605 ASAS Centennial Presentation: The promise of proteomics in animal science. J. D. Lippolis* and T. A. Reinhardt, *National Animal Disease Center, USDA-ARS*, *Ames, IA*.

Proteomics holds significant promise as a method for advancing animal science research. The use of this technology in animal science is still in its infancy. The ability of proteomics to simultaneously identify and quantify potentially thousands of proteins is unparalleled. In this review, we will discuss the current state of proteomic technology and cover basic principles of its experimental design. In addition, challenges and limitations of proteomics will be considered, stressing those that are unique to animal sciences. The current proteomic research in animal sciences will be discussed and the potential uses for this technology will be highlighted.

Key Words: Proteomic, Animal Science, Genomics

606 Periparturient liver and mammary tissue-explant gene expression is responsive to bacterial lipopolysaccharide (LPS) in vitro: a model to study tissue-specific genomic responses to infection. M. Mukesh*, D. E. Graugnard, M. Bionaz, and J. J. Loor, *University of Illinois*, *Urbana*.

Periparturient cows experience a state of immunosupression after parturition that renders them highly susceptible to mastitis pathogens. Objectives were to evaluate the suitability of an in-vitro mammary (MG) and liver (LIV) tissue explant model to study dose and temporal effects of bacterial LPS on gene expression profiles. Percutaneous biopsies of MG and LIV were obtained simultaneously from Holstein cows (7 DIM) that were bacteriologically negative in all mammary quarters. Explants (50-100 mg) were cultured in parallel in DMEM-F12 (MG) or RPMI-1640 (LIV) media at 37 °C and 5% CO_2. In a first study, MG explants were cultured for 2 h with 0 or 20 μg LPS/mL. Similarly, LIV explants were cultured for 2 h with 0, 0.2, 2.0, and 20 μg LPS/mL. In order to evaluate temporal changes in mRNA expression in response to LPS, LIV tissue was cultured with 20 μg LPS/mL and harvested after 2, 4, 6, and 8 h. Genes evaluated included serum amyloid A1 (*SAA1*), tumor necrosis factor-alpha (*TNFA*), complement component 9 (*C9*), haptoglobin (*HP*), and interleukin-6 (*IL6*). LPS challenge of MG resulted in greater ($P < 0.05$) *HP* (2.6-fold), *IL6* (3.9-fold), *SAA1* (3.5-fold), and *TNFA* (22-fold) relative to control. In LIV both *IL6* and *TNFA* increased ($P < 0.05$) markedly in response to incremental LPS, averaging ~5- and 3.5-fold greater expression with 20 than 0 μg LPS/mL. In the time course experiment, an 11-fold increase ($P < 0.05$) in *IL6* mRNA was observed at 4 h post-LPS challenge relative to 2 h, and it continued to increase through 8 h. In contrast, *HP*, *C9*, and *TNFA* mRNA decreased ($P < 0.05$) substantially between 2 and 8 h post-LPS. The present results indicate that mammary and liver tissue explants are suitable to study changes in mRNA expression due to infection. This approach holds promise in terms of allowing researchers to understand transcriptional responses in mammary and liver when stimulated by infectious pathogens. Funded by NRI-USDA project #2007-35204-17758.

Key Words: Nutrition, Immune Response, Inflammation

607 Effects of long-chain fatty acids on concanavalin A-induced cytokine production by bovine peripheral blood mononuclear cells. C. Caldari-Torres*, M. Perdomo, and L. Badinga, *University of Florida*, *Gainesville*.

Fatty acids (FA) are known to modulate immune responses in several mammalian species, including the human, pig, and cow. The objective of this study was to examine the short-term effects of long-chain FAs on concanavalin A (ConA)-induced tumor necrosis factor alpha (TNF-α), interferon gamma (INF-γ) and interleukin-4 (IL-4) production by cultured bovine peripheral blood mononuclear cells (PBMCs). Peripheral blood mononuclear cells were isolated by density gradient centrifugation and then incubated with ConA alone (10 μg/mL) or with a combination of ConA and specific FAs for 48 h at 37 °C in a humidified atmosphere of 5% CO_2. Concentrations of cytokines in cell-conditioned media were determined by specific bovine enzyme-linked immunosorbent assays (ELISAs). Incubation of PBMCs with ConA increased ($P < 0.01$) TNF-α, INF-γ and IL-4 production by 8-, 24-, and 24-fold, respectively. Co-incubation with eicosapentaenoic acid (EPA, $C_{20:5}$n-3) completely abolished ($P < 0.01$) TNF-α, INF-γ and IL-4 responses to ConA. Alpha linolenic acid (ALA, $C_{18:3}$n-3) was less potent in attenuating TNF-α and IL-4 production in vitro. Docosahexaenoic acid (DHA, $C_{22:6}$n-3) and monounsaturated FAs (cis or trans-$C_{9:1}$ and cis or trans-$C_{11:1}$) had minimal effects on ConA-induced cytokine production in cultured bovine PBMCs. Results indicate that ALA and EPA attenuate TNF-α, INF-γ and IL-4 production by bovine PBMCs challenged with ConA. The mechanisms by which these omega-3 FAs modulate cytokine production in bovine PBMCs warrant further investigation.

Key Words: Fatty Acids, PBMCs, Cytokines

608 ASAS Centennial Presentation: Contributions in the *Journal of Animal Science* to understanding cattle metabolic and digestive disorders. J. T. Vasconcelos and M. L. Galyean*, *Texas Tech University*, *Lubbock*.

