T153 Cytokine gene expression patterns of milk from healthy bovine mammary glands in late and early lactation. D. F. R. Bruno*[1], R. G. S. Bruno[3], P. V. Rossitto[2], J. S. Cullor[2], and J. L. Stott[2], [1]*Texas Veterinary Medical Diagnostic Laboratory*, *Amarillo,*[2]*University of California Davis*, *Davis,* [3]*Texas AgriLife Research and Extension*, *Amarillo*.

Cytokines mediate and regulate the immune system and have been studied as an alternative non-antibiotic therapy to treat and prevent mastitis in dairy cows, mainly in critical times as dry-off and early postpartum. The aim was to compare natural levels of expression of 9 cytokines on late lactation (dry-off) and early lactation (first week postpartum) in milk cells from healthy cow mammary glands. Transcriptional levels of expression of interleukin (IL)2Rα, IL4, IL6, IL8, IL10, IL17, interferon (IFN)γ, inducible nitric oxide synthase (iNOS) and tumor necrosis factor (TNF)α were evaluated in both periods by real time PCR. Milk samples from 10 Holstein cows were collected aseptically from 2 quarters/cow at dry-off and again at first week postpartum. Only quarters with somatic cell count ≤200,000 and absence of bacteria in both time points were considered for this study. Significance was defined as $P < 0.10$. Transcripts from IL2Rα, IL8, IL10, IFNγ, iNOS and TNFα were detected in both periods. IL2Rα and IL6 ($P < 0.10$), and IL8 and IL10 ($P < 0.04$) were upregulated in late lactation in comparison with the levels in early lactation. In late lactation, there was a positive correlation of proinflammatory cytokines IL6 and TNFα with anti-inflammatory cytokine IL10, and chemokine IL8 ($P < 0.10$). However, a negative correlation was observed between the proinflammatory cytokine IL17 with IL6 and IL8 ($P < 0.10$). In early lactation, fewer cytokines were correlated. A positive correlation among IL8 and TNFα and IL2Rα was observed. Similar to late lactation, there was a positive correlation between IL6 and IL10, and between TNFα and IL8, which was also correlated to the regulatory mediator iNOS ($P < 0.10$). In conclusion, cytokine mRNA profiles between late and early lactation showed differences, which can be attributed to dramatic changes the mammary gland is subjected to during these 2 stages of lactation. Positive correlation and upregulation of proinflammatory and anti-inflammatory cytokines account for an efficiency of the mammary gland immune system at late lactation and could be used as markers for health control of the udder.

Key Words: cytokine expression, lactation, RT PCR

T154 Intra- and inter-dairy heifer variation of cellular neutrophil functions. L. E. Hulbert*[1,2], L. R. Schwertner[1], J. A. Carroll[2], and M. A. Ballou[1], [1]*Department of Animal and Food Sciences, Texas Tech University*, *Lubbock,* [2]*Livestock Issues Research Unit, USDA-ARS*, *Lubbock, TX*.

Immune competence of dairy cattle is difficult to determine as a healthy immune system requires the resolution of pathogen invasion without excessive host-tissue damage. Neutrophil phagocytosis (PG) is important for eliminating pathogens, but PG induces an oxidative burst (OB), which helps destroy the pathogen but also can damage the neutrophil and surrounding tissue. Neutrophil adhesion molecule L-selectin (L) mediates neutrophil rolling in the periphery, allowing for "surveillance" of pathogens, while its counterpart, β-integrin (β), allows for neutrophil anchoring to epithelial cells and migration into tissue from the periphery. The objectives of these studies were to examine inter-heifer variation (Exp.1, n = 36, 13–16 mo. of age) as well as intra-heifer variation (Exp. 2, n = 12, days = 3) of neutrophil functions. Phagocytosis and OB were determined by 2-color flow cytometry using propidium iodide labeled enterotoxigenic *E. coli* and the oxidation of dihydrorhodamine, respectively. Adhesion molecule expressions were also determined using flow cytometry and expressed as the mean fluorescence intensities. In Exp. 1, neutrophil PG and OB were highly correlated ($R^2 = 0.50$), while adhesion molecules L and β were moderately correlated ($R^2 = 0.39$). Inter-heifer coefficients of variation (CV) were low for PG (19.9%) and β (18.5%), but high for OB (51.1%) and L (38.0%). In Exp. 2, there were day effects ($P \leq 0.01$) for PG, OB and β, but not L ($P \geq 0.10$). Neutrophil PG intra-heifer CV was the least among all immune parameters (11.0%) while OB was the most variable at 22.43%. Adhesion intra-heifer CVs were 17.6% for β and 16.1% for L. These data indicate that neutrophil migration into tissue and subsequent phagocytosis of *E. coli* were more similar between Holstein heifers than either the oxidative killing or surveillance potential. Therefore, the OB and L expression are more likely to contribute to individual heifer variation in immune competence.

