Comparison between conventional culture, MALDI-TOF, and 16S rRNA for test agreement in diagnosis of bacteria in individual cow milk milk samples. D. J. Wilson*, J. Middleton*, P. Adkins2, and G. M. Goodell3, 1University of Missouri, Columbia, MO, 2The Dairy Authority, Greeley, CO.

Comparison of culture, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), and 16S rRNA genomic sequencing to identify mastitis pathogens was the objective. All quarter milks submitted to The Dairy Authority (TDA) laboratory on one day were streaked (10 µL) onto Columbia blood agar and MacConkey agar. Plates with colonies were number coded, paraffin sealed and shipped overnight with cold packs to the University of Missouri (MU). Culture at TDA identified bacteria to genus level except for speciation of S. aureus and E. coli. PCR was negative for Mycoplasma spp. At MU, a MALDI-TOF mass spectrometer tested colonies in duplicate. Comparison with the Biotype database of known bacteria produced scoring between 1.7 and 1.99 for genus level identification and ≥2.0 for species level. 16S rRNA colony lysate PCR products were Sanger sequenced, and sequences were compared with GenBank data using nucleotide-BLAST at MU. All microbiologists were blind to other results. Culture and MALDI-TOF tested 181 isolates; 16S rRNA tested 179 (2 lost during storage). In accordance with culture, S. aureus and E. coli agreement was to species level, all others to genus level. This was a test of agreement, not sensitivity or specificity (no “gold standard”). Overall agreement between the 3 diagnostic methods was 87% (155/179). Agreement between MALDI-TOF and 16S rRNA was 98% (176/179). That assumes agreement for 29 isolates called E. coli by culture and MALDI-TOF that 16S rRNA defined as E. coli or Shigella spp. Most bacteria were identified with good agreement among all 3 methods by McNemar’s test, including 94% (80/85) of isolates defined by culture as Staph spp., with 22 isolates defined by culture as S. aureus, Enterobacter spp., Klebsiella spp., Pasteurella spp., or T. pyogenes showing 100% agreement among all 3 methods. Many members of the dairy industry are comfortable using either bacterial culture or MALDI-TOF for routine milk bacteria diagnosis; 16S rRNA is mainly a research tool. The results suggest that all 3 methods are valuable tools for the dairy industry.

Key Words: mastitis, MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight), 16S rRNA

Investigation of risk factors of subclinical mastitis in large-scale dairy farms. Y. F. Zhong*, Y. M. Wu, and J. X. Liu, Institute of Dairy Institute, Zhejiang University, Hangzhou, China.

Mastitis has been an important disease because of its common occurrence and resulting significant loss of profit. This study was conducted to investigate the risk factors related to subclinical mastitis (SCM) in large-scale farms. Eleven dairy farms with over 1,000 dairy cows were selected in Zhejiang province, China. A questionnaire on management factors potentially associated with SCM was designed and completed with the help of the managers from all the investigated farms. Aspects of management included in the questionnaire consisted of the general farm and stalls, management of diet and water supply, cleaning of stalls, operation and hygiene of calving and milking, strategy of drying off, and detection and treatment of mastitis. The incidence rate of SCM was calculated according to the Dairy Herd Improvement (DHI) data. In this study, the SCM was defined as the SCC over 4 × 10^4/mL milk. Correlation analysis was conducted to identify the herd management factors that were associated with an increased rate of subclinical mastitis. One-way ANOVA was used to analyze the cow factors from DHI data in SPSS 19.0. The average incidence of SCM in these farms was 17.8% in 2015. The incidence rate of SCM increased with the increasing parity and days in milk. Within a year, the incidence of SCM was highest in summer and lowest in winter. Six management factors significantly were identified to be associated with the incidence of SCM, including type of stalls (P < 0.01), milking system (P < 0.01), cleaning frequency of stall (P < 0.01), usage of milking gloves (P < 0.01), individual pen for calving (P < 0.01), and duration staying with lactating cows before calving (P < 0.01). The result of this study indicated that udder health in large dairy farms can be affected by several factors especially the hygiene of milking and calving. It warrants further investigated how mastitis caused by different management methods affects physiological changes of dairy cows.

