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## BREEDING AND GENETICS: MOLECULAR BIOLOGY AND GENOMICS

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**0962 (W066) Associations of the NCAPG I442M and GDF8 Q204X loci on feed efficiency at the onset of puberty in a beef x dairy cattle resource population.** C. Kühn\*, P. Widmann, R. Weikard, and E. Albrecht, *Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany.*

Recent studies highlighted the NCAPG/LCORL region, as well as the GDF8 gene encoding myostatin, being associated with genetic modulation of growth in several mammalian species. Specifically, the NCAPG I442M locus had been associated with pre- and postnatal growth in cattle. Furthermore, it had been demonstrated that the highest effect of this genetic variant is expressed at the onset of puberty, which is suggested as the inflection point of the growth curve. Feed costs account for a large proportion of total costs for production in livestock. Thus, including information about feed efficiency in selection decisions would improve the profitability of livestock production. The aim of our study was to evaluate, if the NCAPG I442M or GDF8 Q204X loci had an effect on feed intake and/or feed efficiency at the onset of puberty in cattle. Our study included 176 F<sub>2</sub> bulls from a Charolais x German Holstein resource population generated by multiple ovulation and embryo transfer to virgin heifers that were kept under standardized environmental conditions. After an initial period of 4 mo on a milk replacer diet, the animals were fed ad libitum with concentrate (11.3 MJ ME/kg dry matter, comprising a mixture of barley, molasses chips, soybean extraction meal, molasses, minerals and a vitamin premix) and hay (9.0 MJ ME/kg dry matter). Feed intake was measured daily and body weight was recorded in monthly intervals. For this study, daily dry matter intake, feed conversion ratio and residual feed intake were investigated between d 183 and d 283 of age. Residual feed intake was calculated from daily dry matter intake, metabolic mid weight, and average daily gain. The alleles at the loci NCAPG I442M and GDF8 Q204X were tested for association with daily dry matter intake, feed conversion ratio and residual feed intake. The model fitted year of birth and season as fixed effects, the additive effect of the NCAPG I442M or GDF8 Q204X allele, and an infinitesimal polygenic animal effect. The analysis revealed that the NCAPG I442M locus was significantly associated with daily feed intake ( $P = 0.03$ ), feed conversion ratio ( $P = 0.00012$ ) and residual feed intake ( $P = 0.00001$ ). In contrast, the GDF8Q204X locus showed only significant effects on feed conversion ratio ( $P = 0.04$ ) and residual feed intake ( $P = 0.0005$ ). Our data indicated that NCAPG I442M is associated with genetic modulation of growth as well as feed efficiency in cattle

**Key Words:** feed efficiency, cattle, NCAPG

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**0963 (W067) Association of DNA methylation levels with tissue-specific expression of adipogenic and lipogenic genes in Longissimus dorsi muscle of Korean cattle.** M. Baik<sup>\*1</sup>, T. T. T. Vu<sup>2</sup>, M. Y. Piao<sup>1</sup>, and H. J. Kang<sup>1</sup>, <sup>1</sup>*Dep. of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, South Korea,* <sup>2</sup>*Chonnam National University, Gwangju, South Korea.*

