T14 Results from the Washington State bovine viral diarrhea virus voluntary control project. J. R. Wenz*, D. A. Moore, H. L. Neibergs, and J. S. Neibergs, *Washington State University*, *Pullman*.

Control of bovine viral diarrhea virus (BVDV) in beef cow-calf herds has been predicated on the identification and removal of persistently infected (PI) animals. Calves are born PI following exposure to BVDV during approximately 40 to 140 d of gestation and are the primary source of BVDV in the herd. The purpose of the BVDV voluntary control project was to educate producers, facilitate herd ear notch testing and implementation of control measures. From January 2008 to December 2009, 12 producer meetings were held at county and state beef producer meetings and a brochure detailing the project was mailed to 1700 members of the Washington Cattlemen's Association. Sixty herds from 18 counties enrolled and completed testing. Ear notch samples were screened by PCR on pools of up to 36 and PI positive samples were identified by a positive commercial antigen capture ELISA test. All PI animals were ELISA positive on 2 ear notch samples collected 2 to 3 weeks apart. Of the 60 herds tested 13.3% had at least one PI. Prevalence of PI animals was 0.81% (80/9881). For calves tested, 0.092% (79/8624) were PI and 1/1257 (0.08%) other cattle (yearlings, bulls, open cows) tested were PI. Only 2/79 (2.5%) dams of PI calves were PI. Two herds contributed 87% of the PI calves. In one herd 12.7% of 213 calves were PI and 52% of 83 calves were PI in the other. Excluding these 2 herds resulted in a PI calf prevalence of 0.12%. The herd prevalence was higher than previous reports from the US and may have been due to enrollment bias (suspected BVD in herd, subsidized testing). The prevalence of PI in calves, excluding the high prevalence herds, was similar to previous reports. A yearling PI was identified, highlighting the importance of testing all animals that may contact pregnant cows in the herd. Except for the herd with 52% PI calves, all PI animals were immediately removed from the herd. Three PI positive herds screened the subsequent year's calf crop and no PI calves were found. Low enrolment in the project suggests cow-calf producers in the state did not perceive sufficient risk posed by BVDV to warrant the cost of whole herd testing.

Key Words: BVDV, persistent infection, beef

T15 Effects of source and level of energy on the immune competence and response to an infectious bovine rhinotracheitis virus (IBRV) challenge in cattle. L. R. Schwertner*[1], L. E. Hulbert[1,2], J. A. Carroll[2], M. L. Galyean[1], 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.*

Objectives were to evaluate how dietary energy level and source affect immune competence and response to an IBRV challenge in cattle. Forty–8 crossbred beef steers were stratified by BW within 2 periods and randomized to 1 of 3 dietary treatments (8 steers/treatment within period). Treatments were: a 70% concentrate diet fed ad libitum (70AL); a 30% concentrate diet fed ad libitum (30AL); and 70% concentrate diet restricted to the NEg intake of 30AL (70RES). Ex vivo immune competences were evaluated after treatments were applied for 28 d, after which cattle were moved into individual pens (d 28 to 40) and intranasally challenged with IBRV on d 30. On d 34, all cattle were offered a 50% concentrate diet ad libitum until d 50. Both energy source ($P < 0.02$) and level ($P < 0.04$) affected peripheral blood mononuclear cell synthesis of tumor necrosis factor-α (TNF- α), with cell culture supernatant concentrations averaging 2,264, 1,887, and 1,241 pg/mL for 70AL, 70RES, and 30AL, respectively. Neither whole blood killing of *Mannheimia hemolytica* nor neutrophil oxidative burst in response to *M. hemolytica* were affected by treatments. Rectal temperature following IBRV peaked 3 d post challenge and returned to baseline by d 6, but it was not affected by treatment. After switching cattle to the 50% concentrate diet on d 4 after the IBRV challenge, there were no differences in DMI while the cattle were individually penned. When cattle were group–penned 10 d after the IBRV challenge, the 70RES cattle had greater DMI ($P < 0.04$). Following the IBRV challenge, serum glucose concentrations did not differ among treatments; however, the 70AL cattle had greater blood urea N concentrations ($P < 0.01$). There was a treatment × time interaction ($P < 0.01$) for NEFA; such that cattle fed the 70AL had elevated NEFA on d 3 and 5 after IBRV. Data indicate that cattle fed higher energy diets and to an extent a higher percentage of concentrates had a more pronounced pro-inflammatory response, but other aspects of innate immune competence were not influenced by level or source of energy.

