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Late-Breaking Original Research

LB1 Identification of a genomic region associated with severe combined immunodeficiency in pigs. E. H. Waide*[1], C. K. Tuggle[1], D. M. Thekkoot[1], N. Boddicker[1], R. R. R. Rowland[2], C. R. Wyatt[2], and J. C. M. Dekkers[1], *[1]Iowa State University, Ames, [2]Kansas State University, Manhattan.*

Severe combined immunodeficiency (SCID) is a genetic defect caused by a recessive mutation in one of several genes and has been identified in humans, horses, and dogs, and artificially created in rats and mice. SCID mice have been extensively used to study immune mechanisms, cancer progression and treatment, autoimmune diseases, and vaccination strategies. We recently reported the first discovery of SCID in pigs based on the postmortem examination of 4 related piglets from a selection line of Yorkshire pigs, which showed severely underdeveloped immune systems, later characterized as being devoid of B and T cells. Repeat matings of the 2 sires and 4 dams of these piglets produced 6 additional litters with confirmed SCID piglets in an approximately 20 to 25% frequency. Piglets with SCID appear normal up to weaning but deteriorate quickly after weaning, typically because of respiratory disease. Affected piglets have been shown to sustain human tumor growth, consistent with SCID. To identify the genomic region associated with this defect, 20 affected piglets, 50 unaffected littermates, the 6 parents, and 96 normal ancestors were genotyped using the Illumina Porcine SNP60 Beadchip. The dfam option of PLINK was used to identify a 5.6-Mb region that is associated with the defect ($P = 2.7 \times 10^{-7}$). The region contains a strong candidate gene associated with the immune system. Haplotype phasing analyses using phase (2.1.1) identified 2 distinct haplotypes that are associated with the recessive defect. Single SNP testing in the region confirmed BeadChip data and co-segregation of the region with the SCID phenotype. Sequencing of the region is underway to identify the causative mutation. This novel finding of SCID in pigs and the associated genetic test are important from several perspectives: (1) this defect may be present in commercial populations, as the line that produced the SCID pigs was originally derived from the US Yorkshire population, and (2) SCID pigs will be valuable for infectious disease research in pigs and as a biomedical model for humans. EHW is a Fellow supported by USDA NIFA National Needs grant #2010-38420-20328.

Key Words: pigs, immune system, genetics

LB2 Characterization of genetic variation within the somatotropic axis in DNA pools of beef and dairy cattle divergent for milk production, size, fertility, and immune response. M. P. Mullen*[1,2], C. Creevey[3], D. P. Berry[4], M. S. McCabe[2], D. J. Howard[1], D. A. Magee[5], M. C. Lucy[6], D. E. MacHugh[5,7], and S. M. Waters[2], *[1]Animal and Bioscience Research Department, Animal and Grassland Research and Innovation Centre, Teagasc, Athenry, Co. Galway, Ireland, [2]UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland, [3]Animal and Bioscience Research Department, Animal and Grassland Research and Innovation Centre, Teagasc, Grange, Co. Meath, Ireland, [4]Animal and Bioscience Research Department, Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Cork, Ireland, [5]UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland, [6]Department of Animal Sciences, University of Missouri, Columbia, [7]UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland.*

The somatotropic axis is well established as a key regulator of postnatal growth and development in mammals with direct effects on agro-economic traits in livestock. The identification of genetic variants affecting performance within this axis is therefore an attractive goal. The aim of this study, using a pooled DNA approach coupled with target enrichment and high-throughput sequencing, was to identify polymorphisms and estimate allele frequencies across 83 candidate genes of the somatotropic axis in (1) dairy bulls of high or low genetic merit for milk protein percent, carcass weight, calving interval, somatic cell count, and tuberculosis susceptibility; and (2) beef bulls representing 6 beef cattle breeds. DNA samples from (1) 750 Holstein-Friesian bulls divided into 10 groups (n = 75) divergent (high and low) for genetic merit for each trait; and (2) 300 beef cattle (n = 50 per breed), were pooled using equimolar quantities from each animal to construct 16 Illumina sequence libraries. A custom Agilent Technologies SureSelect Target Enrichment System was used to selectively capture and enrich approximately 2 Mb of coding, intronic, and regulatory sequences from the 83 targeted genes. These enriched libraries were then sequenced using an Illumina Genome Analyzer II. In total, 7,450 SNPs and 995 indels were identified across all pools. Thirty-five percent (n = 2,637) of SNPs were located within 5′ and 3′ UTRs. Fifty-nine percent (n = 4,351) were intronic and 6% (n = 462) were exonic, including 223 non-synonymous substitutions (NSS) with 11 mutations resulting in a stop codon gain or loss. Significant (≥2-fold; $P < 0.01$) allele frequency differentials between the 5 low and high genetic merit groups were observed for 878 SNPs including 55 NSS (3 stop gain/loss). This study has identified novel variation and allele frequency differentials within key genes involved in growth and development, metabolism and health in cattle, potentially harboring heritable variation in performance and contributing to differences between breeds in somatotropic axis regulation and control.

