rich mash and the number of meals was increased. Devil's Claw (*Harpagophytum Procumbens*) was added on the owner's demand due to its potential anti-inflammatory properties [6]. The pony was also dropping the chewed short fibres but never showed choking symptoms. After one month, the laminitis symptoms improved and blood examination showed a decrease of the basal insulin (182pmol/L) but plasma triglycerides (1.34mmol/L) increased. The pony kept a stable weight.

References: [1] Durham et al. (2019), J Vet Intern Med, 33:335–349; [2] Jarvis et al. (2019), Equine Veterinary Education, 31:102–110. [3] Ireland et al. (2012), Equine Vet J, 44:101–106. [4] Jarvis and McKenzie (2021), Veterinary Clinics of North America - Equine Practice, 37:89–110. [5] Antoniadou et al. (2021), Int J Mol Sci, 22, no.20. [6] Vervuert and Stratton-Phelps (2021), Veterinary Clinics of North America - Equine Practice, 37:207–222.

2B3. Case Report: Nutritional approach to an unbalanced homemade diet and subsequent overcorrection in a female kitten

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Introduction: Feline nutritional secondary hyperparathyroidism (NSH) results from inadequacies regarding dietary calcium (Ca), phosphorus (P), Ca:P ratio, and low vitamin D (vit. D) supply [1], which regularly occur in homemade diets formulated for cats [2]. The growing animal's sensitivity to such imbalances [3], and the difficulty to diagnose NSH without nutritional evaluation, highlight the importance of this case report.

Case history: An intact European shorthair kitten was fed an all-meat diet during the first 5 months of life. Following a femoral fracture at 4 months of age and with normal measured serum parathyroid hormone (PTH), the private veterinarian prescribed vit. D drops at an unknown dosage. At 5 months of age and with 1.5 kg body weight (BW), the dosage was increased to 400 IU per day. After a second pathological fracture at 7 months of age (BW: 2.1 kg), blood work showed elevated alkaline phosphatase activity, as well as low 25-hydroxyvitamin D and vitamin A (vit. A) concentrations. Mineral and vitamin supplementation was therefore recommended (see nutritional assessment below). Due to persisting lameness, skeletal deformities and the development of unspecified neurological symptoms, the patient was presented to the internal medicine clinic of the Vetsuisse Faculty, University of Zurich at 8 months of age. Increased pancreatic lipase activity, PTH, and 1,25-dihydroxyvitamin D concentrations were found. X-rays revealed generalized osteopenia. NSH was diagnosed, and the patient was therefore referred to our nutritional consultation service. The patient weighed 2.2 kg (body condition score (BCS) 5/9 [4], normal muscle condition) and was receiving an online recipe-inspired, homemade diet based on different protein and carbohydrate sources, vegetables, and avocados, supplemented as stated below. Nutritional assessment of the diet showed a Ca:P ratio of 2:1 and excessive Ca, P, vit. A, and D supply (selected nutrients of the resulting diet per kg BW^{0.67}; Ca: 959 mg, P: 473 mg, vit. A: 2535 UI and vit. D: 426 IU).

Recommendations: Above mentioned imbalances were corrected, to approach calculated recommended allowances (CRA) for growth (dietary level per kg BW^{0.67}; Ca: 353 mg, P: 317 mg, vit. A: 333 UI and vit. D: 31 IU) and avocados were eliminated from the diet. Besides an adequate supply of essential fatty acids, a surplus of eicosapentaenoic and docosahexaenoic acid was ensured (209 mg EPA, 369 mg DHA).

Outcome and discussion: One month later, clinical improvement was noted, however no weight gain was achieved and skeletal deformities persisted. Aged 12 months, the diet was adjusted to adult CRA (in diet per kg BW^{0.67}; Ca: 210 mg, P: 192 mg, vit. A: 224 UI and vit. D: 21.3 IU). As symptoms recurred, mineral, and vitamin levels were adjusted near to previous levels (CRA for growth). At 17 and 21 months, a normalization of PTH and decrease (however still above reference) of 1,25-dihydroxyvitamin D blood levels were noted. Osteopenia improved throughout, but the mineralization process is at 21.5 months still considered incomplete. Due to clinical improvement, the diet was however adjusted to CRA for adult cats for all nutrients (in diet per kg BW^{0.67}; Ca: 130 mg, P: 112 mg, vit. A: 218 UI and vit. D: 18 IU). No deterioration was further noted. Early dietary evaluation would have benefitted diagnosis, treatment and prognosis. It remains an essential and reliable assessment when NSH is suspected, since age and species appropriate blood references for parameters such as vit. D and PTH are less sensitive and might lead to misdiagnosis.

References: [1] Corbee (2017) Diseases of the dog and cat. Ettinger SJ 1966-1979; [2] Villaverde & Chandler (2022) J. of Fel. Med. and Surg. 24: 415-428; [3] Gross et al. (2010) Sm. An. Cl. Nu. 429-439; [4] Laflamme D. (1997). Fel. Prac. 25:13-18 [5] NRC (2006) Nutrient Requirements of Dogs and Cats.

2B4. Effects of long-term feeding a diet with moderate protein and phosphorus in cats with CKD IRIS Stage 1 and 2

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Introduction: Renal diets are typically reduced in protein (PROT) and phosphorus (PHOS). However, there is no evidence that reduced PROT intake brings benefits to cats in early stages of chronic kidney disease (CKD). Few studies that had evaluated the effects of a diet with moderate or high PROT and low PHOS to CKD cats [1;2]. The aim of this study was to evaluate the effects of a renal diet with moderate PROT and PHOS in the serum control of PHOS, ionized calcium (iCa) and parathyroid hormone (PTH) in healthy cats and cats with CKD stage 1 (CKD1) and 2 (CKD2) for 330 days.

Animals, materials and methods: This was a case-controlled clinical trial. Healthy cats (n=7; 6.3±3.7 years; 4.7±0.4kg; 1,36mg/dL serum creatine (sCr)); non-proteinuric cats CKD1 (n=6; 10.8±2.6 years; 5.3±0.8kg; 1,50mg/dL sCr); and 2 (n=7; 10.6±4.5 years; 5.1±1.4kg; 1,88mg/dL sCr) were included. The IRIS stage of CKD was determined according to IRIS guidelines [3]. All cats were fed with a pre-trial senior diet for 30 days (10.3g of PROT/100 kcal and 260mg of PHOS/100 kcal) and then assigned to a renal diet (8.7g of PROT/100 kcal and 120mg of PHOS/100 kcal; Ca:P ratio, 1.08) for 330 days. Blood samples were collected at baseline (30 days after senior diet), 30, 60, 150 and 330 days after a renal diet, to evaluate serum concentrations (s-) of total PHOS, PTH and iCa. All procedures were approved by the Ethics Committee on the Use of Animals. Data were analysed using a general mixed linear model for repeated measures in time with, moments, group versus moment as fixed effects. When differences between the averages occurred, Tukey test was used. Significance was established when $p < 0.05$.

Results and discussion: All cats-maintained body weight throughout 330 days. No differences were found among groups and times ($p > 0.05$) of body weight, sPHOS, sPTH and siCa. sPHOS (Figure 1A) remained within laboratory reference range (2.6-6.0mg/dL), although higher values based on IRIS target (< 4.5 mg/dL) [3] were observed in cats with CKD at all time points (4.7±0.63 for CKD 1 and 5.1±0.65 for CKD 2). siCa was within laboratory reference range (1.0-1.4mmol/L) for all groups (1.29±0.03 for healthy and CKD1 and 1.31±0.04 for CKD2; Figure 1B). Healthy cats had PTH concentrations lower than 0.5pmol/L in all time points. At baseline, one cat CKD1 (2.9pmol/L) and one CKD2 (5.8pmol/L) had sPTH concentrations higher than the laboratory reference range (0.4-2.5pmol/L), but at T60 and T330, the concentrations were lower than 0.5pmol/L (Figure 1C). Clinically, the renal diet may be responsible to correct an initial hyperparathyroidism in these two cats.

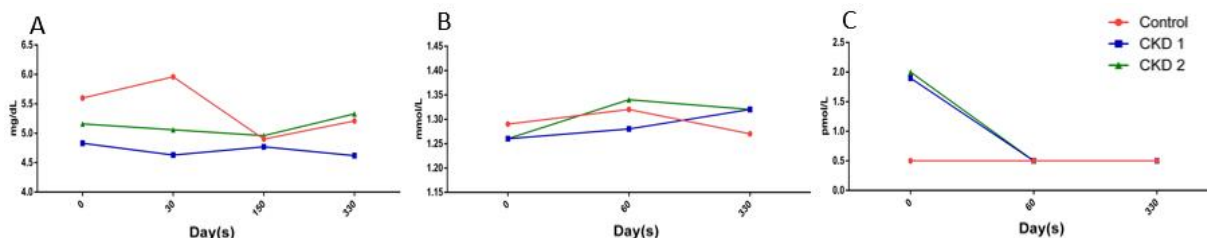


Figure 1. Serum concentrations of phosphorus (A), iCa (B) and PTH (C) observed at different evaluation times.

Conclusion: The renal diet evaluated in this study with a moderate PROT and PHOS can maintain serum PHOS, iCa and PTH in cats with IRIS stage 1 and 2.

References: [1] Hall et al. (2018). *Vet Record*. 184; 1-10; [2] Ephraim et al. (2021). *J. Food Sci. Nutri*. 7:096; [3] International Renal Interest Society. *IRIS Staging of CKD* (2023); [4] Freeman et al. (2006). *J. Vet. Inter. Med*. 30;1661-1666.

Session 2C Clinical cases, Equine, Exotics Posters



2C1. Case report: nutritional management of a protein-losing enteropathy in a senior Weimaraner dog

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Introduction: Protein-losing enteropathy (PLE) in dogs is a syndrome primarily associated with IBD and lymphangiectasia [1]. Dietary management is the cornerstone of PLE treatment [2], while the use of immunosuppressive agents remains controversial [1]. Clinical response to a dietary trial can take up to 14-30 days [3], and even if the initial diet trial is unsuccessful, further trials with low or ultra-low fat/hydrolyzed/home-cooked diets should be conducted [1]. Homemade diets have the advantages of containing highly digestible ingredients and allowing for an easily doable inclusion of MCTs. Dietary management with an ultralow-fat homemade diet is considered the best option for dogs with PLE [3,4]. Although deemed as important factors, BCS and percentage weight loss may not be valid predictors of death after immunosuppressive treatment failure in dogs with PLE caused by IBD or lymphangiectasia [5].

Animals, materials and methods: An intact male purebred Weimaraner (9 years old at the time of presentation, BW 23 kg, BCS 1/9, ideal BW 34 kg) diagnosed with PLE caused by moderate lymphangiectasia and mild lymphocytic plasmacytic gastroenteritis was referred to the Clinical Nutrition Service of the Padua University Teaching Hospital. The patient scored 12 on the CCECAL index, according to which his condition was defined as severe, and his prognosis as guarded. He had lost 6 kg of BW in the previous 4 weeks; both immunosuppressive cortisone treatment and dietary change (from maintenance with fat content (FC) 46 g/Mcal to gastrointestinal moderate-fiber pet food with hydrolyzed fish as main protein source, FC 38 g/Mcal) were ineffective.

Results and discussion: The main nutritional goals were to feed the patient with a home-cooked, highly digestible, high-protein (125 g/Mcal), ultralow-fat (20 g/Mcal), low-fiber diet (1 g/Mcal). The initial formulation consisted of 800 g of chicken or turkey breast, 250 g of egg white, 75 g of ricotta cheese, 150 g of rice (weighed uncooked), 5 ml of sunflower oil, 5 ml of MCT oil, 800 mg EPA and DHA omega-3 and vitamins and minerals supplementation (DER covered: 130 kcal/kg^{0.75}, FC 18 g/Mcal). After 10 days, the patient weighted 22.2 kg (-0.8 kg). Therefore, the diet was changed by increasing the daily amounts of meat (1kg), egg white (500g) and rice (200g) (DER: 165 kcal/kg^{0.75}, FC 20 g/Mcal). Ten days after the diet change, the patient's BW was 23.5 kg (+1.3 kg). One month after the initial consultation, the dog weighted 26 kg, the albumin level was increased up to the physiological range, and the dog's liveliness returned back to normal. Four months later the dog was back at his ideal BW, 34 kg (BCS 5/9). After a failed attempt to go back to a fully hydrolyzed moderate fat diet (27 g/Mcal), that led to the relapse of diarrhoea, the dog was managed with a low-fat (24 g/Mcal) mixed diet (130 kcal/kg^{0.75}) including 60% DER as hydrolyzed kibbles in addition to the home-cooked ingredients, and his BW has been stable ever since.

Conclusion: This case demonstrates that an ultralow-fat homemade diet can successfully manage the signs of PLE also in cachectic dogs. It also suggests that low BCS is not necessarily associated with bad prognosis, and reinforces the idea that at least a couple of weeks should be considered before defining a dietary change as ineffective. Further, it is shown that the immunosuppressive therapy may not be useful in PLE caused by moderate lymphangiectasia/mild IBD. Finally, client's compliance and regular feedback with the veterinarian were crucial to promote the dog's health and the recovery of his BW.

References: [1] Craven & Washabau (2019) J. Vet. Intern. Med. 33(2):383–402; [2] Simmerson et al. (2014) J. Vet. Intern. Med. 28(2):381–337; [3] Jablonski (2021) J. Small Anim. Pract. 69(2):756-764; [4] Nagata et al. (2020) J. Vet. Intern. Med 34(2):659-668; [5] Kathrani et al. (2019) J. Vet. Intern. Med 33(2):536-543.

2C2. Rickets by hypovitaminosis D in a Pit Bull puppy fed imbalanced homemade diet

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Introduction: Rickets is a metabolic bone disorder characterized by decreased bone mineralization and defective bone development during growth, the increased width of the growth plates differentiates it from nutritional secondary hyperparathyroidism (NSH). Occurs due to dietary deficiency in calcium, phosphorus, and/or vitamin D [1,2,3,4]. Dogs are dependent on dietary vitamin D to cover their requirements, due to their inadequate cutaneous synthesis of cholecalciferol [5]. This report presents a case of rickets in a puppy to highlight its diagnosis, treatment, and prevention.

Animals, materials and methods: A 4-month-old male American Pit Bull Terrier dog was referred for nutritional evaluation presenting weakness, major diffuse pain, and apathy. The dog was fed an imbalanced homemade diet consisting of cooked rice and liver. Blood samples were collected at admission (D0), after 15 days (D15) and 90 days (D90), and skeletal radiographs were taken at D0 and D90. At D0 a complete and balanced dog food for puppies was prescribed (27% crude protein, 13% fat, 1-1,6% calcium, 0.9% phosphorus, as fed), and the daily amount was recommended according to FEDIAF (2021) [6], and calculated based on puppies energy requirement (210 kcal/kg^{0.75} or 880kJ/kg^{0.75}). Other food, supplements, or snacks were not provided.

Table 1. Serum values at zero, 15 and 90 days of treatment.

	D0	D15	D90	References	
25(OH)D (ng/mL)	< 4.0	-	80.3	100-150	
1,84 PTH (pg/mL)	16.4	-	12.1	4.0-38.0	
				4-6 months	7-12 months
Calcium (mg/dL)	5.4	11.4	10.7	10-13.2	10.4-12
Phosphorus (mg/dL)	6.6	9.0	7.3	5.6-9.6	3.5-7.8

Results and discussion: At D0, the dog weighed 6.8 kg, 39% under the expected weight (11.2 kg) compared to puppy growth charts [7] for 27 kg predicted adult body weight. At D90, the dog weighed 18.8kg,

still 10% below the curve but closer to the ideal body weight (21kg)At D0 the animal presented with severe hypocalcemia, vitamin D deficiency, normal serum parathyroid hormone concentration (Table 1), and mild normochromic normocytic anaemia (data not shown). Also, severe diffuse osteopenia of the skeletal system, widened and irregular epiphyseal growth plates, and pathological fractures in both femurs were detected. Therefore, the dog was diagnosed with rickets and the severe hypocalcemia and radiographically evident bone changes were likely a result of the low dietary amounts of calcium, vitamin D, and the inadequate dietary calcium-to-phosphorus ratio. At D15, complete normalisation of serum calcium was obtained. At D90, vitamin D increased 20-fold from D0 (Figure 1). General bone mineralization improved and fractures were healed. Anaemia was resolved. A complete nutritional anamnesis was crucial to allow the correct diagnosis.

Conclusion: The clinical findings in this report highlight the risks associated with feeding an imbalanced homemade diet during growth and the importance of obtaining a thorough dietary history.

References: [1] Khundmiri et al. (2016) *Compr Physiol.* 6(2):561-601; [2] Campbell; Douglas (1965) *Br J Nutr.* 19(3):339-351; [3] Pettifor (2005) *Endocrinol Metab Clin North Am.* 34:537-553; [4] Hazewinkel; Tryfonidou (2002) *Mol Cell Endocrinol.* 197:23-33; [5] Hurst et al. (2020) *Vet Clin Pathol.* 49(2):279-291; [6] FEDIAF Nutritional Guidelines (2021); [7] Waltham Petcare Science Institute, UK.

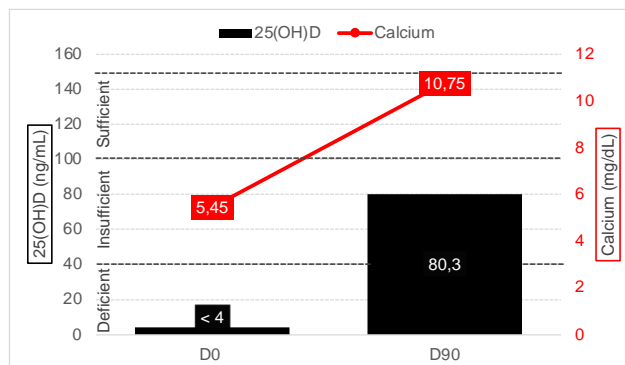


Figure 1. Serum vitamin D and calcium at D0 and D90

2C3. Nutritional strategy in the management of hyperlipaemia and non-alcoholic fatty liver (NAFL) in a Sardinian donkey

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Case presentation: This clinical case reports the nutritional approach in a Sardinian adult jenny suffering from severe malnutrition along with hyperlipaemia (common in donkeys with low feed intake [1-2]), likely following a traumatic event. The jenny came from a regional park in Sardinia (Italy) and showed to be underweight (body weight, BW: 88 kg; body condition score, BCS: 1.75/5), reluctant to eat, with poor fecal score (FS: 2/8, watery/loose feces) and wounds complicated by ulcerations on the elbow, limbs and sternum. The results of analyses showed a systemic inflammatory status, increased liver enzymes and

Table 1: Body weight (BW), body condition score (BCS), fecal score (FS) as well as hematochemical values of the presented donkey, from the beginning of the two-step nutritional treatment to the follow-up 30 weeks later.

Item	Reference range	Day 1	Day 35	Day 210
%NEUT (%)	23.0 – 59.0	81.0	58.7	48.3
%LYMPH (%)	34.0 – 69.0	8.80	30.2	39.6
%BASO (%)	0.00 – 0.50	2.00	1,20	0,10
#NEUT (10 ⁹ /L)	2.40 – 6.30	7.70	5.81	4.99
#LYMPH (10 ⁹ /L)	2.20 – 9.60	0.84	2.99	3.86
#BASO (10 ⁹ /L)	0.00 – 0.07	0.19	0.12	0.04
RBC (10 ¹² /L)	4.40 – 7.10	4.14	3.70	4.76
MCHC (g/L)	310 – 370	404	389	352
PLT (10 ⁹ /L)	95.0 – 384	82.0	315	324
ALB (g/l)	21.5 – 31.6	17.6	20.3	26.3
Ca (mmol/l)	2.20 – 3.40	0.50	2.40	2.60
CREA (μmol/l)	53.0 – 118	44.0	77.0	81.0
P (mmol/l)	0.77 – 1.39	1.81	1.74	1.22
γ-GT (iu/l)	14.0 – 69.0	87.0	37.0	39.0
AST (u/l)	279 – 430	657	324	341
ALT (u/l)	5.00 – 14.0	60.0	22.0	12.0
TG (mmol/l)	0.60 – 2.80	8.70	0.60	0.90

hyperlipidaemia (Table 1; day 1). On ultrasound examination, enlarged and hyperechogenic liver was also observed. The nutritional approach considered a two-step treatment, with subsequent feeding practices.

Nutritional approach: At first, the animal was stimulated to consume feed voluntarily by using highly palatable hay and ad hoc feeding technique (free access to pasture and water, hay 1.80 kg/d and 50 g/d of mash compound feed). The jenny was weighed after one week (BW: 96 kg) and showed increased BCS (2.25/5) and FS (4/8). The second step lasted 4 weeks and was intended to improve the body condition and restore blood parameters. The feeding practice consisted of hay (1500g/d), mash compound (300g/d) and free access to pasture and water. After 4 weeks from the beginning of the second step, the parameters improved markedly and got closer to the physiological range with apparent recovery. Appetite was improved along with overall nutritional conditions. The patient was then moved to graze in a plot with other donkeys to improve animal social and feeding behavior. No changes in BCS, FS, or clinical condition were observed. After 30 weeks, blood parameters were monitored, where the complete blood cell count along with biochemical values were within physiological ranges. The jenny finally weighed 118 kg, with a normal BCS of 3/5 and a FS of 5/8.

Conclusion: The two-step nutritional approach had remarkable results, with improvement of clinical and nutritional conditions of the patient in 4 weeks, beginning from a severe condition and poor health.

References: [1] Burden et al. (2015) J. Equine Vet Sci. 35:376–82; [2] Burden et al. (2011) J Vet Intern Med. 25:1420-5.

2C4. Fluctuation in Body Weight, Body Condition and Muscle Condition of Breeding Stallions during and after the stud season

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Introduction: Stallions entering the stud season are required to face augmented energy demand for semen donation used in artificial insemination or for natural mating. Despite those are established practices in studs, knowledge on variation in body weight (BW), body condition score (BCS), and muscle condition (MCS) of stallions in relation to reproduction performance is limited. We aimed to investigate if variations related to the nutritional status of breeding stallions during and after the stud season could occur.

Animals, materials and methods: Ten stallions (breed, horse number: Anglo-Arabians, n.6, Arabian Thoroughbred, n.2, English Thoroughbred, n.1, Oldenburg, n.1) were enrolled in the study and monthly evaluated during and after the stud season 2022/2023. All animals were fed a diet to cover individual requirements based on BW^{0.75} (good quality hay and compound mixed feeds)[1]. Each stallion was weighed and assessed for BCS (1-9 points [2]), and MCS (1-5 [3] points). The effect of time on selected parameters was analyzed using a one-way ANOVA.

Results and discussion: Stallions displayed a normal BW throughout the period of observation (Figure 1). A significant decrease of BCS during the stud season was observed ($p = 0.046$). MCS increased significantly ($p < 0.0001$) during the stud season (Table 1). Stallions exhibited a higher BCS and lower MCS after the stud season.

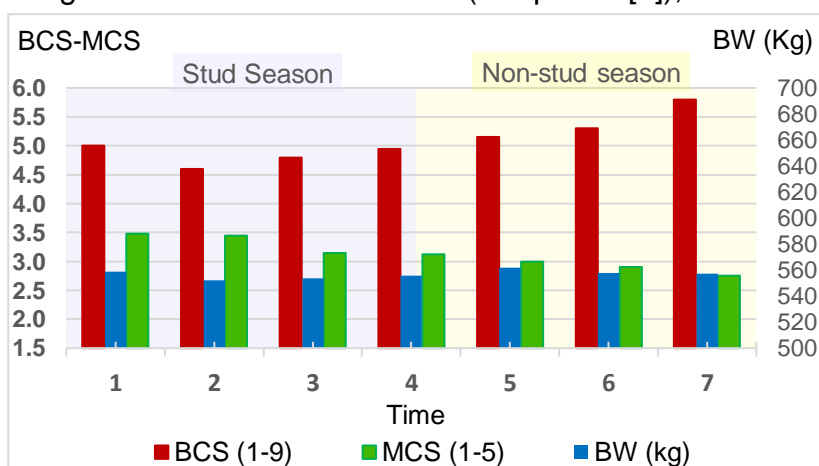


Figure 1. Time-dependent fluctuations in body condition score (BCS), muscle condition score (MCS) and body weight (BW) of breeding stallions.

Table 1: BW, BCS, MCS in relation to sampling time (Mean \pm SD).

Time	Stud Season		BW				BCS				MCS			
	Yes/No	N°	Mean	StDv	p-value	Tukey test*	Mean	StDv	p-value	Tukey test*	Mean	StDv	p-value	Tukey test*
1	Y	10	558.60	39.00		A	5.00	0.67		BC	3.48	0.34		D
2	Y	10	552.20	33.60		A	4.60	0.70		C	3.45	0.37		DE
3	Y	10	553.80	36.60		A	4.80	0.35		C	3.15	0.27		DEF
4	Y	10	555.70	39.90	0.998	A	4.95	0.83	0.046	BC	3.13	0.21	< 0.001	EF
5	N	10	562.10	42.70		A	5.15	0.82		BC	3.00	0.20		FG
6	N	10	557.90	35.50		A	5.30	0.68		BC	2.90	0.13		FG

Conclusion: In conclusion, our study showed that not the BW, but BCS and MCS of breeding stallions varied across stud and non-stud seasons, thus opening to further investigation on the different energy partitioning.

References: [1] National Research Council (US), 2007; [2] Henneke et al., (1984) Theriogenology, 21.6:897-909; [3] Pallesen et al., (2023) Equine Veterinary Education.

2C5. Metabolic shift of nutrition-related parameters in stallions during and after stud season

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Introduction: The nutritional status of stallions along with the metabolic milieu can be conditioning factors of semen quality [1] and appear to be worthy of being monitored throughout the year. The aim of this research was to assess the metabolic fingerprint of plasma parameters selected for nutritional status assessment of stallions according to circannual variation. For this purpose, hormonal profile (leptin, OB; testosterone, T) and oxidative enzymes (catalase, CAT; glutathione peroxidase, GPx; superoxide dismutase, SOD) circulating levels were monitored during and after the stud season.

Animals, material and methods: A total of ten stallions of a same stud center of Agris-DIRIP (breed, horse number: Anglo-Arabians, n.6, Arabian Thoroughbred, n. 2, English Thoroughbred, n.1, Oldenburg, n.1; age, horse number: 8 years, n.1; 10 years, n.2; 12 years, n.1; 13 years, n.2; 15 years, n.1; 16 years, n.1; 19 years, n.1; 22 years, n.1; Body weight, BW; 496 - 625 kg Body condition score, BCS 1-9 points scale: 4-6, at start) were enrolled in the study, as to homogeneous serving frequency. Stallions were housed in individual boxes and were subjected to carousel training 50 minutes per day, for 5 days a week and semen sampling alternatively twice or thrice a week. Animals were fed a diet consisting of high-quality hay and mixed compound feed, following the feeding practice of the stable to meet requirements [3], with *ad libitum* water. Horses were sampled for whole blood at the beginning (T₁), in the middle (T₂) and at the end (T₃) of stud season (Mar-July 2022). A follow up was carried out at the beginning of the negative photoperiod (T₄). Complete blood cell count (CBC), levels of T, OB were determined. Oxidative status was also assessed (GPx, SOD, CAT). All data were analysed using one-way ANOVA (Minitab_18[®]). Age categories were established following previous research. Selected parameters were analysed for correlation by using Pearson correlation test (ρ). Significance was set for P -value<0.05.

Results and discussion: Horses appeared healthy throughout the trial. OB decreased significantly (P -value=0.022) from the beginning of stud season as well as until the end of positive photoperiod (follow-up). OB and T were negatively-correlated with sampling time (ρ =-0.650, P -value=0.000 and (ρ =-0.338, P -value=0.033, respectively). In agreement previous observations [1], horses aged >13 ys. confirmed a progressive decline of circulating T (P -value=0.013). OB and T are positively correlated in horses aged >13 ys (ρ =0.412, P -value=0.046). These results are consistent with Morrone et al. (2023) [4] as to fluctuations of BW and BCS.

Conclusion: In conclusion, the metabolic fingerprint of selected hormonal profile along with nutritional status and activity during the stud season turned out to be significantly affected in stallions older than 13 years, thus pointing at age as an animal conditioning factor, beyond management.

References: [1] Cappai et al., 2021 Biol Trace Elem Res 199, 3287–3296 (2021).

<https://doi.org/10.1007/s12011-020-02447-7> [2] Henneke et al., 1991 [3] NRC (2007), Nutrient Requirements of Horses. Ed. By National Research Council, Washington (USA). [4] Morrone et al., Book of abstract ESVCN 2023.

2C6. Effect of dietary linseed oil and vitamin E supplementation on mRNA expression of genes linked to cell survival in white blood cells of racehorses

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Introduction: Intensive training is a critical component of preparing racehorses for competition, but it can also significantly affect the immune system. Studies conducted so far have suggested that intensive training can lead to decreased white blood cell (WBC) survival, potentially making these animals more susceptible to infection and other health problems [1]. However, there is evidence that WBC apoptosis can be suppressed by antioxidants supplementation. In the present study, we hypothesized that dietary linseed oil (rich in linolenic acid) and vitamin E, due to its antioxidant properties, should positively influence on expression of genes responsible for survival of white blood cells.

Animals, materials and methods: The experiment was carried out on 30 racehorses in training (19 Arabian and 11 Thoroughbred horses, 3 – 5 years old) allocated into 3 groups (blocked by age, breed and stable). During the study their diet consisted of meadow hay (0.014 kg / d / kg body weight (BW)) and concentrate (oats grain with muesli “Livery Mix”, Saracen, UK; 0.009 kg / d / kg BW), with the addition of vegetable oil in amount of 0.5 ml / d / kg of BW. Control group (C) received soybean oil as an energy equivalent, whereas experimental groups received linseed oil without (E1) or with (E2) vitamin E supplementation (20 mg / kg BW^{0.75} / day in a α -tocopherol acetate form). Diets were calculated individually for each horse according to NRC (2007) recommendations, covering energy and nutrients requirement for heavy working animals. The experiment consisted of 74 days, including 14 days of diet adaptation. At day 44 and 74 horses were subjected to physical efficiency test (PET), which consisted of a warm-up and the race distance of 2000 m (canter). Blood samples were collected before (t0), 5 min after (t5) or 120 min after PET (t120). The mRNA expression of nuclear factor NF-kappa-B p65 subunit (RelA), nuclear factor erythroid-derived 2-like 2 (NFE2L2) and baculoviral IAP repeat-containing protein3 (BIRC3) was examined in the white blood cells (WBC) with Real-time PCR. Data were analyzed using the PROC GLIM MIX of the SAS (v.9.4) and pre-planned contrasts were used for scientific hypothesis verification (K vs. E1 & E2 and E1 vs. E2).

Results and discussion: All three investigated genes expression at t5 were affected by the diet ($P \leq 0.03$) and day of study ($P \leq 0.04$). Expression of BIRC3 and NFE2L2 was the highest in E1 and E2 and lowest in C at d 44, with no difference between the groups at 74 d of study (group and time (44 vs 74 d) interaction, $P = 0.06$ and $P < 0.01$, respectively). Similarly, RelA mRNA expression was the highest in E1 and E2 and lowest in C at 44 d of study, but at 74 d of study it was slightly higher in E2 comparing to E1 and C (group and time interaction, $P < 0.01$). There was no effect of the diet at the other sampling times (t0 and t120), except a tendency towards lower expression of NFE2L2 at t0 in the groups E1 and E2 comparing to C ($p = 0.07$).

Conclusion: The effects of intensive training and diet composition on white blood cell survival in racehorses appear to be complex and multifactorial, but it can be concluded that linseed oil supplementation seems to have at least some positive effect on promoting cell survival, mostly right after the training.

The study was financed from statutory activities of University of Agriculture in Krakow (Poland), Faculty of Animal Sciences (project number 020011-D015/2020).

References: [1] Williams (2016). J Anim Sci. 94, 4067-4075.

2C7. Effect of dietary linseed oil and vitamin E supplementation on free radicals scavenging enzymes expression in white blood cells of racehorses

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Introduction: Intensive exercise, especially of young and still growing horses, may lead to muscle damage and inflammation as a consequence of increased oxidative stress and cell apoptosis. Moreover, during the training period, racehorses have greater demand for energy [1]. However, it has been reported that they are able to consume vegetable oil, as a source of energy, without negative effect on nutrients digestion. In this study we hypothesized that due to high ALA content linseed oil supplementation alone or together with vitamin E addition may improve oxidative stress indices and stimulate free radicals scavenging enzymes expression in white blood cells.

Animals, materials and methods: The experiment was carried out on 30 racehorses in training (19 Arabian and 11 Thoroughbred horses, 3 – 5 years old) allocated into 3 groups (blocked by age, breed and stable). During the study their diet consisted of meadow hay (0.014 kg/day/kg body weight (BW)) and concentrate (oats grain with muesli “Livery Mix”, Saracen, UK; 0.009 kg/day/kg BW) with the addition of vegetable oil in amount of 0.5 ml/day/kg BW. Control group (C) received soybean oil, whereas experimental groups received linseed oil without (E1) or with (E2) vitamin E supplementation (20 mg / kg BW^{0.75} / day in a α -tocopherol acetate form). Diets were calculated individually for each horse according to NRC (2007) recommendations [2], covering energy and nutrients requirement for heavy working animals. The experiment consisted of 74 days, including 14 days of diet adaptation. Physical efficiency test (PET) consisted of a warm-up and the race distance of 2000 m (canter). Blood samples were collected at day 44 and 74, before (t₀), 5 min after (t₅) or 120 min after PET (t₁₂₀). The mRNA expression of glutathione peroxidase 1 and 3 (GPX1, GPX3) and superoxide dismutase 1 and 2 (SOD1, SOD2) was examined in the white blood cells (WBC) with Real-time PCR. Data were analyzed using the PROC GLIMMIX of the SAS (v.9.4) and pre-planned contrasts were used for scientific hypothesis verification (K vs. E1 & E2 and E1 vs. E2).

Results and discussion: There were no significant differences between experimental groups in mRNA expression of GPX1 and SOD1. Linseed oil in the diet increased GPX3 expression immediately after exercise (t₅) on day 44, while on day 74 no differences between the groups were observed (group and time interaction; P < 0.01). SOD2 mRNA expression before exercise (t₀) was the highest in the C group and the lowest in the E2 group on day 44. However, on day 74 it was the highest for the C group, and the groups E1 and E2 did not differ from each other (group and time interaction; P = 0.04). In turn, immediately after exercise (t₅) on day 44, the highest SOD2 expression was found in the groups E1 and E2, and on day 74 there was no significant difference between the groups (group and time interaction; P = 0.02).

Conclusion: Based on the results, it can be concluded that dietary linseed oil with vitamin E supplementation may stimulate some free radicals scavenging enzymes expression in white blood cells. On the other hand, the overall decrease in the free radicals scavenging enzymes expression observed during the study may result from adaptation to intensive training.

The study was financed from statutory activities of University of Agriculture in Krakow (Poland), Faculty of Animal Sciences (project number 020011-D015/2020).

References: [1] Geor et al. (2013) Equine Applied and Clinical Nutrition. Saunders Elsevier. [2] National Research Council (NRC), 2007. Nutrient Requirements of Horses. 6th ed., National Academy Press, Washington.

2C8. Body mass, fecal microbiota and fermentation products in zoo felids

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Introduction: Felid species range in their body masses from about 1 kg in the rusty footed cat to about 300 kg in the largest tiger subspecies [1]. The body mass of mammalian carnivores is associated with natural hunting and feeding behaviour[2]. Due to the differences in intake of fibrous animal matter, this may have co-evolved with digestive physiology. A clear relationship between shortened gastrointestinal transit time and primary colonic function has been shown in humans and dogs [3,4], but these mechanisms have not been evaluated across felid species. We hypothesized that differences in fecal microbiota and fermentation products (short-chain fatty acids (SCFA), branched-chain fatty acids (BCFA)) in different zoo felines were related to body mass when fed the same diet.

Animals, materials and methods: 18 felids from Pairi Daiza Zoo (Brugelette, BE) participated in this study, including cheetah (n=1), puma (n=3), clouded leopard (n=2). Lion (n= 2), Javan leopard (n=1), white tiger (n=2), Siberian tiger (n=5) and snow leopard (n=2). All were fed large pieces of beef with bone and whole chicken. During a health check performed under anaesthesia, fresh feces were opportunistically collected from the rectum of each animal and then stored at -80 °C for further analysis. One subsample per animal was used for 16S rRNA sequencing, and one for SCFA (including BCFA). The animals clustered into two body mass groups: those below 100 kg (“small”) and those above 100 kg (“large”). The factor body mass was statistically evaluated using t-tests at $P < 0.05$.

Table 1. Impact of body mass on fecal microbiota and fermentation products in zoo felids.

	Small (<100kg)		Large (>100kg)		P-value
	MEAN	SEM	MEAN	SEM	
Firmicutes (%)	72.8	6.5	60.0	3.9	0.011
Fusobacteria (%)	2.4	1.0	23.0	7.5	0.015
Actinobacteria (%)	17.9	4.9	16.1	5.3	0.085
Shannon	2.9	0.1	2.5	0.1	0.049
InvSimpson	10.6	1.1	6.8	1.0	0.024
Total SCFA (µmol/g DM)	382.4	43.4	407.7	24.8	0.645
Acetate (%)	71.6	1.8	62.8	1.9	0.005
Propionate (%)	14.2	1.2	19.4	2.3	0.066
Butyrate (%)	9.5	0.9	12.8	1.9	0.128
Total BCFA (µmol/g DM)	17.8	3.1	21.9	3.5	0.390
Isobutyrate (µmol/g DM)	6.1	1.1	7.8	1.2	0.304
Isovalerate (µmol/g DM)	11.6	2.3	13.2	2.2	0.586
Valerate (µmol/g DM)	0.7	0.1	0.9	0.1	0.487

Results and discussion:

Table 1 showed the difference in fecal microbiota and fermentation products in two body mass group. The relative abundance of Firmicutes and Fusobacteria were respectively higher and lower in small cats than in big cats. Actinobacteria did not differ. The Shannon index and the Simpson index indicated that the fecal microbial diversity of small cats was higher. Fecal total SCFA and BCFA concentrations increased with higher body mass. Large intestine length is positively

correlated with body mass in carnivores[5]. We assumed that large intestinal transit times in exotic cats increased with body mass. Therefore digesta would reside longer in a larger colon, fermentative capacity (hence SCFA production) should be enhanced.

Conclusion: Microbiome and fermentation may be affected by felid body mass as such. However, ecological niches related to body mass in felids should also be considered.

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2C9. Evaluating the effect of bedding type and feeding level on growth performance and metabolic responses in Pekin ducks

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Introduction: Apart from strain selection, one solution that has been talked about for reducing the negative effects of rapid growth in poultry is reduction of feed intake without affecting final body weight or introducing other welfare concerns such as chronic hunger [1]. Certainly, lower protein levels or higher fibre levels in a diet can slow growth to some degree [2]. Poultry under early feed restrictions can perform varying internal and external coping behavioural and/or physiological adaptation mechanisms to nutritional stress and creating acclimated physiological processes for the later growth stages [3,4]. The present study was therefore carried to evaluate the effect of bedding type and feeding levels on the growth performance, digestive anatomy and histomorphometry, blood metabolites, oxidative stress markers, and body nutrient composition of ducks.

Animals, materials and methods: A total of 240 day-old ducklings (*Anas platyrhynchos domesticus*) were randomly divided over 24 floor pens with 10 ducklings per pen and each pen was randomly attributed to one of the two bedding types and six feeding levels and considered as experimental unit. To evaluate the effect of bedding type on feeding, pens were floor covered (5–10 cm) with either straw bedding or wood shavings, and each group (bedding type) contained six sub-groups: *ad libitum* feed intake and 95, 90, 85, 80, and 75% feed offers of the daily measured *ad libitum* sub-group with a commercially available duck starter (d 0–14) and grower (d 15–42) pelleted feeds. Diets were isonitrogenous and isocaloric. The experiment lasted for 42 days and ducks were fed on pen basis. Performance data were collected on daily and weekly basis. Ducks were sampled for blood before slaughter for acylcarnitine profiling and malonaldehyde evaluations [5] and following post mortem breast muscle was collected from either sides for fatty acids evaluation [6,7]. The entire caeca were excised and caecal contents were collected from either segments for microbial assay [8]. All samplings were done at two points, d 15 and 43, of the experiment and two ducks were sampled per feeding level at each sampling point. A mixed model with feeding level and bedding type was applied to the data.

Results and discussion: As expected, weight gain varied with feeding level ($p < 0.05$) while only the 75% feeding level showed lower feed conversion ratio ($p < 0.05$). Ducks raised on straw bedding showed higher weight gain and lower feed conversion ratio than those on wood shavings ($p < 0.05$) with no effect on feed intake ($p > 0.05$). Straw bedding had increased the ratio of acylcarnitines to free carnitine, in the duck blood indicating elevated metabolic rate ($p < 0.05$) and yielded a better supply of glucogenic substrate to the citric acid cycle (propionyl:acetylcarnitine; $p < 0.05$) [9]. Moreover, straw bedding have also yielded increased breast muscle concentrations of several saturated and monounsaturated fatty acids compared with wood shavings ($p < 0.05$). Caecal microbial evaluations revealed dominating Bacteroides, Firmicutes, and Verucomicrobiota (95.2%) bacterial groups in ducks with straw bedding with Shannon and Simpson diversity indexes of 3.6 and 16.8, respectively ($p < 0.05$).

Conclusion: Bedding type and feeding level both affect the performance of ducks that are associated with changes in the caecal microbiome and host metabolism.

References: [1] Guarino et al. (2022) *Animals*, 12:1354; [2] Hartcher and Lum (2020) *Worl. Poult. Sci. J.*; [3] Li et al. (2011) *Front. Agric.*, 5:94-101; [4] Lippens et al. (2000) *Br. Poult. Sci.*, 41:343-354; [5] Grotto et al. (2007) *J. Pharm. Biomed. Anal.* 43:619-624; [6] AOAC (1990) [7] AOAC (1995); [8] Callahan et al. (2016) *Nat. Methods.* 13:581-3; [9] Xu et al. (2016) *J. Clin. Endocrinol. Metab.* 101:12.

2C10. Comparative analysis of pancreatic amylase activity in laboratory animal species

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Introduction: Pancreatic amylase is the enzyme for starch digestion acting in small intestinal chyme. Carbohydrates are usually the main source of energy in laboratory animal diets for maintenance. Thus, we aimed to get an overview of the pancreatic amylase activity of three common lab animal species to compare their digestive capacity for carbohydrates. Knowledge about such basic functions of digestive physiology are important to be able to choose the most suitable species for animal experiments. **Animals, materials and methods:** Healthy laboratory animals which had not been in any other trial previously were used for the study (SPF, ethical approval): C57Bl/6J mice (8 wks, n=11), Lewis rats (9 wks, n=11), and RjHan:AURA hamsters (11 wks, n=23). All animals were fed the same commercial pelleted breeding diet for rats and mice routinely in the facility ad lib (tested batch fed ≥ 2 wks prior to analysis: 22.7% crd. protein, 5.1% crd. fat, 4.6% crd. fiber, 53.7% NfE, 37.6% starch as fed; degree of starch gelatinization 34.5%). The animals were sacrificed and dissected without fasting. Pancreas and chyme from the anterior part of the duodenum were removed for analysis of amylase activity by the Phadebas© test (Phadebas AB, Sweden). The results were compared between species via Kruskal-Wallis test followed by Dunn's Multiple Comparison test ($\alpha=0.05$; GraphPad Prism).

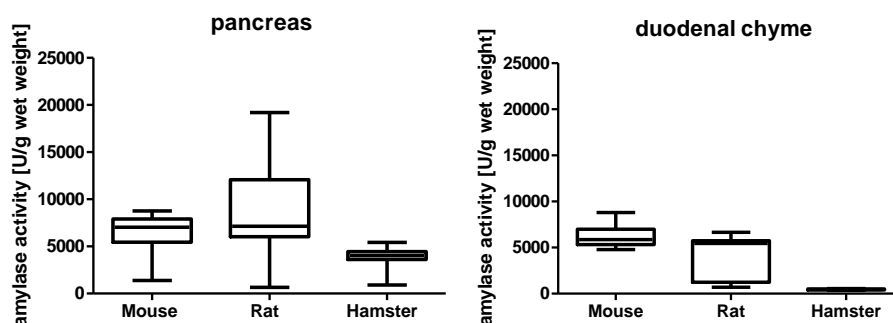


Fig- 1. Amylase activity analysed from pancreatic tissue (left) and duodenal chyme (right) in mice, rats and hamsters.

Results and discussion: Hamsters (3885 ± 964 U/g pancreatic wet weight) showed a significantly lower amylase activity in pancreatic tissue than rats (9167 ± 5680 U/g) and mice (6281 ± 2493 U/g; $p < 0.001$, Fig 1). In the duodenal chyme, the activity pattern was the same with hamsters having significantly lower values (496 ± 76 U/g duodenal chyme wet weight vs. 4027 ± 2367 U/g in rats and 6185 ± 159 U/g in mice).

Pancreatic amylase activity was higher in rats and mice compared to hamsters. In addition to the glandular part of the stomach, hamsters have a pronounced non-glandular forestomach as site of microbial fermentation. A higher degree of allo-enzymatic, microbial degradation of carbohydrates may reduce the need for pancreatic amylase of the host organism. Structural and functional similarities of the hamster forestomach and the rumen of cattle have been shown [1,2]. In mice and rats, the compartmentalisation of the stomach is less pronounced, resulting in less prae-duodenal microbial fermentation. Thus, they may need more amylase to utilize starch from carbohydrate-rich diets. All animals were young adult, so that the results are not likely influenced by age.

Conclusion: Differences in digestive physiology need to be considered for adequate diet formulation and in selecting a lab animal species for a specific experiment. Further investigations comparing different age groups might be valuable to gain insight into the development of enzymatic activity.

References: [1] Ehle & Warner 1978) J.Nutr.108(7),1047-1053; [2] Hoover et al.(1969) J.Anim. Sci.28,349-352.

2C11. Digestibility of crude nutrients and minerals in C57Bl/6J and CD1 mice fed a standard maintenance diet

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Introduction: Basic digestibility data in laboratory mice is scarce. The aim of the present study was to investigate the digestibility of crude nutrients and minerals in mice fed a standard diet. Since there are plenty of different genetic backgrounds and phenotypes, mice of the common CD1 outbred stock and C57Bl/6J inbred strain were used in order to identify potential differences in diet digestibility.

Animals, materials & methods: Ethical approval was obtained (ref. 264-13-04-2021). Male C57Bl/6J and CD1 mice (8-wk-old, n=18 each) were group-housed. They were fed a standard pelleted maintenance diet *ad libitum* (as fed: 20.7% crude protein, 5.5% crude fat, 6.1% crude fibre, 5.0% ash, 52.6% NfE, 0.77% Ca, 0.63% P, 0.20% Mg, 0.73% K, 0.26% Na). The animals had been fed this diet constantly, and for 7d, a digestibility trial with recording of the consumed amount and total faecal collection was conducted. Diet and faecal samples were analysed for gross energy (GE), crude nutrients and minerals with standard methods. For calculation of digestibility, 1 cage á 3 mice was defined as 1 unit. In addition, intake, faecal excretion and apparently digested amount (ADA) of the nutrients were expressed per kg body weight (BW). Lines were compared with Mann-Whitney test ($\alpha=0.05$).

Table 1. Apparent digestibility (%) of nutrients in two mouse lines (means \pm SD).

	C57Bl/6J	CD1	<i>p</i>
GE	80 \pm 0.96	81 \pm 2.1	0.987
Crude protein	82 \pm 1.1	81 \pm 2.1	< 0.05
Crude fat	91 \pm 0.88	92 \pm 1.4	< 0.05
Crude fibre	29 \pm 4.5	30 \pm 6.1	0.400
Crude ash	44 \pm 3.3	34 \pm 7.6	< 0.001
NfE	85 \pm 1.0	85 \pm 1.5	0.579
Ca	27 \pm 5.2	28 \pm 5.2	< 0.05
P	38 \pm 5.2	35 \pm 4.5	< 0.01
Na	63 \pm 4.7	70 \pm 5.8	< 0.01
K	86 \pm 1.2	82 \pm 2.6	< 0.001
Mg	48 \pm 2.7	36 \pm 4.5	< 0.001

Table 2. Regression equations of the nutrients with x=nutrient intake and y=apparently digested amt. of the nutrient.

nutrient	Slope	Intercept	R ²
	Units g/kg BW		
GE	0.91	-0.29	0.98
Crude protein	0.94	-4.05	0.99
Crude fat	0.91	0.08	0.98
Crude ash	0.99	-4.44	0.87
NfE	0.92	-5.78	0.99
	Units mg/kg BW		
Ca	0.65	-425	0.73
P	0.81	-417.45	0.88
K	0.94	-107.32	0.95
Mg	0.97	-164.78	0.84

Results and discussion: Gross energy digestibility did not differ significantly between the C57Bl/6J and CD1 mice. Apparent digestibility (aD) of protein, fat and ash as well as the minerals differed significantly between the mouse lines (Tab. 1). There were remarkably strong linear correlations between intake and ADA of GE, crude protein, crude fat, ash and NfE calculated with data from both mouse lines (Lucas-test [1]; Table 2). The estimated true digestibility (tD) of these nutrients (slope x 100) was >90% according to the regression equations. For crude fibre, the correlation was weaker and better described by an exponential model (R²=0.77). This may be due to the general lower digestibility of fibre and potential individual differences in the microbial fermentation thereof in the intestinal tract. In minerals, the findings were similar as in crude nutrients (Tab. 2). For Na, separate regression equations for CD1 and C57Bl/6J mice seemed a better fit than one for all data.

Conclusion: There seem to be differences in aD of some crude nutrients and minerals in C57Bl/6J and CD1 mice. However, the Lucas tests revealed uniformity over both genetics. The extremely strong linear correlation between intake and ADA

of the crude nutrients, except for fibre, imply a constantly high tD on a standard diet. The same was true for minerals, except Na, where a potential difference in tD between C57Bl/6J and CD1 mice might be worth investigating further.

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2C12. A prebiotic and postbiotic mixture can modulate the immune system and the gut microbiota of elderly mice

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Introduction: In recent years, scientific evidence highlighted the role of gut microbiota in aging [1]. This opens the field of using nutritional solutions to support elderly populations [2]. The objective of the experiment was to describe the effect of a prebiotic (short-chain fructooligosaccharides (scFOS)) and postbiotic (different fractions of *Saccharomyces*) mixture on the faecal microbiota and immune parameters of old healthy mice. The hypothesis was that the addition of the yeast postbiotics could balance the pro- and anti-inflammatory status due to inflammaging process in a stronger way than prebiotics alone.

Animals, materials and methods: Nine-week-old and eighteen-month-old female C57BL/6J mice were randomly assigned into one of the 4 experimental groups (n=12/group) depending on the diet and the age of animals: *i*) control 9 week-old (YOUNG), *ii*) control 18 month-old (OLD), *iii*) 18 month-old fed with the diet containing 10% prebiotic (OLD PRE); *iv*) 18 month-old fed with the diet containing a blend of prebiotic (10%) and yeast-based postbiotics (1%) (Profeed Advanced[®], OLD MIX, n=12) for 8 weeks. Blood and faecal samples were collected 3 times at the start, middle and end of the experiment. At the end of the experiment, the colon and peri-ovarian white adipose tissue were collected and stored at -80 °C. Blood biochemical parameters, cytokines (ELISA) and T-cell subsets were analysed as well as caecal and faecal microbiota composition using amplicon sequencing. Statistical differences between the groups have been assessed using a Kruskal-Wallis test followed by a Dunn's post-hoc test. Linear discriminant analysis (LDA) effect size (LEfSe) was used by default parameters to identify significant differences in taxonomy data. Significance was set as P<0.05.

