Official Journal of the Animal Science and Production Association (ASPA)

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ISSN 1594-4077

eISSN 1828-051X

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http://www.tandfonline.com/tjas

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Italian Journal of Animal Science

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volume 16

supplement 1

2017

italian journal of animal science

ASPA 22nd CONGRESS

Perugia, June 13–16, 2017

Book of Abstracts

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Mycobacterium bovis (Mb), the causing agent of bovine tuberculosis (bTB), is an intracellular pathogen highly adapted to the host condition. In cattle, several studies regarding the influence of host genetic makeup, have highlighted the influence of polymorphisms on progression and/or resistance to bTB. For this reason, we decided to investigate the role of a pleiotropic immune mediator, named interleukin-10 (IL10) on susceptibility to bTB in water buffalo (*Bubalus bubalis*). IL10 is secreted by several kind of cells belonging to the innate immune response and is expressed during TB infection. It is involved in phagosome maturation and in the regulation of pro-inflammatory cytokine induction as well. These two aspects indicate that IL10 gene play a critical role in susceptibility and pathogenesis of bTB. To test our hypothesis, we extracted the DNA from blood samples of 184 buffaloes (59 case and 125 controls) reared in 5 herds located in Campania region (South Italy). A 300bp region spanning the exon 5 of the *IL10* gene (NW_005783511) in 10 cases and 10 controls chosen randomly was amplified and sequenced. Sequence comparisons showed a transversion g.3936G > A responsible of the substitution p.Arg152Lys in the primary protein sequence. To test the possibility that g.3936G > A polymorphism is a bTB associated marker we genotyped all samples by mean of AS-PCR.
Subjects carrying the genotype AA were more represented in the cases group (16 out of 59; frequency: 0.37) compare to controls groups (20 out of 125; frequency: 0.19), Thus, when we compared the AA *vs* GG ratio between case and control subjects by Fisher's exact test, the odds ratio (OR) was 2.26; the 95% confidence interval (CI) was 0.970 – 5.299; *p*-value (two sided) = 0.078. Although we have demonstrated that the polymorphism g.3936 G > A is uncoupled with susceptibility in water buffalo, our work offer a good starting point for further investigate this polymorphism in a wider sample group.

P009

Effects of diets rich in β-glucans on chromosome stability of peripheral blood lymphocytes of pigs

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β-glucans are non-digestible glucose polymers which can be isolated by cell walls of fungi, bacteria, algae and cereal grains and that stimulate the growth of bifidogenic and lactic acid bacteria in the gastro-intestinal tract. It has been demonstrated that they exert a lot of health-promoting effects like immunostimulation and they are often applied as feed additive to enhance the immune response. Aim of this pilot study is to investigate the effects of β-glucans rich diets on chromosome stability of peripheral blood lymphocytes of pigs. 20 Landrace × Large White gilts have been divided in four groups (A, B, C and D) and administered four diets: (A) Corn; (B) β-Glucan enriched barley and Corn; (C) β-Glucan enriched barley, Corn and Faba beans; (D) β-Glucan enriched barley, Corn and peas. All diets were iso-energetic (14.27 ± 0.04 MJ/kg) and iso-protein (14.36 ± 0.16% a.f.) and administered *ad libitum* for 40 days (10 d adapt +30 d treat). At the day 40th of the treatment peripheral blood was collected and per each animal two type of lymphocytes cultures were set up: one for Sister Chromatid Exchanges test (SCE) and one for Chromosome Aberrations (CA) and aneuploidy tests. At least 100, 50 and 35 metaphase plates for each animal were observed for aneuploidy, CA (chromatid and chromosome breaks) and SCE tests, respectively. Average differences were evaluated using SPSS software with Tukey-test and results were confirmed with Bonferroni test. Percentages of cell with aneuploidy were 7.83, 11.86, 8.00 and 8.25 in group A, B, C and D respectively. The mean number of total CAs were 2.83 ± 2.86 (A), 1.14 ± 1.2 (B), 1.33 ± 0.58 (C) and 1.50 ± 1.29 (D), while the mean number of SCEs/cell were 5.37 ± 2.69 (A), 4.41 ± 2.26 (B), 5.54 ± 2.61 (C) and 5.00 ± 2.55 (D). The statistically significant differences are: mean SCEs/cell between group B and A ($p < .001$) and group B and C ($p < .05$). Despite the small number of animals employed in this study results indicate that β-glucans rich diet exert the reduction of mean SCEs/cell value in healthy pigs and a better functionality of the DNA-replication mechanisms in peripheral blood lymphocytes, fundamental for proper immune response. To confirm healthy effects of β-Glucans enriched diets and their benefits on animal welfare it is necessary to test them on a higher number of animals.