Key Words: bovine, granulocytes, immunology

T155 Comparison of the proliferative response of CD8 memory T cells from experimentally and naturally infected cattle shows the response to live *Mycobacterium avium* ssp. *paratuberculosis* stronger than the response to Johnin purified protein derivative (JPPD). H. M. Rihan*[1], G. S. Abdellrazeq[2], M. J. Hamilton[3], A. J. Allen[3], K. T. Park[3], and W. C. Davis[3], [1]*Mansoura University*, *Egypt*, [2]*Alexandria University*, *Egypt*, [3]*Washington State University*, *Pullman*.

Johnin purified protein derivative (JPPD) is the antigen most frequently used to study the T-cell response to *Mycobacterium avium* ssp. *paratuberculosis* (MAP). It has been assumed the response to this antigen can be used to characterize the CD4 and CD8 T cell responses to MAP in experimentally and naturally infected animals. Comparison of the response to PPD and live MAP, however, has revealed a clear difference, especially in the CD8 T cell response. Flow cytometric analysis of the proliferative response of PBMC from experimentally infected calves to JPPD and MAP showed the CD8 memory T cell response to JPPD was low during the first 3 mo post infection. In contrast, the response to MAP was strong and similar to the response of CD4 memory T cells. A comparable difference in the response to JPPD and MAP was observed in PBMC from cows at the late stage of infection. The findings show that further investigation of the mechanisms of immunopathogenesis of paratuberculosis must include a comparison of the response to JPPD and live MAP.

Key Words: *Mycobacterium avium* ssp. *paratuberculosis*, Johnin purified protein derivative, CD8 memory T cell

T156 Tumor necrosis factor-α concentrations from whole blood cultures correlate with isolated peripheral blood mononuclear cell cultures. L. E. Hulbert*[1,2], J. A. Carroll[2], and M. A. Ballou[1], [1]*Department of Animal and Food Sciences, Texas Tech University*, *Lubbock,* [2]*Livestock Issues Research Unit, USDA-ARS*, *Lubbock, TX*.

Many cellular immune assays are impractical because they require labor-intensive isolation of cells from their natural environment. The objectives of this study were to determine the relationship between cell culture supernatant tumor necrosis factor (TNF)-α from isolated peripheral blood mononuclear cells (PBMC) and whole blood (WB) when stimulated with lipopolysaccharide (LPS from *E. coli* O111:B4; 1 and 10 μg/mL for WB; 0.01 and 1 μg/mL for PBMC). Thirty-six dairy heifers (12–16 mo. age) free from any signs of disease were analyzed in

the study. The PBMCs were isolated using a percoll gradient, washed twice with PBS, counted using a hemacytometer, then resuspended to 2 × 10^6 cells/mL in a cell culture RPMI medium with 1% antibiotics, 10% autologous plasma and 5 ng/mL of recombinant bovine interferon-γ. In the WB assay, 200 μL of whole blood were added to 800 μL of RPMI with 1% antibiotics. Samples were incubated with their respective LPS doses for 24-h before supernatants were collected and analyzed for TNF-α using a commercially available sandwich ELISA. Mean TNF-α concentrations from PBMC and WB were moderately correlated (R^2 = 0.40). There were strong correlations between the low and high doses of LPS within each assay (R^2 = 0.67 and 0.87, for isolated PBMC and WB, respectively). The WB data were not correlated with either the number of leukocytes or the percentages of neutrophils (R^2 = 0.15). Inter-heifer coefficients of variation (CV) for the PBMC and WB data were 39.5% and 57.6%, respectively. In another experiment, using samples from 12 Holstein heifers from 3 consecutive days determined that the intra-heifer CVs for PBMC and WB data were 25.33% and 26.51%, respectively. These data indicate the WB assay may serve as a simple but effective ex vivo assay for evaluating bovine pro-inflammatory cytokine synthesis and secretion potential. Additionally, these data elucidate a large population variation, but if a heifer has a reduced response relative to the population at lower LPS concentrations then she will have a reduced response at a higher LPS concentrations, and vice versa.

