ANIMAL HEALTH: **MODELS OF ANIMAL IMMUNE STATUS AND PERFORMANCE**

0827 (M016) Gastrointestinal and hepatic tissue fatty acid composition and interleukin-6 concentration in broiler chickens: Effect of maternal dietary n-3 fatty acids. C. J. Bullock, G. Bobe, and G. Cherian*, *Oregon State University, Corvallis.*

Early exposure to nutrients and fetal programming has gained increased attention because of its association with chick quality and viability. In chickens, the 21-d incubational period contributes to over 35% of a bird's life span. During this period, the egg provides polyunsaturated fatty acids (*P*UFA) to the chick embryo. We hypothesized that early exposure of n-3 and n-6 PUFA through egg lipids can alter tissue fatty acid (FA) composition and interleukin-6 (IL-6) production in the progeny chickens during growth. The objectives of the study were to determine: 1) the extent to which maternal (yolk) n-3 or n-6 FA are retained in the duodenum, jejunum, ileum, and liver tissue of the chicken, and, 2) the effect of maternal FA composition on IL-6 concentrations in serum and hepatic tissue in broiler chickens when fed a diet lacking in long chain (> 20-C) n-3 and n-6 FA during growth. Fertile eggs obtained from Lohman-Brown layer hens (n = 75) fed corn-soy diets supplemented with 3.5% yellow grease, sunflower oil or fish oil were incubated. These fat supplements were selected as sources of saturated, n-6 or n-3 FA. Chicks were raised up to d 14 on a commercial diet lacking long-chain n-6 and n-3 FA. Chick tissues (duodenum, jejunum, ileum, liver, and blood) were collected on Day 1, 7, and 14 and were subjected to FA and IL-6 analysis. The egg yolk arachidonic (20:4 n-6) and docosahexaenoic acid (DHA, 22:6 n-3) content was 3.1, 3.6, 1.0 and 1.6, 1.0, and 6.1 for eggs from hens fed yellow grease, sunflower or fish oil diets ($P < 0.001$). The DHA content in duodenum was highest up to d 14 of growth in chicks from fish oil-fed hens ($P < 0.001$). The long-chain n-6 to n-3 ratio was lowest in the duodenum, jejunum, ileum, and liver in chicks hatched from fish oil-fed hens ($P < 0.001$) up to d 14 post-hatch. A significant maternal diet by age interaction was observed for liver and serum IL-6 concentrations ($P < 0.001$). On the day of hatch, chicks from fish oil-fed hens had the lowest liver and serum IL-6 concentrations, whereas at d 14, chicks from fish oil-fed hens had higher liver and serum IL-6 concentrations than chicks from sunflower oil-fed hens ($P < 0.05$). In conclusion, our results indicate that inflammatory pathways and eicosanoid metabolism of broiler chicks can be altered by the maternal dietary ratio of long-chain n-6: n-3 FA.

Key Words: chicken, interleukin-6, n-3 fatty acids

0828 (M017) Sandwich enzyme-linked immunosorbent assay for detection of *Fasciola gigantica* excretory secretory in goats sera. H. R. Metawi*[1] and E. M. Oudah[2], [1]*Animal Production Research Institute, Agriculture Research Center, Cairo, Egypt,* [2]*Faculty of Agriculture, Mansoura University, Egypt.*

Fascioliasis is considerd a major animals health problem. Many immulogical techniques have been developed over years using the different *Fasciola* antigens for diagnosis of parasitic infection and to replace the parasitological techniques, which are time-consuming and usually proved to be inadequate and unreliable. Viable *F. gigantica* flukes were obtained from infected cows at a local abattoir and kept in suitable meduim inside CO_2 incubators to produce excretory/secretory (E/S) antigenes. The pure E/S was collected, and their protein contents were estimated. The bands of sizes 27, 30, 40, 60, and 60/62 were used to obtain suitable antibody probe to be used in isoloting target antigen of E/S origin from infected Egyptian Nubian goats sera. The 40-kilo Dalton immunogenic of E/S origin sandwich enzyme-linked immunsorbent assay is highly specific in detecting *F. gigantica* infection in Egyptan Nubian goats. It also offers a promissing diagnostic tool.

Key Words: Egyptian Nubian goats, *Fasciola gigantica*, sandwich

0829 (M018) Response of beef cows offered a chlortetracycline fortified mineral and either strip or continuous stocked to stockpiled fescue. M. S. Gadberry*[1], D. S. Hubbell, III[2], J. D. Tucker[2], T. Hess[2], P. A. Beck[3], J. Jennings[1], J. G. Powell[4], and E. A. Backes[4], [1]*Dep. of Animal Science, University of Arkansas, Little Rock,* [2]*University of Arkansas Livestock and Forestry Research Station, Batesville,* [3]*Dep. of Animal Science, University of Arkansas, Hope,* [4]*Dep. of Animal Science, University of Arkansas Division of Agriculture, Fayetteville.*

Food and Drug Administration proposed changes to the U.S. feed law addresses judicious use of medically important antimicrobials. Cattle producers grazing Kentucky 31 tall fescue (*Festuca arundinacea*) routinely feed mineral fortified with chlortetracycline (CTC). Available CTC fortified minerals often contain 3.08 g/kg CTC (350 mg CTC/113.4 g mineral consumed). Fescue management may include fall stockpiling for deferred grazing with forage being allocated using either continuous or strip stocking. This project evaluated the effect of CTC delivered in a free choice mineral supplement with either strip stocking (STRIP) or continuous stocking (CONT) on stockpiled fescue. The study design was a 2 × 2 factorial with three 2.4-ha pasture replications per treatment combination. The same mineral package was used for both the no CTC (CTC-) and CTC (CTC+) supplements. The CTC+ mineral contained 3.08 g/kg CTC. Ninety-six pregnant *Bos taurus* cows

were randomly allocated to the 12 pastures. STRIP pasture was allocated at 0.04 ha/d in 4 and 3 d strips. Grazing occurred from December 4 to January 29. The STRIP:CTC- had the least, per cow mineral intake (7.4 ± 0.43 g/d), differing ($P < 0.1$) only from CONT:CTC- and STRIP:CTC+. All other mineral intakes were similar (8.5 ± 0.43 g). Initial rising plate estimated forage allowance was 2513 ± 129.2 kg/ha. Forage utilization was not affected by either grazing method or CTC addition and averaged 47 ± 3.7%. The numerical difference in forage utilization was 44% (CONT) and 49% (STRIP). CTC+ resulted in a 22 kg greater BW change ($P < 0.05$) from the initial to interim weigh date but not interim to final (7 kg less than CTC-). Final BW and BCS were not affected by grazing management or CTC+. External body temperature and thermocirculatory index (TCI, based on rectal, skin, and ambient temperature) at the rump and ear differed between STRIP and CONT ($P < 0.05$) but was not affected by CTC+. CTC+ had a greater skin temperature between the dew claw ($P < 0.1$) at the interim but not final weigh date. CTC+ had no effect on TCI. In conclusion, grazing method did not significantly affect forage utilization, BW, or BCS but resulted in different skin temperature responses. Adding CTC to the mineral primarily resulted in an initial BW gain response without affecting final BW and BCS. Feeding CTC to cattle grazing stockpiled fescue did not result in a sustained benefit for performance or body temperature.

Key Words: chlortetracycline, fescue, strip grazing, strip stocking

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0830 (M019) Regulation of gene expression and chemotactic and phagocytic function of bovine neutrophils incubated with citrus oil and lipopolysaccharides.

