714 A comparison of six protocols for isolation of high quality and quantity ovine genomic DNA suitable for microarray analysis. A. Psifidi[1], C. I. Dovas[2], G. Bramis[1], G. Arsenos[1], and G. Banos*[1], *[1]Department of Animal Production, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece, [2]Laboratory of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece.*

Isolation of high quality and quantity genomic DNA is a crucial step in large-scale SNP discovery projects using microarrays. Few of the current DNA extraction procedures can be easily adapted to high-throughput operation for microarray genotyping at low cost. In this study, 6 different DNA extraction protocols were evaluated and compared for the isolation of ovine genomic DNA. Blood samples used were taken from 16 ewes of the Chios dairy breed, in 9mL K_2EDTA Vacutainer blood collection tubes. Protocols 1 and 6 were direct applications of commercial kits Nucleospin Blood and Nucleospin Blood L (Macherey-Nagel), respectively. Protocols 2 and 3 were direct applications of extraction kits Nucleospin Blood and Nucleospin Tissue (Macherey-Nagel), respectively, with a buffy coat of 9mL blood being used instead of 200µl whole blood. Protocols 4 and 5 were based on modified protocols of 2 and 3, respectively, aiming at increasing DNA recovery and purity. The key modification was the incorporation of a chloroform extraction step. Spectrophotometer measurements were used as criteria for evaluating quantity and quality (OD260/280 and OD260/230) of the extracted DNA. All samples were subjected to real-time PCR to amplify part of the PRNP gene and test the efficiency of amplification as a measure of DNA sample purity and quality. Processing time and cost were also evaluated. Extraction methods were compared using a model that included the effects of protocol and sample. The model applied separately to DNA concentration, final DNA concentration accounting for volume, OD260/280 and OD260/230. Protocol had a significant ($P < 0.05$) effect on all parameters. Protocols 4, 5 and 6 yielded the highest DNA concentration even after accounting for volume. Method 6 had the lowest values for OD260/280 and OD260/230 suggesting less pure DNA. All samples were amplified with real-time PCR. Incubation time was greater for protocols 2, 3, 4 and 5. Protocol 6 was the most expensive. Based on results, protocols 4 and 5 were selected for further application.

Key words: ovine, DNA extraction, microarrays

715 Association between the ghrelin gene with milk production traits in Murrah buffaloes (*Bubalus bubalis*). F. M. M. Gil, F. R. P. Souza, G. M. F. de Camargo*, P. D. S. Fonseca, D. F. Cardoso, R. R. Aspilcueta-Borquis, G. Stefani, and H. Tonhati, *São Paulo State University, Jaboticabal, São Paulo, Brazil.*

Ghrelin is a gastrointestinal hormone and a potent release stimulator of growth hormone (GH) in the somatotropic cells of the hypophysis and hypothalamus. It also influences the general metabolism of the body. Polymorphisms in the ghrelin gene in dairy cows were associated with milk yield, fat and protein percentage. The characterization of the ghrelin gene (GHRL) in buffaloes is important because it is a gene related to growth, carcass and milk production traits. The aim of this study was to associate a SNP in intron 2 of the GHRL gene in Murrah buffaloes with milk production traits. The SNP is a T/C substitution at position 1456 bp of GHRL. The DNA was extracted from hair of 206 dairy buffaloes from one farm in São Paulo state, Brazil. The animals were genotyped by PCR-RFLP using the restriction enzyme NcoI. For the analysis, the GLM procedure of SAS was used, the model included as fixed effects birth season, birth year and genotype and as a covariable the age of the buffalo. The possible association of the polymorphism with the phenotypic values of milk yield, protein yield, fat yield, protein percentage and fat percentage at a statistical significance of 5% was tested. Three genotypes were obtained. The genotypes CC, CT and TT have the frequencies 0.29, 0.56 and 0.19, respectively. The allelic frequencies of C and T were 0.57 and 0.43, respectively. The results indicate that there is association of the SNP with milk yield ($P = 0.0104$), fat yield ($P = 0.0045$), protein yield ($P = 0.0031$), fat percentage ($P = 0.0403$) and protein percentage ($P = 0.0368$). It indicates that the SNP may be used as a molecular marker for the traits analyzed in dairy Murrah buffaloes. However, further analyses with a bigger number of animals are necessary to confirm the results obtained.

