

## Animal Health II

**T11 Intravaginal administration of lactic acid bacteria modulated selected plasma metabolites in transition dairy cows.** Q. Deng, J. F. Odhiambo, T. Lam, S. M. Dunn, and B. N. Ametaj,\* *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

Transition dairy cows experience dietary changes, negative energy balance, and suppressed immunity after parturition, which enhances their susceptibility to uterine infections. The objective of this study was to evaluate blood metabolite responses of periparturient cows administered intravaginally a mixture of lactic acid bacteria (LAB) around calving. One hundred fifty-two pregnant Holstein cows were randomly (based on parity and BCS) assigned to 3 groups 2 wk before the expected day of parturition. Cows received intravaginal LAB or carrier (sterile skim milk) once a wk at -2, -1 and +1 wk relative to calving as following: treatment 1 (TRT1) - 2 consecutive LAB and 1 carrier dose; treatment 2 (TRT2) - 3 consecutive LAB doses; control (CTR) - 3 consecutive carrier doses. Probiotics were a mixture of 3 LAB including *Lactobacillus sakei* FUA3089, *Pediococcus acidilactici* FUA3138, and FUA3140, which were infused at  $10^8$ - $10^9$  cfu per dose. Blood samples were collected from the tail vein once per wk, before treatment, from -2 to +8 wk. A subset of serum samples from 32 cows were used to evaluate concentrations of glucose, cholesterol, nonesterified fatty acid (NEFA),  $\beta$ -hydroxy butyric acid (BHBA), and lactate. Results showed that concentrations of glucose were  $60.8 \pm 1.1$ ,  $65.2 \pm 1.1$ , and  $59.3 \pm 0.9$  mg/dL ( $P < 0.01$ ) in TRT1, TRT2, and CTR, respectively. Serum cholesterol was  $121.5 \pm 2.3$ ,  $113.8 \pm 2.1$ , and  $106.2 \pm 1.9$  mg/dL ( $P < 0.001$ ) in TRT1, TRT2, and CTR, respectively. Furthermore, concentrations of BHBA in the serum were at  $738 \pm 39$ ,  $555 \pm 37$ , and  $623 \pm 33$   $\mu$ mol/L ( $P < 0.01$ ) in TRT1, TRT2, and CTR, respectively. Treatments had no effect on serum concentrations of NEFA and lactate ( $P > 0.05$ ). Serum glucose, cholesterol, NEFA, BHBA, and lactate were greater ( $P < 0.05$ ) and glucose was lower ( $P < 0.001$ ) in multiparous cows than those in primiparous cows. The concentrations of all metabolites measured changed in relation to wk ( $P < 0.05$ ). In conclusion, data indicated that administration of LAB in the vaginal tract of periparturient dairy cows affected serum concentrations of selected metabolites related to carbohydrate and lipid metabolism and showed differences between multiparous and primiparous cows.

**Key Words:** dairy cow, lactic acid bacteria, serum metabolites

**T12 Indoor versus outdoor housing during the neonatal, weaning, and commingling periods influences innate immune responses in single-housed Holstein calves.** M. D. Sellers,\* C. J. Cobb, D. L. Hanson, A. R. Pepper-Yowell, and B. S. Obeidat, *Department of Animal and Food Sciences, Texas Tech University, Lubbock.*

Objective was to determine if housing environment (indoor vs. outdoor) influences metabolic and innate immune responses during the neonatal, weaning, and commingling periods in single-housed Holstein calves. Forty-three Holstein calves ( $2 \pm 1$  d old) were housed either in indoor pens ( $n = 21$ ) or outdoor hutches ( $n = 22$ ). Weaning started during the wk 7 by removing the PM milk feeding and calves were fully weaned after d 53 when daily starter consumption was 800 g dry matter. Calves were randomly commingled in outdoor group hutches ( $n = 5$ /pen) on d 91. Peripheral blood samples were collected during the neonatal (3, 10, 21 d), weaning (45, 47, 53 d), and commingling periods (91, 94, 99 d) and analyzed for neutrophil oxidative burst (OB) capacity when cocultured

with an *Escherichia coli*, neutrophil L-selectin expression, whole blood secretion of tumor necrosis factor- $\alpha$  (TNF) when cocultured with lipopolysaccharide, as well as plasma concentrations of haptoglobin, glucose, and urea nitrogen. The study concluded on d 99. Data within each period were analyzed by ANOVA with repeated measures. Within the neonatal period, Indoor calves had more neutrophils producing an OB on d 10 and 21 ( $P < 0.05$ ), as well as a more intense OB response on d 3, 10, and 21 ( $P < 0.05$ ). In addition, Indoor calf neutrophils expressed higher L-selectin on d 21 ( $P < 0.05$ ) and had greater TNF secretion on d 10 ( $P < 0.05$ ). During weaning, Indoor calves had greater OB capacity on d 45, 47 and 53 ( $P < 0.05$ ), and lower haptoglobin concentration on d 47 ( $P < 0.05$ ). Urea nitrogen and glucose decreased ( $P < 0.05$ ) over time across both treatments during the weaning period. During commingling, Indoor calves had a lower ( $P < 0.05$ ) percentage of neutrophils positive for OB on d 94 relative to d 91 when compared with Outdoor calves. Indoor housed calves had heightened innate immune responses during both the neonatal and weaning periods compared with Outdoor calves. In contrast, when calves were commingled, Indoor calves had decreased innate immune responses, which could be due to Indoor calves being moved outside during commingling.

**Key Words:** calf, housing, immune

**T13 Prevalence of nematodes eggs of the genera *Cooperia*, *Ostertagia*, and *Haemonchus*, before and after treatment of dairy heifers with commercial anthelmintics in commercial dairy farms in Puerto Rico.** L. López-Soberal,\* A. Ruiz-Lugo, Á. González-Sanabria, M. Pagán, and G. Ortiz-Colón, *University of Puerto Rico, Mayagüez Campus.*

A total of 316 dairy heifers, between 3 and 12 mo of age, were evaluated in 18 commercial dairy farms in Puerto Rico to determine the prevalence of gastrointestinal nematodes eggs before and after anthelmintic treatment. Three grams (3g) of feces were collected directly from the rectum of each heifer in 2 different visits (d0) and (d7). After feces collection on d 7, treatments: Ivomec (ivermectin) ( $n = 111$ ), Dectomax (doramectin) ( $n = 98$ ), and Valbazen (benzimidazole) ( $n = 107$ ) were randomly administered to heifers according to manufacturers' instructions. Fourteen (14) days after treatment, 3 g of feces was collected again and the number of parasite eggs per gram of feces was counted using the Modified McMaster Technique. A total of 1,230 eggs were counted before treatment. Nematode genera were identified by a combination of microscopic egg visual evaluation ( $20\times$ ), and egg measurements. Before treatments, the prevalence of nematode eggs were *Cooperia* (19%), *Ostertagia* (62%), *Haemonchus* (13%); *Strongyloides* (5%) and *Trichuris* (1%). Overall, 489 parasite eggs were counted after the administration of anthelmintics. After treatments, the prevalence of nematode eggs were *Cooperia* (6%), *Ostertagia* (76%), *Haemonchus* (14%), *Strongyloides* (2%) and *Trichuris* (2%). The number of *Cooperia* eggs before treatments was  $109.2 \pm 21$  eggs/gram feces (EGF), and it was reduced in all treatments to  $20.8 \pm 28$  EGF ( $P < 0.03$ ). *Ostertagia* averaged  $166 \pm 23$  EGF before treatments, and the EGF was reduced by Dectomax and Valbazen treatment to  $87 \pm 19.3$  and  $32.4 \pm 18.2$  EGF respectively ( $P < 0.03$ ). Conversely, Ivomec treatment did not affect the number of *Ostertagia* eggs ( $P = 0.63$ ). *Haemonchus* averaged  $76.5 \pm 27.7$  EGF before treatments, and the egg count was reduced by Dectomax and Valbazen treatment to  $30.7 \pm 8.7$  and  $32.4 \pm 11.7$  EGF respectively ( $P < 0.001$ ). Ivomec treatment did not affect the number of *Haemonchus*

eggs ( $P = 0.63$ ). These data suggest that *Ostertagia* and *Haemonchus* populations have developed resistance to ivermectin in Puerto Rico.

