

predicted. Overall, miRNAs were the predominant small-RNA class detected across the tissues (81.1% corresponded to miRNAs, 0.4% rRNA, 0.3% tRNA, 3.02% snoRNA, 0.93% snRNA, 14.25% either mitosRNA, unknown or artifacts). piRNAs were detected in testis, representing the majority of small-RNAs in adult testis and about 30% in neonate testis. Combining the ChIP-seq results from a subset of tissue samples, 73,739 putative active promoter and transcription start site (TSS) states (H3K4me3), 27,408 active enhancer states (H3K27ac and H3K4me1), 12,365 repressive states (H3K27me3) and 68,505 insulators bound by CTCF were detected. The data generated, combined with the new map of TSS already available (CAGE) and ATAC-seq, Hi-C, ChIRP, RRBS, lncRNA and circRNA which will be generated in collaboration with other partners in the BovReg consortium, will yield improved functional annotation of the cattle genome.

**Key Words:** cattle and related species, Functional Annotation of Animal Genomes (FAANG), functional assay, regulatory element

**P188 Seasonal changes in the adipose transcriptomes in semi-domesticated reindeer (*Rangifer tarandus*).** M. Weldenogodguad\*<sup>1,2</sup>, K. Pokhare<sup>1</sup>, L. Niiranen<sup>3</sup>, P. Soppela<sup>4</sup>, I. Ammosov<sup>5</sup>, M. Honkatukia<sup>6</sup>, H. Lindeberg<sup>1</sup>, J. Peippo<sup>1,6</sup>, T. Reilas<sup>1</sup>, N. Mazzullo<sup>4</sup>, K. A. Mäkelä<sup>3</sup>, T. Nyman<sup>7</sup>, A. Tervahauta<sup>2</sup>, K.-H. Herzig<sup>8,9</sup>, J. Kantanen<sup>1</sup>, <sup>1</sup>Natural Resources Institute Finland (Luke), Jokioinen, Finland, <sup>2</sup>Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland, <sup>3</sup>Research Unit of Biomedicine, Faculty of Medicine, University of Oulu, Oulu, Finland, <sup>4</sup>Arctic Centre, University of Lapland, Rovaniemi, Finland, <sup>5</sup>Board of Agricultural Office of Eveno-Bytantaj Region, Batagay-Alyta, The Sakha Republic (Yakutia), Russia, <sup>6</sup>NordGen—Nordic Genetic Resource Center, Ås, Norway, <sup>7</sup>Department of Ecosystems in the Barents Region, Norwegian Institute of Bioeconomy Research, Svanvik, Norway, <sup>8</sup>Research Unit of Biomedicine, Medical Research Center, Faculty of Medicine, University of Oulu, Oulu, Finland, <sup>9</sup>Oulu University Hospital, Oulu, Finland.

Adipose tissues play a vital role in regulating energy homeostasis and thermogenic activity in animals living in northern and Arctic environments by changing gene expressions in their tissues. Here, we conducted transcriptome profiling of 3 adipose tissues from different anatomical depots (metacarpal, perirenal and prescapular) from Finnish and Even reindeer (from Sakha, Russia) at 2 seasons (spring and winter) using RNA-seq technology. A total of 220.5 Gb of clean reads were generated using Illumina HiSeq platform. On average 36.5 million pair-ended clean reads were obtained for each sample, and a total of 16,362 genes were expressed in our data. Gene expression profiles in metacarpal bone marrow adipose tissue were distinct and clustered separately from perirenal and prescapular adipose tissues. Metacarpal adipose tissue seemed to play a key role in reindeer energy metabolism regulation in the spring, when the animals are in their poorest nutritional condition after winter. During spring, the genes associated with the immune system, such as cytokines, chemokines, interferons and interleukin receptors (e.g., *CCL2*, *CCL11*, *CXCL14*, *IGSF3*, *IGHM*, *IGLC7*, *JCHAIN*, and *IGSF10*), were upregulated in perirenal and prescapular adipose tissue, while genes involved in energy metabolism (e.g., *ACOT2*, *APOA1*, *ANGPTL1*, *ANGPTL8*, *ELOVL7*, *PFKFB1*, and *ST3GAL6*) were upregulated in metacarpal adipose tissue. Finnish reindeer revealed relatively higher number of significantly differentially expressed genes irrespective of the season than Even reindeer, possibly owing to climatic and management differences. In summary, the findings of this study revealed several adipose candidate genes potentially involved in immune response, fat deposition, energy metabolism, development, cell growth, and organogenesis. These results provide helpful information on the mechanisms by which reindeer adapt to harsh Arctic conditions.