The epigenetic factor such as DNA methylation status may regulate adipogenesis and lipogenesis, affecting intramuscular fat (IMF) deposition of *longissimus dorsi* muscle (LM) in beef cattle. In steers, the LM consists mainly of muscle tissue. However, the LM in Korean cattle steers also contains IMF. We compared the gene expression levels between the IMF and muscle portions of the LM steers after separation of the tissues. Real-time PCR analysis showed that mRNA levels of both adipogenic peroxisome proliferator-activated receptor  $\gamma$  isoform 1 (PPAR $\gamma$ 1) and lipogenic fatty acid binding protein 4 (FABP4) were higher ( $P < 0.01$ ) in the IMF portion than in the muscle portion of the LM. DNA methylation at specific sites within regulatory regions of gene is known to regulate transcription. We determined DNA methylation levels of regulatory regions of the PPAR  $\gamma$ 1 and FABP4 genes by pyrosequencing of genomic DNA isolated from the IMF and muscle portions of the LM. DNA methylation levels of two CpG sites out of three from regulatory region (+144 ~ +225) of PPAR $\gamma$ 1 gene were lower ( $P < 0.05$ ) in the IMF portion than in muscle portion of the LM. DNA methylation levels of all five CpG sites from regulatory regions (-9664 ~ -9469) of FABP4 gene were also lower ( $P < 0.001$ ) in IMF portion than in the muscle portion. Thus, mRNA levels of both PPAR $\gamma$ 1 and FABP4 genes were inversely correlated with DNA methylation levels of regulatory regions of CpG sites of the corresponding gene. In conclusion, our findings suggest that DNA methylation status regulates tissue-specific expression of adipogenic and lipogenic gene expression in IMF and muscle portions of LM tissues of Korean cattle steers.

**Key Words:** intramuscular fat, adipogenesis, DNA methylation

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**0964 (W068) Changes in the cattle cervical transcriptome between estrus and luteal phase.**

D. Gonzalez-Peña Fundora\*, P. Cardoso, M. B. Wheeler, and S. L. Rodriguez Zas, *University of Illinois at Urbana-Champaign.*

During the periestrous phase, the cow cervix undergoes changes associated with hormonal phases that affect the secretions. The cervical mucus fluctuates from fluid and abundant, allowing the sperm transport at estrus, to dense and thick, creating a barrier that minimize pathogen colonization and infection during pregnancy or luteal phase. The corresponding changes in the transcriptome have not be fully characterized.

The objective of this study was to identify the genes that are differentially expressed between the estrus ( $n = 4$ ) and luteal (7 d postestrus,  $n = 5$ ) phases in the cervical tissue of synchronized beef heifers. An RNA-Seq platform (Illumina Genome Analyzer II) was used to identify and quantify the transcripts. Single-end reads were mapped to the *Bos taurus* reference genome (Baylor Btau\_4.6.1/bos Tau7). In total, 14,419 transcripts from 13,822 genes were tested and 1163 transcripts from 1150 genes were differentially expressed between the luteal and estrus phases (False Discovery Rate adjusted,  $P < 0.05$ ). Among these, angiotensinogen (AGT) and potassium voltage-gated channel, subfamily F, member 1 (KCNF1) were overexpressed, while cartilage oligomeric matrix protein (COMP) and chloride channel accessory 1 (CLCA1) were under-expressed in the luteal relative to the estrus phase. These results are consistent with known gene functions. AGT produces the enzyme angiotensin II that has been associated with the corpus luteum. KCNF1 regulates the epithelial electrolyte transport and COMP plays a role in cell proliferation, apoptosis, and regulation of cell movement and attachment. Also, the activity of CLCA1 decreases during the luteal phase when mucus thickens. Functional analysis of the differentially expressed genes using DAVID identified six category clusters (enrichment score  $> 3$  equivalent to average category  $P < 0.001$ ). These categories included cartilage development and condensation, inflammatory responses, defense responses, and sterol-cholesterol biosynthetic. These functional categories suggest that changes during the estrus cycle are associated with changes in molecular pathways that in turn may affect the morphology, function and penetrability of the cervix.

**Key Words:** RNA-seq, cervix, cow

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**0965 (W069) Physical and chemical and fatty acid profile in the steers beef with different genetic predominance fed with diets containing substitutions levels of corn by pearl millet.**

R. M. D. Silva<sup>1,2,3</sup>, J. T. Pádua<sup>2</sup>, J. J. R. Fernandes<sup>2</sup>, R. Z. Taveira<sup>1</sup>, R. L. Missio<sup>4</sup>, P. S. Pacheco<sup>5</sup>, D. A. Fausto<sup>6</sup>, and J. Restle<sup>2</sup>, <sup>1</sup>Universidade Estadual de Goiás, São Luis de Montes Belos, Brazil, <sup>2</sup>Universidade Federal de Goiás, Goiânia, Brazil, <sup>3</sup>FAPEG, Goiânia, Goiás, Brazil, <sup>4</sup>Universidade Tecnológica Federal do Paraná, Pato Branco, Brazil, <sup>5</sup>Universidade Federal de Santa Maria, Rio Grande do Sul, Brazil, <sup>6</sup>ESALQ/USP, Piracicaba, São Paulo, Brazil.

