Graduate Student Competition: ADSA Production Division
Graduate Student Poster Competition, PhD

T198   Effects of estrus number on milk yield and estrus expression in Holstein cows managed for extended lactation. Charlotte Gaillard*, Mogens Vestergaard, and Jakob Sehested, *Aarhus University, Foulum, Tjele, Denmark.*

Cows managed for a 16-mo extended lactation have, at least, 8 estrus periods before being inseminated. The aims were to determine the effect of estrus number on milk yield and estrus expression, and to compare conception rates with the rates obtained during the previous 10 mo lactation. It was hypothesized that (1) milk yield will decrease during the day of estrus, and this loss will diminish with increasing estrus number; (2) postponing the insemination (AI) will increase the expression of estrus leading to a better detection and a higher conception rate. A total of 62 Holstein cows (30% 1st parity) were managed for 16 mo extended lactation. The 8 first estrus periods were determined by milk progesterone levels (<3 ng/L) and the day of estrus by visual observations (mucus consistency, blood in genital area, cow jump/stand/lay). Milk yield at 'estrus day' was compared with 'normal day' yield (average yield of d −4, −5 and −6 before 'estrus day'). The percentage of cows expressing estrus was calculated using the behaviors mounting, being mounted or standing as criteria. Milk yield was analyzed using a linear mixed effects model while a chi-squared test was used for evaluating estrus expression and conception rate. The results showed a milk loss of 0.56 ± 0.19 kg per estrus day ($P < 0.001$) with no differences between the 8 estruses ($P = 0.3$). This negative estrus effect on milk yield is small compared with the well-known pregnancy effect. Moreover, 70% of the cows expressed the 8th estrus compared with 37% for the 1st estrus ($P = 0.02$) and 40% for the 2nd estrus ($P = 0.06$). However, the conception rates of the 1st and 2nd AI did not differ from those of the previous 10 mo lactation (respectively + 2.9%, $P = 0.8$ and + 3.4%, $P = 0.7$). To conclude, milk yield decreases similarly during each of the 8 estrus days studied whereas the proportion of cows expressing estrus nearly doubles from the 1st and 2nd to the 8th estrus.

Key Words: estrus number, milk yield, mounting

T199   Effects of canola meal as a source of rumen-undegraded protein on ruminal fermentation using a dual-flow continuous-culture system. Eduardo Marostegan de Paula*[1], Lorrayny Galoro da Silva[1], Pedro Del Bianco Benedeti[1,2], Hugo Monteiro[1,3], Yenling Yeh[1], Teshome Shenkoru[1], Glen A. Broderick[4], and Antonio Faciola[1], [1]*University of Nevada, Reno, NV,* [2]*Federal University of Viçosa, Viçosa, MG, Brazil,* [3]*Maringa State University, Maringa, PR, Brazil,* [4]*Broderick Nutrition & Research, Madison, WI.*

Previous research indicated that there were significant differences in rumen-undegraded protein (RUP) among canola meals (CM). These differences could influence the nutritional value of CM. The objective of this study was to evaluate the effects of feeding CM with different RUP on ruminal fermentation, nutrient digestion, and microbial growth using a dual-flow continuous-culture system. Diets were randomly assigned to 6 fermenters in a replicated 3 × 3 Latin square with three 10-d experimental periods consisting of 7-d for adaptation and 3-d for sample collection. Treatments were solvent soybean-meal (SBM); low-RUP CM (LCM); and high-RUP CM (HCM). Fermenters were fed 72 g/d, divided in 4 feedings. Diets were prepared as 3 concentrate mixes that were combined with orchardgrass hay and wheat straw. Liquid and solid flow rates were adjusted to 11 and 5.5%/h, respectively. Samples were collected for digestibility, ruminal fermentation, and microbial growth. Statistical analyses were performed using SAS. Orthogonal contrasts were used to compare effects of different protein sources (SBM vs. LCM + HCM), and (LCM vs. HCM). Partial data are presented in the table below. Ruminal NH_3-N, total VFA concentration, molar proportion of acetate, propionate, butyrate, and isobutyrate were not affected by treatments. Molar proportion of valerate was greater when SBM was fed, whereas molar proportions of isovalerate, and total BCVFA were lower for CM diets (Table 1). Isovalerate, an intermediate product of leucine degradation, is associated with protein degradation; therefore, this may indicate that CM diets had higher RUP than SBM.

Table 1 (Abstr. T199). Effects of feeding canola meals (with different RUP LCM and HCM) on ruminal fermentation

Item	Treatment				Contrast *P*-values	
	SBM	LCM	HCM	SEM	SBM vs. LCM + HCM	LCM vs. HCM
NH_3-N, g/d	0.51	0.57	0.53	0.02	NS	NS
Total VFA, mmol	122.6	116.2	116.6	2.54	NS	NS
Acetate, %	64.6	65.4	66.5	1.25	NS	NS
Propionate, %	19.9	20.7	21.1	0.86	NS	NS
Butyrate, %	11.7	10.9	9.4	0.64	NS	NS
Isobutyrate, %	0.44	0.48	0.44	0.02	NS	NS
Valerate, %	1.5	1.6	1.6	0.03	0.02	NS
Isovalerate, %	1.3	1.1	1.1	0.08	0.02	NS
Acetate: Propionate	3.3	3.2	3.2	0.18	NS	NS
Total BCVFA, mmol	2.1	1.8	1.8	0.11	0.04	NS

Key Words: canola meal, continuous culture, RUP

T200   Comparison of lying times of lame versus sound dairy cattle using a leg-based accelerometer. Barbara A. Wadsworth*, Lauren M. Mayo, Nicky I. Tsai, Amanda E. Stone, Denise L. Ray, Joey D. Clark, and Jeffrey M. Bewley, *University of Kentucky, Lexington, KY.*

Lameness is a painful disease that affects cattle performance. Lame cattle may lay down longer than sound cattle. The objective of this study, conducted at the University of Kentucky Coldstream Dairy from March 18 to December 10, 2014, was to determine lying times of lame cows versus sound cows using a leg-based accelerometer. All cows (n = 96) were housed in 2 freestall barns and were balanced for parity and days in milk. Each individual freestall barn stocking density never exceeded 100%. One barn was equipped with sawdust covered Dual Chamber Cow Waterbeds (Advanced Comfort Technology, Reedsburg, WI) and another with sawdust covered rubber-filled mattresses (Promat Inc., Woodstock, Ontario, Canada). All cows were equipped with an AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel), which recorded daily lying time. The AfiMilk milking system (Afimilk) recorded daily milk yield. The Hobo U23 Pro V2 External Temperature Relative Humidity Data Logger (Onset, Bourne, MA) determined temperature and relative humidity (THI) in each barn every 15 min. Ambient conditions were categorized as low and high if THI was below or above 68, respectively.

J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2

383

Cows gait was assessed weekly using a 1 (sound cow) to 5 (severely lame cow) scale for general symmetry, speed, head bobbing, spine curvature, tracking, and abduction and adduction. Final gait score was calculated as the mean of all gait aspects for each cow. Cows scored ≥3 were determined as lame. The MIXED procedure in SAS (Version 9.3, Cary, NC) was used to evaluate factors influencing lying times. Stepwise backward elimination was used to remove non-significant interactions ($P \geq 0.05$). Lying times of lame cows were greater than sound cows ($P < 0.01$; 10.96 h/d and 9.51 h/d, respectively). All cows lied down longer in the cold season versus the warm season ($P < 0.01$; 10.43 h/d and 10.04 h/d, respectively). Milk yield was not different ($P \geq 0.05$) between lame and sound cows.