Key Words: large-scale dairy farm, risk factors, subclinical mastitis

Use of electrical conductivity for the differentiation of mastitis-causing pathogens. S. Paudyal*, P. Melendez2, D. Manriquez1, A. Velasquez2, P. Pinedo1, and G. Pena3, 1Colorado State University, Fort Collins, CO, 2University of Missouri, Columbia, MO, 3Zoetis, Parsippany, NJ.

Mastitis is one of the most prevalent and costly diseases in dairy operations. Key components for adequate mastitis control are the detection of early stages of infection, as well as the selection of therapy based on the causal pathogen associated with infection. Our objective was to characterize the pattern of electrical conductivity (EC), provided by an in-line mastitis detection system, considering specific mastitis-causing pathogen group involvement. Cows (n = 200) identified by the system with a deviation >15% in the manufacturer’s (Afimilk Ltd., Kibbutz Afikim, Israel) proprietary algorithm for EC (HEC) were considered cases and enrolled in the study. One control (CON), defined as within normal ranges, for EC, was matched to each case and monitored for milk yield (MY) and EC for ±10 d. A sterile pooled milk sample was collected from each cow for bacteriological culture. Pathogens were categorized into gram-positive (GP), gram-negative (GN), other (OTH), and no growth (NO). Data were submitted for repeated measures analysis (PROC MIXED, SAS), with EC as dependent variable. EC status (HEC or CON), bacterial categories, and milking relative to d of enrolment were considered independent fixed variables and farm was included as a random effect in the model. For HEC animals, EC was greater in NO than in GN (P = 0.036) but EC was not different among other pathogen groups. For CON animals, EC was greatest in OTH compared with NO (P = 0.03), GP (P = 0.03), and GN (P = 0.07). However, EC was not different when comparing between GP and GN. For HEC animals, MY was not significantly different among pathogen groups. For CON, GN had greater MY than NO (P = 0.006) and GP (P = 0.02). For HEC and CON animals, EC was greater in multiparous cows than in primiparous cows (P < 0.001). State of lactation had no effect in CON animals whereas, for HEC cows, EC was greater in animals in early (P < 0.001) and late lactation (P = 0.0015), compared with mid lactation cows. Thus, it is concluded that EC variation cannot solely be attributed to pathogen groups and multiple factors should be considered in developing mastitis pathogen detection models based on EC.

Key Words: electrical conductivity, mastitis
46 Flax oil supplementation affects systemic blood biomarkers and polymorphonuclear leukocytes mRNA expression in neonatal dairy calves. F. Rosa*1, C. R. Schossow1, N. A. Carpinelli2, E. Trevisi2, J. L. Anderson1, and J. S. Osorio1, 1Dairy and Food Science Department, South Dakota State University, Brookings, SD, 2Department of Animal Sciences, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy.

Polysaturated fatty acids have been observed to reduce inflammatory response. The adaptation of neonatal calves to an extraterine environment is commonly associated with inflammatory-like conditions. Thus, our objective was to evaluate the effects of supplementing flax oil on immune function reflected in the profiles of systemic blood biomarkers and mRNA in polymorphonuclear leukocytes (PMNL). Thirty-six Holstein dairy calves housed in individual hutches were used in a randomized complete block design from birth until 12 wk of age. A subset of 16 calves (n = 8/treatment) was used for immune function analysis. Treatments were control (CON) with no supplement or 80 g/d of flax oil (FLAX) with the milk. Calves were fed 2.8 L/d of pasteurized milk 2 × /d during wk 1 to 5. Starter pellets and water were fed ad libitum. Blood samples were taken at 1, 7, and 14 d of age for biomarker profiling of metabolism, oxidative stress, and inflammation as well as PMNL isolation. RNA was extracted from isolated PMNL, and concentration and viability were assessed through flow cytometry (Attune NxT; Invitrogen). Target genes evaluated in PMNL function involved inflammation, cellular receptors, and oxidative stress. Data were analyzed using the MIXED procedure of SAS. There was a significant (P = 0.04) diet by time (D × T) interaction in the mRNA expression of SELL (L-Selectin), a cell surface receptor, where an upregulation (P = 0.03) of SELL in FLAX calves at 7 d of age was observed. A trend (P = 0.14) for a D × T effect was observed in IL1B (interleukin 1B), an inflammatory cytokine, which resulted in a trend (P = 0.06) for lower expression of IL1B in FLAX calves at 7 d of age. The overall MPO (myeloperoxidase) mRNA expression tended (P = 0.07) to be upregulated in FLAX calves, which is commonly associated with PMNL activity. Results suggest that early life supplementation of flax oil to neonatal calves may benefit their transition into an extraterine environment not only by mediating the inflammatory response, but enhancing the PMNL ability to detect potential infection sites through cell receptors such L-Selectin.