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Antibiotic use is coming under increasing public scrutiny due to the possible development of resistant pathogens and risk of residues appearing in the milk. Therefore, new strategies to control or treat mastitis are warranted. Recent studies have shown that citrus derived oil (CO) inhibited growth of major mastitis-causing bacteria. However, the effect of CO on the function of bovine blood neutrophils (BBN) is currently unknown. The objective of this study was to identify the effect of CO and lipopolysaccharide (LPS) endotoxin on BBN by evaluating function and relative expression of genes in vitro. Jugular blood (~150 mL) was collected from 11 healthy Holstein cows in mid-lactation (> 100 DIM). BBN were isolated and incubated with or without 0.01% CO and 50 µg LPS/mL for 2 h at 37°C, 95% humidity, and 5% CO_2. After incubation, BBN chemotaxis and phagocytosis capabilities were determined in vitro, the cell pellet was recovered, and relative gene expression was analyzed via qPCR using the $2^{\wedge-\Delta\Delta Ct}$ method. Three pre-planned non-orthogonal contrasts were evaluated for gene expression. Non-LPS challenged BBN incubated with CO had a 47% increase ($P = 0.03$) in migration in response to IL-8 and a moderate increase in phagocytic capacity (15.9 vs. 14.2%, $P = 0.02$). This effect indicates that the CO was not impairing the function of BBN. However, the pattern of gene expression did not reflect the functional response, where BBN incubated with CO, regardless of LPS challenge, reduced expression ($P < 0.05$, fold-change (FC) $\leq$ -1.57) of several pro-inflammatory genes (IL1B, NFKB, SOD2, TNFA, and TLR2) with the exception of IL8, which tended to be up -regulated ($P < 0.06$, FC = 1.92) when compared to controls. For controls, expression of TLR4, critical for LPS recognition, was downregulated ($P = 0.03$, FC = -1.66) due to LPS although expression of *TNFA* was up-regulated ($P = 0.01$, FC = 2.62). In addition, the anti-inflammatory mechanism of CO at the gene-level does not appear to be mediated by IL10, where IL10 was downregulated ($P < 0.01$, FC = -3.78) in BBN incubated with CO when compared to controls. In conclusion, CO downregulated the expression of pro-inflammatory genes in BBN. However, CO does not appear to be inhibitory for overall BBN function in vitro. Future studies examining the effect of CO on BBN during mastitis in dairy cattle are warranted.

Key Words: neutrophil, citrus oil, genes

0831 (M020) Effect of *Penicillium* mycotoxins on bovine macrophage (BoMac) function.

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Penicillium mycotoxins (*P*M) are natural contaminants that are commonly found in improperly stored animal feeds. Although exposure to certain PMs has been reported to affect immune function, little data are available for ruminant species. Therefore, in this study bovine macrophages (BoMacs) were exposed to the following PM: citrinin (CIT), ochratoxin A (OTA), patulin (*P*AT), mycophenolic acid (MPA) and penicillic acid (*P*A), and macrophage function was assessed by measuring cytokine gene expression, the production of reactive oxygen species (ROS), and phagocytosis of *Mycobacterium avium* ssp. *paratuberculosis* (MAP), which is the causative agent of Johne's disease. Real-time PCR analysis of pro-inflammatory cytokines interleukin (IL)-1α and IL-6, anti-inflammatory cytokines IL-10 and transforming growth factor-β (TGF)-B, as well as neutrophil stimulating cytokines IL-12 and IL-23 was assessed following 6 and 24 h of PM exposure at concentrations that inhibited BoMac proliferation by 25% (IC25). The mycotoxin treatments altered the gene expression of cytokines at 24 h. Ochratoxin A induced IL-1α expression ($P < 0.05$), while IL-6 expression was suppressed ($P < 0.01$). Mycophenolic acid induced the IL-1α expression

($P < 0.05$) and reduced the expression of IL-12α ($P < 0.01$) and IL-10 ($P < 0.01$). Patulin suppressed the expression of IL-23 ($P < 0.01$), IL-10 ($P < 0.05$), and TGF-b ($P < 0.05$). Neither CIT nor PA affected the expression of these genes. The mycotoxins also affected BoMac intracellular ROS production and phagocytosis at the higher concentrations. Pretreatment with CIT at 300.0 μM increased pathogen associated molecule (*P*AM)-3-induced ROS production, which appeared to contribute to cell death. In contrast, PAT and PA significantly decreased the ROS production at concentrations ranging from 1.3 μM to 10.0 μM and from 31.3 μM to 125.0 μM, respectively; these two PMs simultaneously increased BoMac viability at 10.0 μM and 125.0 μM, respectively, even though they caused the cell death at higher concentrations. Although OTA did not affect the ROS production, an increasing trend in the phagocytosis of MAP was observed from 3.1 to 12.5 μM. In contrast, a decreasing trend in phagocytosis was observed for PAT concentrations from 2.5 to 10.0 μM. These findings suggest that exposure to sublethal concentrations of PM can alter immune function, which could affect innate antimicrobial resistance and immunoregulation.

Key Words: *Penicillium* mycotoxins, bovine macrophages, immunomodulation

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0832 (M021) The Mycobacterial Diseases of Animals (MDA) Multistate Initiative— A cooperative effort addressing animal diseases. K. E. Olson[*1], V. Kapur[2], P. Coussens[3], and D. H. Lein[4], *[1]KEO Consulting, Schaumburg, IL, [2]Pennsylvania State University, State College, [3]Michigan State University, East Lansing, [4]Cornell University, Ithaca, NY*.

Johne's Disease Integrated Program (JDIP) efforts are well-known and documented. Primary funding was through USDA grants that allowed leveraging of additional public and private resources to expand the effort. The grants have come to an end, so a plan for the future was needed. JDIP addressed many knowledge gaps, but much work remains, so a range of options for the consortium was considered. Primary objectives were to maintain the networking, collaboration and basic infrastructure developed through JDIP, allowing participants to identify, obtain, and share resources needed to address Johne's and other mycobacterial diseases. To this end, a proposal was, developed and later, approved by USDA's National Institute for Food and Agriculture (NIFA) to begin operation as Multistate Initiative: NE1201, Mycobacterial Diseases of Animals (MDA). The multi-state initiative (MI) is focused on two mycobacterial disease complexes— paratuberculosis (Johne's disease; JD) and the tuberculosis complex of diseases (TBc; i.e bovine tuberculosis). The initiative includes five objectives: 1) increase understanding of the epidemiology and transmission of MDA, including predictive modeling; 2) develop and implement new generations of diagnostic tests for JD and TBc; 3) improving our understanding of the biology and pathogenesis of MDA, as well as the host response to infection; 4) develop programs to evaluate and develop new generations of vaccines for JD and TBc; and 5) develop and deliver JD and TBc education and outreach material in electronic and print form for use by producers and other stakeholders. Use trade media, producer organizations, and other outlets to aid in dissemination of information. Projects within each objective, with cross-cutting contributions, are designed to address major animal, human, and societal issues surrounding detection and control of mycobacterial infection, including how these organisms move and spread within cattle, small ruminant, and wildlife populations.

Key Words: mycobacterial disease, Johne's, tuberculosis

0833 (M022) Up-regulation of fetal cardiac genes following persistent and transient bovine viral diarrhea virus infection. *S*. W. Hahm[*], T. R. Hansen, and H. Han, *Colorado State University, Fort Collins.*

Transplacental infection by non-cytopathic (ncp) bovine viral diarrhea virus (BVDV) during early gestation results in persistently infected (PI) fetuses with lifelong viremia. Conversely, infection of ncp BVDV later in gestation (~day 150) or after birth leads to transient infection (TI). We hypothesized that ncp BVDV infection of the dam would alter gene expression related to development of fetal heart and vascular remodeling. Gene expression in the right ventricular heart (RV) of uninfected bovine fetuses was compared to PI and TI fetuses. Naïve pregnant heifers were challenged with 2 mL of ncp BVDV (4.4 log10 TCID50/mL) on d 75 (*P*I fetus; $n = 6$) or Day 175 (TI fetus, $n = 6$) or kept uninfected (Control fetus; $n = 6$). Maternal blood ncp BVDV RNA increased in concentration following PI and TI and then diminished. Fetuses were collected via caesarean section and necropsied on d 190 of pregnancy. To examine fetal cardiac gene expression, quantitative real-time PCR was completed. Data were analyzed using PROC GLM procedure of SAS. BVDV RNA concentration in the RV was greater in PI fetuses when compared to uninfected control and TI fetuses ($P < 0.05$). BVDV was not detected in TI fetal heart because of clearance of virus between d 175 and 190. Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) act to reduce blood pressure. The mRNA concentrationsfor ANP and BNP were greater ($P < 0.05$) in RV of the TI fetuses, when compared with controls. Vascular endothelial growth factor (VEGF; angiogenic) and cyclin D1 (marker for left ventricular hypertrophy) mRNA concentrations were upregulated ($P < 0.05$) in TI fetuses. Fibroblast growth factor receptor1 (FGFR1; angiogenic) mRNA concentration was greater in RV of PI and TI fetuses ($P < 0.05$). Chemokine ligand 12 (CXCL12) and its receptor, chemokine receptor4 (CXCR4) facilitate fetal cardiac development and may assist in remodeling/repair following acute myocardial infarction. Concentrations of the mRNAs encod-

ing CXCL12 and CXCR4 tended to be upregulated in RV of TI fetuses ($P = 0.0860$, $P = 0.0951$; respectively). Maternal ncp BVDV infection may impaired fetal cardiac development via up-regulation of vascular regulatory, cardiac remodeling, and angiogenic genes regardless of TI or PI. *USDA NIFA AFRI 2008–35204–04652.*