**Key Words:** ivermectin, doramectin, benzimidazole

**T14 Eicosapentaenoic acid and NF- $\kappa$ B inhibitor pyrrolidine dithiocarbamate attenuate prostaglandin production by bovine endometrial cells treated with lipopolysaccharide.** L. Badinga,\* M. S. Gulay, and A. D. Ealy, *University of Florida, Gainesville.*

The recognition that n-3 polyunsaturated fatty acids (n-3 PUFA) possess anti-inflammatory properties has prompted a series of studies investigating their efficacy in animal and human models of inflammatory disease. Although their action in antagonizing arachidonic acid metabolism is a key anti-inflammatory effect of n-3 PUFAs, these FA have several other anti-inflammatory effects which might occur downstream of altered eicosanoid production or might be independent of this activity. The objective of this study was to examine the effects of  $\alpha$  linolenic (ALA, C18:3; 100  $\mu$ M), eicosapentaenoic (EPA, C20:5n-3; 100  $\mu$ M) and docosahexaenoic (DHA, C22:6n-3; 100  $\mu$ M) acids on prostaglandin E2 (PGE2) and F2 $\alpha$  (PGF2 $\alpha$ ) production by bovine endometrial (BEND) cells treated with lipopolysaccharide (LPS, 10  $\mu$ g / mL). Concentrations of PGE2 and PGF2 $\alpha$  in the culture medium increased ( $P < 0.01$ ) in a dose and time-dependent manner following treatment with LPS. Addition of omega-3 PUFAs to the incubation medium decreased ( $P < 0.01$ ) PGE2 response to LPS by 70.2%. Alpha linolenic acid and EPA were equipotent in reducing PGE2 response to LPS (89% reduction). A smaller but statistically significant reduction of PGE2 production was detected ( $P < 0.01$ ) when BEND cells were co-treated with LPS and DHA. ALA and EPA decreased ( $P < 0.01$ ) PGF2 $\alpha$  response to LPS by 63 and 79%, respectively. In contrast, DHA increased ( $P < 0.01$ ) PGF2 $\alpha$  response to LPS by 42%. Addition of an NF- $\kappa$ B inhibitor (50  $\mu$ M pyrrolidine dithiocarbamate) to the culture medium decreased ( $P < 0.01$ ) PGE2 response to LPS to a greater extent than did EPA. Results indicate that omega-3 FA and the NF- $\kappa$ B inhibitor attenuate LPS effects on endometrial PG secretion and that these molecules may act through distinct signaling pathways. Additional studies are needed to fully characterize the mechanism by which omega-3 FA modulate pro-inflammatory eicosanoid biosynthesis in the bovine endometrium

**Key Words:** eicosapentaenoic acid, prostaglandin, cattle

**T15 Plane of milk replacer nutrition influences the acute phase response of weaned Jersey calves to an oral *Salmonella typhimurium* challenge.** D. L. Hanson\*<sup>1</sup>, M. D. Sellers<sup>1</sup>, C. J. Cobb<sup>1</sup>, T. J. Earleywine<sup>2</sup>, and M. A. Ballou<sup>1</sup>, <sup>1</sup>*Department of Animal and Food Sciences, Lubbock, TX*, <sup>2</sup>*Land O'Lakes, Animal Milk Products Co., Shoreview, MN.*

Objective was to determine if previous plane of milk replacer nutrition influences the acute phase response to an oral *Salmonella typhimurium* (ATCC14028) challenge in weaned Jersey calves. Twenty Jersey bull calves (77  $\pm$  1 d old) that were previously fed either a Low Plane (LP; 409 g/d of a 20/20 milk replacer) or a High Plane (HP; 610 and 735 g/d of a 28/25 milk replacer for wk 1 and wks 2–6, respectively) of milk replacer were enrolled in the study. All calves were offered ad libitum access to calf starter after the 1st wk of life. Calves began weaning by removing the PM feeding during wk 7 and were completely weaned when they consumed 600 g of calf starter / d after the 7th wk. Calves were orally challenged with  $1.5 \times 10^7$  colony forming units of *Salmonella typhimurium*. Indwelling rectal temperature probes collected a measurement every 5 min. from -16 h to the conclusion of the study at 236 h

post-challenge. Peripheral blood samples were collected daily at 0800 throughout the study and analyzed for oxidative burst capacity when cocultured with an *Escherichia coli*, whole blood secretion of tumor necrosis factor- $\alpha$  when cocultured with lipopolysaccharide, as well as plasma concentrations of haptoglobin, glucose, and urea nitrogen. The percentage of neutrophils producing an oxidative burst was greater ( $P < 0.05$ ) among HP calves from d 1—5 after the challenge. Similarly, the intensity of the oxidative burst tended to be greater ( $P < 0.10$ ) among HP calves on d 2 and 3 after the challenge. In addition, the secretion of tumor necrosis factor- $\alpha$  tended ( $P < 0.10$ ) to be greater on d 1 and was greater ( $P < 0.05$ ) on d 5 and 6 after the challenge among HP calves. Median ranks of haptoglobin concentrations were lower ( $P < 0.05$ ) among HP calves throughout the study; however, there was no difference in rectal temperature ( $P = 0.99$ ) between LP and HP calves. Data was analyzed using ANOVA with repeated measures. These data support that calves fed a HP of milk replacer nutrition have a more aggressive innate immune response to an oral *Salmonella typhimurium* challenge, which may improve resistance to disease.

**Key Words:** calf, immune, nutrition

**T16 Intravaginal probiotics expedited uterine involution in postpartum dairy cows.** Q. Deng, J. F. Odhiambo, T. Lam, S. M. Dunn, and B. N. Ametaj,\* *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

Uterine involution and resumption of ovarian cyclicity are good indicators of uterine status and reproductive performance of postpartum dairy cows. The objective of this study was to investigate the effect of intravaginal probiotics on uterine involution and resumption of ovarian cyclicity in postpartum dairy cows. 154 pregnant Holstein cows were randomly assigned (based on parity and BCS) to 3 groups 2 wk before the expected day of calving. Cows received intravaginally a mixture of lactic acid bacteria (LAB) or carrier (skim milk) once a wk at -2, -1 and +1 wk relative to calving as following: treatment 1 (TRT1) - 2 consecutive LAB and 1 carrier dose; treatment 2 (TRT2) - 3 consecutive LAB doses; and control (CTR) - 3 consecutive carrier doses. LAB used in this study were a mixture of 3 bacteria: *Lactobacillus sakei* FUA3089, *Pediococcus acidilactici* FUA3138, and FUA3140 and infused at a dose of  $10^8$ - $10^9$  cfu per dose. Uterine involution was evaluated by rectal ultrasonography using a Sonosite ultrasound fitted with a 7.5MHz probe once a wk at +2, +3, +5, +7 wk to obtain images of the cervix, uterine body and horns, follicles, and corpus luteum. Data were analyzed with the mixed procedure of SAS including treatment, parity, and wk. Results showed differences among treatments in terms of the cross-sectional areas of gravid horn and uterine body. TRT1 had smaller cross-sectional areas of the gravid horn than TRT2 (3.98  $\pm$  0.13 vs. 4.48  $\pm$  0.14 cm<sup>2</sup>;  $P < 0.01$ ) and CTR (3.98  $\pm$  0.13 vs. 4.23  $\pm$  0.12 cm<sup>2</sup>;  $P > 0.05$ ). The cross-sectional areas of uterine body were smaller in TRT1 compared with TRT2 (5.41  $\pm$  0.16 vs. 5.90  $\pm$  0.17 cm<sup>2</sup>;  $P < 0.05$ ) and CTR (5.41  $\pm$  0.16 vs. 5.95  $\pm$  0.15 cm<sup>2</sup>;  $P < 0.01$ ). Multiparous cows had larger gravid horn cross-sectional areas than primiparous cows (4.57 vs. 3.90 cm<sup>2</sup>;  $P < 0.001$ ). Parity also affected cervix and uterine body cross-section areas ( $P < 0.05$ ). Week had an effect on the cross-sectional areas of gravid horn, cervix, and uterine body ( $P < 0.001$ ). Based on the presence of corpus luteum, data showed that the number of cows that had resumed their cyclic activity by wk +7 were 57%, 67%, and 55% ( $P = 0.08$ ) in TRT1, TRT2, and CTR, respectively. Overall, intravaginal LAB treatment expedited involution of the uterine gravid horn and body, and had a tendency to hasten the resumption of ovarian cyclicity in postparturient dairy cows.

**Key Words:** dairy cows, lactic acid bacteria, uterine involution

**T17 Plane of nutrition during the pre- and post-weaned periods influences the innate immune activity of Holstein calves.** B. S. Obeidat<sup>1</sup>, C. J. Cobb<sup>1</sup>, M. D. Sellers<sup>1</sup>, A. R. Pepper-Yowell<sup>1</sup>, D. L. Hanson<sup>1</sup>, T. J. Earleywine<sup>2</sup>, and M. A. Ballou\*<sup>1</sup>, <sup>1</sup>Department of Animal and Food Sciences, Texas Tech University, Lubbock, <sup>2</sup>Land O'Lakes, Animal Milk Products Co., Shoreview, MN.