Ruminal acidosis, bloat, liver abscesses, and polioencephalomalacia (PEM) were reviewed with respect to contributions published in the *Journal of Animal Science* (JAS) regarding these metabolic and digestive disorders in beef cattle. Increased grain feeding and expansion of the feedlot industry in the 1960s led to considerable research on acidosis. The concept of subacute acidosis was developed in the 1970s. Significant research was published during the 1980s and 1990s on adaptation to high-grain diets, effects of ionophores, and the development of model systems to study ruminal and metabolic changes in acidosis. Recent publications JAS on acidosis have largely focused on individual animal variability in response to acid loads and the role of management strategies in controlling acidosis. Increased grain feeding also was associated with an increase in the incidence of liver abscesses, which were quickly linked to insults to the ruminal epithelium associated with acidosis. The role of antibiotics, particularly tylosin, in decreasing the incidence and severity of liver abscesses was a significant contribution of JAS publications during the 1970s and 1980s. Papers on bloat were among the earliest published in JAS related to metabolic and digestive disorders

in cattle. Noteworthy accomplishments in bloat research chronicled in JAS include the nature of ruminal contents in legume and feedlot bloat, the efficacy of poloxalene, ionophores, and more recently, condensed tannins, in decreasing the incidence and severity of bloat. Although less has been published on PEM, early publications highlighting the association between PEM and ruminal acidity and the role of thiaminase in certain forms of the disorder, as well as more recent publications related to the role of S in the development of PEM, are noteworthy contributions. The JAS has played a significant role as a repository for information pertaining to metabolic and digestive disorders in cattle, particularly through the publication of ASAS-sponsored symposia, and it will no doubt continue to be a premier resource for information on these conditions.

Key Words: Acidosis, Bloat, Liver Abscesses

609 Neutrophil function in response to level of dietary energy pre-partum and post-partum inflammatory challenge in dairy cows.
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Dairy cattle experience a state of immunosupression after parturition that renders them highly susceptible to mastitis pathogens as well as metabolic diseases. We hypothesized that plane of dietary energy pre-partum is a management tool that can affect neutrophil and metabolic tissue function and, thus, transition success. Thirty-two Holstein cows with average SCC of ~128,000 ± 108,000 in the previous lactation were assigned (n = 16/diet) to a control (high-straw; NE_L = 1.33 Mcal/kg) or moderate-energy (ME; NE_L = 1.58 Mcal/kg) diet during the entire dry period. All cows were fed a common lactation diet post-partum. At 7 DIM, cows were assigned to receive an intrammamary bacterial lipopolysacharide (LPS) challenge (200 μg; n = 8/pre-partum diet) or served as controls (n = 8/pre-partum diet). Cows used were bacteriologically-negative in all mammary quarters. Blood for neutrophil isolation was collected on -14, 7 (prior to LPS), 14, and 30 d relative to parturition. Neutrophils for RNA, phagocytosis, and chemotaxis were promptly isolated by centrifugation after lysis of erythrocytes. Neutrophil migration (3×10^6 isolated cells/mL) was assessed using IL-8 (100 ng/mL) and C5a (10^{-6} M) as chemoattractants. Phagocytic capacity of neutrophils (2×10^5/mL isolated cells) was assessed with 1.75 μm fluorescent microspheres followed by quantification via flow cytometry. Differentials for phagocytic cells were assessed by sorting via flow cytometry. Phagocytosis did not differ between late pre-partum (-14 d) and early post-partum (7 d) or due to pre-partum diet. Analysis of LPS-effects (d 7, 14, and 30) showed that phagocytosis in cows fed the control diet pre-partum increased ~22% by d 14 compared to a ~19% decrease in cows fed ME pre-partum (interaction $P < 0.01$). At 30 d post-partum, phagocytosis was ~30% lower than 7 d (time $P < 0.01$). Results show that neutrophil activity during an infection challenge following parturition might be affected by pre-partum dietary energy intake. Funded by NRI-USDA project #2007-35204-17758.

Key Words: Periparturient, Mastitis, Metabolic Disease

610 An immuno-evaluation system for anti-inflammatory probiotics using originally established porcine epitheliocyte (PIE) cells.
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Intestinal epithelial cells (IECs) are exposed to a variety of antigens, such as pathogenic and commensal bacteria, and are involved in the regulation of the mucosal immune responses. Although probiotics are believed to protect the host from pathogenic inflammation of the IECs, the precise mechanism of the anti-inflammatory effect of probiotics is not well understood. IECs recognize bacterial components through pattern recognition receptors, such as Toll-like receptors (TLRs), to initiate immune responses for pathogen elimination. Despite the importance of swine as a human model for some disease, little is known about the regulation of the immune response of porcine IECs. In the present study, we established and characterized an original porcine intestinal epitheliocyte (PIE) cell line in order to develop a novel immuno-evaluation system for anti-inflammatory probiotics based on the pathogenic inflammatory response of PIE cells. First, we isolated and cloned PIE cells from an unsuckled neonatal swine. These PIE cells expressed TLR1-9 and MD-2 mRNAs, and preferentially expressed TLR4/MD-2. In addition, we confirmed that TLR4 was expressed at the protein level. Upon stimulation with LPS, an antagonist for TLR4, the PIE cells upregulated the expression of several TLRs (TLR2, 3, 4, 5 and 8), Th1 cytokines (IL-1α, IL-6), and chemokine (MCP-1). Furthermore, Enterotoxigenic *Escherichia coli* (ETEC), a major pathogen of neonatal swine, induced severe inflammatory cytokines through the TLR4 on the PIE cells. Interestingly, pretreatment with several probiotics significantly reduced inflammatory cytokine expression (IL-6, TNF-α) without affecting the TLR4 expression level. These results indicate that PIE cells are a useful cell line for studying the anti-inflammatory mechanism of probiotics with a view to developing new physiologically functional foods with intestinal anti-inflammatory effects.

Key Words: Probiotics, Intestinal Epithelial Cells, Anti-inflammation