M24 Natural resistance-associated macrophage protein (Nramp1) and goat health. Y. Ahmed, M. Worku*, H. Mukhtar, and R. Noble, *North Carolina Agricultural and Technical State University*, *Greensboro*.

The objective of this study was to determine how Nramp1 expression may be associated with disease states in the goat. Natural resistance-associated macrophage protein (Nramp1) is expressed exclusively in professional phagocytes. Expression of Nramp1 affects host innate immunity to intracellular bacteria because of its ability to transport divalent cations in late endosome/lysosomes. Studying the association of the NRAMP1 gene and innate immune response to pathogens may aid in understanding and enhancement of goat genetic resistance to pathogens. Clinically healthy Boer, Spanish and Spanish-Boer cross goats (n = 60) from the NC A&T Small ruminant unit were used. Fecal samples were evaluated for levels of *Haemonchus* and *Coccidia* using McMaster slides. Blood samples were evaluated for packed cell volume and white blood cells Differential count. The FAMACHA score body condition score, body weight, body temperature, and age were recorded. Genomic DNA was extracted from blood (n = 12 goats)on FTA cards according to the manufacturer's protocol. RNA was extracted using the ZR Whole-Blood Total RNA Kit. RNA samples were reverse-transcribed and the cDNA was obtained using the Ambion-Retroscript as per the manufacturer protocol. Specific primers for Nramp1 and GAPDH as loading control were used for RT PCR. Data were analyzed using SAS ANOVA and an independent sample *t*-test. Nramp1 was expressed with variability among animals. Identification of Nramp1 in genomic DNA was not affected by breed. However, Nramp1 expression was significantly increased with increased fecal coccidia egg counts ($P < 0.05$) but was not affected by fecal *Haemonchus* egg counts ($P = 0.0677$).

Key Words: NRAMP-1, goat, pathogen

M25 Identification of serum biomarkers in poultry with leg problems. K. S. Rasaputra*[1,2], R. Liyanage[1], J. O. Lay Jr.[1], and N. C. Rath[2], [1]*University of Arkansas*, *Fayetteville,* [2]*Agricultural Research Service/ USDA*, *Fayetteville, AR*.

Disease induced changes in tissue metabolism is often reflected in the blood; therefore, serum chemistry is conventionally used for diagnosis of health problems. Being the structural and functional basis of tissues, changes in the serum protein profiles are of considerable interest as biomarkers. Tibial dyschondroplasia (TD), caused by the failure of growth plate to form bone results in lameness causing significant economic loss to the poultry industry. Serum markers could identify poultry susceptible to these problems aiding in better genetic selection. Thus, the objective of our study was to identify serum protein differences in normal and diseased birds for use as biomarkers. Similar to other applications involving serum, identification of serum protein biomarkers is hindered because of few high abundant proteins. We used combinatorial peptide ligand library based "Proteominer" beads to enrich low abundant proteins and study their differential expressions in serum. Serum was collected from 6 wk-old chickens with and without leg problems and processed through the proteominer column to deplete the high abundant and enrich the low abundant proteins. Equal amounts of proteins from both groups were subjected to 2D gel electrophoresis. The Coomassie blue stained protein spots in the gels were analyzed using Melanie software to identify differentially expressed proteins in both groups. The protein spots were subjected to in gel trypsin digestion followed by MALDI peptide mass fingerprinting for protein identification. The results showed that proteominer resulted in enrichment of several new protein spots and depletion of some of the high abundant proteins. The Coomassie stained gel showed 50 protein spots of which 21 spots were identified. Melanie analysis showed 4 differentially regulated proteins in the diseased condition. Two spots corresponding to immunoglobulin G (IgG) were upregulated while the other 2 protein spots were down-regulated. It is not known whether the pathogenesis of TD is related to immunity; however, our results show that the IgG levels are upregulated suggesting the role of immunity in TD.

Key Words: tibial dyschondroplasia, serum, proteomics

M26 The detection of bovine respiratory disease in low risk cattle using infrared thermography. A. L. Schaefer*[1], N. J. Cook[2], C. Bench[3], J. Colyn[1], B. Chabot[1], T. Liu[1], P. Lepage[1], D. Froehlich[2], L. Holt-Klimek[1], S. Marchand[1], J. Basarab[2], and E. Okine[3], [1]*Agriculture and Agri-Food Canada, Lacombe Research Centre, Lacombe, Alberta*, [2]*Alberta Agriculture, Lacombe Alberta*, [3]*Department of AFNS, University of Alberta, Edmonton, Alberta*.

Bovine respiratory disease (BRD) causes significant harm to the cattle industry. Clinical methods can identify BRD once the symptoms are advanced. However, identifying symptoms earlier in low risk cattle is more challenging yet important to avoid missing false negatives. The objective of the present study was to examine whether infrared thermography (IRT) of the eye could identify BRD in low risk calves more effectively than conventional methods. Sixty 5 crossbred calves averaging 220 kg were used. The calves were from Agriculture and Agri-Food Canada (AAFC) herds. The calves were weaned and transported approximately 1h to an auction and held over night. The animals were transported back to an AAFC feedlot facility, offloaded, weighed, blood sampled, examined for rectal temperature (RT) and placed into a feedlot pen with free access to cereal silage and water. Daily clinical scores were conducted by pen checkers and an automated, RFID triggered IRT camera (FLIR S60) was mounted at the water station to record eye temperatures. All calves were again monitored 2 weeks later. Hematology analysis included white blood cell counts (WBC) and neutrophil/lymphocyte ratios (N/L). A true positive animal (TP) for BRD needed to display 2 or more of; RT > °104 C, WBC >11 or <7 X 1000 /μL, Clinical Score values (CS) > 3 or N/L ratios >0.8 or <0.1 . No calves were identified by clinical pen checking as in need of treatment. However, hematology, CS and RT identified 11 of the 65 calves as TP with 7 of these TP at both blood sampling dates. Values for TP calves for RT, CS, WBC and N/L ratios were 103.6 ± 0.74 (SD), 4.5 ± 1.1, 11.16 ± 2.65 and 0.13 ± 0.7 respectively while for calves displaying true negative values (TN) at both sample periods were 102.1 ± 0.5, 2.11 ± 1.02, 8.97 ± 1.15 and 0.21 ± 0.1 ($P < 0.01$). Of interest was the observation that the average IRT value for the TP calves was higher throughout the 2 week period (35.44 ± 0.58 C) compared with the TN calves (34.7 ± 0.57 C, $P < 0.01$). Data from this study suggests that the use of non invasively collected eye IRT data may be useful in early identifying calves displaying BRD.

Key Words: bovine respiratory disease, infrared thermography, cattle

M27 Feeding *Lactobacillus* spp. and *Bacillus* spp. does not improve growth or survival of channel catfish experimentally challenged with *Edwardsiella ictaluri*. B. C. Peterson*[1], M. L. Wood[1], N. J. Booth[1], M. Morgan[2], N. Pumford[2], G. Tellez[2], and B. M. Hargis[2], [1]*USDA/ARS, Stoneville, MS,* [2]*University of Arkansas, Fayetteville*.

A major problem in the channel catfish industry has been high disease loss to enteric septicemia of catfish, caused by the bacterium *Edwardsiella ictaluri*. Feeding probiotics may prove beneficial in improving disease resistance. The first study examined the effects of a *Lactobacillus* probiotic (FloraMax-B11; Ivesco, LLC, Springdale, AR) (poultry origin) on growth and resistance to *E. ictaluri*. Two hundred catfish (23.5 ± 0.3 g) were assigned to 2 treatments with 5 replicates each: 1.) Control (36% CP diet) and 2.) FloraMax-B11 (11 *Lactobacillus* spp., sprayed on feed at 10^6 cfu/g of diet). The fish were fed for 6 wks and then experimentally challenged with *E. ictaluri*. The second study examined the effects of 3 spores of *Bacillus* spp. on growth and disease resistance. *Bacillus subtilis*-1 (environmental sample) and *B. subtilis* -2 (catfish origin) were previously shown to have antibacterial in vitro activity against *Escherichia coli* and *E. ictaluri*. *Bacillus pumilus* of catfish origin, which did not show in vitro antibacterial activity, was also tested in this study. Five hundred catfish (11.2 ± 0.1 g) were assigned to 4 treatments with 5 replicates each: 1) Control (36% CP diet); 2) BS-1 (*B. subtillis* at 10^7 cfu/g of diet); 3) BS-2 (*B. subtilis* at 10^7 cfu/g of diet); and 4) BP (*B. pumilus* at 10^6 cfu/g of diet). Fish were fed for 9 wks and challenged with *E. ictaluri*. Data were subjected to ANOVA with tank as the experimental unit. Addition of *Lactobacillus* spp. and *Bacillus* spp. to the diet of catfish had no effect on weight gain, FE, or survival after challenge with *E. ictaluri* ($P > 0.10$). The results of these studies suggest that neither the *Lactobacillus* based probiotic nor any of the 3 spore base probiotic candidates improved growth performance or resistance against *E. ictaluri* . Identifying other catfish specific bacteria and understanding how they may manipulate the microflora of the GI tract will be key in determining whether probiotics have a benefit in improving catfish aquaculture.