Key Words: calves, inflammation, flax oil

47 Validation of methods to practically evaluate failure of passive transfer in calves arriving to a veal facility. D. L. Renaud*, T. F. Duffield, S. J. LeBlanc, and D. F. Kelton, Department of Population Medicine, University of Guelph, Guelph, ON, Canada.

Providing a sufficient quantity of good quality colostrum is critical for male and female calves to reduce the risk of disease and mortality. Practical tests have not been validated at arrival to veal facilities to determine failure of passive transfer (FPT). There are many challenges to validation including the lack of information regarding the age of the calf and the high prevalence of dehydration. The objective of this study was to validate a semiquantitative IgG test using whole blood and a digital refractometer using serum to determine passive transfer status. A total of 149 Holstein calves were evaluated at arrival to a milk-fed veal facility for dehydration status and had blood drawn to evaluate passive transfer. Serum IgG was determined by radial immunodiffusion (RID), whereas, serum total protein (STP) was evaluated using a digital refractometer (Misco Palm Abbe) and a semiquantitative test (ZAPvet Bovine IgG test) was used on whole blood to determine failure of passive transfer. A nonparametric receiver operating characteristic (ROC) curve was generated to compare STP and IgG levels. Sensitivity, specificity, positive predictive values and negative predictive values were calculated for STP and the semiquantitative IgG test using RID as a gold standard test. A total of 31 calves (21%) had a level of serum IgG <1,000 mg/dL. The level of total protein was well correlated with the level of IgG yielding an R² value of 0.75. The cut point to determine passive transfer on serum was ≥5.1 g/dL, yielding a sensitivity of 84% and specificity of 90%. The semiquantitative test performed poorly on whole blood with a sensitivity of 77% and a specificity of 44%. This study demonstrates that serum total protein is a reliable measure to evaluate passive transfer status and can be used despite high levels of dehydration and the inability to determine the calf’s age.

Key Words: male calf, colostrum, total protein


Mycotoxins, secondary metabolites produced by molds, can occur in many types of grains and forages as well as other feedstuffs such as fruits and nuts which are consumed by livestock and humans. These toxic substances frequently negatively affect animal health and performance. A survey was conducted to determine the occurrence of mycotoxins in the 2017 US corn crop to assess the potential risk posed to livestock. A total of 442 corn-based samples (grain, fermented feeds, and by-products) were collected from August 2017 to January 2018 from 29 states. The majority of samples originated from the Midwest. Samples were analyzed at Romer Labs (Union, MO) for the presence of 17 mycotoxins by the liquid chromatography tandem mass spectrometry (LC-MS/MS) method. These mycotoxins were categorized into 6 groups: Type B trichothecenes including deoxynivalenol (DON), fumonisins (FUM), zearalenone (ZEN), aflatoxins (Afla), Type A trichothecenes including T-2 toxin (T-2), and ochratoxin A (OTA). Eighty-eight percent of samples had at least one mycotoxin detected while 45% of samples were contaminated with more than one mycotoxin. The percent of positive samples, mean of positives [ppb], standard error of the mean (SEM) of positives [ppb], and maximum of positives [ppb] for the 6 major mycotoxin groups are presented in Table 1. Deoxynivalenol was detected in 75% of samples (−14 percentage points vs. 2016 Biomin survey data) while FUM were present in 43% of samples (−29 percentage points vs.