Key Words: bovine, dietary concentrate, immune

T16 The effects of dam parity and antibiotics on immune parameters and gastrointestinal bacterial diversity in weanling pigs. E. E. Hinkle*, H. Tran, J. W. Bundy, R. Moreno, P. S. Miller, J. Walter, and T. E. Burkey, *University of Nebraska*, *Lincoln*.

The progeny of first parity (P1) dams may have reduced growth performance compared with the progeny derived from mature dams (≥P2). A 42-d experiment was conducted to evaluate the effect of dam parity and antibiotics on immune parameters and the gastrointestinal (GIT) microbiota among progeny derived from P1 or P4 dams. Weaned pigs (n = 96, 6.02 ± 0.07 kg) initially derived from P1 or P4 dams were allotted to 2 dietary treatments: control (CTL) or antibiotic (50 g/ton Mecadox; AB). This created a 2 × 2 factorial with the following treatments: 1) P1, CTL; 2) P1, AB; 3) P4, CTL; and 4) P4, AB (6 pigs/pen, 4 pens/trt). Blood samples were collected on d 0 and weekly thereafter for quantification of serum immunoglobulin (Ig) G and A via porcine specific ELISA. There was no effect of parity or AB, or their interaction ($P > 0.05$) for IgG concentrations; however, a parity × AB interaction ($P < 0.05$) was observed for circulating IgA where P1 pigs had decreased serum IgA concentrations when fed AB, while P4 pigs had greater serum IgA concentrations when fed AB. The fecal microbiota at d 7 and 42 was characterized by denaturing gradient gel electrophoresis. Bionumerics software was used to calculate similarity and diversity indices. The similarity index represents the percentage of the microbial fingerprint that is similar within a group (P1 vs. P4). The diversity indices (Shannon′s and Simpson′s) of the gut microbiota can be inferred from DGGE fingerprints by using Shannon′s and Simpson′s indices. A greater Shannon′s index signifies a more diverse microbial population, while a lower Simpson′s index indicates a greater microbial diversity. No differences were observed in bacterial population similarity on d 7 or 42. Significant differences (Shannon′s, $P = 0.03$; Simpson′s, $P = 0.09$) in bacterial species diversity were observed on d 7 and 42 among progeny derived from P1 compared with P4 dams, while AB administration had no effect. The results obtained in our study suggest that immune parameters and gastrointestinal bacteria may be affected by dam parity.

Key Words: bacterial diversity, dam parity, immunoglobulins

T17 Serum IgG concentrations and performance, incidence of diseases, and risk of death in pre-weaned Holstein calves. M. C. Perdomo* and J. E. P. Santos, *University of Florida*, *Gainesville*.

Holstein calves, 782 females and 200 males, housed in individual hutches received 5.7 L of frozen-thawed colostrum in the first 24 h of life. From d 1 to 21 of age, calves were fed 1.9 L of pasteurized whole milk 3 times daily, and twice daily thereafter until 60 d of age, when they were weaned. Calves were fed grain ad libitum, and amounts offered and refused were measured daily during the first 70 d of age. Body weights (BW) were measured on d 1, 30 and 70 of age. Blood was sampled 48 h after colostrum feeding and serum was analyzed for concentrations of total immunoglobulin (Ig) G using a single-radial immunodiffusion assay. Rectal temperature was taken daily in the first 10 d of age, and again at every diagnosis of disease. Feces were scored daily for detection of diarrhea. Respiratory disease was evaluated based on respiration rate, nasal discharge, and coughing reflex. Duration of disease events and treatment costs were measured. Hazard of death was analyzed using the Cox′s proportional model using IgG and gender as predictors for survival. Failure of passive transfer (PT; IgG < 1.5 g/dL) was used as predictor of grain intake, BW, grain conversion into BW, and treatment cost. Rate of death decreased ($P < 0.01$) 29% for every 1 g/dL increase in serum IgG concentration (hazard rate = 0.71, 95% CI = 0.56–0.90). Calves with failure (IgG < 1.5 g/dL) or adequate (IgG > 1.5 g/dL) PT had, respectively, 11.9 and 5.7% mortality ($P < 0.02$), and 45.2 and 35.9% more than 1 disease event ($P < 0.05$). Disease treatment costs ($/calf) were 6.05 and 4.90 for failure and adequate PT ($P = 0.08$). For calves with failure and adequate PT, BW gain in the first 30 d (295 vs. 330 g/d, $P = 0.02$) and BW at d 60 (73.4 vs. 75.5 kg; $P = 0.05$) differed; however, failure of PT did not influence ($P > 0.10$) BW gain from 30 to 60 d (693 vs. 666 g/d), grain dry matter intake in the first 30 d (104 vs. 107 g/d) or from 30 to 60 d (901 vs. 914 g/d), or efficiency of conversion of grain into BW. Improving IgG absorption decreased the risk of mortality and treatment costs, and improved weaning weight, but it did not influence grain intake or feed conversion.