**Key Words:** energy metabolism, immune process, metacarpal adipose tissue, perirenal adipose tissue, prescapular adipose tissue

**P189 Genes related to chemotaxis of the immune system underlie ongoing indicine-aurine cattle domestication at copy number vari-**

**ation hotspots.** V. H. da Silva\*<sup>1</sup>, L. Correia De Almeida Regitano<sup>2</sup>, A. Zerlotini Neto<sup>3</sup>, G. Barreto Mourão<sup>1</sup>, and L. Lehmann Coutinho<sup>1</sup>, <sup>1</sup>Department of Animal Science, University of São Paulo (USP), Luiz de Queiroz College of Agriculture (ESALQ), Piracicaba, São Paulo, Brazil, <sup>2</sup>Embrapa Pecuária Sudeste, São Carlos, São Paulo, Brazil, <sup>3</sup>Embrapa Informática Agropecuária, Campinas, São Paulo, Brazil.

The domestication of aurochs (*Bos primigenius*) gave rise to different taurine (*Bos taurus*) and indicine (*Bos indicus*) breeds. The beef production is either based on taurine, indicine or even mixed breeds, but the choice of the best breed, for a specific production system, depends on different factors such as climate. Indicine breeds are more resistant to tropical parasites whereas taurine breeds are adapted to temperate environments. Genes underlying taurine-indicine domestication can be uncovered by the sequences that are dissimilar between their genomes, and among them are the copy number variation (CNV) hotspots. To extend the knowledge about the biological pathways associated with ongoing cattle domestication we (i) inferred the synteny between taurus and indicine-hybrid reference genomes, (ii) overlapped the regions of synteny break with polymorphic CNVs from a population of 765 Nelore (*Bos indicus*) animals and (iii) performed an enrichment analysis with the genes at the regions of overlap. We found 51 polymorphic CNV regions, i.e., hotspots located at regions of synteny break, encompassing 219 genes that are significantly enriched (FDR adjusted *P*-value <0.05) for 3 different immune-related KEGG pathways (S. aureus infection, NOD-like receptor and IL-17 signaling). Moreover, several gene ontology (GO) biological processes associated with chemotaxis, which is essential to immune system function and homeostasis, were significantly enriched (FDR adjusted *P*-value <0.01). Our results indicate that CNVs on genes associated with the immune system chemotaxis are linked to the current taurine-indicine differentiation and are still a relevant source of variation for a genomic selection for disease resilience, at least in widespread indicine breeds such as Nelore. FAPESP process number 20/00340-0.

**Key Words:** synteny, evolution, structural variation

**P190 Liver RNA-seq expression analysis in cattle supplemented with rumen-protected choline.** D. Hernández Maizón<sup>1</sup>, P. Alvarez Cecco<sup>1</sup>, H. Morales Durand<sup>1</sup>, L. H. Olivera<sup>1</sup>, M. E. Fernandez<sup>1</sup>, P. Peral Garcia\*<sup>1</sup>, G. Giovambattista<sup>1</sup>, and A. Rogberg-Muñoz<sup>1,2</sup>, <sup>1</sup>IGEVET – Instituto de Genética Veterinaria “Ing. Noel Dolout” UNLP-CONICET LA PLATA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina, <sup>2</sup>IMPA - Instituto de Mejoramiento y Producción Animal, Facultad de Agronomía (UBA-CONICET), Ciudad Autónoma de Buenos Aires, Argentina.

Nutrition plays a major role in animal performance, while nutrigenomic studies how food affects gene expression. Several researches were done over feeding systems including diets, feedstuffs, essential nutrients, vitamins and cofactors. Within them, choline is a water-soluble essential nutrient that serves as methyl donor and cofactor; as a precursor of acetylcholine, phospholipids and betaine; required for growth, neural development and fat metabolism. Choline is de novo synthesized but a dietary intake is required, but in ruminants has been used as rumen-protected choline (RPC) as it is easily degraded in rumen. Despite its demonstrated metabolic effect, little is known about the regulation of gene expression in the liver of choline supplement in beef cattle. The objective of this study was to evaluate the differential gene expression and enriched pathways when adding RPC to the diet of fattening beef cattle. A total of 11 Braford steers were fed during 85 d with a formulated diet (89% DM, 15.1% CP, 31% NDF; 5.6% EE, 43.3% starch, 2.9 Mcal ME/kg DM), whereas 6 of them receive 6 g/animal/day of RPC. At slaughter, a liver sample was taken, preserved in RNA-later, and whole expression NGS sequencing was performed. Quality control of sequences, alignment to reference genome (UMD3.1) and raw count matrix data was conducted using FASTQC, hisat2, samtools and featureCounts softwares. Differential expression analysis was carried out by edgeR and DESeq2, R packages. DAVID, FAANG