Key words: molecular marker, SNP, PCR-RFLP

716 Relationship between horn fly infestation and polymorphisms in cytochrome P450 and prolactin promoter genes in beef cows. A. R. Boyer*[1], M. A. Brown[2], M. L. Looper[3], A. H. Brown, Jr.[1], C. D. Steelman[1], and C. F. Rosenkrans Jr.[1], *[1]University of Arkansas, Fayetteville, [2]USDA-ARS, Grazinglands Research Laboratory, El Reno, OK, [3]USDA-ARS, Dale Bumpers Small Farms Research Center, Booneville, AR.*

Individual animal variation occurs regarding external parasite infestation in beef cattle. Our objective was to determine if horn fly infestations present on beef cattle are associated with the single nucleotide polymorphism (SNP; T318C) in the cytochrome P450 gene (CYP3A28) and the prolactin (PRL) promoter gene (C1286T). Unrelated beef cows of Angus (A), Brahman (B), and crossbred (BA and AB) breed types were used, and genotyped for the T318C SNP (n = 64) and the C1286T SNP (n = 43). Cattle were on either common bermudagrass (BG) or endophyte-infected tall fescue (E+) throughout the study. Individual horn fly counts on cows were recorded for 21 wks beginning in May. Horn flies were counted by walking around each cow at a set distance of 5 to 10 m. Horn fly numbers ≤25 were counted individually, while numbers >25 were counted in groups of 5. Chemical fly control containing coumaphos was used wk 9 and 16 of the study. Genomic DNA was prepared from buffy coat, and 3 genotypes for the CYP3A28 gene and PRL promoter gene were identified. Results found an effect of week occurred for both the T318C ($P < 0.0001$) and the C1286T ($P < 0.0001$) SNP. Homozygous thymine cows (TT) for T318C SNP had greater ($P < 0.0001$) numbers of horn flies (180.1 ± 10.9 flies) than heterozygous thymine-cystine (TC; 96.7 ± 12.4 flies) and homozygous cystine (CC; 53.5 ± 35.0 flies) cows. Cows homozygous CC for the C1286T SNP had greater ($P = 0.0025$) numbers of horn flies (207.7 ± 21.6 flies) than heterozygous CT (127.9 ± 14.6 flies) and homozygous TT (87.6 ± 27.3 flies) cows. A genotype x week interaction occurred for both the T318C ($P < 0.0001$) and C1286T ($P = 0.0018$) SNP. Cattle grazing E+ tall fescue tended to have fewer horn flies than those grazing BG for both the T318C (86.2 ± 22.4 vs. 134.0 ± 12.8 flies; $P = 0.06$) and C1286T (120.0 ± 18.5 vs. 162.1 ± 17.1 flies; $P = 0.10$) SNP. Different genotypes appear to have an effect on external parasite infestation observed on beef cows, and genetic selection of cows with these SNP could improve external parasite resistance in beef cattle.

Key words: horn fly, genotype, single nucleotide polymorphism

717 Gene expression analysis and fatty acid profiling in concentrate and pasture based beef finishing systems. J. W. Buchanan*[1], A. J. Garmyn[1], G. G. Hilton[1], D. L. VanOverbeke[1], Q. Duan[2], D. C. Beitz[2], and R. G. Mateescu[1], [1]*Oklahoma State University, Stillwater,* [2]*Iowa State University, Ames.*

Expression analysis of genes involved in lipogenesis and lipid desaturation provides understanding about the genetic component controlling intramuscular fatty acid profile in different finishing systems. The health advantages of grass finished beef compared with concentrate finished beef include increased monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), conjugated linoleic acid (CLA), and an improved omega-3 to omega-6 fatty acid ratio. Intramuscular (IM) samples were taken at slaughter from 100 heifers finished on a concentrate (CO) diet and 60 heifers finished on pasture (PA). Across treatments, CO heifers had a higher hot carcass weight and higher marbling score ($P < 0.05$) than PA heifers, but no significant differences were found in frame size and fatness due to hot carcass weight, rib eye area, marbling score, and yield grade within treatment. IM fatty acid profiles were characterized using gas chromatography fatty acid methyl ester analysis and data were analyzed using the PROC GLM procedure in SAS (SAS Institute Inc., Cary, NC). IM samples were sorted into healthy and unhealthy groups based on the atheroindex (AI) ($P < 0.001$), and 10 samples from each treatment (n = 20) were selected for gene expression analysis. Differences in fatty acid profile across treatments included a higher AI, and a lower omega-3 to omega-6 ratio, lower percent PUFA, and lower percent omega-3 fatty acids in CO compared with PA ($P < 0.05$). Total RNA was extracted from each sample and gene expression was quantified in real-time using SYBR Green RT-PCR detection. Data were normalized against the geometric mean of the most stable housekeeping genes β actin and RPS15A as determined by the BESTKEEPER software. The transcriptional regulator PPAR gamma was upregulated in PA vs. CO heifers ($P < 0.05$) by 5- and 6-fold in healthy and unhealthy AI groups, respectively. Significant upregulation in PA vs. CO heifers was also detected across AI groups ($P < 0.05$), where DGAT2, ADIPOQ, and FABP4 were upregulated 3-, 7-, and 26-fold in unhealthy vs. 1-, 2-, and 7-fold in healthy samples.