Key Words: calf, health, IgG

T18 Effects of live and killed *Mycoplasma gallisepticum* vaccines prior to an F-strain *Mycoplasma gallisepticum* overlay on the reproductive and digestive organ characteristics of commercial layers. R. Jacob*[1], E. D. Peebles[1], J. D. Purswell[2], and S. L. Branton[2], [1]*Mississippi State University*, *Mississippi State,* [2]*USDA-ARS, Poultry Research Unit*, *Mississippi State, MS*.

The effects of prelay vaccinations of ts-11 strain *Mycoplasma gallisepticum* (MG), MG-Bacterin, or their combination, when overlaid with F strain MG (FMG) post-peak production, on the digestive and reproductive organ characteristics of commercial layers were investigated. A total of 160 Single Comb White Leghorn layer hens were used. In each of 16 isolation units (pens), 10 birds were housed, with 4 replicate units in each of 4 treatments. The following treatments were utilized at 10 wk of age (woa): 1) Control (no vaccinations); 2) ts-11 strain MG, (Mycoplasma Gallisepticum Vaccine); 3) MG-Bacterin (MG-Bac); and 4) ts-11 strain MG /MG-Bacterin combination. At 45 woa, the birds in 2 replicate pens were challenged with a 99th passage of FMG, increasing the number of treatments to 8. A completely randomized experimental design was used. PCR tests using choanal swab samples confirmed the presence of MG in vaccinated birds and its absence in non-vaccinated birds. Necropsies were performed at the end of the trial (58 woa), using 2 birds per replicate pen (4 birds per treatment). Parameters examined included BW; liver, ovary, oviduct and small intestine weights; ovarian follicular hierarchy; and the lengths and weights of the components of the oviduct and small intestine. Results indicated that there were no significant treatment differences ($P > 0.05$) for any of the parameters investigated. Mean relative oviduct weight in control birds with and without FMG and birds given the combinatorial treatment with and without FMG were 4.18, 4.00, 4.00, 4.23% (SEM = 0.24), respectively. In conclusion, the individual or combinatorial use of ts-11 strain MG vaccine and MG-Bacterin, when administered during prelay, are effective in preventing possible adverse effects on the reproductive and digestive organs in response to a post-peak production challenge of FMG.

Key Words: layers, *Mycoplasma gallisepticum*, vaccine

T19 Discovery of differentially expressed microRNAs in porcine reproductive and respiratory syndrome (PRRS) virus infected alveolar macrophages. J. A. Hicks, N. Trakooljul, and H. C. Liu*, *North Carolina State University*, *Raleigh*.

Porcine reproductive and respiratory syndrome (PRRS) has a major impact on the swine industry. PRRS is characterized by abortions in pregnant sows and respiratory disease, particularly in young pigs. The causative agent is the arterivirus, porcine reproductive and respiratory syndrome virus (PRRSV). Determination of alterations in host gene expression upon PRRSV infection will provide a better understanding of the pathogenesis of the virus. It is now well-established that small RNAs are an important class of gene regulators. MicroRNA (miRNA, ~22nt) is a family of small RNAs that post-transcriptionally regulate gene expression. Studies have found that viral infections induce changes in the expression of miRNAs of infected host cells. These miRNAs often target genes associated with the immune response. The goal of the present study is to determine changes in host miRNA expression during PRRSV infection. Alveolar macrophages (SAMs) were isolated from seven 8-wk-old pigs. SAMs were maintained for 24hrs in RPMI 1640 with 10% FBS and then infected with PRRSV strain VR-2332 at an m.o.i. = 10. Infected and uninfected SAMs were collected at 24 or 48 h p.i. MiRNA expression analysis was carried out using the miRScript RT-PCR system. Over 40 miRNAs were found to be differentially expressed at either 24 h or 48 h p.i. upon PRRSV infection compared with non-infected cells. The expression of these miRNAs is dynamic, as some miRNAs are altered early (24hrs p.i.) while other miRNAs are differently expressed late (48hrs p.i.) upon infection. Among these are known immune miRNAs, including miR-146a and members of the miR-17–92 cistron, as well as miRNAs which have not previously been associated with viral infections, such as miR-130b and miR-342–3p. Target prediction and subsequent validation via luciferase assay of selected miRNAs suggest that these miRNAs target not only immune genes but also genes associated with intracellular signaling and trafficking. Our data indicates that miRNAs play a role(s) during PRRSV infection by affecting gene expression associated with PRRSV pathogenesis.