Key Words: bovine, innate immunity, immune competence

T157 Effect of a *Bacillus*-based direct-fed microbial on cytokine gene expression in the IEC-6 rat intestinal epithelial cell line. C. A. Wehnes*, K. N. Novak, M. Duersteler, E. Davis, and A. H. Smith, *Danisco USA, Inc.*, *Waukesha, WI*.

Enhanced immune maturation was observed in a previous study where calves were treated with a 3-strain *Bacillus*-based direct-fed microbial (DFM). The objective of this study was to examine the immunomodulatory effects of the combination of bacilli strains in an intestinal epithelial cell line. Treatments were vegetative or sporulated bacilli, applied at 1 × 10^7 cfu per well, compared with untreated rat IEC-6 cells (3 × 10^5 cells per well). Expression of interleukin (IL)-1, IL-6, IL-10, tumor necrosis factor-α (TNF-α), and macrophage-inflammatory protein-2 (MIP-2) was analyzed by quantitative PCR and expressed relative to untreated IEC-6 cells. Sporulated bacilli increased IL-6 and MIP-2 gene expression compared with control cells; whereas, vegetative bacilli increased IL-10 gene expression (Table 1). To determine whether the bacilli would affect inflammation caused by LPS, IEC-6 cells were co-incubated with bacilli and 10 ng LPS per well. LPS increased IL-6, TNF-α, and MIP-2 gene expression compared with control cells (Table 1). Vegetative bacilli reduced ($P \leq 0.05$) elevated TNF-α gene expression caused by LPS 4-fold; whereas, sporulated bacilli did not ($P > 0.05$). These data demonstrate that cytokine gene expression differs depending on whether bacilli are vegetative or sporulated in both the presence and absence of LPS stimulation; furthermore, expression of inflammatory cytokines induced by LPS was reduced by vegetative bacilli, but not by spores.

Table 1. Fold change in gene expression, relative to unstimulated cells, of various cytokines in a rat intestinal cell line (IEC-6) exposed to vegetative or sporulated bacilli with or without lipopolysaccharide (LPS) to stimulate inflammation

Bacilli	None	Spores	Spores	Vegetative	Vegetative
LPS	+	–	+	–	+
IL-1B	1.0	–0.5	3.1	1.9	1.0
IL-6	3.3*	3.4*	5.8*	2.4	2.6
IL-10	2.9	1.7	1.5	5.5*	3.3
TNF-α	25.5*	17.7	35.9*	8.3	7.4
MIP-2	43.1*	42.2*	73.0*	9.7	21.4

*Means are significantly different to untreated control cells ($P \leq 0.05$).

Key Words: probiotic, immunity, DFM

T158 Post-weaning intestinal mucin dynamics is influenced by cereal grain type and commensal microbiota. G. Malik*, M. D. Drew, and A. G. Van Kessel, *University of Saskatchewan*, *Saskatoon, SK, Canada*.