This study aimed to evaluate the steers crossbred meat fatty acid profile with European (E) or Zebu (Z) genotypic predominance fed with high concentrated diets (80%) containing levels of ground millet grain in replacement of ground corn grain (0, 33, 66, and 100%). Twenty-four steers of each genetic predominance were feedlot during 96 d and slaughtered at 24 mo of age. The experimental design was completely random-

ized design with treatments in a 4x2 factorial arrangement using six replicates. The initial pH (6.68) and final carcass pH values (5.9), the final carcass temperature (9.72°C), the fluid loss during thawing (9.7%) and cooking (26.6%), color (3.7 points), texture (3.1 points), marbling (4.5 points), the shear strength of the muscle fibers (8.1 kgf) and moisture (72.9%), crude protein (23.0%), and ether extract content (1.5%) of the meat were not affected by the substitution of corn grain by millet grain ( $P > 0.05$ ). Meat characteristics were not influenced by genetic predominance, except the marbling, which was higher (4.99 vs. 3.95 points) in European animals. The final pH was correlated with thawing liquid loss ( $r = -0.36$ ) and meat color ( $r = -0.62$ ). The replacement of corn grain by millet grain in diet did not affect the steers meat quality ( $P > 0.05$ ). Increasing the proportion of millet in the diet it linearly increased the arachidic (C20:0), heneicosanoic (C21:0),  $\alpha$  linolenic (C18:3 n-3), and di-homo  $\gamma$  linolenic (C20:3 n-6) fatty acids. European bulls meat showed less content of myristic (C14:0), heneicosanoic (C21:0), and  $\gamma$  linolenic (C18:3 n-6) fatty acids. The total concentration of saturated (45.2%), monounsaturated (41.2%), and polyunsaturated (8.7%) fatty acids and monounsaturated/saturated (1.09) and polyunsaturated/saturated (0.18) relationship were not affected by the factors studied. Zebu cattle fed high concentrate produce meat with higher levels of hypercholesterolemic fatty acids that European cattle. The millet grain percentage increase in the diet of European and Zebu crossbred steers improves the ratio between  $n-6/n-3$  fatty acids (0% = 13.90, 33% = 13.79, 66% = 11.67, 100% = 10.58) ( $P = 0.042$ ). These results indicate that the millet grain used in a complementary way for other strategies, such as providing protected polyunsaturated fatty acids (PUFA) ruminal fermentation, may allow getting a closer relationships  $\omega 6/\omega 3$  to the recommended.

**Key Words:** intramuscular fat, meat tenderness, nutrition

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**0966 (W070) Major loci associated with growth traits on BTA14 in Hanwoo (Korean cattle).** S. W. Lee<sup>\*1</sup>, K. Y. Chung<sup>1</sup>, U. H. Kim<sup>1</sup>, B. W. Choi<sup>2</sup>, D. Lim<sup>2</sup>, Y. M. Cho<sup>2</sup>, C. G. Dang<sup>1</sup>, H. C. Kim<sup>1</sup>, S. H. Yeon<sup>1</sup>, H. S. Kang<sup>1</sup>, and C. Gondro<sup>3</sup>, <sup>1</sup>Hanwoo Experiment Station, NIAS, RDA, Pyeongchang, South Korea, <sup>2</sup>Animal Genomics & Bioinformatics Division, NIAS, RDA, Suwon, South Korea, <sup>3</sup>University of New England, Armidale, Australia.