and Panther tools were used to get the Gene Ontology (GO) enriched terms. Results showed 945 genes with significantly modified expression ( $P < 0.05$ ), and 13 of them remained after Holm-Bonferroni correction. The GO analysis identified terms related to the enhancement of immunity (innate immunity, T-cell activation and B cell receptor signaling pathway), cellular transportation (exosome, scavenger receptor activity and LDL transport), protein binding and collagen trimer. This agreed with several studies that showed an increment of immune response when choline is supplemented, and support the reported hepatic protection. Finally, could introduce information about the mechanism behind the liver fat reduction and enhanced milk production observed in dairy cattle.

**Key Words:** liver, choline, RNA-seq, expression, cattle

**P191 Influence of fetal weight on liver transcriptome in purebred and crossbred Iberian pig fetuses.** Y. Núñez<sup>\*1</sup>, C. García Contreras<sup>1</sup>, M. Vázquez Gómez<sup>3</sup>, S. Astiz<sup>1</sup>, R. Benítez<sup>1</sup>, A. Heras Molina<sup>1</sup>, B. Isabel<sup>2</sup>, A. Rey<sup>2</sup>, A. González Bulnes<sup>1</sup>, and C. Óvilo<sup>1</sup>, <sup>1</sup>INIA CSIC, Madrid, Spain, <sup>2</sup>UCM, Madrid, Spain, <sup>3</sup>Sorbonne Université, Paris, France.

Iberian is a local pig breed, traditionally produced in extensive systems, subjected to scarce selection and less efficient than commercial breeds, but highly valued for its high-quality products. This breed is also characterized by low uterine capacity and high variability in birth weight within the same litter, which is accentuated when the mother's nutrition is deficient. The objective of this study was to evaluate how the body weight of the fetuses of undernourished sows, belonging to the same litter and to 2 different genotypes, affected the liver transcriptome. Pure Iberian sows were inseminated with heterospermic semen from Iberian and Large White males. Samples were obtained from 51 purebred and crossbred fetuses at d 77 gestation. The weight of the fetuses was employed to select 32 individuals phenotypically divergent for body weight, to perform liver RNA-seq analyses (16 from each genotype and from each sex). Transcriptome differences between fetuses with high and low weight were analyzed within each genotype. A great effect of weight was observed in purebred fetuses [747 differentially expressed genes (DEGs) with  $q < 0.05$ ], but, in contrast, negligible effect was observed in crossbred fetuses (2 DEGs). The functional analysis of DEGs in purebred fetuses (high vs low weight) showed the activation in the high weight group of functions related to cellular movement, cell-to-cell signaling and interaction, immune cell trafficking and inflammatory response while functions related with organismal survival and apoptosis were increased in the low weight group. Potential upstream regulators were identified in both groups, with cytokines such as IL4, IL5 or PRL being activated in the high weight group, while PPAR $\alpha$ , a regulator activated under conditions of energy deprivation, was activated in the low weight group. Transcriptome differences between low and high weight fetuses confirmed that low weight Iberian animals had signals of compromised viability from very early stages while in crossbred fetuses absence of transcriptomic differences between the small and large fetuses may suggest a more advanced and balanced developmental and metabolic status in this genotype.

**Key Words:** transcriptome, fetuses, pig, weight, liver

**P192 Expression of taste receptor genes in growing Iberian and Duroc pigs.** R. Benítez<sup>\*1</sup>, R. Peiro<sup>1</sup>, Y. Nunez<sup>1</sup>, F. Garcia<sup>1</sup>, E. de Mercado<sup>3</sup>, E. Gomez-Izquierdo<sup>3</sup>, J. Garcia-Casco<sup>1</sup>, and C. Lopez-Bote<sup>2</sup>, <sup>1</sup>INIA-CSIC, Madrid, Spain, <sup>2</sup>Faculty of Veterinary Medicine, UCM, Madrid, Spain, <sup>3</sup>Pig Test Center ITACYL, Hontalbilla, Segovia, Spain.

