



ASPA 24th Congress Book of Abstract

Roberto Mantovani & Alessio Cecchinato

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Italian Journal of Animal Science

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ASPA 24th Congress

Padova, September 21-24, 2021

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September 21-24, 2021**

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“Fiore di Botta”

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Via del Pescarotto, 8-Padova

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Copper-associated liver disease in a Bergamasco Sheepdog: a case report

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Hepatic Cu accumulation in dogs may be the result of the impaired biliary excretion occurring in several liver diseases or a primary metabolic defect. An inherited Cu-associated hepatopathy has been documented in a variety of breeds, however, it is still unclear why most of them accumulate Cu consuming regular maintenance diets. Aim of the treatment is to reduce the excessive Cu content in the liver as well as to prevent further build-up, and it involves dietary changes and Cu-binding treatments. Case report. A 5-year-old, spayed female Bergamasco Sheepdog of 23.7 kg and BCS 2/9 was referred to the Veterinary Teaching Hospital of the University of Padova on 5 May 2020. The dog looked slightly icteric, and blood tests, urinalysis and abdominal ultrasonography showed: coagulation disorders, increased plasma enzymes, hyperbilirubinemia, marked bilirubinuria, hepatomegaly and a diffuse echogenicity variation. Therapy consisted of maropitant, lansoprazole, prednisolone, vitamin K, and tranexamic acid; after resolution of the coagulopathy, liver cytology was performed and Cu toxicosis was revealed by the rubeanic acid staining on May 18. Nutritional intervention was required as the dog showed hyporexia, severe weight loss, and chronic diarrhoea despite numerous dietary changes (all maintenance dry pet food-based); therefore, a home-prepared diet was formulated. Daily energy requirements (DER) were calculated using the ideal body weight (BW) and the formula $110 \text{ kcal} \times \text{BW}^{0.75}$ recommended by FEDIAF, and all nutrient requirements were determined according to the same guidelines. A gradual increase in the energy intake (up to $1.2 \times \text{DER}$) was applied over three weeks to promote weight gain. The diet was moderate-protein (60 g/Mcal) and moderate-fat (35 g/Mcal); the composition included non-meat, low Cu-containing protein sources (i.e. egg and ricotta cheese), a medium-chain triglyceride oil, omega-3 pearls and a multivitamin/mineral supplement specific for canine hepatic diets (Cu: 30 mg/kg, Zn: 5550 g/kg); as a result, maximum daily Cu intake was less than half of the recommended allowance (1.3 vs. 2.8 mg). In addition, the diet was supplemented with silymarin and therapy was complemented by ursodeoxycholic acid. After seven months (December 18), at latest check-up, the dog was in overall good health status and reached 34 kg BW (BCS 5/9); plasma enzymes drastically decreased (halved or more), bilirubin levels fell within range, and no bilirubinuria was present.

O158

Disorders of sexual development in 9 dogs: clinical, cytogenetics and molecular findings

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Reproductive health plays a key role in animal husbandry, in livestock it impacts mainly on the productive ability while in pets in addition to affecting veterinary expenses, it is also often a cause of apprehension in the owner of the affected subject. Among the reproductive problems of greatest interest there are undoubtedly the Disorders of Sexual Development (DSDs): congenital conditions in which chromosomal, gonadal or anatomical sex development are not coordinated each other. Currently, there is an increasing attention to these disorders because they are often diagnosed when the malformed organs develop pathologic conditions like strong inflammations or cancer lesions. Identifying causative mutations would allow the development of diagnostic tools for early identification of individuals who may develop these pathologies. Aim of this work is to describe clinical, cytogenetic and genetic aspects of nine dogs with different type of DSDs. All cases were submitted to clinical evaluation for the abnormal external genitalia conformation observed by the owner (enlarged clitoris, abnormal vulva opening, rudimentary penis, hypospadias or cryptorchidism), except for Case 5 that was examined for the sudden increase in volume of the abdomen. Gonads were surgically removed in 8 of the 9 cases and were found to be histologically testis in cases 1, 2, 3, 4, 5, 6 and 7 and ovo-testis in case 9. In Case 8, ultrasound examination allowed to observe ovo-testis like structures caudally to the kidneys. Cases 1, 2, 3 and 4 were classified as 78, XY DSD – cryptorchids. Case 5 showed the coexistence of testes and uterus and was classified as Persistence of Mullerian Ductus Syndrome (PMDS). Cases 6 and 7 were classified as 78, XX testicular DSDs and Cases 8 and 9 as 78, XX ovo-testicular DSDs. Based on this classification Cases 1, 2, 3 and 4 were submitted to the analysis of MAMLD1, SRD5A2, and AR genes, Case 5 to the analysis of AMRH2 gene and Cases 6, 7, 8 and 9 to array-CGH analyses with a custom Agilent dog CGH microarray K133. Polymorphisms of the MAMLD1, SRD5A2, and AR genes observed in the four dogs analysed did not show an association with the observed ambiguities of the external genitalia, the analysis of AMRH2 showed the presence of the homozygote mutation 241C T exon 3 nt5 confirming the diagnosis of PMSD. Finally,