Objective was to determine the influence of plane of nutrition during the pre- and post-weaned periods on the performance and innate immune activity of Holstein calves. Thirty 9 (2 ± 1 d old) calves were randomly assigned to 2 treatments. Treatments were a low (LPN; n = 18) and high plane of nutrition (HPN; n = 21). Calves in LPN treatment were fed 409 g/d dry matter (DM) of a 20% CP/20% fat milk replacer; whereas calves in HPN treatment were fed 756 and 910 g/d DM of a 28% CP/20% fat during the 1st wk and wk 2–6, respectively. Weaning was initiated during the 7th wk by removing the PM feeding and calves were completely weaned when they were consuming 800 g DM of calf starter after d 53. Calves were fed their respective calf starter until the end of the study at 91 d. Peripheral blood samples were collected on d 3, 10, 21, 45, 47, 53, and 91 for ex vivo immunological and biochemical analyses. Data were analyzed by ANOVA with repeated measures. Metabolizable energy intake, crude protein intake, and average daily gain were greater ( $P < 0.001$ ) for HPN calves compared with LPN calves. No difference between treatments was observed for secretion of tumor necrosis factor- $\alpha$  when whole blood was cocultured with lipopolysaccharide. On d 3 and 21 neutrophil L-selectin expression was greater ( $P < 0.05$ ) in LPN than HPN. The percentage of neutrophils producing an oxidative burst when cocultured with *Escherichia coli* tended ( $P = 0.06$ ) to be greater and was greater ( $P < 0.05$ ) in LPN than HPN on d 10 and 21, respectively. In addition, neutrophils from LPN had greater ( $P < 0.01$ ) oxidative burst intensity throughout the study. On d 3, 10, 21, and 45 plasma glucose concentrations were greater ( $P < 0.02$ ) in calves fed the HPN. The innate immune responses of calves fed a LPN were more active during the pre-weaning period than calves fed a HPN; however, this response was not observed during the immediate post-weaned period. The exact mechanism underlying the more active innate immune responses of the LPN during the pre-weaned period is not known, but could be due to either increased immunogenic stimulation or decreased stress.

**Key Words:** calf, immune, plane of nutrition

**T18 Pathophysiological response to an oral *Salmonella typhimurium* challenge is influenced by the inoculum dose in newborn colostrum-fed Jersey calves.** A. R. Pepper-Yowell\*, D. L. Hanson, M. D. Sellers, C. J. Cobb, B. S. Obeidat, and M. A. Ballou, Texas Tech University, Lubbock.

Objective was to determine how the pathophysiology of newborn Jersey calves changed over time at increasing doses of an oral *Salmonella typhimurium* (ATCC14028) challenge. Twenty Jersey bull calves (4 ± 1 d old) were orally inoculated with one of 5 doses (n = 4) of *Salmonella typhimurium*: saline,  $1 \times 10^4$ ,  $1 \times 10^6$ ,  $1 \times 10^8$ , or  $1 \times 10^{10}$  colony forming units. Peripheral blood samples were collected daily from -2 to 11 d and on d 14 relative to the challenge and analyzed for glucose, urea nitrogen, and haptoglobin. Indwelling rectal temperature probes collected hourly measurements throughout the study. Both  $10^8$  and  $10^{10}$  were 100% lethal doses (LD<sub>100</sub>) occurring between 2 and 8 d post challenge; whereas  $10^6$  was an LD<sub>50</sub> occurring between 10 and 11 d post challenge. None of the  $10^4$  calves showed any visual signs of disease during the observation period. All values are expressed as differences from the mean of the saline at each time point. Time to reach nadir glucose values for  $10^8$  and  $10^{10}$  was faster than  $10^4$  and  $10^6$  ( $P < 0.01$ ; 3.5, 1.0, 9.0 and 7.8 ± 1.04 d). Average nadir glucose value for  $10^8$  was

lower than  $10^4$  ( $P < 0.05$ ; -44.9 and -28.6 ± 4.91 mg/dL). Time to peak urea nitrogen was faster for  $10^{10}$  than  $10^4$ ,  $10^6$  and  $10^8$  ( $P < 0.01$ ; 1.0, 6.0, 6.0, and 3.8 ± 0.94 d). Peak urea nitrogen was lower for  $10^4$  and  $10^{10}$  than  $10^6$  and  $10^8$  ( $P < 0.05$ ; 8.2, 5.8, 14.9 and 15.6 ± 2.12 mg/dL). Time to peak haptoglobin was faster for  $10^8$  and  $10^{10}$  than  $10^4$  and  $10^6$  ( $P < 0.01$ ; 1.8, 1.0, 4.0 and 4.0 ± 0.63 d). Time to peak rectal temperature was faster for  $10^8$  and  $10^{10}$  than  $10^4$  and  $10^6$  ( $P < 0.01$ ; 1.5, 1.0, 3.5 and 3.3 ± 0.33 d). There was no treatment effect on peak haptoglobin values and peak rectal temperature ( $P = 0.22$  and  $P = 0.13$ ). However, when the animals were classified by survivability the calves that died had higher average haptoglobin than calves that survived ( $P < 0.01$ ; 3.5 and 1.8 ± 0.38 OD). Increasing the dose of *Salmonella typhimurium* in newborn Jersey calves shortened the timeframe that calves reached a peak immune reaction, and increased the magnitude of the reaction for metabolic parameters.

**Key Words:** bacteria challenge, calf, health

**T19 Effect of dietary supplementation with *Curcuma longa* (turmeric) during *Eimeria maxima* and *Eimeria tenella* infection of chickens.** D. K. Kim\*<sup>1</sup>, H. S. Lillehoj<sup>1</sup>, S. H. Lee<sup>1</sup>, S. I. Jang<sup>1</sup>, M. S. Park<sup>1</sup>, and D. Bravo<sup>2</sup>, <sup>1</sup>Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, United States Department of Agriculture, Beltsville, MD, <sup>2</sup>Pancosma SA, Geneva, Switzerland.

The effects of orally administered *Curcuma longa* (turmeric) on the classic criteria of coccidiosis in chicken was evaluated during experimental *Eimeria maxima* (EM) or *Eimeria tenella* (ET) infection and the modification of genome-wide profile was analyzed in uninfected condition. Broiler chickens continuously fed from hatch with *Curcuma Longa* supplemented diet and orally challenged with live EM or ET had increased body weight gain, greater antibody responses to microneme protein 2 from ET (EtMIC2) compared with chickens fed a non-supplemented diet. However, the chickens fed turmeric-supplemented diet shed significantly decreased fecal oocyst excretion only in ET infected chickens, not in EM infection group. Differences between groups were considered statistically significant at  $P < 0.05$  by one-way ANOVA. Differential gene expression by microarray hybridization using a cutoff of >2.0-fold changes identified 601 transcripts whose levels were significantly altered in intestinal lymphocytes of turmeric-fed birds without *Eimeria* infection compared with non-supplemented controls (287 upregulated, 314 downregulated). Biological pathway analysis identified the most significant function belonging to the categories of "Disease and Disorder" as "Nutritional Diseases." The levels of local cytokine transcripts of IL-1 $\beta$ , IL-6, IL-15, and IFN- $\gamma$  were consistently greater in the turmeric-fed no-infection group compared with the controls fed only the standard diet. These results suggest the immunologic and genomic changes that occur in chickens following dietary supplementation of turmeric that are relevant to protective immunity during avian coccidiosis.

**Key Words:** *Curcuma longa*, turmeric, coccidiosis

**T20 Detection of neutralizing antibody titration against rabies virus in dogs.** A. E. Gazi\*<sup>1</sup> and S. Ak<sup>2</sup>, <sup>1</sup>Tarim Ilce Mudurlugu, Bozyazi, Turkey, <sup>2</sup>Istanbul University, Veteriner Fak. Microbiology, Istanbul, Turkey.

The aim of this study was to determine the titers of neutralizing antibodies against rabies virus in 5 hundred vaccinated owned and stray dogs within the Istanbul province. The levels of the neutralizing antibody titration in the blood serum were evaluated by ELISA method. Sixty-five

(13%) of the dogs examined had adequate level (0.5 IU/ml and over) of antibody titration. In addition, the effects on the formation of the neutralizing antibody levels following the vaccination of some factors, such as being owned or stray, the number of repetition of vaccination, the age, living area, sex, interval between the last vaccination and sampling, have been evaluated. The statistical comparisons of the ages, number of repetition of vaccination, interval between the last vaccination and the sampling dates of the groups were analyzed by using one way ANOVA. *t*-test procedure was used to evaluate rest of the data. As a result of the statistical evaluations, it has been determined that interval between the last vaccination and sampling had an important effect on formation of the neutralizing antibody titration ( $P < 0.001$ ). Moreover, the effect of the age of the dogs was also significant for the neutralizing antibody levels ( $P < 0.05$ ). In contrast, being owned or stray, sex, living area and the number of repetition of the vaccination had no effects on the neutralizing antibody levels.