Key words: fatty acid profile, gene expression, grass finished beef

718 Expression analysis of key genes of bovine fat metabolism indicated correlated trans regulatory mechanisms in a bovine resource population segregating for two major genes affecting growth and lipid deposition. Ch. Kuehn*, C. Kalbe, R. Brunner, T. Goldammer, and R. Weikard, *Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

Recently, the mutations NCAPG I442M and GDF8 Q204X were identified as major loci affecting growth and lipid deposition in an F2 resource population from Charolais and German Holstein. Therefore, the effects of both mutations on the expression of key genes in lipid deposition (ADIPOR1, FABP4, SCD and DGAT2) could be comparatively investigated on an identical genetic background. Additionally, genome-wide linkage studies were performed to identify further genomic loci with impact on the expression of those 4 genes involved in regulation of lipid metabolism, fatty acid transport and desaturation and triglyceride synthesis. Our study comprised 150 bulls, which had been generated by consistent application of embryo transfer during generation of the resource population and had been fed a semi ad libitum feed ration of concentrates and chaffed hay until slaughter at 18 mo of age. Tissue samples of skeletal muscle (M. longissimus) were taken immediately after slaughter and snap frozen for further gene expression analysis by quantitative real-time PCR analysis. All F2 bulls and all P0 and F1 ancestors were genotyped for the NCAPG I442M and GDF8 Q204X mutation as well as for 565 further genetic markers (SNPs, microsatellites) distributed across the entire genome. Association studies revealed a significant effect ($P < 0.05$) of the GDF8 Q204X mutation on the expression of the ADIPOR1, SCD, and DGAT2 gene. For the NCAPG I442M mutation, a significant effect was restricted to the SCD gene. This fits the previous observation that the GDF8 Q204X mutation had a larger effect on intramuscular fat deposition compared with NCAPG I442M. Further genome-wide linkage analyses searching for other QTL affecting the expression of the 4 investigated key genes of fat metabolism indicated a trans regulation of their transcription, because none of the QTL detected (most significant QTL for ADIPOR1: BTA12, FABP4: BTA22, SCD: BTA6, DGAT2: BTA1) was located on the chromosome harboring the respective target gene.

Key words: lipid deposition, eQTL, NCAPG

719 Sound and efficient designs and models for RNA-seq experiments with application in animal genomics. J. P. Steibel* and P. Reeb, *Michigan State University, East Lansing.*

Next generation RNA sequencing (RNA-seq) will become the method of choice for high throughput quantification of transcriptomes. In order for an RNA-seq experiment to produce meaningful results, a sound experimental design has to be matched with the corresponding analysis model. Consequently, the objective of this work is to introduce experimental designs and models of RNA-seq experiments with application in animal functional genomics. We separate the experimental design into 2 parts: a) the sampling scheme and b) the sequencing scheme and we show how to efficiently combine the 2 parts. Following features common across RNA-seq platforms, we consider lane effects and barcode effects as blocking factors. We show general design rules that result in efficient designs, but illustrate their use with 3 sampling schemes widely used in animal functional genomics. 1) Tissue differential expression, where RNA is extracted from T tissues in each of N animals, 2) Complete block design where K treatment are applied to N animals, but they are blocked in groups of size K and 3) Repeated measurement experiments, where K treatments are applied to each of N animals and RNA is extracted at S subsequent time points. We show sequencing schemes for the 3 experiments with or without resorting to bar coding. We present the appropriate analysis model using overdispersed Poisson likelihoods. When using a single level sampling scheme (experiments 1 and 2), publicly available R packages (e.g., edgeR, DGEseq) can be used to fit the required models. But in case of multilevel sampling designs (example 3), there is currently no software package available that would produce the required fit. We discuss alternatives (e.g., using SAS or R functions) and their main advantages and disadvantages to perform the analysis. While this technology is still novel and there are not many experimental data sets available, this paper addresses basic issues that should be considered before such data sets are generated to ensure that experimental and nuisance factors can be appropriately accounted for and disentangled to produce meaningful conclusions.

Key words: RNA-seq, experimental design, gene expression