Key Words: PRRSV, microRNA, gene regulation

T20 Development of mouse monoclonal antibodies specific for chicken interleukin-18 (IL-18). Y. H. Hong*[1], H. S. Lillehoj[2], S. H. Lee[2], M.-S. Park[2], J. LaBresh[3], D. Tompkins[4], and C. Baldwin[4], [1]*Department of Animal Science and Technology, Chung-Ang University*, *Anseong, Gyeonggi-do Republic of Korea*, [2]*Animal and Natural Resources Institute, Agricultural Research Service-USDA*, *Beltsville, MD*, [3]*Kingfisher Biotech, Inc.*, *St. Paul, MN*, [4]*Department of Veterinary and Animal Sciences, Paige Laboratory, University of Massachusetts*, *Amherst*.

Two mouse monoclonal antibodies (mAbs) which are specific for chicken interleukin-18 (chIL-18) were produced and characterized

by enzyme-linked immunosorbent assay (ELISA), Western blotting, quantitative real-time PCR and functional assays. The mAbs specific for chIL-18 identified a 23 kDa yeast-expressed chIL-18 and a 66 kDa *E. coli*- MBP fusion protein by Western blot analysis. Bioassays for chIL-18 were carried out to evaluate its ability to induce IFN-γ production in primary chicken spleen cells, and nitric oxide (NO) secretion in the HD11 macrophage cell line. Two mAbs showed neutralizing activity. Taken together, we have developed mouse monoclonal antibodies specific for chicken IL-18. These immune reagents will be useful tools to analyze IL-18 secretion during infections and to do basic and applied research for poultry.

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Key Words: chicken, IL-18, monoclonal antibody

T21 Influence of two different doses of infectious bovine rhinotracheitis virus (IBRV) on immune and physiological parameters in steers. S. M. Falkenberg*[1], T. B. Schmidt[1], T. Elsasser[4], J. L. Sartin[3], J. O. Buntyn[1], and J. A. Carroll[2], [1]*Mississippi State University*, *Mississippi State*, [2]*Livestock Issues Research Unit, USDA-ARS*, *Lubbock, TX*, [3]*Auburn University College of Veterinary Medicine*, *Auburn, AL*, [4]*Bovine Functional Genomics, USDA-ARS*, *Beltsville, MD*.

To evaluate the effects of IBRV on immunological and physiological parameters of cattle, 18 Holstein steers (450.11 ± 75.70 kg) were randomly assigned to either a Control group (Cg) or 1 of 2 IBRV challenged groups. Prior to the challenge, steers were fitted with indwelling rectal probes, BW recorded, and a blood sample obtained. On d 0, steers received either an intra-nasal dose of IBRV [3 mL/nostril (IBR1) or 4mL/nostril (IBR2); Cooper strain, 1 X 10^7 PFU/ml] or saline (3 mL/nostril; Cg). IBRV steers were placed in a paddock that was isolated from the Cg as well as all other cattle on the research farm. Blood samples were collected via jugular venipuncture every 24 h on d 1 and 2, and every 12 h on d 3, 4, 5, 6, and 7 post-challenge. All IBRV steers had elevated rectal temperatures ($P < 0.05$) as compared with Cg by d 2, returning to baseline on approximately d 5. Serum was analyzed for interleukin-6 (IL-6), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), growth hormone (GH) and insulin-like growth factor-1 (IGF-1). An increase in IFN-γ for the overall effect of IBRV as compared with the Cg ($P < 0.05$) was observed though no differences were found for IFN-γ in IBR1 vs. IBR2. Where a numerical increase in the mean concentrations of TNF-α and IL-6 were measured in IBRV vs Cg, the response was not different ($P > 0.05$). Furthermore, no differences ($P > 0.05$) in mean concentrations of GH and IGF-1 were found between Cg and IBRV. Results indicate that both doses of IBRV elicited immune responses; however there were no measured differences between the 2 dose concentrations. Collectively, the data suggest that measurable immune responses to IBRV at the doses chosen may be highly selective in regard to cytokine and metabolic parameters.

Key Words: cytokines, IBRV, dose response

T22 The effect of thymol on reactive oxygen species production by bovine neutrophils. L. M. Nemec*, C. Wu, S. Cordova, K. Davison, and T. F. Gressley, *University of Delaware*, *Newark*.

