Ruminant Nutrition: Using Molecular Techniques to Advance Research in Ruminant Nutrition

482 Introduction to molecular techniques currently used in ruminant nutrition research. J. R. Knapp*, *Fox Hollow Consulting, LLC*, *Columbus, OH*.

Molecular techniques have evolved rapidly over the past 20 years, and in conjunction with the expansion of knowledge provided by genome sequencing projects and improvements in computing resources, have provided new research tools and approaches for ruminant nutritionists, physiologists, and microbiologists. Polymerase chain reaction (PCR) and gel electrophoresis are the basis of many molecular techniques; the principles of these techniques will be presented. From these principles, extension of the applications to quantitative and real-time PCR, restriction-fragment length polymorphisms, denaturing gradient gel electrophoresis (DGGE), and ribosomal profiling of rumen microorganisms will be discussed, including advantages, disadvantages, and limitations. Also, an overview of information management and computing resources used in ribosomal profiling and the development of gene networks will be given.

Key Words: molecular biology, microbial genomics, gene expression

483 Integration of microbial profiling techniques to improve the efficiency of nutrient usage in ruminant production. J. L. Firkins* and Z. Yu, *The Ohio State University*, *Columbus*.

Further improvement in the efficiency of conversion of nutrients into ruminant products requires a better ability to explain dietary interactions and reduce unknown variability among studies. Although molecular approaches have expanded the boundaries of microbial diversity in the rumen, key aspects of the microbiome (e.g., community structure and population dynamics) have not been well linked to efficiency of nutrient usage. Emerging technology is expanding our ability to analyze archived samples from nutrition experiments that have important metabolic changes in order to profile important microbial populations over time, among animals, among particulate versus fluid fractions, or their interactions. Profiling techniques such as denaturing gradient gel electrophoresis (DGGE) using universal primers in PCR can be combined with banding analyses and sequencing of DNA recovered from specific bands to identify likely population shifts among treatments. Species- or group-specific PCR-DGGE can provide more detailed information, and dynamics of the population(s) identified can be examined by real-time PCR. Such approaches revealed the importance of previously unrecognized groups of bacteria and archaea, greatly expanded (and sometimes disagreed with) observations made from pure and mixed cultures, shown how protozoal abundance can influence prokaryotic populations, and demonstrated that a change in population need not be requisite for a change in function. In the latter regard, stable isotope profiling can help identify the populations that directly metabolize an isotopically labeled dietary component of interest and for which their RNA or DNA can be fractionated prior to microbial profiling. Thus, the combination of community profiling (diversity and functionality) and quantification of populations identified during the profiling will provide an interface to improve our ability to predict accumulation of trans fatty acids responsible for depression of milk fat secretion, lessen the negative associative effects of feeding mixed diets, and decrease intraruminal nitrogen recycling.

Key Words: rumen microbial profiling, microbiome, efficiency of nutrient usage

484 Metagenomics of the rumen microbial ecosystem. D. Krause*, *University of Manitoba*, *Winnipeg, Canada*.

Rumen microbiology has a rich history of innovation, beginning with the pioneering work of Robert Hungate and Marvin Bryant in the 1940's and 1950's. The questions for rumen microbiologists have always been the same: what species are present; how many of each species are present; and what are each of the species doing? In the 1940's it was possible to look at rumen fluid under a microscope and morphologically identify microorganisms that could be cultured, but the many bacteria that could not be cultured was troubling. The work of Hungate and Bryant gave us anaerobic techniques so that we could now culture important anaerobic species like *Ruminococcus albus* and *Fibrobacter succinogenes* which were important fiber digesting species. In the late 1970's and early 1980's Carl Woes demonstrated that ribosomal genes, present in all life forms, could be used to classify microorganisms. In the late 1980's David Stahl published a manuscript illustrating how ribosomal genes could be used to monitor rumen populations when supplemented with monensin. This was a breakthrough because it was not possible to evaluate microbial population changes without having to culture them. In the late 1990's and early 2000 sequencing technology had advanced to the point where it was possible to obtain a catalogue of all the genes in a single species of bacteria. Sequencing technology has since advanced even further, and new platforms based on pyrosequencing are not available. It is now possible to sequence all the genes of all the species of the rumen ecosystem to produce a metagenome. This presentation will discuss the development of this technology and its application to practical problems in ruminant production.