Results and discussion: Body weight and peri-ovarian adipose tissue (%BW) were significantly lower in YOUNG than in other groups. Blood NEFA concentration was lower, while leptin concentration was higher in the OLD group vs YOUNG group (P<0.05). Blood concentrations of IL22 decreased while IL6:IL10 increased in OLD group vs YOUNG and increased or decreased in OLD MIX vs OLD group for IL22 and IL6:IL10, respectively. The concentration of acute phase proteins was higher in OLD and OLD PRE groups compared to the YOUNG. The Th17: Treg was significantly decreased in OLD MIX group vs OLD and OLD PRE. In the colon, the phospho mTOR:mTOR, p62 and LC3b decreased, while the phosphoULK1:ULK1 increased in OLD PRE group vs the 3 other groups. TLR2 and TLR5 decreased in OLD PRE and OLD MIX groups vs the OLD and YOUNG one; while the gene expression of TLR4 increased in OLD vs YOUNG groups and decreased in OLD PRE and OLD MIX groups vs the OLD group. Faecal microbiota was affected by the treatment: the supplementation with the mixture and prebiotic resulted in a decreased relative abundance of Proteobacteria and Firmicutes, and increased relative abundance of Bacteroidetes, *Bacteroides* and Bifidobacteriaceae.

Conclusion: Young and old animals exhibit different biochemical, immune and apoptosis parameters. A combo of prebiotic and postbiotic can trigger immune response and modulate faecal microbiota in a different way than the prebiotic used alone.

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Session 2D Clinical cases II

Chair: Niels Blee



2D1. A case of fatal poisoning with chinaberry tree berries in a dog

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Introduction: the chinaberry tree (*Melia azedarach* L.), also known as White Cedar, Persian, or Cape Lilac, is native to Persia, China, and India. Despite the toxicity of its leaves, bark, flowers, and drupes¹⁻³, it has been medicinally used to treat various human disorders⁴. The tree was recently introduced to France as an ornamental tree, growing widely in the southeast. It spreads quickly thanks to its seeds being dispersed by animals.

Materials and methods: in January 2023, one spayed female dog (6 years old, 24 kg BW, BCS 8/9) was found with acute vomiting and diarrhoea. The owner reported finding the dog near a tree and suspecting eating its fruits. The tree was identified as a chinaberry tree. On admission, she presented mild anemia (31.4% hematocrit), but amylase, lipase, creatinine, and urea were markedly elevated (amylase > 2500 U/L, normal <1400; lipase 315 U/L, normal [10-160]; creatinine 135.2 mg/L, normal [4-14]; urea > 3 g/L, normal [0.2-0.62]). The treatment consisted of aggressive infusion therapy and forced diuresis, which led to a slight improvement in the next three days. However, the renal parameters were not improved, and the dog's medical condition deteriorated rapidly (amylase > 1204 U/L; lipase 280 U/L; creatinine 114 mg/L; urea 2.63 g/L). She was euthanized six days later. The histopathological evaluation of the kidney confirmed an acute epithelial injury with severe hemosiderosis and abundant hyaline and granular casts usually seen on chronic interstitial nephritis and glomerulonephritis.

Results and discussion: Cases of poisoning with Chinaberry tree were recorded in cattle, sheep, goats, pigs, poultry, horses, rabbit, rats, guinea pigs, humans, and even in dogs where poisoning is potentially fatal⁵⁻⁷. Digestive disorders (nausea, vomiting, constipation, bloody diarrhoea) and acute nervous symptoms (excitement or depression) were the main symptoms. Several potentially toxic components were isolated. Among them, meliatoxins, toxic tetranortriterpenes of the limonoid class, appeared in high concentrations in fruits². No neurologic signs were observed in this dog, but she presented acute pancreatitis and kidney failure. Unfortunately, it was impossible to know the number of ingested berries the dog consumed and then estimate the toxic dose.

Conclusion: The rapid spreading of the Chinaberry tree in the southeast of France requires alerting dog owners about the risks associated with consuming the berries.

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2D2. Evaluation of two elimination diets using the canine atopic dermatitis extent and severity index (CADESI) in dogs with suspected food allergy – preliminary data

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Introduction: Adverse food reaction (AFR) manifesting as severe pruritic skin disorders are a common problem in veterinary practice. Diagnostic therapy includes an elimination diet, the aim of which is to exclude the intolerance-triggering factors from the food [1]. The overall objective of this study is to evaluate two elimination diets, an insect-based food and a conventional hydrolyzed diet, regarding their effects on the clinical symptoms of dogs with AFR. Our working hypothesis is that an insect-based elimination diet leads to clinical improvement in skin appearance comparable to a hydrolyzed diet in dogs.

Material and methods: Healthy, adult dogs suspicious for AFR were included. All other reasons for pruritus were ruled out and no previous treatment had been carried out. An elimination diet was performed over a period of 8 weeks. Patients were randomly assigned to a treatment based either on a hydrolyzed diet (consisting mainly of rice and hydrolyzed soy protein isolate) or on a diet with *Hermetia illucens* (consisting mainly of potatoes and insect meal). Patients were clinically evaluated at study entry and 2, 4 and 8 weeks after diet change using the CADESI-03 score [2]. Skin problems and their severity were further assessed by the owners using a questionnaire. Localization of lesions and itching were evaluated by owner and veterinarian. Bonferroni post-hoc test was used for comparison between groups.

Results: Twelve dogs were included between two and eleven years old. Two were female neutered, ten were male (four intact; six neutered). Breeds included were two Labrador Retrievers, one Magyar Vizsla, one Chowchow, one Miniature Poodle, one Patterdale Terrier, one Maltese and five crossbreeds. Since the age of the dogs varied considerably, also the time suffering from AFR varied from months to years. After eight weeks of elimination diet, all dogs had an improved severity scale score of the skin ($p < 0.001$). Dogs fed with insect based food showed the similar improvement between week 0 and 8 ($p = 0.036$) as dogs fed hydrolyzed diet ($p = 0.057$) (see Fig 1).

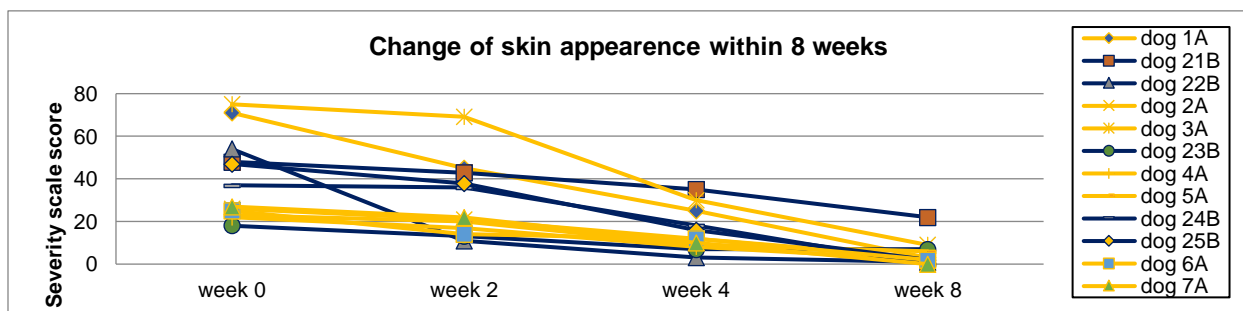


Fig. 1: Evaluation of 12 dogs according to CADESI within 8 weeks of elimination diet; feeding group A/ hydrolyzed diet (yellow), feedings group B/insect-based diet (blue)

The CADESI scores were in accordance with the owner surveys regarding frequency of itching and skin problems noticed.

Conclusion: Our preliminary results suggest that insect-based foods can be used as an alternative to hydrolyzed diets as an elimination diet in dogs suspected for AFR. More participants need to be examined for a statistical validation of the results.

References: [1] Hall EJ (2002) Prakt. Tierarzt, 83: 30-36; [2] Olivry et al. (2007) Vet Dermatol. 18:78-86

2D3. Case report: Hypocobalaminemia and coat discoloration in a dog

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Introduction: For absorption of cobalamin, a complex process is in place. Firstly, cobalamin is separated from dietary protein by gastric pepsin and bound to haptocorrin for protection from bacterial fermentation. Secondly, cobalamin is separated by pancreatic proteases in the small intestine and forms a complex with intrinsic factor (IF), in dogs primarily produced in the pancreas. Third, the complex is absorbed in the distal ileum. Due to the essential role of the exocrine pancreas and the limited absorption area, hypocobalaminemia can be initiated by gastrointestinal disease, specifically by exocrine pancreatic insufficiency and severe chronic ileal disease. Cobalamin is essential for myelinogenesis, haematopoiesis, and DNA synthesis via methionine synthase and methylmalonyl CoA mutase. Consequently, cobalamin deficiency can result in signs of vomiting and diarrhoea. Due to this vicious circle, veterinarians should be alert to the possibility of vitamin B12 deficiency in case of chronic diarrhoea, both primary and secondary [1].

Animal: A 10 year-old male intact Frisian pointing dog (18.9 kg; BCS 4/9) was presented to the primary care veterinarian with acute small bowel diarrhoea. Other signs included: vomiting, reduced appetite and lethargy.

Materials and methods: First treatment by the primary care veterinarian was symptomatic. Six months after first presentation, the gastrointestinal problems worsened: daily vomiting and diarrhoea. Additionally, a clear discolouration of the coat appeared (see picture 1A). For further examination, extensive blood analysis was performed, apart from symptomatic treatment. Blood level of both cobalamin (145 pmol/L; reference 173-599) and folic acid (18.5 nmol/L; reference 21.1-54.0) were reduced. Consistent with hypocobalaminemia, a typical nonregenerative anaemia was found.

Result and discussion: The dog was treated weekly with 1 mg vitamin B12 injections (6 weeks) and supplementation per os (3 months). Despite reduced folate levels, which can contribute to the same symptoms as hypocobalaminemia, folate was not supplemented. The treatment resolved the diarrhoea and coat discoloration (see picture 1B). To the authors' knowledge, the coat discoloration as a result of hypocobalaminemia has not yet been described in dogs.

In humans, reversible hyperpigmentation of the skin and depigmentation of the hair has been reported in some

cases of vitamin B12 deficiency. Histopathologically, melanin was increased in the basal layer of the epidermis in these patients. However, the underlying mechanism remain unknown. [2].



Picture 1A. Before treatment



Picture 1B. After treatment 6 months after start of treatment

References: [1] Kather S, Grützner N, Kook PH, Dengler F, Heilmann RM. Review of cobalamin status and disorders of cobalamin metabolism in dogs. *J Vet Intern Med.* 2020 Jan;34(1):13-28. doi: 10.1111/jvim.15638. Epub 2019 Nov 23. PMID: 31758868; PMCID: PMC6979111.

[2] Mori K, Ando I, Kukita A. Generalized hyperpigmentation of the skin due to vitamin B12 deficiency. *J Dermatol.* 2001 May;28(5):282-5. doi: 10.1111/j.1346-8138.2001.tb00134.x. PMID: 11436369.

2D4. Nutritional Consultation for a growing Australian Kelpie with hereditary Intestinal Lipid Malabsorption – a Case Report

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Introduction: Intestinal lipid malabsorption (ILM) in Australian Kelpies is an autosomal recessively inherited condition, caused by a deletion of the ACSL5 (Acyl-CoA synthetase long chain family member 5) gene [1]. ACSL5 plays an important role for lipid metabolism and fat deposition in carnivores [2; 3]. Australian Kelpies with ILM show diarrhoea and steatorrhea from early age onwards and are smaller than their unaffected littermates [1].

Animals, materials and methods: A 12-weeks-old male, intact Australian Kelpie was referred to the Institute of Animal Nutrition and Dietetics of the Vetsuisse Faculty, University of Zurich after being identified as a double carrier for ILM. At the timepoint of referral, the dog's body weight (BW) was 4.0 kg which was 1.3 kg below the ideal growth curve for the expected adult weight of 12.5 kg. The dog had soft faeces and was supplemented with a pancreatic enzyme supplement (Lypex®). The nutritional contents of the patient's diet and the ideal growth curve were calculated with DietCheck Munich ©2005 Version 3.0. Diet A (fed prior to referral) consisted of a commercial dry food and treats. The goal was to formulate a highly digestible diet (diet B) with a low fat content while covering the recommendations for linoleic acid (LA), alpha-linolenic acid (ALA), eicosapentaenoic and docosahexaenoic acid (EPA + DHA) for a growing dog [4].

Results and discussion: Diet A met the recommendations for protein, calcium and phosphorous at the time of referral, however, did not meet the criteria of a fat-reduced diet (20% on a dry matter (DM) basis or 11.5g fat/MJ metabolic energy (ME)). Except for zinc, no nutritional additives with a legal maximum were listed on the label of the dry food, therefore it is highly questionable whether the dry food is a complete diet. The manufacturer did not provide further analytical data. Diet B was a home-made diet prescribed to support the nutritional requirements of a growing dog with impaired fat absorption and was adapted according to the different growth phases. The main ingredients according to their contribution to the ME requirement were horse meat, rice, dried deer meat (treat) and corn germ oil, as well as carrots, fennel and a fishoil-based EPA/DHA supplement. Minerals and vitamins were supplemented with bone meal, a commercial mineral vitamin supplement and salt. The initial ME intake with diet B was 2.9 MJ ME up to 14 weeks of age and matched the daily ME intake with diet A (88% of the recommended ME intake according to DietCheck). Diet B included 10% fat on DM basis (5.2g fat/MJ ME) and the recommendations for LA, ALA; EPA+DHA [4] were met. As the dog did not gain weight according to the growth curve, ME content of the diet was increased in week 16 (98% of the recommended ME intake according to DietCheck). In week 20 the BW was slightly below the growth curve, however, the owner only carried out the offered ME reduction in week 26, resulting in BW exceeding the growth curve. At the age of 8 months, pancreatic enzymes were discontinued, which resulted in decreased faecal quality. A test for *Giardia spp.* was positive at the same time complicating interpretation of enzyme discontinuation. Pancreatic enzymes were added again and are still supplemented up to adult age (last follow up at the age of 21 months).

Conclusion: To our knowledge this is the first case report highlighting the nutritional management of an Australian Kelpie with ILM. The patient reached his growth potential while fed a low-fat homemade diet covering the nutritional recommendations for growing dogs and being supplemented with pancreatic enzymes. However, it remains to be determined if pancreatic enzyme supplementation is generally necessary in adulthood.

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2D5. Case report: Nutritional management of an overweight dog with Myasthenia Gravis

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Introduction: Acquired Myasthenia Gravis is an autoimmune disease of the neuromuscular system. The pathology can present itself in different forms, including a local form that affects the oesophagus and oropharynx with mainly symptoms as dysphagia, regurgitation, vomiting, and megaesophagus [1, 2].

Case history: A 3-year-old male Golden Retriever, body weight (BW) 45 kg, BCS 7/9 was diagnosed with Myasthenia Gravis and concurrent hypothyroidism after an episode of severe muscle weakness. Since the age of 11 months, he suffered from chronic vomiting and hypersalivation. A barium radiography revealed mild stasis in the cranial oesophagus with absence of megaesophagus. The dog was fed 350 g of a complete dry petfood for adult dogs (Crude protein 25%, Crude fat 16%, Crude fibre 2.6%, Crude ash 7.8%), 250 g of pork meat and an unquantifiable quantity of cooked potatoes, administered every time the dog had nausea. The estimated daily ME intake was around 7.53MJ.

The dog was referred to the veterinary nutritionist because several months after the diagnosis and the beginning of pharmacological therapy (pyridostigmine and levothyroxine, domperidone, pantoprazole), he continued to show nausea, reflux and chronic vomiting.

The aim of the nutritional therapy was to reduce the symptoms caused by Myasthenia Gravis and to reach the ideal BW.

Nutritional therapy: The energy requirement was calculated to achieve a 20% BW loss, and was 30% lower than the actual intake.

The dry food already used was maintained, but the amount was reduced to 200 g; 400 g of a complete wet petfood (Crude protein 10.1%, Crude fat 6.4%, Crude fiber 0.4%, Crude ash 2.4%, Moisture 79%) and 8 g of sunflower oil were added. Main features of the diet are shown in table 1.

Table 1. Main features of the new diet

Features	New diet
ME content	5.2 MJ ME
Energy density	0.008 MJ ME/g as is
Moisture	55%
Crude protein	17.3 g/MJ
Crude fat	12.57 g/MJ
Crude fiber	1.29 g/MJ

The food was prepared to obtain a slurry: the kibbles were soaked in hot water for 2-3 hours, then partially drained and mixed with the wet food and the oil and administered divided into 6 meals per day, one spoon at a time, to the dog in upright position, thanks to the use of a customized step stool. After 2 months the owner tried to eliminate the use of the step but after a few days the dog started to show nausea, which stopped again after the step's reintroduction. After 9 months since the diet change the dog lost 10% of the BW (40 kg, BCS 6/9) and has not been vomiting in the last 7 months.

Conclusion: Strategies typically used for nutritional therapy of megaesophagus from various causes, such as meal in the form of a slurry, reduced speed of intake and use of the step, can also be useful in early stages of Myasthenia Gravis.

References: [1] Sang-Gwan et al. (2005) J Vet Clin 22(4):392-395; [2] Forgash et al. (2021) J Vet Intern Med 35:2315-2326.

2D6. Dietary intervention in the management of Corpus Callosum dysgenesis in a young English bulldog

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Introduction: Sodium (Na) is the third most abundant body cation, playing a major role in the osmotic pressure regulation. Dietary Na absorption is quite high, especially in canine colon [1]. Serum Na concentration ([Na]) is strictly regulated by the antidiuretic hormone (promotes renal water reclamation), aldosterone (promotes renal sodium reabsorption), influencing also the water intake, driven by thirst [2]. True hypernatremia always results in a hyperosmolar/hypertonic condition, indicating a water deficit [3]. Among the main causes of hypernatremia, salt toxicity, water loss, pseudohypernatremia (hypoproteinemia), hypodipsia and endocrine dysfunction (hyperaldosteronism and hyperadrenocorticism) could be enumerated.

Animals, materials and methods: A 8 months old female English Bulldog puppy was referred to the clinical nutrition service for hypernatremia, likely due to reported adipsia. A magnetic resonance imaging performed because of the neurological symptoms (obtundation, paresis) revealed the Corpus Callosum dysgenesis. After the stabilisation of the hypernatremia (patient range 159–193 mmol/L; ref. range: 144-150 mmol/L), the diet and water requirement have been evaluated. At the physical examination the puppy weighed 12.2 kg and showed a BCS 5/9, but appeared smaller than siblings. She was receiving a commercial dry diet (Na: 1.04 g/Mcal), previously selected by the owner.

Results and discussion: In order to stabilise the serum [Na], water needs have been calculated in terms of energy intake and amount of water derived from pet food. Due to the lack of interest in spontaneous water intake, 1400 ml of fresh water, calculated on the basis energy requirement estimated on the ideal adult body weight, were offered in several occasions during the day, in order to avoid a rapid drop in [Na]. A mixed dry and wet diet solution has been proposed to the owner to guarantee a better hydration using a reduced volume of free water. In particular, the substitution of extruded pet food was based on the lowest [Na] (0.56 g /Mcal), able to satisfy the puppy requirement and to avoid clinical symptoms. In the following weeks the [Na] was regularly checked (Table 1).

Table 1: Follow up serum sodium concentration.

Post Discharge	Na (mmol/L)
7 days	158
18 days	171
27 days (6 days after the introduction of the new diet)	149
2 months	155
8 months	154

In literature several cases of absence or dysgenesis of the Corpus Callosum in canine breeds have been reported [4,5,6], but the usual clinical approach was only based on water intake, whereas dietary approach has been never considered in maintaining [Na] within normal limits (<155 mmol/L). The choice of the appropriate diet could be helpful to maintain the correct water balance, reducing the risks connected to the administration of large volume of free water. However, further investigations are need in order to confirm the role of diet.

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2D7. Case-control study of the prevalence and etiology of ataxia in asiatic lions (*Panthera leo persica*) between 2002 and 2020

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Introduction: The Asiatic lion (*Panthera leo persica*) is an endangered species, with a slowly increasing captive and wild population. Several zoos from within the EAZA (European Association of Zoos and Aquaria) Ex situ Programme (EEP) reported an increased number of Asiatic lions with neurological signs such as (progressive) ataxia, abnormal gait and stargazing. The aim of this study was to evaluate the prevalence and etiology of these clinical signs within the EEP Asiatic lion population.

Animals, materials and methods: The dietary and medical history of 74 Asiatic lions (36 healthy and 38 affected) was retrieved from the EEP records and reviewed for blood work (biochemical, hematological and retinol), diagnostic imaging (MRI and CT-scans) and post-mortem pathology examinations. The data of the affected lions was compared with those of healthy lions. Statistical analyses were performed in R. Pearson's Chi-Square test of Independence with Yates' continuity correction was performed to assess the relationship between ataxia presence and vitamin A status.

Results and discussion: The prevalence of ataxia in the EEP population was considered estimated to be 13-36% and stiffness (9/38) and lameness (7/38) were the most described symptoms coinciding with ataxia. Diets varied between zoos but consisted of prey feeding and/or processed meat. 34/38 of the affected lions, and 23/36 of the non-affected lions did receive supplements (i.e. Calfosonic, Carmix, Carnizoo, or Mazuri), however the amounts were not always specified. Specific amounts or in depth nutritional details were not well documented. The lack of supplementation was reflected by the plasma retinol levels, which were lower in affected lions (95% CI = 0.26-0.52 $\mu\text{mol/L}$ (n=14)) compared to controls (0.59-0.81 $\mu\text{mol/L}$ (n=21)), p=0.009. Blood results showed lower albumin and higher monocyte counts in affected lions. Diagnostic imaging and necropsy revealed caudal fossa hyperostosis with in some occasions cerebellar herniation, and calvarial hyperostosis syndrome, all of which could be caused by vitamin A deficiency [1]. Leucomyelopathy, syringomyelia (in one case combined with cerebellar herniation), occipital abnormalities of the calvarium and incidental mineralizations of the dura mater were also found, which were thought to have a congenital/hereditary component. Most likely this condition is either multifactorial or has multiple different causes. Vitamin A supplementation should be given as soon as clinical signs are present to treat the lions that are possibly affected by vitamin A deficiency. Nutritional management of lions in zoos can and should be optimized. Future research should focus on whole genome sequencing and genetic analyses to distinguish if a genetic defect (giving neurological symptoms) is present in the population. These genetic studies as well as a follow up on vitamin A intake versus requirements and measurement of other metabolites of vitamin A are currently conducted by our team.

References: [1] O'Sullivan B, Mayo F, Hartley W. Neurologic lesions in young captive lions associated with vitamin a deficiency. Australian Veterinary Journal. 1977;53(4):187-189.

Plenary session Friday
Chair: Ronald Jan Corbee



Speaker: Dr. Javier García Alonso

Dr. Javier García Alonso is Professor at the Technical University of Madrid. His research lines are focused on rabbit nutrition: feed evaluation (mainly fibrous ingredients), nutritional requirements, gut health, additives evaluation, and improvement of feed efficiency. He participated in 21 national projects, 22 contracts with private companies and supervised 6 PhD Thesis. He published 53 SCI papers, presented 140 conferences/communications to research congress and technical meetings. He was President of the Spanish Association of Rabbit Science (2012-2016), editor of the nutrition section of the journal 'World Rabbit Science' (2007-2016), and he is the Secretary of the Fundación Española para el Desarrollo de la Nutrición Animal (FEDNA).



El Dr. Javier García Alonso es profesor de la Escuela Técnica Superior de Ingeniería Agronómica Alimentaria y de Biosistemas de la Universidad Politécnica de Madrid. Sus líneas de investigación se centran en la nutrición del conejo: evaluación nutritiva de alimentos (mayoritariamente ricos en fibra), necesidades nutricionales, salud intestinal, evaluación de aditivos y mejora de la eficiencia alimenticia. Ha participado en 21 proyectos nacionales, 22 contratos con empresas privadas y dirigido 6 tesis doctorales. Ha publicado 50 artículos SCI, presentado 140 conferencias / comunicaciones a congresos de investigación y reuniones técnicas. Fue Presidente de la Asociación Española de Cunicultura (2012-2016), editor de la sección de nutrición de la revista 'World Rabbit Science' (2007-2016), y es Secretario de la Fundación Española para el Desarrollo de la Nutrición. Animal (FEDNA).

Understanding pet rabbit nutrition and feeding

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Introduction: The number of pet rabbits is increasing every year, as well as the training and knowledge of their keepers. However, there is still a significant proportion of animals whose welfare is compromised due to imbalances in feeding, housing and/or social contact. It may explain why the average lifespan of pet rabbits is 4.5 years when they could live up to 13 years [1,2]. Rabbits are non-ruminant herbivores with a complex digestive physiology, independently if they are wild, farm or pet rabbits, which makes their diet very different from other pets, and usually unfamiliar to new keepers. There is scarce scientific data regarding the feeding of pet rabbits in the long term. The nutritional standards used in the formulation of diets for pet rabbits derives directly from those developed for rabbits for meat production [3,4,5]. However, there are great differences among these two groups, both in their nutritional requirements and the way to manage the diet. The meat rabbits are bred in rather standard conditions, and are fed and managed as a group, whereas pet rabbits live in heterogeneous environments depending on the keeper and are managed individually. In this paper we will do an approach to better understand how can be fed pet rabbits to enhance their welfare status.

Digestive physiology: The rabbit is a small herbivore with high nutritional requirements per unit live weight. Therefore, this species has had to improve the efficiency of using very fibrous diets to meet its nutritional requirements and has developed two strategies. The first one was to increase its intake capacity (> 85 g/kg body weight, BW), and the second one was the recycling of caecal microbial protein through the caecotrophy. The high intake level was made possible by the large capacity of the stomach and the short mean retention time of the digesta through the digestive tract, especially in the hindgut. Digestion in the stomach and small intestine is like that of other non-ruminants. Then, the ileal digesta come into the caecum and when it leaves the caecum it undergoes a selection process at the ileo-colic-caecal junction. Small particles (< 0.1 mm) and both soluble and endogenous substances are pushed back into the cecum by antiperistaltic movements of the proximal colon and peristaltic movements of the cecum. Large particles (> 0.3 mm), are not retained any longer in the cecum, continues to the colon and rectum and are excreted as hard faeces [6,7]. The secretion of water in the proximal colon combined with the antiperistaltic movements prevents that soluble and fine particles from being lost in the hard faeces. This mechanism allows the rabbit to retain the most fermentable and least voluminous part of the digestive contents to be used as a substrate for caecal microbial growth and fermentation, considering the limited time available for fermentation in the caecum and proximal colon (10 h). In order to utilise the soluble and fine digesta, the rabbit has a highly developed caecum where bacteria ferment almost all endogenous substances as well as part of the soluble and insoluble fibre, producing microbial protein and volatile fatty acids. Once the feeding period finished (before sunrise or light is switched on) the caecal contents came out from the caecum, are packed into small spheres covered by a mucus film secreted in the colon which form the soft faeces or caecotrophs. These are ingested by the animal directly from the anus, passing without being chewed into the stomach, where the digestion of the microbial protein begins.

The rabbit follows a circadian rhythm in the intake of soft faeces and excretion of hard faeces. During the darkness period carries out most of its feed and water intake and hard faeces excretion, while soft faeces intake (caecotrophy) is carried out in the morning during daylight hours (a period which in natural conditions would remain in the burrow). In nature, this strategy allows it to enrich its fibrous and low protein (of poor quality) diet with microbial protein that has a high proportion of essential amino acids, as well as B vitamins.

Nutritional requirements: Pet rabbits have mainly maintenance requirements, except the first 3-6 months of age, where they are still growing, or in the case of pregnant and/or lactating females. The daily digestible energy (DE) requirement for maintenance in the thermoneutral zone is 0.430 MJ DE/kg BW^{0.75}·d, but it may increase depending on the activity/exercise, and on the environmental temperature, which are closely influenced by the type of housing. Some energetic requirements are expressed in metabolizable energy, that also considers the urinary energetic losses. However, there are really few studies using this unit to evaluate either requirements or energy value of ingredients, while most of them used DE, that nowadays is a more accurate one for rabbits. The minimal dietary DE concentration that allows a chemostatic regulation of feed intake is ≥ 9 MJ DE/kg, and below it a physical-type regulation dominates and factors like diet presentation (pellet vs. roughage) or fibrous particle size are relevant. Dietary DE concentrations around this value allow rabbits to maximize feed intake. However, if the pet rabbit is fed *ad libitum*, a lower DE value (higher fibre content) might be used as long as feed intake be enough to meet DE requirements, as it may help to extend the feeding time, produce satiety, and reduce abnormal behaviour, improving welfare [8,9,10]. The daily digestible protein (DP) requirement for maintenance is 2.9 g DP/kg BW^{0.75}·d [11], suggesting a ratio DP/DE for maintenance of 6.7 g DP/MJ DE, which is much lower than the one recommended for growing rabbits or breeding does: 10.2 and 12.0 g DP/MJ DE, respectively [5]. The amino acid requirements for growth and breeding does are defined for lysine, sulphur amino acids and threonine. There is no data for maintenance, but the recommendations of FEDIAF [3].

One of the essential components of the diet of an herbivore animal is the insoluble fibre that in rabbits has an essential role on caecal physiology, daily feed intake and rate of passage, and its content is inversely correlated to DE. It is important the unit used to measure insoluble fibre and those derived from the Van Soest's scheme are preferred: neutral detergent fibre, NDF, acid detergent fibre, ADF, and lignin detergent fibre, ADL, instead of crude fibre like in other herbivores [12,13]. The minimal dietary insoluble fibre content required by growing rabbits is 30% NDF, but the insoluble fibre content of diets for pet rabbits should be higher, and more similar to the diets recommended for rearing does (> 40% NDF) [14]. It is also important the fibre particle size [15,16,17,18], as large fibrous particles (> 1.25 mm) may delay the stomach emptying, while short fibrous particles (< 0.3 mm) accumulate in the caecum, and in excess might lead to a caecal dysbiosis. In both cases feed intake might be limited, although it might be not negative for rabbits at maintenance. However, it is difficult to produce a feed with a good pellet quality using a high proportion of large fibre, whereas fibrous sources with short particle size are not common. It is not usual to control the dietary particle size, and even less common to measure the fibrous particle size, that is the one that influences the digestive physiology, but its determination is time-consuming [18], and because it is not a problem when using the most frequent ingredients. In practice, the most usual is to include a minimal ADL level of 4% to warrant the adequate caecal function [19,20].

The use of a low lignified diet (including a low proportion of insoluble fibre, or a high proportion

low lignified fibrous sources like sugar beet pulp or soybean hulls with a higher water holding capacity) might lead to the accumulation of the digesta in the caecum which favours the digestive troubles. The combination of fibrous sources with different particle sizes and lignin contents might favour an adequate peristalsis and caecal filling that may help to produce a satiety feeling.

The mineral and vitamin requirements are reasonably well defined for growing rabbits [21], although there is no differentiation between requirements for maintenance and production. The requirements of the two major macrominerals, calcium and phosphorus are 0.5-0.6 g Ca/MJ DE and 0.4 g P/MJ DE, maintaining a Ca/P ratio around 1.5-2 [3,5]. The micromineral/vitamins of the ingredients are not usually analysed, and all the requirements are met with the mineral vitamin premix included at 0.2-0.5% in the pelleted diet. Dietary vitamin D in adult rabbits should not be higher than 800 IU/kg to avoid calcification of heart valve and kidneys [22].

Type of diet and diet presentation: Growing rabbits in farms are fed 2-3 different pelleted diets that help to supply all the essential nutrients required, and that are adapted to the nutritional requirements at each age. In contrast, pet rabbits are rarely fed only a pelleted diet, which would help to supply better their nutritional requirements, but a mixture of pellets, grains (processed or not as muesli, biscuits, nuggets, croquettes...), vegetables, and forages (pelleted, hay). These types of mixtures favour rabbit selection, always towards the more energetic/proteinic part of the diet at the expense of the most fibrous fraction (pellets, forage), resulting in the intake of an unbalanced diet. The main problems related to an unbalanced diet are usually **i)** a low fibre intake that might derive in severe digestive troubles, reduction of water intake (that may favour urolithiasis), hair intake, less chewing and incisors attrition leading to dental disease, **ii)** the insufficient intake of mineral and vitamins (that usually are included in the pelleted feed) which might favour the dental disease, **iii)** the intake of a reversed calcium to phosphorus ratio which might favour the metabolic bone disease, **iv)** an excess of sugars/starch intake that might lead to obesity [23,24,25,26,27,28]. For these reasons it is difficult to supply a balanced diet when concentrates/grains can be eaten separately. The use of a single pelleted diet, containing all the nutrients required, combined with a roughage seems to be the safer option to assure a correct pet rabbit nutrition.

The diet presentation has other implications. The feeding of alfalfa pellets instead of chopped (with much larger particle size and then bulkier) reduced the time dedicated to feeding (by 57%) and those rabbits used more intensively the gnawing stick available [29]. It indicates that fibrous and bulky diet may lead to extend the feeding time and seemed to be even better than the provision of a tool/toy to avoid stereotyped behaviours [30]. Pellet quality (diameter: 3-4 mm, length: 8-10 mm, hardness: > 8 kg, and durability: > 98%) is important to be palatable for the rabbits (e.g. avoiding fines: < 2%, during transport, storage, and feeding) [31,32].

Ingredient/diet processing: The use of processed ingredients is interesting to improve the starch/protein ileal digestibility (by starch gelatinization, breakdown of endosperm cells, destruction of antinutritive factors) that increase the nutritive value and reduce the ileal flow of starch and protein, minimizing the risk of digestive diseases after weaning [33,34], that finally reduces total the faecal output. However, when feeding adult rabbits there is not so interest in the use of such refined ingredients to prevent digestive diseases, and the reduction of the number of faecal pellets is not so an important issue considering they do not produce the problems of other pet's faeces (they are quite dry and almost odourless). Besides, these types of ingredients are specially desired by rabbits, that are a fruit and foliage selectors adapted in nature to look for the scarce concentrates available [35] and lead them to eat an unbalanced diet. The election of the type of forage depends on the age of the rabbit. For growing rabbits, alfalfa hay is better than grass hay, due to its higher protein and calcium content, but grass hay would be usually the one selected for adult rabbits at maintenance because of their lower calcium requirements [36].

Other considerations: Rabbits have an ingestion of hair during grooming that is retained and could be accumulated in the stomach and cause obstructions in the small intestine, especially in long-haired rabbit breeds (like Angora rabbits). To avoid the formation of hair balls (trichobezoar) it is recommended fasting one day a week (maintaining the forage supply), to let the rabbit expel it from the stomach to the small intestine [27,37]. The supply of low fibrous diets may also increase the hair intake, although this effect was not properly measured (unpublished results).

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Session 3A Companion animals I

Chair: Tomás Magalhães

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3A1. Bioavailability of collagen hydrolysates in healthy dogs after single and prolonged intake

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Introduction: Although there is a known beneficial effect of some nutraceuticals on osteoarthritis (OA) symptoms, the reported effects of collagen hydrolysates (CH) on OA pain and patient mobility vary between studies [1,2]. The bioavailability of CH in dogs is unknown and most used CH doses were extrapolated from studies in people. This study aimed to 1) determine the effects of a single dose of CH on concentrations of a representative marker, hydroxyproline (Hyp), and to 2) determine the effects of repetitive (7 consecutive days) CH intake on Hyp baseline and peak concentrations.

Animals, materials and methods: Three healthy Beagle dogs received CH supplementation (Hydro-P Premium, Darling Ingredients) in their diet at incremental doses (0, 250, 500, 750 mg/kg). The supplement was provided as unique administration with a 3 day-washout period between doses. Plasma free Hyp levels were measured at 0, 30, 60 and 90 minutes after CH intake using liquid chromatography/mass-spectrometry, and analyzed using Friedman tests. To investigate the bioavailability of long-term CH intake, eight healthy Beagle dogs were supplemented with CH at different doses (0, 250, 500, 750 mg/kg) for 7 consecutive days in a randomized order, with a 7 day-washout period between doses. Plasma Hyp levels were measured at 0 (T0, baseline) and at peak concentration (resulting from the previous trial) and analyzed using a mixed model.

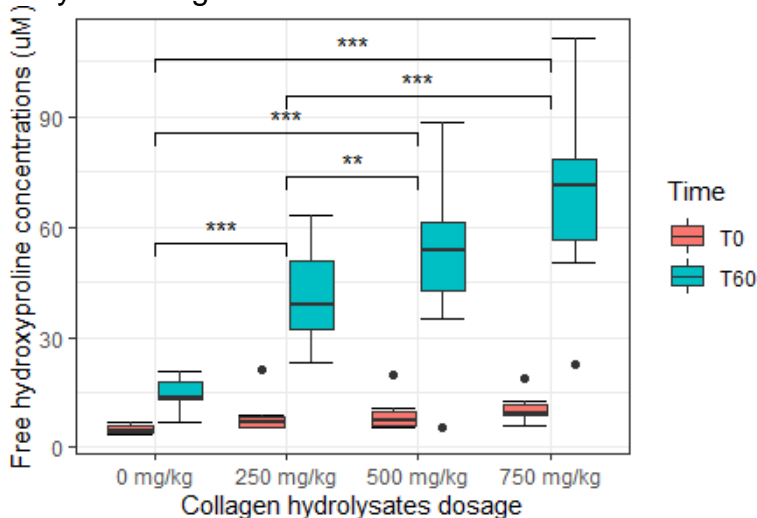


Figure 1. Plasma free Hyp concentrations at baseline (T0) and 60 minutes (T60) after prolonged intake of incremental CH doses

Results and discussion: Hyp peak concentrations occurred 60 minutes after single CH intake for each dose compared to baseline ($P=0.03$ for 250 mg/kg, $P<0.01$ for 500 mg/kg, and $P=0.01$ for 750 mg/kg). At this timepoint, Hyp concentrations of 500 mg/kg and 750 mg/kg CH intake, were significantly higher than the control diet (no CH supplementation) ($P=0.027$ and $P=0.011$, respectively). Repetitive intake of all CH doses resulted in significantly higher free Hyp concentrations than control diet ($P<0.001$ for 250, 500 and 750 mg/kg) at T60 (Figure 1).

Furthermore, at T60 Hyp concentrations of 500 and 750 mg/kg CH were significantly higher than those of 250 mg/kg CH ($P=0.045$ and $P=0.005$), but did not significantly differ between them ($P=0.2496$). Whether these plasma Hyp concentrations, and the large interindividual differences, correspond to clinical efficacy is currently unknown and needs to be elucidated in further studies.

Conclusion: Administration of a single intake of CH causes a peak Hyp concentration at T60 at any tested doses. Further, incremental doses of CH enhances their absorption in dogs after prolonged intake; however, the intake of 750 mg/kg CH did not result in significantly higher Hyp concentrations than the intake of 500 mg/kg CH.

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3A2. Impact of dietary supplement combinations on glycemic control in overweight colony beagle dogs

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Introduction: Chronic canine obesity leads to a reduction in lifespan, and metabolic disturbances such as lower insulin sensitivity and development of oxidative and inflammatory stress [1]. In dogs, short-term gain of up to 20% excess weight results in modification of metabolic parameters, including a trend to insulin resistance [2], while loss of ~20% excess weight is generally accompanied by improved insulin sensitivity [3]. Even if some overweight dogs do not achieve ideal body condition, improvements in metabolic parameters could still be envisaged. Some dietary supplements have proved beneficial in this regard: white bean extract (WBE) to reduce glucose absorption in dogs undergoing weight loss [4]; Nutriose[®] to improve insulin sensitivity in humans [5]; curcumin to improve the obesity-related inflammatory state in obese cats [6]. The aim of this study was to examine the effects of supplementation with active ingredients on insulin sensitivity in colony dogs maintained in an overweight state.

Animals, materials and methods: Thirty overweight, but otherwise healthy (notably no glucose metabolism disturbances), male and female neutered beagle dogs (5.5±0.2 years (mean±SEM), BW 16.5±1.7 kg, all BCS 7 (on 9-point scale) were fed a high-fat diet (30% fat, 32% protein, 20% carbohydrate; 19707 kJ/kg (4710 kcal/kg) NRC06 as fed [7]; Endurance 4800[®]; Royal Canin, France) [n=10; control] supplemented with either 0.2% WBE+0.015% Curcuma longa [n=10; WBE-C] or 1% Nutriose[®] +0.015% Curcuma longa [n=10; N-C]. Energy allocation was modified to maintain dogs at initial BW±10%. A glucose challenge test was performed in 24h-unfed dogs, before and at the end of 12 weeks' intervention. Blood samples were taken, before and 15 and 30 min, then every 30 min until 3 h, after oral administration of glucose (3 g/kg BW^{0.75}). Blood glucose concentration was assayed using AlphaTRAK; insulin was assayed using a radioimmunoassay. Positive incremental glucose and insulin AUC were calculated. One-way ANOVA was used to analyze changes from baseline between groups for normally distributed data, otherwise, the Kruskal-Wallis test was used. Dunn's multiple comparison test was used for post-hoc analyses. Statistical analyses were completed using Graphpad Prism 8.3.1[®] (San Diego, CA).

Results and discussion: A trend toward a higher basal glucose concentration was observed in supplemented groups compared to the control group (Control vs WBE-C p=0.09; Control vs N-C p=0.06). No differences were found for basal insulin (p>0.5 for both). The changes from baseline were -6%, +29% and -6% for glycemic AUC (overall p=0.28), and +5%, +68% and +37%, for insulinemic AUC for the control, WBE-C and N-C groups, respectively (overall p=0.26) (Fig. 1). Possible limitations of the study are the relatively short supplementation period of 12 weeks and the homogenous population studied.

Conclusion: Unexpectedly, the dietary supplements increased either glucose and/or insulin AUC and did not appear beneficial for glycemic control in overweight dogs.

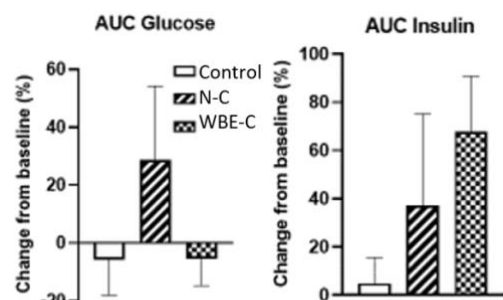


Figure 1. Change from baseline of glycemic and insulinemic AUC. Data: mean ± SEM.

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3A3. Effects of two diets on the clinical assessment and lipid profile of diabetic dogs on insulin therapy

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Introduction: Next to adequate insulin therapy, consistent dietary support is the mainstay of canine diabetes mellitus (DM) management [1]. This randomized cross-over study aimed to compare the effects of a commercial diet (Monge Vetsolution Diabetic, CD) and a home-cooked diet (HCD) in terms of the clinical assessment and lipid profile of client-owned dogs with DM on insulin therapy with good glycemic control.

Animals, materials and methods: Ten dogs with DM treated with daily insulin injections (4 porcine lente, 6 glargine 300U/ml) were prospectively enrolled. At the time of inclusion, a flash glucose monitoring system (GMS) was placed and dogs were randomly assigned to receive either the CD (chicken, tapioca, potato and pea, on dry matter basis: crude protein (CP) 33%, ether extract (EE) 12%, starch 25%, crude fiber (CF) 6.6%, dietary fiber (TDF) 18%, ash 9%) or the HCD (on dry matter basis, chicken, barley, pea and potato, CP 35%, EE 15%, starch 16%, CF 2.4%, TDF 8.6%, ash 5%) in a 2x6-week period (CD-HCD/HCD-CD, 4days transition). The daily energy requirement was calculated according to the current needs, to avoid any body weight (BW) change. Dogs were re-evaluated every 2 weeks (T1-T2-T3) and BW, BCS, and mean serum glucose (GLU) were recorded. Clinical score was assessed using the scoring system ALIVE [2], and GMS data guided the insulin dose and type variations. At T3, serum cholesterol (CHO), triglycerides (TRG), fructosamine (FR), and beta-hydroxybutyrate (β HBA) concentrations were measured. Differences between diets on BW, BCS, GLU, clinical score, and insulin dose, were tested by a repeated measure ANOVA fitting a cross-over design with pairwise test; CHO, TRG, FR, and β HBA were assessed by pairwise tests. Statistical significance was set at $p < 0.05$. Data are shown as mean \pm SD.

Results and discussion: GLU tended to be lower with HCD vs. CD ($p=0.07$, Table1), while neither other data from Table1, nor TRG and β HBA differed between diets or over time. Comparing HCD vs. CD at T3, FR tended to be lower (497 ± 92 vs. 584 ± 106 $\mu\text{mol/l}$, $p=0.06$), and CHO was significantly lower (306 ± 130 vs. 382 ± 141 mg/dl, $p < 0.01$), despite the higher fat content of HCD.

		T1	T2	T3	p-value
BW (kg)	CD	13.9 \pm 9.3	13.9 \pm 9.2	13.9 \pm 9.1	0.69
	HCD	13.9 \pm 9.7	13.7 \pm 9.3	13.7 \pm 9.3	
BCS 9-scale	CD	5.1 \pm 1.1	5.2 \pm 1.1	5.2 \pm 1.1	0.78
	HCD	5.1 \pm 1.4	5 \pm 1.2	5.1 \pm 1.1	
GLU (mg/dl)	CD	256 \pm 50	263 \pm 55	252 \pm 45	0.07
	HCD	240 \pm 57	207 \pm 39	212 \pm 52	
Score ALIVE [2]	CD	1.1 \pm 0.7	1.2 \pm 1.3	1.4 \pm 1.35	0.87
	HCD	1 \pm 0.8	1.1 \pm 1.2	1.1 \pm 1.2	
Insulin UI/dog/day	CD	14 \pm 8	15 \pm 8	15 \pm 8	0.32
	HCD	15 \pm 8	14 \pm 8	14 \pm 8	

Starch sources, in addition to fat content, have been thought to influence the lipid profile in dogs with DM [3]; β -glucans found in barley may explain the lower-CHO effect exerted by HCD here and elsewhere [3]. Both CD and HCD resulted in good glycemic control of diabetic dogs, with no significant changes in terms of clinical score and insulin dose and type over time. In agreement with our results, diets with different starch and fiber content had already been proven as equally valid in dogs with controlled DM [4].

Conclusion: Dogs with controlled DM on insulin therapy were successfully fed either CD or HCD, with a tendency of HCD for better glycemic control. However, the consistency and reproducibility of HCD might be challenging in daily practice.

References: [1] Rand et al. (2004) J. Nutr. 134:2072–80; [2] Niessen et al. (2022) Vet. J. 289:105910; [3] Teixeira et al. (2020) BMC Vet. Res. 16:2; [4] Fleeman et al. (2009) JSAP 50:604-14.

3A4. Effect of TEKRO Cardiodiet on electrolytes in cats with HCM

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Introduction: Cats with hypertrophic cardiomyopathy (HCM) frequently suffer from electrolyte imbalances due to either the disease or as an adverse effect of the treatment [1]. The aim of this study was to evaluate the effect of a new commercial diet (TEKRO Cardiodiet) for cats with HCM on serum electrolyte concentrations.

Animals, materials and methods: Client-owned non-azotemic cats diagnosed with HCM were included in this study. Cats were diagnosed based on ultrasonographic examination and were treated (ACE inhibitors alone or in combination with torasemide and clopidogrel) for HCM at least one month prior to study enrolment. Cats were fed the commercial cardio diet TEKRO Cardiodiet *ad libitum* for three months. Control echocardiography was performed and blood for hematological and biochemical examination was collected at the beginning of the study and as a part of the routine monthly check-up. Cats in which azotaemia developed or their clinical state required changes in medication were excluded from this study and from statistical evaluation. At each time point, the proportion of cats with electrolytes (sodium, chlorides, potassium, phosphorus, magnesium, calcium) outside of laboratory reference range was recoded and electrolyte concentrations were compared to baseline values using Wilcoxon paired test.

Results and discussion: Ten from 13 included cats in this study were used for final statistical evaluation. Three cats were excluded due to development of azotaemia (n=1) and/or change in medication (n=2) during the study. At the beginning of the study, 40% of cats exhibited hyponatremia (146.6-149.6 mmol/l) and hypochloremia (105.8-108.3 mmol/l) and 10% of cats experienced hypokalemia (3.3 mmol/l). After one month of diet intake, only 10% of cats were hypochloremic and all other cats showed levels of potassium, chloride, sodium, magnesium, phosphorus and calcium within reference ranges. After three months, all cats had all parameters within reference ranges except one cat, which was exhibited borderline hypercalcemia (3.1 mmol/l). Sodium level increased significantly after the first month (158.6 mmol/l, p=0.03) and third month (159.1 mmol/l, p=0.02) in comparison to baseline value (153.5 mmol/l). No other parameters were significantly different from baseline values.

Table: Median (minimum-maximum) values of selected electrolytes in cats with HCM during the study. Asterisks mark group where cat(s) outside the reference range were present, † indicate significance difference from baseline.

	Sodium (mmol/l) Ref. range : 150-165	Chlorides (mmol/l) Ref. range: 112-129	Potassium (mmol/l) Ref. range: 3.5-5.8	Calcium (mmol/l) Ref. range: 2.0-3.0
Baseline	153.5 (146.6-163.9)*	115.0 (105.8-123.9)*	4.4 (3.3-4.9)*	2.6 (2.4-2.7)
1st month	158.6 (155.4-164.3)†	117.1 (111.1-124.7)*	4.4 (3.6-5.1)	2.7 (2.4-2.8)
2 nd month	157.2 (154.2-161.2)	118.8 (114.9-119.9)	4.7 (3.7-5.3)	2.5 (2.2-2.9)
3rd month	159.1 (156.6-161.7)†	118.0 (112.6-123.8)	4.6 (3.8-4.8)	2.7 (2.4-3.1)*

Electrolyte disturbances in treated or untreated cats with HCM are anticipated, and might have direct impact on progression of HCM and responsiveness to therapy. Sodium level is important for maintaining renin-angiotensin-aldosterone balance and water homeostasis and hyponatremia is associated with poor outcome in patients with heart failure.[2,3]

Conclusion: TEKRO Cardiodiet as a part of the treatment in cats with HCM might have potential in correcting hyponatremia, but larger studies are needed. Reason of hypercalcemia in one cat remains unclear.

References: [1] Cote (2017) Vet. Clin. North. Am. Small. Anim. Pract. 47:1055–1064; [2] Dargie (1990) Am. J. Cardiol. 65:28E-32E.[3] Rodriguez et al. (2019) Curr Cardiol Rev, 15:252-261.

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3B1. Can recyclable retort pouches improve wet pet food shelf life?

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Introduction: Recyclable packaging is the most popular claim in the EU and the fastest growing one. Between July 2016 and June 2021, pet food launches that carried the recyclability claim grew from 5% to 14% of all packaging launches. While flexible pouches are the most common pack type for European pet food launches, they are the least likely to claim recyclability [1]. While retort pouches have historically been one of the most difficult packaging solutions to make recyclable. Indeed, the retorting process requires flexible materials, usually multi-materials, able to absorb pressure deformation without content leakage [2]. The aim of this study was to determine the effect on 48 months' shelf-life of retorted cat food packed in recyclable flexible pouch compared to standard pouch.