Neutrophils produce intracellular and extracellular reactive oxygen species (ROS) to destroy engulfed and extracellular bacteria, respectively. However, extracellular ROS production during mastitis can also damage healthy tissue and impede recovery. This study evaluated the effect of the antioxidant thymol on intracellular and extracellular ROS production by bovine neutrophils. Neutrophils were activated with phorbol myristate acetate (PMA) or zymosan in the presence of 0, 0.0001, 0.001, 0.01 or 0.1 g/L thymol. Neutrophil ROS production was assessed by measuring luminescence or fluorescence (arbitrary units) every 5 min for 75 to 120 min following addition of 1 of 4 reagents: luminol (LUM) for measurement of total ROS (intracellular+extracellular); isoluminol (ISO) for extracellular ROS; methyl cypridina luciferin analog (MCLA) for extracellular superoxide; 5-(6)-chloromethyl-2′, 7′-dichlorodihydrofluorescein diacetate, acetyl ester (CMH2) for intracellular ROS. All activator/thymol combinations were assessed using neutrophils isolated from 4 different cows. Area under the curve (auc) for each activator/thymol/cow combination was calculated and log-transformed before statistical analysis. The MIXED model of SAS included fixed effects of thymol concentration and activator and random effects of assay date and cow. Least squares means and *P*-values for overall thymol effect and linear and quadratic thymol contrasts are presented in the table. Overall, addition of thymol to activated neutrophils decreased extracellular ROS and increased intracellular ROS, which may reduce tissue damage during mastitis without negatively impacting neutrophil killing.

Table 1. Effect of Thymol on Neutrophil ROS Production

	LUM, log(auc)	ISO, log (auc)	MCLA, log (auc)	CMH2, log (auc)
Thymol, g/L				
0	5.61	4.82	4.05	5.35
0.0001	5.56	4.88	4.25	5.35
0.001	5.54	4.73	4.30	5.35
0.01	5.38	4.12	4.49	5.41
0.1	4.80	3.38	3.24	6.20
P-values				
Thymol	0.001	0.001	0.94	0.001
Linear	0.001	0.001	0.06	0.001
Quadratic	0.001	0.002	0.002	0.001

Key Words: neutrophil, thymol, reactive oxygen species

T23 Bovine hepatic and adipose retinol binding protein gene expression. P. Rezamand*, K. M. Hunt, R. D. Schramm, and M. A. McGuire, *University of Idaho*, *Moscow*.

Retinol is primarily transported in circulation to target organs by retinol-binding protein (RBP). The protein is relatively small (21 kDa), has one binding site for retinol in the all-trans form, is bound to transthyretin (TTR), and primarily synthesized in the liver. Circulating TTR and RBP may decrease in response to inflammation. Induction of transcription factor NF-IL6 by pro-inflammatory cytokine TNF-α results in down-regulation of the hepatic synthesis of proteins such as TTR. Our previous findings, however, indicated that circulating RBP concentration was greater in cows with a new intramammary infection (IMI) as compared with cows without a new IMI. Our objective was to determine whether there was a relation between hepatic and adipose mRNA expressions of RBP with that of TNF-α. Liver and intestinal adipose tissues were sampled from dairy cows (n = 28) at slaughter and frozen in liquid nitrogen. Total RNA was extracted from each tissue sample and cDNA was generated using the High Capacity Reverse Transcription Kit. Gene expressions of RBP, TNF-α, and β-actin, as a housekeeping gene, were measured in relative quantification using real time rt-PCR. Data were analyzed using original Ct values, adjusted to β-actin expression, in the MIXED and CORR procedures of SAS, and significance was determined at $P \leq 0.05$. Expression of RBP and TNF-α was detected in

all samples and for both genes, variations among cows were significant ($P < 0.001$). Relative to β-actin expression, RBP and TNF-α were more expressed in intestinal adipose than in liver ($P < 0.001$). Across tissues, RBP and TNF-α expressions were positively correlated (r = 0.66; $P < 0.001$). Within intestinal adipose, RBP and TNF-α expressions were weakly correlated (r = 0.38; $P < 0.001$). In the liver, however, mRNA expressions of RBP and TNF-α were strongly correlated (r = 0.64; $P < 0.001$). This implies that regulation of RBP at the transcription level may be independent from that of TTR, which is downregulated by pro-inflammatory stimuli via induction of transcription factor NF-IL6.

Key Words: bovine, retinol-binding protein, TNF-α