Acknowledgements

The research was funded by DMVPA01/POR Campania "Prebiotics in Animal feeding".

P010

Complex transcripts pattern identified at river buffalo DGAT1

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DGAT1 has been recognised as strong functional QTL for the milk fat content in cattle. In river buffalo, *DGAT1* has been investigated mainly for the characterization of the gene itself and for the identification of the K232A mutation similarly to what has been done in cattle. No investigation has been carried out at transcripts level so far. Aim of this study was to analyse the transcript profile of *DGAT1* in lactating buffaloes.

Milk samples were collected from 8 unrelated buffalos (at the 3rd lactation and 120 days from calving), reared in Piedmont region and belonging to one farm. Total RNA was isolated from milk somatic cells using TRIzol. The reverse transcription was performed using an oligo dT_{18}, whereas the PCR reaction was accomplished using the following primers 5′-ATGGGCGACCGCGGCGG-3′ and 5′-TCAGGTGCCGGC-TGCCGG-3′, corresponding to the nucleotides 1-17 (exon 1) and complementary to the base pairs 1453-1470 (exon 17) of the buffalo *DGAT1* cDNA (EMBL ID: DQ120929). The PCR products were purified and cloned. Recombinant clones were randomly chosen and screened by PCR. All amplicons different in size (bp) were purified and sequenced in both directions. The obtained sequences were compared with NCBI sequences by BLAST.

A total of 147 recombinant clones were analysed. The sequence analysis showed a complex mRNA pattern with a total of at least 6 transcripts. The most represented mRNA was that correctly assembled (86 out of 147 clones, 58.5%), 1470bp long and coding for a functional protein of 489aa (amino acids). The following mRNA population (1425bp) was skipped of the exon 12 (21.09%). Despite this deletion, the mature mRNA did not undergo any frame-shift and the termination codon was kept as in the normal isoform. The putative protein 474 aa long is different from other predicted buffalo *DGAT1* isoforms available in NCBI. Part of the transcripts were deleted of the last 66bp of the exon 8 (1404bp; 7.48%). This alternative splicing is consequence of the incorrect identification of a splice donor site directly in the exon 8 and it is responsible of a protein isoform 22 aa shorter. Minor transcripts are represented by mRNAs with the insertion of the intron 13 (1557bp; 5.44%), populations skipped of the exon 16 (1407bp; 4.76%) and transcripts characterised by the contemporary out-splicing of the exons 6 and 7 and the insertion of the intron 13 (1337bp; 2.72%). The investigation at DNA level will likely clarify the variability found at mRNA level.

Acknowledgements

The authors would like to thank the Azienda Agricola Moris and the buffalo owner Dr. Ivan Morisiasco for providing milk samples.

P011

Establishment of a crossbreed Simmental × Holstein experimental herd and first assessment of heterosis effects on technical and biological parameters

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Parallel to the large increase in productivity, dairy bovine breeds show deterioration of functional traits such as fertility, health, and longevity. Crossbreeding may help to overcome these problems because crosses express heterosis. A long term research has been established at CREA-PCM with the aim to implement a rotational breeding scheme between the Holstein and the Simmental breeds in order to evaluate both the genomic basis of heterosis and the possibility to increase meat production from dairy farms. The experimental herd is made by three groups of lactating cows: purebred Holstein, purebred Simmental and Crosses, in the expected relative proportions of 1:1:2. During the project the following data have been recorded: weight at birth, weight, price and morphological scoring at sale for male calves, health treatment on all calves, weight and age at puberty for heifers, reproductive events (inseminations, calving, abortions, early embryonic losses). Cows have been monitored by an activity meter system to detect heats. Since the beginning of the experiment, four years ago, a total of 276 calves were born and, out of those, 103 females have been kept as replacement: 53 crossbred, 18 purebred Simmental and 32 purebred Holstein. Among calves requiring veterinary treatments, crossbreed animal sicked less and required less time to resolve illness compared to purebreds. Male calves of the three genetic groups showed statistically significant differences for weight at birth, weight at sale and morphological scores. Following the experimental design, the first F1 heifers were artificially inseminated with either Simmental or Holstein semen. Weight at birth, weight and age at puberty of F1 heifers showed intermediate values between Holstein and Simmental heifers. Moreover the pregnancy rate at first insemination indicated an heterosis value of +8% compared to purebreds. Effects of