Key Words: probiotics, disease, catfish

M28 Effects of intravenous *Escherichia coli* (*E. coli*) dose on the pathophysiological response of colostrum-fed Jersey calves. M. A. Ballou*[1], J. W. Dailey[2], L. E. Hulbert1,2, C. J. Cobb[1], and J. A. Carroll[2], [1]*Texas Tech University, Department of Animal Science, Lubbock,* [2]*Livestock Issues Research Unit, USDA-ARS, Lubbock, TX*.

Objective was to determine the effects of *E. coli* dose on the pathophysiological response of dairy calves following an intravenous challenge. Eighteen 3-wk-old colostrum-fed Jersey calves were completely randomized to 1 of 6 doses of *E. coli*. The challenge doses included 0, 10^5, 10^6, 10^7, 10^8, and 10^9 colony-forming units (CFU) given as a bolus in 5 mL of sterile isotonic saline. Peripheral blood samples were collected at 0, 2, 4, 8, 12, 24, and 48h relative to the challenge for blood metabolite, total white blood cell count and differential analyses. Rectal temperatures were collected via indwelling rectal probes at 1-min intervals, and hourly averages calculated from 2d before the challenge till 2d after the challenge. All calves survived the 48h observation period following the challenge. The attitude of calves given 10^8 and 10^9 CFU was altered ($P < 0.01$) beginning 0.5h after the challenge and returned to that of the control calves by 8 and 48h for calves challenged with 10^8 and 10^9 CFU, respectively. There were treatment x time interactions ($P < 0.01$) on total white blood cell counts and plasma glucose concentrations. Calves administered 10^8 and 10^9 CFU had leucopenia and neutropenia beginning 2h after the challenge and returning to counts similar to the control calves within 24h. Additionally, those calves were hypoglycemic from 4 to 12h after the challenge with the degree of hypoglycemia inversely related to the dose of the *E. coli*. There were treatment x time interactions ($P < 0.01$) on rectal temperatures following the challenge. All calves challenged with *E. coli* developed a febrile response, but the intensity and duration of the response were dependent on the challenge dose. These data indicate that calves intravenously challenged with 10^8 and 10^9 CFU of an *E. coli* showed immediate clinical and biochemical signs indicative of septicemia. However, calves administered 10^7 or less of the *E. coli* had febrile responses, but did not develop septicemia.

Key Words: calf, *E. coli*, septicemia

M29 *Eimeria tenella* oocyst output in cecal or fecal material following challenge in restrict-fed broilers. A. Jordan*[1], D. Caldwell[1], J. Klein[1], J. Coppedge[1], S. Pohl[1], K. Jessen[1], S. Fitz-Coy[2], and J. Lee[1], [1]*Texas A&M University, College Station*, [2]*Intervet/Schering-Plough Animal Health, Summit, NJ*.

The current experiment was conducted to compare *Eimeria tenella* oocyst shedding in fecal or cecal contents in restrict-fed broilers following experimental challenge in brooder batteries. Ninety-six Cobb 500 by-product males were placed in battery pens with 8 chicks per pen. On d 14, restrict feeding was initiated and chicks were challenged with one of 3 challenge levels (1,000, 5,000, and 20,000 oocysts). Total 24 h collections of cecal and fecal material were obtained separately beginning on d 4 post-challenge through d 10 post-challenge for oocysts per gram and total output determination. Six days post-challenge, 4 broilers from each pen were removed and subjected to necropsy for lesion assessment. Data was subjected to a one-way ANOVA using the GLM procedure. Means were deemed significantly different at $P < 0.05$ and means were separated using Duncan's Multiple Range Test. Body weight gain was not affected due to challenge level throughout the experimental period. Increases ($P < 0.05$) in both gross and microscopic lesion development were observed with each increase in challenge level. Oocyst output peaked on d 8 post-challenge. Oocyst concentration was higher ($P < 0.05$) in cecal droppings as compared with fecal material throughout peak shedding. However, total output of *Eimeria tenella* oocysts was similar ($P > 0.05$) in fecal and cecal material. These data indicate that total oocyst output during an experimental challenge are similar in fecal and cecal material, however concentrations are significantly lower in fecal material compared with cecal material when compared on a output per gram basis.

Key Words: *Eimeria*, broiler, oocyst shedding

M30 Effect of aqueous iodine supplementation on growth and dental condition of newly weaned piglets. A. L. Tucker* and R. M. Friendship, *University of Guelph, Guelph, Ontario, Canada*.

Weaning is a stressful event and many pigs experience poor growth in their first weeks in the nursery. The use of antibiotics to improve growth and health has come under increasing scrutiny and alternative means of reducing bacterial load are desirable. Iodine, a broad-spectrum antiseptic, shows potential as a therapeutic agent because it has low potential for resistance and is inexpensive. It is also used to prevent oral disease, a condition that can develop in nursery age piglets. Recently, premolar eruption and the presence of poor oral conditions have been shown to affect weight gain in weaned piglets. The aim of this study was to examine the efficacy of an aqueous iodine supplement for improving piglet weight gain and oral condition. Across 3 trials, 624 piglets on a commercial farrow-wean farm were examined. An iodine-based sanitizer containing 1.75% titratable iodine was added to the water supply to a final concentration of 1 ppm. Water was supplied to via nipple drinkers

with testing confirming mean concentrations of 0.96, 0.68 and 0.91 ppm in trials 1, 2 and 3. Piglets were weighed and given oral exams within 24 h of weaning and again 3 and 6 wks later. Deciduous teeth were recorded as being erupted (vs not erupted) if any portion of the tooth crown penetrated the gingiva. Dental staining and caries (cavities) on incisors, gingivitis around any teeth and oral lesions on the gingiva, tongue, cheeks or throat were recorded. A repeated measures ANOVA (SAS Proc MIXED) was used to examine how iodine supplementation influenced growth and oral condition. Overall, the presence of staining and caries on the primary incisors increased between weaning and wk 3 ($P < 0.05$), while the incidence of both oral lesions and gingivitis increased between wks 3 and 6 ($P < 0.05$). There were no significant treatment differences in piglet growth or oral condition during the period that iodine was supplemented ($P > 0.05$). These results indicate that deleterious oral conditions do develop and increase throughout the weaning period, but, at 1 ppm, an aqueous iodine supplement does not provide an advantage for weight gain or oral condition in nursery piglets.

Key Words: growth, dentition, swine

M31 Interaction of breed and quantity of milk replacer on innate immune competence of dairy calves. M. A. Ballou* and C. J. Cobb, *Department of Animal and Food Sciences, Texas Tech University*, *Lubbock*.

Objective was to determine the influence of breed and quantity of milk replacer fed on the immune competence of dairy calves. Forty 2 bull calves (n = 20 Holstein and n = 22 Jersey, 2 ± 1 d old) were studied in a 2 × 2 factorial arrangement. Holstein and Jersey calves on the lower plane of nutrition (LPN) were fed 454 g /d of a 20/20 milk replacer. Holstein calves on the higher plan of nutrition (HPN) were fed 810 and 1,180 g / d of a 28/20 milk replacer for wk 1 and wk 2 – 6, respectively. Jersey calves on the HPN were fed 568 and 680 g / d of a 28/25 milk replacer for wk 1 and wk 2 – 6, respectively. On d 4, 42, and 77 peripheral blood was collected for ex vivo immunological analyses, and on d 7 all calves were challenged subcutaneously with LPS (4 μg/kgBW) and clinical and biochemical responses evaluated at 0, 1, 2, 3, 4, 5, 6, 9, 12, 24, and 72 h. There was a breed x plane of nutrition interaction ($P < 0.04$) on serum glucose concentrations following the LPS challenge; whereas Holsteins on the HPN had higher glucose concentrations than the other treatments. There were no breed or plane of nutrition interactions with time following the LPS challenge. Isolated mononuclear cells from Holstein calves when stimulated ex vivo with LPS on d 42 and 77 synthesized more tumor necrosis factor-α than cells from Jersey calves (2149 and 1527 pg/mL ± 149.9; $P < 0.01$). There were breed x day ($P < 0.05$) and plane of nutrition x day ($P < 0.07$) interactions on the intensity of the oxidative burst produced in response to a pathogenic *E. coli*; whereas Jersey calves fed LPN had a reduced intensity on d 77 when compared with the other treatments. Additionally, on d 77 Jerseys fed LPN had a reduced ($P < 0.04$) whole blood killing capacity when incubated with the *E. coli* for 60 min. When whole blood was incubated with the *E. coli* for 10 min, Jersey calves consistently had reduced ($P < 0.03$) killing capacities over the entire study period. These data indicate that Jersey calves had lower measures of many innate immune variables and HPN may improve aspects of the innate immune competence in a breed and immune variable specific manner.