Genome-wide single marker regression using Bovine 50K BeadChip was performed on growth traits from 1012 Hanwoo steers in Hanwoo (Korean cattle). SNPs were excluded from the analysis if they failed in over 5% of the genotypes, had median GC scores below 0.6, had GC scores under 0.6 in less than 90% of the samples, deviated in heterozygosity more than three standard deviations from the other SNPs and were out of Hardy-Weinberg equilibrium for a cutoff p-value of  $1E^{-5}$ . Unmapped and SNPs on sex chromosomes were also

excluded. A total of 32,696 SNPs were used in this analysis. To test an association between SNP and QTL, single marker regression analysis was implemented in this study. SNP was assumed to be in LD with QTL in close proximity and the effect evaluated was additive effect (QTL allele substitution effect). The Bonferroni-corrected genome wide significant association ( $P < 1.5 \times 10^{-6}$ ) was applied to detect significant SNPs for the GWAS. The GWAS identified one major QTL for body weight at 6, 12, 18, and 23 mo ranging 23Mb to 25Mb on BTA14. The most significant SNP was Hapmap32241-BTC-054753 (24Mb,  $P = 1.8 \times 10^{-6}$ ) for BW6, Hapmap27934-BTC-065223 (25Mb,  $P = 1.2 \times 10^{-10}$ ) for BW12, BW18 and BW23 in Hanwoo. The most significant SNPs accounted for 8 to 10% of additive genetic variance, which is quite large proportion against total additive genetic variance. The Hapmap27934-BTC-065223 has 12.97 kg of allele substitution effect in body weight at 12 mo (BW12). The results revealed that growth traits was affected by major QTL with large effect and many other SNP with small effects with the normal distribution.

**Key Words:** GWAS, major loci, BTA14

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**0967 (W071) SNP located on three candidate genes influencing growth, performance and carcass traits in a population of steers sired by Braunvieh, Charolais and Simmental bulls.** M. D. Garcia\*, S. Mizell, and T. Page, *Louisiana State University, Baton Rouge.*

The objective of the current study was to evaluate the association of single nucleotide polymorphisms (SNP) on three candidate genes for growth, performance, carcass traits in 39 steers sired by Braunvieh, Charolais, and Simmental bulls. Single nucleotide polymorphisms from three candidate genes including the Thyroglobulin gene (TG), the Adiponectin (ADPOQ) gene, and the Insulin-like growth factor 1 (IGF-1) gene were utilized for association analyses. Single nucleotide polymorphisms were selected utilizing SNP that were evenly distributed and represented the total length of the candidate gene. Of the 67 SNP genotyped, 20 were chosen for TG, 20 for ADPOQ, and 27 for IGF-1. Linkage disequilibrium (LD) was not evaluated in the current study due to the small population size; however, LD will be calculated as the genotyped population becomes larger in future studies. The growth traits that were evaluated included, birth weight (BW), hip height (HH), and weaning weight (WW). Carcass quality and composition traits included marbling score (MS), back fat thickness (BF), ribeye area (REA), hot carcass weight (HCW), and yield grade (YG). The PROC MIXED of SAS was utilized to evaluate associations of the 67 SNPs and measured traits. Sire breed and SNP genotype were fit in the model as fixed effects with performance and carcass traits fit as random effects. Associations between traits and SNPs were reported as significant if  $P < 0.05$ . Multiple SNP from all three candidate genes