Key Words: lying time, lameness, accelerometer

T201   Survey of management of reproduction on Canadian dairy farms. José Denis-Robichaud*[1], Ronaldo L. A. Cerri[2], Andria Jones-Bitton[1], and Stephen J. LeBlanc[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada.*

The objective of this study was to survey attitudes and management practices for reproduction in a representative sample of Canadian dairy farms. A questionnaire was distributed online and by mail to Canadian dairy farmers from March to May 2014. Respondents were asked to give their percentage of artificial inseminations (AI) based on visual heat detection, timed artificial insemination (TAI) program, or automated activity monitoring (AAM), for first and subsequent AI separately. The main management practice was the one that used for >50% of inseminations, or "combined" if no one practice was used for >50% of AI. Out of the approximate 9,000 invitations to participate to the survey, a total of 833 surveys were completed (response rate estimated at 9%). The average number of lactating cows per herd was 77 (median = 55, interquartile range = 40–88), and the herds were located in all Canadian provinces. Lactating cows were housed in tie stall (61%) and freestall barns (39%). Visual heat detection was reported to be the main management practice in 51% and 44% of herds for first and subsequent AI, respectively. Respondents reported observing cows for heat signs 3.3 times per day for a total of 36 min, on average. Timed AI was the main management practice in 21% and 22% of herds for first and subsequent AI, respectively. Although 74% of respondents agreed that reproductive hormones were safe for consumers of dairy products, fewer (64%) agreed that routine use of synchronization programs was acceptable to them, or to consumers (44%). Ten percent of respondents, mainly housing lactating cows in free stall barns, reported using AAM as their main management practice for all AI. They reported using the system to flag cows in heat, but also using observed signs of estrus to decide to inseminate. Sixteen and 20% of all respondents used combined practices for first and subsequent inseminations, respectively. Bulls were used by 4% of respondents. These results suggest that visual heat detection is the principal management practice in many Canadian herds, but that TAI and AAM systems are also important in the management of reproduction.

Key Words: dairy cow, reproduction management, survey

T202   Lactational performance of early lactation, high-producing dairy cows fed corn silage produced by different seed corn hybrids. Ishwary Acharya*[1], Mark Kirk[2], and David Casper[1], [1]*Dairy Science Department, South Dakota State University, Brookings, SD,* [2]*Masters Choice, Anna, IL.*

Twenty-one (6 primiparous and 15 multiparous) high producing early lactation Holstein cows were fed one of 3 experimental corn silages harvested from the planting of 3 different seed corn hybrids from wk 4 through wk 14 postpartum. Corn hybrids (Control (C): Dekalb and Masters Choice: MC1 and MC2) were planted and harvested as corn silage during the 2012 growing season. Corn silage was harvested using a kernel processor silage harvester, inoculated, and ensiled in either a bunker or individual Ag Bags. Total mixed rations were formulated to be isonitrogenous at 17.5% CP consisting of 15.9% alfalfa hay, 35.1% concentrate mix and 48% of the respective experimental corn silage (DM basis). Cows were blocked by calving date and parity and randomly assigned to 1 of 3 treatments in a randomized complete block design. Data collected the third wk postpartum was used as a covariate in least squares analysis of covariance via the PROC MIXED procedure (SAS Institute). Dry matter intake (22.9, 23.5, and 22.4 kg/d for C, MC1, and MC2, respectively), milk yield (35.6, 34.8, and 36.1 kg/d), 3.5% fat-corrected milk (FCM) yield (38.7, 36.5, and 37.6 kg/d), energy corrected milk yield (38.2, 36.1, and 38.1 kg/d), feed efficiency (1.79, 1.61, and 1.67 kg/kg; 3.5% FCM/ DMI), milk fat (4.17, 3.94, and 3.71%), milk protein (3.12, 3.09, and 3.03%), lactose (4.93, 4.92, and 4.92%), solid-not-fat (8.96, 8.92, and 8.85%), body weight change (−0.10, −0.06, and −0.08 kg/d), and body condition score change (−0.05, −0.04, and −0.05 score/d) were similar for early lactation dairy cows fed all corn silage hybrids. Milk urea nitrogen was significantly ($P < 0.05$) lower for cows fed C (13.64) compared with cows fed MC1 and MC2 corn silage (15.0, and 15.0 mg/dL). This study demonstrated that different seed corn hybrids for the production of corn silage were similar in lactational performance.

Key Words: corn silage, corn hybrid, high-producing dairy cow

T203   Nitrogen utilization and growth effects in Holstein dairy calves fed a moderately high protein or conventional milk replacer. Colleen E. Chapman*[1], Thelton M. Hill[2], and Peter S. Erickson[1], [1]*University of New Hampshire, Durham, NH,* [2]*Provimi North America, Brookville, OH.*

Studies have shown that calves fed milk replacers (MR) with crude protein (CP) concentrations greater than 20% typically found in conventional MR have higher dry matter intakes (DMI) and greater average daily gains (ADG), but consume less starter which can lead to stress during weaning and reduced rumen development. The greater amount of CP being fed to preweaned calves may alter their nitrogen (N) balance and excess N may be excreted in the urine. The objective of this study was to determine N utilization in preweaned calves fed diets varying in the amount of CP and MR fed. This study used 24 newborn dairy heifer calves blocked by birth and randomly assigned to 1 of 3 treatments: (1) 454 g of a conventional MR (C; 20% CP, 20% fat), (2) 680 g of a moderately high protein MR (MMR; 26% CP, 17% fat), or (3) 908 g of a MMR (HMR; 26% CP, 17% fat). All calves had free choice access to starter and water. Both MR and starter were medicated with decoquinate. During weaning (d 43–49), AM MR feeding ceased. On d 50, all MR feedings ended; however, starter and water intakes were continuously recorded until d 56. At 5 wk of age, urine was collected using urinary catheters for 3 d and chromium oxide was administered by bolus at 2 g/d for 7 d to estimate N efficiency. Calves fed MMR and HMR had similar starter intakes, feed efficiencies (FE), and ADG; with the combined treatments having reduced starter intakes (258 g/d vs. 537 g/d), greater ADG (674 g/d vs. 422 g/d), and improved FE (0.57 vs. 0.45) compared with the C calves preweaning ($P < 0.05$). However, DMI and water intake were similar across all treatments. Results from the N utilization phase showed that MMR and HMR treatments had

similar but lower N efficiency than C calves (45.5% vs 52.7%; $P < 0.05$). This could be due to MMR and HMR fed calves having greater urine volume; and thereby, greater combined urine N output compared with C calves (17.6 g/d vs 12.1 g/d; $P < 0.05$). In summary, feeding a moderately high protein MR increased ADG and improved FE during the preweaning period, but reduced starter intake and lowered N efficiency.