Key Words: breed, calf, immune

M32 Effects of neomycin and oxytetracycline (N/T) fed at treatment rate for 14 days in calf milk replacer (CMR) on calf performance and health. D. Shields*[1], R. Blome[2], D. Wood[2], and J. Sowinski[2], [1]*Merrick's, Inc.*, *Middleton, WI*, [2]*Animix*, *Juneau, WI*.

New government regulations for the treatment of bacterial scours levels of the new combination (1:1) for neomycin/oxytetracycline of 10mg/lb BW for 14 d are now in effect. There is minimal calf data for feeding this new level. The objective of this study was to evaluate calf health and performance under the new federal guidelines. Auction sourced Holstein bull calves (n = 48, 3–5 d old) were stratified by weight into 2 treatment groups (24 calves/trt). Calves were weighed 1×/wk during the 8 wk trial. Calves were housed in individual hutches and fed 284 g reconstituted CMR (20% C.P., 20% fat, all milk protein) via individual nipple bottles at 0600 and 1700 h. Trt 1 received the treatment rate for bacterial scours for 14 d, and trt 2 received a non-medicated control formula. Water and a commercial textured calf starter (18% CP, 2.5% fat, Decoquinate) was offered daily for ad libitum consumption. Orts were collected and weighed daily and no hay fed. Straw-bedded hutches held a consistent nesting score ≥ 3. Subjective fecal scores (FS) were recorded 1×/d using 1–4 scale with 1 being normal and 4 severe scours. FS ≥ 3 were given electrolyte therapy. Weaning occurred at 42 d if starter intake (SI) was ≥ 454 g for 3 d and non-weaned calves were reduced to 1×/d CMR to promote SI. During the first 3 wks, ADG tended ($P \leq 0.09$) to improve with N/T (0.40 vs. 0.34 kg/d) and total SI was higher (3.7 vs. 2.1 kg). From wk 3– 6 opposite trends were observed as ADG tended ($P \leq 0.09$) to be higher for control calves (0.81 vs. 0.89 kg/d) and F/E was improved 11.7% ($P \leq 0.02$) at 0.58 vs. 0.65. Overall in the 56 d study no differences noted in growth, SI or F/E. However, N/T reduced calves that scoured 38% ($P \leq 0.06$), calves that required health treatments 36% ($P \leq 0.07$) and treatment d 28.6% ($P \leq 0.07$). Under conditions of this study N/T reduced incidence of scours, treatment days of calves treated and improved early SI but did not affect ADG, total SI or FS.

Key Words: calf, medication

M33 The effect of adding the organic complex of zinc, copper, manganese and cobalt on hoof health and performance in feedlot cattle. G. R. Noori[1], H. Amanlou[1], D. Zahmatkesh[1], E. Mahjoubi*[1], and Y. Mokhtabad[2], [1]*Zanjan University*, *Zanjan, Iran*, [2]*Azad University*, *Mazandaran, Iran*.

It has been shown that Zn, Mn, Cu and Co are essential for protein synthesis, vitamin metabolism, connective tissue synthesis and immune system function. There is, however, little evidence in finishing cattle. A study was conducted to investigate the effects of micro-mineral organic complex on lameness occurrence and performance in Holstein bull calves. Ninety-three Holstein bull calves (250.21 ± 4.21) were used in a completely randomized design. Calves were group fed a similar basal diet during 42 d experimental period. Used treatments included 1) control treatment without feed additive, and 2) experimental diet that calves consumed 7 g/d micro-mineral complex (Availa 4, an amino acid mineral complex available from ZinPro Corporation, Eden Prairie, MN), that supplied 360 mg Zn, 200 mg Mn, 125 mg Cu and 12 mg Co. Results showed that the effect of experimental diet on dry matter intake was significant (7.02 vs. 7.22 kg/d, $P < 0.001$) but feed conversion ratio was not significant (7.36 vs. 7.23, $P < 0.21$). A tendency was detected for average daily gain (0.96 vs. 1.04 kg/d, $P < 0.11$) and weight gain during the trial (40.3 vs. 43.6 kg, $P < 0.11$). The consumption of micro-mineral organic complex influenced plasma concentration of globulin (2.96 vs. 3.98 g/dL, $P < 0.03$) and albumin (3.26 vs. 2.44 g/dL, $P < 0.002$) significantly. The prevalence of lameness was higher in control group than organic complex supplemented treatment (23% vs. 11%; odds ratio

= 2.5). Generally, our results show that feeding micro-mineral organic complex can have an efficient role in reducing lameness occurrence and increasing profitability in feedlot farms.

Key Words: lameness, trace mineral, Holstein bull calves

M34 The effect of early feeding on blood factors, immune system, digestive tract and intestinal morphology of broiler chicks. M. Asgari[1], S. Rahimi*[1], M. Kiaei[2], and M. A. Karimi Torshizi[1], [1]*Tarbiat Modares University*, *Tehran, Iran*, [2]*University of Tehran*, *Tehran, Iran*.

In this study 560 male broiler chicks (ROSS 308) were used to study the effect of early feeding on blood factors, immune system, digestive tract and intestinal morphology. Birds were divided into 5 treatments with 4 replicates (112 chicks per treatment) as follows: 1. Control group (were fed starter diet 48 h after hatch); 2. Vitagel group (received 5g per bird jelly feed for the first 48 h after hatch and then starter diet); 3. Chick-mix group (were administered with starter diet); 4.Wet feed (with10% moisture for first 48 h after hatch and then starter diet); 5. Saline (1mL sterile saline were injected via subcutaneous in neck of each bird in this group after hatch and they were fed starter diet 48 h later). Body weight, feed intake, feed conversion, weight of liver, Proventriculus, gizzard, small intestine, bursa of Fabricius, spleen, pancreas, yolk sac, digestive tract, and length of small intestine were measured for all groups. Newcastle disease (NDV) and SRBC antibody titer, glucose, triglyceride, and cholesterol of serum were determined. The number of villi, length and width of villi, depth of crypt and percentage of different villi (leaf, tongue, bridge and finger shape) in small intestine were measured for all groups. At end of the experiment (40 d), weight of different parts of carcass and productive index were measured. The production index, GUT weight, intestine length, villi height and crypt depth were higher in early fed groups compared with other groups ($P < 0.05$). There were no significant differences in BW, FCR, and antibody titer against NDV and SRBC between different treatments. Serum glucose, relative weight of liver, proventriculus, pancreas, intestine, bursa of Fabricius, spleen, length of small intestine were higher in early fed groups ($P < 0.05$). In conclusion, early feeding of broiler chicks can be recommended to have better performance and immune response in these birds.

Key Words: broiler chicks, early feeding, blood factors

M35 Evaluation of effect of sodium bicarbonate as a top-dress on preventing laminitis and performance in feedlot cattle. G. R. Noori[1], H. Amanlou[1], D. Zahmatkesh[1], E. Mahjoubi*[1], and Y. Mokhtabad[2], [1]*Zanjan University*, *Zanjan, Iran*, [2]*Azad University*, *Mazandaran, Iran*.

Sodium bicarbonate is known as a pH modulator in cattle. Acidosis and, subsequently, laminitis are common in feedlot cattle. The effect of sodium bicarbonate as a top-dress has not been investigated. Therefore, our objective was to investigate the effects of sodium bicarbonate on laminitis occurrence and performance. One hundred Holstein bull calves (251.75 ± 5.75) were used in a completely randomized design. Calves were group fed a similar basal diet during 42 d experimental period. Used treatments included 1) control treatment without feed additive, and 2) experimental diet that calves consumed 50 g/d sodium bicarbonate as a top-dress. Results showed that the effect of experimental diet on dry matter intake (7.02 vs. 7.36 kg/d, $P < 0.001$), average weight gain through trial (40.3 vs. 46.6 kg, $P < 0.03$) and feed conversion ratio (7.36 vs. 6.53, $P < 0.02$) were significant. A tendency was detected for average daily gain (0.96 vs. 1.11 kg/d, $P < 0.07$). Compared with control group, occurrence of laminitis in experimental group was less (22% vs. 9%; odds ratio = 2.6). Generally, results showed that feeding of sodium bicarbonate as a top-dress can have an efficient role in reducing laminitis occurrence and increasing profitability in feedlot farms.

Key Words: laminitis, feedlot, sodium bicarbonate

M36 Expression of members of the wingless gene family in goats. M. Worku*, H. Mukhtar, and N. Mikiashvili, *North Carolina Agricultural and Technical State University*, *Greensboro*.