Key Words: bovine viral diarrhea virus, fetus, heart

0834 (M023) OmniGen-AF supplementation inclusion rate independently promotes immune function in a rat model. J. A. Branson*[1,2], D. J. McLean[1], N. E. Forsberg[1], S. A. Armstrong[1], T. H. Schell[1], and G. Bobe[2], *[1]OmniGen Research, Prince Agri Products, Corvallis, OR, [2]Oregon State University, Corvallis.*

Feeding OmniGen-AF (OG; Prince Agri Products, Inc., Quincy, IL), a branded proprietary product, supports immune function in many domestic animals. Targeted profiling of immune-associated genes in whole blood is an established methodology to evaluate the efficacy of feed additives with immune-altering properties. We hypothesized that higher daily inclusion rate of OG than 0.5% may be required to optimize immune function. The objective of this study was to evaluate the effect of dietary OG inclusion rate (0.5% vs. 1.0%) on the expression profile of immune-associated genes. Male CD rats (5/treatment) weighing 180 to 200 g had ad libitum access to a diet with 0 (control), 0.5 (1×), or 1% (2×) of OmniGen-AF for 28 d. At the end of the feeding period, whole blood was collected. RNA was purified from whole blood samples and used to generate cDNA that acted as template in the Rat Innate and Adaptive Immune Responses RT^2 Profiler PCR array (SABiosciences). Using PROC GLM, we compared cDNA abundance of immune-associated genes between control and supplemented groups (0.5 or 1%) with a $P < 0.05$ cut-off value for significance. Of the 79 immune-associated genes that were expressed above the detection limit in all samples, 16 (seven up -regulated) and 13 genes (eight up-regulated) were altered by 0.5% and 1% OG supplementation, most of which (11 with six up-regulated) were altered at both OG inclusion rates. Genes that were up-regulated at both rates include IL13 (0.5%: +3.16, 1%: +3.70-fold-change), IL5 (0.5%: +2.64, 1%: +2.62), Irak1 (0.5%: +2.50, 1%: +1.98), Nod2 (0.5%: +1.83, 1%: +2.02), IFNa1 (0.5%: +1.81, 1%: +2.10), and Cd80 (0.5%: +1.77, 1%: +2.47). Genes that were downregulated at both inclusion rates include TLR3 (0.5%: -2.22, 1%: -2.39), CxCL10 (0.5%: -2.19, 1%: -2.26), STAT1 (0.5%: -2.07, 1%: -1.99), STAT3 (0.5%: -2.05, 1%: -1.92), and NFκb1 (0.5%: -1.84, 1%: -1.75). In conclusion, our results suggest that OG supplementation promotes immune function through various pathways including pathogen recognition, adaptive immune cell activation, and various transcription factors, independent of dietary inclusion rate.

Key Words: gene profiling, immunity, OmniGen-AF

0835 (M024) Effects of betaine on growth performance, carcass characteristics, and meat quality of broilers. J. Ma, W. Chang*, G. Liu, H. Cai, S. Zhang, and A. Zhen, *Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing.*

This study was to evaluate the effects of betaine on growth performance, carcass characteristics and meat quality of broilers. A total of 240 1-d-old healthy Arbor Acres male broilers were randomly allotted to four groups with six replicates per group and 10 birds per replicate by a single factor completely randomized design. The four diets included a basal diet and three experimental diets supplemented with 0.05, 0.10, and 0.20% betaine, respectively. Birds were slaughtered at 42 dold. The results showed that 0.20% betaine supplementation significantly increased ADG of broilers by 6.53% ($P < 0.05$) but did not significantly affect ADFI and F/G ($P > 0.05$), compared with the control group. Betaine supplementation at the 0.20% level improved breast muscle percentage 9.23% and decreased the values of drip loss 36.36% in breast muscle of broilers ($P < 0.05$). There was no significant difference in dressing percentage, eviscerated yield percentage and thigh muscle percentage between control group and experimental groups ($P > 0.05$). Supplementing betaine of 0.05, 0.10, and 0.20% in diets resulted in significant increase of IMP contents of 13.94, 17.94, and 12.10%, and increase of IMF content of 44.70, 42.92, and 53.14% in breast muscle of broilers, compared with the control group, respectively ($P < 0.05$). The IMF content of 0.20% betaine group was significantly higher than that in 0.05% and 0.10% betaine supplementation groups($P < 0.05$). The IMF content in thigh muscle of broilers in 0.05, 0.10, and 0.20% betaine supplementation group were improved 13.46, 28.53, and 13.75% than that in control group, respectively($P < 0.05$). The content of IMF of 0.10% betaine supplementation group was significantly higher than that in 0.05 and 0.20% betaine group($P < 0.05$). In conclusion, supplementing 0.20% betaine in diet could significantly improve ADG, carcass characteristics, and meat quality of broiler, with an exception of higher IMF content in thigh muscle in 0.10% betaine supplementation group.

Key Words: betaine, carcass characteristic, meat quality

Table 0835.

Treatment	control	0.05% betaine	0.10% betaine	0.20% betaine	*P*–value
ADFI(g/d)	104.26 ± 0.83	104.76 ± 5.85	103.31 ± 4.18	104.13 ± 2.44	0.335
ADG(g/d)	58.39 ± 1.63[a]	60.22 ± 1.47[ab]	57.73 ± 4.22[a]	62.47 ± 1.03[b]	0.029
F/G	1.84 ± 0.14	1.74 ± 0.11	1.79 ± 0.10	1.73 ± 0.02	0.224

0836 (M025) Effects of dietary polyphenols on inflammatory processes, nutrient digestibility, and microbiota in the intestine of piglets. A. Fiesel[1], D. K. Geßner[1], B. Eckel[*2], and K. Eder[1], *[1]Institute of Animal Nutrition and Nutrition Physiology, Universität Gießen, Gießen, Germany, [2]Dr. Eckel GmbH, Niederzissen, Germany.*

The weaning period of piglets is a stressful event characterized by an increased occurance of enteric infections and a pro-inflammatory intestinal condition with negative effects on feed consumption and animal growth. Recent studies have shown that polyphenolic compounds exert anti-inflammatory effects in the intestine. This study investigated the hypothesis that feeding the polyphenol-rich dietary supplements grape seed and grape marc meal extract (GSGME) or spent hops has the potential to suppress inflammatory processes in the intestine of piglets. Besides, the influence on nutrient digestibility and fecal microbiota composition should be investigated for the first time. A feeding trial with 48 5-wk-old piglets was performed. The control group received the basal diet mainly based on wheat, barley, and soybean meal; the GSGME group received the diet supplemented with 1% GSGME (AntaOx, Dr. Eckel GmbH, Niederzissen, Germany); and the hop group received the basal diet supplemented with 1% spent hops (AntaPhyt H, Dr. Eckel GmbH). Statistical analysis was done by one-way ANOVA. There were no differences in average daily gains, daily feed intake, and final body weights between the three groups. However, the gain:feed ratio was increased in the hop group ($P < 0.05$) and the GSGME group ($P = 0.15$). Moreover, both treatment groups had lower expression levels of pro-inflammatory genes (e.g., TNFα, IL-1β and IL-8) and nutrient transporters (SGLT1, GLUT2, GLUT5, PEPT1) in the mucosa of duodenum, ileum and colon ($P < 0.05$). In line with this, decreased digestibilities of crude protein and crude fiber have been observed in the hop group ($P < 0.05$). Supplementation of GSGME and hop revealed an increased fecal pH value, lower levels of volatile fatty acids (acetate, propionate, butyrate) together with changes in the fecal microbial composition with a lower amount of *Streptococcus* spp. and Clostridium cluster XIVa ($P < 0.05$). In conclusion, this study confirmed the anti-inflammatory effect of polyphenol-rich dietary supplements that can be a useful dietary strategy during weaning. It is suggested that the improved feed efficiency results from decreased inflammatory processes and might be also due to an interaction with the gut microbiota and their metabolites.