Key Words: high protein, milk replacer, nitrogen efficiency

T204   Blood calcium changes after prophylactic subcutaneous treatment with calcium. Cynthia L. Miltenburg*, Elizabeth Scholtz, Dorothee Bienzle, Todd F. Duffield, and Stephen J. LeBlanc, *University of Guelph, Guelph, ON, Canada.*

Prophylactic calcium supplementation immediately after calving is a common strategy to prevent clinical and subclinical hypocalcemia in parturient dairy cows. The objective of this study was to evaluate the effect of prophylactic administration of Theracalcium on blood calcium concentration at 24 and 48 h after treatment, in cows without clinical hypocalcemia. Cows (n = 128) from 4 farms were blocked by parity and randomly assigned to receive either calcium gluconate (35% w/v) in combination with calcium glucoheptonate (10% w/v; Theracalcium, Vétoquinol Canada Inc., Lavaltrie, Quebec) or a placebo (medication vehicle solution with no calcium) at first contact with each cow after calving and again 12–24 h later when available for lockup. Each dose was 120mL injected subcutaneously over 2 sites. Total serum calcium concentration (tCa) was measured from coccygeal blood samples before (time 0) and 24 and 48 h after first treatment. There was no significant difference in tCa at time 0 (2.05 ± 0.02) between groups ($P = 0.18$). Serum tCa at time 0 was highly correlated with parity (r = −0.7) therefore pre-treatment tCa but not parity was used as a covariate in the models. A mixed model was constructed to measure tCa at 24 h after first treatment. For cows that had received 1 injection of calcium before the blood sample at 24 h (n = 95), tCa was significantly higher in the treated cows ($P = 0.01$): mean ± SE 2.03 ± 0.03 versus 1.90 ± 0.03 mmol/L, accounting for tCa at time 0 and a treatment by tCa at time 0 interaction. At 48 h there was no significant difference in tCa between treatment and control (mean ± SE 2.12 ± 0.02 and 2.10 ± 0.03 mmol/L, respectively) accounting for tCa at time 0 and farm as a fixed effect. With this subcutaneous prophylactic calcium treatment regimen, blood calcium levels were temporarily increased at 24 h after treatment. Further studies of disease and production in the postpartum period are required to determine if such a rise in blood calcium translates into improvements in transition cow health.

Key Words: calcium supplementation, hypocalcemia

T205   Moved to Ruminant Nutrition: General II (page 475)

T206   Quantification of select ruminal bacterial in Holstein bull calves treated with daily oral sodium bicarbonate. Taylor T. Yohe*[1,2], Rene R. Delgado-Peraza[1], Hannah L. M. Tucker[1,2], and Kristy M. Daniels[1,2], [1]*The Ohio State University, OARDC, Wooster, OH,* [2]*Virginia Tech, Blacksburg, VA.*

Dairy calves commonly have a rumen pH below 5.5 and it is unknown if this is problematic or normal for rumen development. The objective was to test if oral administration of sodium bicarbonate ($NaHCO_3$) to young calves can affect ruminal: pH, lactate concentration, and populations of select ruminal bacteria. We hypothesized that administration of $NaHCO_3$ would increase rumen pH, decrease lactate concentration, and

affect populations of lactate-producing (*Lactobacillus* spp.) and lactate-utilizing (*Megasphaera elsdenii*) bacteria. Twelve Holstein bulls (40.2 ± 1.6 kg BW) were randomized into treatments, arranged factorially. The factorials were treatment and route of treatment. The treatments were: CON-drench (water), BICARB-drench (1 *M* $NaHCO_3$ in water), CON-bolus (gelatin capsule), BICARB-bolus ($NaHCO_3$ gelatin capsule). $NaHCO_3$ was administered twice daily, adjusted weekly, and reached a maximum of 48 g/d. Calves were fed 543g DM/d of a 22% CP, 20% fat milk replacer and 20% CP starter. Rumen contents were sampled at wk 1 and 8 for pH, lactate, and bacteria measurement. Rumen pH did not depend on treatment or route, but was different by week (wk 1, pH = 6.00 ± 0.15; wk 8 pH = 5.19 ± 0.14; $P = 0.007$). Overall *D-* and *L-* lactate concentrations did not differ (7.45 ± 2.15 m*M* and 7.33 ± 1.98 m*M*, respectively); although, concentrations of each were numerically higher at wk 8 as opposed to wk 1. Populations of *Lactobacillus* spp. and *M. elsdenii* were calculated as a percentage of total 16S rDNA gene copies. *Lactobacillus* spp. abundance was unaffected by treatments or their interactions, but abundance of *M. elsdenii* was affected by the interaction of treatment and week (wk 1 CON = 0.05%, wk 1 BICARB = 0.00%, wk 8 CON = 0.19%, wk 8 BICARB = 0.26% of total bacteria, respectively; $P = 0.05$). Administration of $NaHCO_3$ by one of 2 oral routes did not affect rumen pH, lactate, or *Lactobacillus* spp. numbers, but in combination with time, did have an effect on *M. elsdenii* abundance. This suggests that *M. elsdenii* was able utilize lactate as a substrate and can withstand low rumen pH. These results support the idea that low rumen pH may be part of the normal rumen development of a dairy calf.

Key Words: dairy calf, rumen, bacteria

T207   Cost of days open equations accounting for variable market and dairy herd conditions. Karmella A. Dolecheck* and Jeffrey M. Bewley, *University of Kentucky, Lexington, KY.*

The objective of this study was to develop equations for estimating farm-specific cost of days open. The equations were constructed using a whole farm stochastic simulation model previously described by Bewley et al. (2010) and Liang (2013). Ten thousand iterations were run for lactations 1 to 5 with the mean cost per day open as an output. Stochastic variables expected to have potential effects on cost per day open were collected from each iteration. Those variables included: rolling herd average milk production, age at first calving, mature cow live weight, heifer calf value, bull calf value, semen cost, days in milk dictating an open cow as a reproductive cull, milk production level dictating an open cow as a production cull, veterinary costs, discount rate, milk price, feed price, replacement price, cull cow price, voluntary waiting period, estrus detection rate, and conception rate. The GLMSE-LECT procedure of SAS 9.3 (SAS Institute, Inc., Cary, NC) was used to analyze the effect of stochastic variables and 2-way interactions on the mean cost per day open for each lactation. Variables remained in the model when significant at $P < 0.05$. The R^2 of the resulting models were 0.57, 0.54, 0.64, 0.85, and 0.63 for lactations 1 to 5, respectively. The models were used to develop deterministic, lactation-specific equations for cost of days open. These equations are available in an online spreadsheet at: http://afsdairy.ca.uky.edu/CostOfDaysOpen. To demonstrate use, mean US Holstein herd data from 2015 DairyMetrics (Dairy Records Management Systems, Raleigh, NC), 2014 Food and Agricultural Research Policy Institute (Columbia, MO), and published literature were entered into each equation. Mean cost per day open for lactations 1 to 5 was $2.44, $2.82, $4.42, $4.54, and $3.32, respectively. These new, robust regression equations for cost of days open account for the complexities of varying market and herd conditions. The equations

J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2

385

can estimate cost of days open in partial budgets without the costs or computing time required for stochastic simulations.