The objectives of this study were to identify members of the wingless (Wnt) signaling pathway (Wnt gene family) in the goat genome and to evaluate expression of genes involved in Wnt signaling in blood. The Wnt gene family encodes secreted members of the wingless family of signaling molecules that control cell fate, specification, proliferation, polarity, and movement in animal development and diseases. Wnt gene expression impacts production traits in farm animals as well as meat quality and is reported to be crucial for myogenesis and adipogenesis. Blood was collected from meat (4 Boer) and milk (4 Alpine) goats on FTA elute cards (Whatman Inc.) for DNA isolation. For RNA isolation PAXgene Blood RNA tubes were used. Genomic DNA was extracted from the FTA card according to the manufacturer's protocol. RNA was extracted using the ZR Whole-Blood Total RNA Kit. RNA samples were reverse-transcribed and the cDNA was obtained using the Ambion-Retroscript as per the manufacturer protocol. Specific primers for Wnt1, Frizzeled and secreted frizzeled were used for PCR amplification. Amplified products were run on a 1% agarose gel with PCR markers. Gels were stained with ethidium bromide and visualized using a Gel documentation system. GAPDH was used as loading control and primers in the absence of DNA were used as negative controls. Variability was observed among animals in detection of Wnt-1 in genomic DNA. Genes were detected in blood. Wnt-1 and Frizzeled genes were expressed in goats. Differential gene expression and animal to animal variability were observed. Both Wnt and Frizzeled family genes appear to be conserved in goats. This simple method for detection of Wnt-1 gene expression in goat blood will aid in the deciphering of the underlying mechanisms involved in animal development and disease impacting production traits in farm animals.

Key Words: goat, Wnt family, gene expression

M37 Dynamic changes in physiological responses to heat stress in cattle of different geographic origins. P. A. Eichen*, H. L. Vellios, B. S. Scharf, J. S. Johnson, D. K. Kishore, E. A. Coate, and D. E. Spiers, *University of Missouri*, *Columbia*.

Response to heat stress may differ in *Bos taurus* cattle originating in different US regions. Hourly measurements at specific heat stress levels may enable further understanding of these regional differences. Angus steers from Oklahoma (OK; n = 6) and Missouri (MO; n = 6) were compared with heat-tolerant Romosinuano steers (RO; n = 5) from Florida in the University of Missouri Brody Environmental Center. Initially, the 3 groups were exposed to air temperature (Ta) treatments that included a constant thermoneutral temperature (TN) of 20°C for 8 d, followed by a daily heat cycle (HS1) of 28 to 38°C for 8 d, and a greater heat cycle (HS2) of 30 to 40°C for 8 d. Twenty-four hour measurements were made after stabilization at each temperature. Hourly measurements included rectal temperature (Tr), respiration rate (RR), and skin temperatures averaged at trunk and peripheral sites. Linear regression was used to determine animal and ambient relationships, with ANOVA for group differences. There was no difference in Tr between groups at TN, but Tr was lower in RO compared with Angus during HS1 and HS2 ($\alpha = 0.05$). Relationships between Tr and Ta were similar for MO and OK during

HS1 and HS2. However, there was no relationship between Tr and Ta for RO during HS1 and HS2. RO had lower RR during TN and HS1 ($\alpha = 0.05$), but were not different from Angus during HS2 to suggest an adaptive response. Interestingly, relationships between RR and Ta for RO were equal to Angus during HS1 and HS2, indicating no group difference in the RR relationship. Skin temperatures at trunk or peripheral sites were affected by hour of day during all 3 temperature treatments ($P < 0.03 - 0.0001$). There was also an hour x group effect on trunk temperature during all 3 periods ($P < 0.05$), with no effect on peripheral sites. Geographic origin of Angus did not affect the heat stress response. Romosinuano steers exhibited characteristic heat tolerance, but showed some temporal shifts in response to prolonged heat exposure.

Key Words: heat stress, cattle, diurnal

M38 Patterns of heat response and adaptation on summer pasture: A comparison of heat sensitive (Angus) and tolerant (Romosinuano) cattle. J. S. Johnson*, B. Scharf, R. L. Weaber, P. A. Eichen, and D. E. Spiers, *University of Missouri*, *Columbia.*

Heat stress in *Bos taurus* cattle is a problem that affects many regions of the world. Numerous studies have focused on heat stress in feedlots or environmental chambers; but few have looked at undisturbed cattle on pasture. The present study followed 2 cattle breeds throughout a mid-Missouri summer to determine thermoregulatory responses to fluctuating summer air temperature (Ta). Breeds included 22 Angus grouped into 10 Missouri Angus (468 ± 11 kg BW) and 12 Oklahoma Angus (490 ± 9 kg BW). These were compared with 11 heat tolerant Romosinuano (RO; 352 ± 6 kg BW) steers from Florida. Animals were monitored on 16 d from June 8 through August 9 of 2009 on endophyte free tall fescue at the University of Missouri South Farm. For analysis, the data was divided into 2 periods that consisted of the first 11 d (Period 1; 9 d; Ta range = 19.8–34.3°C) and the last 27 d (Period 2; 7 d; Ta range = 15.5–33.4°C). Periods were determined by comparing respiration rate (RR) to Ta. Periods 1 and 2 were the times at which RR response to Ta was significantly different at ($P < 0.05$). RR was measured (counting 1 min flank movement) at 0800 and 1500 h, and ruminal temperature (Trum) was monitored hourly as an indication of core body temperature using a telemetric temperature transmitter (SmartStock, Pawnee, OK). Relationships between RR, Trum, and Ta were determined using linear regression for both breeds and groups within breeds. RR and Trum showed no significant differences between Angus groups ($P = 0.05$), however breed differences were found between Angus and RO ($P < 0.05$) steers. Slopes of RR to Ta from Periods 1 to 2 decreased from 2.55 to 1.13 bpm/°C and 2.27 to 0.49 bpm/°C for Angus and RO, respectively. Slopes of Trum to Ta also decreased from Periods 1 to 2 from 0.12 to 0.01°C /°C and 0.02 to 0.01°C /°C for Angus and RO, respectively. Correlations of Trum to Ta in Period 2 were insignificant ($P > 0.05$). Although Romosinuano have a lower respiration rate and ruminal temperature than Angus, they share a similar pattern of adaptation from early to late summer periods.

Key Words: cattle, heat stress, adaptation

M39 Taguchi approach for anti-heat stress prescription compatibility in mice spleen lymphocytes in vitro. X. Zhu*[1], G. Cheng[2,3], F. Liu[2,3], J. Yu[2], Y. Wang[1], T. Yu[2], J. Xu[1], and M. Wang[1], [1]*TCVM Laboratory, CAU-BUA TCVM Teaching & Research Team, College of Veterinary Medicine, China Agricultural University, Beijing, China*, [2]*Department of Animal Science and Technology, Beijing University of Agriculture*, *Beijing, China*, [3]*Beijing Key Laboratory of TCVM, CAU-BUA TCVM Teaching & Research Team*, *Beijing, China*.

The study was to evaluate the possible immune function of Agastache rugosa, Atractylodes lancea, Cortex Phellodendri, and Gypsum Fibrosum on spleen lymphocytes under heat stress. The Taguchi Design, which allows rapid and high efficiency to select the best conditions for the composition, was used to investigate the compatibility of the herbs. The extracts from herbs in various dilutions were added to previously cultured cells with final concentrations of 10, 50, 100, 200 μg/mL to give a total volume of 100 μL/well. Then lymphocytes exposed with extracts were incubated at 37°C for 24 h, heated at 42°C for 2 h and recovered to 37°C for 22 h. As a heat shock group (HS), lymphocytes (with ConA, 10 μg/mL or LPS, 2.5 μg/mL) were treated at 37°C for 24 h, heated at 42°C for 2 h and recovered to 37°C for 22 h. As a control, lymphocytes (with ConA, 10 μg/mL or LPS, 2.5 μg/mL) were treated at 37°C for 48 h. Lymphocyte proliferation was evaluated by MTT assay. The optical density (OD, 570nm) of HS was significantly lower than the control group ($P < 0.05$). Under heat stress the OD of higher concentrations of Agastache rugosa (200 μg/ml and 100 μg/ml), Atractylodes lancea (200 μg/ml and 100 μg/ml), Cortex Phellodendri (100 μg/ml and 50 μg/ml) and Gypsum Fibrosum (200 μg/ml and 100 μg/ml) caused a significant increase on ConA/LPS-induced proliferation of lymphocytes than HS ($P < 0.05$). The Taguchi results demonstrated that Agastache rugosa (200 μg/ml), Atractylodes lancea (200 μg/ml), Cortex Phellodendri (100 μg/ml) and Gypsum Fibrosum (100 μg/ml) were the optimal conditions for the composition of herbs. The validation experiment results confirmed that our composition in optimum extraction conditions has enhancing effects on ConA or LPS-induced lymphocytes under heat stress. The 4 herbal extracts may recover from the immunosuppression induced by heat stress. Taguchi optimization approach is a suitable method for optimization of the composition of herbs.