Key Words: grape seed and grape marc meal extract, hop, anti-inflammatory

0837 (M026) Effects of CO_2 and filter pore size on bovine neutrophil chemotaxis. A. M. Barnard*, R. Nebenhaus, S. Polukis, and T. F. Gressley, *University of Delaware, Newark.*

In vitro chemotaxis assays are an efficient and cost-effective way to assess neutrophil (PMN) function, but variations in the procedure may impact assay results. The aim of this study was to evaluate the effects of CO_2 and membrane pore size on chemotaxis of PMN from lactating cows ($n = 9$; 4–163 DIM). Neutrophils were isolated and adjusted to 2×10^6 cells/mL. Media consisted of HBSS supplemented with 5% FBS. The bottom wells of 48-well chemotaxis chambers (Neuro Probe Inc., Gaithersburg, MD) contained media supplemented with 50 ng/mL of complement component 5a (C5a) or 100 ng/mL of Interleukin 8 (IL-8). Polycarbonate membranes with 3, 5, or 8 μm pores separated the bottom and top wells. Neutrophil suspension (50 μL) was added to the top wells, and chambers were incubated at 37°C for 1 h in the presence or absence of 5% CO_2. Negative controls contained no chemoattractant in either well, and positive controls contained 50 ng/mL of C5a in both top and bottom wells. All combinations of cow PMN, CO_2, pore size, and chemoattractant were evaluated in triplicate wells over 6 different test dates. Raw adherence (RawAd) was determined by counting PMN adhered to the bottom of the membrane in five microscope fields per well. Relative adherence (RelAd) was calculated as (RawAd test well)/(RawAd negative control well) × 100%. Data were analyzed using the Glimmix procedure of SAS with the fixed effects of CO_2, pore size, chemoattractant, and all interactions and random effects of date and cow within date. Both RawAd and RelAd were affected by CO_2, pore size, chemoattractant, and CO_2 × pore size ($P < 0.001$), and RawAd was affected by CO_2 × chemoattractant ($P < 0.001$). Both RawAd and RelAd decreased in the presence of CO_2. RawAd increased and RelAd decreased with increasing pore size. For both RawAd and RelAd, chemotaxis to C5a and IL-8 did not differ but both were greater than chemotaxis to controls. The CO_2×pore interaction for both RawAd and RelAd was driven by a reduced increase in chemotaxis with increasing pore size when CO_2 was present. The CO_2 × chemoattractant interaction for RawAd was due to muted effects of C5a or IL-8 on chemotaxis in the presence of CO_2. Of the conditions evaluated, we propose that the use of a 3 μm membrane and incubation without CO_2 may be preferable because this combination resulted in the greatest increase in adherence relative to the negative control.

Key Words: neutrophils, chemotaxis, bovine

0838 (M027) Preliminary evaluation of the effect of a mushroom (*Coriolus versicolor*) probiotic on gene expression in goat blood. K. A. Ekwemalor*, *North Carolina Agricultuiral and Technical State University, Greensboro.*

Gastrointestinal parasites pose a serious threat to the global goat industry due to resistance of parasites to anthelmintic drugs. Oral administration of anthelimintics may activate genes in peripheral blood and impact goat health and production. *Coriolus versicolor* is a mushroom with immunostimulant properties used as a dietary supplement as an immunostimulant. CorPet biomass (Mycology Research Laboratories Ltd, UK) is a mushroom (*Coriolus versicolor*) based feed that is being used as a probiotic in horses and small animals as an immunostimualnt. White-rot fungi such as *Coriolus versicolor* are efficient lignin degraders and have been studied for their ability to ferment different crop residues to produce improved animal feed for ruminants such as goats. Although the impact of white rot fungi on animal feed has been studied the effect of their use as feed supplements on the animal needs further study. The objective of this study was to evaluate the effect of aqueous extracts of CorPet on gene activation in adult Boer goats infected with gastrointestinal parasites. Following initial screening for infection, goats were assigned to three groups of five ($n = 15$). Powdered CorPet was soaked in hot or cold water with stirring. Sterile filtered extracts were prepared. Goats were drenched daily with 10 mL of the hot (treatment I) or cold extract (treatment II) daily for a 4-wk period, and a control group of five age-matched goats received sterile water (treatment III). The groups were reversed for a further 4 wk. Body weight, PCV, fecal sample, and blood were collected in PAXgene tubes. Total RNA was isolated using the Zyomed kit. Haemonchus and coccidi were counted using a 3069 stereo microscope. There was no significant difference between the hot and cold treatment. There was an effect treatment on weight of the animals due to treatment ($P > 0.0041$). The Nanodrop spectrophotometer was used to evaluate RNA concentration and purity. The average concentration and purities of the different treatment groups for each week revealed some variation over time. Administration of Corpet as an oral drench may stimulate gene expression in peripheral blood and may impact rumen microorganisms. Further studies using more samples are needed to assess the impact on diversity and feed efficiency.

Key Words: anthelmintics, immunostimulant, CorPet

0839 (M028) Current colostrums management practices on Jersey farms in Vermont and New York State. K. M. Morrill*[1], M. M. Spring[2], and H. D. Tyler[2], [1]*Cornell University, Ithaca, NY,* [2]*Iowa State University, Ames.*

The objective of this study was to evaluate current colostrum management practices on Jersey farms in New York and Vermont. Colostrum management surveys consisting of seven general farm questions and 24 colostrum management questions were mailed to 75 dairy farms in New York and Vermont in June 2013. A total of 38 farms responded to the survey (50.66%). Of the 38 farms that responded, 10 provided calf serum for IgG analysis. Farms represented conventional (56%), organic (3%), and combinations of conventional and grazing (41%) operations. Farm size ranged from < 100 cows (67%), 100 to 199 (15%), 200 to 500 (10%), 501 to 1000 (5%), 1001 to 2000 cows (3% of respondents). Colostrum collection occurred within 1 h on 16% of farms and within 6 h on an additional 58% of farms. Fresh cows were milked most often in the same parlor as the rest of the herd (69%) and were frequently milked last (52%). Colostrum was transferred to an average of 2.32 containers (SD = 0.47) before feeding. Mean time to first colostrum feeding was 7.79 h (SD = 7.62); 24% of farms surveyed fed calves within 1 h, 33% within 2 h of birth, 35% within 6 h of birth and 8% of calves were fed within 12 h of birth. Mean colostrum consumption within the first 24 h was 3.00 L (SD = 1.11) with a range of < 1 (3% of farms) to > 4.5 L (13% of farms). Colostrum quality was a concern on 55% of the farms and was assessed on 78% of the farms. The most common methods of assessment were to evaluate color and consistency of colostrum; only one farm was using a refractometer to measure colostrum quality. The majority of farms surveyed (82%) would discard unacceptable colostrum. The following conditions led to discarding colostrum on greater than 20% of farms surveyed: mastitis, sick cow, positive for Johne's or Leucosis, watery appearance, or bloody appearance. Only one farm routinely monitored passive transfer in newborn calves. These data suggests that farms in this study are willing to discard colostrum from sick cows or visible altered (bloody); however, colostrum management practices on Jersey farms in New York and Vermont have room for improvement, primarily in timing of feeding, amount of quality colostrum fed within 24 h and assessment of passive transfer.