Key Words: days open, cost of days open, stochastic model

T208   Checks and balances: Evaluating reliability of dairy nutrient management data to better protect groundwater resources. Christine Miller* and Deanne Meyer, *University of California, Davis, Davis, CA.*

To protect groundwater from further nitrate contamination, California regulations prohibit dairy producers from applying more than 140% of the nitrogen (N) that their crops remove. The regulations require copious annual reporting of crop field management, farm infrastructure, and animal population. The data collected in these annual reports could be integral to evaluating and improving both farm practices and the regulations themselves. Data reliability and accuracy must be assessed to use the information responsibly. Annual Reports from 18 dairies were obtained to assess reliability. Mass balance calculations were preformed to check the self-consistency of data within a facility. The results of mass balance calculations show that the data do not account for a remarkably large percentage of the nutrients being produced on the farms. Literature suggests that over 60% of N and 90% of P should be recovered; however, a median of only 25% of both N and P in cattle manure was recovered based on annual report data. This could be due to many different causes including inaccurate nitrogen sampling and analysis techniques, systematic reporting errors, or fraudulent reporting. Given that the accuracy of the majority of the recommended sampling and analysis protocols has not been assessed, it is likely that these methods are a significant source of error. Projects that should improve data collection protocols in both the short and long-term are in progress. Online decision trees are being developed to help farmers self-assess their current data collection practices, and provide personalized suggestions for improvement. Additionally, I will use a statistical modeling approach paired with in-field measurements to examine the uncertainty in these recommended protocols (and thereby the overall uncertainty in regulations). By separating the various sources of measurement error, the model will identify the best ways to improve data collection and regulation efficacy. Results of this and future studies will influence future nutrient management regulations in California and other states with active livestock industries.

Key Words: dairy waste management, nitrate leaching

T209   Distribution of quarter-level SCC across the dry and early post-partum period. Stephanie A. Metzger*, Laura L. Hernandez, and Pamela L. Ruegg, *University of Wisconsin, Madison, WI.*

The aim of this study was to characterize changes in quarter-level SCC for cows at dry off and the first 2 weeks postpartum. Milk samples were collected at dry off (S1) and postpartum wk 1 (S2) and wk 2 (S3) from quarters (n = 660) of 185 cows at the UW dairy farm. Microbiological analysis of milk samples was performed at the UW Milk Quality Laboratory. The SCC at each sampling period was used to determine postpartum categories: (1) Healthy (SCC < 150,000 at S1,S2,S3), (2) DryCure (S1 ≥ 150,000; S2 & S3 < 150,000), (3) Chronic (S1,S2,S3 all ≥ 150,000), (4) PostChronic (S < 150,000; S2 & S3 ≥ 150,000), (5) LateCure (S1 & S2 ≥ 150,000, S3 < 150,000), (6) LateInf (S1 & S2 < 150,000, S3 ≥ 150,000),(7) ShortNew (S1 & S3 < 150,000, S2 ≥ 150,000). Univariate analyses were performed to determine risk factors associated with Chronic quarters. Average DIM was 6.1 and 13.6 for S1 and S2, respectively. Median SCC were 117,000, 42,000, and 18,000 cells/mL for S1, S2 and S3, respectively. The prevalence of SCC ≥ 150,000 cells/ml was 43.6%, 13.5%, and 9.4% for S1, S2, and S3, respectively. The distribution of quarters was: Healthy (n = 321; 48.6%), DryCure (221; 33.5%), Chronic (20; 3.0%), PostChronic (13, 2.0%), LateCure (33, 5.0%), LateInf (29, 4.4%), and ShortNew (23, 3.5%). The occurrence of a clinical case in the lactation before dry off was associated with postpartum category (*P* = 0.004). Fewer quarters that had previously experienced a clinical case were categorized as healthy and a greater proportion were categorized as LateCure and PostChronic. Parity was associated with postpartum category (*P* < 0.001). As compared with younger cows, quarters of cows in parity ≥ 3 were 10.9 (3.6–34.6) times more likely to be categorized as chronic (rather than healthy). The number of days dry was greater for quarters categorized as Chronic (66.8d) and LateCure (66.4) as compared with quarters categorized as DryCure (59.1), PostChronic (57.6), LateInf (54.2), Healthy (53.1), or ShortNew (50.5) (*P* < 0.001). Only 8.6% of Chronic quarters were microbiologically positive at dry off. Results of this study demonstrate the dynamic nature of quarter SCC values during the dry and immediate postpartum period.

Key Words: dryoff, fresh cow, SCC

T210   Protein nutrient supply and feed milk value of two newly developed genotypes of transgenic alfalfa compared with non-transgenic alfalfa in dairy cattle. Xinxin Li*[1,2], Yonggen Zhang[2], and Peiqiang Yu[1], *[1]Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, [2]College of Animal Science and Technology, Northeast Agricultural University, Harbin, China.*

Two newly developed transgenic alfalfa with transformed *TRANSPARENT TESTA 8* (*TT8*) and *HB12* genes were developed by scientists at Agriculture and Agri-Food Canada. Our study was conducted to (1) predict the extent of ruminal and intestinal digestion of protein, (2) evaluate the nutrient supply to dairy cattle and (3) determine the feed milk value in dairy cattle. *HB12* transgenic alfalfa, *TT8* transgenic alfalfa and unmodified control genotype alfalfa were cultivated and harvested from the greenhouse of Agriculture and Agri-Food Canada. There were 2 experimental samples for each treatment. Total rumen degraded protein (TRDP), total rumen undegraded protein (TRUP) and intestinal digestible protein fractions were predicted. The parameters evaluated included: truly absorbed rumen microbial protein (AMCP), truly absorbed rumen undegraded feed protein in the small intestine (ARUP), endogenous protein loss in the small intestine (ENDP), total metabolizable protein (DVE) and degraded protein balance (OEB). The PROC MIXED procedure of SAS 9.3 was used for data analysis. Significance was declared at *P* < 0.05 and trends at *P* ≤ 0.10. The results showed that *TT8* alfalfa had highest TRDP value compared with HB12 and control alfalfa (*P* < 0.05). *TT8* alfalfa trended to be higher in TRUP than *HB12* alfalfa, with control alfalfa intermediate (*P* < 0.10). Intestinal digestible PB1 was higher in *TT8* alfalfa than the other 2 alfalfa plants (*P* < 0.05). *TT8* alfalfa had a greater content of microbial protein production (MCP$_{RDP}$, *P* < 0.05). No significant difference of OEB value was found between *HB12* alfalfa and control alfalfa, but the OEB value was higher in *TT8* alfalfa (*P* < 0.05). No significant differences in DVE and feed milk value (FMV) were found among 3 different genotypes of alfalfa plants (*P* > 0.05). In conclusion, altering the expression of lignin-related *TT8* and *HB12* genes in alfalfa plant could affect its nutrient supply to dairy cattle.