Key Words: heat stress, spleen lymphocytes, taguchi approach

M40 Effect of heat stress on the rat small intestine: A morphological and gene expression study. A. Lu*[1], G. Cheng[1,2], W. Luan[1], B. Zhou[1], F. Liu[1,2], and J. Xu[3], [1]*Department of Animal Science and Technology, Beijing University of Agriculture, Beijing*, *China*, [2]*Beijing Key Laboratory of TCVM, CAU-BUA TCVM Teaching & Research Team*, *Beijing, PR China*, [3]*TCVM Laboratory, CAU-BUA TCVM Teaching & Research Team, College of Veterinary Medicine, China Agricultural University, Beijing, PR China.*

The aim of the current study was to investigate changes in morphology and gene expression in the rat small intestine in response to heat stress. Forty-eight male SD rats (200 ± 20 g) were randomly divided into control or heat-stressed groups. Rats in control group housed with the environment of 25°C, while rats in heat-stressed group were subjected to 40°C for 5 h each day for 10 successive days. Rats were sacrificed on 1st, 3rd, 6th, and 10th day after heat treatment and sections of the small intestine epithelial tissue were excised for morphological examination and microarray analyses. During the microarray examination, each group contain 3 chips, 3 rats' cRNA were pooled and hybridized to each chip. The rat rectal and body surface temperature and serum cortisol levels were all significantly increased after heat treatment ($P < 0.01$). The duodenum and jejunum were significant damaged after 3

d of treatment, and the damage was recovered gradually as time went on. Microarray analysis found 289 genes upregulated (log 2ratio > 1, $P < 0.01$) and 133 genes downregulated (log2ratio < −1, $P < 0.01$) in response to heat stress. Subsequent bioinformatic analysis (including gene ontology and KEGG pathway analysis) revealed the genes altered in response to heat stress mainly related to apical part of cell, oxidation reduction, response to stress, tetrapyrrole binding, rhythmic process, oxidoreductase activity, oxygen binding, transcription factor activity. The pathway mainly involve in Antigen processing and presentation, MAPK signaling pathway, Circadian rhythm-mammal, "Glycine, serine and threonine metabolism" and Retinol metabolism. Heat stress caused significant damage to the rat small intestine and altered gene expression in the rat jejunum. The results of the bioinformatic analysis from the present study will be beneficial to further investigate the mechanisms involved in heat stress-induced damage in the rat small intestine.

Key Words: heat stress, morphology, microarray

M41 Study of immune expression profile of heat stress-induced rat using gene microarray. A. Lu*[1], G. Cheng[1,2], W. Luan[1], J. Yu[1], B. Zhou[1], F. Liu[1,2], and J. Xu[3], [1]*Department of Animal Science and Technology, Beijing University of Agriculture*, *Beijing, China*, [2]*Beijing Key Laboratory of TCVM, CAU-BUA TCVM Teaching & Research Team*, *Beijing, China*, [3]*TCVM Laboratory, CAU-BUA TCVM Teaching & Research Team, College of Veterinary Medicine*, *Beijing, China*.

The aim of the current study was to investigate the changes in morphology of intestine mucosa and gene expression of mucosal immune in the rat small intestine in response to heat stress. Forty-eight male SD rats (200 ± 20 g) were randomly divided into control or heat-stressed groups. Rats in the control group were housed with the environment of 25°C and 60% humidity daily for 10days; while heat-stressed group were housed under control group conditions, but exposed to 40°C and 60% humidity for 5h each day for 10 consecutive days. Rats were sacrificed on 1st, 3rd, 6th, and 10th day after heat treatment. Hematoxylin and eosin (HE) and periodic acid-Schiff (PAS) staining method was used to observe the Morphology changes and the numbers of immune cells. The gene expression profile was analyzed using Agilent microarray. Quantitative RT-PCR (qRT-PCR) was carried out for interesting genes that upregulated and downregulated more than 2 times to validate the reliability of microarray analysis. Structural changes of the mucosa included atrophy of some villi and a reduction in the size of crypts. In contrast to control group, the numbers of lymphocyte and chalice cell were both significantly decreased ($P < 0.01$). There were approximately 3152 expressed genes ($P < 0.01$) among total 41012 genes set in the microarray plate, and 56 immune related genes including 14 upregulated and 42 downregulated genes ($P < 0.01$). Some genes were assayed by qRT-PCR and demonstrated the same alteration tendency as in microarray analysis. Subsequent bioinformatic analysis (including gene ontology and KEGG pathway analysis) revealed the immune genes altered in response to heat stress were related to Positive regulation of immune system process, regulation of immune system, immune system development, leukocyte activation and migration, and genes involved in signal transduction. Heat stress caused significant effect to the rat small intestine mucosa and altered immune related gene expression in the rat jejunum.

Key Words: heat stress, immune, microarray

M42 Study of the mechanism of heat stress-induced IEC-6 cell apoptosis. W. Luan[1], K. Guo[1], G. Cheng[1,2], J. Yu[1], F. Liu*[1,2], and J. Xu[3], [1]*Department of Animal Science and Technology, Beijing University of Agriculture*, *Beijing, China*, [2]*Beijing Key Laboratory of TCVM, CAU-BUA TCVM Teaching & Research Team*, *Beijing, China*, [3]*TCVM Laboratory, CAU-BUA TCVM Teaching & Research Team, College of Veterinary Medicine, China Agricultural University*, *Beijing, China*.

The aim of the study was to study the effects of heat stress on apoptotic of IEC-6 in rat. The IEC-6 cell were divided into control group and heat stress group, both cultured for 4 h with the condition of 37°C and 42°C respectively. The morphological changes of IEC-6 was observed by acridine orange (AO) fluorescence staining; reverse transcription-polymerase chain reaction (RT-PCR) was used to determine the mRNA expression of apoptosis-related gene, such as caspase-3, caspase-8, caspase-9, bax and bcl-2; the early and late apoptotic rate of the 2 groups was detected by flow cytometry; the activity of terminal execute enzyme caspase-3 was detected by the UV spectrophotometry. In contrast to control group, the apoptotic cells were stained by AO and showed densely green yellow or fragment in heat stress group. The percentage of early and late apoptotic cells in heat stress group was significantly higher than that of control group ($P < 0.05$). After heat stress, mRNA expression level of the bax, caspase-3, caspase-9 were significantly increased ($P < 0.01$), mRNA expression level of the bcl-2 was significantly decreased ($P < 0.01$); and activation of Caspase-3 was significantly enhanced ($P < 0.01$). In conclusion, our results revealed that heat stress could trigger IEC-6 cell apoptosis via the mitochondrial pathway of apoptosis, depending upon the activation of caspase-9.

Key Words: IEC-6 apoptosis, mitochondrion, bax, bcl-2, caspase-3, caspase-9

M43 Coagulase-negative staphylococci mastitis management. T. E. Quirk*, L. K. Fox, J. L. Capper, D. D. Hancock, and J. R. Wenz, *Washington State University*, *Pullman*.

Coagulase-negative staphylococci (CNS) are the most common pathogens associated with intramammary infections (IMI) in dairy cows. We hypothesize that post-milking teat disinfection, teat dip, would reduce the microbial colonization of the streak canal and thus reduce the prevalence of IMI caused by CNS species. The efficacy of iodine post-milking teat dip was tested against CNS colonization of the streak canal, and incidence of IMI was measured. Using an udder-half model, 43 Holstein cows at the Washington State University Dairy were enrolled in the trial; teat dip was only applied after milking to one udder-half. Streak canal swab solutions and mammary quarter milk samples were taken in duplicate once a week for 16 weeks for microbial culture. A CNS IMI was identified when 2 of 3 consecutive duplicate quarter milk samples were identified with the same CNS species containing > 120 cfu/ml. The isolates were speciated using PCR-RFLP and gel electrophoresis. Colonization of the streak canal and IMI by CNS were assessed. Twenty-five CNS IMI were diagnosed; prevalence of IMI in control quarters and treated quarters were 8.1% and 19.7%, respectively. Isolation of CNS in milk was less likely in treated than control quarters (Odds Ratio = 0.704, $P = 0.0113$, 95% CI = 0.536–0.925). The majority of CNS IMI was by *Staphylococcus chromogenes* (44%) and appeared to be linked to streak canal colonization. Conversely, the second most prevalent cause of CNS IMI was by *Staphylococcus xylosus* (36%), but did not appear to be linked to colonization of the streak canal. In conclusion, post-milking teat dip was efficacious for reducing IMI caused by CNS, but CNS IMI was not necessarily linked to streak canal colonization.

Key Words: coagulase-negative staphylococci, mastitis, teat dip

M44 Morphometric evaluation of udders in Gir cows and the prevalence of subclinical mastitis. M. A. F. Porcionato[1], M. V. Santos*[1], C. B. M. Reis[1], M. M. Stradiotto[2], C. S. Cortinhas[1], and W. V.B Soares[3], [1]*Department of Nutrition and Animal Production, FMVZ/USP, Pirassununga, Brazil*, [2]*Department of Basic Science, FZEA/USP, Pirassununga, Brazil*, [3]*Institute of Zootecny, IZ/APTA, Mococa, Brazil*.