Key Words: colostrum, management, survey

0840 (M029) Effect of 2,4-thiazolidinedione treatment in milk production and leukocytes phagocytosis after sub-clinical mastitis induction in lactating dairy goats. *S. G. Richards*, L. Robertson, D. Dahl, L. Johnston, C. T. Estill, and M. Bionaz, Dep. of Animal and Rangeland Sciences, Oregon State University, Corvallis.*

Mastitis is one of the most costly diseases for the dairy industry. There is indirect evidence suggesting that peroxisome proliferator-activated receptor γ (PPARγ) regulates milk fat synthesis and may help to prevent mastitis. We postulate that continuous activation of PPARγ in the mammary gland can improve response to sub-clinical mastitis and increase milk fat synthesis. To test this, 25 lactating Saanen goats received either 8 mg/kg BW daily intravenous injections of the PPARγ

activator 2,4-thiazolidinedione (TZD) or saline. Following 1 wk of treatment, half of the TZD and half of the saline treated goats received intramammary infusion (IMI) of 1.7×10^8 CFU/mL of *Streptococcus uberis* (*S. uberis)* in both mammary halves (MTZD and MCTR), while the remaining goats in each group received IMI of saline (CTZD and CTRL). Animals were monitored for 12 d after IMI. Milk was collected daily during the trial to measure yield and composition, including somatic cell count (SCC). Bacteriological analysis of the milk was performed before infusion and 24 h after IMI. Rectal temperature (RT) was assessed daily after IMI. Blood was collected during the trial to assess leukocytes phagocytosis. Body weight and body condition score (BCS) were assessed weekly. Data were analyzed using GLIMMIX of SAS with TZD, IMI, time and all interactions as main effect and goat ID as random. Significance was determined with a Tukey-adjusted $P < 0.05$. Milk SCC increased significantly after IMI in goats infused with *S. uberis,* but the goats receiving TZD had an overall lower SCC. There was a significant decrease in milk yield in mastitis groups but no effect of TZD. There was a decrease in milk fat (mg/d) after IMI in MCTR, while the MTZD group maintained milk fat comparable to non-mastitis groups. The mastitis groups had a much higher percentage of milk protein than non-mastitis groups, but no TZD effect was observed. Compared to 2 d before, after IMI we observed a decrease in % blood neutrophils and increase in % lymphocytes, which was larger in goats infused with *S. uberis*. The phagocytic activity of monocytes increased more in groups infused with saline compared to *S. uberis*. No effect on body weight, BCS, and rectal temperature was observed. The results confirmed successful induction of sub-clinical mastitis, uncovered a positive effect of TZD on SCC and in preventing milk fat depression due to sub-clinical mastitis, but TZD treatment did not affect leukocytes.

Key Words: PPARγ, goat, subclinical mastitis

0841 (M030) Cross-talk between liver and mammary tissue after experimental *Escherichia coli* mastitis in Holstein dairy cows using RNaseq.

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Using RNaseq combined with bioinformatics tools, our objective was to identify cross-talk between liver and mammary tissue and key pathways altered during intramammary (IMI) challenge with *Escherichia coli* (*E. coli*). Six cows were inoculated with ~20 to 40 CFU of live *E. coli* into one mammary quarter at ~4 to 6 wk in lactation. Biopsies were performed at -144 and 24 h relative to challenge in liver and at 24 h in both rear quarters (i.e., infected and non-infected) of the mammary gland. Each sample was sequenced using a 100 bp paired-end approach. Sequence reads were aligned to the Bovine genome and the number of reads that mapped to each of the 24,616 Ensembl genes was determined. A generalized linear model was fitted for the read count of each gene and differential expression was assessed using a likelihood ratio test statistic after adjustment for multiple testing (FDR). Ingenuity Pathway Analysis coupled with the Dynamic Impact Approach analysis of differentially expressed genes (overall time effect FDR $\leq$ 0.05, post-hoc $P \leq 0.05$) indicated that IMI induced a large biological response in the liver and mammary tissue with a strong inhibition of metabolism, especially related to lipid, glucose, and xenobiotic metabolism, in the former and induction of inflammatory response/immune cells activation in the latter. Analysis of upstream regulators indicated a prominent role of several cytokines, growth factors, and transcription regulators in the two tissues' transcriptomics adaptation to IMI, clearly lipid-related and inhibited in the liver and inflammatory-related and activated in mammary tissue. The analysis uncovered a substantial cross-talk between the two tissues during IMI with a communication almost unidirectional (i.e., from mammary to the liver) via the induction of the hepatic proliferation, regeneration, and inflammatory response due to a large number of cytokines with an increased expression in the mammary gland and able to interact with highly induced hepatic receptors. The analysis indicated that only three proteins (SPP1, EPO, and GRP) with an increased hepatic expression due to IMI could potentially interact with receptors involved in leukocytes differentiation/proliferation with an increased expression due to IMI in mammary tissue. The larger enrichment of immune cell-related functions in the data from the mammary tissue suggests increased recruitment of active immune-cells to the mammary tissue. The analysis uncovered a large communication from the mammary to the liver to coordinate the inflammatory response with very few factors potentially released by the liver to control mammary gland response.

Key Words: dynamic impact approach, liver, mastitis, RNaseq

0842 (M031) Identifying the major bacteria causing intramammary infections in individual milk samples of sheep and goats using traditional bacteria culturing and Real-time Polymerase Chain Reaction. M. Rovai[1], G. Caja[1], A. Salama[*1,2], A. Jubert[3], B. Lazaro[4], M. Lazaro[3], and G. Leitner[5], *[1]Group of Ruminant Research (G2R), Universitat Autonoma de Barcelona, Bellaterra, Spain, [2]Animal Production Research Institute, Dokki, Giza, Egypt, [3]Laboratori Interprofessional Lleter de Catalunya (ALLIC), Cabrils, Spain, [4]Vacunek, Ibaizabal Bidea 800, Parque Científico y Tecnológico de Bizkaia, Derio, Spain, [5]National Mastitis Reference Center, Kimron Veterinary Institute, Bet-Dagan, Israel.*

Milk provides the major source of income in dairy farms, while one of the main causes of milk production losses is intramammary infection (IMI). Depending on the bacteria type involved, some bacteria cause clinical infection, while the majority of the bacteria cause subclinical with no visible signs of infection. Consequently, to identify infected animals, as well as the bacteria, milk sampling and laboratory diagnosis are needed. Use of DNA based methods such as real-time PCR has increased sensitivity and shortened time for bacteria identification, compared to traditional bacteriology; however, results should be regarded with caution. One-hundred eight lactating dairy ewes (Manchega, 56; Lacaune, 52) and 24 Murciano-Granadina dairy goats were used for identifying the main bacteria causing IMI using traditional bacterial culturing and real-time PCR and their effects on milk performances. Milk samples were taken aseptically from each udder-half for bacterial culture and somatic cell count (SCC) three times throughout lactation. The IMI was assessed based on bacteria isolated in $\geq$ 2 samplings accompanied by increased SCC. Mammary gland infection was caused mainly by *S. aureus* and various CNS species, and resulted in lowering milk yield and decrease of its quality as indicated by coagulation. Prevalence of subclinical IMI was 42.9% in Manchega, 50.0% in Lacaune and 41.7% in goats, estimated milk yield loss being 13.1, 17.9, and 18.0%, respectively. According to bacteriology results, 87% of the identified single bacteria colonies or culture-negative growths were repeatable throughout samplings, and bacteriology and PCR had 100% agreement. Nevertheless, the study emphasized that one sampling may not be sufficient to determine IMI, and therefore, other inflammatory responses such as increased SCC should be monitored to identify true infections. Moreover, when PCR methodology is used, aseptic and precise milk sampling procedure is the key for avoiding false positive amplifications. In conclusion, both PCR and bacterial culture methods proved to have similar accuracy for identifying infective bacteria in sheep and goats. Final methods choice will depend on their diagnosis time and analysis cost according to the farm management strategy (treatment and/or prevention of new infection).