Mechanisms by which diet composition and commensal microbiota influence post-weaning intestinal mucin dynamics were studied using conventional and gnotobiotic pigs in a 2x2 factorial design. Caesarean-section derived germ-free pigs (n = 16) were reared in HEPA-filtered isolator units (4 pigs/unit) and fed sterilized sow colostrum (120 mL/pig) followed by infant formula (2:1; formula: water) ad libitum. Conventional (CON) pigs (n = 32) were vaginally delivered and sow-reared. At 14 d of age all pigs were weaned to diets formulated to meet nutrient requirements using corn or wheat/barley. At 24 d of age, pigs were killed and tissue collected at 75% (cranial to caudal) of small intestinal (SI) length. Contamination of germ-free pigs resulted in monoassociation with *Enterococcus faecium*. Acidic, neutral and total numbers of goblet cells were determined in villi and crypts using stained formalin-fixed tissue cross-sections taken at 75% of SI length. Expression of membrane associated mucin genes Muc 1, Muc 13 and secreted type Muc 2 was also determined. Data were analyzed as a 2x2 ANOVA using GLM procedure (GLM, SPSS software v. 12.0, SPSS Inc., Chicago IL, USA) with main effects of cereal grain (corn vs. wheat/barley) and microbial status (conventional vs. monoassociated) plus interactions as sources of variation. Monoassociation reduced ($P < 0.01$) neutral, acidic and total goblet cells in crypts and neutral goblet cell in villi and mucin gene expression. Monoassociation tended to increase ($P < 0.01$) acidic mucin cells in the villi. Interactive effects were observed only as trends ($P < 0.1$) such that Muc 2 expression was lower only in monoassociated pigs fed wheat-barley. In conclusion, as expected, monoassociation markedly influenced intestinal physiology. Limited effects of cereal grain type were observed.

Key Words: microbiota, mucin, swine

T159 Mannan oligosaccharide (MOS) modulates ileal gene expression in pigs experimentally infected with porcine reproductive and respiratory syndrome virus (PRRSV). T. M. Che*[1], R. W. Johnson[1], K. W. Kelley[1], W. G. Van Alstine[2], K. A. Dawson[3], C. A. Moran[3], and J. E. Pettigrew[1], [1]*University of Illinois*, *Urbana,* [2]*Purdue University*, *West Lafayette, IN,* [3]*Alltech Biotechnology Center*, *Nicholasville, KY*.

The objective of this study was to investigate ileal gene expression in control- or mannan oligosaccharide (MOS) (Bio-Mos)-fed pigs with or without porcine reproductive and respiratory syndrome virus (PRRSV) at d 14 postinfection (PI). Weaned pigs (3 wk old) fed 0% or 0.2% MOS

diets were intranasally inoculated with PRRSV or medium at 5 wk old. Total RNA was extracted from ileal tissue including Peyer's patches. Double-stranded cDNA was amplified, labeled, and further hybridized to the Affymetrix GeneChip Porcine Genome Array consisting of 23,937 probe sets representing 20,201 genes. Microarray data were analyzed in R using packages from the Bioconductor project. The MOS x PRRSV interaction and PRRSV main effect were not significant. Therefore, gene expression data from control-fed pigs and MOS-fed pigs were pooled (4 pigs/dietary treatment) for statistical analysis using the LIMMA package. Dietary MOS affected ($P < 0.05$) the expression of thousands of non-immune probe sets (1151 up and 1571 down). Using a 2-fold change difference and *P*-value cutoff of <0.05, we identified that MOS increased the expression of 134 non-immune genes and reduced the expression of 25 non-immune genes. The greatest mRNA upregulation was observed in many important genes involved in absorption of lipid, glucose, and glutamate, cellular protection from endogenous or external proteolysis, and d-amino acid oxidation. With respect to immune genes, MOS altered ($P < 0.05$) the expression of 23 immune probe sets (16 up and 7 down). Using a 2-fold change difference and P-value cutoff of <0.05, MOS upregulated 8 genes and downregulated 5 genes. The greatest increases were seen in genes encoding antimicrobial peptide, intestinal lymphocyte recruiting chemokine, and complement component 5. In short, PRRSV infection did not affect the ileal gene expression at d 14 PI, but feeding MOS to pigs may be beneficial by enhancing intestinal uptake of nutrients and mucosal defense against enteric infection.

Key Words: ileal gene expression, mannan oligosaccharide, nursery pigs

T160 Differential gene expression in subcutaneous and visceral adipose depots in response to lipopolysaccharide in the Sinclair minipig. S. L. Booker*, C. J. Kojima, J. S. Gouffon, and N. Moustaid-Moussa, *The University of Tennessee*, *Knoxville*.

