M42  Transient versus persistent subclinical hypocalcemia: Association of calcium status with early lactation disease and production in Holstein cows. J. A. A. McArt*1 and R. C. Neves*, 1Cornell University, Ithaca, NY, 2Texas Tech University, Lubbock, TX.

Our objectives were to evaluate the association of subclinical hypocalcemia (SCH) duration with the risk of early lactation negative events and milk production. Data from a cohort of 407 Holstein cows in 2 herds in NY were used. Primiparous (P) and multiparous (M) cows were classified into 1 of 4 groups based on postpartum plasma total Ca ([tCa]P): normocalcemic (NC; [tCa]P > 2.15 mmol/L at 1 and 2 DIM, n = 67; [tCa]P M > 1.77 at 1 DIM, > 2.20 mmol/L at 4 DIM, n = 109); transient SCH (tSCH; [tCa]P ≤ 2.15 at 1 DIM, > 2.15 mmol/L at 2 DIM, n = 25; [tCa]P M ≤ 1.77 at 1 DIM, > 2.20 mmol/L at 4 DIM, n = 50); persistent SCH (pSCH; [tCa]P ≤ 2.15 mmol/L at 1 and 2 DIM, n = 33; [tCa]P M ≤ 1.77 at 1 DIM, ≤ 2.20 mmol/L at 4 DIM, n = 34); or delayed SCH (dSCH; [tCa]P > 2.15 at 1 DIM, ≤ 2.15 mmol/L at 2 DIM, n = 19; [tCa]P M > 1.77 at 1 DIM, ≤ 2.20 mmol/L at 4 DIM, n = 70). Evaluated outcomes were development of a negative event (NEG; hyperketonemia at 3, 5, 7, and/or 10 DIM, metritis, or displaced abomasum or herd removal within 60 DIM) and average milk yield per d across the first 10 wk of lactation. Multivariable Poisson models were used to analyze the NEG outcome and generalized linear mixed models for milk yield. Both P and M cows with tSCH were no more likely to have a NEG event than NC cows (risk ratioP (RR) = 1.3, 95% CI = 0.5 to 3.2; RRM = 1.4, 95% CI = 1.0 to 2.1). However, P and M cows with SCH were 4.1 (95% CI = 2.1 to 7.9) and 1.8 (95% CI = 1.2 to 2.7), and dSCH cows 3.2 (95% CI = 1.5 to 7.0) and 1.8 (95% CI = 1.4 to 2.6) times more likely to have a NEG event than NC cows, respectively. Both P and M cows with SCH produced more milk per d than NC, pSCH, or dSCH across the first 10 wk of lactation. Primiparous cows averaged 28.5 ± 0.7, 31.9 ± 1.1, 29.7 ± 0.9, and 28.7 ± 1.2 kg per d for NC, tSCH, pSCH, and dSCH cows, respectively, and M cows averaged 44.6 ± 0.7, 49.1 ± 1.1, 46.4 ± 1.3, and 41.4 ± 0.9 kg per d for NC, tSCH, pSCH, and dSCH, respectively. Our results suggest that cows with SCH adapt better to early lactation, develop fewer NEG events, and produce more milk than NC, pSCH, or dSCH cows. Primiparous or M cows with SCH or dSCH are at an increased risk for early lactation NEG events and reduced milk production.

Key Words: dairy cow, hypocalcemia, transition cow

M44  The effect of prepartum negative dietary cation-anion difference and serum calcium concentration on blood neutrophil function in the transition period. R. Couto Serrenho*, E. I. Morison, O. Bogado Pascottini, and S. J. LeBlanc, University of Guelph, Guelph, ON, Canada.