Key Words: lignin-related gene, alfalfa, feed milk value

T211 Probiotic *Enterococcus faecium* increased the propionate and total volatile fatty acids on in vitro rumen fermentation.
Lovelia L. Mamuad, Ashraf A. Biswas, and Sang Suk Lee*, *Sunchon National University, Suncheon, Jeonnam, South Korea.*

Enterococcus faecium had been widely used as direct fed microbial due to their capability to produce antibacterial compounds. Thus, this study was conducted to determine the beneficial effects of *E. faecium* on in vitro rumen fermentation, methane concentration, microbial diversity and population. Ruminal samples were collected from ruminally cannulated Holstein Friesian cattle and 40:60 rice straw to concentrate ratio were used as substrate at 1g dry matter (DM) per 100mL buffered rumen fluid. Fresh culture of *E. faecium* (7.5×10^8 cfu/ml) at different inclusion rates were investigated using in vitro rumen fermentation. The following treatments were non addition, 0.1%, 0.5% and 1.0% of *E. faecium* and, hereafter referred to as control, treatments 1, 2, and 3, respectively. Increased in total gas production and ammonia nitrogen concentration over time were found in control and all treatments while the opposite was observed in pH. Addition of *E. faecium* had significant effect on total gas production after 48h of incubation having the highest ($P < 0.05$) in T2 followed by T4, T3 and control with 93.33, 91.75, 89.50 and 82.20 ml, respectively. pH was not affected by addition of *E. faecium* and lactate was only detected at 0h and was not detected after 12, 24 and 48h of incubation. Propionate was found highest ($P < 0.05$) in T1 after 12h of incubation with 14.15 mM/L. Higher ($P < 0.05$) concentrations in treated than control were found in acetate after 12h, propionate after 48h, butyrate after 12, 24 and 48h and total volatile fatty acids (TVFA) after 12 and 48h of incubations. Pyrosequencing, methane concentration, and methanogen quantity are still on going. So far, addition of *E. faecium* did not affect the pH but increased the propionate and TVFA concentrations.

Key Words: *Enterococcus faecium*, in vitro, pyrosequencing

T212 Effects of replacing soybean meal with canola meal or treated canola meal on performance of lactating dairy cows.
E. M. Paula*[1], M. A. C. Danes[2], N. E. Lobos[2], G. I. Zanton[3], G. A. Broderick[4], and A. Faciola[1], *[1]University of Nevada, Reno, NV, [2]University of Wisconsin, Madison, WI, [3]USDA-Agricultural Research Service, US Dairy Forage Research Center, Madison, WI, [4]Broderick Nutrition & Research, Madison, WI.*

Canola meal (CM) has been shown to be a more effective CP source than soybean meal (SBM) for lactating dairy cows. Treating CM may increase its RUP fraction and improve the amount of absorbable AA. The objective of this study was to evaluate the effects of feeding treated CM (TCM) on performance of dairy cows. Forty-five Holstein cows were blocked by parity and DIM and used in a study of randomized complete block design. Cows were fed a control diet for a 2-week covariate period and then switched to the experimental diets for a 12-week study. Treatments differed only in CP source and were SBM, CM, and TCM. All diets contained (DM basis) 30% alfalfa silage, 30% corn silage, 4% soy hulls, 2.4% mineral-vitamin premix and 16% CP. SBM diets contained 25% high moisture corn (HMC) and 8.6% SBM; CM diets contained 22% HMC and 11.4% CM. Data were analyzed using the mixed procedure of SAS. Orthogonal contrasts were used to compare effects of different protein sources (SBM vs. CM + TCM) and (CM vs. TCM). There were no statistical differences in DMI and milk yield among diets; however, CM diets had numerically higher milk yields (Table 1). Compared with SBM both CM diets decreased MUN concentration ($P = 0.02$). There were no significant differences in milk composition and yields among treatments. Results from this study suggest that CM diets may improve N utilization, as indicated by reduced MUN. Although the large numeric differences in milk production were not statistically significant, these differences may be important from a practical standpoint.

Table 1 (Abstr. T212). Dry matter intake, milk production, and composition results

	Treatment				Contrast probability	
Item	SBM	CM	TCM	SEM	SBM vs. CM + TCM	CM vs. TCM
DMI, kg/d	25.9	26.5	27.1	0.90	0.44	0.70
Milk, kg/d	39.4	40.3	41.9	1.34	0.32	0.46
FCM, kg/d	41.2	45.2	43.7	1.96	0.16	0.66
FCM/DMI	1.64	1.67	1.70	0.06	0.53	0.79
Fat, %	4.16	4.18	4.14	0.15	0.99	0.93
Fat, kg/d	1.55	1.60	1.67	0.08	0.33	0.63
Protein, %	3.18	3.12	3.22	0.06	0.95	0.34
Protein, kg/d	1.18	1.21	1.30	0.05	0.26	0.33
Lactose, %	4.83	4.91	4.89	0.05	0.26	0.80
Lactose, kg/d	1.77	1.85	2.00	0.10	0.20	0.41
SNF, %	8.91	8.95	8.95	0.07	0.72	0.99
SNF, kg/d	3.32	3.43	3.68	0.17	0.25	0.41
MUN, mg/dL	14.0	12.9	12.5	0.40	0.02	0.49

Key Words: canola meal, MUN, soybean meal

T213 Transport of a fluorescent analog of glucose (2-NBDG) by rumen bacteria.
Junyi Tao*, Rebecca K. Diaz, and Timothy J. Hackmann, *University of Florida, Gainesville, FL.*

Our objective was to determine if cultured strains of rumen bacteria would transport a fluorescent analog of glucose (2-NBDG) with the same specificity and kinetics as glucose. Our rationale was that 2-NBDG could be used to identify uncultured, glucose-utilizing bacteria if it were transported similarly to glucose. Pure cultures of bacteria were harvested in the mid-to-late log phase, washed, and dosed with 2-NBDG or radio-labeled sugar (0 to 100 µM). Transport was halted by adding −5°C stop buffer and filtering through a membrane. The membrane was taken for fluorometry or liquid scintillation counting. Transport of 2-NBDG could be detected within 2 s of 2-NBDG addition for *Streptococcus bovis* and *Selenomonas ruminantium* (2 strains each), but it was not detected at any time for 6 other glucose-fermenting species. Genomes of *S. bovis* and *S. ruminantium* strains were found to possess genes for the mannose phosphotransferase system, whereas the other species had genes for other glucose transporters. For *S. bovis* JB1, the Michaelis constant (K_m) for 2-NBDG transport was 10.6-fold lower than that for [^{14}C]-glucose transport ($P = 0.006$). The maximum velocity (V_{max}) was 2.9-fold lower than that for [^{14}C]-glucose, but this difference was not significant ($P = 0.100$). In another set of experiments, transport of 2-NBDG at a single concentration (100 µM) was compared with that of [^{14}C]-glucose, [^{3}H]-mannose, and [^{14}C]-deoxy-2-glucose. For *S. bovis* JB1, transport of 2-NBDG was 3.2-fold lower than that of [^{14}C]-glucose ($P = 0.002$) but similar to that for [^{3}H]-mannose ($P = 0.992$) and [^{14}C]-deoxyglucose ($P = 0.955$). 2-NBDG could identify uncultured, glucose-utilizing bacteria, but only those with a mannose phosphotransferase system (not other glucose transporters). Its transport may more closely reflect that of mannose and deoxy-2-glucose than glucose.