Materials and Methods: A commercial complete wet cat food (crude protein 9.5%, fat 5%, starch 3.5%, formulated without preservatives) was hermetically sealed and retorted into 2 different types of flexible pouches: 1) recyclable multi-layer mono-material doypack with gusset 100 g (RP) composed by OPP (oriented polypropylene) 30µm/OPP-HB/PP 70µm and 2) traditional multi-layer multi-material doypack with gusset 100 g (TP) composed by PET 12µm/ALU 9µm/OPP (not oriented polypropylene) 60µm. An accelerated shelf-life test was performed based on the Arrhenius model using the equation of 1 week at 37°C corresponding to 1 month at ambient temperature [3]. Four TP and 4 RP were stored at 37°C in a thermostatically controlled incubator for 0, 12, 24, 48 weeks. At each timepoint, one of both recyclable and traditional pouches were opened and the cat food was assessed for its physicochemical characteristics (total acidity and moisture), sensory parameters, oxidative stability (peroxide value, total volatile base nitrogen (TVBN), p-anisidine), nutritional values (vitamins A-D3-E), and microbiological parameters (biogenic amine index, absence of *Salmonella* and *Enterobacteriaceae*). Significant differences were analyzed by means of one-way ANOVA or Wilcoxon test.

Results and discussion: All parameters at the end of accelerated shelf life were compliant with pet food safety standards (Table 1). No significant differences were found between RP and TP at all testing points, except for moisture and vitamin E. RP showed a progressive and significant decrease of moisture compared to the more stabilized value of TP, meanwhile TP showed a progressive and significant decrease of Vitamin E compared to the more stabilized value of RP. This result can be explained by a low oxygen transmission rate and high-water vapor transmission rate of RP barrier.

Table 1	T0	RP-T12	TP-T12	RP-T24	TP-T24	RP-T48	TP-T48
Total acidity g/100g fat	5	5.9	7.1	7.6	8.9	14.27	15.7
Moisture % *	81.6	80.2	81.9	78.6	81.5	78.0	81.9
Peroxides meqO ₂ /kg fat	2.9	1.5	1.8	0.6	0.5	1	1
TVBN mg N/100 g	44	25	24	32	26	35	30
p-anisidine number	5.1	7.9	5.2	7.8	5.2	4.4	5.2
Vitamin A (IU/kg)	57,300	56,700	53,400	52,800	51,300	44,100	37,700
Vitamin E (mg/kg) *	28.5	28.9	21	27.1	22.8	29.7	20.9
Vitamin D3 (IU/kg)	307	319	273	304	231	212	387
Salmonella /25 g	absence	absence	absence	absence	absence	absence	absence
Enterobacteriaceae UFC/g	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Biogenic amine index mg/kg	1.3	1.4	1.4	1.5	1.4	1.5	1.4

LOD:limit of detection(<10UFC/g).*superscripts: significantly different results (p<0.05) between RP-TP at all timepoints.

Conclusion: The present study suggests that tested sustainable pouches offer high-barrier properties and a better vitamin E recovery performance on the 48 months' shelf life of retorted cat food compared to traditional multi-layer multi-material pouches and it could offer a feasible sustainable alternative to standard pet food packaging. **Acknowledgment:** Monge & C. S.p.a. to fund the study.

References: [1] Mintel GNPD Pet Food Market Survey (2022); [2] Holdsworth et al. (2007) Thermal processing of packaged foods:1-13; [3] Phimolsiripol et al.(2016) Reference Module in Food Science 1:1-8.

3B2. Correlation between dog breed size and processing and starch cooking requirements of diets

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Introduction: Large dog breeds are more commonly implicated to produce soft not adequately shaped stools than medium and small dog breeds [1]. This might be due to differences in anatomy, gastrointestinal transit time (GTT), digestibility or microbial fermentation in the colon [2]. Taken these in considerations, it was hypothesized that foods for large breed dogs need a better processing and higher starch gelatinization (SG) to reduce organic matter flow to colon. The present study evaluated the apparent total tract digestibility (ATTD), faeces traits and fermentation products, GTT, and Na, K, and Cl apparent absorption of a same formulation processed to obtain three SG levels, fed to a large (Rottweiler; Ro), medium (Beagle; Be) and small (Shih Tzu; ST) dog breed.

Animals, materials and methods: A single formula balanced to maintenance ($28\pm 1.2\%$ crude protein [CP]; $17\pm 2.2\%$ fat; $30\pm 2.5\%$ starch; $1.9\pm 0.1\%$ crude fibre) with sorghum as the starch source was processed to obtain: SG90 (91.3% of SG; extruded with high specific mechanical energy [SME] = 13 ± 2 kWh/ton; finely grind, raw material mean geometric diameter [MGD] = $275\pm 1.5\mu\text{m}$); SG50 (50.7% of SG; extruded with moderate SME = 9 ± 1 kWh/ton; coarse grind, MGD = $326\pm 1.5\mu\text{m}$); GA30 (27.9% of SG; pelletized, SME = 2.3 ± 0.5 kWh/ton; MGD = $326\pm 1.5\mu\text{m}$). Fifteen Ro (49.4 ± 6.1 kg), 18 Be (12.1 ± 1.7 kg) and 20 ST (4.6 ± 1.1 kg), all health and dewormed were used. A 3 (SG levels) x 3 (dog breed sizes) factorial arrangement was used, totalling 9 treatments. Dogs were fed each diet for 23d: 10d for adaptation; 5d for faeces collection to ATTD (chromic oxide method); 3d for fresh faeces collection to measure fermentation products; 5d to evaluate GTT. Results were submitted to ANOVA considering SG, breed size, and their interaction effects, and means compared by the Tukey test ($P < 0.05$). Approval number: 3527/21.

Results and discussion: All dogs readily ate the foods, without episodes of refusing, including the pelletized treatment. Faeces score was lower for Ro ($P < 0.05$), being inadequate for SG50 (2.7 ± 0.4) and SG30 (2.2 ± 0.3 ; adequate is > 3 in the system [3]). Faeces was adequately formed for Be and ST to all diets (score > 3.5 ; $P > 0.05$). Faeces pH and moisture, regardless of breed were higher for SG90 than SG50 and SG30 ($P < 0.01$), which was similar each other. Faeces moisture was higher for Ro ($P < 0.05$). No diet effect was observed for GTT ($P > 0.05$), but it was lower for Ro (41.7 ± 6.2 h) than for ST (48.7 ± 8.6) and Be (46.8 ± 6.2) ($P < 0.05$), which did not differ each other. The ATTD of nutrients and energy was lower for SG30, intermediary for SG50, and higher for SG90 ($P < 0.01$), showing important effect of processing. The ATTD of DM, OM, and crude energy was higher for Be than ST and Ro ($P < 0.05$), that did not differ from each other, but the ATTD of CP was lower for Ro, intermediary for ST, and higher for Be ($P < 0.01$). The apparent absorption of Cl and K was lower for Ro ($P < 0.05$), and of the Na lower for SG30, intermediary for SG50, and higher for SG90 ($P < 0.01$). Faecal short-chain fatty acids concentrations (SCFA) were lower in dogs fed SG30 than SG50 and SG90 ($P < 0.01$), with exception of propionate and butyrate that was higher for SG50, and lower for the others ($P < 0.05$). The total SCFA (mMol/g faeces DM) was higher in the faeces of Ro (643 ± 16), intermediary for Be (556 ± 15), and lower for ST (506 ± 14) ($P < 0.01$), indicating higher fermentation in the colon for Ro.

Conclusion: Raw material particle size, SME application and SG was important only for Ro faeces formation. Greater fermentation on colon, and lower protein ATTD, electrolytes absorption, and GTT may be involved in the soft unformed faeces formation of Ro.

References: [1] Goudez et al. (2011). *Brit. J. of Nutr*, 106(S1):S211-S215; [2] Deschamps et al. (2022). *Int. J. Biol. Sci.*, 18(13): 5086. [3] Carciofi, et al. (2008). *J Anim Physiol Anim Nutr*, 92:326–336.

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3B3. Determination of digestibility and urinary pH in client-owned cats in a domestic environment

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Introduction: Digestibility and urinary pH trials in cats are performed in laboratory facilities with metabolic cages that cause stress and compromises the animals' welfare [1]. The "In-home" methodologies, using client-owned animals, can be performed to reduce these effects. However, the technique requires validation and owners training. In this sense, this study aimed to compare the results of digestibility and urinary pH of dry extruded diets for cats in laboratory *versus* a domestic environment.

Animals, material, and methods:

Thirty adult cats (\pm 7 years old) were housed in two environments:

laboratory (LAB) (n= 24 laboratory cats) and domestic (n=6 client-owned cats) (In-Home). A completely randomized design was used and four experimental foods were evaluated in four periods of 10 days each, being 5 for adaptation, and 5 for feces and urine collection. For the sample collection, the LAB cats were housed in individual metabolic cages, while for the In-Home, the animals were kept in their own houses and a litter box was adapted for the collection of feces and urine separately (Figure 1). For the In-Home, the traditional sanitary granulate was replaced by polypropylene spheres, which do not adhere to the feces and even absorb urine, and a glass was coupled at the bottom of the box to collect the urine. For the determination of urinary pH, regardless of the method, thymol was used as a preservative of fresh samples until pH analysis [2].

Figure 1. A and B: 3D model of the litter box used. C: Polypropylene spheres used instead of the traditional sanitary granulate.

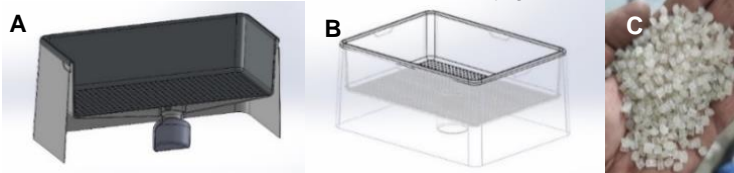


Table 1. The total tract apparent digestibility coefficients (% in dry matter) and urinary pH for both environments.

Variables	LAB	In-Home	SEM	p-value
Dry matter	81.91	80.11	0.979	0.2400
Organic matter	85.23	84.05	0.818	0.3473
Crude protein	85.33	83.44	0.598	0.0677
Fat	92.40	89.27	0.712	0.0206
pH	6.58	7.08	0.510	0.0458

SEM: standard error of the mean.

of dry matter, organic matter, crude protein, and fat were determined [3]. For the statistical analysis, the results of both methods were compared by the t Student test where $p < 0.05$ was significant and $p < 0.1$ a tendency. **Results and discussion:** Some differences were found for TTADC, especially for the digestibility of fat ($p < 0.05$) and crude protein ($p < 0.1$), which were higher for the LAB. On the other hand, the urinary pH was higher for In-Home (Table 1). The differences observed in TTADC were not expected but it is possible that some factors like frequency of defecation, the smaller sample size of animals used In-Home or food amount consumed In-Home has been influenced the results, but this was not evaluated in the study. For the urinary pH, the inadequacy of litter box cleaning or failure in urine preservation is the possible factor that influenced the higher pH observed in owned cats. These results show the importance of some improvements and cautions when owned animals are used for scientific studies, more specifically related to the owner's training.

Conclusion: The results for TTADC were similar between methods, but some improvements are needed to get In-Home results closer to the LAB. Concerning pH, the difference found may have occurred due to failure in the litter box cleaning or urine preservation by the owners. In addition, the apparatus developed and used was viable, requiring only improvements to facilitate cleaning procedures.

References: [1] Ellis et al. (2014), Vet Rec Open 1:e000056; [2] ABINPET, M. Pet Food; [3] AOAC (2006) Official Met. of Ana.

3B4. The effect of isoenergetic reduction of dietary macronutrients on physical activity, energy expenditure, and body composition in cats

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Introduction: Commercial extruded cat foods have greater carbohydrate content and less protein than the typical prey of feral or wild cats¹. The greater carbohydrate intake has been suggested as a risk factor for feline obesity². However, carbohydrate inclusion as a risk factor for, or treatment of, obesity is inconclusive³⁻⁶. This study investigated the effects of different dietary protein, fat and carbohydrate density on physical activity, energy expenditure (EE) and body composition in cats.

Animals, materials and methods: Three extruded diets were formulated for adult maintenance according to AAFCO in an isoenergetic approach using the same ingredients in varying levels for either a low-protein (LP; protein 28%ME, fat 40%ME, NFE 32%ME), low-fat (LF; protein 42%ME, fat 30%ME, NFE 30%ME), or low-carbohydrate (LC; protein 36%ME, fat 41%ME, NFE 23%ME), (LC) diet (Table 1). Adult male neutered cats were divided into two groups based on body condition score (lean: n=9; BCS=4 or 5/9 or obese: n=8; BCS>8/9) and fed each diet to maintenance for 4-weeks each in a 3x3 Latin square design. Daily food intake and weekly body weight were recorded throughout the study. Physical activity was measured via actigraphy on day 15-21 of

Table 1. Proximate analysis (% as fed) of three diets formulated for adult maintenance using an isoenergetic approach with varying levels of the same ingredients

% As Fed	LP	LF	LC
Moisture	8.3	5.2	6.1
Crude Protein	31.2	42.5	40.9
Crude Fat	18.4	12.9	19.1
NFE*	31.4	27.2	22.0
Crude Fibre	4.4	4.8	4.7
Ash	6.3	7.4	7.2
ME (kcal/kg)#	3,756.8	3,535.3	3,826.8

LC, low-carbohydrate; LF, low-fat; LP, low-protein; ME, metabolizable energy; NFE, nitrogen free extract. *Calculated as: $NFE=100-(Crude\ Protein+Crude\ Fat+Crude\ Fiber+Ash)$ #Calculated as: $ME=10*(Crude\ Protein*3.5)+(Crude\ Fat*8.5)+(NFE*3.5)$

each period. On day 22 of each period, EE was measured via indirect calorimetry and on day 23, total tissue mass (TTM), body fat mass (BFM), lean soft tissue mass (LSTM), and bone mineral content (BMC) were assessed by dual energy x-ray absorptiometry. Data were statistically analysed in SAS Studio as repeated measures via the GLIMMIX procedure. Tukey post-hoc analysis was performed to assess multiple comparisons when significant ($P<0.05$).

Results and discussion: Body weight, TTM, BFM, LSTM, and BMC were greater in obese compared to lean cats ($P_{bc}<0.05$), but no effect of diet was observed. Daily physical activity was similar between groups; however, obese cats were more active during dark hours when fed LC and LF ($P_{bc*diet}=0.03$). Obese cats had lower fasted EE when adjusted for body weight ($P_{bc}=0.023$), and, regardless of BCS, cats fed LC had greater EE in the fed state than cats fed LF ($P_{diet}=0.04$).

Conclusion: Short-term feeding of a LP, LF, or LC diet did not affect cat body composition. Obese cats fed a LF or LC diet may show greater night-time activity and LC diets may increase EE regardless of BCS. Future research should explore metabolic changes resulting from different macronutrient distributions to further build on the physiological findings presented here.

Acknowledgements: Funding provided by Champion Petfoods; Mitacs Accelerate

References: [1] Plantinga et al. (2011) Br. J. Nutr. 106:S35-48; [2] Buffington (2008) Can. Vet. J. Rev. 49:561-3; [3] Verbrugghe et al. (2017) Vet. Sci. 4:55; [4] Backus et al. (2007) Br. J. Nutr. 98:641-50; [5] Nguyen et al. (2004) Am. J. Vet. Res. 65:1708-13; [6] Coradini et al. (2011) Br. J. Nutr. 106:S64-77.

3B5. Validation of the reduction of days of total faeces collection for apparent digestibility protocol in dogs

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Introduction: Total faeces collection is a method currently validated by the Association of American Feed Control Officials (AAFCO) [1], which recommends 5 days of food adaptation and 5 days of total faeces collection. The reduction on days of total faeces collection can optimize time and improve the welfare of dogs that participate in the experimental protocols. The present study aim was to validate the reduction of days of total faeces collection in dogs to improve welfare and time optimization.

Animals, materials and methods: Days of total faeces collection in dogs (3, 4 and 5 days) were considered treatments. Nine *in vivo* evaluations of coefficient of total tract apparent digestibility (CTTAD), with at least 6 dogs each test, were performed, totalizing 67 observations (sum of the number of dogs it participated of the 9 *in vivo* evaluations) per treatment. Different dry extruded complete foods for dogs were tested (moisture: 7.5% (6.7-9.4%); crude protein (CP): 29% (26.5-33.1%); ether extract (EE): 16.3% (13.2-19.2%); mineral matter (MM): 6.8% (3.9-7.1%); crude fibre 2.4% (1.4-3.4%); median (min-max)). Statistics were performed comparing treatments for the parameters: faeces volume, CTTAD of dry matter (DM), organic matter (OM), CP, EE, MM and non-nitrogen extractive (NNE). All the procedures were approved by the Ethics Committee on the Use of Animals. The studies were grouped into three clusters based on their similarities in the variables, using the Hierarchical Clustering on Principal Components method of the FactoMineR package [2]. Data were analysed using a mixed linear model [3], with the random effect of the studies and the fixed effects of clusters (C1, C2 and C3), treatments (3d, 4d and 5d) and sex (male and female), using several R packages. The significance level was $P \leq 0.05$.

Results and discussion: The institution that regulates the use of animals in research in Brazil (CONCEA), recommends the optimization of tests regarding the collection of biological materials and

days of confinement so that conditions of animal welfare are always improved [4]. In this study, it was demonstrated that there was no significant difference either in faecal volume or in any of the evaluated CTTAD values ($P > 0.05$) among treatments (Table 1). It must be considered that the analysed faeces were only from the pool of 5 days of total faeces collection. The difference between treatments was related to the food consumption and excreted faeces used in the calculation to determine the CTTAD. This may be a limiting factor for the study, however, in healthy animals adapted to a given food, no difference between digestibility from one day to the next is expected. Fewer days of total faeces collection were not tested due to the minimal amount of faeces to do the analyses.

Conclusion: In conclusion, a reduction from 5 to 3 or 4 days of total faeces collection can be applied to assess apparent digestibility in dogs, and this protocol is recommended due to animal welfare and time optimization.

References: [1] AAFCO Official Publication (2022); [2] Lê, S et al. (2008) J. Stat. Softw. 25:1-18; [3] St-Pierre, NR (2001) J. Dairy Sci. 84(4):741-755; [4] CONCEA (2016) Normativas do Conceca 3 Ed.

Table 1. Comparison of faeces volume and CTTAD with 3, 4, and 5 days of total faeces collection in dogs.

Variables	Treatments*			SEM	P value
	3 days	4 days	5 days		
Vol, (gFz/gR)	0.445	0.444	0.444	0.006	0.990
CTTAD DM (%)	87.59	88.06	88.08	0.435	0.870
CTTAD OM (%)	88.27	88.33	88.26	0.209	0.990
CTTAD CP (%)	85.57	85.65	85.58	0.272	0.990
CTTAD EE (%)	95.20	95.23	95.18	0.073	0.940
CTTAD MM (%)	31.07	31.31	30.77	1.482	0.990
CTTAD NNE (%)	90.78	90.83	90.81	0.216	0,990

* Values are the means of least squares.

3B6. Comparison between conventional and marker methods for fecal collection in the digestibility trials in cats

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Introduction: Fecal collection to determine the Total Tract Apparent Digestibility (TTAD) can be performed by the conventional or marker methods. For cats, the total collection of feces should take at least 120 hours [1]. However, to carry out digestibility trials in cats, they must be housed in metabolic cages, which can lead to stress and fecal retention in some cases, and then overestimated results. Markers, such as ferric oxide (FeO), are generally used in digestibility studies to limit the exact moment to start and finish the collection [2]. Therefore, this study aimed to compare two methods of fecal collection in digestibility tests (marker vs conventional) in adult cats.

Animals, material, and methods: This study was approved by the Ethics Committee of the State University of Maringá (nº 7966170123). Thirty-six adult cats (2 to 7 years old) were included and housed in metabolic cages. The study was a split-plot design, with six diets and two methods tested. Six commercial extruded dry foods for adult cats were used. The diet acclimation lasted 5 days, and the fecal collection period was variable (5.97 ± 0.56 days). During the collection period, two methods were used simultaneously. All the cats were fed twice a day. The first method was carried out by collecting feces for 120 hours (conventional) [1]. The second one was developed by using FeO as a marker. For this latter method, in the first meal of the collection period, all cats received 0.1g of FeO added into their diets by coating. This marker was also used in the last meal of the fifth day of collection. The fecal weighing started when the feces acquired a reddish color after the first meal supplemented by FeO and ended when this color disappeared after the last meal supplemented by FeO [2]. Total collection of feces was performed by both methods. The dry matter, organic matter, crude protein, acid-hydrolyzed fat, crude fiber, ash, and nitrogen-free extract were determined in the fecal and food samples [3]. The TTAD coefficients were determined for each method according to the weight of feces counted in each one. Data were analyzed considering the effects of diet, marker, and their interaction, at 5% of probability.

Results and discussion: There were no effects of method and interaction ($p > 0.05$) between diet and method on the TTAD coefficients considered in the study. On the other hand, the effect of diet was observed for all the TTAD coefficients analyzed. This result was expected since the diets were from different commercial market segmentation (standard, premium and super premium) [4].

Cats can change their behavior in stressful situations, such as housed in metabolic cages for digestibility trials. For instance, they can retain feces due to the stress generated by the constrained environment and the absence of a litter box [5]. This behavior can overestimate the digestibility results. However, despite that cases of fecal retention may have occurred in this study, this effect did not affect the results, since no differences ($p > 0.05$) were observed between methodologies. The cats used in the present study were animals adapted to be housed in individual metabolic cages, which may have contributed to the reduction in the behavior of fecal retention.

Conclusion: The TTADs determined by the marker method with FeO were similar to those determined by the conventional method. The marker method can be helpful when fecal retention in caged animals is observed, but this was not tested in this study.

References: [1] FEDIAF (2021) European pet food industry federation; [2] Jagger, et al. (1992) Brit. J. Nutri., 68, 729-739; [3] AOAC (2006) Official Methods of Analysis; [4] Carciofi, et al. (2009) Rev. Bras. Saúde Prod. An., 10, 489-500; [5] Graham, et al. (1996) Zoo. Bio., 12, 71-82.

3B7. Association of Cat Owners' Nutritional Habits with Cats' Body Condition Score in Türkiye

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Introduction: Obesity and overweight (OWO) can lead to diseases such as type-II diabetes, cardiovascular disease, osteoarthritis, high blood pressure, and cancer. On the other hand, having a thin body condition score (BCS) may occur due to chronic disease and malnutrition [1, 2]. Cat obesity risk factors have been reported in the literature on a global scale; however, there is little information regarding the cat's BCS distribution in Türkiye [3, 4]. The association between cat owners' eating habits, exercise frequencies, and the BCS of their cats is still not clearly documented. This study aimed to investigate the status of cats with thin and OWO BCS compared to normal BCS, as well as the association between the nutritional habits of cat owners and their cats' BCS.

Animals, materials and methods: This study was planned to be analytical and cross-sectional. An online, 46-question questionnaire distributed via social media and private clinic e-mail lists were used to collect answers from cat owners between February and April 2022. Cat owners with at least one non-pregnant, older-than-six-month cat attended the study. Cat owners' and cats' demographics, nutritional habits, cats' five stage visual BCS according to owner, weight, and owner's body mass index were recorded. Female cats were ordered as thin: 3.0 kg, normal: 3.0-4.5 kg, OWO: >4.5 kg, while male cats were ordered as thin: 4.5 kg, normal: 4.5-5.5 kg, OWO: >5.5 kg. All parameters were grouped as categorized variables; cats with normal BCS were accepted as a reference category, and the association of thin and OWO cats with the determined risk factors was evaluated by multinomial logistic regression. SPSS V.23, JASP 0.16.3, and Jamovi 2.2.5 were used in statistical analyses, the type 1 margin of error was accepted as 0.05 (bidirectional).

Results and discussion: Valid survey data (n=1875) collected without pregnant cats (n= 9) or incomplete surveys. Owner reports indicated 30.8% five-grade visual BCS and 33.8% OWO cats based on cat weight. Normal BCS was found to be 4.9 ± 1.4 kg in male cats and 4.0 ± 1.21 kg in female cats. The odds ratio of a cat becoming OWO was 1.6 (95% CI = 1.20-2.13) when living with three cats or more compared to a cat living alone. It is founded that the odds of a cat becoming OWO increase in parallel with the age referenced for 6 months-2 years old cats ([3-6 years: OR= 1.65(95% CI = 1.29-2.10); 7-10 years OR=2.84 (95% CI = 2.02-4.02); >10 years: OR= 2.74(95% CI = 1.61-4.66)]). It was determined that the cats that stole feed from the feed package were more likely to have OWO BCS [OR= 1.66 (95% CI = 1.26-2.20)]. The odds ratio of a cat becoming OWO was 1.51 (95% CI = 1.06-2.12) when its drinking water was changed every other day compared to a cat that had a daily water change. Only the grain eating habits of the cat owners were associated with the BCS of the cats. The cats whose owners occasionally ate grain were weaker than those who always ate [OR=1.68 (95% CI=1.03-2.72)]. It has been determined that having knowledge about the negative effects of obesity reduces the percentage of OWO BCS in cats [OR= 1.45 (95% CI = 1.05-2.03)].

Conclusion: Although there was no clear link between the cat owners' dietary habits and workout practices, there was an association between the cats' feeding and watering practices. Since the feed and water for the cats are provided by the cat owners, it can be mentioned that the cat owners' behaviors play a crucial role in the cat's BCS. Awareness-raising studies for cat owners could generate positive outcomes in this area and pave the way for further research.

References: [1] German (2010) In Practice 32(2): 42–50. [2] Miyake et al. (2020) Int. J. of Envr. Res. and Pub. Health 17: 3498. [3] Kocabağlı et al. (2017) Acta Scie. Vet. 45(1): 5.[4] Kocabağlı et al. (2019) Int. VETEXPO-2019 Vet. Sci. Cong.: 103–110.

3B8. Preference of Diets Containing Clove, Blueberries or Kelp in Dogs

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Introduction: A growing interest in the pet food industry is to keep high nutritional and quality standards by using natural ingredients [1]. Ingredients like clove containing eugenol (being aware of safety levels), blueberries providing anthocyanins, or kelp algae rich in fucoidans, could represent natural antioxidant alternatives [2, 3, 4]. Though incorporated into pet food products following trends, the effect of these compounds on palatability for dogs has not yet been investigated. The objective of this work was to assess the effect of the inclusion of clove, blueberries, or kelp algae on the preference of wet diets for dogs.

Animals, materials and methods: Twelve Beagle dogs (6 males and 6 females, 3-5 years old, 13.8 ± 1.96 kg), were included in this study. One complete and balanced wet diet was formulated (CON), and also supplemented with powdered clove (CLO, 0.45%), fresh blueberries (BLB, 3%) or grounded dehydrated kelp algae (KLP, 1.5%).

Using two-bowl preference tests [5], the 6 possible comparisons were tested for 2 consecutive days each. The same amount of both tested diets was offered each time. The weight of the bowl with food was registered before and after the test to calculate the consumed/presented ratio; the bowl that the animal first approached and the bowl where the animal first ate from for 15 seconds continuously (first election) were also recorded. Diet preference was determined by comparing the ratio of consumed/presented of each bowl using a one-way ANOVA test, whereas for the first approach and first election analysis, a Fisher exact test was used (RStudio, 1.4.1106 version).

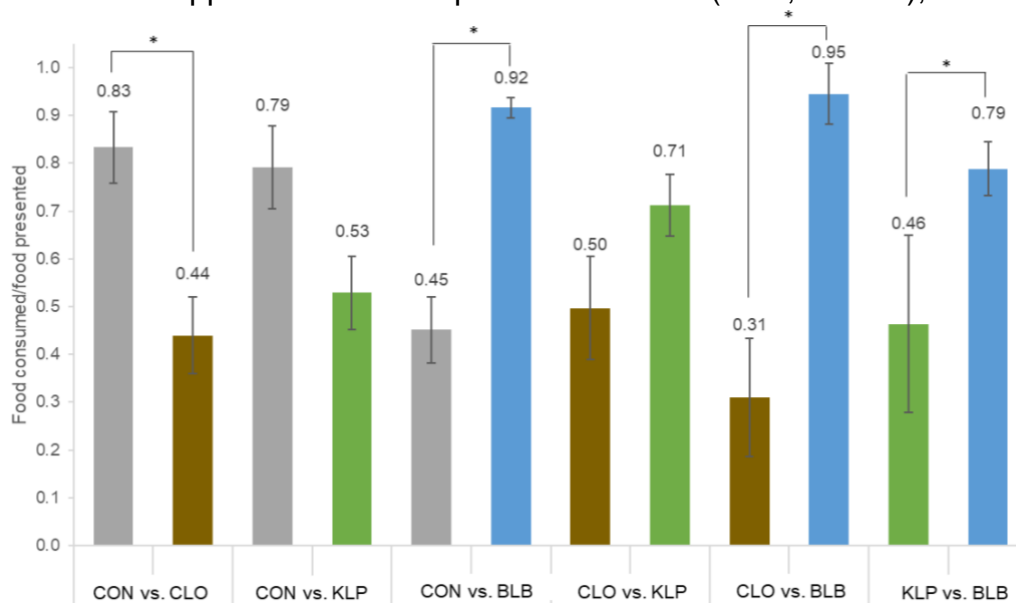


Figure 1. Food consumed/presented for each test. Average values are shown. Error bars correspond to SEM. * indicates statistically differences (p-value < 0.05). CON: control (grey), CLO: clove (brown), KLP: kelp (green), BLB: blueberries (blue).

consumed/presented ratio; the bowl that the animal first approached and the bowl where the animal first ate from for 15 seconds continuously (first election) were also recorded. Diet preference was determined by comparing the ratio of consumed/presented of each bowl using a one-way ANOVA test, whereas for the first approach and first election analysis, a Fisher exact test was used (RStudio, 1.4.1106 version).

Results and discussion: The average ratio of consumed/presented food for each test is given in Figure 1. Dogs showed a higher preference for the BLB diet when compared to the rest of diets. First approach did not show any significant differences, but first election showed again a clear preference for BLB when compared to the other diets.

Conclusion: Dogs showed a preference for the diet including fresh blueberries as observed through the ratios of consumed/presented and the first election. These results can be helpful in the development of new formulations with natural ingredients that can both increase the health of our pets and be attractive from a sensory point of view.

References: [1] Buff et al. (2014). JAS. 92 (9): 3781-91. [2] Mendes et al. (2021). Arch Anim Nutr. 75(5): 389-403. [3] Dunlap et al. (2006). Comp Biochem Physiol A Mol Integr Physiol. 143(4): 429-34. [4] Dutot et al. (2012). App Biochem and Biotech. 167: 2234-40. [5] Aldrich et al. (2015). Animals. 5: 43-55.

3B9. Unconventional feeding methods in Norwegian hunting dogs

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Introduction: In recent years, pet owners' feeding management and trends have shifted. The goal of this survey was to investigate Norwegian hunting dog owners' motivation behind their current feeding regime, mainly the decision of using alternative feeding methods such as raw meat-based (RMBD) or plant-based diets (PBD), as these diets are becoming more popular [1, 2, 3]. We also aimed to investigate the knowledge of owners regarding the potential risks of RMBD and PBD.

Materials and methods: An online survey which consisted of 42 questions was made and published in a Norwegian hunting dog group ("Beagle Norge" with 5200 members). The data were collected between 2021 November and 2022 January.

Results and discussion: Eighty-five responses were collected. The descriptive statistics showed that the majority of the respondents (69.4%) said that nutrition plays a very important role in the dog's health. Owners trusted the advice of veterinarians "very much" (64.7%) and "somewhat" (32.9%) only a few people said, "not very much" (2.4%). On the contrary, the trust in veterinarians concerning the dog's nutrition decreased dramatically: "very much" (46.9%) and "somewhat" (38.3%), "not very much" (7.4%), and "a little" (7.4%). Thirty-four owners applied RMBD as the main diet, and three of them combined it with other diets (e.g.: raw feeding was used only during hunting season). None of the respondents used only PBD, two owners applied PBD as the dog's main diet, but not as an only diet. The majority (n=19/34, 55.9%) of raw feeders had people at risk in the household such as elderly people, children, chronically ill/immune suppressed, or pregnant women. Commercially prepared RMBD was used by 70.8% (n=17/34) of the participants. In the group of non-raw feeders veterinarians or online veterinary information sites helped to establish the diet (n=20/51). While among the raw feeders, these were less relevant (n=3/34), and they mainly relied on non-professional sources (internet, other pet owners, family, friends, and breeders). In general, ("very much" 48.6% vs. 72.5%; "not very much" 5.7% vs. 0%), and in terms of nutrition ("very much" 28.6% vs. 56.9%; "not very much" 14.3% vs. 1.0%) raw feeders had lower trust in veterinarians. The most important reason for choosing a raw diet was to "improve skin and coat" (n=14/34) of the dog and it was considered to be "more natural" (n=13/34). More than 60% (n=21/34) of the raw feeders did use a dietary supplement which was much higher than in the "non-raw" feeder group (n=2/51). Beef (25.9%, n=21) and chicken (18.5%, n=15) were the most popular raw products. The minority of raw feeders were aware of the hygienic risks of raw meat such as bacterial infections (n=14/34). Dental problems (n=4/34) and nutritional deficiencies (n=5/34) were also mentioned only by the minority. Almost 60% of respondents handled the dog's food in the same place but with different instruments than human food. Only seven owners handled the dog's food in a different place than human food. The technique "I handle the dog's food in the same place with the same instruments as human food" was mentioned by three respondents. "Washing hands after preparing food or handling pet" and "cleaning and disinfecting all bowls and surfaces that have come into contact with the pet and its food daily" were equally important (n=17/34).

Conclusion: Most probably raw feeding is more popular among hunting dog/sport dog owners than among pet dog owners, as also shown by Kluess et al. [4]. Based on the results, increasing RMBD users' trust in veterinarians regarding pets' nutrition would be very important. Education of owners about the hygienic risks of RMBD should also be emphasized as most owners were unaware of it.

References: [1] Michel et al. (2008) JAVMA 233: 1699–1703; [2] Dodd et al. (2019) Plos One 17; [3] Dodd et al. (2020) Vet Rec 186; [4] Kluess et al. (2021) Animals 11:1752.

3B10. Dog feeding practices and supplement use with regard to exercise routine in Switzerland – a survey

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Introduction: Regular exercise is related to owner awareness of their dog's body condition and feeding habits [1]. We hypothesized that owners seem to overestimate the impact of their dog's working regimen on its energy and nutritional requirements. The goal of this survey was to explore dog owners' feeding habits depending on sport/ working intensity (WI) of their dogs.

Animals, materials and methods: This survey was conducted at an annual dog exhibition in Switzerland. A set of 14 questions were asked: 4 about the dog, 3 about the WI routine and 7 about feeding routine and supplement use. 247 owners completed the survey. The obtained data were descriptively evaluated using Microsoft Excel (Excel 365, NM, USA). Data regarding feeding habits were evaluated with respect to work use (C group = no work, W group = work or sport use). Text answers regarding the category of W for which the dog was used were categorized in 13 different categories.

Results and discussion: Characteristics of the study population are displayed in Table 1.

Table 1. Description of the study population

	C group (n=104)	W group (n=143)
Age (years, mean ± standard deviation)	5.8 ± 4.5	5.8 ± 3.5
Male (% of total)	23	29
Female (% of total)	19	29
Body weight category ≤ 5 kg (% of total)	5	5
Body weight category 6 to 10 kg (% of total)	7	7
Body weight category 11 to 20 kg (% of total)	12	16
Body weight category 21 to 30 kg (% of total)	13	19
Body weight category 31 to 45 kg (% of total)	5	8
Body weight category > 45 kg (% of total)	0	3

Overall, 57% of the dogs were classified in the W group of which agility (n = 29), companion/ guard dog training (n = 28), and mantrailing (n = 20) were the most common. 36% of dogs in the W group were used twice per week, 28% once per week and 26% 3 to 4 times per week for W.

Most activities of W dogs lasted for 1-2 h (38% of W dogs), followed by activities lasting for 30 min to 1 hour (34% of W dogs) and activities shorter than 30 min (18% of W dogs). In W dogs, 54% of the owners believed that their dog does not have additional nutritional needs due to WI while 29% believed that their dog has additional nutritional needs due to WI. Feeding preferences were dry food (52% of W, 49% of C), wet food (25% of W, 27% of C), and BARF in the W group (13%) and home cooked in the C group (14%). Regardless of WI, dog owners found a protein rich diet important (58 and 57% in W and C, respectively). Most owners thought that the nutritional needs of their dog can be met with a diet with few components (58% in W, 63% in C group) while 23 and 21% in the W and C group thought that a large variety of foods is necessary to cover the nutritional needs of their dog. Most participants rated the use of supplements to the diet as necessary depending on the diet (36% in W group, 26% in C group). The use of supplements was rated as "always healthy but not necessary" by 25% in the W and 21% in the C group. The most used supplements in the W group were oils (23%), green-lipped mussel products (13%) and vitamin and mineral supplements (12%). In the C group oils (26%), vitamin and mineral supplements (11%) and green-lipped mussel products (9%) were the most used. 11% of the W group and 19% of the C group did not report any supplement use.

Conclusion: In conclusion, this survey suggests that feeding habits of dog owners mainly differ regarding the use of supplements. Further studies therefore should investigate the quantitative impact of the supplements on dietary nutrient composition.

References: [1] Kluess et al. (2021) *Animals*, 11:1752

3B11. Survey of motivations of dog owners and veterinarians seeking nutritional counselling at Naples University VTH

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Introduction: Awareness of the importance of nutritional treatment of companion animals is growing among veterinary doctors and companion animal owners, who refer to the dietetics service of the Veterinary Hospital (VTH) of Naples University. The aim of this investigation was to evaluate the clinical nutrition patients at VTH.

Animals, materials, and methods: In order to assess the main motivations for veterinarians and dog owners to seek nutritional counselling, the medical records database for the period January 2021-March 2023 was examined. Eighty-six dogs seen at the VTH were evaluated. Through the hospital's management system, information was collected from patients' medical records regarding reporting, history, diagnosis, and treatment. Specifically: age, sex, body condition score (BCS) on a 9-point scale, reason for visit, nutritional history, choice of nutritional therapy, whether cases had been referred from other departments in the hospital or were being visited for the first time, and on follow-up.

Results and discussion: Most of the dogs visited (about 55%) belonged to the adult age group (1-7 years old). In contrast, young and old subjects accounted for 18% and 27%, respectively. Male dogs accounted for 64% of the patients, however, neutered subjects were mostly females [1]. Evaluation of the BCS indicated that overweight subjects (BCS >6/9) outnumbered underweight subjects (BCS <3/9). The reason for the visit (Figure 1) included various diseases, but also healthy subjects for whom the prescription of an appropriate diet plan was requested. The nutritional history showed that 45% of the subjects were fed a mixed diet, partly commercial and partly homemade, often unbalanced and formulated by the owners themselves. The homemade diet was the most requested and consequently prescribed type of diet, which considered the needs of the animal and the owner [2]. The typical patient referred to the nutrition department was often a patient who already had a medical condition and was referred from another outpatient clinic or other departments at the

VTH. In addition, it was common to find patients with multiple pathologies at the same time. In fact, patients with BCS >6/9 often also suffered from osteoarticular, cardiac, liver, and/or urinary tract diseases. However, over time, cases of healthy individuals who visited for the prescription of a balanced diet plan, often a homemade diet, had also become more frequent. There was no relation between dog

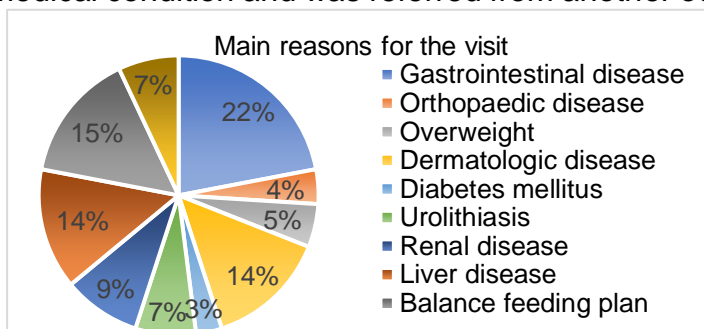


Figure 1. Main reasons for the visit

signalment data and diagnosed diseases.

Conclusion: Although the total number of registered cases has been affected by the COVID-19 pandemic period, it could be seen that to date companion animal owners grasp the importance of nutritional examination almost exclusively for therapeutic purposes and little for preventive reasons [3]. Moreover, follow-ups were not always consistent and stopped at the disappearance of symptoms, as was often the case during diagnostic courses, such as during the deprivation diet.

References: [1] Vandendriessche et al. (2017) *J Anim Physiol Anim Nutr*, 101: 4-14; [2] Dodd et al. (2020) *Veterinary Record*, 186: 643-643; [3] Blees et al. (2022) *Veterinary Medicine and Science*, 8,52-68.

3B12. Single versus group housing and feeding practices of pet rabbits: results from a survey in Germany

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Introduction: There is increasing interest in the optimal caring practice of pet rabbits [1], while little research has been carried out on how they are commonly fed. This survey study aimed to identify aspects of housing, feeding practices, and frequency of diarrhea and constipation in German pet rabbit owners and amateur breeders, and to explore associations between feeding aspects and diarrhea and constipation.

Animals, materials and methods: Two surveys, with closed and open multiple-choice questions, were developed to investigate answers from owners and breeders recruited in Germany through Facebooks groups and breeders' local clubs. Common parts of the surveys included animal characteristics - single or group housing - feeding aspects such as frequency and type of feed used, and self-reported diseases. The survey for breeders differed from that for owners by including additional questions regarding diarrhea and constipation in does, kits, and young rabbits. Data generated were submitted to frequency analysis of the explanatory variables, and a Chi-squared test was performed to compare diarrhea and constipation with feeding aspects. The relation was considered significant at $p < 0.05$ and data are presented as percentages (%).

Results and discussion: A total of 431 responses (owners=221 and breeders=210) were received. The majority of owners owned 2 or 3 rabbits (48% and 28%, respectively), mostly group-housed (94%), while 60% of breeders owned up to 50 rabbits, mostly single-housed (93%). The difference in housing conditions is explained by the need for breeders to keep rabbits used as breeders in single housing, despite the evidence of reduced animal welfare [2]. Regarding feeding habits, results showed that 74% of owners fed rabbits at least twice daily, while 67% of breeders fed once daily. Hay was not fed daily by 7% of owners and breeders, while 80% and 4% of owners, and 11% and 22% of breeders, did never feed high-fiber pellets and green leafy vegetables, respectively. Feeding hay *ad libitum* and a certain amount of pellets and green leafy vegetables daily are well-established concepts in rabbit nutrition [3]. Notably, more than 99% of both owners and breeders depicted their animals' body condition as normal, despite recent data on obesity prevalence in pet rabbits being 5-35% [4]. Regarding self-reported diseases, diarrhea was described as occurring at least one time in 23% and 76% of owners and breeders, respectively; regarding constipation, the same was true for 12% and 51% of owners and breeders, respectively. Breeders fed different diets to does during pregnancy in 48% of cases, with no association with the proportion of diarrhea or constipation ($p > 0.05$). Among people owning a minimum of a pair of rabbits, 87% of owners did not differentiate diet type and daily amount, while 71% of breeders fed differently, and this had a significant association with diarrhea ($p = 0.014$) and constipation ($p < 0.01$) in young rabbits. In fact, weaning has been linked to a higher risk of digestive disorders in young rabbits [5]. Data from owners and breeders showed that the daily use of concentrates ($p < 0.001$), and no daily use of green leafy vegetables ($p < 0.05$), resulted in an increased association with diarrhea and constipation.

Conclusion: This study gathered data on pet rabbits' housing and feeding practices and identified concerns that should be addressed, including the importance of feeding an appropriate diet at different life stages, which had been shown to influence the frequency of diarrhea and constipation occurrence.

References: [1] Bradbury & Dickens (2016) J. Small Anim. Pract. 57:503-9; [2] Verga et al. (2007) Horm. Behav. 52:122-9; [3] Clauss & Hatt (2017) Vet Clin North Am Exot Anim Pract. 20:871-84; [4] Adji et al. (2022) J. Exot. Pet. Med. 41:20-37; [5] Bivolarski & Bachkova (2014) J. Anim. Physiol. Anim. Nutr. 98:9-18.

Session 3C Companion animals II

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3C1. Assessment of nutrient concentrations and labeling adequacy of commercial insect-based pet food products

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Introduction: Over the past few years, the use of edible insects as a protein source has been promoted and several insect-based pet foods (IPF) are now available on the market [1]. Although some concern has been raised about possible nutritional challenges related to the use of insects as feed sources [2], the adequacy of IPF products has not yet been evaluated. The present study aimed to assess the nutrient concentration and labeling adequacy of commercially available IPFs.

Materials and methods: A total of 29 IPFs declared as complete diets for dogs (20 dry; 4 wet) and cats (5 dry) were purchased through online sources. Eight diets were suitable for puppies and three for kittens. Five diets declared other animal protein sources on their label. Ca, P, and Mg were declared in 24, 20, and 6 products, respectively. Dry matter (DM), crude ash, ether extract (EE), crude fiber (CF), and crude protein (CP) were analyzed according to AOAC [3]. Calcium (Ca), phosphorous (P), potassium (K), magnesium (Mg), copper (Cu), iron (Fe), and zinc (Zn) were determined by ICP-OES. Selenium (Se) was determined by ICP-MS. All products were measured as single samples. Dry and wet reference materials were used as a control with a coefficient of variance of <15%. The label information was compared to the analytical results and the legal regulations [4] when appropriate. Measured nutrient concentrations were compared to the minimum requirement (NCR) [5], recommended, and maximum allowances (FEDIAF) [6] in DM for the intended species and life stages.

Results and discussion: In total 4/29 dry diets (2 adult dogs; 1 puppy; 1 adult cat) were compliant with the label regulation and were nutritionally adequate for the intended species and life stage [6]. Differences between label information and nutritional analyses, according to the legally allowed differences [4], were observed in 4/29 diets for EE (below minimum: 2 adult dog and 2 puppy diets); 1/29 for crude ash (below minimum: 1 cat diet); 5/24 for Ca (above maximum: 1 adult dog, 1 puppy; below minimum: 2 puppies and 1 kitten diet) and 2/6 for Mg (below minimum: 1 adult dog and 1 adult cat diet). Those differences may be due to an inconsistent production of insect meal since the insect's life stage and feeding can change its nutritional composition. All products were compliant with the declared amounts of CP and P. When comparing the analyzed nutrient results with FEDIAF recommendations, 24/29 diets did not present values in the range for one or more nutrients. Some products presented nutrients below FEDIAF recommendation: 1 for EE (1 cat), 5 for Ca (2 puppies; 3 kittens), 1 for K (1 cat), 3 for P (2 puppies; 1 kitten), and 13 Se (9 dogs; 3 puppies; 1 kitten). Other products had nutrients above the maximum legal limit: 1 for Cu (1 dog), 1 for Zn (1 dog), and 6 for Se (5 dogs, 1 puppy). Ca:P ratio was high than the nutritional maximum limit in 1 dog's diet and low in 2 kitten's diets, which could lead to nutritional problems (e.g., nutritional secondary hyperparathyroidism) [5]. 27/29 exceeded the minimum amounts recommended by NRC, and two puppy diets were low in Se.

Conclusion: Despite the popularity of insects as an ingredient in pet food, several commercially available diets presented mismatched guarantee analyses on their labels. Some products did not meet the minimum amounts for NRC and recommended amounts for FEDIAF, and some even exceeded the legal and nutritional maximum limit for FEDIAF.

References: [1] Siddiqui et al (2023) J. Asia Pac. Entomol 26: 102020; [2] van Huis (2022) Entomol. Res. 52(4), 161-177; [3] AOAC. Official methods of analysis (2006); [4] COMMISSION REGULATION (EC) No 767/2009; [5] National Research Council. (2006) ; [6] FEDIAF Nutritional Guidelines (2021)

3C2. Environmental impacts of extruded dry dog food produced in Brazil, determined by the Life Cycle Assessment (LCA)

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Introduction: Despite of the importance that dogs and cats have for humans and the notable increased number of these animals, strategies focused on the sustainable development of the pet food industry must be prioritized. The first step to developing strategies to mitigate the environmental impact of an activity is the characterization of its key points. LCA (Life Cycle Assessment), adopted by ISO 14040-14044 [1] and recommended by FEDIAF [2], is an important tool for this purpose. The objective of this study was to quantify by LCA, the environmental impacts related to the production of an extruded Brazilian dry dog food in order to identify the main critical points.

Animals, materials and methods: The study was based on data obtained from a Brazilian Pet food company. The system boundaries were "cradle to gate" (activities of extracting raw materials, transport and processing, pet food production, packaging, and distribution), in the amount necessary to meet the energy requirements of a dog with 10 kg of body weight (Functional unit = 2.59 MJ/day; Reference Flux = 177.3 g/day). The method for the environmental impact calculation was EP 3.0 (v.1.00, SimaPro v. 9.1.1.1). The ingredients used in the formulation and packing material were modeled for Brazilian conditions (Ecoinvent

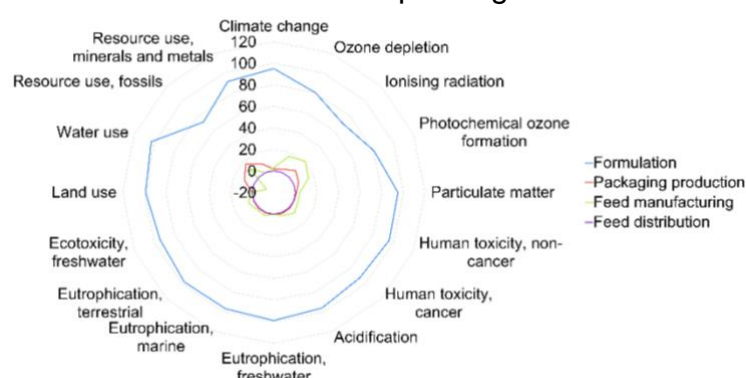


Figure 1. Environmental contribution of the main stages related to the Pet food system.

3.7.1 and Agri-footprint 5.0 Databases). Data regarding transport, processing, distribution, electric and thermal energy, water use, and waste were extracted from the company records from 2019 to 2020.

Results and discussion: The formulation was the most relevant phase, contributing with 70% to 90% of the total environmental impacts (Figure 1). The main impact categories were terrestrial and marine Eutrophication, Acidification, Particulate Matter, and Climate Change (80% of the total impacts). In this study, the environmental profile of the production of dry extruded food for medium-sized dogs in Brazil was associated with the emission of 88.73 kg of CO₂ Eq-/year, similar to what has already been described (106.52 kg of CO₂ Eq-/year) [2]. The choice of ingredients for the pet food formulation is an important decision when the environmental impact is considered. In this study, the animal meals (poultry byproduct meal and meat and bone meal) and vegetable coproducts (wheat bran and rice bran) contributed to the low environmental impact of the formulation under study, as described by other authors [4]. However, it is also important to take into account the profile and bioavailability of the nutrients, to make the right decision in the choice of ingredients when the environmental impact is targeted.

Conclusion: The formulation is the most relevant step to control the environmental impact in the production of dry extruded pet food. The main impact categories are terrestrial and marine Eutrophication, Acidification, Particulate Matter, and Climate Change.

References: [1] ABNT (2009) NBR ISO 14040-14044 ACV; [2] European Com. 2018. Product Environmental Footprint Category Rules (PEFCRs) Prepared Pet Food for Cats and Dogs; [3] Alexander, et al. (2020) G. Env. Chan. 65:102153; [4] Acuff et al. (2021) Vet. Clin. S. Ani. 51:563–581.