485 Basal expression of 27 nucleoside and amino acid transporter mRNA by small intestinal epithelia of forage-fed growing beef steers is differentially affected by increased luminal substrate or energy supply. J. C. Matthews*, S. F. Liao, and J. A. Boling, *Department of Animal and Food Sciences, University of Kentucky*, *Lexington*.

In many cool-season forage systems, microbial-derived nucleosides and amino acids typically supply adequate or surplus sources of N, whereas energy supply is typically low enough to limit growth. This talk summarizes a series of relative quantitative real-time RT-PCR experiments that collectively tested the hypotheses that (a) expression of mRNA (normalized to 18S rRNA) for 5 nucleoside (NT), 21 amino acid (AAT), and 1 dipeptide (PepT1) transport proteins differs among duodenal (D), jejunal (J), and ileal (I) epithelia of beef cattle, and (b) expression of these transporter mRNA is affected by increased supply of microbial-derived substrate, starch-derived energy, or both. Total RNA was extracted from a single set of epithelial tissues collected at slaughter from 18 ruminally and abomasally catheterized Angus steers (BW ~ 260 kg) consuming an alfalfa-cube based diet at 1.3× NE_m. Cattle were infused with either (n = 6) water (basal) or ruminal (substrate) or abomasal (energy) corn starch hydrolysate (SH; at 20% ME intake). All 27 mRNA were expressed. Of these, 23 were differentially ($P \leq 0.07$; 1.0- to 49-fold) expressed among D, J, or I epithelia. Substrate and energy treatments affected ($P \leq 0.08$; -0.27- to +2.10-fold) basal mRNA expression. More specifically, increased substrate supply increased D expression of 3 NT mRNA and decreased J content of 4 cationic AAT mRNA, whereas expression of 18 AAT and PepT1 mRNA were not affected. In contrast, increased luminal energy increased 2 D NT mRNA, decreased 2 J cationic AAT,

and increased 6 I neutral AAT and PepT1 mRNA. For a given class of transporter, mRNA expression was altered such that potential capacities of apical and basolateral membranes were changed in a parallel manner. These findings provide an initial understanding of the basal expression of bovine NT and AAT genes and their responsiveness to increased substrate or energy supply.

Key Words: amino acid transport, nucleoside transport, nutrient-gene interaction

486 Molecular adaptations in transition dairy cows. J. J. Loor*, *University of Illinois*, *Urbana*.

The periparturient period is characterized by dramatic alterations in metabolism and function of immune cells and key tissues such as liver, adipose, and mammary. Understanding the molecular adaptations of tissues during this physiological state remains a great challenge. Development of transcriptomic technologies has dramatically accelerated the rate at which biological information can be collected from agricultural animals. Use of oligonucleotide bovine microarrays on liver, adipose, and mammary tissue from transition dairy cows has demonstrated the potential of high-throughput technologies for identifying genes involved in regulating and coordinating function and crosstalk among tissues. Work examining the role of prepartum level of dietary energy has revealed unique clusters encompassing functional categories including signal transduction, cell-to-cell signaling, molecular transport, insulin signaling, lipid metabolism (synthesis and beta-oxidation), immune or inflammatory processes, and cell death in adipose as well as liver. High-throughput technologies and associated bioinformatics tools are especially well-suited for studying the complex regulation of transcriptional networks in tissues from transition cows. Major advances in understanding biology of the transition cow may come from coupling existing knowledge of enzyme kinetics, biochemistry, and hormone action with transcriptomics, proteomics, and metabolomics approaches. Using a systems biology approach to integrate data generated at the mRNA, protein, metabolite, and tissue level can allow the assembly of the important components needed to model the transition cow. Such models will prove useful in determining how we can manipulate complex processes that could have significant long-term economic impact, e.g., lactation persistency, fertility, and efficiency of conversion of feed to milk. An important goal of the future will be to apply additional experimental tools (e.g., gene silencing, chromatin immunoprecipitation) and bioinformatics (e.g., transcription factor binding site identification) to studies focused on periparturient cows.

Key Words: transcriptomics, energy balance, systems biology

Small Ruminant: Production, Management, Lactation

487 Effects of kid genotype on carcass traits of meat goats from a three-breed diallel. R. Browning, Jr.*[1], W. Getz[2], O. Phelps[3], and C. Chisley[4], [1]*Tennessee State University*, *Nashville*, [2]*Fort Valley State University*, *Fort Valley, GA*, [3]*USDA-AMS*, *Lakewood, CO*, [4]*Southern University*, *Baton Rouge, LA*.