Key Words: rumen bacteria, 2-NBDG, transport

J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2

387

T214   Characteristics of dairy cows with a greater or lower risk of subacute rumen acidosis: Volatile fatty acid absorption, rumen digestion kinetics and consistency of the risk. Xiaosheng Gao* and Masahito Oba, *University of Alberta, Edmonton, AB, Canada.*

The objectives of this study were to examine if lactating dairy cows with a greater or lower risk of subacute ruminal acidosis (SARA) have differences in volatile fatty acid (VFA) absorption rate, rumen digesta passage rate, and in situ rumen digestion, and determine if cows identified to have a greater risk of SARA in mid-lactation consistently have lower rumen pH in late-lactation than cows with a lower risk of SARA. Fourteen ruminally-cannulated dairy cows (DIM = 119 ± 47.2; BW = 640 ± 47.9 kg) were fed a high-grain diet consisting of 30% forage to induce SARA. Eight cows with the lowest acidosis index (area below pH 5.8 normalized for DMI; $0.10 ± 0.16$ pH·min/kg) and 5 with the highest acidosis index ($3.72 ± 0.19$ pH·min/kg) were classified as animals with lower (LOW) and higher (HIGH) risk of SARA, respectively. All response variables were evaluated for the group effect using the PROC TTEST procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). Minimum (5.75 vs. 5.33; $P < 0.01$) and mean ruminal pH (6.33 vs. 5.98; $P < 0.01$) was higher for LOW compared with HIGH animals. However, there were no differences in VFA absorption rate, rumen digesta passage rate, and in situ runminal digestibility of starch and NDF between HIGH and LOW cows. Nine of these 14 animals (4 HIGH and 5 LOW) were fed the same high-grain diet at a later stage of lactation (DIM = 243 ± 19.6) for 21 d. Similar to the mid-lactation, minimum (5.73 vs. 5.32; $P = 0.02$) and mean ruminal pH (6.32 vs. 6.07; $P = 0.01$) was higher for LOW compared with HIGH animals. These results suggested that variable risk of SARA among lactating dairy cows fed a high grain diet cannot be attributed to the differences in VFA absorption rate, rumen digesta passage rate, and ruminal digestibility, and that stage of lactation does not affect the risk category of animals in developing SARA.

Key Words: acidosis, VFA absorption, rumen digestion kinetics

T215   Systems nutrition in dairy cattle: Integrating hepatic metabolomics and transcriptomics in late pregnancy to better understand postpartal ketosis. Khuram Shahzad*[1], Johan Osorio[2], Daniel Luchini[3], and Juan J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*Oregon State University, Corvallis, OR,* [3]*Adisseo NA, Alpharetta, GA.*

'Omics' and bioinformatics were used to identify unique signatures characterizing liver of cows with postpartal ketosis relative to healthy cows fed rumen-protected methionine during late-pregnancy. Transcriptomics and metabolomics data were generated from liver tissue (n = 8/group, d −10 relative to parturition) of cows overfed a higher-energy diet during the dry period and classified as follows based on postpartal health: healthy (OVE), ketosis (K), or OVE plus Smartamine M (SM) or MetaSmart (MS). Data integration was via Ingenuity Pathways Analysis. Network construction included transcription regulators (TR) within the transcriptomics database and metabolites obtained through GC/MS-LC/MS. By comparing the different groups we obtained 21, 6, 10, 3, 11 and 15 transcription regulators (TR) out of 2908, 832, 1261, 922, 1573 and 1033, respectively, differentially expressed genes from K vs. OVE, K vs. SM, K vs. MS, SM vs. OVE, MS vs. OVE and SM vs. MS. Out of 313 known biochemical compounds, we detected 25, 34, 33, 20, 21, and 48 affected metabolites in the respective comparisons. As an example, using the TR (*HIF1A, HIF3A, SIRT1, HDAC4*) along with affected metabolites (cholic acid, D-erythro-dihydrosphingosine, lactic acid, malic acid, xylitol) in K vs. OVE the bioinformatics analyses revealed alterations in pathways related to tissue growth, and glucose and lipid metabolism. In regards to the methionine-supplemented groups,

using the TR (*HDAC2, SOX10, STAT1*) along with affected metabolites (arginine, inosine) in SM vs. OVE the bioinformatics analyses revealed alterations in pathways related to regulation of liver regeneration and metabolism. Unique patterns also were detected between SM and MS, analysis of the TR (*CREBBP, GATA2, NFKB2, STAT1*) along with affected metabolites (arachidonic acid, arginine, chenodeoxycholic acid, docosahexaenoic acid, oleic acid) revealed alterations in pathways involved in cell signaling, immune response, and cholesterol synthesis. Results indicate that 'omics' data integration could be helpful in better understanding the link between nutrition and incidence of disorders after calving.

Key Words: systems biology, ketosis, network reconstruction

T216   Laboratory validation of a prototype cow-side instrument for the measurement of blood ionized calcium concentrations in dairy cattle. Rafael C. Neves*, Tracy Stokol, and Jessica A. A. McArt, *Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY.*

There is currently no efficient and inexpensive method for field measurement of blood calcium concentrations. Ionized calcium (iCa) is the homeostatic form of the mineral and is thought to have greater biological relevance over that of total calcium. The objective of this study was to evaluate the linearity and precision of a prototype cow-side instrument (Horiba, Japan) for measuring blood iCa concentrations. Blood (300 mL) was collected from the right jugular vein of a multiparous dairy cow (4 d-in-milk) into lithium heparin tubes immediately before (T0) and 5 min after (T5) intravenous administration of 500 mL of 23% calcium borogluconate. The iCa concentrations were determined using a blood-gas analyzer (ABL-800 FLEX, Radiometer) as a gold-standard. The T0 sample was diluted using 0.9% saline to create a sample with low iCa (reference interval = 1.10 to 1.35 μmol/L). The diluted T0 sample was then mixed with the T5 sample in different ratios (100/0, 75/25, 50/50, 25/75, 0/100) to obtain 5 levels of iCa concentrations (0.69, 1.0, 1.28, 1.58, and 1.82 μmol/L). Each mixture was then analyzed in triplicate using 3 different prototypes under one-point (1P) and 2-point (2P) calibration with the means compared with results from the blood-gas analyzer. Cumulative sum tests for linearity from Passing and Bablok regressions showed no deviation from linearity for the combined results of all 3 prototypes under 1P vs. the gold-standard ($P = 0.19$) and under 2P vs. the gold-standard ($P = 0.19$). Instrument precision (coefficient of variation; CV) was determined by 10 repeat measurements of the diluted T0 sample, T0, and T5 samples under 1P and 2P calibrations. The CV ranged from 1.3 to 5% for the 3 prototypes. Laboratory results indicate good accuracy and precision for a cow-side instrument at the tested iCa concentrations. Investigation of the instrument under field conditions is warranted.