Taste receptor genes are expressed in sensory cells located in the tongue taste buds. In pigs, these genes comprise 2 families: Tas1r (sweet and umami taste perception) and Tas2r (bitter taste perception). Besides, fatty acids receptors are involved in fat taste perception. Taste perception is related to feed intake and may differ between breeds showing differences in appetite. We analyzed the expression of a panel of 10 taste receptor genes by qPCR in the circumvallate papillae of growing Iberian and Duroc pigs fed diets with different energy sources. We employed 30

Iberian and 19 Duroc males kept under identical management conditions except the nutritional treatment (HO diet with 6% high oleic sunflower oil and CH diet with carbohydrates), killed after 47 d of treatment, with 51.2 kg of average LW. Differences in gene expression between breeds were observed for all analyzed genes. *TAS1R1*, *TAS1R2*, *TAS1R3*, *TAS2R4*, *TAS2R38*, *TAS2R39*, *GPR84*, and *CD36*, were overexpressed in Duroc pigs and *GPR40* gene showed a similar trend. Only *GPR120* gene was overexpressed in the Iberian pigs. Diet effect was small with only *TAS1R3* gene being overexpressed in HO diet. In Iberians, *TAS1R* and *TAS2R* gene families were positively correlated among them and negatively correlated with *GPR40* and *CD36* genes. However, in the Duroc pigs, the opposite occurred. Correlations between gene expression and main phenotypic traits differed between breeds and between diets within each breed. In Iberian pigs, *TAS1R1* expression correlated to IMF content in *Biceps femoris*. In Duroc pigs, *GPR40* expression was negatively correlated to feed intake. SNP detection was performed from *Biceps femoris* muscle RNA-seq data obtained from the same animals, with 7,512 common variants being detected in the 2 breeds. Moreover, in the studied genes we detected 3 SNPs and 1 INDEL located in *TAS1R3* and *CD36* genes, respectively, specific for Iberian pigs and 1 Duroc-specific SNP located at *CD36* gene. Also, 1 common INDEL was identified at *CD36* gene. The taste receptor genes characterization could contribute to improve the knowledge on the genetic basis of voluntary feed intake and other relevant traits.

**Key Words:** taste receptors, gene expression, taste buds, appetite, pig

**P193 Investigation of bovine leukemia virus (BLV) proviral DNA integration in cattle genome.** M. Polat<sup>\*1,2</sup>, S. Saito<sup>2</sup>, K. Hosomichi<sup>3</sup>, and Y. Aida<sup>1,2</sup>, <sup>1</sup>The University of Tokyo, Tokyo, Japan, <sup>2</sup>RIKEN, Saitama, Japan, <sup>3</sup>Kanazawa University, Ishikawa, Japan.

Bovine leukemia virus (BLV) is the etiological agent of B-cell leukemia/lymphoma in cattle. BLV infects cattle worldwide, and cause huge economic lost. BLV integrates into host genome and remains in cellular genome as provirus. The mechanism behind the BLV-induced leukemia is still unclear and it is believed that BLV integration has potential role in leukemia progression. The aim of this study is to investigate BLV integration to understand of oncogenic mechanisms of BLV. For this purpose, we choose monoclonal originated 2 B-cell lines (Ku-1 and Ku-17) derived from BLV-infected lymphoma cattle and one clinical leukemic cattle. BLV integration into host genome is investigated by high throughput next-generation sequencing (NGS). DNA library were constructed using KAPA Hyper Plus Library Preparation Kit. BLV LTR specific biotinylated probes were used to enrich libraries and sequenced using Miseq Reagent kit V3 (600 cycle). NGS paired-end sequences were mapped and aligned to BLV and host genome separately. Sequences of 40 to 300 bp were obtained centering the viral integration site (IS). BLV proviral ISs were detected in the Chr1 and the intron of *CHEK2* gene in Chr17 of leukemic cattle. *CHEK2* is a tumor suppressor gene involved in DNA repair, apoptosis and linked to cancers. We successfully found that BLV was integrated into intron of *RPTOR* gene of Chr19 of KU-1 cell line and upstream of *ATG5* gene in Chr9 of KU-17 cell lines. *RPTOR* gene evolves in mTOR pathway and signaling in cancer. In addition, *ATG3* plays important role in autophagy, apoptosis and cell cycle arrest. Proviral ISs were further confirmed by both inverse PCR and standard PCR, plus Sanger sequencing. Defective provirus in KU-1 and whole BLV proviral sequences in Ku17 were also confirmed and obtained by long PCR. Current study provides comprehensive information about the proviral structure and the viral integration in tested sample. BLV integration were detected in the intron of *CHEK2*, *RPTOR* and *ATG* genes which are playing key roles in DNA repair, apoptosis, cell signaling and autophagy. Detailed analysis concerned the biological impact of BLV into host genome, especially on nearby genes will be carried out.

**Key Words:** bovine leukemia virus, next-generation sequencing, viral integration, PCR