Key Words: DNA pools

LB3 Imputation of microsatellite alleles from dense SNP genotypes for paternal verification. M. C. McClure[1], T. S. Sonstegard[1], G. R. Wiggans[1], A. Van Eenennaam[2], K. Weber[2], C. Penedo[2], and C. P. Van Tassell*[1], *[1]USDA-ARS, Beltsville, MD, [2]University of California, Davis.*

Microsatellite (MS) markers have recently been used for parental verification and are still the international standard despite higher cost, error rate, and turnaround time compared with single nucleotide polymorphism (SNP)-based assays. Despite domestic and international interest from producers and research communities, no viable means currently exist to verify parentage for an individual unless all familial connections were analyzed using the same DNA marker type (MS or SNP). This requirement is a major limitation for historic animals where a DNA source does not exist due to culling, death, or change in ownership of animals. Moreover, the additional cost of MS genotyping is difficult to justify when increasing numbers of commercial dairy cows are SNP genotyped and nearly every dairy sire is SNP genotyped. A simple and cost-effective method was devised to impute MS alleles from SNP haplotypes within breeds. This approach has been verified

(>98% accurate) for imputing the International Society of Animal Genetics (ISAG) recommended panel of 12 MS for cattle parentage verification across 479 dairy cattle representing 4 dairy breeds (Brown Swiss, Guernsey, Holstein, and Jersey). Some MS-haplotype associations held true across these phylogenetically diverse breeds, implying that some combinations were present before modern breed formations and have been preserved across breeds. Results from the dairy breeds were used to impute MS in 117 beef animals from 7 breeds (Belgian Blue, Devon, Angus, Maine-Anjou, Charolais, Dexter, Texas Longhorn, Ankole). While the beef MS imputation call rate was lower (69%), of those called the imputation was still fairly accurate (78%) although for most loci multiple (2–4) alleles were imputed. MS markers are currently the international standard parentage verification for exported semen; this work represents a tool to quickly migrate toward SNP based verification in 1 generation. Finally, these imputation methods can be implemented in any species with available high-density SNP genotypes flanking MS allele genotype.

Key Words: microsatellite, SNP, impute

LB4 Improvement of feed efficiency through diet and breed-dependent genetic polymorphisms. N. V. L. Serão,* J. E. Beever, D. B. Faulkner, and S. L. Rodriguez-Zas, *University of Illinois at Urbana-Champaign.*

The consideration of different production systems in genome-wide association studies may provide insights into the effects of markers interacting with other factors. The objective of this study was to identify SNPs with diet and breed-dependent associations for feed efficiency traits in beef cattle. A total of 1,321 steers obtained from 5 ranches (2005 to 2008) were used in this study. Animals from 5 breeds, using Angus (AN) and Simmental (SM; purebred Angus [AN], 3/4AN, 1/2AN:1/2 SM, 3/4 SM and purebred SM) were fed with 5 diets, with different ingredients, and levels of total energy and non-degradable fiber. Traits analyzed were: average daily gain (ADG), dry matter intake (DMI), residual feed intake (RFI), residual average daily gain (RADG) and residual intake and gain (RIG). For RFI, DMI was predicted using ADG, back-fat depth (BF), rib-eye area (REA) and mid-test metabolic weight (MW), whereas for RADG, ADG was predicted using DMI, BF, REA and MW. The sum of RADG and −1*RFI defined RIG. Associations were performed using Qxpak5 v.5, in a model including the fixed effects of individual SNPs, breed, diet, breed/diet by SNP interaction, initial weight (covariate), and the random effects of contemporary group (29 levels), and additive polygenic effect. A total of 33 and 26 SNPs interacting with, respectively, breed and diet, were significantly associated ($P < 0.0001$), in which 10 markers were overrepresented across all traits. In addition, 19 markers are harbored on gene regions. The SIPA1L1 gene showed diet-dependent effect for RFI. Higher efficiency is observed in animals presenting "CC" genotypes when fed diet with medium energy and non-degradable fiber content, compared with those fed a low-energy diet containing stored wet distiller grains. For the PTPRT gene, the additive effect for allele "A" was 0.3143 g higher in 3/4 AN steers compared with SM for RADG. This indicates that 3/4 AN steers present higher gain than SM steers with similar intake. Our results show the importance of identifying diet and breed-dependent genomic markers to be used for selection on different production systems.