Key Words: intramammary infection prevalence; small ruminant; real-time polymerase chain reaction

0843 (M032) Antibiotic dry-off therapy for intramammary infections in dairy sheep and goats. *M*. Rovai[1], G. Caja[1], A. Salama[*1,2], C. L. Manuelian[1], X. Such[1], M. Cervino[3], and G. Leitner[4], *[1]Group of Ruminant Research (G2R), Universitat Autonoma de Barcelona, Bellaterra, Spain, [2]Animal Production Research Institute, Dokki, Giza, Egypt, [3]Boehringer-Ingelheim España S.A., Barcelona, Spain, [4]National Mastitis Reference Center, Kimron Veterinary Institute, Bet-Dagan, Israel.*

Mammary glands are susceptible to new infections especially at the end of lactation and before parturition and control of subclinical udder infections is still an issue in small ruminants. Intramammary infections (IMI) during the dry period are likely to remain from the previous lactation to the next or develop during this period, which can lead to reducing milk production in the ensuing lactation. Ninety-four lactating dairy ewes (Manchega, 47; Lacaune, 47) and 20 Murciano-Granadina dairy goats were used to evaluate the effectiveness in reducing the prevalence of IMI with a dry-off antibiotic therapy (one syringe/gland of Mamyzin containing 100 mg of penethamate hydriodide, 280 mg of benethamine penicillin, and 100 mg of framycetin sulphate; Boehringer-Ingelheim, Sant Cugat del Vallès, Spain). At drying-off animals were classified into three groups according to the gland bacteria status: 1) N0 (non-infected animals without treatment), T0 (non-infected animals with dry-off therapy), and T1 (infected animals with dry-off therapy). The goat herd was classified into T0 and T1 groups due to the number of available animals. Prevalence of bacterial isolation from milk was determined at the following lactation (15, 40, and 60 DIM in sheep; 20 and 60 DIM in goats). Coagulase negative staphylococci were the most common isolates at dry-off. Incidence of new IMI was determined taking udder halves, previous infection information and results from all samplings after parturition. Rate of new infection was 20 and 18% for N0 and T0 sheep group, respectively. Mammary gland healthy status did not differ between N0 and T0 groups, showing that in this study, dry-off therapy did not protect against new infections in early lactation. Cure rate for the ewes infected and treated at dry-off was 84% after parturition. For goats, the used of antibiotic cured 67% of T1 group while there was no infection found in T0 does. The use of Mamyzin at dry-off showed to be effective to treat infected glands of sheep and goats, reducing IMI prevalence in the subsequent lactation.

Key Words: dry-off therapy, intramammary infection, dairy ruminants

0844 (M033) Tissue protein nitration and peripheral blood endotoxin activity are indicative of the severity of systemic organ compromise in naturally occurring clinical cases of bacterial mastitis in Holstein dairy cows. S. Kahl*, T. H. Elsasser, and G. Sample, *USDA, Agricultural Research Service, Beltsville, MD.*

The objective of this survey study was to determine a relationship between the intensity of tissue protein tyrosine nitration measured in samples of mammary gland, liver, pancreas, and lung compared to endotoxin (LPS) activity estimated in blood. Blood was collected from nine multiparous Holstein cows on confirmation by the State Diagnostic Laboratory of Maryland of mastitis and the relevant causative pathogen. In addition, control blood was collected from 17 healthy animals (cows and steers). Blood LPS activity (BLA) was estimated using an autologous neutrophil chemiluminescence-based assay (EAA, Spectral Diagnostics, Inc., Toronto, Canada) and expressed as a ratio of chemiluminescence of blood samples without and with an added reference quantity of LPS. In accordance with an approved USDA Animal Care Committee protocol, mastitis animals were subsequently euthanized and tissue collected for immunohistochemical (IHC) quantification (pixel density from digital image analysis) of antigens representative of protein tyrosine nitration (PNT) and inducible nitric oxide synthase (iNOS). Resolved pixel densities for pNT and iNOS were compared to a standardized panel of similar tissues previously obtained from healthy animals. Estimated BLA was higher ($P < 0.01$) in cows diagnosed with clinical mastitis than in healthy cows (0.354 ± 0.068 vs. 0.058 ± 0.010). All mastitic cows presented IHC evidence of pNT and iNOS in the designated lobulo-alveolar mammary tissues from both infected and noninfected quarters and peripheral presence of pNT and iNOS in liver, pancreatic islets, and lung alveolae and bronchiolar epithelial cells ($P < 0.03$ vs. control). Across the spectrum of BLA levels, correlation assessments suggested that the greatest levels of tissue pNT were associated with the higher levels of BLA. Given the known relationship between the presence of pNT in pathologic tissues and organ dysfunction, the data here suggest that mastitis generates perturbations in peripheral organs essential to proper metabolic and pulmonary function.

Key Words: mastitis, endotoxin, protein tyrosine nitration

0845 (M034) Proinflammatory responses of a hTERT-transformed, immortalized line of cultured bovine mammary epithelial cells (BME). T. H. Elsasser*[1], S. Kahl[1], D. E. Kerr[2], E. Zudaire[3], and F. Cuttitta[3], *[1]USDA, Agricultural Research Service, Beltsville, MD, [2]University of Vermont, Burlington, [3]NIH-NCI, Bethesda, MD.*

Cell Characterization: Primary cultures of BME were generated from healthy mammary glands as described (Vet Immunol Immunopath 101(3–4):191–202, 2004). Towards immortalization, BME from four cows were pooled and transfected with pCI neo-hEST2-HA, a human telomerase segment containing a neomycin/Geneticin resistance selection cassette (Cell 90:785–95, 1997). Cells were grown in DMEM +10%FBS and Geneticin (800 μg/ml) and followed through the ensuing selection growth lag to full proliferation capacity. Following 50+ passages, cells were further subcloned to increase epithelial and decrease myoepithelial cell content; the resulting culture was called ELS-321-Clone2B. For function studies and to achieve hormone and cytokine receptor access by the apical-lumenal polarized cells, cultures experiments were conducted on porous (0.4 μm) hanging well inserts coated with laminin-111. At confluence, cells had the following characteristics: tight junctions (electron microscopic confirmation of desmosomes, EpCAM-1 and E-cadherin immunostaining), expression (immunohistochemical localization) of prolactin receptor PRLr, xanthine oxidase (XO), inducible nitric oxide synthase (iNOS), and cytokeratin-18 (< 10% cells displayed myoepithelial smooth muscle actin). Proinflammatory Modeling: Confluent cells were challenged with 20 ng/ml recombinant bovine TNF-α and 200 ng/ml IL-17a. Media was collected at 0, 1, 4, and 24 h relative to challenge with respective cells on inserts fixed in paraformaldehyde for immunofluorescence analysis of nitrated proteins (PNT) and PRLr. Media content of lactate dehydrogenase progressively accumulated past time 0. The first noticeable cell response to challenge was the redistribution of PRLr followed by a reduction in numbers to < 10% T0 values. Cell pNT content was increased at T4, and progressively increased through T24. The data suggest that ELS-321–2B are well-suited to serve as an in vitro model to characterize BME responses to proinflammatory conditions.

Key Words: mammary, cell, proinflammatory

0846 (M035) A snapshot of multi-toxin contamination in feed— Summary of 37+ Analysis results for 2012–13. A. Yiannikouris*, *Center for Animal Nutrigenomics and Applied Animal Nutrition, Alltech, Nicholasville, KY.*

The large number and structural diversity of mycotoxins has impeded rapid quantification using LC-MS/MS owing to varying extraction efficiencies and interferences from feed and food matrices. Alltech researchers have successfully tackled

these challenges by developing the 37+ Analysis. This novel method provides simultaneous and accurate quantification for more than 37 mycotoxins in feed in a cost-effective manner. This approach normalizes losses during extraction and matrix suppression/enhancement by using labeled mycotoxins as surrogates and internal standards. In this analytical setting, four isotopologues were used to normalize the MS signals of known concentrations of 10 mycotoxin groups. During 2012–13, 3322 feed samples were received from across the world and subjected to the 37+ Analysis. The mycotoxin population followed a Gaussian distribution with measurable concentrations of mycotoxins detected in 99.6% of the 3322 samples (average = eight different mycotoxins/sample), with only 14 samples containing no detectable mycotoxins. The number of mycotoxins per sample at measurable concentrations ranged from 2 to 20, with ~87% of samples contaminated with 3 to 11 mycotoxins. Fumonisins closely followed by trichothecene B were the most prevalent. Trichothecenes, ergot alkaloids and other toxins, such as *Aspergillus* and *Penicillium* toxins found in stored feed accounted for 30% of the balance. Interestingly, hot spots of contamination accounted for 10s to 100s of ppm for certain mycotoxins. For the first time, analysis of the distribution of *Penicillium* toxin as well as potential synergistic compounds such as fusaric acid has been made possible. Mycotoxin concentrations were further interpreted and normalized according to known species-specific sensitivities. The latter were evaluated using principles of toxic equivalent factors used to perform risk assessment for PCBs, dioxins and furans and adapted to mycotoxins. This approach allowed evaluating the toxicological risk associated with levels of mycotoxins found in samples and normalized according to the impact of the distribution of the toxin for mixed animal species. The data showed that trichothecenes B accounted for the highest risk to animal performance or health, followed by aflatoxins (despite only accounting for 2% of mycotoxins found), ochratoxin and *Penicillium* toxins. In conclusion, the 37+ Analysis shows that the spectrum of mycotoxins that naturally contaminates feed commodities is exceedingly broad. For the first time, we are proposing a holistic strategy for accounting and reporting multiple mycotoxin contamination trends that were often neglected using other analytical approaches that focused only on a small number of contaminants.