11 Copy Number Variations were found in the Cases 6, 7, 8 and 9 and one of them seems to be included in SOX9 promoter region that could therefore be the genetic cause of the DSD observed in these cases.

SESSION 33 ANIMAL EFFICIENCY – II

O159
Production and egg quality in brown hens kept in a cage-free system: effects of hen age and nest lighting

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The study evaluated the effects of hen age and nest lighting on egg production and quality, and oviposition pattern from 26 to 45 weeks of age in 1800 Lohmann Brown-Classic hens housed in 8 pens of an aviary system. From 17 to 26 weeks of age, half pens had the nest opened with the inner led light turned on 1.5 h before turning on the installation light (5:30); the other half had the nests closed until turning on the installation light and without any inner led light. Measurements were taken weekly and monthly.

As hen age increased, the oviposition rate ($p < .001$) increased from 89.1% housed hens (on average of 26–34 weeks) to 92.8% (34–45 weeks). Both the oviposition rate of broken and dirty eggs decreased from the first period (5.13% and 7.65% on average, respectively) to the second period (2.28% and 4.21%) ($p < .001$). The weight and width of the eggs increased and the shape index decreased as age increased ($p < .001$). As for oviposition pattern from 26 to 45 weeks, eggs laid in the first hours (5:30–7:30) decreased (76.4–45.8% of eggs laid in 24 h), whereas eggs laid in the rest of the day increased ($p < .001$). Moreover, eggs laid in the nest (as % eggs laid in each time interval) between 5:30 and 7:30 (77.9–86.4%) and between 7:30 and 9:30 (68.3–84.7%) increased from 26 to 45 weeks ($p < .001$). A significant decrease of broken eggs (% eggs laid in each time interval) was also recorded for the eggs laid within 9:30 when the hen age increased. The use of nest lighting from 17 to 26 weeks increased oviposition rate in the following period (26–45 weeks) from 90.1% to 92.3% ($p < .001$), decreased egg weight, width, and surface ($p < .001$) as well as the rate of broken (4.22–3.43%; $p < .001$) and dirty eggs (5.98–5.65%; $p = .10$), whereas the rate of defective eggs was not

affected. The oviposition pattern (i.e. distribution of eggs laid in the different daily time intervals) did not change. In the first time interval (5:50–7:30), eggs laid in the nest were higher (83.2% vs. 80.2% eggs laid in the time interval; $p < .01$) and broken eggs lower (4.83% vs. 7.01%; $p < .01$) in the case of the presence of nest lighting.

Based on the above results, changes in the oviposition during the day according to hen age must be considered in cage-free systems to assure a correct use of nests. As for early nest lighting, further investigation is worth in view of the positive effects on egg production and quality, and on nest use.

O160
In vitro protein degradability in fish: a tool to evaluate novel protein sources

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Sustainability issues drove the interest towards alternative protein-rich raw or processed feed materials to include in aquafeeds. Besides chemical composition, digestibility is a basic parameter to be considered. It is usually determined through in vivo methods. Anyway these novel ingredients are characterized by high variability due to their origin and the technological process applied. To overcome these issues, in vitro methods are widely used in the evaluation of the nutritional quality of feeds in land-animals but, recently, their application for aquafeeds has increased. These methods have been useful for ranking ingredients according to their potential digestibility, but also to establish the ability of different fish species to utilise them.

This study was aimed at estimating the nutritive value of a range of novel protein-rich ingredients to explore their suitability as protein sources in the diet for rainbow trout (*Oncorhynchus mykiss*): (i) poultry by product meal composed by chicken and turkey leftovers (PBM), (ii) *Hermetia illucens* pupae meal (HM), (iii) three dried biomasses of *Tetrasemis suecica* (TETRA), *Tisochrysis lutea* (TISO) and *Arthrospira platensis* (ART). The ingredients were characterized for their chemical composition, in vivo protein digestibility (ADC) and in vitro enzymatic protein hydrolysis based on an assay that used rainbow trout digestive enzyme extract. Protein degradation was monitored after 120 min by electrophoretic techniques using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to obtain a quantitative coefficient of protein degradation (CPD).