Table 1 (Abstr. 48). Summary of mycotoxin analyses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DON</th>
<th>FUM</th>
<th>ZEN</th>
<th>Afla</th>
<th>T-2</th>
<th>OTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive samples (%)</td>
<td>75</td>
<td>43</td>
<td>29</td>
<td>4</td>
<td>0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Mean of positives (ppb)</td>
<td>1,026</td>
<td>2,998</td>
<td>248</td>
<td>12</td>
<td>—</td>
<td>600</td>
</tr>
<tr>
<td>SEM1 of positives (ppb)</td>
<td>179</td>
<td>397</td>
<td>55</td>
<td>4</td>
<td>—</td>
<td>NA</td>
</tr>
<tr>
<td>Maximum contamination (ppb)</td>
<td>54,149</td>
<td>64,500</td>
<td>5,556</td>
<td>67</td>
<td>—</td>
<td>600</td>
</tr>
</tbody>
</table>

1Standard error of mean.
2016 Biomin survey data). Zearalenone was the third most frequently occurring mycotoxin, detected in 29% of samples (−28 percentage points vs. 2016 Biomin survey data). Deoxynivalenol, with additional potential concerns from FUM and ZEN, pose the greatest mycotoxin threats to animal health and productivity due to their frequent occurrence in 2017 US corn sourced predominantly from the Midwest.

Key Words: mycotoxin, deoxynivalenol, fumonis

49 Feeding NutriTek reduces linear scores and clinical mastitis cases. J. D. Ferguson1, M. A. Sattler2, D. L. Hanson*2, C. P. Davis2, T. S. Edrington2, and I. Yoon3, 1University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA, 2Diamond V, Cedar Rapids, IA.

The objective of the current research was to examine the association of feeding NutriTek, a Saccharomyces cerevisiae fermentation product (Diamond V, Cedar Rapids, IA) in close-up and lactating cows with udder health. Production records and clinical mastitis event data were collected from 25 herds from 5 regions of the US using back-up files from herd DairyComp305 collected from June to September 2017. This provided production and event records that ranged from 400 to 890 d following NutriTek feeding. Herds ranged in 305-d milk production from 7,800 to 12,700 kg, and the number of cows in the adult herd ranged from 880 to 10,727 cows. Test day records were extracted for herds that recorded linear scores (LS) by cow, event data for mastitis, date of calving, and date of herd removal for an equal period of time before and after feeding NutriTek. Fifteen herds had test day records with LS data and all 25 herds had event data. A total of 1,444,450 production records were available for analysis: 702,449 records were for the period before, and 742,000 records post NutriTek feeding. Lactation records were categorized by herd, cow, lactation number (where available), date fresh, and date of test. Data for LS were analyzed using SAS statistical software and analyzed by herd using Proc Mixed with period of NutriTek feeding, test period, and month of test as independent variables. Cow was treated as a repeated observation. Least squares means for LS (NutriTek or no NutriTek) were estimated for each herd. Clinical mastitis prevalence was tested by herd using Proc Logistic in SAS. Overall BW was not affected (P = 0.05) in the independent variables. Sixteen herds had a reduction (P < 0.05) in the prevalence of clinical mastitis cases and 8 herds had a tendency (P < 0.10) for a reduction in mean LS during the NutriTek feeding period. A purely random sampling should have resulted in only one-third of herds improving their linear scores and mastitis cases indicating that the herd fed NutriTek improved more than expected (odds ratio (OR) = 0.77) by maternal milk production.

Key Words: antibiotic, treatment frequency, dairy

51 Residual effects of maternal consumption of metal amino acid complexes in offspring inflammatory and oxidative status during the weaning period. R. C. B. Grazziotin*1, C. B. Jacometo2, M. Socha3, E. Trevisi1, J. J. Loor5, and J. S. Osorio1, 1South Dakota State University, Brookings, SD, 2Universidad de la Salle, Bogota, DC, Colombia, 3Zinpro Corporation, Eden Prairie, MN, 4Università Cattolica del Sacro Cuore, Piacenza, Italy, 5University of Illinois, Urbana Champaign, IL.