This trial aimed to evaluate the relation between morphological teats characteristics of Gir cows evaluated by ultrasound and the prevalence of subclinical mastitis. Eighty lactating Gir cows with 90 to 200 d of the 2nd or 3rd lactation were grouped according with their milk flow: fast or slow and milked twice a day with mechanical milker. The teats characteristics were measured by ultrasound and external morphometrical measurements. Somatic cell count (SCC) was determined by the fluoro-opto-electronic method (Somacount 300, Bentley Instrument Inc., Chaska, MN, USA). Samples were considered positive for subclinical mastitis with SCC > 200,000 cells/mL and the pathogens identified in microbiological culture. Data were analyzed using GLM procedure (SAS, version 8.2) and differences were considered significant at $P < 0.05$. Ultrasonography images showed higher ($P < 0.05$) teat channel in slow flow (25.68 mm) than in fast flow (22.31 mm) groups. No significant correlation ($P > 0.05$) was observed between Log(SCC) and morphological teats characteristics. The infrared thermography technique was used to the evaluate udder temperature variation in cows with subclinical mastitis, but no differences ($P > 0.05$) were observed for type of microorganism or Log(SCC). The channel length and the distance for the teat to floor had influence on the prevalence of subclinical mastitis, as well as the mastitis-causing pathogens in Gir cows.

Key Words: mastitis, morphology, thermography

M45 Comparison of 16S rRNA gene sequencing and aerobic culture results performed on milk samples from cows with clinical mastitis. J. R. Wenz*, T. E. Besser, and L. K. Fox, *Washington State University*, *Pullman*

The hypothesis tested in this study was that 16S rRNA gene sequencing (16S) performed on DNA from quarter milk samples of cows with clinical mastitis (CM) would identify the same bacteria found by aerobic milk culture. To test this hypothesis duplicate milk samples were collected from cows with CM. Aerobic milk culture was performed on 100 μL of milk for presumptive bacterial identification and cfu/ml determination. Cows with the same milk culture result on duplicate milk samples (n = 31) were included. Common mastitis pathogens were cultured from 24 samples and 7 were bacteriologically negative (BN). DNA was harvested from each 2 mL milk sample and quantitated. A variable region of the small ribosomal subunit gene was amplified using PCR primers complementary to flanking regions shared among eubacteria and cloned. Sequences of 12 clones from each sample were determined and GenBank searches used to identify bacterial species with the most similar sequences (>99% identity). For 18 of 24 (75%) of the samples, 16S results agreed with culture results. These samples all had >250 cfu/mL (median 13,650; range 290 to > 30,000 cfu/mL) bacteria on culture and >40ng/mL of DNA (median 444; range 45.4 to 1730 ng/mL). In contrast, the remaining 6 samples had lower bacterial counts (median 40 cfu/mL; range 10 to 520) and lower DNA yields (median 44 ng/mL; range 0.70 to 756). In 4/6 samples where culture and 16S results differed, culture identified low numbers of coagulase negative staphylococci and no consistent clone was revealed by 16S. Similarly, BN samples had low DNA yields and no consistent clone identification (and no clone consistent with common mastitis pathogens) was revealed by 16S. The results of this study suggest 16S and culture results are consistent when bacterial numbers are ≥ 300 cfu/mL. Furthermore, these results do not support the hypothesis that BN milk samples result from infection with unculturable bacterial agents.

Key Words: 16S r RNA gene sequence, clinical mastitis, aerobic culture

M46 Hyphenated mass spectrometry investigations applied to the characterization of organic chelates. A. Yiannikouris*[1], C. Connolly[2], R. Power[1], and R. Lobinski[3], [1]*Alltech Inc.*, *Nicholasville, KY,* [2]*Alltech Ireland*, *Dunboyne, County Meath, Ireland*, [3]*CNRS UMR 5254*, *Pau, France*.

This work focused on the development of a method to screen for the presence of metal-complexes in organic chelates using hyphenated techniques of inductively coupled plasma-mass spectrometry (ICP-MS). Identification and characterization of metal-peptide interactions was achieved using hyphenated techniques of mass spectrometry (ESI-MS). The ICP-MS detection in HPLC formed the basis of the methods developed because of its sensitivity regardless of the matrix, which allowed the optimization of the analyte purification before ESI-MS/MS. Analyses indicate that using size-exclusion chromatography (SEC) 5 peaks were characterized for the organic mineral. Chromatogram intensity was a linear function of the total area under the curve indicating the reproducibility of extraction (R2 = 0.999 and RSD ≤3.25%). Application to batch-to-batch reproducibility on 6 samples injected in triplicate showed that the morphology of the Cu elution pattern was identical and reproducible with significant overlapping of the chromatograms ($P \leq 0.05$). Coefficient of variation of the area under the curves ranked between 0.007 and 0.012. SEC ICP-MS detection is thus a valid technique to monitor the molecular mass distribution of metal-binding molecules to assess product quality and the unique fingerprinting per proteinate investigated, with a detection limit below 1 μg/g. This procedure offers a novel application to the detection of the complexes in premixes and feed samples. The fine characterization of the metal-peptides complexes formed through parallel ICP MS and ESI MS/MS detection enabled the identification of the ones stable enough to survive the ionization process. The precursor proteins were identified by means of the ExPASy Proteomics Server of the Swiss Institute of bioinformatics data on soybean genome. In silico molecular modeling using a force-field adapted to the protein and ligand interactions studies (CVFF) clarified the coordination of the amino acids surrounding the mineral by the evaluation of a potential energy according to environmental settings.

Key Words: organic minerals, chelates, speciation

M47 Methods to predict true disease prevalence in beef cattle. C. M. McAllister*[1], B. W. Brigham[1], R. K. Peel[1], H. Van Campen[1], G. H. Loneragan[2], R. L. Weaber[3], J. L. Salak-Johnson[4], and C. C. L. Chase[5], [1]*Colorado State University*, *Fort Collins*, [2]*West Texas A&M University*, *Canyon*, [3]*University of Missouri*, *Columbia*, [4]*University of Illinois*, *Urbana*, [5]*South Dakota State University*, *Brookings*.

Inherent complications arise during the evaluation of disease data for the genetic improvement of disease susceptibility and immune response including the imperfect diagnosis of infection, time of infection, severity of disease challenge, level of disease challenge of clinically normal individuals, and variation in prevalence across years. The objective of this study was to identify alternative methods for classifying diseased versus healthy individuals to improve the accuracy of which bovine respiratory disease (BRD) prevalence (Pr) is estimated. Data for this study included feedlot treatment records (TR), lung lesion scores (LS), early (eADG) (80 d.) and overall average daily gain (ADG) on 2,434 crossbred steers. Disease prevalence was 24.9 and 58.6% for TR and

LS, respectively. Cluster analysis was performed to group animals with similar performance based on TR, mean lung score (MLS), and eADG or ADG to estimate prevalence. A k-means method was implemented in R which utilizes an Euclidean distance matrix to form cluster groups. Principal components (PC) of the individual cluster components were used to determine the point variation explained by TR, LS, and clustered groups. Cluster 1 (C1) grouped animals on TR, MLS, and ADG. The Pr for C1 was 39.2%, recategorizing 725 animals between TRT and C1 and 477 animals between LS and C1. The first 2 PC were able to explain 72.94% of the point variation associated with TR, LS, and C1. Cluster 2 (C2) was formed by replacing ADG with eADG. The Pr of C2 was estimated to be 58.6%, recategorizing 1,202 animals between TR and C2, and no animals between LS and C2. The first 2 PC from the C2 analysis were able to explain 74.8% of the point variation of TRT, LS, BRD, and C2. The amount of point variation explained by the first 2 PC suggest that eADG is more accurate than ADG in predicting true BRD prevalence. The increased predictive power of eADG can be attributed to the majority of animals being diagnosed with BRD occurring early in the feeding period. Comparison of categorical groups indicates that LS is most sufficient at estimating true BRD prevalence during the postweaning phase.

Key Words: beef cattle, bovine respiratory disease, health

M48 A research model for inducing leg problems in broilers. R. F. Wideman*[1], F. Khajali[2], K. R. Hamal[1], A. F. Wideman[1], and H. Lester[1], [1]*University of Arkansas Division of Agriculture*, *Fayetteville,* [2]*Shahrekord University, Faculty of Agriculture*, *Shahrekord, Iran*.

Leg problems increasingly affect fast growing broiler chickens worldwide. Studies investigating practical methods for reducing lameness have been hampered by the sporadic onset and variable incidence of leg problems within experimental flocks. Our objective was to develop a model for inducing a reliably high incidence of lameness in fast growing broilers. To accomplish this we constructed wire floors to create sporadic unstable footing in broiler pens. Rectangular frameworks were constructed from 5 cm x 5 cm lumber. Each frame was 3.05 m long and 1.52 m wide, with 5 cm x 5 cm cross members added for support. Hardware cloth (1.3 cm x 2.54 cm mesh) was fastened to the frame and cross-members. Ten pens (3.05 m x 3.05 m) were set up with floor litter only and 10 pens were set up with half litter and half wire-frame floors. Initially the wire frame was placed flat on the pen floor. Tube feeders were positioned on one side of the pen and the nipples were positioned above the wire frame on the opposite side of the pen. When the chicks reached 2 weeks of age the wire floor was elevated to a 20% slope (Experiment 1) or a 30% slope (Experiment 2), forcing the chicks to walk up and down the sloping wire to drink. Chicks were placed at densities of 50 or 100 per pen in Experiments 1 and 2, respectively. Cumulative incidences of lameness were compared for 2 to 8 wk old broilers using a z-test, with significance declared at $P \leq 0.05$. The incidence of lameness induced in Experiment 1 by the 20% sloping wire floor (6.8%; 34/500 birds) did not differ from the spontaneous occurrence of lameness on litter alone (5.8%; 29/500 birds). The incidence of lameness induced in Experiment 2 by the 30% sloping wire floor (26.7%; 111/416 birds) was significantly higher ($P = 0.01$) than the spontaneous occurrence of lameness on litter alone (10.7%; 43/400 birds). Sloping wire floors can be used to reliably induce reasonably high incidences of lameness, thereby permitting future assessments of practical strategies for reducing leg problems during broiler production.