Our objectives were to assess the effects of a negative dietary cation-anion difference (DCAD) on phagocytosis (P) and oxidative burst (OB) function of circulating neutrophils and to determine the associations of serum ionized (iCa) and total calcium concentrations ([Ca]T) with P and OB. We hypothesized that multiparous cows fed a negative DCAD prepartum would have greater serum ionized (iCa) and total calcium concentrations ([Ca]T), and thus improved P and OB. From 3 wk before expected parturition until calving, 38 healthy multiparous cows from 3 farms were randomly assigned to negative DCAD (NEG; −100 meEq/kg DM; n = 21) or a control diet (CON; 12 meEq/kg DM; n = 17). Urine pH was measured weekly and in NEG was 6.3 ± 0.8 with 76% of 21 samples <7 and 62% < 6.5. Each farm was on 1 treatment or the other. P, OB, iCa, and [Ca]T were measured at −7, 1, and 4 DIM. Mean fluorescence intensity for P (MFIP) and OB (MFIOB), and the shift of percentage of active cells for P (PP) and OB (POB) were measured in isolated, stimulated neutrophils via flow cytometry. Outcomes were assessed with mixed linear regression models accounting for repeated measures. There were no significant differences between treatments in the 4 neutrophil function outcomes. Although MFIOB varied over time, there were no interactions of treatment with time for any outcome. iCa and [Ca]T did not differ between NEG and CON. The LSM ± SE for iCa were: −7 DIM, 1.23 ± 0.02 vs. 1.21 ± 0.02; 1 DIM, 1.07 ± 0.02 vs. 1.02 ± 0.02; 4 DIM, 1.16 ± 0.02 vs. 1.17 ± 0.02 mmol/L, respectively; and for [Ca]T: −7 DIM 2.39 ± 0.04 vs 2.44 ± 0.05; 1 DIM, 2.01 ± 0.04 vs 1.97 ± 0.05; 4 DIM, 2.33 ± 0.04 vs 2.32 ± 0.05 mmol/L, respectively. The proportion of blood samples with iCa <2.15mmol/L at −7, 1 and 4 DIM was 5%, 76%, and 13%, respectively, with no differences between NEG and CON (P > 0.7). However, iCa tended to be weakly positively correlated with MFIOB at −7 DIM. There was no association between

Key Words: dairy cow, hypocalcemia, transition cow
tCa and P or OB. We did not observe the hypothesized differences in aspects of innate immunity in multiparous cows fed a negative DCAD.

Key Words: hypocalcemia, immune function

M45  Factors that contribute to ketosis in early lactation Holstein dairy cattle. C. Sousa* and H. Rossow, School of Veterinary Medicine, University of California, Davis, Davis, CA.

Ketosis in early lactation dairy cattle can negatively impact health events, milk yield and reproductive performance causing a loss in profit for dairy producers. The objective of this retrospective observational study was to identify factors such as management, metabolic profile and milk production that contribute to ketosis in early lactation. One hundred and thirty-two multiparous dry and lactating Holstein cows from a California commercial dairy herd were bled weekly for 3 wk before calving to the dry period via coccygeal venipuncture into evacuated sterile tubes containing sodium fluoride and potassium oxalate. Milk yield, fat and protein percentage were measured monthly by Tulare County Dairy Herd Improvement Association using a Bentley Instrument ChemSpec 150 (Chaska, MN). Blood metabolite data including nonesterified fatty acids (NEFA) using a commercial kit from Wako Chemicals Inc. (Richmond, VA), glucose and BHB using a Precision Xtra handheld meter (Abbott Diabetes Care Inc., Alameda, CA), and metabolic data using an Abaxis Vet Scan analyzer Large animal profile rotor (VetScan®, Abaxis, Inc., Union City, CA) were measured weekly. Management variables and milk production from Dairy Comp 305 (Valley Agricultural Software, Tulare, CA) were collected monthly in the previous and current lactation. Using the General Linear Model Procedure of SAS (SAS Institute v.9.4, 2018), BHB were regressed on blood metabolites, management data and milk production. Variables were eliminated from the regression if \( P > 0.05 \). The significant management variables that remained in the regression were total days in milk in previous lactation and days dry in previous lactation \( (P < 0.05, R^2 = 0.70) \). When metabolic variables such as aspartate amino transferase, globulin, blood urea nitrogen, NEFA, and glucose from 7 to 14 d relative to calving were added, and milk production variables such as total solids and total fat in the previous lactation were added to the regression, \( P < 0.05 \) and \( R^2 = 0.92 \). Residuals were normally distributed and unbiased. These equations indicate that cows at risk for ketosis could be identified before calving to help dairy producers manage ketosis before it negatively affects health and production performance.