Key Words: mycotoxin, mass spectrometry, UPLC-MS/MS, feed, contamination

0847 (M036) Identification of immune response markers to OmniGen-AF supplementation in a rat model.

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OmniGen-AF (OG; Prince Agri Products, Inc., Quincy, IL) is a branded proprietary product shown to augment immune function in ruminants and other species. Targeted profiling of immune-associated genes in whole blood is an effective platform for identification of multiple immune response markers to feed additives. The objective of this study was to identify multiple immune response markers that are increased by dietary OG throughout a 28-d supplementation period. We hypothesized that several immune-associated genes in whole blood are consistently up-regulated in a 28-d supplementation period. Fourteen male CD rats weighing 180 to200 g had ad libitum access to a diet containing 0 (control; $n = 5$, only 28 d) or 0.5% OG for 7 ($n = 4$) or 28 d ($n = 5$). Whole blood was collected at the end of the feeding period. RNA was purified from whole blood samples and used to generate cDNA that acted as template in the Rat Innate and Adaptive Immune Responses RT^2 Profiler PCR array (SABiosciences). Using PROC GLM, we compared cDNA abundance of immune-associated genes between control and supplemented groups (7 or 28 d) with a $P < 0.05$ cut-off value for significance. Of the 77 immune-associated genes that were expressed above the detection limit in all samples, six genes were up-regulated after 7 d of OG supplementation with only four genes up-regulated after 28 d of OG supplementation. Of these genes, three were up-regulated on both d 7 (Cd80: +2.40; Irak1: +2.25; Nod2: +2.08-fold-change) and 28 (Cd80: +1.77; Irak1: +2.50; Nod2: +1.83-fold-change). In conclusion, our results suggest Cd80, Irak1, and Nod2 as immune response markers are increased by dietary OG throughout a 28-d supplementation period.

Key Words: biomarker, immunity, OmniGen-AF

0848 (M037) Effects of recombinant bovine somatotropin treatment during the transition period on serum growth hormone and insulin-like growth factor 1 concentrations and liver content of lipid, triglyceride, and glycogen.

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Objectives were to evaluate the effects of treatment of peripartum Holstein cows with recombinant bovine somatotropin (rbST) on serum growth hormone (GH) and insulin-like growth factor (IGF)-1 concentrations and liver total lipid

(TL), triglyceride (TG), and glycogen (GLY) contents. Cows were assigned to one of three treatments at 253 ± 1 d of gestation: CON (0 mg/d rbST, $n = 53$), rbST87.5 (87.5 mg of rbST every 7 d, $n = 53$), or rbST125 (125 mg of rbST every 7 d, $n = 53$). Treatments were given weekly from d −21 to 28 relative to calving. Milk production data was collected weekly until 150 d postpartum. Blood was sampled weekly from d −21 to 21 relative to calving for determination of GH and IGF-1 concentrations. Liver biopsies were performed in a sub-group of cows ($n = 10$/treatment) at −21, −7, and 7 d relative to calving for determination of liver contents of TL, TG, and GLY. Continuous data were analyzed by ANOVA using the PROC MIXED. Treatment did not ($P = 0.75$) affect milk production (CON = 45.9 ± 1.3, rbST87.5 = 45.8 ± 1.4, rbST125 = 47.1 ± 1.3 kg/d) but there was an interaction between treatment and day ($P < 0.01$). Treatment affected GH concentration (CON = 13.0 ± 0.9, rbST87.5 = 16.1 ± 0.9, rbST125 = 18.2 ± 0.9 ng/ml; $P < 0.01$). Concentration of GH of rbST125 cows tended to be higher than rbST87.5 cows ($P = 0.11$), and rbST87.5 ($P = 0.02$) and rbST125 ($P < 0.01$) cows had higher GH concentrations than CON cows. Treatment tended ($P = 0.06$) to affect IGF1 concentration (CON = 84.6 ± 3.5, rbST87.5 = 92.9 ± 3.5, rbST125 = 96.2 ± 3.5 ng/ml). IGF-1 concentration was not ($P = 0.50$) different between rbST87.5 and rbST125 cows, but rbST87.5 cows tended to ($P = 0.10$) and rbST125 cows had ($P = 0.02$) greater IGF1 concentration than CON cows. The interaction between treatment and day ($P = 0.01$) demonstrated that the rbST treatment effect on IGF-1 concentration was observed primarily before calving. There was no effect of treatment on TL ($P = 0.80$) and TG ($P = 0.24$) liver content. Although GLY liver content was not ($P = 0.19$) affected by treatment, rbST125 cows tended to have greater GLY liver content compared to rbST87.5 ($P = 0.10$) and CON ($P = 0.13$) cows at −7 and 7 d relative to calving. Peripartum treatment with rbST increased serum GH concentration during the peripartum period and serum IGF1 concentration prepartum. The increased GLY liver content in rbST treated cows may indicate a more efficient use of fatty acids and sparing of glycogen as a consequence of rbST treatment.

Key Words: transition cow, recombinant bovine somatotropin, metabolism

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0849 (M038) Vitamin D signaling enhances expression of antibacterial β-defensin genes in bovine monocytes.

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Vitamin D contributes to immunity of cattle via an intracrine vitamin D pathway that is activated in macrophages in response to recognition of pathogens. The 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) that is produced in that pathway activates the vitamin D receptor and regulates the transcription of vitamin D-dependent genes. RNA sequence analysis of $1,25(OH)_2D_3$–treated monocytes compared to control-treated monocytes identified bovine β-defensin 3 (DEFB3) and β-defensin 6 (DEFB6) as potential targets of the activated vitamin D receptor. The DEFB3 and DEFB6 genes encode for small antibacterial peptides and are located on bovine chromosome 27 along with DEFB1, DEFB4A, DEFB5, DEFB7, DEFB10, enteric β-defensin (EBD), lingual antimicrobial peptide (LAP), and tracheal antimicrobial peptide (TAP). The objective of this study was to evaluate the effects of $1,25(OH)_2D_3$ on expression of the β-defensin genes in resting and stimulated monocytes. Peripheral blood monocytes from eight Holstein cows were treated with 0 or 100 ng/mL lipopolysaccharide (LPS) in combination with 0 or 10 nM $1,25(OH)_2D_3$ and cultured for 24h. The mRNA transcripts for each of the β-defensin genes were measured with real-time PCR and normalized to ribosomal protein S9 transcript abundance. The effects of $1,25(OH)_2D_3$ and LPS on expression of each of the β-defensin genes was analyzed with a general linear model that accounted for effects of cow and treatment. In the non-stimulated monocytes, the $1,25(OH)_2D_3$ treatment increased DEFB3, DEFB6, DEFB7, and DEFB10 gene expression ($P < 0.05$; 10 ± 3, 17 ± 8, 4 ± 1, and 5 ± twofold change ± SE, respectively). Similarly, the $1,25(OH)_2D_3$ treatment increased DEFB3, DEFB6, DEFB7, and DEFB10 gene expression in the LPS-stimulated monocytes ($P < 0.05$; 10 ± 3, 60 ± 28, 7 ± 2, and 20 ± sixfold change ± SE, respectively). DEFB1, DEFB4A, DEFB5, DEFB13, EBD, LAP, and TAP were not affected by $1,25(OH)_2D_3$ in either resting or LPS-stimulated monocytes. In addition, LPS treatment alone did not significantly increase any of the β-defensin genes evaluated in this study ($P > 0.05$). In conclusion, $1,25(OH)_2D_3$ induces expression of the DEFB3, DEFB6, DEFB7, and DEFB10 genes in bovine monocytes. Upregulation of these β-defensin antimicrobial genes in response to $1,25(OH)_2D_3$ suggests vitamin D is needed in cattle to support innate host defense mechanisms.