Key Words: lameness, model, broilers

M49 Microbial diversity in the ileal and cecal contents of broilers using pyrosequencing. S. J. Eom*[1], H. J. Kim[1], C. J. Cha[2], and G. B. Kim[1], [1]*Department of Animal Science and Technology, Chung-Ang University*, *Anseong 456-756, South Korea*, [2]*Department of Biotechnology (BK21 Program), Chung-Ang University*, *Anseong 456-756, South Korea*.

The microbiota in the gastrointestinal (GI) tract of animal plays a pivotal role in the animal′s overall health. However, there is a scarcity of information on the microbial diversity in the gut of livestock animals including broilers and layer hens. Recent developments in microbial ecology have utilized rapid sequencing technologies such as pyrosequencing to investigate the microbial diversity of the human and animal gut. The present study was designed to evaluate differences in the ileal and cecal microbial communities of adult broilers (5 wks old, n = 6) using a bacterial barcoded pyrosequencing strategy. The V1-V3 region of the 16S rRNA gene was amplified by PCR using bar-coded universal primers of 27F and 518R. The amplicons were combined in a single region of the picotiter plate such that approximately 5,000 sequences were obtained from each animal. Taxonomic assignment was performed using the EzTaxon database (http://www.eztaxon.org) and the quantitative analyses was carried out based on the number of sequence reads of each bacterial taxon. Lactobacilli were found to be predominant in the upper gastrointestinal tract and the most abundant *Lactobacillus* species in the ileum were *L. salivarius, L. crispatus*, and *L. aviarius*. Another 12 *Lactobacillus* species were also detected at different levels. In addition, *Enterococcus cecorum* were also abundant in the ileum of adult broilers. In the ceca, the microbial community was highly diverse and *Lactobacillus* species were not found. Clostridia were the most abundant, representing 79% of the total reads. The most common genus detected in the ceca was *Clostridium* and other genera found in the ceca were *Dorea, Alistipes, Bacteriodes*, and *Roseburia*. Pyrosequencing used in this study was proven to be a useful tool for the evaluation of the microbial diversity in the GI tract. Further studies using this tool should be done to better understand the normal microbiome associated with efficient productivity, as well as the impact of changes made in the diet including probiotics supplementation for hens or broilers.

Key Words: broilers, microbiota, pyrosequencing

M50 Use of infrared thermography to monitor risk factors in newborn piglets. J. Morales[1], A. Manso[1], M. Aparicio[1,2], and C. Pineiro*[1], [1]*PigCHAMP Pro Europa*, *Segovia, Spain*, [2]*Centro de Experimentación y Formación en Porcino*, *Segovia, Spain*.

Hypothermia is one of the most important factors affecting perinatal mortality. In this study, infrared thermography was used as a new tool to determine hypothermia and its evolution in newborn piglets. Thermography might provide high advantages in swine clinical practice, revealing lesions and risk factors that would remain hidden with other diagnostic systems. Twenty-two piglets from 2 different litters entered the study at birth time. In each litter, half of the piglets were immediately dried with an absorbent material (cut paper). The other half was not manipulated at birth. Skin temperature on the back was recorded from each piglet at birth and every 10 min time during 1.5 h using a thermographic camera (Fluke Ti45). Piglets were weighed at birth and at 2, 4 and 9 d of life. Data were analyzed using the MIXED procedure of SAS (v 9.00). The statistical model for the temperature analysis included the fixed effects of treatment (control vs drying), time (10 min intervals) and their interaction and the block effect of litter within treatment. Drying piglets immediately after birth increased the skin temperature in the first 90 min of life (39.3°C vs 37.8°C in dried and control piglets, respectively; $P < 0.05$). Evolution of the skin temperature was also different

between treatment (P treatment x time = 0.0001). Initial temperature was 39.3 ± 0.79°C and was kept almost constant in the dried-piglets in the 90 min-interval, while in the control group immediately decreased after birth and then increased progressively until 90 min after farrowing, when skin temperature did not differ between groups (P = 0.27). Body weight evolution did not differ between treatments (2.71 kg at 9 d of life). Drying piglets immediately after birth was effective to keep the body temperature, confirming it as a good management practice to prevent perinatal hypothermia. Infrared thermography demonstrated enough accuracy to be considered as a new tool to complement other diagnostic tools.

Key Words: infrared thermography, newborn piglet, hypothermia

M51 Relationship between lying patterns, feeding management, and incidence of intramammary infection in dairy cows milked in an automated system. T. J. DeVries*[1], K. E. Leslie[2], H. W. Barkema[3], J. Rodenburg[4], and G. Seguin[5], [1]*University of Guelph, Kemptville Campus*, *Kemptville, ON, Canada*, [2]*University of Guelph*, *Guelph, ON, Canada*, [3]*University of Calgary*, *Calgary, AB, Canada*, [4]*DairyLogix Consulting*, *Woodstock, ON, Canada*, [5]*Dairy Farmers of Ontario*, *Casselman, ON, Canada*.

The objectives of this study were to investigate whether feed manipulation affects post-milking standing time in cows milked in an automated milking system (AMS) and to determine if this time relates to the incidence of coagulase-negative staphylococci (CNS) intramammary infection (IMI). Over a 4-mo period, 111 lactating Holstein dairy cows, kept in a sand-bedded freestall barn with 2 pens, each with a free traffic AMS, were monitored. Feed was delivered once daily, and pushed up 2–3 times per day. Quarter milk samples were collected for bacteriological culture from each cow, once every 4 wks. A new IMI was defined as a positive culture sample following a negative culture. For 7 d before each of the last 3 milk samplings, lying behavior, and times of milking and feed manipulation (feed delivery and push up) were recorded. A logistic regression model was used to assess the relationship between post-milking standing time and occurrence of a new CNS IMI. Feed manipulation around the time cows were milked (1 h before 2 h after) resulted in the longest post-milking standing times (86.9 ± 4.3 min; P < 0.001). The shortest post-milking standing times (50.9 ± 4.6 min) were seen in those cows that were milked >4 h before feed manipulation. Over the study period, 58 new CNS IMI were detected, resulting in a herd incidence rate of 0.94 CNS IMI/quarter-year at risk. A non-linear relationship between post-milking standing time and CNS IMI incidence was found (P < 0.04). Compared with those cows that lie down <120 min after milking, those cows that lie down for the first time 120–150 min after milking had lower risk (OR = 0.26, 95% CI = 0.04, 1.98), while those cows that lie down for the first time >150 min after milking had higher risk (OR = 2.70, 95% CI = 1.08, 6.78) of a new CNS IMI. These results suggest that despite being able to manage post-milking standing time of cows milked in an AMS by providing fresh feed, as well as by pushing up feed, frequently throughout the day, the use of such a feeding management strategy in AMS will not necessarily prevent new CNS IMI.

Key Words: intramammary infection, automated milking, lying behavior

M52 Proteomics analysis of plasma and milk protein between healthy dairy cows and *Staphylococcus aureus* infected-subclinical cows. Y. X. Yang*, G. L. Cheng, H. L. Zhao, X. C. Jiang, and S. Chen, *Anhui Academy of Agricultural sciences*, *Hefei Anhui, China*.

Mastitis caused by *Staphylococcus aureus* that are most often the contagious type remains the largest problem on dairy farms. The purpose of our study was to investigate the dynamic changes of plasma and milk protein from healthy and *S. aureus* infected cows. Plasma and milk was collected from dairy cows on d 8 ± 2 and d 50 ± 2 with following diagnosis of acute subclinical mastitis (n = 6) and negative control cows (n = 10) according to the bacteriological culture of milk from all 4 quarters and somatic cell count. Plasma and milk proteins were separated by 2-dimensional electrophoresis; differentially expressed proteins were analyzed by PDQuest 8.0 software, and identified by HPLC equipped with ion trap mass spectrometer. Expression of haptoglobin was abruptly upregulated on d 8 ± 2, and similarly on d 50 ± 2, while α 1 acid glycoprotein was upregulated on d 50 ± 2 in plasma of cows subclinically infected with *S. aureus* mastitis. Expression of albumin and β-casein variant was increased on d 8 ± 2 and continuously on d 50 ± 2 in milk protein of *S. aureus* infected cows, in addition, albumin and β-casein fragments were more variation on d 50 ± 2 than control milk and on d 8 ± 2 in milk protein. The results indicated that expression abundance of plasma and milk proteins were altered and participated in the principal effects of the inflammatory response during dairy cows infected with *S. aureus* subclinical mastitis. Moreover, milk proteins from *S. aureus* infected cows had much larger variation as time goes on. The findings may be useful to provide evidence for treatment the *S. aureus* subclinical mastitis in the early stage of infection to minimize production losses.