3C3. Effects of choline or L-carnitine supplementation on plasma fatty acids in lean and obese cats during weight maintenance

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Introduction: Obesity is a risk factor for feline hepatic lipidosis, and essential fatty acid (EFA) deficiencies might play a role [1]. Dietary choline (Cho) and L-carnitine (Lca) could prevent these conditions by increasing hepatic very low density lipoprotein secretion and fat oxidation, respectively [2]. This study assessed the effects of dietary Cho or Lca supplementation on plasma fatty acid (FA) profiles in lean (Ln) and obese (Ob) cats.

Animals, materials and methods: All procedures were approved by the University of Guelph Animal Care Committee (AUP#4496). Ln (n=9; body condition score [BCS] 4-5/9) and Ob (n=9; BCS: 8-9/9) adult (1-2-years-old) male neutered cats were split into three groups: Cho – 6 x recommended allowance [3]: 378 mg/kg body weight (BW)^{0.67}; Lca – 200 mg/kg BW; and Control (Con) – no supplement. A replicated 3 x 3 complete Latin square design was used with periods of 6 weeks, and 2-week washout. Cho or Lca was added on top of a commercial dry cat food (4100 mg choline/kg diet) and cats were fed for BW maintenance [3]. Blood collections occurred at the end of each 6 week-period after 24-h fasting. Total FA content was determined by gas chromatography [4] and the relative composition in percentage was calculated for each FA. Data were analysed by ANOVA with group and period as random effects, and treatment (Trt), BCS, and their interaction as fixed effects. Tukey's post-hoc test was used for comparisons (p<0.05).

Results and discussion: BCS and Trt effects are presented in Table 1. No interaction effect was observed. Lca led to higher linoleic acid (LA), while Cho led to higher arachidonic acid (AA), eicosapentaenoic acid (EPA) and n-6 FAs. Ob cats had higher AA, docosahexaenoic acid (DHA) and n-3 FAs, and lower LA, n-6 and n-6:n-3 than Ln cats. This differs from obese women that, even with adequate intake, had low blood n-3 index, measured as the ratio between EPA, docosapentaenoic acid and DHA to total FAs [5], though this is likely due to differences in feline FA metabolism [1,3].

Table 1. Plasma fatty acid composition (% total fatty acids) of lean and obese male neutered cats (n = 9 x 2 = 18) fed diets supplemented with choline (Cho) or L-carnitine (Lca) compared to control (Con).

Fatty acid	BCS		Trt			SEM ²	P-value	
	Lean	Obese	Con	Cho	Lca		Trt	BCS
Linoleic acid	35.3 ^A	33.7 ^B	34.4 ^b	33.5 ^b	35.7 ^a	0.032	<0.001	<0.001
Arachidonic acid	11.5 ^B	12.4 ^A	12.2 ^b	13.4 ^a	10.3 ^c	0.256	<0.001	0.005
Alpha-linolenic acid	1.11	1.14	1.15	1.08	1.15	0.028	0.519	0.599
Eicosapentaenoic acid	0.19	0.19	0.20 ^a	0.23 ^a	0.16 ^b	0.007	<0.001	0.884
Docosahexaenoic acid	0.85 ^B	1.04 ^A	0.93	1.02	0.90	0.034	0.192	0.002
Total n-6	48.8 ^A	48.1 ^B	48.5 ^{ab}	49.0 ^a	47.8 ^b	0.156	0.010	0.027
Total n-3	2.51 ^B	2.78 ^A	2.64	2.72	2.58	0.041	0.308	<0.001
n-6:n-3	19.7 ^A	17.5 ^B	18.6	18.2	18.9	0.315	0.659	<0.001
n-3 index	0.013 ^B	0.015 ^A	0.014	0.015	0.013	0.0004	0.097	0.003

¹BCS: body condition score; ²SEM: standard error of the mean.

Row means that do not share a capital (for BCS) or lowercase letter (for Trt) are different.

Conclusion: Obesity as well as Cho or Lca supplementation affect feline plasma EFAs. Remarkably, n-6:n-3 was lower in obese cats. More studies are warranted to better understand feline FA metabolism and mechanisms behind obesity, Cho, Lca as well as their combined effects.

References: [1] Verbrugge & Bakovic. (2013) *Nutrients*. 5:2811-35; [2] Verbrugge et al. (2021) *Animals*. 11:2196; [3] Nat. Res. Council. *Nutr. Req. Dogs and Cats* (2006); [4] Folch. (1957) *J. Biol. Chem.* 226(1):497-509. [5] Young et al. (2020) *Nutrients*. 12:1480.

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3C4. Study of the Potential Antioxidant Effect of a Diet Containing Fresh Blueberries on Exercise-Induced Stress in Dogs

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Introduction: Blueberries' anthocyanins are known to possess antioxidant properties, which could counteract the free radicals associated with oxidative stress in dogs [1, 2], which is linked to cell and tissue damage, and to several diseases related to aging [3]. The aim of this study was to investigate the effect of supplementing a diet with fresh blueberries on exercise-induced oxidative stress in dogs with no previous training. **Animals, materials and methods:** Eight Beagle dogs (4 males and 4 females, 3-5 years old and 13.6 ± 1.94 kg) were offered a maintenance control diet for 4 weeks before performing a first sub-maximal exercise session on an inclined treadmill (30 min running at 70% of their reserve heart rate) [4]. Then, 4 randomly selected dogs continued with the control diet (CON, n = 4), whereas the rest received the same diet containing blueberries (3%) (BLB, n = 4). After 4 weeks, dogs performed a second exercise session. Blood samples were collected before (t₀), just after (t₁) and 24 h after (t₂) each exercise. Creatine kinase (CK), C-reactive protein (CRP) and urea were measured as exercise-stress indicators whereas malondialdehyde (MDA), total antioxidant status (TAS), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured as antioxidant/oxidant status indicators. Two-way ANOVA tests were used to analyze the data (RStudio, 1.4.1106 version).

Results and discussion: The first sub-maximal exercise induced increases in CK (p = 0.045), urea (p = 0.003) and MDA (p < 0.001) at t₁, returning to basal concentrations after 24 hours, except MDA which was still significantly higher than at t₀. When assessing the effect of diet on the antioxidant/oxidant status, the most noteworthy variation was in TAS. Whereas in the first session TAS tended to be higher in CTR compared to BLB, this trend disappeared in the second session (Table 1). When comparing TAS levels between the first and the second session for each time, the only relevant increase was observed with the BLB diet at t₁, suggesting a higher ability in these animals to cope with oxidative stressors. Regarding other parameters, no differences related to diets were found.

Conclusion: Submaximal exercise induced immediate increases in CK, CRP and MDA,

Table 1. Total antioxidant status values (mmol/L) ± SEM for each timepoint and group in the two sub-maximal sessions. G1/G2 = group 1/2; CON = control; BLB = blueberry. ^{xy} indicate 0.05 < P < 0.10. n = 4.

		t ₀	t ₁	t ₂	\bar{x}	p-value
Session 1	G1: CON	1.18 ± 0.028	1.18 ± 0.038	1.12 ± 0.014	1.16 ± 0.018^x	0.057
	G2: CON	1.06 ± 0.050	1.09 ± 0.039	1.14 ± 0.028	1.09 ± 0.023^y	
	\bar{x}	1.12 ± 0.035	1.14 ± 0.031	1.13 ± 0.015		
Session 2	G1: CON	1.12 ± 0.021	1.15 ± 0.030	1.12 ± 0.030	1.13 ± 0.015	0.245
	G2: BLB	1.07 ± 0.037	1.13 ± 0.021	1.04 ± 0.072	1.08 ± 0.028	
	\bar{x}	1.09 ± 0.022	1.14 ± 0.018	1.08 ± 0.039		

No significant differences were found related to time nor the interaction diet x time.

reflecting muscle damage and lipid peroxidation, and validating the submaximal exercise model. The lower changes in the TAS concentration (average of t₀, t₁ and t₂) between sessions in the BLB group compared to CON, would suggest that the dietary inclusion of 3 % blueberry for 4 weeks could potentially provide a better antioxidant status to the animals in front of oxidative stressors. However, more research including larger number of animals and/or longer terms are required to manifest more clearly this potential.

References: [1] Kalt et al. (2020). American Society for Nutrition. 11: 224-36. [2] Dunlap et al. (2006). Comparative Biochemistry and Physiology, Part A. 143: 429-34. [3] McMichael MA. (2007). JAVMA. 231(5): 714-20. [4] Tipton et al. (1974). Journal of Applied Physiology. 37: 271-5.

Session 3D Companion animals III

Chair: Marco Fantinati

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LALLEMAND ANIMAL NUTRITION



3D1. Nutritional considerations of the diet of Neolithic dogs: a literature review and scenario analyses

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Introduction: The ancestors of our modern-day pet dogs diverged from carnivorous wolves around 40,000 years ago in the Palaeolithic era when humans had a hunter-gatherer lifestyle. During the following Neolithic era, the period that humans became more sedentary agriculturalists, the food sources available for the (proto) dogs living with humans also changed. The dog's genome shows signatures of an enhanced digestive capacity to digest starch, which reflects a profound shift in the dog's diet with increased amounts of cereal grains throughout this era [1]. The foraging strategies these dogs had to develop to meet energy and nutrient requirements are, however, understudied. We therefore aimed to advance our understanding of the nutrition of Neolithic dogs.

Materials and methods: Data from various publications that included stable isotope, coprolite and observational analyses were collected and compiled into a literature review. Hypothetical feeding strategies (scenarios) were formulated based on commonly reported food sources and it was investigated how dogs could manage their dietary needs in terms of metabolizable energy (ME), nitrogen and indispensable amino acids. Reference tables for feedstuff and human food sources were used for nutrient composition. The composition of human faeces (including indispensable amino acids) was based on data from total tract digestibility studies reported in the literature. The ME contents were estimated based on the chemical composition using modified Atwater factors.

Results and discussion: Stable isotope and coprolite analyses suggest that Neolithic dogs had a highly variable diet, with a large variety in food items and differing ratios of plant-based food sources (e.g. millet, maize) to animal-based food sources (e.g. livestock, rodents). Observations support these findings with e.g. plant-based sources contributing 24 or 66% of the biomass, animal-based sources 17 or 54%. Moreover, human faeces are often noted in the literature as a food source for dogs with estimates 17 or 24% of the dietary biomass. The ME requirement is shown to be easily met with the addition of animal-based feedstuffs and nitrogen requirements were always met if the dietary intake was sufficient to cover for minimum ME. However, several amino acids (i.e. Met+Cys) were limiting if diet contained too little quantities of animal-based feedstuffs. For example, 0.38 kg (as is) of millet per day seems to be sufficient to cover energy needs of an active 15 kg dog (energy requirements based on FEDIAF [2]), but does not provide sufficient Met+Cys (1.23 vs 1.73 g/d; NRC [3]). In contrast, consumption of 0.84 kg goat meat would be sufficient to meet both the ME and the Met+Cys requirements. Supplementing 0.31 kg millet with 0.29 kg human faeces per day would be sufficient to meet the ME and Met+Cys requirements, assuming 100% bioavailability of these amino acids. Data from various publications and these scenarios provide context for the nutrition of present-day dogs and coprophagy commonly observed.

Conclusion: Neolithic dogs developed an opportunistic foraging strategy resulting in diverse omnivorous diets. To support indispensable amino acids, dogs likely could not thrive solely on cereal-based diets but required to supplement these with animal-based food sources or human faeces.

References: [1] Axelsson *et al.* (2013) The genomic signature of dog domestication reveals adaptation to a starch-rich diet. *Nature* 495: 360-364; [2] FEDIAF (2021) Nutritional guidelines for complete and complementary pet food for cats and dogs; [3] NRC (2006). Nutrient requirements of dogs and cats.

3D2. Untargeted serum metabolomic analysis comparing healthy and diabetic dogs

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Introduction: The objective of this research was to evaluate the metabolomic profiles of blood serum of diabetic dogs and compare them to those of healthy dogs using a study model for DM1 in humans [1].

Animals, materials and methods: The procedures were previously approved by the Committee on Ethics in the Use of Animals (protocol: 4668091214 and 7943060716). Ten healthy adult dogs [control group (CG)] fed a maintenance diet (standardized) and ten diabetic dogs [diabetic group (DG)] fed a hypocaloric diet for maintenance (standardized) and under insulin therapy (NPH type), were included. Metabolites were identified on the high-resolution ¹H Nuclear Magnetic Resonance (NMR) spectra and statistical multivariate analysis was realized using SIMCA-P software (version 16.0, Umetrics). Principal Component Analyses (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) models were constructed using Pareto scaling pre-processing of the animals' blood serum to discriminate and identify the metabolites that differentiated groups (53 observations x 236 variables).

Results and discussion: In the PCA, most of the CG was grouped into negative principal component 1 (PC1) and principal component 2 (PC2) (Figure 1). The majority of DG clustered in negative PC1, but disseminated in PC2. The PC1 load graph revealed that the metabolites that influenced the separation on axis 1 were α - and β -glucose. Furthermore, DG distributed in positive PC2 showed higher concentrations of glucose and lactate. The column graph for PLS-DA axis 1 showed that glucose was the metabolite with the highest concentration in the DG, and this group also had higher concentrations of lipid metabolites, lactate and branched-chain amino acids (BCAAs) (Figure 2). Increased lactate may be associated with decreased insulin action [2]. Diabetic dogs had higher valine levels related to increased BCAA [3], but the increase in lipids is characteristic of type 2 diabetes mellitus (DM2) in humans [4] and metabolic changes in diabetic dogs [5].

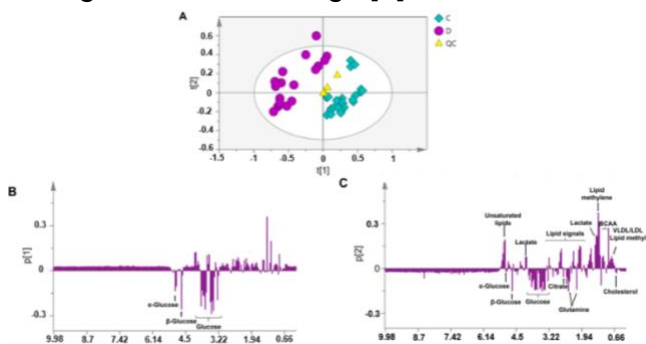
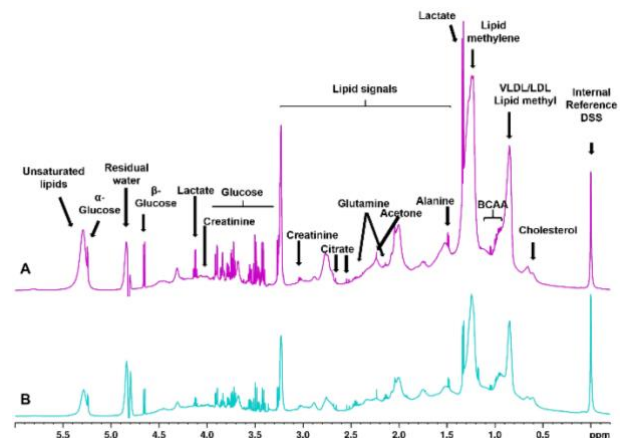


Figure 1. PLS-DA of ¹H NMR data of dogs' serum (A) PLS-DA score scatter plot of axis 1 versus axis 2; (B) loadings column plot of axis 1 of PLS-DA, showing the main metabolites that influenced the separation; (C) loadings column plot of axis 2 of PLS-DA, showing the main metabolites that influenced the separation. Label: C: Control group; QC: quality control pool of samples; D: diabetic group

Figure 2. 1D NOESY-presat ¹H NMR spectra highlighting the main detected metabolites of (A) diabetic dogs; (B) control group



Conclusion: The metabolomic profiles of diabetic dogs differed from healthy dogs. However, more studies are needed to understand the increase in lipids in diabetic dogs and how specific metabolites influence the pathophysiology of diabetes in dogs.

References: [1] Moshref et al. (2019) Stem. Cell. Trans. Med. 5:450-455; [2] Vangipurapu et al. (2020) 6:1319-1325; [3] O'Kell et al. (2021) Metab. 17:1-13; [4] Jin et al. (2021) Cells. 11:1-38; [5] Teixeira F.A. (2020) 106 p. Thesis USP

3D3. Mixed feeding practice reduces weight loss time compared to dry food in obese dogs

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Introduction: Obesity is the most common nutritional disorder in pets [1], so strategies to improve weight loss, such as mixed feeding (combination of dry and wet food) should be investigated. The aim of this study was to evaluate the use of mixed feeding in a weight loss protocol for obese owned dogs and its effects on biochemical, inflammatory, and hormonal profiles.

Animals, materials and methods: Dogs with body condition score (BCS) of 8 and 9/9 [2] without comorbidities were selected and divided into: Group DF composed of eight obese dogs fed 100% of the energy requirement for weight loss (ERWL) as dry hypocaloric food (HF); and Group MF, composed of 11 obese dogs receiving its ERWL as 80% of dry HF and 20% of wet HF (mixed feeding). The estimated ERWL was calculated according to $ERWL=292.6x(TW)^{0.75}(kJ/day)$, where TW (target weight) is the initial body weight (BW) reduced by 20%. Follow-up occurred monthly, weekly weight loss rate (WWLR) was calculated, and diet amount adjustments were made if necessary [3]. The weight loss protocol ended when a BCS of 5/9 or TW was achieved. The effectiveness of the protocol was evaluated through its duration (months), WWLR (%), weight (kg), BCS, and caloric intake (kJ/day). Blood samples were collected at the beginning and at the end of the protocol to analyze biochemical (total protein, albumin, ALT, AP, urea, creatinine, triglycerides, and cholesterol); inflammatory (C-reactive protein, IL-1 β , IL-6, IL-10, and TNF- α), and hormonal profiles (amylin, ghrelin, GLP-1, glucagon, insulin, leptin, and PYY).

Table 1. Weight loss variables of groups DF and MF.

Variable		DF	MF	p value
Weight loss time (months)		7.6 \pm 1.5	5.5 \pm 2.0	0.020 ⁽¹⁾
WWLR (%)		0.83 \pm 0.10	0.98 \pm 0.39	0.302 ⁽²⁾
Weight (Kg)	Initial	29.0 \pm 13.2	23.0 \pm 12.7	0.355 ⁽³⁾
	Final	22.8 \pm 10.5	18.6 \pm 10.2	
p value		0.001 ⁽⁴⁾		
Caloric intake (kJ/day)	Initial	3030.0 \pm 1100.0	2545.1 \pm 1022.6	0.489 ⁽³⁾
	Final	2744.3 \pm 943.5	2546.0 \pm 1113.4	
p value		0.060 ⁽⁴⁾		
BCS	Initial	8.9 \pm 0.4	8.6 \pm 0.5	0.138 ⁽²⁾
	Final	5.5 \pm 0.8	5.3 \pm 0.5	
p value		0.010 ⁽²⁾	0.002 ⁽²⁾	

(1) Student's t test (2) Non-parametric Mann-Whitney test (3) Comparison Between Groups (ANOVA) (4) Comparison between times (ANOVA)

weight loss, confirming the effectiveness of caloric restriction. No difference was noted in the initial and final mean caloric daily intake between groups, but the MF group presented a minor variation along the program, representing fewer adjustments throughout the process. For the biochemical and inflammatory profile, there was no difference between groups ($p>0.05$, data not shown). After weight loss, MF and DF serum leptin levels were reduced ($p<0.001$) and serum ghrelin levels increased ($p=0.002$), expected results for appetite-related hormones when adipose tissue reduces and in negative energy balance [5].

Conclusion: Mixed feeding practice reduced weight loss time compared to dry food in obese owned dogs.

References: [1] GERMAN et al. (2010) Vet Jour, v.185 n.1, p. 4–9; [2] LAFLAMME (1997). Can Prac, v. 22, n. 4, p. 10-15, 1997; [3] BROOKS et al (2014). Jour of the Amer Ani Hosp Assoc, v. 50, n. 1, p. 1–11, 2014 [4] SERISIER et al. (2014) Jour of Nutri Sci, vol. 3, e. 59, p. 1-5; [5] JEREMIAS et al. (2020) Brit Jour of Nutri, v. 113, n. 1.

Session 4A Farm animals I
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4A1. Association between 16S RNA-based ruminal microbiota composition and ruminal lesions in grazing cattle slaughtered in southern France: a pilot study

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Introduction: In 2020, the official veterinarian of the slaughterhouse in Aurillac, France, observed numerous ruminal lesions, mostly attributed to rumenitis, in cattle from a grassland system. Rumenitis is often secondary to subacute ruminal acidosis (SARA), which is commonly observed in cattle fed high-concentrate diets [1]. However, grazing cattle can also develop rumenitis from excess ryegrass or clover intake, leading to imbalanced microbiota and butyric acid production [2]. This pilot study aims to investigate whether microbial dysbiosis in the rumen is associated with rumenitis-like lesions.

Animals, materials and methods: During summer 2021, rumen samples from 20 randomly selected cows previously grazing on grasslands were collected at the slaughterhouse of Aurillac, France. Bacterial DNA was extracted and sequenced using 16s rRNA gene with Illumina Miseq technology. Bioinformatic analysis of sequences was implemented with FROGS [3], resulting in an abundance table of amplicon sequence variants (ASVs). Abundance values were transformed using Centered Log-Ratio (CLR) after replacing zeros with the GBM method. Rumenitis-like lesions were defined based on color (normal/abnormally dark), inflammation (absence/presence), and papillary modifications (no/yes). We used PLS-DA (R version 4.1.0, package Mixomics) to discriminate between normal and abnormal groups for each type of lesion, based on the analysis of bacterial communities. ASVs were identified as discriminant between the groups when their Variable Importance in Projection (VIP) score exceeded 1. The efficacy of our multivariate analysis was evaluated using the area under the ROC (Receiver Operating Characteristic) curve, and overall BER (Balanced Error Rate) which were found to be respectively close to 1 and 0, indicating excellent results. We limited the number of ASVs through Selbal prior to the PLS-DA analysis.

Results and discussion: We obtained 222,572 sequences regrouped in 2544 ASVs, representing 63 bacterial families. The dominant phylum was *Firmicutes* (56% of total sequences), followed by the phylum *Bacteroidota* (38% of total sequences). The most abundant families within these phyla were Lachnospiraceae (20% of total sequences) and Prevotellaceae (17% of total sequences), respectively.

Abnormal color concerned 13 cows and was associated with a higher abundance of an ASV belonging to *Oscillospiraceae NK4A214 group* genus and lower abundances of three ASVs belonging to *Treponema*, an unknown from *Muribaculaceae* family and *Ruminococcus* genera, compared to the seven normal color rumen. Inflammation was present in seven cows and was associated with higher abundances of two ASVs belonging to *Prevotella* and *Rikenellaceae RC9 gut group* genera and a lower abundance of an ASV belonging to the *Christensenellaceae R-7 group* genus, compared to the 13 non-inflamed rumens. Papillary modifications were observed in nine cows and were associated with a higher abundance of an ASV belonging to *Prevotella* genus and a lower abundance of an ASV belonging to an unknown genus from the [*Eubacterium*] *coprostanoligenes group* family.

Compared to SARA's impact on ruminal microbiota, our results are closer to SARA induced by alfalfa pellets than that induced by a high-grain diet [4].

Conclusion: This pilot study confirms an association between dysbiosis and rumenitis-like lesions in grassland system. The causal relationship is yet to be determined, but it may involve phenomena close to SARA.

References: [1] Fu et al. (2022) *Microorganisms*, vol. 10, no 8, p. 1495; [2] Kleen et al. (2003) *J. Vet. Med. Ser. A*, vol. 50, no 8, p. 406-414; [3] Escudié et al. (2017) *Bioinformatics*. 34-8:1287–1294; [4] Khafipour et al. (2009) *Applied and Environmental Microbiology*, p.7115-7124.

4A2. Potential association between Negative Energy Balance and thyroid cysts in milking dairy cows: preliminary results

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Introduction: Thyroid metabolism is poorly studied in ruminant medicine, although it is strongly involved in many metabolic pathways, such as milk and meat production [1]. The purpose of this study is to investigate the potential link in dairy cows between thyroid cysts (TC) observed on thyroid ultrasound (TU) [2] and their energy metabolism, assessed by blood assays and Body Condition Score (BCS).



Figure 1. Ultrasound images of the right lobe of the thyroid gland of a cow with cysts. (a) transversal view; (b) sagittal view. T= trachea, Th= thyroid; c= cysts.

Animals, materials and methods: TU (Mindray, DP50-Vet; convex probe, 5 mHz) [2] and blood sampling were performed on 176 randomised milking cows from 5 different farms. Beta-hydroxybutyrate (BHB) and glucose were directly measured on fresh blood (Precision Xceed®, Abbott). In addition, urea, cholesterol, albumin, non-esterified fatty acids (NEFA), plasma inorganic iodine, selenium, total thyroxine were assayed in 14 cows with TC and 28 healthy cows without TU anomalies. Healthy cows are defined via a clinical examination and blood test results.

Results and discussion: Out of these 176 cows, 43 had TC (Figure 1). Compared with healthy cows, those with TC had a BCS significantly lower (mean± standard deviation=2.25±0.3; t-test p value <0.01), a NEFA blood concentration significantly higher (0.3±0.2 mmol/L; t-test p value <0.05), and a trend for a lower serum selenium concentration (67±16.5 µg/L; t-test p value=0.056). When

comparing the percentage of TC in the 5 farms, this percentage was significantly negatively correlated with the blood glucose/BHB ratio of all cows examined in each farm (Pearson correlation; $r = -0.92$; p value < 0.05; Table 1). In human medicine, a risk factor for the development of thyroid nodules (including cysts) is obesity and metabolic syndrome [3,4,5]. Measurements of BCS, BHB, glucose, NEFA allow to evaluate the energy balance of cattle [6,7].

TC would rather appear in cows that have lost weight, in negative energy balance. This study shows a relationship between these energy parameters and the incidence of TC in cattle. The potential role of selenium should also be studied, although its role in the development of thyroid nodules is controversial in human medicine, despite its involvement in synthesis of thyroid hormones [8,9].

Conclusion: These preliminary results represent an interesting path in the future evaluation of a feed ration in cattle and the detection of farms with energy imbalances, although more work is needed to validate TU in a nutrition monitoring context.

Table 1. Diet information in relation with the thyroid cysts (TC) percentage.

FARM	UFL/KG	g PDI/KG	PDI/UFL	% TC
1	0.92	77.9	84.7	22.2
2	0.92	84.6	92	8.9
3	0.94	83.1	88.7	20.6
4	0.94	80.8	86	46.2
5	0.92	97	105.4	51.6

References: [1] Huszenicza et al. (2002) *Veterinari Medicina* 47(7):199-210; [2] Eppe et al. (2023) *Vet Sci, in press*; [3] Ross et al. (2017). *UpToDate*. [4] Sousa et al. (2013) *Endocrinol Metabol* 57(2): 120-5. [5] Shin et al. (2016) *J Intern Med*. 31(1):98-105.[6] Dubuc et al. (2018), *J Dairy Sci*. 101(4); 3374-3379. [7] Nicola et al. (2022) *J Dairy Sci*. 105(11):9098-9106. [8] Samir and El-Awady (1998). *Clin Otolaryngol Allied Sci*. 23(6):512-4; [9] Sakiz et al. (2016). *Biol Trace Elem Res*. 174(1):21-26.

4A3. Effects of chitosan-loaded manganese nanoparticles on the production performance, serum metabolites, meat quality, and intestinal histomorphometry in broilers

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Introduction: Manganese (Mn), though a fundamental trace mineral in poultry nutrition, may cause environmental concerns due to increased excretion rate when consumed at high levels. The use of Mn in the form of nanoparticles (NPs) is considered to enhance its bioavailability with limited excretion in the environment. However, there is limited information regarding the dietary inclusion of smaller safe dosages of Mn-NPs in broilers. The present study, therefore, aims to investigate whether the use of varying doses of dietary chitosan-loaded Mn-NPs would positively affect the growth performance, serum metabolites, meat quality parameters, and jejunal microarchitecture in broilers.

Animals, materials, and methods: Day-old Ross broilers (n=200) were randomly divided into five treatments, each containing forty birds, with five replicates of eight birds. Birds were fed on a corn-based diet supplemented with 20, 40, 60, or 80 mg/kg chitosan Mn-NPs (average particle size 80nm) or only 80 mg/kg MnSO₄, called bulk MnSO₄, for 35 days. Zootechnical parameters were measured weekly. On day 35, 10 birds from each treatment (2 birds/replicate) were slaughtered to measure the relative weights of viscera. At the time of slaughtering, blood samples were collected to harvest serum for biochemical metabolites determination. Selected indices of meat quality of breast muscles (pH, cooking loss, and water holding capacity) and jejunal histomorphometry were measured. Data were analyzed by one-way ANOVA (SPSS Inc., USA), and when the means were significant at P<0.05, Tukey's test was employed.

Results and discussion: Growth performance was not influenced by the Mn-NPs supplementation except higher FCR (P<0.01) in the 80-Mn-NPs group compared to the other groups on week 2. Relative weight of the gizzard was lower (P<0.05) in the 40-Mn-NPs group compared with the other treatments. The pH_{15min} of breast muscle determined at 15 minutes post-slaughtering was lower (P<0.05) in the 80-Mn-NPs compared with birds in the Mn-NPs 20 and 40 treatments. However, pH_{24hrs} of the same breast muscle, measured 24 hours post slaughtering, was lower (P<0.01) in the 40-Mn-NPs and the bulk-MnSO₄ group than in the 80-Mn-NPs fed birds. The inclusion of Mn-NPs did not affect the lipid profile, hepatic profile, renal profile, water-holding capacity, and cooking loss of meat. Jejunal villi were taller (P<0.001) in the 20-Mn-NPs compared with the other groups. On the other hand, birds supplemented with 80 mg/kg of Mn-NPs had shorter villi (P<0.001) and lesser crypt depth (P<0.001) when compared with the other treatments suggesting that a high level of Mn-NPs might have interfered with the absorption of other micro-minerals that needs to be investigated.

Conclusion: Replacing the bulk form of manganese sulfate with reduced levels of Mn-NPs in the broiler's ration may improve jejunal microarchitecture and breast muscle pH without any negative effects on growth performance and serum health markers. Further studies are warranted to investigate the bioavailability of Mn-NPs in lower doses and their possible interactions with other trace elements.

4A4. Effects of dietary supplementation of pomegranate peel in laying hens on egg quality and nutritional parameters

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Introduction: Pomegranate peel by-product (PPB) is used as a feed additive in poultry diets to improve birds' health and productivity. It contains bioactive compounds, including high molecular weight phenolics, ellagitannins, proanthocyanidins, flavonoids and microelements [1]. In broilers, previous studies showed that dietary inclusion of PPB reduced the incidence of ascites [2] and improved the antioxidant and quality indices of breast meat [3]. In laying hens, PPB alleviated the oxidative stress induced by dexamethasone [4]. However, literature about how PPB affects egg quality is limited. The objective of the study was to evaluate if egg quality could be improved following dietary supplementation of a PPB in laying hens.

Animals, materials and methods: Forty-eight Isa Brown laying hens, about 36-weeks-old, were allotted to four groups and fed on the following treatments for 8 weeks: Control (0% PPB); PPB-1 (1% PPB); PPB-2.5 (2.5% PPB); PPB-5 (5% PPB). Eggs were analyzed for quality parameters, yolk malondialdehyde (MDA) content, fatty acid profile, lipidomic profile, total phenolic content, α -tocopherol content and cholesterol content. Statistical analysis was performed using one-way ANOVA (IBM SPSS Statistics). Post hoc comparisons were investigated by Tukey's test. For parameters not following normal distribution, the data were analyzed with Kruskal-Wallis and Mann-Whitney tests.

Results and discussion: Eggshell weight and thickness were higher in PPB-5 group compared to PPB-2.5 ($P < 0.05$). Specific gravity was higher in PPB-5 group compared with Control and PPB-1 ($P < 0.05$). Probably the Ca and P found in PPB are responsible for these effects. Eggshell color was lighter in PPB-2.5 and PPB-5 groups compared with Control and PPB-1 ($P < 0.001$). All treatments enhanced ($P < 0.001$) yolk color compared to Control, as reported previously [5]. MDA content in egg yolk was lower in PPB-2.5 and PPB-5 groups compared to Control and PPB-1 ($P < 0.001$), indicating reduction of lipid peroxidation, which agrees with a recent study [6]. Yolk fatty acid profile, total phenolic and α -tocopherol contents were not affected by the treatments. Yolk Cholesterol content was higher ($P < 0.05$) in PPB-1 and tended ($P = 0.062$) to increase in PPB-2.5 group compared with Control. For lipidomic analysis, lipids belonging mainly to the group of ceramides were found statistically significant for the differentiation of the samples (Control vs PPB-2.5 and PPB-5) in the positive and negative ionisation modes.

Conclusion: Conclusively, PPB supplementation at 5% level in laying hens diet improved eggshell weight, eggshell thickness and egg specific gravity with concomitant reduction in the yolk lipid peroxidation. All PPB levels enhanced yolk color. However, 2.5% and 5% PPB levels resulted in lighter eggshell colors, while the inclusion of lower doses of PPB increased yolk cholesterol contents.

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Session 4B Farm animals and companion animals Posters



4B1. Fiber composition in sows' diets influences bile acid profile in colostrum

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Introduction: Colostrum is an important source of nutrients and bioactive compounds to newborn piglets [1]. Dietary fibre has been reported to modify colostrum and milk composition in sows [2]. Bile acids aid in fat digestion and lipid absorption and are important signaling molecules for the digestive function, cell proliferation, immune system and they exert antimicrobial properties [3]. Whether colostrum could be a carrier of bile acids for the offspring remains a question. Thus, the aim of this study was to determine bile acid composition and concentration in colostrum obtained from sows fed different dietary fibres during gestation and lactation.

Animals, materials and methods: Twenty sows (6 pluriparous, 14 gilts) were fed gestation and lactation diets enriched with either 15 % high-fermentable sugar beet pulp (SBP, n=10) or 15 % low-fermentable lignocellulose (LNC, n=10). The diets were isocaloric and isonitrogenic. Colostrum was collected once the afterbirth was excreted, which was within 10 h after beginning of farrowing, and assessed for bile acids using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). Data were analysed using Student t-test ($\alpha=0.05$). The animal trial was approved by the LAGeSo Reg. G0112/19.

Results and discussion: Altogether, five primary and seven secondary bile acids were identified in colostrum. Colostrum from the sows fed SBP vs. LNC had significantly higher concentrations of lithocholic acid (0.44 $\mu\text{g/mL}$ vs. 0.10 $\mu\text{g/mL}$, $p=0.0007$), hyodeoxycholic acid (1.60 $\mu\text{g/mL}$ vs. 0.89 $\mu\text{g/mL}$, $p=0.0144$), taurodeoxycholic acid (0.04 $\mu\text{g/mL}$ vs. 0.03 $\mu\text{g/mL}$, $p<0.0001$), oxolithocholic acid (0.31 $\mu\text{g/mL}$ vs. 0.08 $\mu\text{g/mL}$, $p=0.0016$), and tauroolithocholic acid (0.12 $\mu\text{g/mL}$ vs. 0.03 $\mu\text{g/mL}$, $p<0.0001$). These secondary bile acids are products of colonic microbial biotransformation i.e. deconjugation and dehydroxylation of the primary bile acids [3]. On the contrary, colostrum from the sows fed LNC vs. SBP had significantly higher concentration of trihydroxycholestanic acid (0.16 $\mu\text{g/mL}$ vs. 0.08 $\mu\text{g/mL}$, $p=0.0099$), a precursor of cholic acid, while a trend for a higher glycohyodeoxycholic acid (0.28 $\mu\text{g/mL}$ vs. 0.14 $\mu\text{g/mL}$, $p=0.0605$) was observed. There was no difference in the concentration of chenodeoxycholic acid, hyocholic acid, taurochenodeoxycholic acid, glycohyocholic acid and taurohyodeoxycholic acid in colostrum in either of the sows' dietary groups.

Conclusion: Porcine colostrum contains bile acids. Addition of certain dietary fibres to sows' feed may differentially influence (through the microbial biotransformation) the colonic composition of primary and secondary bile acids, which are then transferred to colostrum. This can have potential consequences on piglet intestinal health.

References: [1] Quesnel (2011) *Animal* 5:1546-53; [2] Grześkowiak (2022) *Animal*. 17:100697; [3] Keating and Keely (2009) *Curr. Gastroenterol. Rep.* 11:375–382.

4B2. Oat hulls in the diet of newly weaned piglets modified gastric conditions and morphology

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Introduction: Insoluble dietary fibre can modify gastrointestinal conditions and morphology of pigs with effects depending on its particle size [1]. Previously, in suckling piglets offered a diet with oat hulls (OH), subtle intestinal maturational effects were observed when compared to a low fibre control diet whereas no effects were reported for the stomach [2], contrasting data in older pigs. The current study reports the results in newly weaned pigs fed similar diets in order to investigate the effect of OH on the developing stomach.

Animals, materials and methods: Piglets originating from 10 litters had access to a finely milled, nutrient dense control diet (CON; 10.38 MJ NE, 12.49 g/kg SID Lys) or diets containing 15% OH, either finely or coarsely milled, replacing corn starch (OH-f and OH-c, respectively) from 14 days prior to weaning onward. Piglets were weaned at 24 days of age (7.2 ± 1.04 kg) and individual feed intakes and body weight gains were recorded. On day 13-14 after weaning, per treatment twelve randomly selected piglets were sacrificed and stomachs were collected. The *pars non-glandularis* (PNG) was visually scored for hyperkeratosis as an early sign of ulceration [2]. Gastric pH and dry matter (DM) concentrations were determined for the proximal and distal region. Data were evaluated using the GLM and GENMOD (PNG data) procedures of SAS Studio. Body weight at dissection and feed intake were retained as covariates when their P-value was <0.200.

Results and discussion: The supplemental feed intake of suckling piglets and their post-weaning dry feed intake and body weight gain did not differ between treatments ($P > 0.10$).

Table 1. Gastric characteristics of piglets (9.4 ± 1.69 kg) fed either a control diet (CON) or a diet with fine (OH-f) or coarse oat hulls (OH-c)

	CON	OH-f	OH-c	SEM	P-value
Empty weight, g	73.9 a	88.1 b	97.6 c	1.99	< 0.001
Contents weight, g	225.0 a	360.1 b	335.4 b	29.49	0.006
Proximal pH	3.40 a	3.64 a	4.33 b	0.188	0.004
Distal pH	3.52	3.40	3.39	0.222	0.904
Proximal DM, %	14.4 a	19.0 ab	25.8 b	1.34	< 0.001
Distal DM, %	23.8	23.1	28.1	1.98	0.170

consumption of diets with OH resulted in increased empty stomach ($P < 0.001$) and contents ($P = 0.006$) weights (Table 1). Moreover, proximal pH ($P = 0.004$) and proximal DM% ($P < 0.001$) were greater in piglets fed a diet with coarse OH. The PNG scores differed between treatments with piglets from OH-c having intact mucosa (12 out of 12 with score 0) whereas hyperkeratinisation was observed in 5 and 10 out of 12 observations for OH-f and CON, respectively ($\chi^2 = 22.15$, $P < 0.001$). These early mucosal aberrations, also reported in older piglets [3], may be related to the fibre content and particle size of the diet affecting intra gastric conditions.

Conclusion: The dietary inclusion of oat hulls resulted in heavier stomachs. In addition, coarser oat hulls increased proximal gastric pH and DM % and improved the *pars non-glandularis* condition.

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4B3. The action of zearalenone on enzymes of phase I (CYP1A2) and phase II (COMT, UGT) mycotoxin metabolism in the liver of healthy and ketotic cows *in vitro*

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Introduction: Ketosis is a metabolic disorder occurring in dairy cows in early lactation which can result in significant economic losses in dairy farming [1]. Zearalenone (ZEA), on the other hand, is a mycotoxin produced by *Fusarium sp.* that can contaminate animal feeds such as corn silage. ZEA has been shown to exacerbate the negative effects of ketosis by impairing liver function and increasing oxidative stress. This can lead to a variety of health problems, including decreased milk production, reproductive issues, and even death in severe cases [2]. The study focuses on investigating whether the impact of this mycotoxin affects the detoxification capacity of the liver cells for zearalenone by examining the level of expression of enzymes of the I and II phase of mycotoxin metabolism by cows suffering from ketosis in relation to healthy cows.

Animals, materials and methods: Bovine hepatocytes were isolated from 0.5 g liver biopsies taken from Holstein Friesians cows in their second or later lactation divided into two groups: healthy cows (level of ketone bodies in blood < 1.4 mmol/L, control group, n = 3 cows) and from animals with clinical ketosis (level of ketone bodies in blood > 3 mmol/L; n = 3 cows) being in lactation (0-14 days in milk). All samples were collected with permission of Local Ethics Committee for Animal Experimentation. Liver explants (4 for each dose and control) were cultured in William's E medium, maintained at 38.5°C and exposed to zearalenone in doses of 0-100 µM for 1, 3, or 6 hours. mRNA expression of selected genes (CYP1A2, COMT, UGT) was analysed by the qPCR method. Protein expression was then confirmed by additional Western blot analysis. Data were analysed by two-way analysis of variance (ANOVA), with ketotic state and ZEA dose as fixed effects followed by Tukey's HSD multiple-range test. Statistical significance was assumed at p < 0.05

Results and discussion: ZEA did not affect gene expression of phase I enzyme CYP1A2 in liver samples from healthy cows, however, it decreased gene expression in ketotic liver samples down to 19% and 34% in doses 50 µM and 100 µM, respectively, after 6 hours of exposition. Our data also revealed an increase of mRNA expression of phase II enzyme COMT under the influence of ZEA in healthy cows' samples even up to 219% in the highest dose after 6 hours of exposition, while in ketotic samples we observed a decrease in COMT mRNA expression down to 20% and 24% in doses 50 µM and 100 µM, respectively. Another phase II enzyme UGT mRNA was not affected by zearalenone among healthy liver samples, however, ZEA decreased its level in the liver of ketotic cows.

Conclusion:—These results suggest that ZEA has different effects on liver enzyme gene expression in healthy and ketotic cows, which may have implications for the metabolism and detoxification of xenobiotics in these animals. Therefore cows in the state of ketosis may be more susceptible to the harmful effects of zearalenone, and proper management and prevention of ketosis are crucial to minimizing the risk of zearalenone toxicity. Further research is needed to fully understand the interactions between ketosis and zearalenone and to develop effective management strategies for dairy producers.

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4B4. Nutritional emulsifiers in sows and piglets' diets are an effective tool to improve intestinal morphology and metabolism during the post-weaning period

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Introduction: Weaning is a stressful event for piglets due to environmental, nutritional and social challenges. Post-weaning stress can impact health and performance of weaned piglets. Several nutritional tools are used to support piglets during this period, such as emulsifiers. Nutritional emulsifiers are surfactants promoting intestinal enzymatic digestion and fat absorption. This study's objective was to evaluate the effect of a nutritional emulsifier on intestinal morphology and metabolism in weaned piglets.

Animals, materials and methods: Thirty sows were followed from 90 days of gestation onwards. Randomly divided, half of the sows received a basal diet (control sows), while the other half received the same diet with a nutritional emulsifier (350 ppm of Excential Energy Plus, Orffa, based on glyceryl polyethylene glycol ricinolate and diatomaceous earth) on-top (treated sows) throughout the study period. Sows' basal diet was corn-based (2837 kcal/kg metabolizable energy (ME), 15% crude protein (CP), 3% crude fat). Three hundred eighty piglets with similar bodyweight were randomly selected: 94 piglets from the control sows and 96 from the treated sows received a basal diet (control piglets), and 92 piglets from the control sows and 98 from the treated sows received the basal diet with a nutritional emulsifier (350 ppm of Excential Energy Plus, Orffa) on-top (treated piglets). Piglets received a commercial diet (Biofarma Perfecto Nursery, 3400 kcal/kg ME, 22% CP, with soybean oil). At weaning (21 ± 2 days of age), 20 piglets were randomly selected from each group for blood sampling by puncture of the anterior vena cava, at 4, 8, 12 and 15 days post-weaning. Plasma citrulline concentrations were analysed by HPLC-FLD and the area under concentration vs time curve (AUC) was calculated. On day 15 post-weaning, 6 piglets were randomly selected from each group and killed, and samples of the middle jejunum and ileum were collected. The mucosa-serosa amplification ratio and intestinal absorption area (IAA) were determined according to the model proposed by Kisielinski et al. (2002) [1]. All parameters were analysed with a two-way ANOVA considering the effects of the sows' and piglets' treatments, and their interaction (95% confidence interval, P < 0.05).

Results and discussion: The results are represented in Table 1. Treated piglets showed significantly higher AUC plasma citrulline (biomarker for metabolic activity and functional mass of enterocytes, Berkeveld et al. 2008 [2]), and jejunum and ileum IAA but similar weight gain, without any effect of the sows' treatment and interaction (P-values > 0.05). Several studies already demonstrated that emulsifiers improve nutrient digestibility and performance in several species. For instance, Sun et al. (2019) [3] showed that the dietary supplementation of a nutritional emulsifier improved growth, and nutrient digestion and absorption when fed to growing pigs.

Conclusion: In conclusion, supplementing a weaned piglet diet with the nutritional emulsifier resulted in an overall significant improvement in intestinal morphology and metabolism. This shows the potential for nutritional emulsifiers to support piglets during the post-weaning period.

Table 1. Intestinal morphology and metabolism parameters of control and treated piglets. Different superscript letter within the same row indicates statistical significant differences between the piglets' groups (P<0.05). Standard deviation (SD) between brackets.

	Control piglets	Treated piglets	P-values
AUC citrulline (mmol*day/L)	563.8 ^a (206.0)	661.9 ^b (210.0)	0.046
Ratio jejunum IAA	5.83 ^a (0.66)	6.71 ^b (0.62)	0.004
Ratio ileum IAA	4.86 ^a (0.66)	5.47 ^b (0.74)	0.033
Piglets weight gain (kg/day)	0.24 (0.09)	0.25 (0.08)	< 0.05

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4B5. Reducing the protein content in broiler diets – performance-based effects on environmental impacts

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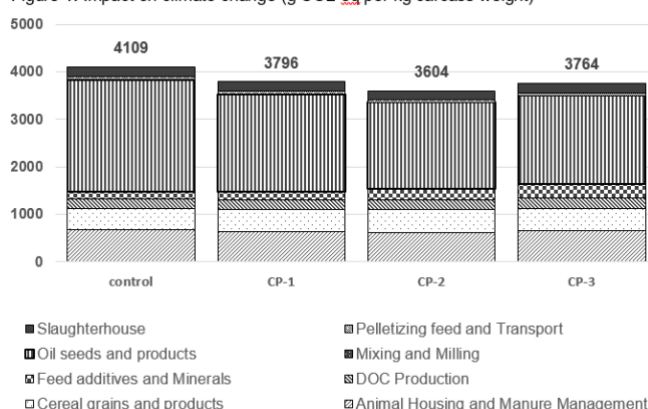
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Introduction: The use of soybean meal (SBM) from South America results in high environmental impacts (EI) in poultry production in Europe [1]. Studies have already shown that a reduction of the protein content is partly possible, when amino acids are added [2]. The aim of the study was, to find out, how a reduction in the content of SBM/crude protein (CP) affects the environmental impacts of the produced product (chicken carcass), taking into account the performance of the animals.

Animals, materials and methods: Day-old broilers (n=360) were randomly divided into four treatment groups (6 replicates each/24 boxes) and kept for 35 days. The fed diets (starter/grower/finisher) containing increasing amounts of corn and decreasing amounts of SBM / and therefore reduced contents (in steps of 1%) of CP (analysed CP levels: group CP-C [control]: 22.2/20.5/19.9%; group CP-1: 21.5/19.4/19.2%; group CP-2: 20.2/18.6/18.1%; group CP-3: 19.2/17.7/17.0%) while contents of essential amino acids were held at the same level by supplementation of single amino acids (meeting animals requirements). Starter and grower phases lasted 7 days each, followed by 21 days finisher phase. The zootechnical parameters were measured weekly. For body weight (BW) the individual and for feed intake as well as the feed conversion ratio (FCR) the boxes were basis of the observation. The monitoring of the environmental impacts (10 categories) was carried out by a life cycle assessments (LCA) according to ISO 14040/14044 using the software Opteinics® (BASF Lampertsheim GmbH, Germany), based on the Global Feed LCA Institute database. Group comparisons were performed by ANOVA resp. REGWQ and $p < 0.05$ considered to be significant.

Results and discussion: On day 35, animals of the group CP-2 showed the highest ($p < 0.05$) BW, followed by CP-1, CP-C and CP-3. No significant differences were found regarding the FCR (CP-C: 1.47 ± 0.036 ; CP-1: 1.46 ± 0.010 ; CP-2: 1.45 ± 0.017 ; CP-3: 1.49 ± 0.016). The highest decrease in SBM content led to a numerical reduction of the impact on climate change (kg CO₂ eq per t feed) of the diet by 18.5% in the starter, 15.1% in the grower and 20.4% in the

Figure 1: Impact on climate change (g CO₂ eq per kg carcass weight)



finisher diets. The impact on climate change throughout the production (g CO₂ eq per kg carcass weight) was significantly ($p < 0.0001$) reduced and was 4109 ± 78.9 g for CPC, 3796 ± 119 g for CP-1, 3604 ± 50.9 g for CP-2 and 3764 ± 41.4 g for CP-3. For CP-2, the lowest values were also found in the following categories: eutrophication (marine / freshwater), land use, particulate matter and use of resources and water.

Conclusion: When using single amino acids, the reduction of SBM to a certain proportion can be appropriate in terms of increasing sustainability. More studies are needed to determine to what extent the reduction of other mainly used protein components, while using single amino acids in the diets, may increase the sustainability of poultry production.

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4B6. Effects of iron oxide nanoparticles supplementation on the growth performance, viscera development, physiological health markers, and meat quality in broilers

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Introduction: Among trace minerals, iron (Fe) is an important component of poultry nutrition. Supplementation of Fe in bulk form or in higher doses causes less retention and more excretion resulting in environmental pollution. One of the possible solutions to this problem is the inclusion of Fe as nanoparticles (NPs) in poultry diets due to its higher bioavailability. However, less information is available regarding the use of iron as NPs in broiler's nutrition. Therefore, we assessed the effects of dietary supplementation of various levels of iron oxide nanoparticles (IO-NPs) compared to the bulk form of iron on the production performance, health biomarkers, and meat quality in broilers.

Animals, material and methods: The study was ethically approved by the institutional ethical committee. Day-old Ross broilers (n=300), housed on litter, were randomly divided into six groups (n=50/group), each group having five replicates with 10 birds per replicate. Birds were fed on a corn soyabean-based diet supplemented with either 0, 20, 40, 60, or 80mg/kg IO-NPs or 80mg/kg of FeSO₄ (called as bulk form) for 35 days. The growth performance was assessed by recording daily feed intake, weekly body weight, body weight gain and FCR. On day 35, ten birds per group were killed to determine the relative viscera weights and lengths. Blood was collected to harvest serum to analyze glucose, lipid profile, liver enzymes, renal function, and serum proteins concentration. For meat quality attributes (pH, color, drip loss, and cooking loss), pectoral muscle was collected. Data were analyzed using one-way ANOVA and Tukey's test was employed if group means were significant at P<0.05 using the SPSS software.

Results and discussion: Growth performance attributes, relative viscera weights, and lengths remained unaffected with the supplementation of IO-NPs or FeSO₄. Similarly, inclusion of IO-NPs in the diet also did not affect the serum glucose, lipid profile, and serum proteins levels. However, serum AST was higher (P<0.05) in birds supplemented with 20 and 40mg/kg IO-NPs compared to the FeSO₄ and without iron-supplemented groups. The uric acid level was decreased (P<0.05) by IO-NPs supplementation at the levels of 60 and 80mg/kg compared to the FeSO₄-supplemented group. The pH_{15min}, drip loss, and cooking loss of the pectoral muscle remained unaffected by IO-NPs or FeSO₄ supplementation. While the pH_{24hrs} was lower (P<0.05) in birds supplemented with 40mg/kg IO-NPs compared to the non-iron supplemented group. Considering the meat paleness categorization as described earlier [1], the meat color in the birds supplemented with IO-NPs remained within normal range and was best exhibited in the 20mg/kg and 40mg/kg supplemented groups compared with the other groups.