Key Words: vitamin D, innate immunity

0850 (M039) Effects of genotype and transportation stress on cytokine gene expression in steers.

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The objective of this study was to determine effects of transportation stress and single nucleotide polymorphisms (SNP) in the coding sequence of two stress indicators, cytochrome P450 and the prolactin promoter region (C994G and C1286T, respectively), on gene expression of the prolactin receptor (PRLR) and three cytokines: monocyte chemoattractant protein (MCP-1 or CCL2), interleukin-8, and tumor necrosis factor (TNF)-&α. Relative gene expression was quantified

by real-time reverse transcription PCR using custom-made TaqMan assays for each gene of interest (Applied Biosystems, LifeTechnologies, Carlsbad, CA) in peripheral blood mononuclear cells of Gelbvieh x Angus crossbred steers (n = 47; mean body weight = 303 ± 39 kg; mean age = 294 ± 19 d). Three time points relative to transport (T): 27 h before (T−27), and 6 and 20 h after transport (T+6 and T+20, respectively) from Booneville, AR to Stillwater, OK (386 km) were evaluated. Time relative to transport, genotype, and their interaction affected ($P < 0.05$) gene expression. A C1286T x time interaction affected ($P < 0.01$) CCL2 expression: lowest level was in CT steers at T+6. Time affected ($P < 0.01$) PRLR and TNF-&α; expression in both C994G and C1286T genotypes: highest level was at T+20, whereas expression at earlier time points was similar. Genotype affected CCL2 expression on both C994G and C1286T genotypes: expression levels were lowest ($P < 0.0001$) when steers were CT for the C1286T SNP, or CC ($P < 0.01$) for the C994G SNP. Genotype effects on PRLR and TNF-&α expression were evident only in C1286T genotypes: greatest ($P < 0.01$) expression of both genes was in CC steers. Our findings suggest that genotypes at C994G and C1286T SNP sites influence cytokine gene expression and may therefore be used as biomarkers for animal tolerance to shipping stress. Investigating effects of other stressors before and/or after transport on the expression of other stress response genes will help identify critical control points when developing practical and/or therapeutic measures to reduce transport stress.

Key Words: cytochrome P450, cytokine, prolactin

0851 (M040) prevalence and molecular identification of *Cryptosporidium* spp. in lambs on the Huasteca Alta region, State of Veracruz, México. S. S. Gonzalez[1] and I. Vitela-Mendoza*[2], [1]*Colegio de Postgraduados, Montecillo Estado de México,* [2]*Instituto Tecnologico El LLano, Aguascalientes, México.*

Cryptosporidium is a protozoan parasite that causes enteric infection in several mammalian species, including humans. This infection has a major impact in immunocompromised domestic mammals and public health because the parasite oocysts are resistant to environment and can contaminate food and water. In sheep, cryptosporidiosis is presented with mild to severe yellowish diarrhea, plus weight loss, depression, abdominal pain, and eventually the animal may die; usually, it is more common in lambs 1 to 30 d old. Therefore, the objective of this study was to determine the prevalence of *Cryptosporidium* spp., and identify the species of the oocysts in lambs maintained in extensive grazing systems at the Huasteca Alta region, Veracruz, México. From March to June 2012, 210 fecal samples were collected from Blackbelly x Pelibuey lambs 7 to 21 d old from 21 flocks located in seven locations at the Huasteca Alta region. The samples were processed by performing a fecal smear and then dyed by the Kinyoun acid-alcohol resistant staining, and then were observed with a microscope (LCD Digital Leica) at 100 X. A molecular diagnosis was performed using nested PCR to amplify the region of the 18S rRNA gene of the parasite (830 bp), and the positive samples were sequenced. The overall prevalence of *Cryptosporidium* spp. infection in lambs was 19.5%: 10.5% in 7 to 14 d old, and 9% in 15 to 21 d old. The prevalence in flocks ranged from 10 to 40%, and in 62% of them there was at least one infected lamb. In the seven locations positive lambs were detected, and the highest prevalence was observed in Tamiahua (36.67%). The sequenced samples had a homology between 96 and 100% with the 18S rRNA region of *C. parvum*. This is the first report of the presence of *C. parvum* in lambs in the Mexican tropic.

Key Words: *Cryptosporidium*, sheep, genotyping.

0852 (M041) Bacteriological culture and California Mastitis Test results of non-clinical quarters from cows with clinical mastitis. A. Lago[1] and N. Silva-del-Rio*[2], [1]*DairyExperts, Tulare, CA,* [2]*VMTRC, University of California, Tulare.*

The economic value of lactation therapy of subclinical mastitis with antibiotics is generally considered to be limited because of the cost of milk discarded during the withdrawal period. However, the treatment of quarters affected with subclinical mastitis when treating other quarters affected with clinical mastitis does not result in additional discarded milk. The objectives of this study were: a) describe bacteriological culture results of non-clinical quarters in cows affected with clinical mastitis; and, b) validate the California Mastitis Test (CMT) to identify subclinically infected quarters in these cows. A total of 109 cows with clinical mastitis from two dairy herds were identified with clinical mastitis at the general parlor and moved to a hospital pen, where they were milked at a hospital parlor. At the first milking at the hospital parlor, the CMT was performed and a milk sample was aseptically collected for culture from all quarters (clinical and non-clinical). Bacteria were isolated from 52% of the 110 quarters affected with clinical mastitis and from 35% of the other 319 non-clinical quarters. Coliforms (35%), non-agalactiae Streptococcus (25%), and coagulase-negative Staphylococcus (23%) were the bacteria most commonly isolated from clinical quarters. In non-clinical quarters coagulase-negative Staphylococcus (49%), *Bacillus* spp. (21%) and non-agalactiae Streptococcus (13%) were the bacteria most commonly isolated. Of all cows with clinical mastitis, 67% of them had bacteria isolated from at least one of the three non-clinical quarters. If also considering the quarter affected with clinical mastitis, 82% of the cows had at least one quarter infected. The CMT was evaluated for three diagnostic interpretations: identify bacterial growth, identify gram-positive bacterial growth, and identify non-agalactiae streptococci infections. The sensitivity of the CMT was 38, 43, and 29%, and the specificity 71, 70, and 68% for each one of the diagnostic interpretations, respectively. Therefore, the

CMT was not a sensitive enough tool for the identification of subclinically infected quarters in cows with clinical mastitis. The high proportion of infected non-clinical quarters in cows with clinical mastitis may warrant the need to evaluate the efficacy and cost-benefit of treating clinical and subclinical quarters simultaneously.

Key Words: clinical mastitis, subclinical mastitis, California Mastitis Test

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0853 (M042) Effect of early feed restriction programs on IgY production of broiler chickens. M. L. Moraes*, F. M. Butzen, M. M. Vieira, C. M. M. Pimente, and A. M. L. Ribeiro, *Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.*

It is known that feed restriction increases antibody production in poultry, but very little research exists exploring the after effects of feed restriction on the adaptive immune system. The objective of this experiment was to evaluate the effects of different programs of early feed restriction on IgY production in broiler chickens. A total of 384 Cobb 500 male broiler chicks were randomly assigned to 24 pens at d 1 of age. The study lasted 42 d. After a week adaptation period, four treatments were applied from d 8 to 16: T1– control group, standard diet (SD); T2– quantitative feed restriction (80% of ad libtum feed intake of the SD, according to the breeder management guide); T3– feed restriction by time (SD offered during 8 h/day); and T4– qualitative feed restriction (80% of the limiting nutrients; SD was diluted by the addition of 10% kaolin and 10% rice husk). At d 7 and 21, birds were injected with bovine serum albumin (BSA), and blood samples were collected weekly from d 7 to 42 for assessment of IgY anti-BSA production. There was no effect ($P > 0.05$) of the first BSA inoculation between treatments; however, at 28 d of age, birds in all the three early feed restriction programs had higher IgY anti-BSA than the control group ($P < 0.05$). At d 35, the quantitative and the by time feed restriction groups still showed residual effects of the BSA injection, but no difference ($P > 0.05$) was observed between the four treatments at 42 d. It is concluded that the three different programs of early feed restriction had beneficial effect on the humoral immune system after the restriction program had ended. Overall, the quantitative and the by time feed restriction programs promoted the longer lasting responses.

Key Words: chicken, feed restriction, IgY