Key Words: ketosis, dairy cattle


Transition diseases result in considerable economic losses for dairy producers. The purpose of this study was to evaluate the technical consequences of subclinical ketosis (SCK), their economic impact on dairy farm, and the profitability of prevention strategies used in practice. Data from current literature was collected and summarized, and used to model the impact of different incidences \((20-40-60\%)\), and its effect on milk production \((\text{loss of } 5-10\% \text{ for } 60 \text{ d})\), fertility \((\text{decrease of } 25-50\% \text{ for } 120 \text{ d})\), and increased risk of displaced abomasum, clinical ketosis, metritis and the culling risk of 6, 3.6, 2 and 3.7 times, respectively. Two systematic prevention strategies applied to all cows at calving were used: propylene glycol (PG) with a total cost of €12/cow and a 47% reduction of incidence; or a slow-release monensin bolus (MON) with a total cost of €30/cow and a 60% reduction of incidence. Four simulations were carried out at 2 milk prices (€300 and €360/l): 1) a control without SCK incidence; 2) with SCK (40% incidence, a 10% loss of milk production and a 50% decrease in fertility); 3) with SCK and PG; and 4) with SCK and MON. The SCK impact was evaluated in a farm of 300 dairy cows with average 12,000 L/cow/yr and average technical performance indexes for dairy farms in Spain, using a dairy farm stochastic dynamic model (www.dairyfarm.es; Calsamiglia et al., 2018). The cost per case was €339, ranging from €277 to €373 among incidences. Economic losses due to decreased milk production (64%) were larger that losses attributed to reduced reproductive performance (36%). The PG (€63) was a more profitable strategy compared with MON (€33). Although the cost of the case was similar across incidences, when the incidence was reduced (fewer cases) or milk price decreased, the economic return of prevention treatment was lower. Subclinical ketosis has important effects on cow performance and farm profitability. The adequacy and type of prevention strategy depends on the SCK incidence, the cost of treatment and the price of milk.

Key Words: ketosis cost, propylene glycol, monensin


Our objective was to evaluate the effect of a health monitoring program based primarily on alerts generated by automated health monitoring (AHM) systems on disease detection and performance of dairy cows. Parous and nulliparous Holstein cows at 245 ± 3 d in gestation were stratified by parity and randomly assigned to a CON (n = 622) or TRT group (n = 621). Cows from both groups were commingled. For cows in CON, clinical examination (CE) was conducted daily up to 10 DIM and in response to daily milk yield reduction (≥15% negative deviation) alerts (Afirmilk) or visual observation of clinical signs of disease from 11 to 30 DIM. For cows in TRT, CE up to 30 DIM was conducted in response to one or more of the following: an alert generated with a combination of rumination time and physical activity (HID score, SCR Dairy), milk yield deviation, or visual observation of clinical signs of disease. Daily after the morning milking, cows eligible for CE were evaluated following similar procedures. Binomial and quantitative data were analyzed by logistic regression and ANOVA with repeated measures, respectively. The proportion of cows with at least one event of metritis \((P = 0.43; \text{CON} = 12.5\%; \text{TRT} = 11.1\%)\), displaced abomasum \((P = 0.45; \text{CON} = 1.1\%; \text{TRT} = 1.6\%)\), indigestion \((P = 0.73; \text{CON} = 2.9\%; \text{TRT} = 3.2\%)\), pneumonia \((P = 0.3; \text{CON} = 1.5\%; \text{TRT} = 0.8\%)\), and mastitis \((P = 0.4; \text{CON} = 10.3\%; \text{TRT} = 8.9\%)\) did not differ. The proportion of cows with ketosis \((P = 0.09; \text{CON} = 8.7\%; \text{TRT} = 6.1\%)\) and the total proportion of cows with at least one event of disease \((P = 0.05; \text{CON} = 30.4\%; \text{TRT} = 25.3\%)\) tended to differ. There was no difference in the combined proportion of cows sold and dead \((P = 0.35; \text{CON} = 15.2\%; \text{TRT} = 12.3\%)\). No difference \((P = 0.29)\) was observed for average weekly milk \((\text{CON} = 41.6 ± 0.3 \text{ kg} ; \text{TRT} = 41.2 ± 0.3 \text{ kg})\) for up to 35 DIM. Cows inseminated at detected estrus \((P = 0.17; \text{CON} = 41.7\%; \text{TRT} = 40.1\%)\). We conclude that a health-monitoring program based primarily on alerts generated by AHM systems was an effective strategy to identify cows with health disorders and did not negatively affect herd production and reproductive performance outcomes. Supported by New York Farm Viability Institute project 017-014.