Key Words: ionized calcium, cow-side instrument, dairy cattle

T217   Validation of a hand-held meter for measuring β-hydroxybutyrate in plasma and serum of dairy cows. Arnulfo Pineda* and Felipe C. Cardoso, *University of Illinois, Urbana, IL.*

The aim of this study was to compare serum (sBHBA) and plasma (pBHBA) BHBA concentrations analyzed using either a laboratory method (Randox Laboratories Ltd., UK, Cat # RB1007 as "gold standard"; LM) or a hand-held meter (PX; Precision Xtra). Results from 187 samples taken from Holstein cows from 11 d before to 5 d after parturition were used for the analysis. Statistical analysis was performed using the MIXED, REG, and LOGISTIC procedures of SAS (v9.4). A

388

J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2

linear mixed model with repeated measures was performed for LM and PX. A regression analysis was completed to estimate the relationship between the 2 methods. Cross-validation by randomly splitting the data in model building and validation sets was performed to estimate and validate the equation that predicted the LM results using PX. Receiver operation characteristic (ROC) curves were made to estimate the sensitivity and specificity of PX at different threshold levels. The PX yielded higher ($P < 0.01$) pBHBA and sBHBA than LM, 1.45 vs. 0.95 and 1.63 vs. 1.00 mmol/dL, respectively. Adjusted R^2 between both methods for pBHBA was 97.1% and 97.8% for sBHBA. The equation from the model building data set that predicted pBHBA results using PX was y = −0.05353 + 0.67842x, while the equation that predicted sBHBA results was y = 0.05542 + 0.56862x, where y = predicted LM BHBA and x = PX BHBA. Mean square error (MSE) yielded by the model building data set for pBHBA and sBHBA were 0.006 and 0.005, respectively. Mean squared predictor error (MSPR) from the validation data set was 0.007 for pBHBA and 0.006 for sBHBA. Highest sensitivity and specificity for PX was achieved when threshold was set to 1.8 for pBHBA and 2.1 mmol/dL for sBHBA. The area under ROC curve (AUC) was 0.97 for pBHBA and 0.96 for sBHBA. In conclusion, the small difference between MSE and MSPR suggest that PX can be used to predict pBHBA and sBHBA by using the aforementioned equations. However, the threshold for ketosis requests to be higher than the commonly used in the field (1.2 mmol/dL) when using PX. The AUC close to 1 suggests greater ability of PX in predicting ketosis under the indicated thresholds.

Key Words: BHBA, dairy cow, Precision Xtra

T218   Effects of feeding calcium hydroxide-treated corn stover on milk production and milk composition in lactating Holstein cows. Brittany A. Casperson*[1], Aimee E. Wert-Lutz[2], and Shawn S. Donkin[1], [1]*Purdue University, West Lafayette, IN,* [2]*ADM Alliance Nutrition, Quincy, IL.*

Chemical treatment may improve the nutritional value of corn stalk residues and their potential use as an alternative forage source for lactating dairy cows. The objectives of this study were to determine the effect of prestorage hydration and treatment with 6.6% Ca(OH)$_2$ on feeding value of corn stalks as an alternative forage source on milk production, milk composition, and DMI. Mid-lactation multiparous Holstein cows (n = 30) were stratified by parity and milk production and randomly assigned to 1 of 3 diets. Corn stalks were chopped, hydrated, and treated with 6.6% Ca(OH)$_2$ (DM basis) and stored in Ag-bag silos. Treated corn stover was fed in a TMR at 0, 15, and 30% of the diet DM. Treated corn stover replaced either alfalfa haylage (15% stover diet) or replaced alfalfa haylage and an additional portion of corn silage (30% stover diet). Cows were individually fed in tie stalls for 10 weeks. Milk production was not altered by treatment ($P = 0.80$). Compared with 0% stover diet, DMI was reduced when the 15% stover diet was fed (25.9 vs. 22.7 ± 0.88 kg/d, $P < 0.05$) and tended to be reduced (25.9 vs. 23.1 ± 0.88 kg/d, $P = 0.08$) when cows were fed the 30% stover diet. Milk production per unit DMI (kg/kg) tended to increase for cows fed 15% stover diet compared with the 0% stover diet (1.41 vs. 1.62 ± 0.07, $P = 0.08$) but was not different between cows fed the 0% and 30% stover diets (1.41 vs. 1.50 ± 0.07, $P = 0.62$). Milk composition, energy corrected milk production, and energy corrected milk produced per unit of DMI (kg/kg) was not different ($P > 0.05$) among treatments for the 10-week feeding period. Cows fed the 15% and 30% diets had stable DMI and daily milk production over the 10-week treatment period but DMI for cows fed 0% stover increased slightly (time x treatment effect, $P < 0.05$). These data indicate that corn stover processed through prestorage hydration with Ca(OH)$_2$ results in an alternative forage source

for lactating dairy cows that when fed to mid-lactation cows tends to improve the efficiency of conversion of feed to milk without altering milk production or milk composition.

Key Words: corn stover, alternative forage, milk fat

T219   Increased stocking density at the feed bunk may affect the welfare and productivity of dairy cows and growth of their heifer calves. Jessica A. Pempek*, Maurice L. Eastridge, Kathryn L. Proudfoot, Gregory G. Habing, Lohendy M. Muñoz Vargas, and Danielle N. Coleman, *The Ohio State University, Columbus, OH.*

Overstocking the feed bunk during late-gestation has behavioral and physiological consequences, both of which negatively affect the welfare of the cow. However, it is unknown as to whether overstocking the dam may also affect the growth of the developing calf. The aim of the present study was to investigate the effect of increased stocking density at the feed bunk during different stages of the dry period on metabolic health and productivity of dairy cows, as well as the postnatal growth of their calves. One hundred twenty nonlactating Holstein dairy cows were blocked and assigned to 1 of 4 treatment groups with different stocking densities at the feed bunk (Overstocked (OS): 0.88 headlocks/cow; Understocked (US): 1.17 headlocks/cow). The 4 treatments were: OS from 60 to 1 d (OS), OS from 60 to 26 d and US from 25 to 1 d (OS-US), US from 60 to 26 d and OS from 25 to 1 d (US-OS), and US from 60 to 1 d (US) before calving. The heifer calves' treatment reflected the treatment assignment of their dam (n = 13, 18, 16, and 11, respectively). Blood samples were obtained from the cow at −60, −30, −14, −7, and +7 d relative to calving to determine concentrations of nonesterified fatty acids (NEFA). Colostrum quantity and quality were recorded from the cow's first milking. Calf body weight (BW) was measured once per wk through weaning at 5 wk of age. Data were analyzed using repeated measures and the Mixed model procedure of SAS (2012). NEFA concentrations were similar among treatment groups across periods (0.36, 0.42, 0.37, and 0.42 mEq/L, respectively; $P > 0.05$). There was a tendency for colostrum quantity to differ among treatments (7.52, 5.70, 7.03, and 8.76 kg, respectively; $P = 0.11$), primarily due to the difference between OS–US and US. However, there was no difference in colostrum quality (weighted Brix value of 7.24%). Using initial BW as a covariate, calf BW was similar among treatment groups (53.7, 51.9, 52.2, and 54.1 kg, respectively; $P > 0.05$), and there was no treatment by week interaction. In conclusion, moderate increases in stocking density at the feed bunk did not appear to compromise the metabolic status of dairy cows or the growth of their heifer calves.