Maternal diets fed during late pregnancy (close-up) to dairy cows have been observed to have a significant effect on the offspring’s immune status and health during the neonatal stage. Our objective was to evaluate the effect of maternal consumption of organic trace minerals supplemented during late pregnancy on offspring’s immune and oxidative status during the weaning period. Forty multiparous Holstein cows were supplemented for 30 d prepartum to supply 40, 20, 5, and 1 mg/kg of Zn, Mn, Cu, and Co from either metal amino acid complexes (AAC) or sulfate (INO) sources (total diet contained supplemental 75, 65, 11, and 1 mg/kg of Zn, Mn, Cu, and Co, and additional Zn, Mn, and Co provided by sulfates). A subset of calves (n = 8/ht) born to dams enrolled in the trial was used for growth and performance data and blood immunometabolic markers. Calves were housed in individual hutches from birth to 7 wk of age and intakes of milk replacer and starter were measured daily. Calves were fed the same nutritional program with 2 × 2 feeding milk replacer from wk 1 to 5, 1 × 1/d feeding during wk 6 (36 to 42 d of age), and weaning at wk 7 (43 d of age). Starter grain was offered ad libitum. Fecal and respiratory scores were recorded daily. Blood samples were taken pre-weaning and post-weaning at 40 and 50 d of age, respectively. Body weight (BW) and withers height (WH) were recorded weekly. Data were analyzed using the MIXED procedure of SAS. Overall BW was not affected (P = 0.77) by maternal feeding.
AAC, however, there was a trend ($P = 0.07$) for greater withers height in AAC calves. The AAC calves had overall lower paraoxonase ($P = 0.02$) and myeloperoxidase ($P = 0.03$), while greater ferric reducing antioxidant power (FRAP; $P = 0.04$) in comparison to INO calves. A trend for lower glutamic-oxaloacetic transaminase (GOT; $P = 0.06$), $\gamma$-glutamyltransferase (GGT; $P = 0.06$), and reactive oxygen metabolites (ROM; $P = 0.10$) was observed in AAC calves than INO. These data suggest that maternal consumption of AAC can have long-lasting effects in the offspring by reducing oxidative stress and potentially alter liver function during stress periods such as weaning.

**Key Words:** weaning, trace minerals, calves

52 Experimental *Staphylococcus aureus* mastitis teat-dip infection model for evaluation of efficacy of vaccine against *Staphylococcus aureus* intramammary infection. O. K. Dego*, R. Abdi, and R. Almeida, The University of Tennessee, Knoxville, TN.

*Staphylococcus aureus* is the most frequent and major pathogen of mammary glands of dairy cows. There is increasing need to develop protective vaccine against *S. aureus* IMI. A good experimental infection model is required to evaluate vaccine efficacy. The intramammary infusion of *S. aureus* is a reliable method in terms of causing infection. However, intramammary infusion is unrealistic method in terms of mimicking natural IMI because it overwhelm the host immune mediated defenses since large number of bacteria were directly delivered into the intramammary area bypassing innate and acquired immune defenses at teat opening. Therefore, a challenge model that is similar to natural infection is required to evaluate protective effect of new vaccine against *S. aureus* mastitis. The objective of this study was to develop experimental *S. aureus* mastitis teat-dip infection model. A total of 8 dairy cows at early dry period were divided into group 1 (n = 5 cows) and group 2 (n = 3 cows). Cows in Group 1 were challenged with *S. aureus* strain SAUT1 by dipping all 4 teats in a suspension of *S. aureus* at a concentration of $10^6$ cfu/mL Tryptic soy broth (TSB) medium. Similarly, all 4 teats of cows in the group 2 (Control) were dipped in PBS. The challenge strain was grown to mid log phase in TSB at $37^\circ$C and cows were challenged after morning milking. Results showed that out of 5 *S. aureus* challenged cows 80% (n = 4 out of 5) and 20% (n = 1 out of 5) were infected subclinically and clinically respectively. At d 3 of challenge, at least one quarter of each cow was shedding *S. aureus* in milk resulting in 100% infection. At d 6 of challenge, 100% of challenged quarters were shedding *S. aureus* in milk. We also further evaluated the protective effects of experimental vaccine against *S. aureus* intramammary infection (IMI) using this model and found promising results. We concluded that teat dipping in the *S. aureus* bacterial suspension at cell density of $10^6$ cfu/mL of growth medium is good experimental intramammary infection model to induce *S. aureus* IMI.

**Key Words:** intramammary infection, model, dairy cow