**Key Words:** rabies, vaccines, immunogenicity of rabies vaccine

**T21 Dairy health records use and management by producers in Washington and Idaho.** J. R. Wenz\*<sup>1</sup>, D. A. Moore<sup>1</sup>, R. A. Jus-saume<sup>1</sup>, S. Giebel<sup>1</sup>, S. Poisson<sup>1</sup>, and C. S. Schneider<sup>2</sup>, <sup>1</sup>Washington State University, Pullman, <sup>2</sup>University of Idaho, Moscow.

Nationally, more than 90% of large dairy herds use computerized records, making it possible to use epidemiologic techniques to evaluate management of the herd. However, accuracy and consistency of these data are often lacking. Dairy management software prescribes the way reproduction records are entered but health data entry is user-defined and highly variable within and between farms. Therefore, the ability to meaningfully summarize and evaluate health data are limited. The purpose of this study was to evaluate the use and perceived value of health data on dairies. Mail and online survey that focused on perception of health data utility and how health data were recorded was pre-tested and then provided to 951 dairy producers in Washington and Idaho. Comparisons of proportion of respondents to a question were made using a chi-squared test for homogeneity of proportions. A total of 242 (25.4%) surveys were returned. About 65% of producers used commercial dairy management software and just over half recorded some health events in the computer. Of the 116 that used commercial software and recorded some health events, 85% indicated that their disease and treatment records were most useful for making culling decisions for individual cows but were less confident about the ability of their computerized health records to track drug withdrawal times, monitor disease in the herd or evaluate the effectiveness of management practices for groups of cows. Of all the respondents, 45.6% (108) agreed that better guidelines on how to record disease and treatment records are needed while 16.5% (39) disagreed. Few respondents agreed that disease and treatment data are recorded the same way on most dairies (11.4%). A minority of respondents (16.5%) were dissatisfied with the quality of their records. A higher percentage of dissatisfied respondents agreed that better guidelines for recording health data were needed versus the need for better guidelines on reproduction data (90 vs 51%;  $P < 0.001$ ). Most producers saw value to their health data but primarily for making individual cow decisions. Improvements in health data quality allowing epidemiological evaluation to inform herd-level decision making will require on-farm health data entry industry standards as currently exist for milk production and reproduction records. Such standards would be best implemented by a change from user-defined to prescribed data entry in dairy management software.

**Key Words:** dairy, health, records

**T22 Effect of method of detection and uterine dimensions in the diagnosis of endometritis in lactating dairy cows.** R. L. A. Cerri\*<sup>1</sup>, D. M. Veira<sup>2</sup>, A. M. Tabmasbi<sup>3</sup>, A. M. L. Madureira<sup>1</sup>, S. A. Balios<sup>1</sup>, A. H. Souza<sup>4</sup>, and J. L. M. Vasconcelos<sup>5</sup>, <sup>1</sup>University of British Columbia, Vancouver, BC, Canada, <sup>2</sup>Agriculture and Agri-Food Canada, Agassiz, BC, Canada, <sup>3</sup>Fedowski University of Mashhad, Iran, <sup>4</sup>University of Wisconsin, Madison, <sup>5</sup>Sao Paulo State University, Botucatu, SP, Brazil.

Objectives of this study were to 1) identify the agreement among different methods to detect endometritis and, 2) determine interactions among uterine measurements, parity, BCS and endometritis diagnosis. Three-hundred and forty-seven diagnoses were performed in lactating Holstein cows from the UBC Dairy Centre. Cows were diagnosed at 30 and 44 ± 3 d in milk by gloved hand (GH) and Metriceck (MC) exams based on vaginal discharge that were scored on a 1–5 scale (>3 considered positive), and ultrasonography (US) of the uterus (presence of ecogenic fluid in lumen) as the gold standard. Blood samples and BCS (Low <2.75 > Moderate) were collected on the same dates of uterine exams. Major US measures of the uterus were diameter of the endometrium in both horns (Small <18mm > Large), asymmetry between horns (Moderate <3mm > High), and diameter of the inner layer of cervix (Small <18mm > Large). Data was analyzed by logistic regression using SAS (significant if  $P < 0.05$ ). Results show that endometritis was diagnosed in 10.6% of the time, whereas MC and GH were diagnosed in 15.7% and 13.4% of the time. Considering US as the reference method, agreement with MC and GH was 35.3% and 32.3%, respectively. A large uterine horn (22.8% vs. 12.8%), high asymmetry of uterine horns (16.3% vs. 6.2%) and a large cervix (15.5% vs. 5.8%) measured by US were more likely to be diagnosed with endometritis. When diagnosis was done with MC or GH, the high asymmetry between uterine horns proved significant (20.3% vs. 9.8%) to predict endometritis, but only in the GH a large cervix was more likely to predict endometritis (18.9% vs. 8.2%). Primiparous cows were also more likely to be diagnosed with endometritis based on vaginal discharge (20.2% vs. 12.6%) compared with multiparous cows. BCS had no influence in the diagnosis of endometritis. In conclusion, methods that used solely vaginal discharge to detect clinical cases of endometritis overestimated the number of endometritis cases and were inaccurate compared with US diagnosis. Methods using vaginal discharge are likely diagnosing vaginitis or cervicitis more accurately than endometritis.

**Key Words:** dairy cows, endometritis, ultrasound

**T23 Effects of phytoncide supplementation on growth performance, nutrient digestibility, blood profiles, diarrhea score, and fecal microbial shedding in weaning pigs.** S. Zhang\*<sup>1</sup>, J. H. Jung<sup>1</sup>, H. S. Kim<sup>2</sup>, B. Y. Kim<sup>2</sup>, and I. H. Kim<sup>1</sup>, <sup>1</sup>Department of Animal Resource & Science, Dankook University, Cheonan, Choongnam, South Korea, <sup>2</sup>Phylus Co. Ltd., Chungbuk, South Korea.

A total of 140 weaning pigs [(Landrace × Yorkshire) × Duroc, BW = 6.47 ± 0.86 kg] were used in a 5-week growth trial to determine the effects of phytoncide supplementation on growth performance, apparent total tract digestibility (ATTD), blood profiles, diarrhea score, and fecal microbial shedding. Pigs were randomly assigned into 1 of 5 treatments. Dietary treatments were: NC, basal diet (without antibiotics); PC, NC + 0.05% tylosin; EO, NC + 0.1% essential oil; PP, NC + 0.2% PP (phytoncide with 2% citric acid), and PA, NC + 0.2% PA (phytoncide). Each treatment had 7 replicate pens with 4 pigs per pen. All pigs were housed in pens with a self-feeder and a nipple drinker, and allowed ad libitum access to feed and water throughout the experiment. Statistical analyses were conducted in accordance with mean separations performed by the Duncan multiple range test using the GLM procedure of the SAS

statistical software package (1996). During 0–2 weeks, the gain to feed (G/f) ratio in EO and PP treatments were increased ( $P < 0.05$ ) compared with PA treatments. During 2–5 weeks, PA treatment had the highest ( $P < 0.05$ ) G/f among treatments. Pigs fed the PC and PP diets had higher ( $P < 0.05$ ) ATTD of gross energy than those fed the NC and EO diets at 2 week. The ATTD of dry matter (DM) was improved ( $P < 0.05$ ) in PC, PP and PA treatments compared with NC treatment at 5 week. The PP treatment increased ( $P < 0.05$ ) the concentration of immunoglobulin G (IgG) in blood compared with NC, PC and EO treatments at 2 week. These pigs fed PP diet showed greater ( $P < 0.05$ ) amounts of fecal *Lactobacillus* compared with NC treatment. However, no difference ( $P > 0.05$ ) was observed in diarrhea score among treatments. In conclusion, dietary supplementation with 0.2% PP may improve nutrient digestibility, increase blood IgG concentration, and the amounts of fecal *Lactobacillus* in weaning pigs, which indicated that phytoncide with 2% citric acid could be used as an alternative for antibiotics in weaning pigs.

**Key Words:** blood profiles, phytoncide, weaning pigs

**T24 Influence of tannins extract supplementation on feedlot performance of receiving bull-calves naturally infested with gastro intestinal parasites.** R. Barajas<sup>1</sup>, B. J. Cervantes<sup>2</sup>, M. A. Espino<sup>1,3</sup>, A. Camacho<sup>1</sup>, I. Enriquez<sup>1</sup>, C. Barraza<sup>1</sup>, L. R. Flores<sup>1</sup>, J. J. Lomeli<sup>1</sup>, and J. A. Romo<sup>1</sup>, <sup>1</sup>FMVZ-Universidad Autónoma de Sinaloa, Culiacán, Sinaloa, México, <sup>2</sup>Ganadera Los Migueles S.A. de C.V., Culiacán, Sinaloa, México, <sup>3</sup>Pronutrient Developers, León, Guanajuato, México.