Protein ADCs were higher for PBM, HM and TISO (92.0–96.5%) than ART and TETRA (83.1–86.2%) ($p < .05$). Despite CPD values

discriminant analysis significantly separated LRCI from HRCI. Then the Bayesian GWAS (B-GWAS) was developed by using the BayesR software and, at the end of the procedure, markers were ranked according to their posterior inclusion probability and the first 698 SNPs were selected. Only markers simultaneously selected in the two procedures (11) were considered associated with RCI. The subsequent gene discovery analysis highlighted 50 genes in the neighborhood (250 kb downstream and upstream) of the 11 SNPs with 17 genes directly associated with RFI and average daily gain.

P100

Application of single-step GBLUP in Italian Comisana sheep

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Comisana is a dairy sheep breed reared in central and southern Italian regions used to produce PDO cheeses. Since 2000 a nucleus flock has been established at the breeders association experimental station aiming at improving both milk yield and composition using pedigree-based EBVs and fitting a lactation model. Recently, within the national CHEESR project, ewes of the experimental station were genotyped (Illumina OvineSNP50 Beadchip) and a repeated test-day model was implemented using both pedigree-based (TD-PBLUP) or single-step genomic best linear unbiased prediction (TD-ssGBLUP). The main objective of this study was to compare results from TD-PBLUP and TD-ssGBLUP. Data consisted of daily milk production and weekly fat and protein contents for 1138 Comisana sheep, 50K SNP genotypes for 309 animals and 2062 animals in the pedigree. Overall, a total of 169 individual – dam pairs were available. After a quality check 45,343 SNPs were retained. A multiple-trait repeatability test-day model was fitted including year of birth, month of calving, class of days in milk as fixed effects, and flock-test-date, permanent environmental and additive genetic as random effects. Phenotypes of 100 ewes (50 with genotypes) were masked to create a validation group. Heritability ranged from 0.09 (fat content) to 0.33 (daily milk yield). The average accuracy of TD-PBLUP and TD-ssGBLUP EBVs for the validation group was 0.43 and 0.45 for milk yield, 0.48 for fat content and 0.62 for protein content. When considering only ewes with genotypes the average accuracy

of TD-ssGBLUP was higher than that of TD-PBLUP only for milk yield (0.40 vs. 0.45) while for protein and fat content did not change being 0.60 and 0.48, respectively. Results from the current research confirm the usefulness of a single-step approach but they depend on the trait and its heritability, the percentage of genotyped individuals and the population structure. The upcoming inclusion of ram genotypes is expected to increase the accuracy of the TD-ssGBLUP EBVs.

P101

Genetic structure at CSN1S1, CSN2 and CSN1S2 loci of four autochthonous goat breed reared in Lazio and Campania regions

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One main aspect of safeguard plans for livestock Autochthonous Genetic Types (AGTs) is to ensure economic sustainability of their farming. This encourages farmers to contribute to the preservation of AGTs. The aim of this study was to characterize the genetic structure of four AGTs of endangered goats at the α S1, β and α S2 calcium sensitive caseins encoding genes CSN1S1, CSN2 and CSN1S2, respectively. The peculiar polymorphisms detected at these loci are associated with milk qualitative characteristics and have been largely investigated in widespread dairy goat breeds. On the contrary, there are very few studies carried out in AGTs goats. This study was performed on 50 Neapolitan goats, typical of the Campania region, and on 61 goats which included the three breeds Monticellana, Capestrina and Grigia Ciociara, reared in Lazio region (Lazio AGTs goat). Genotyping of quantitative alleles at CSN1S1, CSN2 and CSN1S2 loci was performed according to previous studies. At the CSN1S1 locus a high variability was observed in all four AGTs. In particular, a higher frequency of the null allele N was observed in Neapolitan goats than Lazio AGTs goats (0.420 vs. 0.008). On the contrary a higher frequency of F allele, associated with a low α S1 casein content in milk, was observed in Lazio AGTs goat (0.442) than Neapolitan goat (0.120). All four AGTs do not carry the null alleles CSN1S1 01, CSN2 01 and CSN1S2 0 and the alleles associated with an intermediate content of α S1 (CSN1S1 E) and α S2

(CSN1S2 D) caseins in milk. The genotyping data obtained provide useful information regarding the productive aptitudes of the four AGTs studied. In fact, the high frequency of CSN1S1 N allele in Neapolitan goat, predisposes this breed to produce low-protein milk for fresh consumption while the high frequency of the allele CSN1S1 F in the Lazio AGTs goat addresses their milk to processing for the production of typical cheese with unique organoleptic and flavor characteristics. Developing safeguard plans that take these results into account will have a higher chance of success, with a consequent increase in the number of animals raised.

