regions jointly identified by the five statistic methods, since each approach tends to detect different signals. However, a total of 7 genomic regions on four different chromosomes were jointly identified by at least two statistical approaches, which can provide clues to potential new targets of selection. In particular, we found a congruence among the EHH-based methods (iHS, Rsb and XP-EHH), but also betweeen iHS and ROH approaches. Several candidate genes for milk production were identified within the genomic regions of the samples under selection, corroborating the polygenic nature of this trait. We also found that some of our candidate regions putatively under selection spanned several genes related to growth traits and innate immunity. Overall, the identified genes may explain the effect of selection to improve the performances related to milk production traits in the breed. The genomic regions here identified corroborate with previously reported studies carried out in other livestock species. The different approaches did not detect genomic regions known to contain strong functional candidate genes for milk production traits, such as casein clusters or *DGAT1*. This could be a consequence of a low density of the adopted SNP array in these regions. Therefore, further studies using the high density array data, would be particularly relevant to refine and validate these results.

O130

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

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

O334

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

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Genomic evaluations are routinely used in most livestock breeding programs and Single–Step Genomic Best Linear Unbiased Prediction (ssGBLUP) is the most popular methodology. Saanen and Camosciata delle Alpi are the most important dairy goats in Italy and they have similar breeding programs in which estimated breeding values (EBVs) for productive traits are calculated using BLUP. Developing a new genomic evaluation in these breeds using ssGBLUP is one of the aims of CHEER and SHEEP&GOAT projects, managed by the Italian Sheep and Goat Breeders Association (Asso.Na.Pa.) and funded by the Italian Rural Development Plan (sub-measure 10.2). In this study, we present the first results of this activity, for which genomic breeding values were estimated using BLUP, GBLUP, and ssGBLUP methods. The following data were included for Saanen and Camosciata delle Alpi breeds, respectively: 1139 and 2472 animals genotyped with the Illumina GoatSNP65 Bead Chip; 8881 and 2589 lactations of 210 days belonging to them and used within official genetic evaluations, with milk (MY), protein (PY) and fat (FY) yields and protein (PP) and fat (FP) percentages; and three generations of parents loaded from the official herdbooks, checked, and corrected with genomic data using seekparentf90. Variance components estimated with blupf90+ on the corrected pedigrees were used for all the three EBV's estimation methods. Furthermore, we calculated the correlation between EBVs and genetic trends for all the three methods. Results were validated with the linear regression method (LR).
Moderate heritabilities were estimated for all the traits: 0.30 for MY, 0.33 for PY, 0.28 for FY; 0.33 and 0.52 for PP, and 0.20 and 0.34 for PP in Saanen and Camosciata delle Alpi, respectively. EBVs estimated with BLUP, GBLUP, and ssGBLUP showed very high correlations (>0.90); the genetic trends were very close for all methods and generally increasing from 2015 to 2020. EBVs were more accurate under ssGBLUP than BLUP and GBLUP (around +9% and +1% across traits).
In conclusion, our results show that ssGBLUP improves the accuracy of EBVs, especially when animals do not have phenotypes; thus, it is likely to be the best method to improve genetic gain in Saanen and Camosciata delle Alpi goats.

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O188

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

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Alpha-lactalbumin (α-La), encoded by *LALBA* gene, is a Ca^{2+} binding whey-protein whose key function is to facilitate lactose synthesis by the galactosyltransferase component, serving as a regulatory subunit. Other biological functions have been demonstrated including immune modulation, cell growth regulation, antimicrobial activity, etc. Gene promoters have transcription factor (TF) binding sites necessary for gene expression regulation. Mutations in the promoters may modify the transcription rates or the mRNA stability, thus affecting the protein yield.
This study aims to sequence the *LALBA* promoter in alpacas, identify putative TFs and detect genetic diversity affecting gene expression.
A DNA fragment (800 bp) spanning the gene promoter until the exon 1 was amplified and sequenced for 20 alpacas. Multiple alignments and SNP discovery were accomplished by DNAsis software, whereas Transfact 7.0 was used for the TF sites search. Three independent gene reporter assays were achieved by pGL3 specific contructs to test luciferase expression in HEK 293T cells. Data elaboration was performed using JASP software ($p < 0.05$, students's *t*-test).
TF binding sites analysis evidenced 16 putative consensus sequences, including 3 C/EBPα, 3 Sp1, one NF-1, etc. Seven polymorphic sites were found. Taking as reference the first nucleotide of the exon 1, one SNP (g.15C > G) was found in the signal peptide, but it is a silent mutation. The other 6 SNPs were detected in the promoter (g.-553A > G, g.-428C > T, g.-308C > G, g.-236A > T, g.-73C > G, g.-51A > G). The SNP g.-553A > G creates a putative binding site of the TF Sp1. This motif is a well-known enhancer element for the basal expression of many genes, including milk proteins.
To assess the SNP effect on the *LALBA* promoter, we amplified and cloned a DNA region of 178bp in the pGL3-basic vector from four homozygous individuals (two g.-553AA and two g.-553GG). The two different constructs (g.553A and g.-553G), with the pGL3 vector as a control were used to transiently transfect HEK293T cells. After 48 h, the reporter activity of the variants was measured using the luciferase assay system. The G variant of this SNP enhances the promoter activity of the alpaca *LALBA* ($p < 0.01$). Therefore, we suppose an effective role of this binding site in the