Key Words: stocking density, feed bunk, dry period

T220   Unraveling the mechanisms that regulate activation of β-defensin antimicrobial peptide responses in cattle. Mercedes F. Kweh*, Kathryn E. Merriman, and Corwin D. Nelson, *University of Florida, Gainesville, FL.*

Bovine β-defensin peptides exhibit antimicrobial properties against bacterial pathogens, and expression of several β-defensin genes has been reported in the udder during mastitis. In cattle, toll-like receptor (TLR) and vitamin D signaling pathways induce expression of multiple β-defensin genes; however, β-defensin expression in mammary epithelial cells is only activated via the TLR pathway and is much lower compared with neutrophils and macrophages. The β-defensin gene cluster on bovine chromosome 27 contains multiple potential DNA methylation sites, suggesting the hypothesis that epigenetic mechanisms contribute to induction of the β-defensin response in cattle. The objective of this

J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2

389

study was to investigate the effects of DNA methylation and histone deacetylation inhibitors, 5-aza-2'-deoxycytidine (5-Aza) and Trichostatin A (TSA), respectively, on TLR and 1,25-dihydroxyvitamin D_3 (1,25D)-induced expression of β-defensins in primary bovine mammary epithelial cells (bMEC). Primary bMEC cultures obtained via mammary biopsy were treated with 5-Aza (1uM, 72 h), TSA (80 nM, 16 h) or control in combination with lipopolysaccharide (LPS; 100 ng/mL; 16 h) and 1,25D (10 nM, 16 h). Expression of β-defensin 3 (*BNBD3*), *BNBD4, BNBD7, BNBD10,* and lingual antimicrobial peptide (*LAP*) genes was determined using qPCR. The 5-Aza treatment resulted in a > 10-fold increased expression of each gene ($P < 0.001$). There was also an interaction between LPS and 5-Aza for expression of *BNBD3, BNBD10*, and *LAP* genes ($P < 0.001$). The TSA treatment also increased expression of the *BNBD7, BNBD10,* and *LAP* genes approximately 5-fold compared with control ($P < 0.01$), and there was TSA by LPS interaction for each of the genes ($P < 0.05$). In contrast, there were not interactions between 1,25D and 5-Aza or TSA for any of the β-defensin genes. These data suggest that DNA methylation and histone acetylation both contribute to β-defensin expression, and that epigenetic mechanisms may affect TLR activation of the β-defensin antimicrobial response of mammary epithelial cells.

Key Words: β-defensins, mammary immunity, vitamin D

T221   Ellipsoid equation improves accuracy and efficiency of estimating protozoal volume. Benjamin A. Wenner*, Brooklyn K. Wagner, and Jeffrey L. Firkins, *Department of Animal Sciences, The Ohio State University, Columbus, OH.*

Previous observations of protozoa in cultures treated with essential oils or ionophores indicated possible cell shrinkage due to deleterious effects on cell function. Cell volume reduction by formaldehyde preservation combined with visually flattened or tapered morphology of rumen protozoa limited our ability to detect volume differences using common cylindrical derivations for protozoa. The advent of affordable, high definition imaging equipment enables recording of live protozoa from cultures treated with various additives that potentially shrink cells. We hypothesized that using still frames from video of protozoa swimming would improve accuracy of volume predictions by optimizing an approach to measure one maximal longitudinal measurement and both minimum and maximum diameter measurements perpendicular to the longitudinal axis, thus yielding a 3-dimensional estimation of protozoal volume. An ellipsoid formula (E, $\frac{4}{3}\pi abc$) was compared with previously published estimations using cylindrical (C, $L\pi(^W/_2)^2$) or species coefficient (SP, $X\text{LW}^2$) calculations. Testing this method on inanimate objects shaped similarly to protozoa demonstrated that the ellipsoid is more accurate in predicting volume as measured by displacement. True displacement was 11.8 mL for 10 large particles, and estimated volumes were 12.7 to 27.1, 7.7 to 16.6, and 12.3 mL for C, SP, and E, respectively. For smaller particles with more surface area, true displacement was 4.5 mL, and estimated volumes were 5.6 to 13.1, 3.2 to 7.5, and 5.6 for C, SP, and E, respectively. Rumen fluid sampled from 2 lactating Jersey cows was flocculated and wet-mounted on a microscope fitted with an

HD (1080p) camera. Mean entodiniomorphid (ENTO) volumes were 109,665 (±55,912), 99,145 (±51,704), and 79,830 (±39,859) μm³ for C, SP and E, respectively. Mean epidinium (EPI) volumes were 1.74×10^5, 2.47×10^5, and 1.45×10^5 for C, SP, and E, respectively. Regression of SP on E demonstrated that SP more likely overestimates volume for ENTO (Y = 1.187X + 0.8567) or EPI (Y = 1.467X + 38528) than for teardrop-shaped isotrichids (Y = 0.833X + 346,973). This ellipsoid method offers potential to advance prediction of treatment effects on protozoal viability and volume.

Key Words: protozoa, imaging, rumen

T222   Milk yield at dry-off and other factors affecting risk of intramammary infections at calving. Paige N. Gott*[1], Päivi J. Rajala-Schultz[1], Gustavo M. Schuenemann[1], and Joseph S. Hogan[2], *[1]The Ohio State University, Columbus, OH, [2]The Ohio State University, Wooster, OH.*

Increased milk yield at dry-off has been associated with increased risk of intramammary infections (IMI) at calving. Abrupt cessation of milking is widely practiced although gradual cessation has been shown to significantly decrease milk yield. The objective of this study was to evaluate the effect of milk cessation method (MilkCess) and Dairy Herd Improvement Association final test day (FTD) milk yield on IMI at calving. Cows in 8 Ohio dairy herds were enrolled 7 to 14 d before expected dry-off and randomly assigned either to ABRUPT or to GRADUAL cessation of milking. The GRADUAL group was milked once daily for the final week of lactation while the ABRUPT group kept the farm's normal milking schedule. Aseptic quarter foremilk samples were collected at enrollment, at the final milking (DRY), and within 7 d of calving (CALV). Samples were cultured following NMC guidelines. Isolation of ≥ 10 colonies of similar morphology was considered an IMI while isolation of ≥3 morphologies was called contamination. Coagulase-negative staphylococci and *Corynebacterium* spp. were considered minor (MIN) pathogens. Other species were considered major (MAJ) pathogens. The association between quarter IMI status at CALV and MilkCess and FTD milk yield was evaluated using PROC GLIMMIX in SAS. Separate models were run with MAJ and MIN IMI at CALV as the outcomes. Data from 392 cows were analyzed. MilkCess was not significant, but was forced into each model. For every 4.5-kg increase in FTD milk yield, the odds of IMI at CALV increased (MAJ = 39%, $P = 0.0007$; MIN = 18%, $P = 0.0498$). The odds of MAJ IMI at CALV for quarters with MAJ IMI at DRY were 17.2 times the odds of quarters uninfected at DRY ($P < 0.0001$). The odds of MIN IMI at CALV for quarters with MIN IMI at DRY were 1.9 times the odds of quarters uninfected at DRY ($P = 0.0316$). For every 7d increase in DIM at dry-off above 305 DIM, the odds of MIN IMI at CALV increased 3.1% ($P = 0.0328$). On average, dry periods lasted 58 d and for every 7-d increase in days dry above 58 d, the odds of MIN IMI increased 28% ($P = 0.0125$). In conclusion, decreasing milk yield before dry-off could help improve udder health at calving.

Key Words: milk yield, IMI, dry-off

390

J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2