Conclusion: Supplementation of IO-NPs may improve the meat quality yet had no discernible detrimental impact on growth efficiency and health parameters.

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References:[1] Lee et al. (2022),Poult. Sci. J.101(3): 101627.

4B7. Effect of increasing dietary vitamin D₃ contents on bone breaking strength of young layer pullets of different genetic origin

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Introduction: Bone damages in laying hens, up to fractures, are a problem even in modern egg production. In the past it was hypothesized frequently that high performing laying hens might be more prone to bone damages [1], but the relevance of a dietary support on optimal bone development during the rearing period was hardly addressed. Thus, in the present study an increasing dietary vitamin D₃ content was tested in high and low performing genotypes during rearing, to test the hypothesis that high performing genotypes might be more demanding regarding vitamin D₃ supply than moderate performing ones. The presented results are part of an investigation regarding performance and health.

Animals, materials and methods: An animal model described by [2] with two high and two low performing genotypes was used (Fig. 1). In total, 940 animals were part of this study. At hatch, 40 animals were killed to receive comparative data for subsequent measurements. Remaining chicks were divided into 12 groups (4x3 factorial design: 4 genotypes, 3 diets). The diets (2-phase-feeding, based on soybean meal and cereals) differed only regarding the added vitamin D₃ content (300/1000/3000 IU/kg). After every four weeks three male and three female randomly chosen animals per group were slaughtered for bone dissection. Bone breaking strength was measured at the left tibiotarsus (Model 4301, Instron, High Wycombe, United Kingdom). Statistical evaluation was performed by SAS 9.4, Institute Inc. ($p < 0.05$, 4-factorial ANOVA: genotype, diet, sex, age) with PROC MIXED. All procedures were reviewed and approved by the relevant authorities (LAVES, Germany).

Results and discussion: Body mass development differed regarding genotype as expected (week 12, means (g): WLA / BLA / R11 / L68 = 1041 / 1029 / 836 / 1142). No effect of dietary vitamin D₃ content on bone breaking strength could be verified, but age, sex, genotype and

Table 1: Bone breaking strength (Newton) of tibiotarsus in chicks/pullets depending on dietary vitamin D₃ content, genotype, age and sex

	IU	Week of age 4			Week of age 8			Week of age 12		
		300	1000	3000	300	1000	3000	300	1000	3000
WLA	♂	63.3	73.7	59.3	122	116	134	149	150	166
	♀	56.0	53.3	64.0	92.3	104	84.0	110	131	122
BLA	♂	38.0	77.3	64.7	114	128	113	156	173	152
	♀	57.7	55.7	48.7	84.0	81.3	82.7	126	123	112
R11	♂	51.3	47.3	64.7	85.0	87.3	87.7	148	112	134
	♀	49.7	51.7	43.7	67.7	61.7	69.7	100	102	100
L68	♂	86.3	79.0	86.3	132	144	135	179	212	200
	♀	76.0	87.7	86.0	109	119	117	155	143	151

P values: Genotype (G), Age (A), Sex (S), A*S: < 0.001; Diet (D): 0.358; G*A: 0.035; G*S: 0.721; G*D: 0.370; A*D: 0.990; S*D: 0.595; G*A*S: 0.770; G*A*D: 0.968; G*S*D: 0.349; A*S*D: 0.994; G*A*S*D: 0.267

age*sex as well as genotype*age had an impact (Table 1). Notable is the high bone breaking strength in L68 compared to BLA (both brown layer lines).

Conclusion: During the rearing period of layer pullets (up to 12 weeks of life) the different dietary vitamin D₃ contents did not influence breaking strength of the tibiotarsus in the four genetically diverse purebred layer lines, neither in high nor in moderate performing ones.

References: [1] Kittelsen et al. (2021) PLOS ONE 16(7): e0255234. <https://doi.org/10.1371/journal.pone.0255234>; [2] Lieboldt et al. (2015) European Poultry Science, 79, DOI: 10.1399/eps.2015.89.

4B8. Evaluation of dog owners' perception as to the physical activity performed by their animals associated with the quantity of food supplied

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Introduction: Physical activity (PA) is essential for dogs. Knowing the frequency, intensity, and time of PA performed daily is necessary to calculate the correct amount of food provided to these animals. It avoids the excess or deficiency of ingesting energy components in the diet. The purpose of this study was to evaluate, through an online questionnaire, different factors associated with dogs' PA and how this can affect the feeding of these animals based on the perception of their owners.

Animals, material, and methods: An online questionnaire was developed on the Google Forms platform. In total, 20 questions were asked and divided into 4 sections. In section 1 was asked if the person agreed to participate in the research. Section 2 was related to personal information of owners and PA performed with their dogs, such as: what is the frequency of daily exercises performed, what time of exercise performed on average, what is the body condition of dogs according to the perception of their owners, and which type of food is provided to the dog. Section 3 was intended only for owners that provided extruded dry food, with the objective to evaluate where information is taken from the amount of food provided daily to the animal (label, professional recommendation, or quantity the owner finds necessary). Section 4 was intended to assess whether the owner who uses the information available on the food label regarding the amount of daily supply uses the recommended amount for animals with low, moderate, or high PA. Fisher exact test and chi-square were used to evaluate the associations. Statistical analysis was obtained by Graphpad prism. $P < 0.05$ were considered significant.

Results and discussion: 1044 dog owners answered the questionnaire. Associations between the average time spent during PA ($p < 0.0001$) and the frequency of weekly PA ($p < 0.0001$) performed by the dog with the amount of food that the owners provide based on prescriptions of extruded dry food labels were observed. In the literature, many definitions are found for what is PA performed by dogs [1,2]. In the FEDIAF nutrition guidelines [3] a definition is described in hours considering the impact of PA that the animal needs to perform so that it is considered as low, moderate, or high intensity. It was not observed standard for providing the recommended amount of food in animals that practice PA of different intensities with the average time spent in each PA and the weekly frequency of this activity. For example, regarding people who responded that their dogs practice on average 1 or 2 hours of PA, some of them use the recommendation of the low-intensity label, but there are also those who use moderate or high intensity.

Associations were also found between the animal's body condition score with the ingestion or not of snacks ($p = 0.0229$) and the type of food ($p = 0.0330$). Other authors have already reported these associations [4,5].

Conclusion: It was observed a lack of consensus among owners to define the different levels of PA, what demonstrated the importance of questioning during nutritional consultations about the time and intensity of PA performed by the animals. As owners also based their decisions on prescribed dietary recommendations on the labels, Pet Food companies could provide information about the time and intensity of PA for each guidance to assist owners.

References: [1] Thorpe, et al. (2006) J. Amer. Ger. Soc., 54 :1419-1424; [2] Coleman, et al. (2008) Prev. Med., 47:309-312; [3] FEDIAF (2021) European pet food industry federation; [4] Lund, et al. (2006) Int. J. Appl. Res. Vet. Med., 4 :177. [5] Porsani, et al. (2020) Sci. Rep., 10.1:14082

4B9. Possible nutrient deficiency in cats at 10% of total calories from snacks and treats

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Introduction: The World Small Animal Veterinary Association (WSAVA) guidelines recommend that snacks calories be up to 10% of a dog's daily energy requirement [1,2]. The aim of this research was to evaluate whether the inclusion of treats of up to 10% is safe for dogs and cats [1-3].

Animals, materials and methods: 226 labels of dry extruded complete diets for healthy adult dogs and 124 labels of dry extruded complete diets for healthy adult cats were evaluated. The equations used for determining the maintenance energy requirement (MER) was $95 \times BW^{0.75}$ for dogs and $75 \times BW^{0.67}$, for cats, according to FEDIAF (2021). The MER included 10% inclusion of snacks, considering all types of treats. In addition, the nutritional values of the snacks were disregarded, since the snacks offered are very diverse and depend on the profile of tutors in the world.

Results and discussion: 97.3% of the dog diets (n=218/224) met or exceeded the minimum protein recommendations (FEDIAF, 2021) with 10.0% of snacks. For cats, 60.5% (n=75/124) of the diets did not reach the minimum protein recommendation when 10% of MER was considered

Table 1. Minimum protein and fat recommendations in cats and percentage of diets meeting requirements after 10% reduction

*FEDIAF (2021); DNM^{10MER}: diets not met the recommended of 10% on MER

Nutrient	Cat			
	Adult – based on MER of			
	75kcal/kg ^{0.67*}	DNM ^{10MER}	100kcal/kg ^{0.67*}	DNM ^{10MER}
Protein	83.3g/1000kcal	60.48%	62.5g/1000kcal	2.40%
Fat	22.5g/1000kcal	51.61%	22.5g/1000kcal	8.87%

Regarding the fat levels present in the diets, all dog foods provided or exceeded the specified minimum. However, 51.6% (n=64/124) of cat foods did not supply the minimum fat recommendation. The variety

between types of snacks encourages the possibility of nutritional imbalance. Despite the offer of foods with a high protein and fat levels, these nutrients vary between sources and types of snacks [4]. Protein and fat deficiency can lead to amino acids and essential fatty acids deficiency and can cause various health risk [5].

Figure 1. The minimum requirements of protein (6.25 kg BW^{0.67}) and fat (2,25 kg BW^{0.67}) were 10% of snacks inclusion.



Conclusion: It was verified that reducing the amount of food provided for the inclusion of snacks implies a reduced intake of essential nutrient. Despite being recommended by WSAVA, caloric intake of up to 10% of total calories ingested in snacks should be reviewed. Mainly for neutered and/or indoor cats, as this can lead to nutritional imbalance in these animals.

References: [1] Cline et al. (2021) J. Amin. Hosp. Assoc. 57:153–178; [2] Freeman et al. (2011) J. Fel. Med. Surg. 13:516–525; [3] Pars et al. (2014) Vet. Clin. Small. Anim. 44:667–688; [4] Dodd et al. (2020) Vet. Rec. 19:643–643. [5] NRC, 2006.

4B10. Does the season influence the food intake of adult cats?

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Introduction: Food intake in cats is influenced by diet composition, body weight [1], and life stage [2]. Studies have shown that water intake and urinary excretion were influenced according to environmental temperature but did not evaluate on food intake [3]. Given the scarcity in studies comparing environmental temperature and food intake, this study analysed these data in young adult cats.

Animals, material and methods: This study was approved by the ethics committee. Twelve healthy neutered adult cats, of both sexes, with a body condition score of 5/9 [4] and 2.11 ± 0.05 years old were used. The animals received isonutrient diets for 12 months, with three meals a day (7:00 am, 1:00 pm, 3:30 pm) in individualized cages for 30 minutes. Cats had access to water *ad libitum* and access to an outdoor area for socialization and activity after meals. A digital wall thermometer (Minipa Capela AGZ) was installed in the feeding and resting areas. The collection of temperatures was done during the first daily meal, after which the equipment was reset. The animals were distributed in randomized blocks, according to the total daily consumption. At the end of the day, the leftovers were weighed on semi-precision scales. To evaluate the effects of seasons on the percentages of leftovers, mixed model regression analyses were performed considering the same random effects and the mean temperature as an

Table 1. Food leftovers (%) of adult cats per season.

Seasons	Leftovers %	p value
Summer	9.68 ^A	<.0001
Fall	2.26 ^C	
Winter	2.18 ^C	
Spring	3.70 ^B	

^{A,B} Averages in the same column followed by different capital letters differed by 5.0% in the Tukey test.

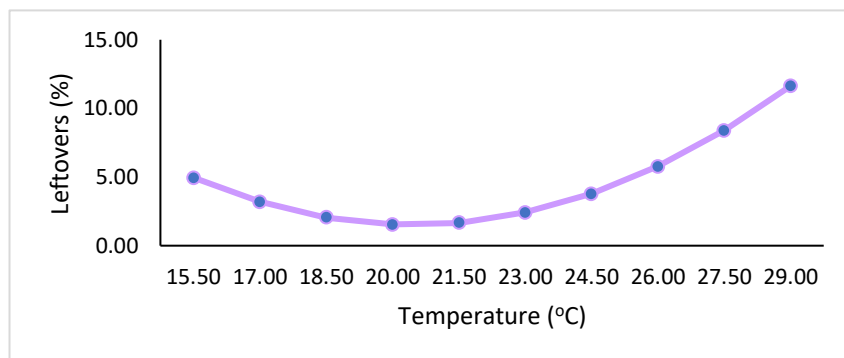


Figure 1. Cat food leftovers (%) in relation to temperature.

independent variable. All analyses were performed using the MIXED procedure of the Statistical Analysis System, version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion: The cats had the lowest consumption in the season with the highest temperature (Table 1), summer, which corroborates with Tauson [3], followed by spring. In the cooler seasons, fall and winter, we obtained the lowest amount of leftovers (Figure 1), which resulted in the highest food consumption. Despite not having evaluated the weight in the different seasons, the reduced food intake will influence the loss of weight, which may impair the health of the animals.

Conclusion: Environment temperature influences the food intake of adult cats and can impair animal health due to weight loss.

References: [1] Alegría-Morán et al. (2019). *Animals*, 9(6):372; [2] Bermingham et al. (1999). *Ener Prot Met Nutri*, 513; [3] Tauson (1999). *Ani Sci*, 69(1):171-181; [4] Laflamme (1997). *Fel pract*, 25:13-18.

4B11. Determination of nutrients in veterinary diets marketed in Brazil for weight loss in cats

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Introduction: Obesity is one of the major health and welfare problems in cats worldwide and this is due to the excessive food intake or insufficient energy expenditure, which causes a positive energy balance. The study evaluated veterinary diets for weight loss in cats and compared whether they meet the nutritional requirements or not.

Animals, material and methods: Six veterinary diets for obese cats available in the Brazilian market were evaluated. In these, a bromatological analysis for crude protein, fat (acid hydrolysis), crude fiber, calcium, phosphorus and ash was performed. The nutrients provided by the diets per kg metabolic body weight (kg BW^{0.67}) of an obese cat were compared to the recommendations of FEDIAF considering the three most used equations for weight loss program (WLP): WLP1: 85 x (BW)^{0.4}[1], WLP2: 0.8 x (70 x BW^{0.75}) [2], and WLP3: 70 x BW x 0.6 [3] kcal/day. A 5 kg obese cat was used as an example, with an ideal body weight of 4kg (-20% of current BW). The results

were compared to the label of each diet. Analyses were evaluated by descriptive statistics. **Results and discussion:** All nutrients except fat and crude protein achieved the nutritional requirements of the animals. All

diets did not meet the minimum recommended fat intake per kg BW^{0.67} in all equations (Table 1). Diet A did not meet the minimum recommended protein intake, 6.25g per kg BW^{0.67} (FEDIAF), in all equations (WLP1=5.66g; WLP2=5.54g and WLP3=5.88g). And Diet C had a

Table 1. Amount of crude fat ingested per kg^{0.67} in the different weight loss veterinary diets (A–F) for cats and its comparison with FEDIAF (2021) daily intake recommendations per kg^{0.67}

Diets	Crude fat intake (kg BW ^{0.67})	WLP1	WLP2	WLP3
A	2.25	1.51*	1.48*	1.57*
B		1.60*	1.57*	1.66*
C		1.32*	1.30*	1.38*
D		1.49*	1.46*	1.55*
E		0.94*	0.92*	0.98*
F		2.03*	1.99*	2.11*

WLP1: 85 x (BW)^{0.4} kcal/d [2]; WLP2: 0.80 x [70 x (ideal body weight)^{0.75}] kcal/d [3]; WLP3: 70 x ideal body weight x 0.6 kcal/d [4].

protein deficiency per kg BW^{0.67} in WLP2 (6.20g). Diet C had 7.91g of total fat, below the minimum declared on the label (8.7g of dry matter). Dietary fat is essential in the maintenance and survival of cats due to its provision of energy, palatability, aid in the absorption of fat-soluble vitamins,

Table 2. Amount of crude protein ingested per kg^{0.67} in the different weight loss veterinary diets (A–F) for cats and its comparison with FEDIAF (2021) daily intake recommendations per kg^{0.67}

Diets	Crude protein intake (kg BW ^{0.67})	WLP1	WLP2	WLP3
A	6.25	8.75	8.56	9.08
B		12.65	12.38	13.13
C		9.79	9.58	10.16
D		10.80	10.57	11.22
E		12.19	11.93	12.65
F		11.04	10.80	11.46

WLP1: 85 x (BW)^{0.4} kcal/d [2]; WLP2: 0.80 x [70 x (ideal body weight)^{0.75}] kcal/d [3]; WLP3: 70 x ideal body weight x 0.6 kcal/d [4].

modulation in inflammation, acting as a precursor of eicosanoids and prostaglandins, and aiding in the health of the skin and coat [5]. In addition, fat deficiency can lead to insufficient supply of essential fatty acids, which can cause alterations, such as severe fatty degeneration and dystrophic mineralization of the adrenal glands [6]. Protein deficiency can cause negative nitrogen balance due to the continuation of gluconeogenesis from amino acids, in addition to not meeting the recommendations of essential amino acids that help in homeostasis [7].

Conclusion: All diets for weight loss in cats available in Brazil are deficient in fat and one is deficiency in protein according to recommended calorie intakes for energy restriction.

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4B12. Non-compliances in healthy weight plans at a small animal nutrition clinic: a case series

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Introduction: Owner non-compliance is commonly observed with pet obesity treatment plans and may contribute to the reason these plans fail [1, 2]. The aim of this retrospective case series is to investigate the success of healthy weight clinical meal plans in relation to compliant pet owners.

Animals, materials, and methods: The medical records of 27 dogs and 2 cats presented to the Ghent University Small Animal Hospital nutrition service for obesity treatment between 2019 and 2022 were analysed. The obesity treatment meal plans outlined the amounts of the specified diet and treats which were to be fed for healthy weight loss. The estimated ideal body weight was also indicated. For inclusion in the case series, patients must have had at least one follow-up consultation. A follow-up consultation is recommended to be held at 1 month after the initial consultation. The clinical assessment at this time included the evaluation of body weight (BW), body condition score (BCS), and compliance to the prescribed plan.

Results and discussion: Average age of the patients was 6.2 years \pm 3.5 and ranged from 1 to 14. Average starting BCS was 8.1 \pm 1 and ranged from 6 to 9 on a 9-point scale.

Out of the 29 cases in the series, 18 owners (62%) were non-compliant (NC) with the obesity treatment plan. The proportion of NC owners is greater than what has been previously reported by Yassle and colleagues [2]. After accounting for dogs who dropped out of the study, 32% of dog owners (n=47) were NC with a weight loss plan [2]. In the present case series, the most common non-compliance seen (11/18) was the feeding of additional treats. Despite non-compliance, an average of 0.74% BW was lost per week by the follow-up consultation. The follow-up consultation for this group occurred an average of 2 months \pm 1.47. Out of the 18 NC cases, 3 patients failed to lose weight by the follow-up consultation. Non-compliances included not weighing meals before serving and giving patient access to the food of another pet in the home. The average time taken for the follow-up consultation in this group was almost 3 months. Two of these patients, eventually achieved weight loss with further review of the obesity treatment plan and individualised support. Notably, the one patient in this series who dropped out of the plan without evidence of weight loss had the first follow-up visit at close to 4 months. In all cases that pet owners were compliant (C) with the meal plan, weight loss was achieved. When compared to the NC group, average weight loss in the C group achieved was greater; 1.1% BW per week. This weight loss is also well-within the stated target for weight loss for cats and dogs in the literature of 0.5-2% body weight loss per week [3]. The follow up for these patients occurred, on average 2 months \pm 0.95. A monthly follow-up is recommended as best practice in the literature [3]. Interestingly, 73% of all patients who lost weight had the first follow up appointment scheduled no later than 2 months after the first consultation.

Conclusion: Over 80% of patients managed to lose weight by their follow-up appointment even though the owners were not compliant with the prescribed obesity treatment plan. It is important the veterinary practitioner be aware of common non-compliances with such plans and correct them during follow-up to ensure success with weight loss plan goals.

References: [1] German et al. (2007). Dietary energy restriction and successful weight loss in obese client-owned dogs. *Journal of Veterinary Internal Medicine*, 21(6), 1174-1180. [2] Yaissle et al. (2004). Evaluation of owner education as a component of obesity treatment programs for dogs. *Journal of the American Veterinary Medical Association*, 224(12), 1932-1935. [3] Brooks, D., Churchill, J., Fein, K., Linder, D., Michel, K. E., Tudor, K., & Witzel, A. (2014). 2014 AAHA weight management guidelines for dogs and cats. *Journal of the American Animal Hospital Association*, 50(1), 1-11.

Session 4C Farm animals II

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4C1. Performance of sows and piglets receiving diets enriched with coarse or finely ground hay or sugar beet pulp

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Introduction: It has been frequently suggested that swine diets rich in dietary fibre reduce pig performance due to a dilution effect and a decrease digestibility of nutrients. However, recent findings suggest a more diverse picture, revealing both fibre source and fibre particle size as important factors [1]. The study aimed to investigate if coarse or finely ground hay- (low fermentable fibre source) and sugar beet pulp- (SBP; highly fermentable fibre source) enriched diets result in sow and piglet performance differences from insemination until weaning, considering two different weaning ages.

Animals, materials and methods: German landrace gilts (3/group) were inseminated (German landrace semen) at 128 ± 7.5 kg bodyweight (BW) and randomly allocated to one of the 4 feeding groups: meadow hay finely ground (Hf), hay coarse ground (Hc), SBP finely ground (SBPf) or SBP coarse (SBPc). The sows were fed isocaloric and iso-nitrogenic diets containing the respective dietary fibre options throughout gestation (restricted, 254 ± 6 g TDF/kg DM) and lactation (*ad libitum*, 238 ± 3 g TDF/kg DM). Their piglets received creep feed from d 14 of life until weaning (*ad libitum*), containing the same dietary fibre treatment as their dams' diet (122 ± 2 g TDF/kg DM). At 26 ± 2 d (early) and at 40 ± 2 d (late), 1 male and 1 female piglet were weaned from each sow and fed isocaloric and iso-nitrogenic diets (146 ± 3 g TDF/kg DM) enriched with the same fibre source and fibre particle size as their dams and the creep feed. The litter size was adjusted to 6.1 ± 1.1 piglets after the first weaning. Both sows and piglets were weighed weekly and individual feed intake (FI) was obtained from sows, while the piglets' milk intake was estimated according to the bodyweight gain (BWG) [2]. Always 2 weaned piglets from the same dam were housed together in flat deck pens and their joined FI was measured weekly. The piglets were sacrificed 2 wk postweaning and intestinal weight, gut fill, and jejunum length were calculated in relation to the BW and the pH of digesta and faeces were measured. The animal trial was approved by the Regional Office for Health and Social Affairs Berlin (LAGeSo, StN 014/22). Two-factorial ANOVA was applied to calculate statistical differences ($p < 0.05$).

Results and discussion: Sows fed Hc or SBPf had the largest litters ($p = 0.045$), but the mean piglet birthweight and litter weight at birth were not affected by the maternal gestation diet. Sows fed SBP showed a higher total litter weight gain than sows fed Hc or Hf (trend, $p = 0.053$). Here, coarse fibre particles led to the highest weight gain ($p = 0.028$). In early weaned piglets, the stomach fill was lower when the diets contained SBP compared to piglets fed diets containing hay ($p = 0.027$). An interaction between fibre type and particle size was observed in these piglets regarding caecal content, with more digesta in piglets fed fine hay or coarse SBP ($p = 0.026$). Piglets weaned at a later timepoint and fed diets containing SBP had higher BW at dissection compared to Hc and Hf ($p = 0.045$), possibly because of the microbial fermentation with a production of short chain fatty acids as energy supply in the hindgut [1]. The jejunum length and ileum weight of these piglets was smaller than when hay was added to the diets ($p = 0.021$; $p = 0.006$, respectively). In early weaned piglets, the caecal content pH was lower with coarse fibre diets ($p = 0.026$), while no significant differences in pH were observed in late weaned piglets.

Conclusion: Chemical and physical features of dietary fibre sources can influence performance in sows and piglets. Hence, a future dietary fibre evaluation system should consider both factors.

References: [1] Grzeskowiak et al. (2023) Anim. Health Res. Rev.1 – 29; [2] Hojgaard et al. (2020) J. Anim. Sci. 98.

4C2. Intake of starch in first weeks of life affects lambs behaviour and has a medium-term impact on ruminal fermentation

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Introduction: Although ruminants evolved to efficiently use forages and structural carbohydrates as a main source of energy, livestock ruminants are commonly fed diets high in cereal grains that are abundant sources of nonstructural carbohydrates, mostly starch. Such a feeding strategy increases the energy content of the diet and hence the efficiency of meat or milk production. However, a high intake of starch may have a long-term impact on the animal behaviour, rumen microbiota and the functions of the rumen epithelium, and consequently on the efficiency of the digestion process and the use of feed at further stages of production. The hypothesis of this study was that a high starch intake early in life affects the behaviour of lambs, feed intake and response of the rumen to a high starch intake at latter stages of the growth.

Animals, materials and methods: Twenty three lambs (males and females 7-14 days old) were divided into two groups and fed with milk replacer and solid feed consisting mainly of cereal grains (S group; n = 11) or meadow hay (C group; n = 12). The milk replacer was fed for the first 6 weeks of the experiment. In the first 8 weeks of the experiment, the animals from the S group were fed ad libitum with a concentrate mixture containing mainly barley, while the animals from the C group received meadow hay. From week 3 to 7 lambs were video recorded to evaluate their behaviour. Starting from the 9th week of the experiment until the age of 7 months, the animals of both groups were fed with a diet based solely on roughage (hay, haylage). After reaching the mentioned age, the feed intake (meadow hay) of the animals was determined. Subsequently, males (n = 5 and 6 for S and C group, respectively) were fed diet in which 50% of dry matter (DM) intake with hay was replaced with barley. After 7 days, the males were euthanized 3 hours after feeding in order to collect rumen fluid samples for further analyses (pH, volatile fatty acids [VFA], ammonia concentration). Behaviour parameters were analysed by two way ANOVA with the PROC GLIMMIX of SAS (ver 9.4) allowing to determine impact of treatment and time (week of the study); whereas, rumen fermentation parameters were analysed using one way ANOVA with PROC MIXED of SAS.

Results and discussion: Lambs fed mainly concentrate mixture (S) spent less time ruminating and more time on stereotypic behaviours ($P \leq 0.05$), which indicate that high starch intake early in life may affect the behaviour and welfare of young ruminants. Once reaching 7 months of age, voluntary DM intake of hay did not differ between the experimental groups (3.60 vs. 3.57% of body weight for the S and C groups, respectively; $P = 0.81$). However, after exposure to a high starch diet the pH of the rumen fluid in sheep was lower for the S group compared to the C group (5.80 vs. 5.98; $P < 0.01$). The molar proportion of valeric, isobutyric and isovaleric acids in the total VFA was lower for the S group ($P \leq 0.03$). The ammonia concentration in the rumen did not differ between the groups. The lower pH of the rumen fluid in S animals indicates that high starch intake early in life may predispose the animals to rumen acidosis later in life.

Conclusion: A high starch intake early in life may affects the behaviour and welfare of lambs, as well as affects rumen fermentation later in life. Sheep fed a ration rich in starch in the first weeks of life had a lower pH of the rumen fluid after exposure to such a diet at the age of 7 months. As a result, this feeding practice may predispose animals to rumen acidosis later in life.

4C3. Age-dependent short-chain fatty acid absorption in the proximal colon of piglets fed high- or low- fermentable fibre sources

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Introduction: The intestinal microbial community ferments non-digestible carbohydrates and releases short-chain fatty acids (SCFA), which can be absorbed and contribute to the energy supply of the host. While various dietary fibre sources affect SCFA concentrations in the colon digesta differently [1], little is known about the capacity of piglets to absorb SCFA in the colon. The study aimed to investigate the effects of dietary fibre and animal age on the colonic SCFA absorption in weaned piglets fed diets containing high- and low-fermentable dietary fibre sources.

Animals, materials and methods: Piglets (German Landrace, DanBred × Duroc) were fed diets containing 15.5 % apple pomace (n=8; 226 g TDF/kg DM) or 8 % sugar beet pulp (SBP; n=16; 144 g TDF/kg DM) as high fermentable and 8 % meadow hay (n=16; 147 g TDF/kg DM) or 14 % dried hemp (*Cannabis sativa* L., n=8; 166 g TDF/kg DM) as low fermentable dietary fibre sources. Besides the fibre-rich feedstuff, diets were based on the same ingredients and isocaloric and formulated to be isonitrogenous within age groups. Proximal colon digesta and tissue were collected from piglets aged 6 (n=16), 8 (n=16) and 11 (n=20) weeks. SCFA concentrations were measured (gas chromatography) in the digesta and the tissue was used for Ussing chamber experiments. In the Ussing chambers, acetate, propionate and n-butyrate were added to the mucosal side and their concentrations were measured in both mucosal and serosal chambers directly and 60 min after addition. The relative change in concentration from timepoint 0 to 60 min was calculated and evaluated. The Mann-Whitney-U and Kruskal-Wallis test were applied to determine statistical differences ($p < 0.05$). The animal trial was approved by the Regional Office for Health and Social Affairs Berlin (LaGeSo StN 023/21, StN 014/22).

Results and discussion: Acetate and propionate concentrations were higher in colon contents of 6-week-old piglets fed diets with hay compared to SBP ($p=0.020$ and $p=0.005$, respectively) as a fibre source. In 8 week old piglets, acetate concentrations were higher when SBP was added to the diet, compared to hay ($p=0.020$), while propionate concentrations were enhanced in 11-week-old piglets fed dried hemp compared to apple pomace ($p=0.036$). Diet-dependent differences in SCFA absorption were only detectable at 6 weeks of age. Here, serosal appearance of acetate ($p=0.033$) and propionate ($p=0.049$), as well as n-butyrate disappearance from the mucosal side ($p=0.049$) were higher in animals fed SBP-containing diets, compared to piglets fed diets with hay. Age had a major impact on SCFA concentration changes. The highest disappearance from the mucosal side and largest accumulation on the serosal side were observed in 11 week old piglets ($p<0.001$). Six and 8 week old piglets only differed in their propionate accumulation, with higher rates in 8 week old piglets ($p<0.001$).

Conclusion: Dietary fibre type affects SCFA concentrations in the colon of piglets, with age-dependent differences. The efficiency of SCFA absorption seems to increase with age, which may be related to SCFA transporters and intestinal maturation. Further studies on this topic are warranted.

References: [1] Grzeskowiak et al. (2023) Anim. Health Res. Rev.1 – 29

4C4. Monitoring of environmental impacts of partially substituting soybean meal with rapeseed meal or haemoglobin meal in diets for broilers

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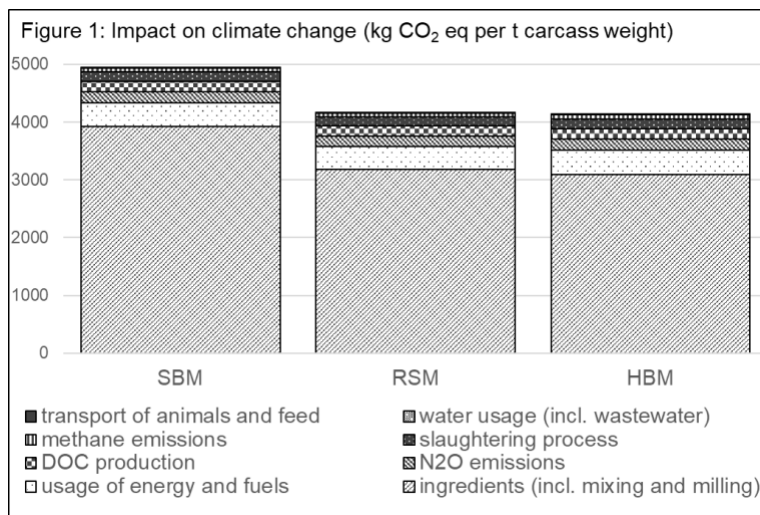
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Introduction: As the use of soybean meal (SBM) imported to Europe in poultry feed is associated with high environmental impacts (EI), alternatives are increasingly being investigated [1]. However, a reduction regarding the EI can only be assessed by taking into account the performance of the animals [2]. The aim of this study was therefore, to assess the effects of a partial replacement of SBM with alternative protein sources in broiler diets on the EI of the feed and the broiler production.

Animals, materials and methods: In total 120 broilers were fed (day 8 to 44) with three different diets containing 32.5 % soybean meal (SBM) as a control a diet where SBM was partly replaced by either 14.5 % rapeseed meal (RSM) or 4.5 % haemoglobin meal (HBM). The diets met the nutritional requirements of the birds. Analyses of the diets showed comparable amounts of crude protein (SBM: 231 g/kg DM, RSM: 228 g/kg DM, HBM: 235 g/kg DM). The animals were randomly allocated into three groups with two replicate pens (20 birds each). The assessment of the EI of 1 t feed and 1 t carcass weight (10 categories, according to the ISO 14040/14044) was performed by a life cycle assessment (LCA) using the software Opteinics® (BASF Lampertsheim GmbH) based on the Global Feed LCA Institute (GFLI) database [3]. Feed and water intakes were measured daily and body weights (BW) were recorded weekly. For BW the individual was basis of the observation. Group comparisons for the BW were performed by an ANOVA ($p < 0.05$). For feed intake, FCR, and LCA data, the boxes were the basis of the observations and therefore could not be statistically analysed.

Results and discussion: The impact on climate change was 1.23 t CO₂ eq per t feed and could be reduced by 15.1% (RSM) and 15.4% (HBM). A numerically reduced EI of the diets

with alternative protein sources could also be found in the categories eutrophication, (marine/freshwater), land use, and water use. The final body weights of the broilers were different (SBM: 2769 ± 319 g; RSM: 2909 ± 325g; HBM: 2478 ± 320g; $p = 0.0003$). The FCR was 1.68 (SBM and HBM) and 1.71 (RSM). The impact on climate change related to carcass weight was 4.9 t per t and could be numerically reduced by 16.4% (HBM) and 15.8% (RSM). A numerically lower EI could be also found in the categories eutrophication (marine), particulate



matter, resource use, land and water use.

Conclusion: The lower EI of the feed allowed the CO₂ footprint of the product to be numerically mostly reduced by the partial replacement of SBM with HBM. In terms of performance, RSM seems to be the more suitable alternative to SBM. Since feeding these protein sources seems feasible, studies with higher inclusion rates should be conducted.

References: [1] Cappelaere, L., et al. *Frontiers in Veterinary Science* 8 (2021): 689259; [2] Wilke, V., et al. *Sustainability* 15.3 (2023): 2210. [3] Bjørn, Anders, et al. "LCA History." *Life cycle assessment: Theory and Practice* (2018): 17-30

Session 4D Equine

Chair: Anne Mößeler



4D1. Changes in the nutritional profile, mycotoxins, and phytoestrogens from spring till late summer in selected horse pastures

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Introduction: The aim of this study was to evaluate the nutritional profile and concentration of common contaminants (i.e., mycotoxins, their metabolites, plant-derived compounds such as phytoestrogens) in selected horse pastures in Austria.

Materials and methods: A total of seven randomly chosen pastures were sampled in the province of Lower Austria for this study. The pastures were either used for horse hay production or grazing and contained mostly grasses (e.g., *Poaceae*), legumes (e.g., *Fabaceae*) and herbs (e.g., *Asteraceae*). Representative sward samples were collected manually by using an electric and a manual grass shear and were performed three times for each pasture between May and August. A quantity of 5 kg grass was collected and dry stored in cardboard boxes until further preparation. The first and the second samplings in May were from the first cut, but at different growth stages (ear emergence and blooming, respectively). The third sampling was between July and August and was from the second cut. Soon after sampling, the grass samples were weighed (fresh weight), dried, pulverised, and analysed for proximate nutrients and water-soluble carbohydrates (WSC; proximate analysis and Anthrone method), minerals and trace elements, as well as for mycotoxins and other fungal and plant-derived compounds by LC-MS/MS. Data were statistically processed with PROC MIXED of SAS, and the significance level was set at $p < 0.05$.

Results and discussion: Grass samples collected at ear emergence (NDF: 45.2 % DM, ADF: 24.2 % DM) had significantly lower NDF and ADF concentrations than those collected in bloom stage (NDF: 53.7 % DM, ADF: 30.9 % DM). Because of a drought-induced stress during summer, the ADF/NDF ratio increased significantly in the 3rd sampling (0.66) compared to the 1st (0.53) and 2nd sampling (0.57). The concentrations of WSC decreased with sampling time, too: 1st sampling: 157 (110.2-314.3) g/kg DM, 2nd sampling: 94.8 (66.8-153.9) g/kg DM, 3rd sampling: 77.7 (47-111.3) g/kg DM. Higher average temperatures during summer facilitated the growth of drought-resistant plant species like *Fabaceae*, resulting in higher average Ca levels in these samples (12.6 g/kg DM) than in the ones from spring (7.4 g/kg DM). In July and August, higher contamination of the grass with metabolites derived from both regulated (1st sampling: 64.6 µg/kg, 2nd sampling: 34.9 µg/kg, 3rd sampling: 1390.4 µg/kg) and emerging (1st sampling: 12.7 µg/kg sample, 2nd sampling: 8 µg/kg, 3rd sampling: 839.4 µg/kg) *Fusarium* mycotoxins, and an increasing number of fungal metabolites (1st sampling: 3656 µg/kg, 2nd sampling: 2827 µg/kg, 3rd sampling: 7537 µg/kg), posing risk for horse health.

Conclusion: The summer pastures showed the lowest water-soluble carbohydrate-concentration, so that the risk for related equine metabolic health disorders is expected to be lowest. However, the drought and high weather temperatures in full summer affected the nutritional and hygienic value of the pasture, as evidenced by higher ADF and Ca contents, as well as the higher contamination with mycotoxins and other metabolites. Further research should evaluate the effects of changes in the nutritional and hygienic status of the pasture on equine nutrient supply and health.

4D2. Effect of live yeast and protected live yeast on the blood parameters of horses at maintenance

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Introduction: Studies evaluating use of yeast supplementation in horses show interesting effects regarding the total apparent digestibility of nutrients, fermentative pattern, and microbiome, but the effects that supplementation has on blood parameters is still controversial. The objective of this study was to evaluate the effect of supplementation on live yeast and protected yeast on the blood parameters of maintenance adult horses.

Animals, materials, and methods: Six mature Arabian Horses geldings (60 ± 5 mo and 457 ± 28 kg of BW) were used. Dietary treatments were evaluated in a replicated 4 x 4 Latin square design experiment. Each experimental period lasted 20 days and consisted of a 15-day dietary adaptation, followed by a 5-day total collection faeces (TFC). The blood parameters were collected in the first day of TFC. After TFC, horses received basal diet for a 15-day washout. Horses were fed a basal diet and a pelleted concentrate (Hay *Cynodon dactylon* L. 'Tifton 85'). Diet was formulated to meet at 1.75 %BW - with a forage-to-concentrate ratio of 57:43. Dietary treatments consisted of a basal diet associated with one of the following yeast supplementation strategies: CTRL: without yeast supplementation ; NPYEA: 15 g/d of non-protected live yeast (*Saccharomyces cerevisiae* 1.5 × 10¹⁰ cfu/g; Procreatin-7); PYEA: 20 g/d of protected live yeast (*Saccharomyces cerevisiae* 1.0 × 10¹⁰ cfu/g; Actisaf HR Plus Sc 47), and COMB: a combination of the daily doses of NPYEA and PYEA (15 and 20 g/d, respectively) total a daily dose of 35 g/d. The NPYEA daily dose was top-dressed onto the pelleted concentrate, divided into 7.5 g per meal, when meals were offered, while the PYEA was incorporated in the pelleted concentrate during the concentrate manufacturing process. The following blood parameters were evaluated: glucose, insulin, cholesterol, triglycerides, VLDL, HDL and LDL using methods validated for equines. Treatment means were compared using orthogonal contrasts (C1: CTRL versus NPYEA, PYEA and COMB; C2: COMB versus NPYEA and PYEA; C3: NPYEA versus PYEA). Data were tested for normal distribution using the Shapiro-Wilk test and submitted to analysis of variance using the MIXED procedure in SAS. Means comparison were adjusted by Tukey's test at the 5% significance level.

Results and discussion: Supplementation strategy did not affect insulin and glucose concentrations (921.5 µU/ml and 80 mg/dL). Supplementation strategy affected blood levels of triglycerides, VLDL and HDL, but not cholesterol and LDL (Table 1). Horses supplemented with yeast, had lower triglycerides and VLDL, but the combination, increased the concentrations of this parameters. We found one study that evaluated blood parameters using yeast in ponies [1], and they did not find differences.

Conclusion: The results show that use of yeast, whether protected or live, improves the triglycerids and HDL and lower the values of VLDL in horses in maintenance.

Table 1. Blood parameters of horses supplementation on unprotected yeast or protected yeast.

Item	CTR	LEV	PROT	L+P	SEM	Trat	C1	C2	C3
Triglycerides	33.7a	26.6b	28.5b	34.9bc	1.55	0.002	0.004	0.009	0.403
Cholesterol	81.3	92.6	85.8	84.3	4.42	0.339	0.158	0.372	0.284
HDL	38.8b	43.6a	42.8a	43.2a	1.59	0.148	0.033	0.974	0.701
LDL	35.8	43.7	37.3	34.2	5.06	0.574	0.453	0.318	0.383
VLDL	6.75a	5.32b	5.70b	6.97c	0.31	0.003	0.004	0.009	0.403

References: [1] Palagi et al. (2017) *Livestock Sci.* Supplementation with live yeasts and essential oils does not alter blood, fecal and digestible parameters in horses, 206: 161-165.

4D3. Perceptions of sustainable horse feeding practices in the Netherlands

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Introduction: The equine industry sits within the agricultural sector in terms of social and environmental sustainability pressures. Within the agriculture sector modification of feed supply and utilization has been shown to be one of the simplest and most practical methods of developing sustainability. The purpose of this study was to determine horse keepers' in the Netherlands perceptions of sustainability, their level of concern around sustainability and their willingness to change to more sustainable feeding practices.

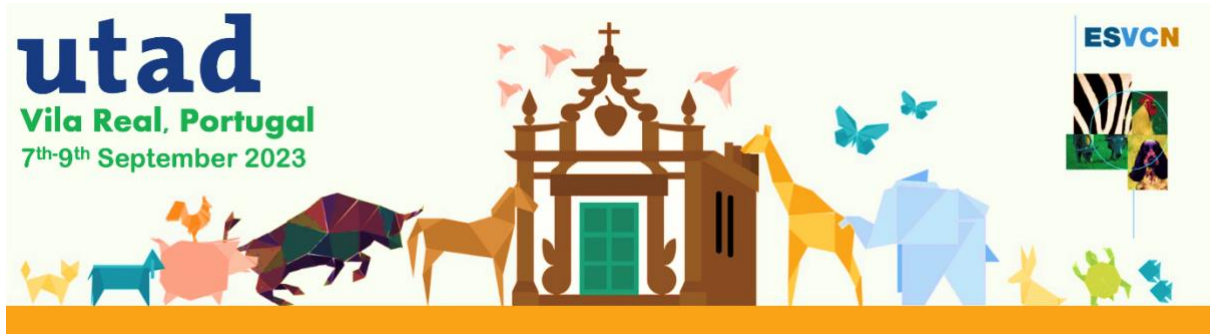
Materials and methods: Data were collected via a cross-sectional online survey (Survey Monkey (2022)) of a convenience sample of horse owners/managers within the Netherlands, recruited via social media (initial Instagram and facebook posts by Pavo and KNHS) from March to June 2022. Respondents were asked to select their top three perceptions of sustainability (out of 9 options), to indicate their concern about sustainability (scale 0-3) and to select their preferences to switch to any of the six sustainable choices provided. Data were stratified based on horse keeping activity and size of the equine operation (small (<20), medium (20-50), and large (>50 horses). Quantitative data were analyzed using descriptive statistics and Chi-square test (SPSS statistical software). Significance was accepted at $P < 0.05$.

Results and discussion: 400 surveys were received, with 338 valid responses representing 5,765 of the 450,000 horses in the Netherlands (estimated response rate of 1.2%). The majority of the yards were livery yards (230/338, 68%) followed by sport horse yards (68/338, 20.1%), riding school yards (35/338, 10.4%) and breeding yards (5/338, 1.5%). The majority of the yards represented were small in size (223/338, 65.9%) with only a few large (12/338, 3.6%) yards represented. The three most frequent terms associated with 'sustainability' were; low environmental impact (61.8%), pesticide and herbicide avoidance (52.7%), and the use of plastic-free packaging products (51.1%). Most respondents (90%) reported consideration of sustainability within the sector with a median score of 2 (moderate concern). However, all participants (338/338) reported willingness to switch to more sustainable feeding practices, the most popular options from 6 provided were; using plastic free packaging products (60.5%), feeding with organic muesli (56.5%), organic roughage (53.3%), stop feeding soy products (50%), feeding with hay rather than haylage (49.7%), and using rotational grazing (42%) regardless of cost impact. Possibly due to less economic and operational constraints small livery yards were more willing to switch to the suggested more sustainable practices than riding schools, sport horse yards or breeding yards ($P < 0.001$) or large facilities ($P < 0.001$).

Conclusion: Small livery yards, which account for the majority of Dutch horse yards, may be the most receptive towards the inclusion of sustainability. A limitation of the study was that economic and operational constraints were not examined as barriers to greater sustainability.

Funding Statement: The SIA Directorate, the Netherlands Organization for Scientific Research (NWO), Taskforce for Applied Research.

Plenary Session Saturday
Chair: Jürgen Zentek



Speaker: Marshall McCue

Dr. Marshall McCue is the Chief Scientific Officer and the head of the Research Department at Sable Systems International, the leading manufacturer of Metabolic measurement systems for animals. He is a broadly trained comparative physiologist with experience studying animal models ranging from insects, fish, amphibians, reptiles, birds, and mammals (including humans).

McCue earned a B.Sc. degree in Zoology from University of Florida, a M.Sc. degree in Biology from the University of California Irvine, a Ph.D. in Biology from University of Arkansas, and completed two years of postdoctoral research at Ben Gurion University in Israel. Between 2010 and 2018 he was a professor in the Department of Biology at St. Mary's University in San Antonio, Texas and simultaneously held visiting researcher positions at Nicholas Copernicus University, Poland; University of North Florida; and Stellenbosch University, South Africa.

McCue has published over 60 peer-reviewed scientific papers, most of which focus on animal nutrition and bioenergetics with emphases on digestive and fasting/starvation physiology. His early research examined the physiological and biochemical mechanisms responsible for the ubiquitous phenomenon called 'Specific Dynamic Action (SDA; also referred to as 'heat increment of feeding' or 'postprandial thermogenesis'), during which he developed expertise in the measurement of metabolic rates (i.e., rates of oxygen consumption and rates of carbon dioxide production in animals). Thereafter, he began exploring the progression of physiological, biochemical, and behavioral changes that occur during periods when animals are not actively digesting meals (known as 'fasting' or 'starvation' depending on the root cause).

These combined experiences examining the discrete influxes of dietary nutrients and the continual need for animals to use stored nutrients led McCue to develop novel methods using stable isotope tracer molecules (notably those containing ^{13}C and ^{15}N) to characterize the allocation of dietary nutrients among organs and tissues as well as approaches to quantify the timing and rates at which animals mobilize and oxidize different classes of macronutrients (lipids, proteins, and carbohydrates) stored within the body. McCue serves on the editorial boards for the Journal of Comparative Physiology and Physiological and Biochemical Zoology and is always on the lookout for new collaborative partners to expand the fields of metabolic measurement and ^{13}C -breath testing.

Using ¹³C-tracers to characterize nutrient use during feeding and fasting in comparative animal models

Marshall D. McCue

Elemental carbon makes up ~45% of the dry biomass of a typical animal. Most metabolic energy used to support the basic functions of life (e.g., growth, survival, and reproduction) was once stored within the carbon-carbon bonds of this biomass. As such an understanding of the carbon fluxes inside an animal as well as between the animal and its environment can provide valuable information about its physiology and mass-energy balance. The carbon atoms found a) in the body of an animal, b) its bulk diet, and c) the CO₂ it exhales naturally occur in two stable isotopic forms (¹²C and ¹³C) that comprise approximately ~99% and ~1%, respectively. The ability to detect differences in ¹³C abundance as small as 0.001% in either tissue or breath samples permits researchers to use trace amounts of ¹³C-labeled molecules to study metabolic processes.

During feeding exogenous carbon enters the body as dietary macromolecules (i.e., lipids, proteins, and carbohydrates) are digested and assimilated before being routed among different tissue compartments. These processes can be costly and are 'paid for' by mobilizing stored [i.e., endogenous] energy reserves as well as oxidizing the newly assimilated nutrients from a meal. ¹³C-tracers can be used to examine internal nutrient routing networks as well as characterize the different energy sources used to support food processing. As physiological and environmental conditions change, endogenous carbon that is already inside the body may be reallocated among different tissues. For example, resources are mobilized and subsequently oxidized by skeletal muscles during exercise. ¹³C-tracers can be used to examine the extent to which different endogenous fuels are used during exercise.

During reproduction endogenous resources may be supplemented with some ratio of exogenous resources as new tissues are deposited. Some mammals may double their food intake as they serve as a conduit to nurse their offspring. ¹³C-tracers can be used to characterize the contribution of stored ('capital') and ingested ('income') nutrients to support pre-natal and post-natal reproduction. When carbon inputs from the diet are halted [either self-imposed (i.e., during fasting) or externally imposed by the environment (i.e., during starvation)] animals must prioritize which resources to oxidize to meet unavoidable energy demands. Endogenous ¹³C-tracers can also be used to characterize the successive changes in metabolic fuels used to survive periods of food limitation.

Session 5A Protein I
Chair: Esther Hagen-Plantinga

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5A1. Influence of the preservation of alfalfa silage on fiber bound protein

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Introduction: Alfalfa has a high protein content, which makes it a valuable, but poorly ensilable crop [1]. Previous trials showed that pre-drying can be effective to ensure protein availability [2], while steam heating contributes to an increase in neutral detergent fibre (NDF)-bound crude protein in ensiled alfalfa [1]. Hence the aim of this study was to investigate whether different drying temperatures and dry matter content of the alfalfa raw materials have an influence on fiber bound protein in alfalfa silage.

Materials and methods: Alfalfa from the 2nd cut, August 2022, was chopped and stored at – 18 °C. It was then dried at 70 °C or 90 °C in a drying oven and a dry matter (DM) content of approx. 55 % or 65 % was targeted. After drying, the alfalfa was ensiled in 1.5 L jars. As a control, wilted silages were created outdoors on tarps (approx. 40 % dry matter). All five variants were made in quintuplicates. Due to the fact that the alfalfa was dried in bowls in the drying oven and on tarps outside, crumb losses can be excluded. After 90 days of storage at room temperature in the dark, the jars were opened. Crude fibre (XF), neutral detergent fibre (aNDFom) and acid detergent fibre (ADFom) were analysed. A part of fiber analysed material was ashed to determine the content of the organic substance. The other part of the analysed material was used to determine the fiber bound protein content by measuring the nitrogen content (Dumas) in the residuals. To estimate the “pure fiber” content, the fiber bound protein content was subtracted from the total fiber content. For the statistical analysis, a multi-factorial ANOVA was performed followed by a post-hoc Tukey test to determine differences ($p < 0.05$) between the silage variants.