were identified as being significantly associated ( $P < 0.05$ ) all traits evaluated in the current study. Association analyses for growth traits revealed seven SNP significantly associated with BW (rs109830314, rs383724494, rs378724414, rs381911082, rs383535987, rs384076273, rs109327701), five with HH (rs110553649, rs132813094, rs210258853, rs109327701, rs137651874), 10 with WW (rs109182502, rs110616947, rs377997897, rs379996188, rs380627374, rs378724414, rs109327701, rs136982429, rs137140434, rs137726884), 10 with BF (rs110501231, rs110616947, rs378567477, rs382644882, rs210258853, rs137140434, rs137374423, rs137601357, rs137662301, rs137726884). Association analyses for carcass quality and composition traits revealed two SNP significantly associated with HCW (rs110501231, rs378567477) four with MS (rs378567477, rs378900777, rs383535987, rs137104571), two with REA (rs109830314, rs137651874), and five with YG (rs379467464, rs382252585, rs386026054, rs378724414, rs137601357). Furthermore, a total of eight SNPs (rs109830314, rs110501231, rs110616947, rs378567477, rs378724414, rs137140434, rs137651874, and rs137726884) representing all three candidate genes were significantly associated with growth, performance and carcass quality and composition traits.

**Key Words:** SNP, candidate genes, beef, growth and performance, carcass traits, snp, growth, carcass, candidate genes

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**0968 (W072) Single nucleotide polymorphisms in the XKR4 and DRD2 genes influence adjusted birth and 205-d weights of calves grazing endophyte-infected tall fescue.** K. M. Ely<sup>1</sup>, C. J. Kojima<sup>1</sup>, A. M. Saxton<sup>1</sup>, and R. L. Kallenbach<sup>2</sup>, <sup>1</sup>University of Tennessee, Knoxville, <sup>2</sup>University of Missouri, Columbia.

Tall fescue (*L. arundinaceum* Schreb.) is the most prevalent forage in the Southeastern United States due to the presence of the endophytic fungus *N. coenophialum*. The fungus enhances the persistence of tall fescue, but decreases the productivity of cow-calf herds grazing it. Single nucleotide polymorphisms in the XK, Kell blood group complex subunit-related family, member 4 (XKR4) and Dopamine Receptor D2 (DRD2) genes both yield the genotypes AA, AG, and GG. The A allele of both XKR4 and DRD2 has shown to increase serum prolactin concentrations in Tennessee beef cattle herds grazing endophyte-infected tall fescue. We evaluated the relationship between genotypes of dam and calf and adjusted 205-d weight (A205) and adjusted birth weight (ABW) in a well-managed fall-calving beef herd in Missouri. The ANOVA model included XKR4 and DRD2 genotype (SAS 9.3, Cary, NC). Genotype and allele frequencies for XKR4 were AA = 0.67, AG = 0.30, GG = 0.04, A = 0.82 and G = 0.18 for the dam; AA = 0.64, AG = 0.34, GG = 0.03, A = 0.81 and G = 0.19 for the calf. Since the G allele was sparsely represented in the

population only AG and AA animals were used for the XKR4 analysis. Genotype and allele frequencies for DRD2 were AA = 0.23, AG = 0.46, GG = 0.31, A = 0.46 and G = 0.54 for the dam; AA = 0.25, AG = 0.50, GG = 0.25, A = 0.50 and G = 0.50 for the calf. Dam genotype for DRD2 influenced ABW such that calves from AA and GG animals had lower ABW than those from AG animals ( $P < 0.0001$ ). This is similar to previous findings in a larger herd of animals from Missouri. The AA genotype for the dam at XKR4 was associated with higher ABW when compared to AG dams ( $P = 0.08$ ). Calf genotype for XKR4 influenced A205, such that AA animals were heavier than AG ( $P = 0.05$ ). The AA genotype for the calf at XKR4 was associated with higher ABW when com-

pared to AG calves ( $P = 0.07$ ). No association was observed between calf genotype for DRD2 on ABW or A205. Similarly, no association was observed between dam genotype for XKR4 and A205. Fall-calving dams are grazing fescue at its peak infection level while in mid-gestation; having a beneficial allele for ABW is important for healthy calf weights. As fall-born calves are grazing endophyte-infected tall fescue before weaning, calf genotype at these loci may influence A205. Taken together with previous work, these results indicate a potential for their use as genetic markers for increased productivity of beef cattle grazing endophyte-infected tall fescue.

**Key Words:** XKR4, DRD2, fescue toxicosis