The goal of this study was to elucidate depot-specific differences in transcriptome response of adipose tissue to a pro-inflammatory challenge. Eight intact male Sinclair minipigs (8 mo of age; 32.3 ± 1.9 kg) were non-surgically cannulated and challenged with 15 μg/kg lipopolysaccharide (LPS; n = 4) or saline (n = 4) delivered IM. Blood was collected every 20 min from −60 to +240 min relative to LPS administration. The following day, pigs received a second IM injection of 5 μg/kg LPS or saline, and were killed 2 h post-injection. Tissues including visceral fat (VF) and subcutaneous fat (SQF) were collected, and RNA was isolated for transcriptome analysis (Affymetrix). Only transcripts for which expression differed between treatments by 2-fold or greater with $P < 0.05$ were noted. In SQF, 541 transcripts were downregulated and 1,117 transcripts were upregulated by LPS relative to saline. Upregulated SQF genes included members of the NFκB inflammation cascade: TLR4 (toll-like receptor 4), TICAM2 (toll-like receptor adaptor molecule 2), TLR9 (toll-like receptor 9), and MIF (macrophage migration inhibitory factor). In VF, only 9 transcripts were downregulated and 7 upregulated by LPS relative to saline. Thrombospondin (THBS1) and adiponectin (ADIPOQ) were upregulated in VF, while in SQF, THBS1 was downregulated and ADIPOQ was not affected by LPS. When transcriptomes from SQF and VF of LPS-treated animals were compared, differentially regulated genes mapped to 3 main pathways: the aryl hydrocarbon receptor (AhR) and transforming growth factor β (TGFβ) signaling pathways (both of which are involved in the inflammatory process), and Type 2 Diabetes (T2D). Genes belonging to the AhR and T2D-related pathways were overall upregulated in VF relative to SQF; genes in the TGFβ pathway were downregulated in VF relative to SQF. These results indicate that both SQF and VF depots are metabolically active but are differentially responsive to an immune challenge. Finally, these data further support the role of visceral adipose in inflammatory processes often associated with metabolic disorders.

Key Words: pig, inflammation, adipose

T161 A comparative analysis of galectin-11 gene expression in ruminants. N. Mikiashvili, M. Worku*, and H. Muktar, *North Carolina Agricultural and Technical State University*, *Greensboro*.

The objective of this study was to assess the expression of galectin-11 in neutrophils isolated from cow and goat blood. Galectin-11 isolated form sheep infected with *Haemonchus contortus* is a member of a family of proteins that consists of β-galactoside binding lectins. Host galectins have been shown to be active participants in the recruitment of cells to sites of inflammation and modulating the effector function of inflammatory cells such as the neutrophil. For example they can serve as negative regulators of lipopolysaccharide (LPS) function, to protect the host from endotoxic shock. The role of galectins in the inflammatory response in ruminants has not been defined. A comparative analysis was conducted to determine expression of galectin-11 in neutrophils and to assess the impact of LPS exposure. Three clinically healthy Holstein Friesian cows and 3 Boer goats pasturing at the North Carolina A&T State University farm were used. Blood was collected from the jugular vein in anticoagulant. Neutrophils were isolated from blood samples by differential centrifugation and hypotonic lysis of red blood cells. Isolated neutrophils in PBS were treated with 10 or 100 ng *E. coli* LPS for 15 or 30 min. Total RNA and DNA isolated using Tri-reagent method. Reverse transcriptase PCR was performed using Oligo (dT) primers. Quantitative one step real time PCR was performed with the intercalating dye SYBR Green. Expression level in target samples was calculated using relative quantification with normalization to a reference gene. Amplified products were run on a 2.5% agarose gel with PCR markers. Primers for GAPDH were used as amplification controls and for calculation with Pfaf method. Neutrophils isolated from cow and goat blood expressed galectin-11. Dose of LPS had an effect on overall transcription in neutrophils as indicated by increased RNA concentration. Control (untreated) and LPS treated samples showed expression of target galectin mRNA transcripts. Our study on ruminant neutrophils may help further understanding of the role of galectins in the inflammatory response following host-pathogen interactions.