Results and discussion: Drying temperature and DM of the silage did not influence the XF content. The dried silages (55 and 65 % DM) had a higher aNDFom content compared to the wilted silage ($p = 0.047$). A higher DM content (65 % DM) also resulted in a significant decrease in the ADFom content ($p < 0.001$), regardless of temperature. For the XF bound crude protein, there was no significant influence by dry matter content or temperature. However, there was a significant increase in aNDF bound crude protein with respect to dry matter ($p < 0.001$). The raw material dried to 65 % DM at 90 °C drying temperature resulted in the highest value, whereas the wilted silage showed the lowest aNDF bound crude protein content. Whereas there was no difference between the variant 70 °C drying to 65 % DM and 90°C drying to 55 % DM in this parameter. For ADF bound crude protein, the high dry matter (65 %) resulted in a reduction of crude protein bound to this fiber fraction compared to the other two DM-contents ($p < 0.001$). An interaction of DM and temperature was observed for the ADF bound crude protein ($p = 0.01$).

Conclusion: The results show that DM-content of the raw material can increase aNDF bound crude protein while ADF bound crude protein can be decreased by the DM-content and drying temperature in alfalfa silages. This could influence the digestibility of the protein.

References: [1] Polan et al. (1998) J. Dairy. Sci 81:765-776. [2] Steinhöfel et al. (2021) Proteinmarkt – Das Infoportal für Fütterung und Management.

5A2. Effect of dehydrated black soldier fly larvae inclusion in the diet on the welfare status of laying hens at 32 weeks of age

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Introduction: Insects have been recognized in poultry farming as a potential alternative to common raw materials such as soybean or fishmeal, because of presence of considerable concentration of nutrients [1]. Insects are also being considered as an environmental enrichment tool in poultry farming, when used alive [2]. However, there is very little information regarding the effect of the use of dehydrated insects in poultry feed on the welfare of birds, especially in laying hens. Therefore, the objective of this study was to evaluate the effect of the inclusion of dehydrated black soldier fly (*Hermetia illucens*) larvae in the diet on the welfare status of laying hens at peak laying.

Animals, materials and methods: Eighty laying hens (Isazul breed) were distributed in 10 floor pens (5 pens per treatment) with 8 hens each. There were 2 experimental treatments: a control feed (2730 kcal AMEn/kg and 16.4 % crude protein); and a control feed supplemented with whole dehydrated *H. illucens* larvae. The feed in both treatments was provided *ad libitum*. For the treatment with insect supplemented, the larvae were offered at 5% of the total dry matter intake (weekly estimated) and deposited in a separate feeder. In the insect-free treatment, an empty feeder was also placed to maintain similar environmental conditions. Nine weeks after the start of the trial, a welfare check-up (32 weeks of age) was carried out according to the Welfare Quality® parameters (using 2 or 3-point scales) for all hens [3]. Adapted tonic immobility test [4] was performed in all pens using 4 hens by pen. A higher score or a longer immobility time (sec) means a worse welfare status. For statistical analysis, the non-parametric Mann–Whitney *U* test was performed, using the SPSS statistical package.

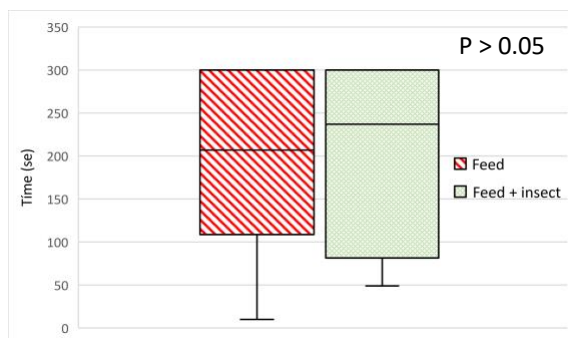


Figure 1. Whisker chart: Effect of larvae inclusion on tonic immobility test in birds

Results and discussion: No hen showed foot pad dermatitis, toe damage, enlarged crops, eye pathologies, enteritis, respiratory pathology or parasites regardless of the treatment. Moreover, larvae inclusion did not affect the keel bone deformation, skin lesions, comb abnormalities or feathering scores in the hens. The value of tonic immobility (Figure 1) of control group, expressed as median and interquartile range, was found to be 207 sec (108.75-300.00), that was similar to the insect included group 237 sec (81.50-300.00)

Conclusion: Inclusion of dehydrated black soldier fly (*H. illucens*) larvae at 5% in the diet of laying hens at peak lay did not negatively affect the studied welfare quality parameters of laying hens.

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5A3. Whole transcriptome analysis in broiler chicken fed mealworm meal

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Introduction: Over the last decades, increasing demand for cost-effective protein sources lead to global research of alternative food and feed sources [1,2]. The aim of this study was to assess the effect of dietary *Tenebrio molitor* meal supplementation on intestinal digestion and absorption of nutrients and the overall metabolism of carbon, nitrogenous and lipidic compounds in hepatic, intestinal and muscular tissues of broilers.

Animals, materials and methods: Ten replicates of day-old broiler chicks (Ross 708; 8 birds/pen) were divided into 2 groups and fed isonitrogenous and isoenergetic diets (12.9 MJ ME/kg and 23.5 % CP from day 1 until day 12, 13.3 MJ ME/kg and 21.3 % CP from day 12 until day 25, and 13.5 MJ ME/kg and 19.6 % CP from day 25 until day 52) containing either 0% (CT) or 15% (TM) mealworm meal. Two broilers/pen were slaughtered on day 53. Liver, jejunal, caecal and breast muscle samples were collected, pooled samples of each treatment and were analyzed for whole RNA-seq. Differential expression of genes (DEG) was carried out using the Cuffdiff. Functional analysis of gene expression was done using Reactome and KEGG database. Significant coding gene expression was defined for DEG below -1 and above 1. Fisher test corrected with the Bonferroni procedure was used and differences were considered significant at $p < 0.05$.

Results and discussion: Hepatic upregulation ($p < 0.001$) of primary bile acid biosynthesis (AKR1D1), steroid hormone biosynthesis (AKR1D1 and CYP1A1) and GLY, SER, THR, CYS and MET metabolism (BHMT) was observed in the TM compared to the CT groups. Birds fed the TM treatment presented upregulation of the jejunal ADH1B ($p < 0.001$) indicating increased glycolysis, gluconeogenesis, pyruvate metabolism along with fatty acid degradation, TYR metabolism and retinol metabolism. Jejunal upregulation of RBP2 ($p < 0.001$) in the TM compared to the CT group indicate higher vitamin digestion and absorption. Downregulation of genes involved in nitrogen metabolism (CA4, $p < 0.001$), pancreatic secretion (CCK, SLC26A3; $p < 0.001$) and protein digestion and absorption (CELA2A, $p < 0.01$) were found in the TM compared to the CT groups. The caecal samples of the TM group were downregulated for genes involved in carbon metabolism (PSPH, $p < 0.001$; AMT, $p < 0.05$), biosynthesis of amino acids (PSPH), GLY, SER and THR metabolism (PSPH, AMT), glyoxylate and dicarboxylate metabolism (AMT), oxidative phosphorylation (COX8A, $p < 0.001$), purine metabolism (HDDC3, $p < 0.05$), butanoate metabolism and VAL, LEU and ILE degradation (HMGC2, $p < 0.01$), glycerophospholipid metabolism, ether lipid metabolism, essential fatty acids metabolism, pancreatic secretion, fat digestion and absorption (PLA2G2E, $p < 0.05$). Upregulation of genes involved in caecal fat digestion and absorption (APOA4 and FABP1, $p < 0.001$), cholesterol metabolism and vitamin digestion and absorption (APOA4) was seen in the TM compared to the CT groups. Muscle downregulation of CHAC1 ($p < 0.001$) involved in glutathione metabolism and upregulation of PHGDH ($p < 0.001$) involved in carbon metabolism, biosynthesis of amino acids, GLY, SER, THR, CYS and MET metabolism was observed in the TM compared to the CT groups. The influence of *T. molitor* supplementation on protein metabolism in poultry [3] was previously reported.

Conclusion: Diet supplementation with *T. molitor* meal seems to stimulate hepatic gene expression of lipid and amino acid metabolism. It also influences intestinal glucose, fat and protein metabolism, and lipid, protein and vitamin digestion and absorption. Finally, *T. molitor* supplementation leads to the upregulation of protein metabolism in muscle.

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5A4. Influence of oral glutamine supplementation on microbial gene abundance and associated metabolic pathways in stomach content of neonatal piglets

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Introduction: The non-essential amino acid glutamine (Gln) has been shown to improve growth and immunological functions in pigs after weaning [1]. Low birthweight piglets (LBW) typically face a higher risk of mortality before weaning, often due to impaired gastrointestinal function [2]. *In vitro* studies have shown, that Gln is used by small intestinal bacteria and also affects the utilization pattern of other amino acids [3]. The aim of the study was to test effects of Gln supplementation on bacterial gene abundances and metabolic pathways in stomach content of LBW and normal birthweight (NBW) piglets under the influence of Gln or alanine (Ala) supplementation.

Animals, materials and methods: For the trial LBW (0.8 - 1.2 kg) and NBW (1.4 - 1.8 kg) male littermates born to gilts were paired at birth (day (d) 0). The piglets received an oral supplementation of either 1 g Gln or Ala 1.22 g per kg bodyweight as a negative control, isonitrogenous to Gln until 12 d. Piglets suckled throughout the study and were euthanized at 5 and 12 d of life ($n = 40$). Stomach content was snap frozen in liquid nitrogen, and stored at -80°C for subsequent analysis. DNA was extracted from the content and full metagenomic sequences were analysed. Gene abundance was estimated with HUMAnN3 and statistical analyses were performed using the R package MaAslin2. Differences in gene abundances and metabolic pathways between supplementation, birthweight and age groups were considered significant at $q < 0.05$. In addition, significant associations for the factors with the metabolic pathways were determined.

Results and discussion: In total 33.157 microbial related genes were detected in stomach content of suckling piglets. In at least 36 out of 40 samples (log fold change (lfc) ± 1.5), 41 genes showed a change in abundance ($q < 0.05$) affected by Gln compared to Ala supplementation. Most of the more abundant gastric content genes affected by Gln supplementation were associated with the most abundant *Lactobacillus* spp., particularly *L. limosilactobacillus mucosae*. The molecular function of the higher abundant genes was mainly associated with DNA and RNA interactions, hydrolase, protease and transferase activity. Significantly upregulated genes were involved in the biological processes of translation, transcription, amino acid and purine/pyrimidine biosynthesis, ATP synthesis and proteolysis. Birthweight affected the abundance of four microbial genes. Age influenced the abundance of 51 genes in at least 38 samples of stomach content (lfc ± 2.5 ; $q < 0.05$). Low birthweight showed a negative association with methionine biosynthesis pathways. Age (5d) had three negative associations with pyrimidine and flavin metabolic pathways. Positively associated with age 5 d compared to 12 d were metabolic pathways mainly related to amino acid and polyamine biosynthesis.

Conclusion: The results demonstrate that Gln compared to Ala supplementation influenced gene abundance associated to mainly *Lactobacillus* ssp. in the stomach content of suckling piglets. Birthweight had little influence on bacterial gene abundance and metabolic pathways, whereas age strongly affected bacterial gene abundance and metabolic pathways. Overall, the metagenomic approach helps to better understand bacterial metabolism in detail, but associations with metabolic pathways should be backed up by measurement of physiological parameters.

References: [1] Wang et al. (2008) J Nutr 138, 1025-1032; [2] Hales et al. (2013) J Anim Sci 91, 4991-5003; [3] Dai et al. (2013) Amino Acids 45, 501-512

5A5. Body composition and protein intake effects on plasma amino acids concentrations in cats

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Introduction: It is known that obesity is a condition that interferes on glucose and energy metabolism, reflecting on energy expenditure (EE) and food intake of cats. However, its implications to protein and amino acid metabolism are less studied, but it may also be influenced by the metabolic alterations induced by this condition [1]. Considering the implications of protein and amino acid intake on protein synthesis, energy metabolism, neoglycogenic pathways and as nitrogen compound donors [2], the present study compared free amino acids plasma concentrations in overweight (OW) or non-overweight (NO) cats fed a high protein (HPr) or a normal protein level (NPr) kibble diets.

Animals, material and methods: The study followed a 2 (diets) x 2 (body condition) arrangement with 4 treatments (approval 9536/18). NPr (40% starch; 38% crude protein [CP], on DM) or HPr (20% starch; 55% CP) kibble diets with similar energy (16.7kJ/g, as fed), fat and fiber content, and 10 NO cats (3.9±0.8 kg; 4.1±0.9 years; Body Condition Score [BCS]: 5.0±0.0; 16% of fatty mass [FM]) and 6 OW cats (4.6±0.8 kg; 5.8±1.2 years; BCS: 7.2±0.1; 27% FM) were used in a crossover design. After 30 days of diet intake, blood was collected after 16 hours of fasting and 6 hours postprandial (PP), and free plasma amino acids (AA) were analyzed by high-performance liquid chromatography. Food was provided to maintain a constant body weight (BW). Data were evaluated with two-way ANOVA considering body composition, protein content, and their interaction effects (P≤0.05 significant; P≤0.10 trend).

Results and Discussion: Amino acids and protein intake (in g/kg^{0.67}/day) was lower for NPr (5.9±1.0 g) than HPr (8.8±1.4g), as expected, and higher for NO (8.1±0.9 g) than OW (6.6±1.0 g) because of the high EE of NO cats (ingested 18% more food than OW to constant body weight; P≤0.05). The AA plasma concentration was within the reference values for cats [3]. The non-essential to essential AA ratio was not influenced by protein intake or body composition (P>0.05), but the glycine:valine ratio was higher for OW than NO cats suggesting higher metabolic consumption of essential AA in OW cats (P<0.05). Few differences at fasting were observed, with higher phenylalanine+tyrosine (phe+tyr), asparagine, glutamine, and hydroxyproline for OW than NO (P≤0.05), without diet effects. In the PP period OW cats showed higher arginine, lysine, aspartic acid, and glutamine plasma concentration than NO cats, and cats fed HPr higher lysine and glutamine (P≤0.05), with a tendency for higher threonine, and lower aspartic acid and phe+tyr than cats fed NPr diet (P≤0.1). It was interesting to observe that even though the large differences in AA intake between diets, body composition did show more implications to plasma AA values than diet. Except for the phe+tyr at the PP period that tended to be higher for NO, all other essential and non-essential AA that showed differences (including phe+tyr at the fasting period) presented higher values in OW than NO cats. Higher phe+tyr plasma concentration were associated with insulin resistance in obese individuals [4], and a possible insulin resistance might explain the higher plasma AA in OW cats in the current study.

Conclusion: Body composition in cats may have a greater impact on free AA concentration in the plasma than high protein intake. The higher plasma concentrations of essential and non-essential AA in OW cats, added to higher glycine:valine ratio, suggest a greater mobilization of these compounds, with possible direction to catabolic pathways for energy production and may also signalize insulin resistance.

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Session 5B Protein II
Chair: Aulus Carciofi



5B1. Weight loss and methionine, tryptophan, valine and threonine supplementation effects on plasmatic amino acid concentrations in dog

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Introduction: Obesity is a common clinical condition related to nutrition in dogs, that compromises life span and quality [1,2]. To lost body weight dogs are induced to a negative energy balance, that promotes adipose tissue mobilization [3,4] but may also interfere on protein and amino acid metabolism [5], aspect less studied. Considering the implications of amino acids in protein synthesis and energy metabolism [6], the present study compared free plasma amino acids (pAA) concentration in non-obese (NO) or obese (OB) dogs (Approval n^o 07196/19) fed a low-calorie kibble diet (CO) or the same formulation supplemented with methionine, tryptophan, valine and threonine (AA). The OB was also compared at start and after a 20% body weight loss (WL).

Animals, materials and methods: The pAA was evaluated in two phases, on phase 1 (Ph1) 20 NO dogs (body condition score [BCS] 4 to 5/9; 23.4±1.3% fatty mass determined with deuterium oxide) and 20 OB dogs (BCS 7-9/9; 43.1±1.3% fatty mass) were fed to maintaining a constant body weight for 14 the CO diet (31.7% crude protein; 25.2% dietary fiber; 3.0 kcal of ME/g, on DM) of the same formulation supplemented with crystalline methionine, tryptophan, valine and threonine (AA). At phase 2 (Ph2), OB dogs were engaged in a weight loss program (medium energy intake of 60 kcal/kg actual weight^{0.75}/day) with both diets, and pAA analyzed at start and after 20% WL. Blood was collected from the jugular vein after 12 hours of fasting and 6 hours of postprandial period (PP), plasma separated and deproteinized, and analyzed by high performance liquid chromatography (HPLC; Biochrom 30 Amino Acid Analyzer, Biochrom, Cambridge, UK). A factorial arrangement were adopted, with 2 diets and 2 (Ph1: body condition; Ph2: before or after WL), with 4 treatments, considering at the F test the effects of diet, body condition or WL, and their interations (P<0.05; P<0.1 as tendency)

Results and discussion: Dogs fed AA diet tended to present higher WL rate (0.83±0.07% per week) than CO dogs (0.63±0.08%; P=0.08), with similar actual ME intake (57±1 kcal/kg actual weight^{0.75}/day) and fatty mass at the end (29±2% of fatty mass). OB dogs presented at PP period lower serine, asparagine, glycine, and higher glutamine, and phenylalanine than NO (P<0.05). Regardless of body condition, dogs fed AA showed higher methionine (P<0.05), with a tendency to lower tryptophan (P<0.1). Body condition x diet interaction was detected, with lower isoleucine, leucine and valine in OB dogs fed AA than the other treatments (P<0.05). After weight loss, regardless of diet, at fasting dogs showed lower aspartic acid, glutamine, and arginine, while at PP period higher taurine, serine, and asparagine (P<0.05), and a tendency to higher valine, isoleucine, and leucine (P<0.1). When WL was induced with the AA diet, lower valine, isoleucine, leucine, and lysine, with higher methionine was observed (P<0.05). In addition, interaction was observed for 3-methyl-histidine (3-MH), with lower concentration after weight loss (at fast and PP period) for dogs which lost body weight fed the CO, but the final values of dogs fed AA diet was similar to initial concentration (P<0.05).

Conclusion: Methionine, tryptophan, valine, and threonine supplementation may induce protein synthesis in OB dogs, due to the observed reduction in plasma branched-chain amino acids, lysine, and tryptophane, which can be attributed to increased plasma methionine. These changes may spare muscle mass loss, as 3-MH did not reduce in amino acid supplemented dogs. The lower arginine suggests an increased urea cycle.

Acknowledgements: Special Dog; BRF Pet Food; BRF Ingredients; ADIMAX Pet Food; CAPES.

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5B2. Effect of extrusion and phytase on the digestibility of plant-based pet foods

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Introduction: Human diets with more plant-based protein sources and less meat has potential benefits for the environment, animal welfare, and human health [1]. Owners therefore increasingly seek plant-based foods for their pets, which stimulates the development of vegetarian and vegan pet foods. These foods can contain various plant protein sources, each with distinct nutritional properties. Extrusion cooking technology is commonly applied to manufacture pet foods. This hydrothermal process may impact the bioavailability of nutrients by changing the molecular conformation of nutrients, activity of anti-nutritional factors and the matrix [2]. Furthermore, phytase also has potential to improve the digestibility of nutrients, suggesting that the addition of phytase can promote the nutritional potential of plant sources. We aimed to evaluate the effect of extrusion and phytase on nutrient bioavailability of pet foods based on various plant protein sources using an *in vitro* digestion assay.

Materials and methods: Six different plant protein sources (pea, faba bean, lentils, kidney bean, chickpea, sunflower, rapeseed) were included at a rate of 50% in formulation and extruded using a twin-screw extruder. The protein, starch and fat levels were standardised by changing inclusion of corn, corn gluten meal and poultry fat. All extruded foods were ground to pass a mesh size of 1 mm. Approximately 0.5 g of each food before and after extrusion was weighed and subjected to a modified *in vitro* digestion procedure, based on Hervera *et al.* [3]. Blanks were used as controls and incubations were performed with two replicates for each sample. Samples were incubated without and with phytase (20000 FTU/kg food), which was added during the simulation of gastric stage. Residues were collected by glass crucibles, washed twice with ethanol and acetone, dried to constant weight and weighed. The dry weight of each sample and residue was used to calculate dry matter (DM) digestibility. Data were statistically analysed by repeated measures ANOVA with Extrusion (E; before, after), Phytase addition (P; yes, no) and the interaction E×P as fixed effects of the model.

Results and discussion: Extrusion increased DM digestibility by 7.81% ($p < 0.001$ for E), while the phytase did not change digestibility ($p = 0.872$ for E×P and $p = 0.759$ for P). According to digestibility data of extruded diet, the digestibility of different protein source without phytase is between 65.4% and 87.9%, which is similar with addition phytase, lentils showed the best potential at 87.9%, while rapeseed was only 65.4% digestible. Besides, pea, faba bean, chickpea also showed great potential on digestibility over 80%. The improvement on digestibility may be due to anti-nutritional factors that are inactivated by extrusion processing [4]. Phytase can increase amino acid absorption in broilers [5], the lack of effect of phytase on the DM digestibility of plant protein sources observed in this study is consistent with a study in pigs [6]. More detailed analyses of nutrients (e.g., minerals) are pending and will provide more insight in the nutritional properties of these plant protein sources as well as the effect of phytase.

Conclusion: This study showed that extrusion can improve the digestibility of plant-based pet foods. The benefit of adding phytase to vegetarian dog diets may be specific to mineral bioavailability, which still needs to be evaluated through further analysis.

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5B3. Changes in faecal microbiota and functional profile in dogs fed diets with higher or lower protein and fibre concentrations

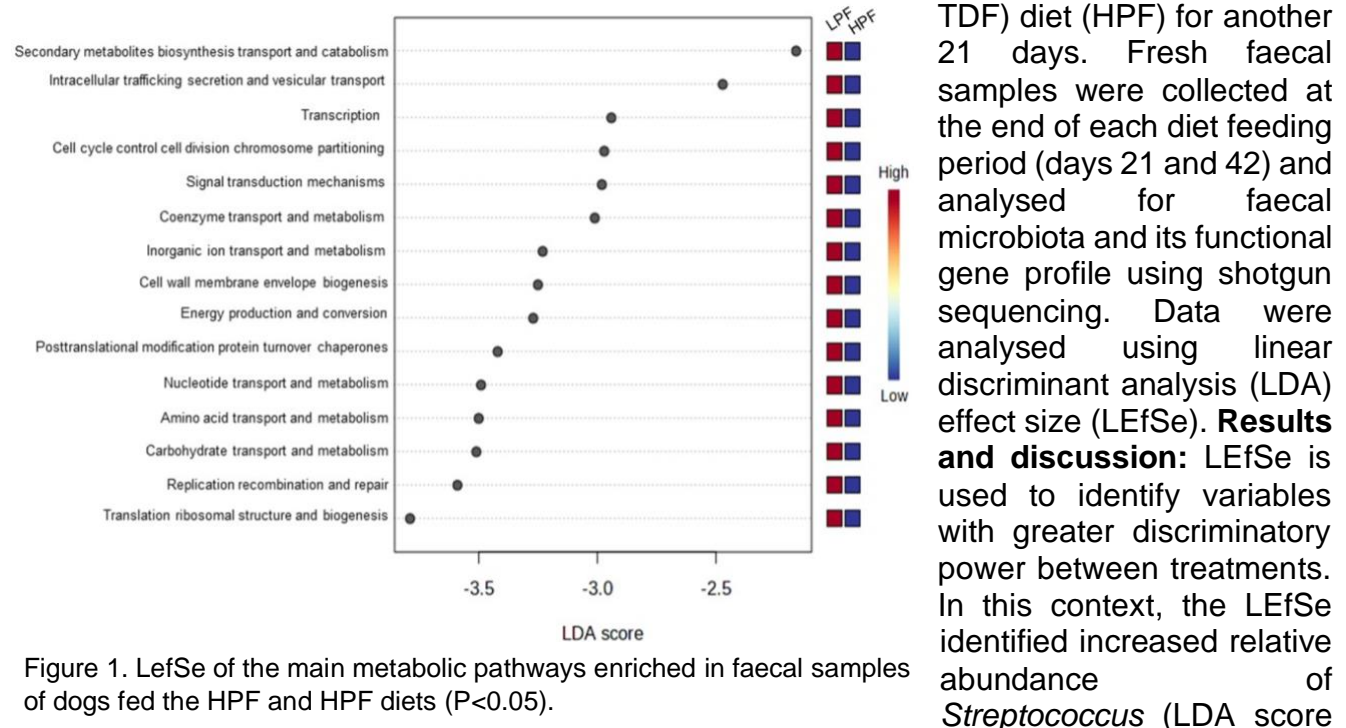
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Introduction: This study aimed to evaluate changes in faecal microbiota and its functional genes in dogs fed diets with varying protein and fibre concentrations.

Animals, materials and methods: 16 adult Beagle dogs (n=16) were fed a lower-protein (20.42% crude protein – CP) and lower-fibre (6.10% total dietary fibre – TDF) diet (LPF) for 21 days, and then abruptly transitioned to a higher-protein (27.52% CP) and higher-fibre (27.20%



TDF) diet (HPF) for another 21 days. Fresh faecal samples were collected at the end of each diet feeding period (days 21 and 42) and analysed for faecal microbiota and its functional gene profile using shotgun sequencing. Data were analysed using linear discriminant analysis (LDA) effect size (LEfSe). **Results and discussion:** LEfSe is used to identify variables with greater discriminatory power between treatments. In this context, the LEfSe identified increased relative abundance of *Streptococcus* (LDA score

= -2.2) in faecal samples of the LPF group and increased *Prevotella* (LDA score = 2.1) and *Bifidobacterium* (LDA score = 2.2) in the HPF group (P<0.05). The HPF diet results are consistent with those obtained by Bermudez et al. [1]. These two genera are correlated with intestinal eubiosis, indicating possible benefits of the HPF diet to dogs [2,3]. Also, enrichment in 15 main metabolic pathways was identified in faecal samples of the LPF group (P<0.05, Figure 1). Among them, are pathways of transport and metabolism of amino acids, carbohydrates, and secondary metabolites, indicating greater fermentative activity in the intestine of the LPF group. Considering the similar soluble fibre content of the diets (HPF, 1.21%; LPF, 1.80%), these results may be related to the lower protein digestibility of the LPF (83.2%) compared to the HPF diet (87.5%), evaluated in a previous study [4], which may have also contributed to the increase of *Streptococcus* [5]. Thus, digestibility plays a role as important as the chemical composition of the diet for the modulation of the microbiota and its functions in the gut.

Conclusion: HPF diet consumption increased faecal *Prevotella* and *Bifidobacterium*. Conversely, LPF diet increased faecal *Streptococcus* and resulted in greater enrichment of carbohydrate, protein, and secondary metabolite metabolic pathways.

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5B4. The effects of hydrolyzed chicken liver on digestibility, faecal characteristics, and faecal fermentative end products of adult dogs

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Introduction: Feeding dogs highly digestible protein sources improve the amount of undigested protein reaching the hindgut reducing proteolytic fermentation [1]. Hydrolyzed proteins are potentially more easily digested and absorbed than usual sources [2]. We hypothesized that high-protein diets based on commercial hydrolyzed chicken liver powder (HCLP) would increase apparent total tract digestibility (ATTD) of crude protein (CP), thereby reducing protein fermentation metabolites in the hindgut of healthy adult dogs compared to poultry by-product meal (PBPM) based diets.

Animals, materials and methods: Twelve healthy adult Beagle (6 of each sex), 3yrs, 11.2±0.85 kg, were distributed in a balanced incomplete Latin square design to assess the effects of protein source (HCLP and PBPM) and concentration (24, 32, and 40% CP DM basis). All the procedures were ethically approved for use of dogs. The experimental design consisted in three blocks of 30-d and 2 dogs per treatment in each block, totalling six dogs (repetitions) per treatment. Treatments were: HCLP24, HCLP32, HCLP40, PBPM24, PBPM32, and PBPM40. Water was supplied *ad libitum*. The ATTD of nutrients and metabolizable energy (ME) were measured using the total faeces collection method. Dogs were adapted to the treatments during 20-d, then faeces were collected for 6-d. On day 30 of each block, fresh faecal samples were collected and analyzed for faecal fermentative metabolites. Short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA) were determined by gas chromatography [3]. Lactate was determined by spectrophotometry [4]. Ammonia was determined in a nitrogen system. Data were analyzed by ANOVA and means were compared using Tukey's test. A p-value <0.05 was considered significant, and >0.05 and <0.10 was considered a trend.

Results and discussion: Both source and concentration of dietary protein affected digestibility. Digestibility of CP was greater for HCLP than PBPM diets (p=0.0025). The same was observed for diets containing 40% CP (p=0.0008). Digestibility of fat, carbohydrates, and ME was decreased for HCLP and high-protein diets (p<0.05). The greatest CP digestibility observed for HCLP diets can be related to the increased availability of small peptides and free amino acids in the protein source that improved digestibility. High-protein diets increased the daily faecal production, moisture, and faecal score (p=0.0039, p<0.0001, p<0.0001 respectively). Although the faecal consistency was affected, it remained within acceptable characteristics [5]. Faecal concentrations of isovalerate, BCFA, and ammonia were increased for the PBPM diets (p<0.05). The PBPM diets tended to increase faecal valerate and isobutyrate (p<0.10). Faecal lactate was greater for HCLP diets (p=0.0164). Lastly, high-protein diets increased the faecal concentrations of valerate, isovalerate, BCFA, and ammonia (p<0.05). The inclusion of HCLP decreased the amount of BCFA and ammonia in the faeces, which may be due to a reduced flow of undigested protein into the colon and decreased protein fermentation [1].

Conclusion: CP digestibility was superior for HCLP diets, which resulted in less protein fermentation in the colon even at higher CP concentrations. Faecal output was the same among diets (g DM/d). However, HCLP increased faecal moisture resulting in greater faecal output (g/d) with no detrimental effects in stool quality.

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5B5. Nutritional and ecological comparison of meat- and plant-based dog feeds

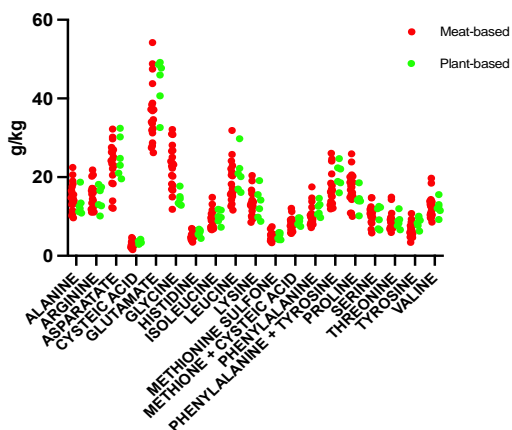
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Introduction: 'Plant-based' is becoming an increasingly popular dietary choice for many people. Pet feed has a surprisingly large carbon footprint [1] and following human diet trends, an increase in availability of plant-based foods for companion animals has become evident. Similar to human, recent studies have suggested multiple health benefits adherence to these diets has on companion animals [2,3,4]. No study has considered whether such vegetarian feeds are nutritionally complete nor how they compare to meat-based diets, particularly with respect to macro/micronutrients, vitamins, amino acids and measures of their carbon footprint.

Animals, materials and methods: 31 complete dry dog foods (n=19 meat-based, n=6 renal and n=6 plant-based) were analysed for minerals (by ICP-MS), amino acids (by LC-MS), fatty acids (by GC-MS), vitamin D and B-vitamins (by HPLC).

Results and discussion: n=3/6 plant-based and n=9/19 meat-based foods reported either



deficient or excess macro-minerals. Amino acid composition was similar (Figure 1). Essential fatty acids were replete in all feeds. Vitamin D analysis revealed n=1 meat-based food above legal limits, with other feeds nutritionally high in Vit D. B vitamins were relatively low in almost all plant-based foods, with Niacin (B3) reaching statistical significance ($P=0.03$). Further analysis on the estimated environmental impact of all foods demonstrated marked reductions in the land use of plant-based versus poultry ($P = 0.03$), beef ($P = <0.001$) and lamb ($P = <0.001$) diets (Figure 2). CO₂ emissions and water use between the feeds

Figure 1. Comparison of amino acid composition between meat- and plant-based dog foods.

showed the same trends.

Conclusion: Plant-based feeds are capable of meeting all nutritional requirements for omnivorous, companion canines, as tested here at an adult life stage. Further supplementation with B-vitamins may be required. Companies that embrace more plant-based foods as part of their portfolio of feeds will reduce their carbon footprint whilst maintained nutritional adequacy.

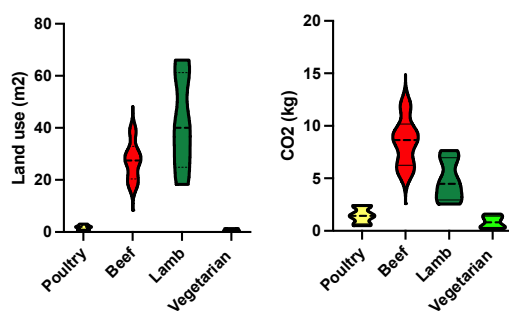


Figure 2. Violin plots showing land use and CO₂ emissions from production of 100g of meat- and plant-based dry dog foods.

References: [1] Okin GS (2017) Environmental impacts of food consumption by dogs and cats. PLoS ONE 12(8): e0181301. <https://doi.org/10.1371/journal.pone.0181301> [2] Knight et al, 2022, Vegan versus meat-based dog food: Guardian-reported indicators of health, PLoS ONE 17(4): e0265662. <https://doi.org/10.1371/journal.pone.0265662> [3] Davies, 2022, Reported Health Benefits of a Vegan Dog Food – A Likert Scale-type Survey of 100 Guardians, bioRxiv 2022.05.30.493980; doi: <https://doi.org/10.1101/2022.05.30.493980> [4] Dodd et al, 2021, Comparison of Key Essential Nutrients in Commercial Plant-Based Pet Foods Sold in Canada to American and European Canine and Feline Dietary Recommendations. Animals 2021, 11, 2348. <https://doi.org/10.3390/ani11082348>

5B6. Acceptance of insects in pet food products – a survey among Belgian dog and cat owners

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Introduction: Over the last few years there has been an increase in the availability of insect-based pet food (IBPF) products. Globally, more than 43 brands sell IBPF, of which 35 brands are located in Europe [1]. The current survey assesses the feeding habits and readiness of Belgian dog (DO) and cat owners (CO) in feeding IBPF to their pets.

Materials and methods: A survey comprising 38 questions was conducted through an online platform. The survey was distributed through social media channels, e-mails, veterinary clinics/hospitals, and pet food stores in Belgium. Individual surveys were excluded if less than 50% of the questionnaire was filled in or if the respondent did not have a dog or a cat. Additionally, the answers to species-specific questions were excluded if the respondent did not report owning the related species. 7-point Likert-scale questions aimed to assess the DO and CO IBPF's acceptance. All analyses were conducted in R version 4.2.2. For paired data, linear mixed models were used, with respondents as random effect. Otherwise, simple linear models were used to assess the various predictors of the dependent variables. In both models, likelihood ratio tests were used to evaluate significance. The analyses were done in parallel for dog and cat owners. Significance was set at $\alpha \leq 0.05$. When appropriate, a Bonferroni correction was applied to correct for multiple testing. Corrected p-values are reported.

Results and discussion: A total of 435 valid responses were evaluated. 82% (n=344) of the respondents were female and 17% (n=71) were male. 66% (n=287) were DO and 58% (n=251) CO. 25% (n=107) respondents were veterinarians, veterinary students, or nurses. The majority of the respondents were between 25-34 years old (34%; n=142), followed by 45-54 (24%; n=101). 43% (n=181) and 38% of the respondents had a university and higher education degree, respectively. 58% (n=160) DO and 66% (n=155) CO were positive about feeding their pets IBPF. This was expected, as over the last few years alternative diets gained popularity among pet owners [2]. Interestingly, CO (5.11±1.688) had a slightly higher acceptance of IBPF ($p < 0.05$) than DO (4.77±1.645). This demonstrates the importance of more studies with edible insects as food for cats. There was a significant increase ($p < 0.01$) in DO and CO IBPF's acceptance rate if it would be proven that these products would provide sustainable and nutritional benefits (DO: 5.1±1.602; CO: 5.47±1.638). 41% (n=119) DO and 53% (n=132) CO were following a specific diet themselves. IBPF's acceptance rate was significantly higher ($p < 0.05$) for CO (CO: 5.44±1.586) compared to the ones that did not follow a specific diet (4.76±1.731). Although DO that would follow a specific diet also scored higher (5.02±1.653) than the ones who didn't (4.6±1.623), the scores were not significantly different. 32% (n=90) DO and 34% (n=86) CO reported having previously eaten edible insect products themselves. An increased acceptance rate of IBPF was observed for the DO (5.04±1.580) and CO (5.44±1.546) that have previously eaten insects compared to the ones that have not (DO: 4.63±1.665; CO: 4.92±1.745), but the difference was not significant. There was no effect of age, gender, or being veterinary-related to the pet owner's IBPF acceptance. It is important to bear in mind the gender and education biases in these responses, as mainly female pet owners answered this survey and most respondents were affluent professionals.

Conclusion: CO had higher acceptance towards IBPF than DO. Increased acceptance of IBPF was seen for CO that were following a specific diet and if DO and CO would know that the IBPF would be sustainable and provide nutritional benefits to their pets.

References: [1] Siddiqui et al (2023) J. Asia Pac. Entomol 26:102020; [2] Dood et al (2019) PLoS One 14:1.

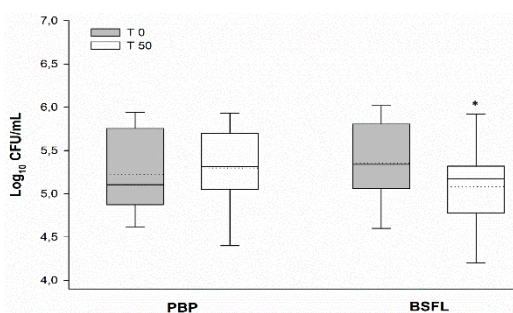
5B7. Potential of an extruded diet based on black soldier fly (*Hermetia illucens*) larvae meal to improve canine oral health

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Introduction: The growth of oral bacteria population can lead to plaque formation, which if continued, can turn into periodontal disease (PD). The black soldier fly larvae (BSFL) is a potential functional ingredient for pet food due to the functional properties including antimicrobial activity¹. The aim of this study was to evaluate the BSFL meal in extruded diets and its potential to improve canine oral health.

Animals, materials and methods: The study was carried out in a cross-over design with two periods of 50 days and two diets: poultry by-product meal based diet (PBP) and BSFL based diet. Eight adult-beagle dogs were selected and submitted to dental cleaning 60 days before the beginning of the study. Samples of dental plaque and saliva were collected using swabs and a syringe², respectively. Dental plaque was collected to evaluate volatile sulfur compound producing bacteria (VSCb) using differential specific medium³ and saliva for DNA extraction and amplification of 16S rDNA gene. Besides, oral malodor was measured by an intensity scale (5 points)⁴. The diets' effect on VSCb was analyzed using ANOVA mixed model with two factors (time x diet) with repeated measures on time and Post-hoc Tukey test. Kruskal-Wallis was used to analyze breath score. Statistical analysis were performed using SigmaPlot 12.0 ($p < 0.05$). The beta diversity was calculated using QIIME2 pipeline, through perMANOVA (10.000 permutations). The alpha diversity was measured through libraries "phyloseq", "vegan" e "microbiome". Mann-Whitney test was used to analyze the relative abundance, alpha and beta diversities.



Results and discussion: The VSCb counting differed between periods ($p=0.047$) and BSFL group had lower counting after 50 days ($p=0.030$). BSFL group also tended to be lower in the oral malodor scale (median BSFL=1; PBP=2) ($p=0.097$). Alpha diversity was similar between groups by Shannon, Simpson, Fisher, OTUs and Chao1 tests ($p > 0.05$) and different in Evenness Pileous analysis ($p=0.035$). Beta diversity was similar between groups by Bray-Curtis ($p=0.902$), Jaccard

Figure 1. Colonies forming units of VSCb in dental plaque of dogs fed PBP and BSF diets before and after 50 days of consuming

Moraxella sp002224245 ($p=0.0378$) was higher in dogs fed BSFL diet. This finding may have been due to the presence of antimicrobial peptides or higher concentration of lauric acid on BSFL, which has an antimicrobial activity against VSCb, such as *Fusobacterium nucleatum*⁵ and *Porphyromonas gingivalis*⁶. Moreover, VSC has been associated with halitosis in dogs⁷ and can be toxic to dental support tissues, leading to PD⁸. VSCb reduction can reflect on *Moraxella*, once it shows a negative correlation between its higher relative abundance and the PD conditions in dogs⁹.

Conclusion: The use of BSFL in extruded diet may perhaps positively modulate oral microbiota of dogs improve oral health condition. However, more research is needed.

References: 1] Kim et al. (2020) Poultry science. 99: 3133-3143; [2] Elliott et al. (2005) Journal of clinical microbiology 43:5470-5476; [3] Allaker, et al. (2008) Archives of oral biology 53:S8-s12; [4] Greenman et al. (2004) Journal of dental research 83:81-85; [5] Fischer et al. (2012) Antimicrobial agents and chemotherapy 56: 1157-1161; [6] Fischer et al. (2013) Int. j. oral science 5:130-140; [7] Iwashita et al. (2019) Fundamental toxicological sciences 6:75-79; [8] Allaker (2010) J. breath research. 4:017103; [9] Oba et al. (2021) J. Anim. Sci. 99:100.

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5C1. Determination of eleven essential amino acids (EAA) by HPLC-DAD/FLD in extruded and retorted grain-free and novel protein diets for dogs

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Introduction: Recent veterinary research has discussed a potential relationship between grain-free (GF) and/or novel protein (NP) diets to non-hereditary canine dilated cardiomyopathy (DCM) [1]. One of the most discussed nutritional etiologies was the limiting sulfur-containing amino acids of GF and NP formulations [2]. Deficiencies in certain nutrients are known to play a role in the development of DCM: taurine and its precursors methionine (Met) and cysteine (Cys), and carnitine and its precursors lysine (Lys) and Met [1]. The objective was to evaluate EAA contents of extruded and retorted GF and NP diets for dogs.

Animals, materials and methods: The samples consisted of 26 complete pet foods for adult dogs, n=15 retorted (7 GF, 8 NP) and n=11 extruded (4 GF, 7 NP). NP diets included 9 novel proteins (trout, duck, rabbit, pork, turkey, buffalo, lamb, tuna and goose). GF diets included 3 alternative starch sources from pulses (green peas, yellow peas and lentils). Samples were analyzed by high-performance liquid chromatography (HPLC) method, equipped with photodiode array (DAD) and fluorometric (FLD) detectors, after an online pre-column derivatisation with o-phthalaldehyde. All EAA, except Tryptophan, were detected. Results were compared with recommendations of FEDIAF for dogs (based on MER: 110 kcal/kg^{0.75}). The comparisons were made in a descriptive manner.

Results and discussion: The EAA composition of GF and NP diets for dogs is reported in Table 1. All foods met FEDIAF nutrient recommendations for canine maintenance [3]. All the EAA were provided at 130%-765% of the FEDIAF requirements for adult dogs. Met and Cys were the most limiting EAA in both retorted and extruded GF and NP diets. Met+Cys were provided at a minimum of 130% of the FEDIAF requirement by extruded GF and NP diets and at a minimum of 285% by retorted GF and NP diets. Lys and arginine were particularly abundant in both retorted GF and NP diets, providing up to 6 to 7 times the minimum requirement. A recent study described the reduction of bioavailability of Met after extrusion temperatures [4]. Meanwhile, in the present study nutrient digestibility and bioavailability in dogs were not assessed.

	his	thr	arg	tyr	val	met+cys	phe	ile	leu	lys
Retorted GF (n=7)	1.2 (0.8-1.5)	1.9 (1.1-2.3)	3.2 (2.2-4.1)	1.6 (1.0-2.1)	2.5 (1.6-3.1)	2.2 (1.2-3.2)	2.2 (1.4-2.6)	1.8 (1.1-2.2)	3.8 (2.4-4.7)	3.2 (2.1-4.1)
Retorted NP (n=8)	1.1 (0.8-1.4)	1.6 (1.1-2.3)	3.1 (2.1-4.2)	1.4 (1.0-1.9)	2.0 (1.5-2.8)	2.3 (1.1-4.1)	1.8 (1.4-2.4)	1.7 (1.1-2.4)	3.2 (2.3-4.4)	3.0 (2.0-4.3)
Extruded GF (n=4)	0.5 (0.4-0.6)	0.9 (0.9-1.1)	1.7 (1.6-1.9)	0.8 (0.7-0.9)	1.1 (1.1-1.3)	0.8 (0.7-0.9)	1.2 (1.1-1.3)	1.0 (0.9-1.1)	1.8 (1.6-2.0)	1.5 (1.3-1.7)
Extruded NP (n=7)	0.6 (0.5-0.6)	1.0 (0.8-1.1)	1.8 (1.5-2.1)	0.9 (0.6-1.2)	1.3 (1.0-1.4)	1.1 (0.8-1.2)	1.3 (0.9-1.7)	1.1 (0.8-1.2)	2.3 (1.4-3.2)	1.4 (1.2-1.8)

Conclusion: All tested extruded dog foods using NP and GF met FEDIAF nutrient recommendations for canine maintenance. Further studies to assess the metabolic bioavailability and digestibility of the most limiting AA (Met and Cys) in extruded GF and NP diets are now required.

Acknowledgment: Monge & C. S.p.a. to fund the study.

References: [1] McCauley SR (2020) J Anim Sci 1; 98(6). [2] Freeman L (2018) J Am Vet Med Assoc 253:1390–1394. [3] FEDIAF Nutritional Guidelines (2021). [4] Crosbie M (2022) ESVCN Congress Proceedings.

5C2. Determination of eleven essential amino acids (EAA) by HPLC-DAD/FLD in protein feed materials for extruded pet food intended for dogs

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Introduction: Lysine (Lys) and Methionine (Met) are often considered limiting EAA in feed materials commonly used for pet food production [1]. However, technology and material composition have changed over time and the pet industry could benefit from new data. Novel proteins have limited data available regarding their nutritional quality [2]. The objective was to evaluate EAA contents of common and novel protein feed materials currently used in extruded pet food manufacturing.

Animals, materials and methods: The samples consisted of 31 protein feed materials: n=4 from vegetal (corn gluten, peas and potato protein concentrates), n=9 from fish (4 species) and n=17 from meat (15 species) origin. Spray-dried whole eggs were used as a reference protein and its EAA content was considered the standard for comparing to the other proteins [2]. Samples were analyzed by high-performance liquid chromatography (HPLC), equipped with photodiode array (DAD) and fluorometric (FLD) detectors, after an online pre-column derivatisation with o-phthalaldehyde. All EAA, except Tryptophan, were detected. The comparisons were made in a descriptive manner, the means of individual and subgroups were compared using descriptive statistics.

Results and discussion: The EAA composition of samples is reported on Table 1.

Table 1 (average values g/100g DM)	his	thr	arg	tyr	val	met	phe	ile	leu	lys	cys
Corn gluten meal (n=1)	1.1	1.8	2.0	2.9	2.3	1.5	3.3	1.9	8.0	1.0	1.1
Peas (n=1)	1.6	2.5	4.8	1.6	3.2	0.4	3.2	2.9	4.6	4.9	0.7
Potato protein concentrate (n=2)	1.5	4.1	3.9	4.0	4.4	1.6	4.6	3.8	7.1	5.3	1.0
Vegetable proteins (n=4)	1.4	3.1	3.6	3.1	3.6	1.3	3.9	3.1	6.7	4.1	1.0
Anchovies meal (n=2)	1.4	2.6	4.2	2.1	2.9	1.9	2.6	2.6	4.5	4.5	0.6
Salmon meal (n=4)	1.4	2.5	4.4	1.9	2.7	1.8	2.4	2.3	4.0	4.0	0.5
Trout meal (n=1)	1.2	2.2	3.5	1.5	2.3	1.6	2.1	2.0	3.1	3.1	0.3
Codfish meal (n=1)	1.1	2.4	3.8	1.8	2.4	1.7	2.2	2.1	3.6	3.7	0.5
Tuna meal (n=1)	2.3	2.6	4.1	1.9	2.8	1.7	2.4	2.6	4.1	4.3	0.5
Fish meals (n=9)	1.4	2.5	4.1	1.9	2.7	1.8	2.4	2.4	4.0	4.0	0.5
Chicken meal (n=5)	1.1	2.3	5.0	1.8	2.8	1.2	2.4	2.3	4.1	3.5	0.7
Rabbit meal (n=1)	0.9	2.0	4.1	1.6	2.2	0.8	1.9	1.5	3.3	2.5	0.7
Bovine meal (n=5)	0.9	1.7	3.9	1.3	2.0	0.9	1.8	1.5	3.0	2.6	0.4
Lamb meal (n=1)	0.8	1.8	3.9	1.2	2.1	0.7	1.7	1.5	3.1	2.6	0.6
Duck meal (n=1)	1.1	2.2	4.4	1.8	2.5	1.3	2.3	2.1	3.9	3.3	0.7
Buffalo meal (n=1)	0.6	1.3	3.5	0.9	1.5	0.6	1.4	1.1	2.3	2.1	0.3
Dear meal (n=1)	0.8	1.3	3.1	0.9	1.8	0.6	1.4	1.2	2.5	2.1	0.2
Goose meal (n=1)	1.5	2.9	5.1	2.2	3.0	1.6	2.9	2.6	5.0	5.1	0.7
Pork meal (n=1)	1.3	2.2	5.1	1.7	2.7	1.1	2.4	2.0	3.9	3.6	0.7
Meat meals (n=17)	1.0	2.0	4.3	1.5	2.3	1.0	2.0	1.8	3.5	3.0	0.5
Spray-dried whole eggs (SDE)	1.5	3.1	4.7	2.7	4.1	2.3	3.6	3.3	5.5	4.5	1.6

Met were particularly abundant in fish meals, potato concentrate and corn gluten meal according to prior studies [2-3]. Met was the first limiting amino acids for rabbit, bovine, lamb, buffalo, and deer meal, and peas. To be considered given a recent study described lower availability of Met after extrusion temperatures [4]. Vegetable proteins had higher mean Tyrosine (Tyr), Phenylalanine (Phe) and Leucine (Leu) content compared to SDE. Phe was the limiting amino acid for fish meals. Higher values of Lys were found in peas, potato protein concentrate, and fish meals compared to meat meals.

Conclusion: New data for novel proteins are now available for pet industry. Further studies to assess the effects of temperature applied during extruded dog food production on the EAA contents are required.

Acknowledgment: funding from Monge & C. Spa. **References:**[1] Bosch et al. (2013) Nutr. Res. Rev 26. 130–148. [2] Donadelli et al. (2019) Poultry Science 98:1371-1378; [3] Yamka et al. (2004) Anim Feed Sci Technol 116:239–248. [4] Crosbie et al. (2022) ESVCN Congress proceedings: 63.

5C3. Analysis of the digestibility of amino acids in complete dog food

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Introduction: Advances in canine and feline nutrition contributed to improved pet longevity and well-being and therefore the protein quality of the feed is becoming increasingly important with respect to the amount of protein fed especially in those physiological stages such as growth where amino acid requirements increase.

The balance between essential (EAAs) and nonessential amino acids improves the biological value of the protein in a feed by promoting the animal's nitrogen metabolism [1]. The energy metabolism of carnivores depends precisely on protein metabolism since it is from certain amino acids (glucogenic) that the animal derives most of the glucose necessary to carry out the main energy functions for the body.