Key Words: genome-wide association study, residual feed intake, residual average daily gain

LB5 Mixed rumen microbes respond to excess carbohydrate by synthesizing glycogen and spilling energy. T. J. Hackmann,* K. L. Backus, and J. L. Firkins, *The Ohio State University, Columbus.*

Some pure cultures of rumen bacteria respond to excess carbohydrate by storing it as glycogen, but others respond by spilling energy. Whereas pure culture studies suggest possible responses of mixed cultures to excess carbohydrate, the response of mixed cultures has not been directly studied. We determined whether mixed rumen microbes direct excess carbohydrate toward glycogen synthesis, energy spilling, or both. Batch cultures of mixed microbes were prepared by centrifuging rumen fluid from 4 dairy cows and washing with N-free buffer. Cultures were maintained under anaerobic conditions (39°C) and contained prokaryotes, protozoa, and fungi. Cultures were dosed with 5 or 20 m*M* glucose. Heat production, free glucose, cell protein, and glycogen were measured using calorimetry, glucose-oxidase peroxidase, bicinchoninic acid, and anthrone. Glycogen was measured enzymatically in preliminary experiments, but these measurements led to lower recovery of cell components than when glycogen was measured using anthrone. Heat production was partitioned across (i) baseline endogenous metabolism (heat production before dosing glucose), (ii) glycogen synthesis (assuming 100 kJ/mol glucose residues accumulated in glycogen), and (iii) energy spilling [total heat production minus (i) and (ii)]. For cultures dosed with 5 m*M* glucose, endogenous metabolism and glycogen synthesis accounted for all (101.4 ± 7.1%) heat production; no spilling occurred ($P \geq 0.152$). For cultures dosed with 20 m*M* glucose, endogenous metabolism and glycogen synthesis accounted completely for heat production, but only initially. Energy spilling became detectable ($P \leq 0.05$) by approximately 30 min, and it eventually accounted for as much as 29.8% of heat production in one culture. After glucose was exhausted, cultures dosed with 20 m*M* glucose degraded glycogen rapidly and continued to spill energy, showing spilling may be mediated by rapid glycogen degradation. As in some pure cultures, mixed cultures can spill some energy. However, mixed cultures respond to excess carbohydrate predominantly by glycogen synthesis, without spilling, when the excess is small.

Key Words: rumen microbes, glycogen, energy spilling

LB6 Fine mapping and discovery of recessive mutations that cause abortions in dairy cattle. P. M. VanRaden[1], D. J. Null*[1], T. S. Sonstegard[2], H. A. Adams[3], C. P. Van Tassell[2], and K. M. Olson[4], *[1]Animal Improvement Programs Laboratory, ARS, USDA, Beltsville, MD, [2]Bovine Functional Genomics Laboratory, ARS, USDA, Beltsville, MD, [3]Institute for Genomic Biology, University of Illinois, Urbana, [4]National Association of Animal Breeders, Columbia, MO.*

Five haplotypes that never become homozygous (HH1, HH2, HH3, JH1, and BH1) were previously confirmed to have recessive effects on dairy cow conception rate. Inheritance is now routinely traced and reported. Regions suspected to contain the mutations were narrowed to 3.2 Mb for HH1 and 0.8 Mb for JH1 where source and crossover haplotypes do not become homozygous. Only crossovers between BovineSNP50 (50k) genotyped animals and a 50K parent are used in narrowing the suspect region. Because pedigrees extend many generations, crossovers of crossovers are used both to designate carrier status and in fine mapping. Sequence data then revealed the causative mutation for HH1 in the APAF1 gene on BTA5 and for JH1 in the CWC15 gene on BTA15. These 2 genes are conserved across many species, but a single nucleotide mutation in each stops the translation of DNA to protein and causes

loss of homozygous embryos and fetuses. Frequency of heterozygous animals among those genotyped is 4.5% for HH1 and 23.4% for JH1. Accuracy of detecting the fertility haplotypes using the BovineLD (LD) chip with 6,909 markers was tested by reducing 50K genotypes to LD for 1,000 animals (500 heterozygous and 500 normal) and then imputing back to 50K. Concordance was 100% between status from the LD imputed and the 50K genotypes, but can be poorer if imputation is not done first or if pedigrees are incomplete. To improve accuracy for such animals and to allow laboratories to determine status without imputation, nearby markers were selected that had lowest frequency within breed for alleles that were present in each fertility haplotype. The best 40 markers per haplotype provided high concordance without requiring pedigrees or imputation and were added to the LD chip by GeneSeek in the Genomic Profiler. For animals genotyped with other chips, inheritance of several previously known recessive defects can also be determined with 95 to 100% accuracy and used in selection and mating programs. If sequence data reveals causative mutations for the remaining fertility haplotypes, these can be added to future chips so that the nearby markers are no longer needed.