Key Words: galectin, neutrophil, ruminant

T162 Analysis of a transient receptor potential channel 3 *(Trpc3)* gene in myotonic goats: A potential model for human cerebellar ataxia. M. M. Corley and J. E. Caviness*, *Virginia State University*, *Petersburg*.

Cerebellar ataxia (CA) is a progressive neurological disorder, manifested by poor coordination of the arms and legs, increased difficulty walking or paralysis, slurred speech, depleted hearing and cold feet. Cerebellar ataxia is caused by the degeneration of Purkinje cells of the cerebellum. More than 50 different inherited forms of CA are known, and evidence suggests common pathological pathways (transcriptional regulation, calcium homeostasis) trigger degeneration of Purkinje cells. Myotonia congenita (MC) is an inherited neuromuscular disorder characterized by the inability of muscles to quickly relax after a voluntary contraction. Mutations in calcium cycling genes have been reported to contribute to the myotonic state. It has been shown that a point mutation in a transient receptor potential channel (Trcp) gene that regulates calcium stores, *Trpc3* causes abnormal Purkinje cell development and CA in moonwalker mice. Although genes in the TRPC family have been linked

to MC, the myotonic goat has not been evaluated as a model for CA, nor has the *Trpc3* gene been isolated in goats. Therefore the objective of this study was to identify and characterize the *Trpc3* gene in the myotonic goat. Total RNA was isolated from whole blood samples and purified. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed using cross species primers designed from the human, bovine, and mouse *Trpc3* gene alignment. The RT-PCR product was visualized via agarose gel electrophoresis. The expected 213 bp RT-PCR product was observed, indicating successful amplification of the goat *Trpc3* cDNA. The RT-PCR product was purified and sequenced. The goat *Trpc3* gene showed 95% and 91% sequence homology to the bovine and human *Trpc3* genes, respectively. Identification and analysis of the myotonic goat *Trpc3* gene will provide insight into the etiology of CA in humans and its relationship to MC.

Key Words: myotonic goat, cerebellar, TRPC3

T163 Simultaneous detection and quantitation of anthelmintic resistance and *Haemonchus contortus* infection in grazing goats. M. M. Corley and A. A. Saeed*, *Virginia State University*, *Petersburg*.

The most prominent factor currently limiting meat goat producers is the blood sucking nematode *Haemonchus contortus*. This gastrointestinal parasite costs the global livestock industry billions of dollars per annum in lost production and drug costs. Resistance to all the major anthelmintic classes is now common worldwide, often leading to failure of treatment and control. Standard methods of gastrointestinal nematode infection (GIN) load are fecal egg counts (FEC), FAMACHA eye color chart score (FAM), and packed cell volume (PCV). Thus far, these detection methods provide the presence of GIN infection, but cannot predict whether the animals will be resistant to the administered anthelmintic after standard method detection. In *Haemonchus contortus*, a single nucleotide polymorphism (SNP) of the β-tubulin gene (TTC to TAC), causing a phenylalanine to tyrosine amino acid substitution, has been shown to be involved in many cases of resistance. This study was conducted to demonstrate simultaneous quantitation of *Haemonchus contortus* load and detection of its resistance to benzimidazole based anthelmintics. Goats exhibiting natural resistance or susceptibility to *Haemonchus contortus* infection were selected based on the standard methods described above. Total RNA and genomic DNA were extracted from stool samples and subjected to both genomic DNA PCR and RT-PCR using primers designed to target the *Haemonchus contortus* β-tubulin 1 gene SNP. Pearson Correlation coefficient analysis showed that there was a negative correlation between FAM and PCV ($P < 0.05$), β-tubulin DNA and a very low positive correlation with FEC ($P < 0.05$). The PCV showed strong negative correlation with FAM ($P < 0.05$), FEC ($P < 0.05$) and β-tubulin DNA ($P < 0.05$). These results demonstrate that the opportunity to detect *Haemonchus contortus* infection by standard methods and at the same time determine whether the animal will be resistant to anthelmintic treatment by DNA detection and quantitation will aid in saving the global livestock industry billions of dollars.

Key Words: *Haemonchus contortus*, anthelmintic resistance, DNA quantitation