Animals, materials and methods: The purpose of the study was to compare the amino acid profile of four dry dog feeds from the same manufacturer (A), one of which is suitable for puppies and three for adult maintenance, with six other dry feeds (two for puppies and four for adults) from competing companies (B) to assess compliance with the minimum claims defined by FEDIAF [2] and whether there are any differences in the amino acid profile between different categories of feeds. Amino acid analysis was carried out in vitro the European Pharmacopoeia [3]: hydrolysis of the proteins; pre-column derivatization of amino acids; and liquid chromatography. We analyzed the amino acids on the digested residue of the protein portion of each individual feed.

Amino acids	FEDIAF requirements	PUPPY fresh ingredients	ADULT fresh ingredients	ADULT mix fresh/flours	ADULT flours
arginine	0.6	1.38	1.10	1.37	1.23
histidine	0.27	0.58	0.43	0.60	0.57
isoleucine	0.53	0.92	0.79	0.94	0.88
leucine	0.95	1.72	1.53	1.71	1.65
lysine	0.46	1.27	0.96	1.35	1.25
methionine	0.46	0.78	0.65	0.86	0.78
cistine		0.45	0.33	0.55	0.47
methionine+cistine	0.88	1.23	0.98	1.41	1.26
phenylalanine	0.63	0.97	0.86	1.05	1.00
tyrosine		0.41	0.35	0.53	0.46
phenylalanine+tyrosine	1.03	1.38	1.21	1.58	1.45
treonine	0.6	1.26	1.07	1.33	1.22
tryptophan	0.2	0.35	0.28	0.36	0.34
valine	0.68	1.05	0.86	1.30	1.13

Table 1. Amino acid profile of feed compared to the minimum requirements suggested by FEDIAF (per 100 g DM)

and valine were found to be below the FEDIAF minimum nutritional values in competitor feeds made from fresh ingredients or flours (Table 2). The cold-pressed feed had insufficient values for histidine, isoleucine, leucine, lysine, methionine+cystine, phenylalanine, phenylalanine+tyrosine, tryptophan, and valine.

Amino acids	FEDIAF requirements	ADULT fresh ingredients	ADULT fresh/flours	ADULT flours	ADULT cold pressed
arginine	0.6	1.23	1.26	0.96	0.70
histidine	0.27	0.53	0.55	0.42	0.25
isoleucine	0.53	0.88	0.83	0.81	0.51
leucine	0.95	1.42	1.20	0.90	0.81
lysine	0.46	0.60	1.02	0.96	0.41
methionine	0.46	0.41	0.40	0.44	0.62
cistine		0.33	0.39	0.51	0.27
methionine+cistine	0.88	0.93	0.79	0.95	0.77
phenylalanine	0.63	0.82	0.78	0.71	0.58
tyrosine		0.47	0.46	0.39	0.31
phenylalanine+tyrosine	1.03	1.29	1.24	1.10	0.89
treonine	0.6	0.94	0.90	0.78	0.70
tryptophan	0.20	0.15	0.24	0.16	0.17
valine	0.68	1.02	0.89	0.56	0.60

Table 2. Amino acid profile of competitive feed for adult dogs compared to the minimum requirements (per 100g DM)

deficiencies. The analysis was performed according to the value of protein digestibility, representing a very reliable and novel picture regarding the metabolic utilization of amino acids.

References: [1] McCusker et al.(2014) Journal of Nutritional Science, 3, E39. doi:10.1017/jns.2014.33

[2] FEDIAF Nutritional Guidelines (2020).

[3] European Pharmacopoeia 5.0-2.2.56, Amino acid analysis -Protein hydrolysis - Method 1, 4, 5 and 7.

Results and discussion: All A feeds provide perfect coverage for EAAs requirements, consequently, result in less nitrogen excretion with urine, demonstrating higher biological value with less overload regarding nitrogen waste disposal by renal glomerular filtration (Table 1).

Some amino acids such as methionine, leucine, tryptophan, methionine+cystine, and valine were found to be below the FEDIAF minimum nutritional values in competitor feeds made from fresh ingredients or flours (Table 2). The cold-pressed feed had insufficient values for histidine, isoleucine, leucine, lysine, methionine+cystine, phenylalanine, phenylalanine+tyrosine, tryptophan, and valine.

Conclusion: Feeds based on fresh ingredients and fresh/flour mix seem to be the best by overall amino acid profile. Failure to cover one or more amino acids poses a very dangerous risk to the health of the animal since numerous proteins are involved in vital processes for the body. Feeding an animal with less digestible dietary principles will not only result in greater excretion of them through the feces but also in dangerous

5C4. Protein intake in horses and the risk of environmental nitrogen overload

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Introduction: Overfeeding of protein is common practice in the equine industry, however excess protein is excreted into the environment via feces and urine. The aim of this study was to quantify the protein intake and nitrogen (N) excretion of horses in the Netherlands.

Materials and methods: The data was collected via an online survey (Survey Monkey) addressed to all horse owners in the Netherlands from March to June 2022. Demographic data and information on feeding (forage, grazing hours, grass quality, complementary feed and supplement brands, respective quantities offered) and workload were collected. Pasture DM intake was estimated at 1.2 g DM/kg^{0.75} for a 500 kg horse [1]. The crude protein (CP) values for pasture and forage were obtained from the NRC [1], while data for feed and supplements was based on product data. CP intake was calculated including workload and compared to horse requirements using NRC [1]. The N assessments were converted using a conversion factor of 6.25 g protein per g nitrogen [1]. The weight of manure produced was assumed to be 5% BW/day [2]. Any estimated excess CP intake was assumed to be excreted as N in the manure. Descriptive data were calculated using Systat 16.0 (SPSS, Chicago, IL). Normally distributed data are presented as mean \pm SD.

Results and discussion: A total of 274 valid responses for horses aged 4 to 14 years were evaluated. The estimated mean BW of the horses was 542.4 \pm 101.9 kg. The horses were 50.5% geldings, 40.6% mares and 8.9% stallions. The most common breed was warmbloods (72.6%). Most horses were from livery yards (68.3%). Most of the horses (85%) had access to pasture, with the mean grazing time 10.6 \pm 7.0 h/day. The majority of the horses were fed grass hay (86%), some were also offered alfalfa (43%), and haylage (35%). The mean forage intake was 6.5 \pm 5.9 kg/d (as fed). Most of the horses were fed complementary feeds (93%), with 46% being fed two or more types. Supplements were also commonly used (79.9%), with 75.6% using two or more types. The mean CP intake (including pasture), was 4 \pm 2 g/kg BW. The estimated CP intake of 96.7% of the horses was above 110% of the NRC recommendations, while 1.1% had an estimated CP intake of below 90% of the NRC recommendations. The mean complementary feed intake (as fed) per day was significantly higher in horses receiving more than 110% CP (3.5 \pm 2.0 kg fed/d) than horses receiving recommended CP levels (1.6 \pm 1.2 kg as fed/d) ($P < 0.001$). It is estimated that each horse produces approximately 27 \pm 5 kg of manure per horse per day with a nitrogen excretion of 228 \pm 134 g, which equates to 8.47 kg N per ton (ref. value 5 kg N per ton [2]). This will result in more than 5.4 million metric tons of manure and 27,400 metric tons of N being produced annually for 450,000 horses in the Netherlands.

Conclusion: Most horses consumed more CP than the daily requirement and therefore N-excretion in manure in the Netherlands can be high, and contribute to environmental impacts. It is necessary to accurately meet protein needs and consider protein quality. Although, the equine digestive tract's complexity and differences in feed types, predicting feed protein digestibility and N-excretion remains a challenge and a research limitation.

Funding Statement: The SIA Directorate, the Netherlands Organization for Scientific Research (NWO), Taskforce for Applied Research.

References: [1] NRC, The nutrient requirement of horses (2007); [2] Westendorf, W.(2004). Horses and manure. Rutgers Equine Science Center Publications: Factsheet 036

5C5. *In vitro* rumen fermentation characteristics of defatted insect meals as compared to conventional plant-based meals

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Introduction: Insect meals are considered sustainable alternatives to traditional plant-based meals in feed formulations [1]. The high fat content of full-fat insect meals is considered a limiting factor for their use in ruminant nutrition [2]. Defatting processes are applied to insect meals to increase their protein content and stability during storage [3]. In this study, the *in vitro* rumen fermentation characteristics of defatted *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) meals were compared with those of soybean (SBM), rapeseed (RPM) and sunflower (SFM) meals.

Animals, materials and methods: The tested defatted insect meals included five ether extract (EE) levels for HI (19.7, 12.8, 9.2, 7.0 and 4.7 g EE/100g dry matter – DM) and two EE levels for TM (8.1 and 5.7 g EE/100g DM). Rumen fluid was obtained from four cannulated sheep. Fermentation parameters after 24h *in vitro* ruminal incubation of the insect and plant meals were measured. A GLM ANOVA was performed including meal type and incubation run as fixed and random effects, respectively. Linear contrasts were used to establish differences between each plant meal and defatted insect meals (INS), and between TM and HI meals.

Results and discussion: Significant lower production of total gas, CH₄ and total volatile fatty acids (VFA) was observed for the insect meals when compared to the plant meals, as well as for HI when compared to TM meals. The NH₃ production was lower for the insect meals when compared to the plant meals, and higher for HI than TM meals. The organic matter digestibility (IVOMD) was significantly higher for the plant meals than for the insect meals, while no differences were observed when comparing the HI and TM meals (Table 1). These results are the consequence of the presence of chitin and high crude protein in insect meals, and of their fatty acid profile (e.g., high C12:0 in HI) [2]. By means of a comparison with results previously obtained for full-fat HI and TM meals [2], it is also observed that defatting increases rumen digestibility of insect meals.

Table 1. *In vitro* rumen fermentation characteristics after 24 h of incubation of defatted insect meals and plant meals (total gas, CH₄ and total VFA: mmol/g DM; NH₃-N: % of initial N; IVOMD: g/g).

Item	Meals						Model SEM	Contrasts <i>P</i> value			
	INS	HI	TM	SBM	RPM	SFM		INS vs SBM	INS vs RPM	INS vs SFM	HI vs TM
Total gas	1.64	1.61	1.72	3.25	3.03	2.09	0.029	0.000	0.000	0.000	0.000
CH ₄	0.39	0.38	0.40	0.69	0.56	0.39	0.011	0.000	0.000	0.000	0.007
NH ₃ -N	50.1	50.9	48.0	70.2	66.5	86.6	1.032	0.000	0.000	0.000	0.003
Total VFA	5.30	5.23	5.45	8.80	7.82	5.98	0.059	0.000	0.000	0.000	0.000
IVOMD	41.1	41.1	41.2	69.8	60.7	46.5	0.629	0.000	0.000	0.000	0.874

Conclusion: When compared to conventional plant protein sources, defatted HI and TM meals exert mitigation effects on enteric gas yield in 24h, but they show lower IVOMD. Defatted insect meals could have a potential use as alternatives to SFM in ruminant diets to lower the environmental impact of livestock production.

References: [1] Gasco et al. (2023) Anim. Front., in press; [2] Renna et al. (2022) J. Anim. Sci. Biotechnol. 13(1): 138; [3] Mishyna et al. (2021) Curr. Opin. Colloid Interface Sci. 56: 101508.

5C6. Comparison of hydrolysed soy versus chicken and egg meals in digestibility and metabolic parameters in adult dogs

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Introduction: Diets based on soy and animal protein result in different crude protein (CP) digestibility and ammonia synthesis [1,2]. However, previous studies did not compare different sources of high-biological value proteins [3]. Therefore, the aim of this study was to evaluate the effects of different protein ingredients on metabolic variables of adult dogs.

Animals, materials and methods: Six male and female English Cocker Spaniels, five Border Collies and one Labrador Retriever, with body condition score 5/9 [4], neutered, aged 4.5±0.65, were randomly distributed in a cross-over design. All procedures were approved by the School's Ethics Committee (protocol 4668091214). A hydrolysed soy protein-based diet (diet A - 26.92% CP DM) and a chicken flour and egg powder-based diet with equivalent CP content (diet B - 25.78% CP) were used. Each experimental period lasted 12 days. Faeces were collected for apparent digestibility coefficients (ADC) of nutrients [5], faecal concentration of short-chain fatty acids (SCFAs), branched-chain fatty acids (BCFAs), and faecal ammoniacal nitrogen (FAN) level. Blood samples were obtained after 5, 6, and 7 hours postprandial for the determination of urea curves. All analyses were performed using the PROC MIXED procedure from SAS (p<0.05).

Table 1. Apparent digestibility coefficients of nutrients (%) of experimental diets.

Nutrient	Diet		P
	A	B	
Crude protein	90.28	89.30	0.6758
Ethereal extract	97.08	95.65	<0.0001
Ash	45.30	27.67	<0.0001

Results and discussion: There were no difference in crude protein ADC between diets A and B (Table 1). However, diet A showed higher ADC for etheral extract and ash. Diet B presented higher faecal concentrations of SCFAs (A=46.22mM; B=72.19mM; p<0.0001)

and lower FAN (diet A=8.03mM; B=5.56mM; p=0.0009). However, there was no difference in BCFAs between the treatments. These results suggest that diet B promoted a healthier intestinal environment than diet A [6], which may have occurred due to the different levels and sources of fibre observed in the two diets. FAN level did not reflect the serum urea concentration (Table 2), which may be related to reduced intestinal ammonia absorption [7]. Time post-feeding did not differ, but the urea peak was reached numerically at 6 hours. Diet B had the highest urea peak, which can be explained by the inclusion of animal source proteins, which are more ammoniagenic than soy protein-based diets [8].

Conclusion: Faecal ammonia nitrogen concentration does not act as a marker for intestinal

Table 2. Urea curve (mg/dL) in healthy dogs after diets with different protein sources consumption.

Time post-feeding	Diet		Treatment	p	
	A	B		Time	Interaction
5 hours	47.97	53.59		0.62	
6 hours	48.18	53.77	<0.0001	45	0.8806
7 hours	47.30	52.52			

ammonia absorption. Hydrolysed soy protein has similar digestibility to animal proteins sources and results in lower urea peaks.

References: [1] Moore et al. (1980) J. Anim. Sci. 50:892-896; [2] Zuo et al. (1996) J. Anim. Sci. 74:2441-2449; [3] Carciofi et al. (2009) Anim. Feed Sci. Technol. 151:251-260; [4] Laflamme (1997) Canine Pract. 22:10-15; [5] AAFCO (2019) Washington, DC; [6] Swanson et al. (2002) J. Nutr. 132:980-989; [7] Condon (1971) Am. J. Surg. 121:107-114; [8] Proot et al. (2009) J. Vet. Intern. Med. 23:794-800.

5C7. Effect of dietary supplementation with 5% black soldier fly larvae (*Hermetia illucens*) on production parameters of laying hens from 23 to 27 weeks of age

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Introduction: About 14 million tons of soybean per year for livestock feed are imported into Europe [1], which increases the impact of the agri-food sector on climate change. Insects are considered as a high-quality, efficient and sustainable alternative protein for the poultry feed [2]. In addition, several insects have been recently approved for use in monogastric feed in the European Union [3]. One of these insects are the larvae of *Hermetia illucens* (black soldier fly), which have a high protein and energy content. Thus, the aim of this work was to evaluate the effect of the inclusion of this insect in the diet of laying hens on their productive performance before peak lay.

Animals, materials and methods: A total of 80 hens (23-week-old Isazul breed) were weighed and randomly distributed into 10 floor pens (8 birds/replicate), and the pens were randomly assigned to treatments. The dietary treatments designed for the experiment were two: a control feed [3047 kcal AMEn/kg dry matter (DM) and 18.30 g crude protein/100 g DM]; and a control diet supplemented with dehydrated *H. illucens* larvae. The feed in both treatments was fed *ad libitum*, and the *H. illucens* was included as a 5% top up of the total DM intake. The *H. illucens* contained 44.09 g crude protein and 34.94 g ether extract per 100 g DM of larvae. The parameters studied were: body weight, egg production, DM intake, egg weight, egg mass, feed conversion ratio (FCR) and mortality, determined between 23 and 27 weeks of age. Student's t-test was performed for data analysis using the SPSS statistical package. A probability level of $P < 0.05$ was considered statistically significant.

Results and discussion: *H. illucens* larvae supplementation did not affect the body weight of the birds during the experiment (Table 1). Laying percentage, egg weight or egg mass were not affected by the treatments. Feed intake and FCR did not increase ($P > 0.05$) despite supplementation with *H. illucens* larvae. This effect was expected since it has been recognized that the energy content of the diet is a major factor regulating the amount of feed intake [4].

Table 1. Effect of insect larvae inclusion on productive parameters of hens from 23 to 27 weeks of age

Item	Control diet	Supplemented diet	P-value
Initial (23 weeks) body weight (g)	1882.34 ± 111.83	1886.43 ± 87.75	0.950
Final (27 weeks) body weight (g)	2059.63 ± 136.08	2040.75 ± 124.70	0.825
Egg production (%)	64.52 ± 8.48	71.29 ± 8.56	0.154
Diet intake (g dry matter/day)	112.03 ± 9.14	113.05 ± 8.66	0.861
Egg weight (g)	62.23 ± 2.19	61.25 ± 2.36	0.514
Egg mass (g/day)	40.1 ± 5.05	43.71 ± 3.98	0.245
FCR ¹ (g dry matter intake/g egg mass)	2.82 ± 0.36	2.59 ± 0.14	0.219

¹ FCR=Feed Conversion Ratio. Data is presented as mean and standard deviation.

Thus, it appears that the *H. illucens* supplementation could replace a part of the feed without affecting the production performance.

Conclusion: Dietary supplementation with *H. illucens* larvae at 5% of DM does not negatively affect productive parameters of laying hens from 23 to 27 weeks of age.

References: [1] European Commission (2019). United States is Europe's main soya beans supplier with imports up by 112%. EU Commission; [2] Veldkamp et al. (2022) *Insects* 13:281; [3] Regulation (EU) 2021/1372 (2021); [4] Harms et al. (2000) *J. Appl. Poult. Res.* 9:535–541.

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5C8. Effect of partial substitution of dietary soybean with other vegetable ingredients on production performance in laying hens

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Introduction: It is currently necessary to reduce imported ingredients for animal feed as the transport has a considerable impact on environment [1]. In Spain, laying hens' diets incorporate high percentages of corn and soybean meal, which are imported from distant countries. Researchers have been exploring ways to decrease the use of soybean meal in animal diets by replacing it with other sources such as insects [2], plant-based sources [3] or synthetic amino acids [4]. The aim of this study was to investigate the effects of partial substitution of corn and soybean meal with ingredients of local/closer origin, resulting in shorter transport distances, and less environmental impact, on the performance of laying hens.

Animals, materials and methods: Eighty 23-week old laying hens (Isazul breed) were distributed in 5 floor pens (8 hens/replicate) per treatment. One group was fed a Control standard layer diet containing corn (41.58 g/100 g) and soybean meal (21.97 g/100 g). The other group, called Alternative group was fed a diet in which soybean and corn meal were reduced by 7% and sunflower meal was increased to 6%, in addition, corn DDG and pea of closer origin (7.46% and 5.56%, respectively) were added, reformulating so that both diets were isoenergetic (2,730 Kcal AME/kg) and isonitrogenous (crude protein 16.4 g/100 g). After four weeks of feeding, production performance (egg production, egg mass and feed to egg ratio) was measured. Data were analysed by independent Student's T-test using SPSS software (Chicago, IL, US).

Results and discussion: The parameters of production performance after 4 weeks of feeding are shown in Figure 1. The 7% substitution of corn and soybean meal with other vegetable protein sources did not negatively affect the production parameters. Although the Control diet contained a higher amount of soybean meal, the Alternative diet was as good as control diet. Possibly, the fact that both diets were isoenergetic and isonitrogenous which could have prevented any negative impact on the production parameters, at least during the first 4 weeks of laying. It would be interesting to continue the study during peak laying to further investigate the effects of the Alternative diet.

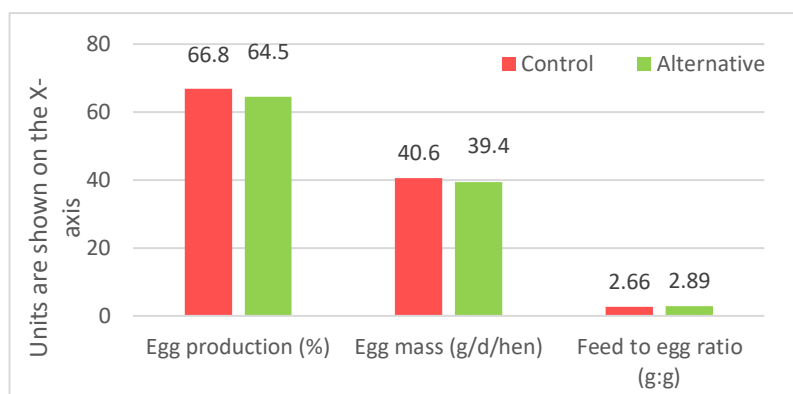


Figure 1. Performance productive (egg production, egg mass and feed to egg ratio) in laying hens fed Control and Alternative diet.

Conclusion: It is possible to replace 7% soybean meal with other vegetable protein sources (sunflower meal, corn DDG, and peas) without affecting the hen's productive performance, at least in the first month of laying.

References: [1] Kebreab et al., (2016) *J. Anim. Sci.* 94(6), 2664-2681. [2] Zawisza et al. (2023) *Animals*, 13(3), 527. [3] Kaminska (2003) *J. Anim. Feed Sci.* 12(1), 111-120. [4] Selle et al. (2020) *Animals*, 10(4), 729.

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Session 6A Exotics
Chair: Angela Gimmel



6A1. Investigations on morphometrics and relative organ mass in *Xenopus laevis* fed two different diets

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Introduction: The African clawed frog, *Xenopus laevis*, is used as a model organism under laboratory conditions e.g. in reproductive biology. There is, however, a lack of data on their nutritional requirements and normal physiological data. Allometric data could be useful for dissections of diseased animals as reference. In lab animal facilities, the frogs are often fed fish feed for logistic/empirical reasons [1] and the feeding is not very standardized. The aim of the present study was to evaluate morphometric measurements and allometric organ mass of *Xenopus* frogs fed either fish feed or a lab *Xenopus* diet.

Animals, materials and methods: Adult, female *Xenopus laevis* (n = 40) were used in the study (ethical reason sacrifice for scientific purpose). Two diet groups (each n = 20) were formed to ensure a similar distribution of frog size in both groups. The diets were fish feed for pond fish (crude protein 21%: crude fat: 3% NfE: 60%; as fed) and a *Xenopus* diet (crude protein: 46%, crude fat: 14%, NfE: 24%). Meals were fed 2x/wk with a defined amount (isoenergetic in terms of gross energy) per tank á 10 animals. After 4 wks on the respective diet, the frogs were sacrificed. A standardized photograph was taken for morphometrics [2] (e.g. surface of triangle describing body form) before the frogs were weighed and dissected. Organ weight was recorded (heart, liver, lungs, gastrointestinal tract, kidneys, ovary, intracoelomic fat body). The diet groups were compared via t-test (GraphPad; $p \leq 0.05$). Correlations between BW and organ weight were calculated. Tank parameters were recorded and monitored to be stable in all tanks.

Results and discussion: In both diet groups, there was considerable variation in body weight (BW), length and accordingly the other parameters. There were no significant differences in

Table 1. BW, length and relative organ weight (selected parameters) of the frogs in both diet groups (means±SD).

	<i>Xenopus</i> feed	Fish feed	<i>p</i>
BW (g)	139.4 ± 68.0	151.9 ± 54.2	0.536
Length (cm)	9.65 ± 1.7	9.63 ± 1.2	1.000
Liver (% BW)	5.6 ± 1.1	5.4 ± 1.2	0.599
Fat body (% BW)	1.5 ± 1.4	2.1 ± 1.7	0.261
Ovary (% BW)	18.7 ± 7.7	19.9 ± 6.5	0.491
Triangle surface (cm ²)	21.65 ± 7.9	21.84 ± 5.5	0.790

BW, body length, morphometrics, and relative organ mass between the diet groups. Selected parameters are given in Table 1. The duration of the feeding period might not have been long enough to result in measureable differences. Due to the wide range of BW and size of the frogs, robust correlations between

parameters could be calculated over all 40 individuals. There was a strong linear correlation between BW and triangle surface ($y = 0.10x + 7.57$; $R^2 = 0.82$) as well as between BW and total ovary weight ($y = 0.30x - 13.52$; $R^2 = 0.81$). The correlation between BW and total fat body weight was weaker ($y = 0.04x - 2.27$; $R^2 = 0.52$). The combination of these findings suggests that in adult female *X. laevis*, body mass and form is dominated by ovary mass more than by intracoelomic fat storage.

Conclusion: Feeding diets with differing crude nutrient composition to adult *X. laevis* for 4 wks did not lead to significant differences in BW, organ weight and morphometrics. The ovary is an important determinant of BW in the female frogs. The present data gives a first orientation on *X. laevis* body composition. Long-term studies are necessary to investigate a potential dietary influence on organ weights and morphometrics.

References: [1] Böswald et al. (2022) *Animals* 12(9) 1163; [2] Böswald et al. (2022) *Animals* 12(21) 2986;

6A2. Ingredient vs nutrient intake in free-ranging European brown bears (*Ursus arctos*)

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Introduction: The intake of nutrients in specific ratios has metabolic significance and has increasingly been studied in free-ranging species to elucidate foraging behaviour [1-3]. Brown bears in particular have recently been described as typically foraging for a low protein to non-protein (carbohydrates and fat) ratio [4,5]. This ratio is considered an optimum since it could be linked with efficient fat mass gain, an important metabolic effect for hibernating bears [4]. Studies reporting on the seasonality of this ratio show that a low protein ratio can occur in fall, whereas high protein intake is typically observed during spring and early summer [6,7].

Animals, materials and methods: We studied the feeding habits of free-ranging Swedish brown bears (n = 55) via faecal analysis (n=886) and determined ingredient and nutrient intake seasonally (spring [n = 28], summer [n = 519], fall [n = 339], annually (2015 [n = 325], 2016 [n = 163], 2017 [n = 157], 2018 [n = 241]) and per social group (adult lone males [n = 95], adult lone females [n = 328], adult females with cubs of the year [n = 147], adult females with yearlings [n = 104], subadult females [n = 160] and males [n = 52]). Ingredients in the faeces (%V) were determined visually and ants were additionally examined with stereoscope and microscope. The estimated dietary content (EDC) [8] was calculated for every ingredient and multiplied with nutrient concentrations (from literature) in order to obtain the nutrient concentration of the diet.

Results and discussion: The diet was dominated by vertebrates and ants in spring and early summer whereas berries were the predominant ingredient in fall. Spring and early summer diets were protein-rich and fall diets were carbohydrate-dominated. Overall, the seasonal and annual variation of ingredients and nutrients are similar to previously published ingredient studies in the Swedish brown bear population [9] and nutrient profiles of bears worldwide [6], and are consistent with the seasonal and annual variation in resources (e.g. berry production, vegetation growth, moose calving). Ingredient intake differed between social groups, indicating that social dynamics can affect resource use [10-12]. However, no difference in nutrient intake was found, suggesting that bears managed to obtain similar nutrient profiles regardless of foraging strategy. The protein to non-protein ratio was low in fall and numerically similar to the 'optimal' protein ratio [4,5], however, this ratio was high in spring and early summer. Considering a low protein ratio as an optimum for ursid species throughout the year seems questionable and should be interpreted in a seasonal context, i.e. it seems an optimum in fall when bears prepare for hibernation and efficient fat mass gain is essential; however, in spring and summer high protein levels appear common in several brown bear populations (this study, [6,7]).

Conclusion: The realized nutrient ratios in the diet of free-ranging brown bears seem to depend on the specific conditions of each bear population, and apart from efficient fat mass gain in fall, other metabolic factors may influence foraging behaviour throughout the year.

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6A3. Species differences in nutrient metabolism in Costa Rican frogs

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Introduction: Amphibians are carnivores as adults, typically opportunistic generalist predators of invertebrates and/or small vertebrates [1]. Digestion of food and metabolism in frogs are little studied at the moment, and such processes could be very particular in the case of amphibians, given their ectothermic and carnivorous nature. The interplay between metabolism and the substrates utilised for the different biochemical pathways involves complex interactions of the macronutrients ingested from the diet, in which animals have to prioritize the target nutrients according to their metabolic capacities and adaptations [2]. The objective of this study was to evaluate metabolic activity in several anurans from Costa Rica, with a focus on glucogenic sources, in order to better comprehend how they utilise the main sources of energy.

Animals, materials and methods: Twenty five adult frogs: n=10 Red-eyed tree frog *Agalychnis callidryas*, n=6 Blue-sided tree frog *Agalychnis annae*, n=6 Green and black poison frog *Dendrobates auratus* and n=3 Canal zone tree frog *Hypsiboas rufitelus* were collected from the wild in two sites in Costa Rica. Morphometric measurements were determined (weight and length), then animals were anesthetized with a solution of isoflurane mixed with distilled water and ultrasound gel, and blood samples were drawn by heart puncture (Ethical Committee authorization No. EC 201/133 and Ministry of Environment of Costa Rica authorization No. SINAC-CUS-PI-R-098-2017). Whole blood samples were spotted onto Protein Saver Cards (Whatman 903) for determination of acylcarnitine and free amino acid profiles, using tandem mass spectrometry [3]. Pearson correlations coefficients were determined to establish relationships among metabolites, declaring significance at $p < 0.05$. In addition, univariate ANOVA analysis and Tukey post-hoc comparisons were performed between species (excepting *H. rufitelus*) to evaluate the effect of the species on amino acid and acylcarnitine profiles, with statistical significance accepted at $p < 0.05$.

Results and discussion: Morphometric measurements were significantly different among species ($p < 0.001$). Body mass showed positive correlations with the ratio of acetylcarnitine:free carnitine, as well as the ratio propionyl:acetyl carnitine ($p < 0.05$). This indicates that smaller species might have high metabolic activity related to lipid metabolism, whereas the *Agalychnis* species appear to use more protein as glucogenic substrate (propionyl CoA) per unit of lipogenic substrate (acetyl CoA). The negative correlation between body mass and the ratio citruline:ornithine (marker of protein catabolism) suggests that smaller species depend less on energy from proteins. A possible explanation would be the higher use of chitin from insect exoskeletons as a glucogenic source. Tyrosine showed significant differences between *A. callidryas* and the other species, with *D. auratus* having the higher levels of the amino acid ($p < 0.05$). This remarkable difference might be related to its synthesis from phenylalanine (which did not vary in this study) and being a precursor from melanin (*D. auratus* has black with green skin).

Conclusion: Body weight is highly linked to metabolic activity in Costa Rican frogs. Although it seems that they also have proteinogenic and lipogenic pathways for energy synthesis as previously reported [4], the present data suggests that *D. auratus* may utilise non-protein glucogenic sources from the prey, whereas *Agalychnis* species depend more on protein as glucogenic substrate, similar to carnivorous mammals [5].

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6A4. Exploration on alternative fibre food of Malay pangolins under human care

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Introduction: Diet is a major barrier for successful pangolin *ex situ* breeding. Due to the high cost of feeding termites only, it is of great significance to explore alternatives for termites in the diet of pangolins under human care. The chitin of the ant exoskeleton might be beneficial for the gut health of pangolins [1,2].

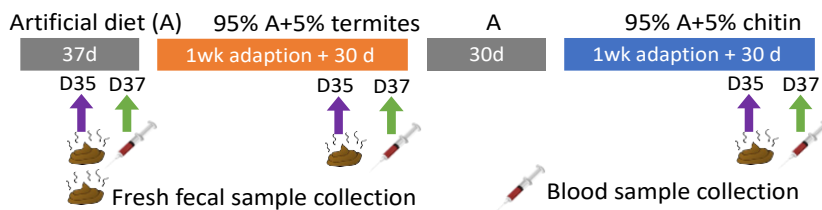


Figure 2. The study design and sample collection

Notes: The artificial diet A included dried black ants, dried yellow mealworms, cat food, milk powder, egg yolk and drinking water

Materials and methods: This study was carried out at the Zhejiang Pangolin Conservation and Breeding Research Base in Jinhua, Zhejiang Province, China. Seven adult healthy Malay pangolins (*Manis javanica*) were included in the study design shown in Figure 1. Faecal score was determined daily [3], high-

throughput sequencing technology was used to study the diversity and composition of faecal bacteria, and blood samples were used for haematology and biochemistry. Data were analysed using one way ANOVA and multiple comparison using SPSS 21.0. All experimental designs and animal handling were approved by the Animal Research Ethics Committee of Zhejiang Normal University.

Results and discussion: 5% chitin was changed to 3% after 3 days of dietary change due to a drop in feed intake. The faecal score of pangolins changed from a normal dry state (1.95 ± 0.54) to a wet state (2.72 ± 0.75) when feeding chitin. In contrast, one study has shown that adding 10% chitin improved the gut health of pangolins [2]. No significant changes were found in haematology. However, the blood albumin (ALB) was decreased with addition of chitin and termites ($P < 0.05$) (artificial diet: 3.14 ± 0.35 g/dL vs termites: 2.67 ± 0.21 g/dL vs chitin: 2.56 ± 0.33 g/dL) (reference range 2.70-4.50 g/dL), and cholesterol (CHOL) also significantly decreased with chitin addition compared to the other two diets (artificial diet: 124 ± 48 mg/dL vs termites: 92 ± 32 mg/dL vs chitin: 30 ± 18 mg/dL) (reference range 104-426 mg/dL). The alpha diversity (Shannon and Simpson index) showed no difference between groups, but the observed OTUs and Chao1 index were significantly higher in the termite group than the artificial diet group ($P = 0.007$), indicating that the microbial community abundance of the termite group was higher than that of the artificial feed group. By principal coordinate analysis (PCoA), the similarity between the termite and chitin groups was higher than the artificial diet group.

Conclusion: Our study could not show a beneficial effect on some pangolin health parameters of adding 3% chitin to an artificial diet. Adding termites to artificial diet promoted the diversity of gut microbiota. Therefore, alternatives for termites in the pangolin diet should go beyond the provision of chitin.

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6A5. Prevalence of metabolic diseases among captive neotropical parrots (Amazona spp.) in São Paulo, Brazil

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Introduction: Neotropical parrots from the Amazona species have been anecdotally described as naturally susceptible to a wide range of metabolic disorders (MD) [1]. Due to the lack of epidemiologic information, this study aimed to assess trends in MD prevalence among these species in captivity.

Animals, materials and methods: A ten-year retrospective study (2009-2019) was conducted on 791 captive parrots (707 alive and 84 dead) that were seen at the University of Sao Paulo Veterinary Hospital (HOVET). The study recorded demographic information, consultation, clinical examination variables, and final diagnoses (FD) of each bird. The study classified FD by their cause, whether infectious, non-infectious, or unknown [2]. The study focused on MDs, which were determined based on the physical examination, caregiver reports, case history, and ancillary exams results [2].

Results and discussion: As a single bird may be submitted to the HOVET for different medical issues at different times throughout its life, each case represents a clinical report and a unique final diagnosis. From the 791 birds, a total of 1033 clinical records were reviewed, from those, 877 FD were identified; from the 84 post-mortem reports, 109 FD were registered.

Nine species from the genera were represented, with the most common species being *A. aestiva* (85.4%). Adults were the most common age category (73.3%). Most birds were coded as unidentified sex (75.2%). the dietary profile arranged in categories according to the elements offered on the regular basis was mostly represented by seeds mixture (61.0%). MD were the most common cause of morbidity (n=189/512; 36.9%) and mortality (n=11/23; 47.8%) from the non-infectious disease group affecting these parrots, with the most common metabolic subprocess being hepatic lipidosis (HL) (n=56/189; 29.6%) among other disorders, which prevalence is evident in table 1. HL is a common issue among companion psittacine birds [3], but its diagnosis is challenging due to nonspecific clinical symptoms [4]. Although biochemical analysis can raise suspicion of HL, liver enzyme concentrations do not necessarily correlate with organ histopathologic changes [4]. Studies have shown that dietary fat can affect cholesterol levels, and there is evidence of genetic differences in cholesterol metabolism among parrot species, as observed in humans [5]. These findings suggest that some parrot species may be more susceptible to metabolic disorders than others [5].

Conclusion: A high prevalence of MD was documented for captive Amazon parrots and brings the importance of considering improving diagnostic methods to assess highly known frequented illness in live birds.

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Table 1. Complete list of MDs diagnosed during the 10-year study period.

Metabolic disease subprocess	Primary diagnoses	% (189)	Live birds	% (166)	Dead birds	% (23)	Cause of death	% (11)
Hepatic lipidosis	56	29.6	50	30.1	6	26.0	1	9.0
HLS	45	23.8	45	27.1	0	0.0	0	0.0
Obesity	29	15.3	29	17.4	0	0.0	0	0.0
Nutritional deficiency	19	10.0	19	11.4	0	0.0	0	0.0
Hypovitaminosis A	14	7.4	13	7.8	1	4.3	0	0.0
Gout	9	4.7	0	0.0	9	39.1	5	45.4
NSH	5	2.6	5	3.0	0	0.0	0	0.0
Atherosclerosis	5	2.6	1	0.6	4	17.3	3	27.2
Diabetes	3	1.5	3	1.8	0	0.0	0	0.0
Haemosiderosis	3	1.5	0	0.0	3	13.0	2	18.1
Hypothyroidism	1	0.5	1	0.6	0	0.0	0	0.0
Total	189		166		23		11	

MDs= Metabolic disorders; NSH= Nutritional secondary hyperparathyroidism; HLS= Hepatic lipidosis suspect.

Session 6B Animal Nutrition II and Minerals

Chair: Isabelle Ruhnke



6B1. Metabolic responses of Nile tilapia to alternative feed resources

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Introduction: In countries like Ethiopia importing and using commercially produced fish feed is too expensive and economically not viable for aquaculture production (1). The evaluation of potential resources through performance studies is not sufficient since it is important to understand the underlying metabolic changes that exert the performance effects. We here evaluated how metabolite analysis can be used to understand the impact on the performance of Nile tilapia (*Oreochromis niloticus*) of three distinct “diets” based on locally available waste streams.

Material and methods: Three treatments were applied to two earthen ponds each at Jimma University (surface area of 20 m² and 70 cm depth). All feed ingredients were collected from Jimma area. Cow dung and rumen liquor were dried in the sun and ground by mortar to mix with the other feed combinations after analysing the nutrient composition. Treatment 1 (T1) was composed of cow dung and rumen liquor while treatment 2 (T2) was composed of brewery waste, poultry manure and wheat bran, and treatment 3 (T3) was composed of noug cake (*Guizotia abyssinica*), poultry manure and rumen liquor. Each pond had 60 Nile tilapia fingerlings, obtained from Sebeta National Fisheries and Aquatic Science. Fish were fed twice daily for 120 days. Twenty fish from each pond were randomly sampled every fourteen days to measure their weight and length. At the end of the experiment dried bloodspot samples of three fish were collected from each pond for the analysis of free amino acids and acylcarnitines. One-way ANOVA was used to compare differences between treatments, using Tukey for post-hoc comparisons. Pearson correlations were calculated between body weight, body length, their ratio and selected ratios of metabolites (free amino acids and acylcarnitines).

Results and Discussion: Fish from T1 had a significantly lower average bodyweight ($W = 51 \pm 8$ g; $p=0.009$) and average body weight:length ratio ($W/L = 3.3 \pm 0.3$; $p=0.005$) than T2 ($W = 63 \pm 11$; $W/L = 3.7 \pm 0.4$) and T3 ($W = 64 \pm 9$ g; $W/L = 3.9 \pm 0.4$). The higher bloodspot malonyl:acetylcarnitine ratio (a fat synthesis marker) in T1 (3.9 ± 2.0 μ M) compared with T3 (2.4 ± 2.5 μ M) ($p=0.046$) suggests that lower growth performance may be linked to insufficient protein provision in the T1 ponds. Body weights indeed negatively correlated to the use of amino acids as glucogenic energy source (valine:leucine; $r=-0.45$, $p=0.046$), relative ketone formation as marker of inefficient energy metabolism ($r=-0.49$; $p=0.032$) and to fat synthesis (malonyl:acetylcarnitine; $r=-0.52$; $p<0.001$). The use of dried bloodspot analysis demonstrated that the poorer performance in T1 may be due to a relative shortage of available protein in these ponds. The combination of cow dung and rumen liquor was the least promising waste stream used in this study although further optimisation and economic evaluation is needed.

Conclusion: The use of this type of metabolite analysis in combination with performance can thus facilitate the development of sustainable aquaculture.

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6B2. The effects of hydrolyzed protein on global metabolomics in adult dogs

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Introduction: Research has shown various hydrolyzed protein possessed beneficial physiological functions [1,2]; however, the mechanism of how hydrolysates influence metabolism is unclear. The rising of the study of metabolomics allows the possibility to gain insights from global level of metabolism. Therefore, the current study aimed to examine the effect of different sources of protein hydrolysates, being the main dietary protein source in extruded diets, on metabolism in healthy adult dogs. The hypothesis was protein metabolism would be altered by different sources of protein ingredient.

Animals, materials and methods: Three complete and balanced extruded canine diets were formulated similarly, except for the main protein source: 1) CONd: control chicken meal diet; 2) CLHd: chicken liver hydrolysate (from chicken heart and liver) diet; 3) CHd: chicken hydrolysate (from mechanically separated chicken) diet. A 3×5 Latin rectangle design was used with 10 adult beagles. Each period consisted of a 7-day washout and a 28-day treatment period. Blood and fresh fecal collection were performed at day 0 and day 28. Plasma and fresh fecal samples were analyzed for global metabolomics with ultra-performance liquid chromatography and high resolution mass spectrometer interfaced with a heated electrospray ionization source and mass analyser. Statistical analyses were performed with Array Studio in Jupyter notebook on log transformed data. Paired t-test was used to compare between treatment groups with statistical significance set at $P < 0.05$ and $q < 0.05$.

Results and discussion: Most of the changes in metabolites were seen in protein metabolism. Plasma samples showed an alternation of amino acid metabolism in dogs fed CLHd when compared with CONd, such as lower phenylalanine and tyrosine but higher 2-hydroxyphenylacetate, lower urea and metabolites from protein degradation, and higher serotonin from tryptophan ($P < 0.05$). There were also changes in microbiome-derived metabolites in the plasma; CLHd group had lower phenol sulfate from tyrosine and higher indole propionate from tryptophan compared with CONd ($P < 0.05$), suggesting a lower oxidative stress level. Dogs in the CHd group had lower plasma bile acid levels when compared with the other groups ($P < 0.05$). Fecal metabolites related to protein and lipid also shifted when dogs were consuming different protein sources. There were higher levels of cholesterol with lower concentrations of dipeptides, metabolites from protein degradation, indole acetate from tryptophan, and lanosterol in dogs fed protein hydrolysate diets in contrast with CONd ($P < 0.05$); these changes could indicate higher protein digestibility of hydrolysates and less absorption of cholesterol in the small intestine. In addition, CLHd group had higher levels of one-carbon metabolites (S-adenosylmethionine, dimethylglycine, betaine, cystathionine, and cysteine) in the feces while CHd group had lower unconjugated bile acid (cholate, chenodeoxycholate, hyocholate, and ursodeoxycholate) and higher taurine conjugated bile acid (taurodeoxycholate and taurocholate) excretion when compared with control ($P < 0.05$).

Conclusion: Protein hydrolysate from poultry origins, serving as the main protein source of extruded diets, could lead to changes in protein and lipid metabolism in healthy adult dogs. Both of the hydrolyzed protein could be more efficient in supplying the need of the animals and result in lower loss of undigested substrates. Chicken meat hydrolysate showed capability in adjusting bile acid metabolism by decreasing unconjugated bile acid and increasing the conjugated form. Moreover, chicken liver and heart hydrolysate demonstrated antioxidant potentials.

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6B3. Gastric ulcers in pigs – the focus could have been too much on average particle size, missing out the effect of the share of coarse particles in the diets?

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Introduction: Gastric ulcers are a widespread problem in swine production (1, 2, 3), not only in fattening pigs but also in sows. Without any doubt feed technology, i.e. fine grinding of ingredients and pelleting, are the most predisposing factors (4, 5), resulting in high prevalence of gastric lesions (keratinization, erosion and ulceration in the pars non-glandularis of swine stomach) (4). Analysing diets' particles size is recommended (6, 7), when there is a higher prevalence of gastric ulcers in herds. The hypothesis of the present field study (8) was that not the hammermill per se is the risk, but the type of sieves within the hammermill.

Animal, materials and methods: Therefore, two types of sieves (common, plate sieve with round holes with a diameter of 3.2 mm vs. a new wire sieve with square holes with a diameter of 3.5 mm) were tested and compared regarding their effects on diets' particles' size, i.e. shares of fractions with different particles size (8 sieves, 3.15 – 0.2 mm). The new type of sieve was implemented on a farm with about 10,000 places for growing, finishing pigs; per week at least 15 slaughtered pigs of this production unit were tested on gastric health by scoring the gastric mucosa visually (0 = healthy up to 5 = ulcer). Regarding the feeding concept of this farm it is worth to be mentioned that the final mash diet was based on ground and afterwards fermented cereals with higher shares of old bread.

Results:

1. Regarding performance (feed intake, daily gains and FCR) there was no obvious effect after implementation of the new sieve in the hammer mill. It has to be underlined that the starch content in faeces of pigs fed the diet ground with the new wire sieve was continuously tested and was always low/negligible (<25 g/kg DM of faeces)
2. Comparing both types of sieves in the same hammermill (common one with round holes vs. a new wire sieve with square holes) resulted - in dry sieve analysis – in markedly different proportions of fines (particles <0.2 mm: 30-31 vs. 14-22 %) and of course particles (> 1.0 mm : 44-47 vs. 51-65 %).
3. After implementation of the new wire sieve the stomachs of 272 slaughtered pigs (15 per week) were tested regarding gastric lesions: The average score was 0.9, there was not a single stomach with an ulcer, in 9 pigs only an erosion was identified. This unexpected highly favourable outcome was achieved in spite of a very high share of fines (wet sieving) in the final liquid diet, (54-58 %), presumably due to the high proportion of coarse particles (~30 % > 1 mm in the final liquid diet) due to the change of sieves. Conclusion:

Besides the share of fines veterinary practitioners should look on the ratio of coarse particles in swine diets when gastric ulcers occur in a high prevalence. It seems that a low or a missing share of particles > 1.0 mm could be the main driver for ulcers – and not the high ratio of fines (here: 54-58 %!). The sieve type in the hammermill offers a chance to improve gastric health in swine.

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6B4. Modulation of bone metabolism in young goats fed a phosphorus and/or nitrogen-reduced diet

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Introduction: Phosphorus (P) and nitrogen (N) are essential for vital processes in the body. Rising feed costs and their negative impact on the environment increase the need to conserve them. Ruminants should be able to compensate well for N- and P-reduction in the diet. This is due to their ability to recycle endogenous phosphate (Pi) and N. However, previous studies have shown that low N and P intake modulated mineral homeostasis in young goats [1, 2]. Mineral homeostasis is maintained in part by storage or mobilization of calcium (Ca) and Pi from bone. N is important for protein collagen in bone. Previous studies have shown that low N and Ca affected bone metabolism in young goats [3]. In cows, a low P diet affected bone metabolism [4]. Therefore, it was hypothesized that a concomitant N and P reduction also influences bone metabolism in young goats. For this purpose, different markers and genes responsible for bone mobilization were investigated.

Animals, materials and methods: Twenty-eight young male coloured German goats were equally divided into four feeding groups. They received either a control diet (0.42% P, 14.5% N), a P-reduced diet (0.1% P, 14.9% N), an N-reduced diet (0.45% P, 7.3% N) or a P- and N-reduced diet (0.1% P, 7.1% N) for six weeks. At the age of 13 weeks, blood plasma concentrations of urea, Ca and Pi were determined colorimetrically using standard photometric methods. Blood plasma concentrations of bone formation marker osteocalcin (OC) and resorption marker CrossLaps (CTX) were determined by competitive ELISA. Blood samples were always taken at the same time to avoid circadian effects. To determine bone-forming activity of osteoblasts, OC, alkaline phosphatase (ALP), calcitonin receptor (CALCR) and runt related transcription factor 2 (RUNX2) mRNA was quantified by qPCR. All data were analysed by 2-way ANOVA with Tukey's multiple comparisons test. $p < 0.05$ was considered significantly different.

Results and discussion: Pi and OC in plasma decreased ($p < 0.0001$; $p = 0.0003$), while Ca concentration increased with P-restriction ($p < 0.0001$). OC, among others, is responsible for bone mineralization. Its decrease could indicate decreased bone mineralization. Pi and OC in blood correlated positively ($p = 0.0002$), suggesting that P-deficiency was responsible for this decrease. N-restriction resulted in decreased blood urea concentration ($p < 0.0001$), hypocalcaemia ($p = 0.0017$) and increased blood concentrations of OC and CTX ($p = 0.0422$; $p < 0.0001$). This increase in OC and CTX indicates increased bone turnover during N reduction. Simultaneous restriction of N and P decreased OC in blood. OC mRNA showed the same results as in blood concentration, confirming these assumptions. CALCR mRNA increased with P-restriction ($p = 0.0158$). The increase in CALCR mRNA inhibits osteoclast activity and therefore bone resorption, probably caused by the prominent hypercalcemia. ALP and RUNX2 were not altered by either N- or P-restriction.

Conclusion: Decreased N and P intake modulated mineral homeostasis in young goats. P reduction is assumed to affect bone mineralization and bone resorption. In contrast, N restriction probably increased bone turnover. Simultaneous N and P restriction decreased OC, indicating a greater effect of P on bone formation than N.

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6B5. Modulation of GCN2/eIF2 α /ATF4 Pathway in the Liver and Induction of FGF21 in Young Goats Fed a Protein- and/or Phosphorus-reduced Diet

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Introduction: Reducing crude protein (CP) and phosphorus (P) in ruminant diets increases feed efficiency, saves expensive feed raw materials, conserves resources, and reduces environmental impact through lower nitrogen (N) and P emissions. However, dietary intervention influences microbial performance, which is closely related to intracellular and extracellular amino acid (AA) homeostasis, once through microbial degradation capacity, but also because the microbial population itself is capable of synthesizing essential AA (EAA) and non-essential AA (NEAA) de novo. Mammals respond to AA imbalance by initiating the GCN2/eIF2 α /ATF4 pathway, the key elements of which are the sensing of AA deficiency by the serine/threonine kinase general control nonderepressible 2 (GCN2), phosphorylation of eukaryotic translation initiation factor 2 α (eIF2 α), and activation of activating transcription factor 4 (ATF4) [1, 2]. To restore cellular homeostasis, a variety of target genes of ATF4 are activated, e.g. those regulating AA biosynthesis and AA transport [3, 4]. FGF21 is a peptide hormone induced by elevated ATF4 levels and is required for adaptive metabolic changes [5]. Information on the GCN2/eIF2 α /ATF4 pathway in ruminants is not yet available and will be investigated in this study.

Animals, materials and methods: Twenty-eight male Colored German Goats, 10 weeks of age, were divided into four feeding groups of 7 animals each with the following CP and P content as a percentage of dry matter: control diet (16.48 % CP; 0.48 % P), N-reduced diet (8.35 % CP; 0.51 % P), P-reduced diet (16.86 % CP; 0.11 % P), and N- and P-reduced diet (8.1 % CP; 0.11 % P). At the end of the experimental period, the AA profile was determined by ion exchange chromatography. Molecular biology studies of hepatic genes involved in the metabolic pathway were performed by quantitative real-time PCR, and Western blot analysis. FGF21 concentrations were determined by an enzyme-linked immunosorbent assay. Data were analyzed by two-way ANOVA followed by post-hoc Tukey test for multiple comparisons. The strength of a linear relationship was tested using Pearson's correlation.