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Key Words: dairy cow, *Staphylococcus aureus* mastitis, proteome

M53 Developmental changes in plasma proteins during the transition period in dairy cows. Y. X. Yang[1,2], S. S. Li[1], J. Q. Wang*[1], D. P. Bu[1], L. Y. Zhang[1], and L. Y. Zhou[1], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*, [2]*Institute of Animal Science and Veterinary Medicine, Anhui Academy of Agricultural Sciences*, *Hefei, China*.

Plasma proteins undergo programmed changes in response to parturition-induced immunosuppression during the transition period. In this study blood samples were collected at 21 d before calving, 1 d and 21 d after calving from healthy Chinese Holstein heifers (n = 6) considered free of mastitis, milk fever, and endometriosis based on the somatic cell count and clinical diagnosis. Developmental changes of plasma were examined using integrated proteomic approaches consisting of minor abundance protein enrichment by ProteoMiner, protein separation by 2-dimensional gel electrophoresis, and protein identification by HPLC equipped with ion trap mass spectrometer. Of the 4 proteins identified, expression of serum amyloid A isoform was altered at parturition, while apolipoprotein E and clusterin were upregulated in minor abundance plasma on d 1 and d 21 after calving compared with d 21 before calving. In addition, IgG, which exerts antimicrobial function, was downregulated on d 1 after calving when compared with other time points during the transition period. Quantitative determination of IgG in plasma was performed using a sandwich ELISA method. Levels of plasma IgG on d 1 after calving were 2.04 ± 0.64 mg/mL, slightly less than IgG concentrations of 2.56 ± 0.87 mg/mL and 3.45 ± 0.96 on d 21 before and after calving, respectively. The IgG concentrations detected

by ELISA were not in complete agreement with the 2-dimensional gel electrophoresis and mass spectrometer expression data. This discrepancy possibly related to IgG polymorphisms. These results may be useful in guiding future studies to investigate mechanisms of the plasma protein secretion during the transition period and to elucidate immune system response at the protein level.

Key Words: periparturient, dairy cow, plasma proteome

M54 Effects of single and combined *Mycoplasma gallisepticums* vaccination on blood electrolytes and acid–base balance in commercial egg-laying hens. H. A. Olanrewaju*, S. D. Collier, and S. L. Branton, *USDA–ARS*, *Starkville, MS*.

Previous study on F-strain *Mycoplasma gallisepticum* (FMG) inoculated layers from our laboratory showed a significant increase in arterial partial pressure of oxygen (pO_2), which is generally associated with an oxygen-dependent improvement in tissue oxygenation to improve the layer chicken's ability to withstand the harmful effects of stressors on their performance and well–being. The aim of this study was to determine whether Bacterin (killed vaccine) and TS-11 (Live vaccine) treatment combination could enhance the arterial (pO_2) levels in layer chickens. The experiment was conducted in 2 trials and arranged in a completely randomized experimental design with 4 treatments. The treatments consisted of Control (MG-Clean), Bacterin, TS-11, and Bacterin + TS-11 combined. In each of the 2 trials, 160 1-day-old *Mycoplasma gallisepticum* (MG) free pullets were raised to 10 week of age (WOA) and were transported to a poultry disease isolation facility. Sixteen isolation units were divided in 4 treatments and each of the 4 treatments had 4 replications with 10 birds/unit (40 birds/treatment). Venous blood samples were collected at the termination of the study. TS-11 vaccinated chickens had significantly ($P \leq 0.05$) higher blood (pO_2) and significantly ($P \leq 0.05$) lower partial pressure of CO_2 (pCO_2) when compared with control and combined MG vaccinated groups. However, no significant differences were observed between Bacterin and TS-11 treatment groups. Hematocrit and blood concentrations of hemoglobin were not statistically affected among treatments, but were slightly higher in TS-11 treatment group. There was a significant ($P \leq 0.05$) effect on blood concentrations of Na^+, Ca^{2+}, and anion, but no significant effect on glucose, cholesterone, triglyceride, and osmolality. These data suggest that inoculation of layers with TS-11 was more effective in elevating (pO_2), than inoculation with Bacterin or TS-11 + Bacterin combined.

Key Words: *Mycoplasma gallisepticum*, vaccine, acid–base balance

M55 Continuously growing chicken liver cell lines for the vaccine production against poultry viruses. J. Y. Lee* and B.-W. Kong, *University of Arkansas*, *Fayetteville*.

A continuously growing, immortal cell line can serve as a stable substrate to produce a cell culture based viral vaccine. The objective of this study is to develop an immortal chicken cell line that can efficiently propagate avian infectious viruses, such as infectious laryngotracheitis virus (ILTV), which causes acute upper respiratory disease in chickens. Primary chicken embryo liver (CEL) cells, which were permissive to ILTV, were transfected with various ectopic expression constructs and/or small interfering RNAs (siRNAs) for cell cycle regulatory genes. As results, 3 immortal chicken embryonic liver (CELi) cell lines were established. The CELi-si-p53 was transfected with the expression construct for siRNA against p53, the CELi-Vector was transfected with the expression vector control, and CELi-im was immortalized spontaneously without transfection. All 3 CELi lines are permissive to ILTV, but the titers produced were low levels (~10 plaque forming units/ml). To further characterize epigenetic states for CELi cells, alterations of mRNA expression for cell cycle regulatory genes were determined at passages of 30, 50, 70, and 90 for all 3 CELi cell lines by quantitative reverse transcription PCR. Throughout all passages in 3 CELi cell lines, the mRNA expression of both p53, (function for cell cycle arrest) and its transcriptional target gene, p21WAF1, were down-regulated showing 10 to 20% expression levels compared with those in primary CEL counterpart. The mRNA expression of E2F1 (function for cell cycle progression), was increased 5.3 - 7.1 fold in all 3 CELi cell lines compared with primary CEL cell counterpart. These results are correlated with mRNA expressions shown in previously established immortal chicken embryo fibroblast cell lines, that efficiently propagate Marek's disease virus (MDV). Though newly established CELi cell lines produced only low ILTV titers, those can be utilized for cell culture based vaccine production against other avian viruses, such as MDV or avian metapneumovirus.

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Key Words: immortal chicken liver cell lines, cell cycle regulation, virus propagation

M56 Effect of rabbit sacculus rotundus antimicrobial peptides on serum antibody titers of AIV and NDV in chicken. R. P. She*[1], K. Z. Wang[2], W. M. Ma[1], Y. Ding[1], and J. Tang[1], [1]*College of Veterinary Medicine, China Agricultural University*, *Beijing,100193,China*, [2]*Research Center of Laboratory Animal*, *Jinan, 250002, Shandong, China*.

Multicellular organisms express numerous antimicrobial peptides (AMP), which have received increased attention over the past decade; over 400 AMP have been identified to date in insects, plants, and animals. AMP have the capacity to kill or inactivate a particular spectrum of bacteria, fungi, and some enveloped viruses in vitro. Our previous research demonstrated that Rabbits Sacculus Rotundus (RSRP) AMP have been shown to improve the structure of intestine and promote intestinal mucosal immunity during the chicken growth period. The purpose of this study was to evaluate the effect of RSRP on serum antibody titers against Avian Influenza virus (AIV) and Newcastle disease (NDV) in chickens and to investigate the potential use of AMP in modulation of the immune response for animal health. Ninety one-day-old healthy Xinza chicks were randomly divided into 2 groups: 40 chickens in the control group, and 50 chickens in the experimental group and control group. AMP from RSRP was injected (I.M.) at doses of 0.1, 0.2, 0.3, 0.4, 0.5, 0.5, 0.5 mg, at the ages of 7, 14, 21, 28, 35, 42, and 49d. Chickens in control groups were given the same doses of sterile saline, respectively. Blood was drawn from chickens at the ages of 7, 14, 21, 28, 35, 42, and 56d, and serum separated. The hemagglutination inhibition titers of NDV and AIV serum antibody were detected in the serum samples of 10 chickens, which were selected randomly from each group. Results: The results were as follows, serum antibody titers of NDV and AIV in the chickens of experimental group were significantly higher than that of the control group ($P < 0.01$) at ages of 21, 28, 35, 42 and 56d. The present observation investigated that AMP of the RSRP could enhance significantly the serum antibody titers of ND live vaccine and AI inactivate vaccine.

Key Words: antibacterial peptide, Newcastle disease, avian influenza, serum antibody titers