Sixty-five bull-calves  $171 \pm \text{SE } 2.77$  kg were used to determine the influence of tannins extract supplementation on feedlot performance of receiving bull-calves naturally-infested with gastro intestinal parasites. Bull-calves were individually weighed, ear-tagged, and grouped in 4 blocks using initial weight criteria for reduction of social interaction, and allotted in 16 ground floor pens ( $6 \times 12$  m). Bull-calves were weighed d 1 and 28 when feces and blood samples were taken from each bull-calf to exploring the presence of gastro-intestinal parasites, blood parasites, and plasma urea nitrogen (PUN) determination. Inside each block, pens were randomly assigned to receive 1 of 2 treatments: 1) Feeding with a 70% concentrate ground sorghum-DDG-canola meal based diet (CTRL;  $n = 32$ ); or 2) Diet similar to CTRL, added with 0.3% (dry matter basis) of tannins extract (TE;  $n = 33$ ). Tannins extract was supplied as TMP-Protein Enhancer (Técnica Mineral Pecuaria; Mexico), a premix that contains 56% of a condensed (Quebracho tree) and soluble (Chesnutt) tannins-blend. Number of infested animals was compared using Chi-squared test. Number of parasites-eggs by gram of feces data (NEGF) was transformed to square-root before analyses. NEGF, PUN, final weight and Average daily gain were analyzed by ANOVA. No blood-parasites were found. Eggs of found parasites corresponded to: *i* (85%), *Eimeria* sp. (72%), *Cooperia* sp. (71%), *Trichostrongylus* sp. (52%), and *Esophagostomus* sp. (32%). In d 1 and 28 the amount of infested-calves and the NEGF were similar ( $P > 0.50$ ) between treatments. TE supplementation decreased ( $P = 0.03$ ) 15.5% PUN concentration (14.06 vs. 11.88 mg/dL). Final weight was similar between treatments ( $P = 0.51$ ). Average daily gain tended to be higher ( $P = 0.07$ ) in bull-calves fed-TE (1.370 vs. 1.191 kg/day for TE and CTRL, respectively). It is concluded that tannins extract supplementation in dose near to 0.3% of dietary DM, did not produce changes on GI parasites population of receiving bull-calves.

**Key Words:** feedlot-calves, parasites, tannins

**T25 Effects of different levels of fermented oat on growth performance, diarrhea score, fecal microbial shedding, and fecal noxious gas emission in weaning pigs.** J. P. Wang,\* J. P. Lee, and I. H. Kim, *Department of Animal Resource & Science, Dankook University, Cheonan, Choongnam, South Korea.*

This study was conducted to investigate the effects of different levels of fermented oat on growth performance, diarrhea score, fecal microbial shedding, and fecal noxious gas emission in weaning pigs. A total of 125 weaning piglets [(Landrace  $\times$  Yorkshire)  $\times$  Duroc, BW =  $7.31 \pm 0.24$  kg] weaned at 21 d of age were assigned to 5 treatments in randomized complete block designs based on the initial BW and sex. This experiment included 2 phases. In phase 1 (0–21 d), there were 5 treatments: T1, basal diet + 15% nature oat; T2, basal diet + 3.7% fermented oat + 11.3% nature oat; T3, basal diet + 7.5% fermented oat + 7.5% nature oat; T4, basal diet + 11.3% fermented oat + 3.7% nature oat, and T5, basal diet + 15% fermented oat. In phase 2 (21–35 d), it also included 5 diets, an half of dose in phase 1 were supplemented in phase 2. Pigs were allowed ad libitum access to feed and water and their BW and FI were measured by each phase for entire experiment. During the phase 1, the use of 7.5%, 11.3%, 15% fermented oat to replace nature oat improved ( $P < 0.05$ ) ADG and G/f compared with T1 and T2 treatments. Use of fermented oat up to 7.5% replacing nature oat showed the highest ( $P < 0.05$ ) ADG and G/f among dietary treatments. During the phase 2, pigs fed the 3.5%, 5.3%, and 7% fermented oat substituted diets showed higher ( $P < 0.05$ ) ADG compared with those fed the basal diet. Overall, ADG and ADFI were higher ( $P < 0.05$ ) in T3, T4 and T5 treatments compared with T1 and T2 treatments. Different levels of fermented oat had no effect ( $P < 0.05$ ) on diarrhea score, nutrient digestibility, fecal microbial shedding, and fecal noxious gas emission. Collectively, we could conclude that supplementation with 50% fermented oat to replace nature oat is an appropriate dose to achieve a better growth performance in weaning pigs.

**Key Words:** fermented oat, growth performance, weaning pigs

**T26 GPR109A mediates calcium mobilization induced by BHBA in isolated bovine monocytes.** L. K. Mamedova\*<sup>1</sup>, E. C. Titgemeyer<sup>1</sup>, G. M. Pighetti<sup>2</sup>, J. Y. Lu<sup>1</sup>, D. H. Hua<sup>1</sup>, and B. J. Bradford<sup>1</sup>, <sup>1</sup>Kansas State University, Manhattan, <sup>2</sup>University of Tennessee, Knoxville.

Ketosis is a common in early lactation dairy cows, and ketosis greatly elevates the risk of subsequent infections. In vitro administration of  $\beta$ -hydroxybutyric acid (BHBA) has inhibitory effects on the function of bovine neutrophils, monocytes, and lymphocytes. However, mechanisms linking BHBA to altered immune function remain unknown. Our objective was to evaluate the potential role of the BHBA receptor GPR109A in leukocytes. Blood was collected from 3 healthy Holstein cows in mid-lactation and peripheral blood mononuclear cells (monocytes) and polymorphonuclear neutrophils were isolated. Protein and RNA were extracted, mRNA abundance of GPR109A was determined by quantitative RT-PCR, and protein abundance was determined by Western blot. Results demonstrated strong expression of GPR109A at the mRNA (mean Ct =  $22.7 \pm 0.6$ ) and protein levels in both cell types. Next, isolated monocytes and neutrophils were exposed to BHBA (1 mM) or niacin (10  $\mu$ M), a potent agonist of GPR109A. Immediately after exposure to treatments, cytosolic calcium concentrations were monitored by fluorescence assay. Both BHBA and niacin elevated cytosolic calcium in both monocytes and neutrophils (all  $P < 0.01$ ). Finally, RNA interference was used to knock down the protein before exposure to agonists. Isolated monocytes were transfected with siRNA (scrambled or GPR109A) and incubated for 24 h. Cells were then treated

with 1 mM BHBA and calcium mobilization was assessed, or cells were harvested to determine knockdown efficiency. Although incubation time was short due to the limited viability of isolated monocytes, densitometry analysis of Western blot revealed that GPR109A protein was significantly knocked down by the siRNA treatment ( $36 \pm 10\%$  decrease,  $P < 0.05$ ). As in the initial study, BHBA caused a significant increase in cytosolic calcium, and GPR109A siRNA decreased this response by  $63 \pm 3\%$  ( $P < 0.001$ ). Collectively, these results suggest that GPR109A at least partially mediates calcium mobilization responses to BHBA in bovine leukocytes. Further research is required to determine whether such mechanisms underlie the effects of BHBA on immune function.

**Key Words:** G protein coupled receptor, ketone, immunity

**T27 Effects of bacteriophage as an alternative for antibiotics on egg performance, egg quality, fecal microbial shedding, and fecal moisture content in laying hens.** P. Y. Zhao,\* B. R. Lee, and I. H. Kim, *Department of Animal Resource & Science, Dankook University, Cheonan, Choongnam, South Korea.*

A total of two hundred forty 40-wk-old ISA-brown laying hens were used in a 6-week feeding trial to evaluate the effects of bacteriophage as an alternative for antibiotics on egg performance, egg quality, fecal microbial shedding, and fecal moisture content. Hens were allotted to 1 of 4 dietary treatments with 10 replications (6 adjacent cages per replication and 1 hen per cage) in a completely randomized design. Dietary treatments included: 1) CON, basal diet; 2) BPS1, CON + 0.020% bacteriophage; 3) BPS2, CON + 0.035% bacteriophage, and 4) BPS3, CON + 0.050% bacteriophage. Bacteriophage used in our study contains *Salmonella gallinarum*, *S. typhimurium*, *S. enteritidis*, *S. pullorum*, *Staphylococcus aureus*. The counts of bacteriophage are  $10^8$  pfu/gram. All diets were formulated to meet or exceed the NRC (1994) requirements for laying hens. Hens were provided with basal diet for a 7 d adjustment period before the beginning of the experiment. All hens were allowed *ad libitum* access to water and feed through nipple drinkers and feeders, respectively. The hens were housed in a windowless and environmentally controlled room that was maintained at 21°C, and had a daily lighting schedule of 16 h light and 8 h dark. During 0–6 weeks, no difference ( $P > 0.05$ ) was observed in egg production and egg weight among dietary treatments. At 4 and 5 weeks, birds in BPS2 group had higher ( $P < 0.05$ ) Haugh unit (HU) than those in CON group. HU in birds fed the BPS1 and BPS3 diets was greater ( $P < 0.05$ ) than those fed the CON diet at 5 and 6 weeks. However, egg weight, egg shell color, yolk height, yolk color unit, egg shell strength, egg shell thickness, egg gravity, fecal microbial shedding, and fecal moisture content were not influenced ( $P > 0.05$ ) by dietary treatments during the entire experimental period. In conclusion, bacteriophage supplementation in our study had beneficial effects on HU, which related to a higher egg albumen contents, thus led to fresher eggs in laying hens.