Purebred and reciprocal crossbred buck kids (n = 275) from a complete diallel mating of Boer (B), Kiko (K), and Spanish (S) breeds were harvested at about 33 wk of age over three yr to assess breed of kid effects on carcass traits in meat goats. Kids were raised post-weaning on summer pasture supplemented with 0.4 kg/d of 16% CP pelleted feed. Each kid and carcass was graded using USDA meat goat standards. Live kid and chilled carcass grades differed ($P < 0.01$) among genotypes. Purebred BB had better ($P < 0.01$) live grades than KK and SS kids; whereas BB and KK had better ($P < 0.01$) carcass grades than SS. Among the crosses, BK and SB graded better ($P < 0.01$) live than KB, SK, and KS; carcass grades were better ($P < 0.01$) for BK than for KB, SB, and SK. Live weight, chilled carcass weight (without kidney-pelvic fat), and chilled dressing percent varied ($P < 0.01$) by genotype. Live and carcass weights among purebreds were heavier ($P < 0.01$) for KK (26.8 ± 0.8 kg; 11.2 ± 0.4 kg) than for BB (23.3; 9.3) and SS (23.2; 9.6). Crossbred live and carcass weights differed ($P < 0.01$) only between SK (27.0 ± 0.7 kg; 11.5 ± 0.4 kg) and SB (24.2; 9.9). Chilled dressing percent was higher ($P < 0.01$) for KK (41.6 ± 0.5%) than for BB (39.9). Among crosses, dressing percent was higher ($P < 0.01$) for SK (42.4 ± 0.4%) and KS (42.2) than for BK (40.9), KB (40.9), BS (40.2) and SB (40.5). Foreleg, hindleg, and combined boneless fore- and hindleg weights were affected ($P < 0.01$) by genotype. Purebred KK were heavier ($P < 0.01$) than SS and BB for each leg trait. Among the crosses, SK had heavier ($P < 0.01$) forelegs than BS and SB and heavier ($P < 0.01$) hindlegs and boneless leg than SB. Kid genotype did not affect lean content (69%) of leg cuts. Genotype affected ($P < 0.01$) loin weights. Loins were heavier ($P < 0.01$) for KK than for BB and heavier ($P < 0.01$) for SK than for SB. Kidney-pelvic fat differed ($P < 0.01$) by genotype. Among purebreds, internal fat was greater ($P < 0.01$) for KK (177 ± 16 g) than for BB (74) and SS (119). Crossbred genotypes did not differ for kidney-pelvic fat. In conclusion, kid genotype has significant effects on carcass yield traits for meat goats.

Key Words: meat goats, breed, carcass

488 Advantages of using electronic identification for automated lambing data and body weight recording in sheep. A. Ait-Saidi, G. Caja*, S. Carné, and A. A. K. Salama, *G2R, Universitat Autònoma de Barcelona*, *Bellaterra, Spain*.

Manual (M), semi- (SA) and fully-automated (A) systems for sheep performance recording were compared in 2 on-farm experiments. System M used visual identification (plastic ear tags), on-paper data recording and manual typing for data uploading. Systems SA and A used electronic identification (20 g mini-boluses, 56 × 11 mm, containing 32 mm half-duplex transponders); performance data were recorded using a reader with keyboard in SA (i.e. lambing data) or automatically in A (i.e. BW), and data uploading was done automatically for SA and A. Each ewe wore an ear tag and a mini-bolus. Exp. 1 compared M and SA lambing recording (1.57 lambs/ewe) in dairy (n = 73) and meat ewes (n = 80) processed in groups of 10. Time for lambing data recording was greater in dairy than meat ewes (P < 0.05), due to the lower operator experience and because half of the dairy ewes needed ear tag cleaning, but when systems were compared, M was greater than SA (P < 0.05) for both dairy (1.11 vs. 0.80 min/ewe) and meat (0.78 vs. 0.68 min/ewe) ewes. Average time for data uploading was greater in M vs. SA (0.54 vs. 0.06 min/ewe; P < 0.001). Consequently, overall time for lambing recording was greater in M than SA in dairy (1.67 vs. 0.87 min/ewe; P < 0.001) and meat ewes (1.30 vs. 0.73 min/ewe; P < 0.001). Data uploading errors were 4.9% in M, but no errors were detected in SA. In Exp. 2, ewes' BW was recorded by M and A systems in dairy (n = 120) and meat ewes (n = 120). Ewes were processed in groups of 20 using an electronic scale which was interfaced to a computer for A. Weighing