Key Words: haplotype, crossover, mutation

LB7 Maternal dietary energy source during gestation affects expression of imprinted genes in fetal tissues in sheep. X. Lan, R. Gambra, M. A. Berg, E. J. Cretney, H. Khatib, and A. E. Radunz,* *University of Wisconsin-Madison, Madison.*

Mature pregnant multiparous Polypay ewes (n = 14) were used in a randomized complete block design to evaluate the effects of maternal dietary energy source during mid- to late-gestation on gene expression in maternal placental tissue and fetal tissue. From d 67 ± 3 of gestation until necropsy (d 130 ± 1), ewes were fed 1 of 3 diets formulated to contain 3.52 Mcal ME/d. The primary energy sources were from alfalfa haylage (HL; fiber), corn (CN; starch), or dried corn distillers grains (DG; protein, fat, fiber). At necropsy, cotyledons were removed and a sample was flash-frozen. Fetuses were dissected and samples were collected from the perirenal and subcutaneous adipose depots as well as longissimus dorsi (LD) muscle. Total RNA was extracted from tissues and used to synthesize cDNA. Expression profiles of 9 ovine imprinted genes (H19, IGF2R, GRB10, MEG8, IGF2, PEG1, PEG3, DLK1, and DIO3) were estimated in placental tissue and the fetal LD using qRT-PCR. No significant differences between sex and treatment were detected among the imprinted genes. In fetal LD tissue, expression of DLK1 ($P = 0.03$) and PEG 1 ($P = 0.04$) were greater in fetuses from dams fed CN vs. HL or DG and expression of IGF2R was greater ($P = 0.01$) in fetuses from dams fed HL vs. CN or DG. Expression of DIO3 in fetal LD tissues from dams fed CN tended ($P = 0.09$) to be greater than fetal LD from dams fed HL or DG although it did not reach significance level. The DLK1 and DIO3 genes are found in a cluster of imprinted genes that is highly conserved in mammalian species. Interestingly, this cluster contains the mutation responsible for the callipyge phenotype (muscle hypertrophy) in sheep. To the best of our knowledge, this is the first report of the effects of maternal diet on gene expression in the callipyge region.

Key Words: fetal programming, imprinted genes, sheep

LB8 Potential for post-extraction algal residue to replace cottonseed meal as a protein supplement to grazing cattle. M. L. Drewery,* J. E. Sawyer, and T. A. Wickersham, *Texas A&M University, College Station.*

Algal biomass has been identified as a second-generation biofuel. Significant quantities of the co-product, post-extraction algal residue (PEAR) result from conversion to biofuel. After extraction, PEAR is concentrated in protein, suggesting it may replace cottonseed meal (CSM) as a protein supplement. Our objectives were to determine the optimal level of PEAR supplementation to steers consuming low-quality forage and compare effects of supplementation on N metabolism and forage utilization to a CSM control. Five steers (BW = 198 kg) arranged in a 5 × 5 Latin square had ad libitum access to oat straw (4% CP). Treatments were infused ruminally once daily and included PEAR (18% CP) at 0, 50, 100, and 150 mg of N/kg of BW daily and CSM (43% CP) at 100 mg of N/kg of BW. Increased provision of PEAR stimulated total digestible organic matter intake (TDOMI) quadratically ($P = 0.05$) from 0.9 to 1.6 kg/d for 0 and 100 mg of N/kg of BW of PEAR, respectively. Organic matter digestibility (OMD) increased quadratically ($P < 0.01$) with supplementation, from 47 to 51% for 0 and 100 mg of N/kg of BW PEAR, respectively. At isonitrogenous levels of PEAR and CSM, TDOMI was similar ($P = 0.22$) as was OMD ($P = 0.50$). Negative N balance was observed with all treatments except PEAR provided at 100 or 150 mg of N/kg of BW. Steers consuming straw without supplemental protein lost 13.6 g of N/d. Nitrogen balance was greatest (1.84 g of N/d) when PEAR was provided at 100 mg of N/kg of BW. There were no significant differences between PEAR and CSM supplementation for plasma urea-N, ammonia, or VFA concentrations. Ammonia concentrations increased quadratically ($P = 0.05$) in response to increased supplemental PEAR, from 0.99 to 1.92 m*M* for 0 and 150 mg of N/kg of BW of PEAR. Overall, comparison of PEAR and CSM at isonitrogenous levels suggests there are no significant differences in forage intake, digestion, or N balance, suggesting cattle provided PEAR utilize forage in a similar manner to those supplemented CSM. These observations indicate PEAR may replace CSM as a protein supplement in forage-based cattle operations.

Key Words: post-extraction algal residue, supplementation