**Key Words:** bacteriophage, egg quality, laying hens

**T28 Effects of bacteriophage as an alternative for antibiotics on growth performance, nutrient digestibility, blood profiles, fecal microbial shedding, diarrhea score, and fecal moisture content in growing pigs.** S. M. Hong,\* H. Y. Baek, and I. H. Kim, *Department of Animal Resource & Science, Dankook University, Cheonan, Choongnam, South Korea.*

A total of 96 growing pigs [(Landrace × Yorkshire) × Duroc, BW =  $28.85 \pm 1.63$  kg] were used in a 6-week feeding trial to investigate the effects of bacteriophage as an alternative for antibiotics on growth

performance, apparent total tract digestibility (ATTD), blood profiles, fecal microbial shedding, diarrhea score, and fecal moisture content. Pigs were randomly allotted to 4 treatments with 6 replicate pens (4 pigs per pen) per treatment according to their initial BW. Dietary treatments were: 1) NC, negative control diet (without antibiotics); 2) PC, NC + 22ppm tylosin; 3) BP1, NC + 0.025% bacteriophage and 4) BP2, NC + 0.05% bacteriophage. Bacteriophage used in our study contains *E. coli* K88, *E. coli* K99, *Salmonella typhimurium*, *S. enteritidis*, *S. cholerae-suis*, *Clostridium perfringens* type A & C, *Staphylococcus aureus*. The counts of bacteriophage are  $10^8$  pfu/gram. No difference ( $P > 0.05$ ) was observed in growth performance and blood profiles among treatments throughout the experimental period. The ATTD of DM was higher ( $P < 0.05$ ) in PC, BP1, and BP2 treatments than that in NC treatment. The ATTD of N was improved ( $P < 0.05$ ) in bacteriophage treatments than that in NC treatment. The ATTD of energy was higher ( $P < 0.05$ ) in PC treatment than that in NC treatment. Fecal *Lactobacillus* counts were increased ( $P < 0.05$ ) in BP2 treatment compared with NC and PC treatments, whereas fecal *Escherichia coli* population was reduced ( $P < 0.05$ ) in PC and BP2 treatments compared with NC treatment. No significant difference ( $P > 0.05$ ) was noted in diarrhea score and fecal moisture content among treatments. In conclusion, supplementation with 0.05% bacteriophage in growing pig diet improved nutrient digestibility, benefited the fecal *Lactobacillus* population, and inhibited the growth of *Escherichia coli*, indicating bacteriophage can be used as an alternative for antibiotics in growing pigs.

**Key Words:** bacteriophage, nutrient digestibility, growing pig

**T29 Effects of bacteriophage as an alternative for antibiotics on growth performance, nutrient digestibility, relative organ weight, blood profiles, and fecal microbial shedding in broilers.** X. Y. Guo,\* J. H. Cho, and I. H. Kim, *Department of Animal Resource & Science, Dankook University, Cheonan, Choongnam, South Korea.*

A total of 720 1-d-old ROSS 308 broilers (BW =  $44.2 \pm 0.2$  g) were used in a 32-d trial to investigate the effects of bacteriophage as an alternative for antibiotics on growth performance, apparent total tract nutrient digestibility, relative organ weight, blood profiles, and fecal microbial shedding. Broilers were randomly assigned to 1 of 4 treatments (12 replicate pens per treatment with 15 broilers per pen). Dietary treatments were: 1) NC, basal diet (without antibiotics); 2) PC, NC + 0.05% tylosin; 3) BP1, NC + 0.025% bacteriophage; 4) BP2, NC + 0.05% bacteriophage. Bacteriophage contains *Salmonella gallinarum*, *S. typhimurium*, *S. enteritidis*, *Clostridium perfringens* type A & C, and *Staphylococcus aureus*. The counts of bacteriophage are  $10^8$  pfu/gram. This trial was separated into starter phase (d 0–14) and finisher phase (d 15–32). Broilers were allowed *ad libitum* access to feed and water. The feed conversion ratio (FCR) decreased ( $P < 0.05$ ) in BP2 treatment compared with NC treatment. No difference ( $P > 0.05$ ) was found in BWG, FI and FCR among treatments during neither period nor overall period. The apparent total tract nutrient digestibility was unaffected ( $P > 0.05$ ) by dietary treatments. The relative liver weight was higher ( $P < 0.05$ ) in BP1 treatment than NC treatment. The meat quality and other relative organ weights were not affected ( $P > 0.05$ ) by dietary treatments. There was no difference ( $P > 0.05$ ) in blood profiles among treatments. The fecal *Lactobacillus* counts were increased ( $P < 0.05$ ) in BP2 treatment compared with NC treatment. The fecal *E. coli* population was reduced ( $P < 0.05$ ) in PC, BP1 and BP2 treatments compared with NC treatment. Collectively, the dietary bacteriophage supplementation at the level of 0.05% could benefit intestinal beneficial

bacteria, and inhibit the growth of pernicious bacteria, thus maintained the intestinal health of broilers.

**Key Words:** bacteriophage, broilers, microbial shedding

**T30 Changes in bacterial communities in bovine milk when comparing low and high somatic cell count quarters using culture independent analysis.** S. M. Buttram\*<sup>1</sup>, K. M. Hunt<sup>1</sup>, L. K. Fox<sup>2</sup>, and M. A. McGuire<sup>1</sup>, <sup>1</sup>Department of Animal and Veterinary Science, University of Idaho, Moscow, <sup>2</sup>Department of Animal Science, Washington State University, Pullman.

Many believe that bovine milk is sterile unless infectious mastitis is occurring. However recent reports indicate the presence of microbial communities in milk of healthy mammary glands, but little is known about these communities when there is an increase in somatic cell count (SCC). The objective of this study was to characterize the microbial communities in bovine milk, to determine if sampling type affected bacterial diversity, and to compare how communities changed with an increase in SCC. Milk was collected once from 6 Holstein cows that had at least one high SCC ( $\geq 400,000$  cells/ml) quarter and one low SCC quarter using 4 sampling techniques: fore strip, needle aspirate of foremilk, residual strip, and needle aspirate of residual milk. To characterize the microbial communities, culture independent pyrosequencing of the 16S ribosomal RNA gene was used to determine percentage relative abundance. Results (mean  $\pm$  SEM) demonstrated that the most abundant bacterial genera in low SCC milk included: *Clostridium* (29.6  $\pm$  4%), *Sporacetigenium* (11.6  $\pm$  1.7%), *Pelomonas* (10.9  $\pm$  2.4%), *Turicibacter* (5.8  $\pm$  1.0%), and *Staphylococcus* (5.1  $\pm$  1.7%). The most abundant genera in high SCC milk included: *Staphylococcus* (63  $\pm$  7.3%), *Peptoniphilus* (7.9  $\pm$  3.7%), *Enterococcus* (6.1  $\pm$  4.2%), *Arcanobacterium* (4.5  $\pm$  2.1%), and *Sporacetigenium* (2.8  $\pm$  1.0). Milk collected from high SCC quarters displayed increased *Staphylococcus* and reduced bacterial diversity (number of genera above 5% relative abundance). This occurred across all sample types. These results suggest that bovine milk is not sterile and contains diverse microbial communities when SCC is low. When an increase in SCC occurs, these bacterial communities are altered as bacterial diversity is diminished.

**Key Words:** microbial communities, somatic cell count, bovine milk

**T31 Group housed Holstein bull calves have suppressed innate immune function compared to individually housed calves during weaning.** L. E. Hulbert\*<sup>1</sup>, M. S. Calvo<sup>1</sup>, M. A. Ballou<sup>2</sup>, K. C. Klasing<sup>1</sup>, and F. M. Mitloehner<sup>1</sup>, <sup>1</sup>Department of Animal Science, University of California-Davis, Davis, <sup>2</sup>Animal and Food Sciences, Texas Tech University, Lubbock.