Results and discussion: Dietary N reduction resulted in a decrease in EAA and an increase in NEAA in plasma. Lack of EAA due to N-reduced feeding resulted in activation of the GCN2/eIF2 α /ATF4 pathway. Moreover, reduced N-intake increased both circulating FGF21 levels and hepatic FGF21 mRNA expression in young goats. NEAA increased in an ATF4-dependent manner, as evidenced by numerous significant correlations between increased ATF4 expression and increased NEAA levels. A remarkable finding of this study is that activation of the described signaling pathway and induction of FGF21 depend on adequate availability of P. P deficiency could lead to a decrease in protein synthesis due to ATP deficiency [6], resulting in accumulation of AA in the blood, thereby preventing activation of the pathway.

Conclusion: N-reduced feed alters the blood AA profile of young goats, despite their complexity of rumen N metabolism. AA deficiency leads to activation of the GCN2/eIF2 α /ATF4 pathway in young ruminants as in monogastric species, but activation depends on adequate P availability. These results illustrate how sensitive studied metabolic pathway is to dietary N and/or P reduction and demonstrate the complexity of different models of dietary restriction in ruminants.

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6B6. Preliminary data on the effects of feeding diets without addition of mineral phosphorus or calcium on performance, serum and bone parameters of growing goats and sheep

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Introduction: The overall goal of this study is to understand the interaction between macro and micro mineral metabolism in growing sheep and goats. The present abstract provides first preliminary data on end points in performance, serum and bone parameters.

Animals, materials and methods: Two consecutive studies were conducted over a period of 28 days on either 18 East Friesian Milk Sheep (mean age 3 months, mix-sex) or 18 Saanen Goats (mean age between 3.5 and 4 months, mix-sex). The animals were fed a diet containing straw and concentrate. The mixed feedstuff was supplemented with either no mineral P (Group P-), or no mineral Ca (Group Ca-) or according to recommendations for both elements (Control group) [1]. Sampling (serum) and data collection (zootechnical data, bone density via pQCT of two points on the left metatarsus) occurred on days 0 and 28, respectively. Data analysis comprised two-way ANOVA (diet, sex, interaction) and the Student-Newman-Keuls-Test (threshold for type I-error $P \leq 0.05$). This study was approved by the Cantonal Veterinary Office of Zurich, based on the animal welfare law of Switzerland (approval ZH 140/2020).

Results and discussion: Mean P and Ca intake were 1.44 ± 0.04 and 4.96 ± 0.13 (P-), 1.78 ± 0.10 and 2.68 ± 0.15 (Ca-), as well as 1.78 ± 0.22 and 4.98 ± 0.62 (Control) g/day in goats, respectively. Associated intake levels for sheep were 1.77 ± 0.14 and 4.82 ± 0.37 , 2.82 ± 0.42 and 2.60 ± 0.39 , as well as 2.76 ± 0.34 and 4.84 ± 0.59 g/day, respectively. Varying mineral supply affected F:G (feed:gain) of goats and sheep, however, the first responded with increased F:G (5.21, 4.3, 4.24 for P-, Ca- and Control) due to compensatory feed intake in P- and Ca- animals (0.73, 0.71, 0.68 kg/d for P-, Ca- and Control) ($P=0.001$, $SEM=0.023$), whereas the latter showed improved F:G (3.34, 3.40, 4.45 for P-, Ca- and Control) via increased gain (0.218, 0.204, 0.167 kg/d for P-, Ca- and Control) at stable feed intake in treatment groups compared to control, though this was not statistically confirmed ($P=0.07$, $SEM=0.029$).

Mean concentration of zinc in serum declined by 0.079 mg/L in both the Ca- and P-sheep from d0 to d28, while it was increased in Control (+0.177) ($P = 0.0026$, $SEM=0.08$) and not affected in any group of the goats ($P=0.772$, $SEM=0.099$).

Mean 1,25OH-vitamin D in serum increased in Ca- goats (+50.1 pmol/l) and decreased substantially in P- goats (-76.38 pmol/l) with a slight decrease in the Control group (-9.48 pmol/l) ($P=0.07$, $SEM=59.17$). In sheep, all groups reacted in an increased 1,25OHVitaminD (+72.74, +190.36, +163.74 pmol/l for P-, Ca- and Control) ($P=0.34$, $SEM=98.27$). An increase in mean trabecular density in the distal metatarsus was detected in goats in Group P- (+44.46 mg/ccm) and Control (+9.18 mg/ccm), while it decreased in Ca- (-18.09 mg/ccm), ($P=0.39$, $SEM=59.249$). In sheep it increased in P-, while it decreased in Ca- and Control (15.97, -37.56, -32.0 mg/ccm for P-, Ca- and Control) ($P=0.01$, $SEM=21.838$).

Conclusion: Reduced supply with mineral P and Ca appeared to initiate a state of deficiency in goats, which was compensated by total feed intake, especially in P- animals. Sheep, on the other hand, appeared to grow with higher efficiency in response to reduced P and Ca supply to control. Further data and laboratory analysis are in progress to understand the observed effects during the experiment, including the reduced serum Zn status in P- and Ca- sheep.

Reference: [1] Agroscope. 2021. Fütterungsempfehlungen für Wiederkäuer (Grünes Buch)

6B7. Effect of a high fat, low carb diet on calcium and phosphorus digestibility in pigs

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Introduction: High fat diets (HFD) are known to influence metabolism and bone mineralization [1]. In rats, the formation of calcium (Ca) soaps contributes to a lower apparent digestibility (aD) of Ca on HFDs, resulting in lower bone mineral density [2;3]. In the present study, the aD of Ca and phosphorus (P) in pigs fed a HFD was investigated to find out whether the effect would be similar to that in rats.

Animals, materials and methods: Six-months-old pigs (body weight 75.9 ± 10.35 kg) were randomly allocated in two groups á 16 pigs, all housed individually. One group was fed a HFD low in carb (crude nutrient content: 14.1 % crude fibre, 13.5 % crude protein, 62.9 % crude lipid 7.0 % crude ash 1.4 % Ca and 1.0 %P (main added Ca and P sources: CaCO₃ and CaHPO₄), as fed; fat source palm fat) for 4 weeks. The other group was fed a control diet (CON, crude nutrient content 5.4 % CF, 18.5 % CP, 4.7 % CL, 4.6 % CA, 0.8 % Ca (CaCO₃) and 0.6 % P (CaHPO₄), as fed) for 4 weeks. The pigs were meal-fed an individual ration in order to avoid obesity, corrected for energy content. During the feeding trial, a 7-day digestibility trial was conducted after a 4-day adaptation period (titanium dioxide (TiO₂) as marker in the diets, 2 faecal samples/ pig/ d). Diet and pooled faecal samples were lyophilized, ground and analysed for TiO₂, Ca and P (by flame emission spectrometry / photometry) to calculate aD. To compare the diet groups, t-tests were used ($p < 0.05$; SigmaPlot software). Further metabolic parameters of the trial are reported elsewhere [4] and partly yet unpublished.

Results and discussion: The pigs fed the HFD had a significantly lower aD(Ca) ($p < 0.05$) than the CON group (Tab. 1), which is similar to the findings in rats [2]. In contrast, aD(P) was significantly higher in the HFD than in the CON group ($p < 0.05$). The lower aD(Ca) may be due to the disruption of Ca absorption by fat in the gastrointestinal tract [2;5]. In combination, the lower aD(Ca) and the high aD(P) are likely to disrupt the balance of Ca and P homeostasis during HFD feeding in pigs.

Tab. 1. Apparent digestibility of Ca and P (mean ± SD).

Parameter	HFD	CON	<i>p</i>
aD(Ca) %	17.4 ± 9.7	23.9 ± 17.5	< 0.05
aD(P) %	53.4 ± 8.5	43.0 ± 10.1	< 0.05

Conclusion: Feeding pigs a low-carb HFD lowered aD(Ca) and increased aD(P) significantly in comparison to a carbohydrate-based CON diet. The fat content is likely to be the cause for this finding. Further investigations are necessary to find out potential effects on the whole organism.

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Session 6C Protein, Animal Nutrition, Clinical cases, Minerals posters



6C1. Processing temperature of full-fat insect meals has limited effects on *in vitro* rumen fermentation characteristics

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Introduction: Insect meals are promising alternatives to conventional protein sources for ruminants [1]. Their composition varies according to insect species. Moreover, to produce full-fat meals, insect larvae can be dried at various temperatures, the increase of which can reduce rumen protein degradability [2]. The aim of this study was to evaluate the effects of processing temperature (T), insect species (S) and their interaction (T × S) on the *in vitro* rumen fermentation parameters of full-fat *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) meals.

Animals, materials and methods: Starting from the same HI and TM larvae, four HI meals and four TM meals were obtained applying different processing temperatures (HI: 40°C, 50°C, 60°C and 70°C; TM: 30°C, 50°C, 60°C and 70°C). Rumen fluid was obtained from four sheep fitted with a rumen cannula. Fermentation parameters after 24h *in vitro* ruminal incubation of the insect meals were measured. A GLM mixed model was performed to test the effects of temperature (regressive factor), species and their interaction (fixed factors). The sheep was used as a random factor.

Results and discussion: Per 1°C increase of processing temperature, a decrease by 0.13% in NH₃-N and a decrease by 0.01% in total volatile fatty acids (VFA) production were observed, irrespective of the insect species. Total gas production and CH₄ production were not affected by the processing temperature or by the insect species. Both the acetate to propionate ratio (C2/C3) and the organic matter digestibility (IVOMD) were not affected by the processing temperature, although a difference between insect species was observed. Previous studies showed a lack of effect of thermal treatment (90°C) of HI larvae on the *in vitro* true dry matter (DM) digestibility in ruminant models [3].

Table 1. Effect of temperature (regressive factor) and species on the *in vitro* rumen fermentation parameters after 24 h of incubation of the insect meals.

Item	S	Model coefficients				P value		
		Intercept	S coefficient	T coefficient	T × S coefficient	S	T	T × S
Total gas (mmol/g DM)	HI	1.72	-	-	-	0.377	0.205	0.811
	TM							
CH ₄ (mmol/g DM)	HI	0.32	-	-	-	0.643	0.149	0.584
	TM							
N-NH ₃ (% of initial N)	HI	57.70	-	-0.13	-	0.214	0.000	0.914
	TM							
Total VFA (mmol/g DM)	HI	4.77	-	-0.01	-	0.855	0.001	0.079
	TM							
C2/C3	HI	3.37	0.79	-	-0.11	0.000	0.721	0.000
	TM		-0.79		0.11			
IVOMD (g/g)	HI	23.90	-7.44	-	0.05	0.000	0.895	0.039
	TM		7.44		-0.05			

Conclusion: An increase of processing temperature of full-fat HI and TM meals in the range from 30°C to 70°C does not influence the enteric total gas and CH₄ production, but negligibly reduces rumen ammonia and total VFA production.

References: [1] Renna et al. (2023) Anim. Front., in press; [2] Son et al. (2023) Animals 13:858; [3] Campbell et al. (2020) Animals 10:682.

6C2. Goat colostrum as supplement for piglets

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Introduction: With today's hyperprolific sow lines, where sows farrow more piglets but do not produce more colostrum [1] and lowest weight piglets have increased probability of mortality [2], the search for porcine colostrum supplements is a priority. On the other hand, goat farms produce more colostrum than they need, which they must eliminate. So, the objective of this work was to study the effects of using goat colostrum as a supplement for newborn low birth weight piglets.

Animals, materials, and methods: 240 piglets with weight at birth between 500-1000g were assigned to two experimental groups according to their body weight: Control group, without supplementation, and Goat group, which piglets were supplemented with two doses of 7.5 g (dry matter) of goat colostrum of the second postpartum day, at 6 and 24 hours after birth. Piglets of both treatments remained with their mothers following the usual suckling. Colostrum was lyophilized and reconstituted at the rate of 75 g lyophilized + 100 ml water at 40°C. The composition of the reconstituted solution was crude protein 16.9%, crude fat 16.2% and lactose 9.5%. All piglets were checked for rectal temperature just before and 2 h after supplementation. In addition, piglets were monitored for the incidence of diarrhea and mortality for 4 days. After 10 days, piglets were weighed again. All data were analyzed with SPSS software. Data of weight and temperatures were analyzed with the T Student Test, and the incidence of diarrhea and mortality were analyzed with Chi-squared test.

Results and discussion: Supplementation with goat colostrum had no effect on average daily gain ($P>0.05$) (Table 1). In addition, no significant differences in rectal temperature or in the mortality rate at 4 days were observed between groups (19 and 13% for Control and Goat group, respectively). The highest number of deaths was recorded on the first day after birth (10 and 7 piglets for the Control and Goat groups, respectively). Thereafter, they decreased progressively until there was 1 death in each group on postpartum day 4. Diarrhea

was not observed in any of the groups. Although numerous supplements for low weight piglets have been studied, not all of them have shown positive results [3,4] and the problem of high mortality of these piglets remains a concern. Our hypothesis was that supplementation with goat colostrum could provide energy

Table 1. Effect of supplementation with goat colostrum to newborn low weight piglet on performance and rectal temperature

	Control	Goat	SEM	P value
Initial weight, g	812.2	814.2	8.423	0.905
Weight at 10 days, g	1821.4	1748.2	46.979	0.437
ADG, g/d	97.6	90.15	4.417	0.403
T2-T1, °C	0.070	0.218	0.053	0.166

ADG: Average Daily Gain; T2-T1: Rectal temperature difference between the temperature 2 hours after supplementation (T2) and the temperature just prior to supplementation (T1).

and increase the competitiveness of these low weight piglets for better sucking, leading to a reduction in mortality. However, those effects have not been observed. Nevertheless, to the authors' best of knowledge, this is the first study using goat colostrum supplement for low-birth-weight piglets and we have observed a good tolerance by the piglets which is encouraging for the study of new supplements based on goat colostrum.

Conclusion: Goat colostrum was well tolerated by low-birth-weight piglets; however, with the protocol used, no positive effects were observed on either growth or mortality of these piglets in the first 10 days of life. It would be necessary to study the long-term effects.

References: [1] Devillers et al. (2007) *Animal*, 1(7), 1033-1041. [2] Farmer and Edwards (2022) *Animal*, 16, 100350. [3] Klaaborg et al. (2020) *Animals*, 10(7), 1–10. [4] Schmitt et al. (2019) *Animals*, 9(5), 227.

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6C3. Profile and prevalence of obesity and other lipid metabolism disorders in captive amazon parrots (*Amazona spp.*)

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Introduction: Obesity in companion birds, like other species, is usually caused by multiple factors such as feeding excessive amounts of inappropriate foods [1], a lack of exercise [2], and increased food intake due to boredom [3]. Although obesity is frequently reported in avian medicine, no large-scale prevalence studies using a representative parrot sample have been conducted [1]. This study aimed to determine the prevalence and characteristics of obesity in captive Amazon parrots at FMVZ/USP Veterinary Teaching Hospital (HOVET) between 2009-2019.

Animals, materials and methods: This study reviewed 1142 health records (HR) of parrots of the *Amazona* species and collected various information such as consultation details, clinical examination results, final diagnoses, and demographic data such as age, sex, weight, and body condition score (BCS). The prevalence of obesity and other lipid metabolism disorders (LMD) in these parrots was also analysed and reported. The diagnoses were made by veterinarians based on pathology, and ancillary test results.

Results and discussion: From the 1142 HR reviewed, 986 final diagnoses of disease were recorded, and from those 127 cases were found to have lipid metabolism disorders (LMD), which prevalence is shown in table 1. Obesity was the second most prevalent LMD, it was identified as the primary cause of morbidity in 22% of the LMD cases (29/127), however, it was mostly diagnosed as a comorbidity, and it represented 53% (44/83) of all the metabolic disorders identified as concomitant, this means

Table 1. Lipid metabolism disorders diagnosed in captive Amazon parrots between 2009-2019.

Process	Primary diagnoses	%Overall prevalence n=986	%Prevalence over lipid related lesions n=127
Atherosclerosis	5	0.51	3.94
Hepatic lipidosis	55	5.58	43.31
Corneal lipid deposition	1	0.10	0.79
Obesity	29	2.94	22.83
Splenic / renal lipidosis	1	0.10	0.79
Xanthoma	6	0.61	4.72
Lipoma	28	2.84	22.05
Liposarcoma	2	0.20	1.57
Total	127	12.88	100.00

the bird was not taken to the HOVET for this reason, but rather for another identified complaint. The species diagnosed with obesity were mainly represented by *A. aestiva* (n=24, 82.7%) however *A. amazonica* (n=4, 13.7%) and *A. festiva* (n=1, 3.4%) were also reported. The profile of the diagnosed obese bird is composed by a household adult pet bird (79.0%), of undetermined sex (68.9%) and feed on a diet of daily seeds (68.9%). Most were coded with BCS as overweight or obese (55.0%, 24.0%) and presented with various symptoms such as respiratory issues (31.0%), coelomic distention (24.0%), and dermatologic problems (20.0%). BCS variation may be attributed to the inconsistencies in filling out clinical records, with different individuals responsible for animal examination and subjective interpretation of body mass with limited anatomical references [4]. Most patients were active (93%), and in three cases, the diagnosis was made during a routine check-up. In the author's experience, bird owners tend to seek veterinary help only when the condition is relatively advanced, and it can be difficult for them to identify when their bird is overweight. Therefore, it is hypothesized that the number of undiagnosed parrots with obesity is high, and the prevalence rate of the disease may be higher than reported [5].

Conclusion: Obesity is prevalent in captive Amazon parrots, indicating the need to begin treating it as an illness and taking it into consideration when discussing epidemiological studies of disease prevalence in these species.

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6C4. Follow-up in 167 homemade diets for maintenance and pathologic conditions in dog

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Introduction: Homemade cooked diets for dogs have gained popularity in recent times because of their high palatability and are considered more natural and healthier. Moreover, cooked diet do not pose parasitological or bacterial concerns like raw diets. The consults with veterinary nutritionist consultations for formulating blanced homemade diets are increasing. The main advantage is related to the total customization, as modifications can be made not only according to actual caloric needs, but especially in case of one or more diseases and/or allergies. To date, few publications in the literature demonstrate the benefits of homemade diets formulated and balanced by a veterinary nutritionist.

Animals, materials and methods: The aim of this study was to evaluate the response to the diet. 167 dogs referred to a nutritional consult and formulation of a personalized home diet. Referrals were in 48 cases for chronic enteropathy, 47 for maintenance diet, 23 for dermopathy, in 29 for both gastrointestinal and dermatological problems, and in 9 for weight loss; the remaining cases were other diseases.

Results and discussion: The median lenght of follow-up was 14 months (minimum 5 - Maximum 24). Of 167 dogs, 104 maintained the homemade diet (62%), while 55 returned to the industrial diet after three months. The reasons for reverting to industrial diets were in 27 cases because the owners considered the cooked diet too much expensive or more time-consuming, especially for large dogs, adverse reactions (15), lack of palatability for the dog (8), onset of new diseases (5). Eight dogs died during the time of follow-up.

Among the 30 dogs referred for maintenance purpose in the 70% the coat improved, in the 46% the frequency of stool decreased, while stool consistency, appetite and energy remained unaltered.

Diet was required for 68 cases with mainly gastrointestinal and/or dermatological conditions as therapeutic support or treatment and 64 of these improved clinically, leaving us to assume that these cases were related to adverse reactions to food. Of the 9 dogs referred for weight loss, 6 were able to achieve a correct Body Condition Score.

Conclusion: The clinical results obtained in this study are promising. The absence of any additional allergens (e.g., mites, preservatives), the customization of the homemade diet by the nutritionist with careful selection of key nutrients and fresh foods with regard to sources that might be allergenic, the selection of functional ingredients and supplementation, and especially the percentages of nutrients to make the diet more digestible or suitable for the animal's living conditions, significantly improve the overall health of the dog both healthy and pathological, confirming the importance of homemeade cooked diet as useful tool in the veterinary practice.

6C5. Vegetable protein sources in kibble diets for cats: extrusion, digestibility, and faecal characteristics

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Introduction: Although cats are strictly carnivores, vegetable proteins are included in feline formulations for sustainability, cost, processing and/or nutritional reasons. These ingredients may have a lower environmental impact, adequate and constant chemical composition, and digestibility, and may favour the extrusion processing. The partial replacement of poultry by-product meal (PBM) by soy protein concentrate (SPC), corn gluten meal (CG) and wheat gluten meal (WG) was compared as protein sources for cats.

Animals, materials and methods: A diet was formulated with only PBM as protein source and unfolded into 9 diets with similar chemical composition (CP 37±0.4%, fat 15±0.4%, starch 22±1.2%), in which SPC, CG and WG replaced 20%, 40% and 60% of the dietary protein, respectively. Diets were produced in a single screw extruder, and processing parameters were adjusted for the PBM diet and kept constant, to observe the impact of the vegetable proteins on processing parameters, specific mechanical energy (SME) and kibble macrostructure. The diets PBM and with 60% WG, CG, and SPC were also tested in 24 cats (6 per diet). After 10 d of adaptation, cats were kept in metabolic cages for 7 d for total faeces collection, apparent total tract digestibility (ATTD) quantification, and 6 d for fresh faeces collection (<15 min) to analyse pH, lactate, and volatile fatty acids. After ANOVA, at extrusion experiment means were compared by polynomial contrasts considering the vegetable protein inclusion level, and in the cat study by the Tukey's test (P<0.05). Animal Ethics Committee approval number: 1044/21.

Results and discussion: SPC and CG inclusion increased SME application and kibble hardness, and reduced kibble density (P<0.05). WG inclusion on the contrary reduced SME, but kibble density also reduced, and hardness increased (P<0.05). The effects on extrusion parameters and kibble formation were higher for SPC, than CG, and lastly WG (P<0.05), showing that although all three protein sources favoured kibble expansion, the extension and mechanism of actions were different. In the cat study, nutrient intake did not differ between treatments (P>0.05). In general, the ATTD of DM, OM, CP, and gross energy were higher and similar for cats fed the WG and CG diets, which values were greater than for PBM and SPC diets (P<0.01). The PBM and SPC diets presented similar DM and CP ATTD, but the PBM higher OM and gross energy ATTD than the SPC treatment (P<0.05). Previous studies had shown that diets with CG and SPC present higher than or comparable ATTD than animal-based rendered proteins [1, 2]. No publications were found about WG ATTD for cats. Faecal score did not change, but moisture and production were higher, and pH lower for cats fed SPC than the other diets (P<0.05). The faeces of cats fed the SPC diet presented higher concentrations of acetate, propionate, butyrate, isobutyrate, and isovalerate than cats fed the other diets (P<0.05). The faeces of cats fed PBM presented lower butyrate, isobutyrate, valerate, and lactate than cats fed the CG and WG diets (P<0.05). During SPC production soluble non-starch poly- and oligosaccharides are removed, but the ingredient has higher proportion of crude fibre (around 4.01%) than CG and WG (around 0.5%), explaining the higher fermentation in the colon of cats fed SPC. As it did not compromise faeces formation, the ingredient is interesting for fibre supplemented formulations.

Conclusion: Vegetable proteins favoured kibble formation. WG and CG showed elevated total tract apparent digestibility of CP, higher than the PBM samples used in the present study. SPC presented comparable CP digestibility than PBM but induced higher fermentation products formation in the colon.

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6C6. Evaluation of calcium, phosphorus, and sodium concentrations in commercial diets for adult dogs and cats

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Introduction: Despite the strong expansion of Brazilian pet food industry (revenue of US\$ 64,52 billion in 2018) [1], there are some studies that observed inadequate calcium (Ca), phosphorus (P), and sodium (Na) concentrations in a small number of commercial pet foods [2,3]. In this context, this study aimed to evaluate Ca, P, and Na levels in a larger amount of commercial pet foods marketed in Brazil.

Animals, materials and methods: In total, 100 commercial pet foods stated as complete and balanced for healthy adult dogs and cats marketed in Brazil were analysed, 75 dry foods (61 for dogs and 14 for cats) and 25 wet foods (14 for cats and 11 for dogs). P and Na were analysed by atomic absorption spectrophotometry [4] and Ca by inductively coupled plasma optical emission spectrometry (ICP-OES) [5]. The results were compared to FEDIAF recommendations [6], descriptively.

Results and discussion:

The results are shown in Table 1. The main findings of this study were the high Ca concentrations and high Ca:P ratio observed, normally because of the protein meals derived from rendering and their associated bone ash. Excessive calcium intake leads to competition with other minerals, such as zinc, which can lead to zinc deficiency [7]. Although it is well known that Ca excess may predispose to developmental osteoarticular diseases, such as osteochondrosis, osteochondritis, and elbow and hip dysplasia in growing dogs [8,9], in adult animals, the effects of excessive Ca intake are poorly studied.

Conclusion: Most pet foods presented nutritional nonconformities when compared to FEDIAF, mainly high Ca:P ratio. Future studies are needed to investigate possible negative effects on small animal health, and manufacturers should review their formulations to ensure the nutritional adequacy of these foods

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Table 1. Results of calcium (Ca), phosphorus (P) and sodium (Na) concentrations in commercial pet foods (in g/100g dry matter).

Minerals	Reference ¹ (g/100gDM)	Mean ± SD	Range	% below minimum	% above maximum
Dry dog foods (n=61)					
Ca	0.58 ² -2.50 ³	2.25±0.77	0.60-4.58	0.00%	27.87%
P	0.46 ² -1.60 ³	0.59±0.12	0.30-0.90	14,75%	0.00%
Ca/P	1/1 ² -2/1 ³	3.75±0.68	1.36-5.32	1.00%	99.00%
Na	0.12 ² -1.50 [*]	0.26±0.15	0.01-0.65	18,03%	0.00%
Wet dog foods (n=10)					
Ca	0.58 ² -2.50 ³	1.56±0.99	0.03-3.26	20.00%	10.00%
P	0.46 ² -1.60 ³	0.59±0.17	0.31-0.82	20.00%	0.00%
Ca/P	1/1 ² -2/1 ³	2.39±1.36	0.10-4.26	20.00%	70.00%
Na	0.12 ² -1.50 [*]	0.53±0.27	0.13-0.91	0.00%	0.00%
Dry cat foods (n=14)					
Ca	0.58 ² -NE	1.72±0.56	1.18-3.06	0.00%	NA
P	0.46 ² -NE	0.59±0.09	0.46-0.76	0.00%	NA
Ca/P	1/1 ² -2/1 ³	2.87±0.62	2.10-4.05	0.00%	100.00%
Na	0.12 ² -1.50 [*]	0.41±0.18	0.13-0.68	0.00%	0.00%
Wet cat foods (n=15)					
Ca	0.58 ² -NE	1.28±0.85	0.03-2.82	20.00%	NA
P	0.46 ² -NE	0.57±0.18	0.27-0.82	6,67%	NA
Ca/P	1/1 ² -2/1 ³	2.11±1.43	0.08-5.45	20.00%	73.33%
Na	0.12 ² -1.50 [*]	0.61±0.33	0.07-1.19	7.00%	0.00%

¹=FEDIAF (2020); ²=minimum recommendation for inactive adult dogs/cats; ³=nutritional limit; * =safe upper limit; NE=not established; NA=not applied.

6C7. Macronutrients, essential minerals, and toxic metals concentrations in commercial unconventional diets for healthy adult cats

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Introduction: There are studies that have demonstrated several nutritional inadequacies in homemade diets for pets [1,2]. Due to the increased demand for unconventional cat diets and the lack of information about their nutritional quality and safety, this study aimed to evaluate the macronutrients, essential minerals and toxic metals (TM) in homemade commercial cat diets commercialized in São Paulo, Brazil.

Animals, materials and methods: Nine homemade commercial diets for adult cats sold in Brazil were analyzed. Bromatological analyses were performed according to AOAC [3]; essential minerals (Ca, P, Mg, Na, Cu, Fe, Mn, and Zn) and toxic metals (Al, B, Ba, Cd, Co, Cr, Mo, Ni, Pb, Sn and V) were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) [2]. For macronutrients and essential minerals, the results were compared with FEDIAF recommendations [4] while the results for TM levels were compared with maximum tolerated levels (MTLs) of FDA (2011) [5], both descriptively.

Results and discussion: Several nutritional inadequacies were observed (Tables 1 and 2). One diet had crude protein levels below the recommended minimum and one diet did not meet the minimum fat recommendation. Levels below the minimum recommendation were observed for: Ca (n=2/9); P (n=1/9); Cu (n=2/9); Fe (n=2/9); Mn (n=2/9), and Zn (n=3/9). The Ca/P ratio did not meet the recommended minimum in five diets. Cu and Zn levels exceeded the legal limit in three and four diets, respectively. Regarding TM, contents above MTLs were

observed for Al in two diets and for Pb in seven diets. Protein deficiency can cause amino acid deficiency, lean body mass loss, and immunodeficiency [6]. Inadequate fat intake results in essential fatty acid deficiency, such as arachidonic acid for cats, and their deficiency can cause impairment of platelet aggregation, thrombocytopenia, and inability to conceive [7]. Ca deficiency and Ca/P ratio below 1 may result in secondary nutritional hyperparathyroidism [8]. Zn deficiency may cause alopecia and lesions on mucocutaneous junctions, as well as parakeratosis in histology [2]. In addition to deficiencies, excessive copper intake, which can cause hepatic accumulation and, consequently, liver disease. Furthermore, high concentration of copper in the cat's diet can inhibit the absorption of zinc, which excess can also be harmful to cats [8, 10, 11]. About TM above the MTLs, there is no information regarding possible adverse effects in cats.

Conclusion: All foods had at least one nutritional inadequacy. Therefore, although these foods are available in the Brazilian market, they should not be recommended for cats.

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Table 1- Nutrients concentrations that did not meet (*) the FEDIAF (2021) recommendations in commercial unconventional diets for cats (in dry matter basis).

	CP (g/100g)	FAT (g/100g)	Ca (g/100g)	P (g/100g)	Cu (mg/100g)	Fe (mg/100g)	Mn (mg/100g)	Zn (mg/100g)	Ca/P
FEDIAF ¹	33.30	9.00	0.53	0.35	0.67	10.70	0.67	10.00	1/1
FEDIAF ²	25.00	9.00	0.40	0.26	0.50	8.0	0.50	7.50	1/1
Dieta 1	48.03	13.35	0.05*	0.55	0.38*	12.93	0.59	4.63*	0.09*
Dieta 2	49.17	20.84	0.06*	0.34	0.31*	21.76	0.91	2.07*	0.18*
Dieta 3	37.88	15.13	0.80	0.67	2.20	8.01	1.86	10.08	1.18
Dieta 4	19.16*	1.60*	0.76	1.08	3.75	78.06	4.19	32.27	0.71*
Dieta 5	41.82	31.38	1.00	0.69	1.49	30.36	1.98	17.69	1.45
Dieta 6	46.20	24.99	0.98	0.88	9.54	9.34	1.37	8.71	1.11
Dieta 7	47.52	27.65	1.69	1.74	0.95	15.77	4.56	22.84	0.97*
Dieta 8	73.39	13.83	0.61	0.60	1.04	20.67	6.35	27.01	1.01
Dieta 9	60.16	21.39	0.83	1.03	3.78	88.02	0.42*	37.36	0.80*

FEDIAF¹= minimum recommendation for inactive cats; ²= minimum recommendation for active cats; Ca= Calcium; P= Phosphorus; Cu= Copper; Fe= Iron; Mn= Manganese; Zn= Zinc; CB= Crude protein.

Table 2- Toxic metals concentrations that exceeded maximum tolerated levels (*) in commercial unconventional diets for cats (in dry matter basis).

Dieta	Al	Pb
1	-	5.38
2	1.55	5.49
3	4.66	11.78*
4	177.59	15.82*
5	155.16	11.33*
6	13.51	12.05*
7	41.86	14.78*
8	338.34*	11.12*
9	206.96*	12.42*
NMT	200	10

Legend: MTL= Maximum tolerated level; Al= Aluminum; Pb = Lead.

6C8. A new teaching tool: Peer- facilitated learning to introduce veterinary students in scientific work – using in-vitro techniques

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Introduction: This project aims to increase students' interest in the scientific workflow and digestive processes by active research. The idea was to impart knowledge on microbial fermentation in rumen (ruminants) and large intestine (hindgut fermenters, omnivores) using practical techniques to investigate the kinetics of microbial fermentation of nutrients and the impact of dietetic measures. Furthermore, this project aimed to enable students to undergo all steps of a scientific study in a fast motion. The fundamental principle of the method is the autonomous experimental work in small groups involving bachelor and master students (peers).

Methods of the project: At the beginning, the students (max. 6 per group, ideally three from each semester) were provided with theoretical knowledge concerning the scientific workflow, didactics and in-vitro techniques. Subsequently, they do a short literature review, design and conduct their own experiment including planning (experimental design, estimation of sample size), realization of the laboratory experiment, data analysis and the presentation of the results. At the end of the course, a feedback round (assessment) is implemented. Didactic support is given by the respective university facilities throughout the project.

During the experiments, on the one hand, the Ankom gas production system (Ankom Technology, Macedon, NY, USA) to measure microbial gas production in light of fermentative activity is used and on the other hand, the Ankom Daisy Inbucator system (Ankom Technology, Macedon, NY, USA) to measure microbial breakdown of feed dry and organic matter (1).

Results: Students were highly motivated and performed the different steps with help from supervisors provided on demand. During the first round of this new course, students experienced that small mistakes in methodology influenced the outcome of the expected results. Due to the fact, that the buffer pH was not adjusted properly the students experienced marked effects on the results and discussed this very carefully and self-critically. By this accidental mistake, the students experienced actively and practically how influences also occurring naturally can destroy microbes and change the digestion sustainably. During the second round of the new course, students performed the method flawless and collected reliable data. The obtained results were not according to expectations from in vivo literature, highlighting to the participants the obvious limits of in vitro systems to predict and in vivo outcome. Although, in these systems feedstuffs are microbial fermented, what is also true for the degradation of feedstuffs in the rumen or hindgut of herbivorous animals.

Conclusion: In conclusion, students participated in the new teaching project with enthusiasm, stating a higher impact of this practical learning compared to the passive learning in lectures or practical exercises in large groups. In the future, necessary background information (e.g. didactic knowledge) should be provided in an e-learning course adapted to peer-facilitated projects on scientific questions by application of in-vitro techniques. Furthermore, this course might be also very valuable for students to prepare for own scientific work.

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Post-congress lectures
Chair: Luis Miguel Mendes Ferreira



Speaker: Josef Kamphues

Prof. Josef Kamphues is a well know expert in the field of animal nutrition. He is a Diplomate of the European College of Veterinary and Comparative Nutrition and emeritus professor at Tierärztliche Hochschule Hannover Germany



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Indeed a challenge: Estimating the amino acid requirements of high producing dairy cows!

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Worldwide (USA, France, and further countries) new recommendations on the nutrient and amino acid supply of dairy cows have been published in the last years (NASEM 2021, INRA 2018). Also in Germany in recent times the work of the AfBN (Committee for Requirement Standards of the Society of Nutrition Physiology; Chair: M. Rodehutschord) was covered mainly by developing new recommendations for energy and nutrient supply of dairy cows, which will come in force in the next months (AfBN 2023).

Like other lactating mammals also dairy cows need amino acids (AA), and are fed crude protein (CP), but the CP is degraded in high shares in the rumen, up to ammonia. Thus, dairy cows depend in a high extent on the synthesis of microbial protein (MP), containing all required essential AA, that are absorbed in the small intestine only, i. e. they do not rely solely on AA present in the ingested diet like pigs or horses. It means, that the dietary AA contribute to the AA supply of the cow in parts only, but the dietary AA are not the main source of AA occurring in the milk finally.

The primary goal of this presentation is to create a deeper understanding of challenges in estimating the AA requirements (and AA amounts occurring in the small intestine) of high performing dairy cows – based on literature as well as on involvement and personal experiences as a member of the committee (AfBN) called above.

The presentation follows the way of crude protein (CP) from the feed/ration to the milk protein at the end. Stepwise the peculiarities in the fate of ingested CP in dairy cows are presented:

1. Contrary to feedstuffs for other species, especially in green fodder and silages the CP contains different proportions of non-protein nitrogen (NPN), for example in grass silages up to 50 % of the nitrogen do not come from AA, due to processes of proteolysis and deamination, resulting in higher ammonia levels in the silage (Hoedtke et al. 2010).
2. The dietary CP is degraded in varying shares in the rumen, thus different amounts of undegraded feed protein (UDP) will reach the small intestine (< 10 up to > 50 %). The UDP contains an AA pattern that is typical for the feed protein. Thus, the protein source and its ruminal degradation are affecting the AA pattern of the protein reaching the small intestine. The CP degradation (rate and extent) will be estimated in the future by standardized in situ-techniques, a standard protocol has been published by the AfBN (2022). It has to be underlined, that the degradation rate is not constant, but affected by the dry matter (DM) intake that results in varying passage rates (faster through the rumen).

3. Concomitantly to the CP degradation there is the synthesis of microbial CP which contains about 85 % protein (MP, sum of all AA). The synthesis of MP in the rumen is quite variable, and primarily dependent on the energy supply of the ruminal microbiota, which is predominantly determined by the intake of digestible organic matter (DOM). According to AfBN (2023) per 1 kg of DOM about 150-160 g MP are produced, when the nutrient supply of the microflora is sufficient/not limited. The higher the intake of DOM, the more MP is formed (linear increase!). It is worth to be mentioned that - due to the urea cycle – nitrogen from cows' own metabolism is contributing to the nitrogen supply of the microflora, i. e. to the synthesis of MP (containing all essential AA required), especially at marginal nitrogen supply/intake.
4. The digestibility rates of protein (sum of all AA) in the small intestine differ markedly: in general high rates (about 85 %) are observed for the microbial protein (MP), but digestibility rates for dietary UDP are quite variable (for example: wheat bran 52 % up to 96 % in corn gluten; AfBN 2023), last but not least due to different feed technologies (protected protein in order to have a higher share of by-pass protein).
5. The digestibility rates of individual AA from the UDP (part of the dietary CP) vary in a large extent, differ from the average protein digestibility rate and differ between the distinct feeds used as protein sources, thus lots of data are required, when a calculation of the sidAA is intended. An estimation of these values is a challenge for the future, presumably in vitro-studies will be the way out to generate them.
6. The AA pattern in the small intestine digesta depends on the ratio between MP and UDP; these both AA sources differ markedly regarding their AA patterns: While the AA pattern of MP is very similar to that of milk protein (about 8.7 and 8.8 Lys/100 g protein, resp.), indicating an evolutionary concept (to cover the need for milk protein synthesis), the AA profile of the UDP is "feed specific". Thus, selecting feeds with a higher share of UDP offers a chance to modulate the AA amounts and patterns reaching the small intestine, the only site of AA absorption.
7. When AA are absorbed from the small intestine, these AA are not available for the milk protein synthesis completely (for 100 %), but only in reduced shares because there are further needs like the metabolism of gut epithelia and provision of energy to the cow (for example in the gluconeogenesis). Thus, the absorbed amounts of AA are not utilized for milk protein synthesis exclusively, but need to be "adjusted" in calculating the requirements, this process is considered via "utilization rate". Respective values have been published by NASEM (2021) and by Lapierre et al. (2007) and (2020): About 70 % of the absorbed protein (sum of all AA) are available to meet the net requirements (for maintenance and lactation), values for Lys are slightly higher (72 %), but for Arg markedly lower (58 %).

8. The AA requirements of dairy cows are finally determined by the milk yield and its protein content (on average 34 g protein per kg). About 95 % of the nitrogen in the milk are represented by protein, about 5 % related to urea. The AA pattern of the milk protein is quite constant in a population, but there might be some breed effects. In general a trend for decreasing protein levels in the milk at high performance (exceeding 30-40 kg/day) is observed.
9. The milk urea concentration is reflecting an unbalanced ratio between energy and nitrogen supply of the rumen microbiota, thus urea levels in the milk are used to optimize the composition of the ration (energy and protein balanced diets), in order to avoid an oversupply with nitrogen. Any excess of nitrogen supply is followed by an almost complete excretion via urea (nitrogen pollution, environmental load).
10. Regarding the characterization/description of the nutritive value of feedstuffs for dairy cows there is a need for data on their potential to deliver sidAA – based on MP, and on the UDP – under defined circumstances. Regarding the ruminal CP degradation from distinct feeds, optimized in situ-procedures/methods are increasingly used to generate the data required for the ration calculation, i. e. a comparison of the expected supply with sidAA and the requirements, determined predominantly by the AA amounts in the milk, but also due to maintenance requirements regarding essential AA.

All the principles described above are - of course - also valid regarding the requirements for maintenance. These are depending on the DM intake predominantly due to the fact that the inevitable nitrogen losses via faeces are determined by the DMI. Estimating the sidAA requirements for maintenance is difficult, too, last but not least due to the state of knowledge regarding the AA content in the different endogenous losses (not considered here).

Assuming that 1 kg milk contains 34 g protein (and 8.8 g Lys/100g protein, it means about 3 g Lys /kg) there is a requirement of about 4.17 g sidLys per kg milk. This amount might be delivered by 4.84 g Lys from MP or by 4.34 g Lys from UDP from soybean meal (soy protein: 6.24 g Lys/100 g protein; Lys digestibility rates are different: 86% for MP vs. 96 % for soy protein). To achieve these amounts there is a need for about 55 g MP or about 77g UDP from soybean meal in the digesta of the small intestine. From this simplified calculation it is obvious, that a comparison of net requirements for AA and the supply at the small intestine (sidAA) is much more difficult than in pigs or horses, due to processes in the rumen that make it so complicated.

Conclusion:

All food producing animals should be fed to meet their requirements as exactly as possible in order to minimize the environmental impact due to excess of nutrients and to ensure high productivity, i. e. to avoid a limitation of performance due to insufficient energy and nutrient supply.

For decades it is well known that dairy cows need – like most mammalian species – essential AA for milk production and that an undersupply of AA occurs not seldom, even in spite of an oversupply with CP (for example fed fresh young grass in the spring), but the specific fate of ingested CP within the rumen accompanied by a variable synthesis of MP have to be considered when an optimized AA supply of high producing dairy cows is intended. But it seems that due to the progress in research a factorial derivation of AA requirements in dairy cows is possible. At high performance of dairy cows the limited DMI is not only a risk factor for the energy supply but also for the required sidAA supply because its interactions with/to the synthesis of MP. Summarizing the efforts to enhance the sidAA supply there are three ways, i. e. to maximize the MP synthesis (especially by increasing DM intake), to feed distinct protein sources with a lower ruminal degradation (higher UDP levels), and to add distinct sidAA (via protected supplements) at high performance/in high yielding dairy cows at peak lactation. The body weight losses / mobilization of energy and protein at lactation start may contribute to milk protein synthesis but it is really complicated to quantify its extent, last but not least due to the variation in the fate of mobilized protein (degradation and deamination or elevating/maintaining AA levels in the blood?).

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The porcine stomach:

a matter of interest for veterinary and comparative nutritionists - a review

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Processes, functions, and dysfunctions in pigs' stomach are worth to be mentioned from different points of view: Those findings are exciting under specific aspects like challenges due to a high feeding intensity or the stomachs role regarding the first line of defense in case of exposure to ingested undesired microorganisms, last but not least they might serve as a model for other monogastric species (including human beings). The most frequently observed disorder of porcine stomachs, i. e. the gastric ulcer - described for the first time about 50 years ago – earns the attention in feed industry and veterinary practitioners as well.

Primary goal of this contribution is a deeper understanding of processes within the stomach of pigs, especially of young ones. Depending on the background, hypothesis and intended information different experimental procedures and techniques were used that could be also of interest, especially to young scientists who are involved in similar subjects (like freezing of the entire stomach with its content at different times after consumption of a meal of varying size or ex-vivo/in-vitro/incubation studies using stomach digesta from dissected pigs).

The acidification of stomach contents needs to be considered because of its effects on the first steps of protein digestion (pepsinogen/pepsin), but also due to its role in preventing (strong acidic milieu) the passage of pathogens into the small intestine.

A huge number of experimental studies and publications was/is dedicated to gastric ulcers in pigs, including experimental studies on diets' physical form for inducing this disorder, but also for its prevention by changes in the technology of feed production (optimizing the grinding process and the diet composition, but also by minimizing factors predisposing for this disorder).

Effects of feeding intensity/meal size on distinct processes within the stomach

- Already in suckling piglets the three different parts of the gastric mucosa (cardia-, fundus- and pylorus region) are found. In very young piglets the acidification of gastric contents results primarily from higher concentrations of lactic acid formed during bacterial fermentation of milk carbohydrates (lactose, but also different oligosaccharides). This mild acidification ensures the absorption of intact immunoglobulins from the milk by neonates.
- With increasing dry feed intake there is a development in stomachs size/tissue mass (g/kg bw) and of the ability to acidify gastric contents by hydrochloric acid (Cl-secretion) as indicated by the strong correlation between chlorine concentration and the pH-value at different sites within the stomach content.
- Also, in elder piglets higher bacterial fermentation occurs in the cardiac part of gastric contents resulting in lactic acid concentrations up to 5 g/kg digesta (more than 55 mmol/kg) and an influx of this organic acid and its producing bacteria into the small intestine (affecting the pH and the microbiota in small intestinal digesta).
- The postprandial acidification of stomachs contents is related to the meal size ingested before and to the time/interval between diet intake and sampling the digesta. The higher the feed amounts consumed, the smaller the share of digesta with very low pH values (only in the pyloric region), indicating the limited capacity of Cl-secretion in piglets.

- At stimulated feed intake (for example due to a longer phase of feed withdrawal) an accelerated emptying is observed, it means that the exposure time of the ingested meal to the gastric acids (hydrochloric acid, lactic acid) and the enzymes activity is reduced.
- The stomach wall, i. e. its tissue mass is related to the physical form of the diet: The coarser structure of diets speeds up the development/maturation, it means that earlier higher values are achieved.
- Feeding intensity (like ad libitum feeding vs. restrictive offer) determines the stomach fill and emptying rate (both favored) as well as the acidification (lowered), especially when the diet has a high buffering capacity (for example due to overdosing of CaCO₃ or MgO).

- **Gastric barrier function against ingested microorganisms**

- Due to the effects of acids (hydrochloric acid and lactic acid) and due to the activity of enzymes within the stomach content the passage of microorganisms through the stomach into the small intestine is markedly reduced, a function called first line of defense.

It can be tested by counting the bacteria occurring in the duodenum after oral infection of pigs (in fistulated ones or by sampling from dissected animals). Another way to measure this capacity of stomach contents is an exposure of bacteria (for example Salmonella, E. coli, Streptokokkus spp.) to the stomach contents ex vivo, i. e. the stomach content of pigs - fed in a standardized protocol - is used for an in vitro incubation and the counts of bacteria are estimated before and after different exposure times. Also the effects of feed additives like organic acids can be tested in this procedure.

- Diets' physical form, i. e. higher shares of coarse particles favor the gastric barrier function, by a stimulated lactic acid formation (cardiac region) and an enhanced hydrochloric acid concentration in the pyloric region of the stomach. These effects are of special interest when dietary strategies against Salmonella are needed (for example due to a higher Salmonella prevalence in the herd or production unit).
- During rearing of piglets there is a widespread use of organic acids in diets (but also administered via drinking water) to support the acidification within the stomach, because the capacity for acidifying is limited in reared piglets. By lower pH-values in stomach contents also an improved proteolysis and protein digestibility are expected/intended.
- At higher contamination of liquid diets by yeasts there is a risk for an enhanced gas formation in pigs' stomach due to yeasts activity, not seldom accompanied by individual losses/dead animals because of stomachs distension and impaired circulatory function. In these cases a deeper investigation regarding yeast species is recommended (distinct yeast species only are able to produce gas within the stomach).

- **Gastric ulcers in pigs**

As observed in many field studies of the last years slaughtered pigs are affected in a high frequency by gastric lesions, their prevalence varies in a range of 10 up to more than 50 % of monitored pigs, last but not least depending on the grade of signs.

- The alterations of the mucosa in pigs' stomach (almost at stomachs' entrance) occur in different grades: in the beginning there is an excessive keratinisation, followed by erosive alterations, ending up in deep ulcers. When the ulceration targets blood vessels in the stomach wall, affected animals may die with signs of bleeding/blood losses (melaena).

- Higher shares of coarse particles only result in forming of layers within the gastric content which prevent, that higher amounts of strong acid gastric contents with proteolytic activity come in direct contact with the unprotected/non covered epithelia at stomachs' entrance.
- Due to higher shares of coarse particles in the diet increased mass of salivary glands were found indicating a stimulated saliva production. Via buffering activity in the saliva (and maybe in esophageal secrets?) the epithelia at stomach's entrance are protected. Also substances like mucines of these secrets could serve as protective agents.
- As demonstrated in multiple experimental studies the main predisposing factor regarding the development of gastric ulcer in pigs is the diets' physical form, i. e. a high grinding intensity followed by pelleting (acts like a secondary grinding).
- Offering one diet in dry or liquid form did not result in any significant effect on prevalence of gastric ulcers, but on farms with a liquid feeding technique there is an enhanced risk when feeds like whey, old bread and by-products of food are fed that are characterized by a very fine structure/missing any share of coarse particles.
- In case of higher prevalence of gastric ulcers in pigs veterinary practitioners need to characterize diets' physical form by sieving the diet (pelleted diets: wet sieving) - as it is ingested. There is a need for a standardized procedure, especially for the wet sieving method, the only way for diets in a pelleted/compacted form.
- Regarding results of the wet sieving the main focus was laid up to now on the fraction/mass of particles < 0.2 mm. The higher the share of this fraction, the higher the risk for developing gastric ulcers (increased risk, when this fraction represents more than 50-60 % of dry matter). But based on a new experimental study it seems that higher shares of very coarse particles might compensate for high amounts of very fine ones.
- For many years also potential effects of the grain type on prevalence of gastric ulcers in pigs were discussed. There are differences between wheat and barley, but these are based on different grinding results at identical grinding conditions/techniques.
- Continuously there is further reason on debate, it means studies on the potential role of Helicobacter spp. in the pathogenesis of gastric ulcers in pigs. Maybe there is an involvement, but as demonstrated repeatedly, Helicobacter was found in affected and non-affected pigs in similar counts or not detected.
- In spite of the predominant role of diets' physical form regarding swine gastric ulcers there is without any doubt a further reason like sudden stress (gut-brain-axis?) due to failure of feeding and/or watering techniques. When pigs are used to a feeding interval of about 4 hours and – due to technical problems – a diet is not available for 20 h and more, one/two days later there is a peak of animal losses – and the affected ones show a deep ulcer frequently (stress-induced ulcers as in horses or human beings?).
- Regarding potential effects/interactions of housing it seems that availability of bedding materials like straw result in lower prevalence and intensity of ulcer formation, maybe due to ingestion of and/or activities with straw (chewing, salivation, buffering, mucosa-protective capacity).
- In case of high prevalence of gastric ulcer in swine production it is recommended to change the diet: a mash diet instead of a pelleted one should be fed, and a part of cereals should be ground in a roller mill instead of a hammer mill (resulting in a more suitable / coarse structure).

Conclusion:

Regarding aspects of comparative nutrition it is worth to be mentioned that in almost all text books for animal nutrition it is underlined that ruminants need a specific physical form of the diet/ration (physically effective fiber, peNDF) to ensure the rumen functions for the health of ruminants, but the time has come to emphasize, that also pigs need a specific structure in their diet, i. e. a distinct share of coarse dietary particles, last but not least to maintain physiological processes within the stomach, to favor its barrier function, to avoid gastric ulcers, and – summarizing - to improve animal's welfare.

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