P102

Breeders' management in a heritage breeds' rabbitry

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Population size reduction and genetic erosion are two critical events in domestic animals' productions. The aim of the present research is to analyze breeders' management data recorded in a heritage breeds' rabbitry to determine breeders' husbandry strategies and their effects on some reproductive parameters. Litters' registrations including buck id, doe id, day of birth, litter size (born alive) and weaned kitten were analyzed. Three breeds' litters were studied in a 11 years time lapse: Belgian Hare (BEHA; $N = 151$), Burgundy Fawn (BUFU; $N = 105$), Californian (CALI; $N = 212$). Inclusion of external breeders was considered too (buck origin 0, 1; doe origin 0, 1; litter origin 0, 1, 2). Data analysis was performed by ANOVA using the General Linear Model procedure of SPSS. In our model dependent variables were litter size (born alive) and percentage of weaned kitten, and sources of variation were breed, year of birth and litters' origin (three levels: both parents internal, one parent internal and one parent external, both parents external). The post hoc Bonferroni test was used to investigate the significant differences ($p \leq .05$). The dependent variables number of litters per buck and number of litters per doe were analysed with breed as source of variation. Frequencies distribution of buck origin, doe origin and litter origin were analysed using SAS PROC FREQ procedure and the Chi Square test was used to investigate significant differences. Breed had no effects on litter size (means \pm SE; BUFU 5.75 ± 0.25 , CALI 5.77 ± 0.18 , BEHA 6.06 ± 0.20) and percentage of weaned kitten on born alive ones (mean \pm SE; BUFU 87.18 ± 2.45 , CALI 88.89 ± 1.74 , BEHA 89.86 ± 1.97). Year of birth affected significantly the litter size and percentage of weaned kittens in all the considered breeds: in the last years, the percentage of weaned kittens was higher than in the first years of recording. The inclusion of external breeders did not significantly influence recorded

reproductive data. The number of litters per buck (mean \pm SE; BUFU 1.91 ± 0.21 , CALI 2.34 ± 0.16 , BEHA 1.94 ± 0.12) and per doe (mean \pm SE; BUFU 1.61 ± 0.17 , CALI 1.77 ± 0.13 , BEHA 2.10 ± 0.16) were not influenced by breed. The frequency of use of external breeders is breed specific. Data recording is a basic step in conservation plans; our results supply some information about breeder's management strategies in a heritage breed rabbitry aimed to produce morphologically typical rabbits limiting genetic variability reduction.

P103

A machine learning approach for the identification of population-informative markers from trout genotyping data

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Cost-effective commercial SNP arrays are now available for several species and this has had a substantial impact on livestock as well as on fields of natural ecology, evolution and conservation biology. Nowadays, genome-wide SNP analysis is the method of choice for the characterization of natural populations. In this context, the identification of a minimum number of SNP with the maximum information to differentiate populations is becoming important but challenging. This may have interesting implications for several downstream applications such as allocation of individuals and comparative analyses of selection signatures. Recently, the use of machine learning approaches and notably of random forest classifier (RF) has been proposed for the identification of the most discriminating genetic markers among thousands of SNP. Here we used the RF algorithm to analyse genotyping data obtained with 57K Trout BeadChip array (Affymetrix) from autochthonous and allochthonous trout populations of Molise rivers and their tributaries. The 48 highest ranked SNP were obtained and compared with the list of the most informative SNP estimated using traditional statistical approaches: Delta, FST and principal component analyses. In total, 103 specimens were enrolled in the study, from a larger cohort of ~300 fishes caught in 30 different sites of Volturno and Biferno basins. The samples were chosen based on results obtained by PCR-RFLP and preliminary fine-scale population structure outcomes. Trout considered in this study were representative of four different native trout subpopulations and one Atlantic species. Four reduced informative panels were obtained and their performances estimated using correct prediction proportions from RF classification. The correct assignment of the

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