Objectives were to (1) determine if housing calves in groups of 3 (Group; n = 9 pens) or individually (Indv; n = 18) influences the innate immune responses of Holstein calves during weaning and (2) determine whether the response of grouped calves is influenced by their ADG ranking within a pen (Low = 0.80  $\pm$  0.116; Mid = 1.27  $\pm$  0.116; or High = 1.68  $\pm$  0.116 kg/d). Calves were fed 227 g of milk replacer (MR) twice-daily until weaning and offered ad libitum calf-starter throughout the experiment. Weaning was initiated at age 53 d by removal of pm MR and was completed at age 64 d by removal of am MR. Peripheral whole blood (WB) samples were collected before the am MR at age 53, 57, 64, 67 and 71 d. In all calves, plasma cortisol and urea nitrogen (UN) increased at 67 d from pre-weaning concentrations ( $P \leq 0.05$ ). Indv-housed calves had greater haptoglobin at 67 d ( $P \leq 0.05$ ). Overall, neutrophil oxidative burst (OB) and phagocytosis (PG) to heat-killed *E. coli* was lower in Group

calves compared with Indv-calves ( $P \leq 0.05$ ). In addition, Group-calves had lower ( $P < 0.05$ ). Tumor Necrosis Factor (TNF)- $\alpha$  concentrations from WB stimulated with endotoxin than Indv-calves at pre-weaning ( $P \leq 0.05$ ). Indv-calves also had greater WB killing (WBK) of *E. coli* than Group-calves at 64 d of age ( $P \leq 0.05$ ). Within Group, Low-ranked calves had the least glucose ( $P \leq 0.05$ ), greatest UN ( $P \leq 0.05$ ), and least OB and PG ( $P \leq 0.05$ ) than other Group-calves. Mid-ranked calves had increased TNF- $\alpha$  responses at 67 and 71 d ( $P \leq 0.05$ ). In addition, secretion Interferon- $\gamma$  by WB stimulated with phytohemagglutinin was greater among Mid-ranked calves at 57 and 64 d ( $P \leq 0.05$ ). Lastly, High-ranked calves had greater WBK at 57 and 64 d than other Group-calves ( $P \leq 0.05$ ). Weaning may have a more negative effect on innate immune function for group-housed calves than individually housed calves, especially the lowest performing calves.

**Key Words:** bovine, commingle, immunity

**T32 Effects of dietary herb supplementation on growth performance, and appearance of diarrhea in weaning-growing pigs.** J. P. Wang,\* X. Y. Guo, and I. H. Kim, Department of Animal Resource & Science, Dankook University, Cheonan, Choongnam, South Korea.

A total of 200 weaning pigs [(Landrace  $\times$  Yorkshire)  $\times$  Duroc, BW = 7.00  $\pm$  0.86 kg] were used in a 10-week feeding experiment to determine the effects of dietary supplementation of herb extract on growth performance, and appearance of diarrhea in weaning-growing pigs. Pigs were randomly allocated into 1 of 5 treatments with 2 replicate pens per treatment and 20 pigs per pen. Dietary treatments were: 1) CON, basal diet; 2) A, CON + 0.1% anti-diarrhea herb seed; 3) B, CON + 0.05% (*Lonicera japonica* + *Japanese atractylodes*); 4) C, CON + 0.1% (*Scutellaria baicalensis* + *Houttuynia cordata*); 5) D, CON + 0.1% anti-diarrhea herb extract. All pigs were housed in pens with a self-feeder and a nipple drinker to allow *ad libitum* access to feed and water throughout the experimental period. The experiment included weaning phase (0–5 weeks) and growing phase (5–10 weeks). In each phase, body weight and feed consumption were recorded to calculate ADG, ADFI, and G/f. Diarrhea pig number was recorded daily from d 0 to 6 in each phase. During 0–5 weeks, ADG and G/f were higher ( $P < 0.05$ ) in B, C and D treatments than those in CON and A treatments. ADFI was increased ( $P < 0.05$ ) in D treatment compared with CON and A treatments. During 5–10 week, pigs fed the B, C and D diets had a greater ( $P < 0.05$ ) ADG and ADFI than those pigs fed the CON and A diets. Overall, pigs fed the B, C and D diets had a higher ( $P < 0.05$ ) ADG than those fed the CON and A diets. From d 2 to 6 in weaning phase and d 1 to 6 in growing phase, the number of diarrhea pigs was decreased ( $P < 0.05$ ) by the application of herbs. In conclusion, dietary supplementation of 0.05% (*Lonicera japonica* + *Japanese atractylodes*), 0.1% (*Scutellaria baicalensis* + *Houttuynia cordata*), and 0.1% anti-diarrhea herb extract could improve the growth performance, and decrease the diarrhea rate in weaning-growing pigs.

**Key Words:** growth performance, herb, weaning-growing pigs

**T33 Efficacy of a yeast cell wall-derived mycotoxin adsorbent on excretion of aflatoxin B<sub>2</sub> in rats.** B. Walters,\* T. Smith, and M. Crump, University of Guelph, Guelph, Ontario, Canada.

Mycotoxins are natural contaminants of livestock feeds and impair production efficiency. One method to reduce exposure of animals to mycotoxins is by using specialized feed additives known as mycotoxin adsorbents (MA). These use nonspecific binding to sequester mycotoxins preventing them from being absorbed into the bloodstream. Current

research has shown that MA can reduce symptoms of mycotoxicoses but quantitative evidence of reduced exposure has yet to be demonstrated. The current study sought to quantify the adsorptive capabilities of a yeast cell wall-derived MA (GMA). In the first experiment, male Sprague-Dawley rats were randomly placed in groups of 10 and were fed either a control diet or a diet containing 1, 3 or 5% w/w GMA (Integral, Alltech Inc., Nicholasville, KY). Rats were fed this diet for 7 d and then exposed by oral gavage to a tracer dose of  $^3\text{H}$ -8,9-aflatoxin B<sub>2</sub>. Feces and urine were collected 24 h after dosing. The rats were sacrificed by CO<sub>2</sub>. The dose collected in each sample was compared between groups using SAS and no significant difference was observed between any GMA group and the control. Due to the kinetics of the digestive pathway, it was thought that total excretion after a set time did not provide as much information as excretion over time. In the second experiment, rats were grouped as before using only the 5% GMA and control diets. Feces and urine were collected for 6, 12, 18, 24, 48 and 72 h after dosing. The amount of excreted dose was higher among the GMA-treated rats compared with the control during the first 18 h after the initial dosing. After 18 h, excretion of dose in both groups was lower. Using ANOVA with a factorial design indicated a significant interaction between diet and time ( $P < 0.05$ ). The natural logarithm of the excreted dose was used for linear regression analysis and showed that the excretion rate in the GMA-treated rats ( $r^2 = 0.88$ ) was faster than the control ( $r^2 = 0.80$ ). Based on this model, it was concluded that GMA reduces transit time of aflatoxin and reduces exposure in rats.

**Key Words:** aflatoxin, adsorbent, glucomannan

**T34 Biotransformation approaches to alleviate the toxic effects induced by Fusarium toxins in swine.** B. Grenier<sup>\*1,3</sup>, A. P. Loureiro-Bracarense<sup>2</sup>, J. Luciolli<sup>2</sup>, A. M. Cossalter<sup>1</sup>, W. D. Moll<sup>3</sup>, G. Schatzmayr<sup>3</sup>, and I. P. Oswald<sup>1</sup>, <sup>1</sup>Institut National de la Recherche Agronomique-ToxAlim, Immuno-Mycotoxicology, Toulouse, France, <sup>2</sup>Universidade Estadual de Londrina, Lab Patologia Animal, Londrina, Brazil, <sup>3</sup>Biomim Research Center, Tulln, Austria.

Mycotoxins are secondary metabolites of fungi that can cause serious health problems in animals, and may result in severe economic losses. Therefore, development of control strategies is necessary. Based on enzymatic reactions, we evaluated the efficiency of detoxifying agents into the gastrointestinal tract of piglets fed a co-contaminated diet with low doses of deoxynivalenol (DON) and fumonisins (FUMO). Four diets were formulated and given to piglets for 5 weeks; a control diet with or without the detoxifying agents (DA), and a co-contaminated diet with both Fusarium toxins (3 mg DON/kg, 6 mg FUMO/kg of feed) with or without DA. At d 4 and 16, the animals were subcutaneously immunized with ovalbumin (OVA) to assess the specific immune response. A decreased proportion of blood neutrophils was observed in the animals fed DON+FUMO diet ( $P = 0.04$ ), and by contrast animals fed this diet with DA were not affected. Microscopical lesions (mild to moderate) were recorded in liver and lungs of piglets exposed to mycotoxins. Addition of the DA did not significantly reduce the hepatic lesion score, but significantly counteracted the effects on the proliferation index of hepatocytes ( $P < 0.01$ ). Similarly, in lung, occurrence and extent of lesions were strongly reduced after inclusion of DA in the co-contaminated diet ( $P = 0.01$ ). Assessment of the specific immune response at d 35 showed a restoration of immune parameters in presence of DA in contaminated feed. Indeed, the ability of lymphocytes to proliferate upon OVA stimulation was significantly enhanced in the DON+FUMO+DA diet (by 2.2 fold,  $P = 0.05$ ). The concentration of IgG anti-OVA was partially increased in plasma of animals fed the co-contaminated with DA (+33%,  $P = 0.28$ ). In spleen, the profile of cytokines expression

(IL-1 $\beta$ , IL-6, IL-8 and IL-12p40) in the group containing both toxins with DA was similar in comparison to both control groups, whereas the association of the mycotoxins significantly depressed the mRNA levels ( $P < 0.05$ ). In conclusion, the adverse effects induced by the ingestion of both DON and FUMO in piglets were partially to totally counteract in presence of DA in this diet.

**Key Words:** Fusarium toxins, biotransformation, immune response

**T35 Impact of cow genetics regarding the osteopontin gene for the immune response to MAP infection.** C. Thibault<sup>1</sup>, P.-L. Dudenaine<sup>2,1</sup>, and N. Bissonnette<sup>\*1,2</sup>, <sup>1</sup>Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Center, Sherbrooke, Quebec, Canada, <sup>2</sup>Université de Sherbrooke, Sherbrooke, Quebec, Canada.

Bovine paratuberculosis is a pandemic infection that affects much of the world's ruminant population. The causative pathogen is *Mycobacterium avium* ssp. *paratuberculosis* (MAP). The goal of this study was to measure the effect of cow genetics regarding the osteopontin (OPN) gene for the immune response to MAP infection. The importance of gaining insight into the unique interactions between host macrophages and mycobacteria is explained. MAP evades host immune responses and is able to persist and multiply in macrophages which generate granuloma formation. Granulomas are characterized by a mononuclear cell infiltration of macrophages and lymphocytes, by the formation of giant cells and epithelioid cells, and by fibrosis, sometimes with calcification. Previous investigations observed that OPN, an early T-cell activating factor, is more abundant in granulomas. In addition, studies confirmed the increased susceptibility of OPN-null mice to intracellular mycobacteria. MAP is believed to grow and survive in macrophages. Thus, the role of OPN in the mycobacteria-macrophage relationship is of considerable interest. In a previous study, our team found that OPN was correlated with bovine mastitis susceptibility and that expression of the OPN gene was correlated with the genotypes (i.e., gene haplotypes). We have undertaken a study of gene expression changes after ex vivo MAP infection using macrophage from different bovine genotypes (SPP1 haplotypes). Four reference genes were also measured; UXT and PPIA were selected by BestKeeper. The qRT-PCR results were analyzed according to the relative quantification method ( $2^{-\Delta\Delta C_t}$ ). Results show that MAP induces a marked increase in cytokines, including interleukin (IL)-1 $\beta$ , -6, -8, -10, and -23 and TNF- $\alpha$  (but not IL-12, SPP1, NOD-2, and SLC11A1 also known as NRAMP1). SPP1 genotype has an effect on IL-6 at 6 h, -8, -10, and -23 gene expression at 24 h ( $P < 0.05$ ) with a tendency for IL-1 $\beta$  at 24 h ( $P = 0.09$ ). The strategy of identifying host genes altered by infection whose expression is linked to animal genetics can provide valuable information for targeting improved disease resistance in genetic selection.

**Key Words:** bovine paratuberculosis, osteopontin, macrophage

**T36 Polymorphisms in the osteopontin gene are associated with *Mycobacterium avium* ssp. *paratuberculosis* infection status.** C. Thibault<sup>1</sup>, P.-L. Dudemaine<sup>2</sup>, G. Fecteau<sup>3</sup>, G. Côté<sup>4,2</sup>, O. Labrecque<sup>5</sup>, and N. Bissonnette<sup>\*1,2</sup>, <sup>1</sup>*Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Center, Sherbrooke, QC, Canada*, <sup>2</sup>*Université de Sherbrooke, Sherbrooke, Québec, Canada*, <sup>3</sup>*Faculté de médecine vétérinaire de l'Université de Montréal, Saint-Hyacinthe, QC, Canada*, <sup>4</sup>*Ministère de l'Agriculture, des pêcheries et de l'Alimentation, Québec, QC, Canada*, <sup>5</sup>*Laboratoire d'épidémiologie animale du Québec, Saint-Hyacinthe, QC, Canada*.

The Canadian dairy industry is aware of the importance of considering resistance to bovine paratuberculosis as a target trait for improvement. The objective of the study was to detect and assess whether DNA polymorphisms in the bovine candidate gene, osteopontin (OPN), are associated with susceptibility to paratuberculosis in dairy cattle. Johne's disease (JD) is a debilitating and chronic enteritis in ruminants caused by an intracellular pathogen called *Mycobacterium avium* ssp. *paratuberculosis* (MAP). JD and bovine paratuberculosis causes significant economic losses to the Canadian dairy industry. Because of the limited sensitivity, diagnosis of paratuberculosis is difficult because animals remain asymptomatic for years. Variability in the susceptibility of cattle to MAP infection is evident. To detect genetic variations (single nucleotide polymorphisms (SNP), the 10-kilobase DNA fragment carrying the encoding OPN gene has been sequenced using 108 Holstein cows from the same group of age. The MAP status of the cows was determined using serum or milk ELISA combined to fecal culture assays. Fifty-seven cows were positive to both tests and considered infected. Fifty-one cows tested negative over a 6-mo period (2 consecutive tests) and were declared negative). Logistic regression was used to determine the association of SNPs and reconstructed haplotypes with MAP infection status. A total of 9 informative SNPs were identified. The SNPs *SPP1c. 1301G>A* and *SPP1c. 1251C>T* (numbering according to GenBank no. AY878328) genotypes were confirmed and found tightly linked ( $P = 0.0007$ , Chi-Square) with MAP infection status. In our previous study, these SNPs were associated with the mammary health status of lactating cows. As OPN is now recognized as an important proinflammatory cytokine with pleiotropic beneficial functions, including intracellular pathogen clearance, the presence of genetic variations and their association with

infection status confirms the importance of this candidate gene in JD disease. It may be possible to use OPN gene in selective breeding strategies to enhance health status including resistance to MAP infection.

**Key Words:** bovine paratuberculosis, osteopontin, susceptibility/resistance

**T37 Phytonutrients affect the integrity of the mucus layer and susceptibility to enteric pathogens.** M. Wlodarska<sup>1</sup>, B. B. Finlay<sup>1</sup>, and D. Bravo<sup>\*2</sup>, <sup>1</sup>*University of British Columbia, Vancouver, British Columbia, Canada*, <sup>2</sup>*Pancosma, Geneva, Switzerland*.

Plant extracts, also known as phytonutrients, are gaining interest for their use as health promoting feed additives in animal production. Their mechanism of action, however, is still unknown. We hypothesize that phytonutrients may function by acting on the large intestine to alter the microbial ecosystem, strengthen mucosal defenses, and stimulate innate immunity. Mucus secretion and thickness is thought to play a major role in both health and disease by providing a protective yet permeable barrier between intestinal contents and host tissue. The mucus layer in the large intestine consists of 2 stratified layers, mainly composed of the secreted mucin, Muc2. The inner layer is of dense composition and devoid of commensal bacteria, while the outer layer is built as a loose matrix housing commensal bacteria. Six phytonutrients: anethol, capsicum oleoresin, carvacrol, cinnamaldehyde, eugenol, and garlicon40, with known antimicrobial and anti-inflammatory properties were evaluated in C57Bl/6 mice for their effect on the mucus layer, microbial composition, and colitis induced by the natural mouse pathogen, *Citrobacter rodentium* (*C. rodentium*). Each phytonutrient was given to mice through their drinking water for 7 d. Of the 6 phytonutrients tested, eugenol-fed mice were found to have increased Muc2 expression ( $P = 0.06$ ) and a significantly thicker inner mucus layer ( $*P = 0.02$ ) in the large intestine compared with untreated mice. Eugenol-fed mice were less susceptible to *C. rodentium*-induced goblet cell depletion in the large intestine ( $***P = 0.0004$ ) and showed a decrease in systemic pro-inflammatory cytokines, TNF- $\alpha$  ( $*P = 0.02$ ), IFN-gamma and MCP-1, 6 d post infection. These results suggest that eugenol acts to strengthen the mucosal barrier by increasing the thickness of the inner mucus layer, which protects against invading pathogens and intestinal inflammation.

**Key Words:** phytonutrient, mucus, pathogen