Key Words: automation, health, dairy cow

24  J. Dairy Sci. Vol. 102, Suppl. 1
Identification of circulating markers that reflect liver triglyceride (lvTG) status would provide valuable insight when liver biopsies are not available. The objective of this study was to determine if metabolites related to energy balance and hepatic health would differ between cows with varying degrees of lvTG accumulation. Liver and blood samples were collected from multiparous Holstein cows (n = 37) at −14, +1, and +14 d relative to calving (DRTC) enrolled in 2 previously reported studies with 2 treatments (TRT) each. During the dry period, cows were fed a ration that met 100% (1 TRT; n = 13) or 120% (3 TRT; n = 7, 6, 11) of energy requirements to yield a range of postpartum lvTG. Postpartum rations were similar, except for 1 ration that included ammoniated lactate (n = 6). Cows were retrospectively grouped based on maximum lvTG% (DM basis) into 3 groups (TGgrp): high (lvTG = 30.3 ± 1.1%; n = 12), med (lvTG = 15.9 ± 1.06%; n = 13), or low (lvTG = 10.4 ± 1.1%; n = 12). Samples were analyzed for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (alb), triglyceride (TG), β-hydroxybutyrate (BHB), and blood urea nitrogen (BUN), and plasma nonesterified fatty acids (NEFA) and glucose (glc). Data were analyzed in SAS (9.4) using Proc Mixed with fixed effects of TGgrp, TRT, and interaction. Concentrations of AST, ALT, alb, BHB, BUN, glc, NEFA, and TG all changed over time (P < 0.001). The milk yield decreased significantly by 11% in SCK (normal: 38.79 ± 4.98, SCK: 34.49 ± 1.64 L/day; P = 0.028) animals. The animals with SCK had significantly higher aspartate aminotransferase (normal: 0.16 ± 1.53, SCK: 110.66 ± 2.29 IU/L; P = 0.035) and total bilirubin (normal: 0.39 ± 0.01, SCK: 0.44 ± 0.02 mg/dL; P = 0.001) concentrations and significantly lower blood glucose (normal: 51.08 ± 0.51, SCK: 46.5 ± 0.86 mg/dL; P = 0.001) concentration than normal animals. This is a recently conducted population based study that shows the nationwide prevalence of SCK in Korea. The results of this study will provide important information to farm practitioners and producers to be aware of the milk yield loss due to SCK during farm management.

Key Words: subclinical ketosis, prevalence, milk yield


The objective was to 1) establish cow-level thresholds for haptoglobin (Hp) concentrations to predict health disorders and 2) to investigate the magnitude of association between elevated Hp and health disorders within 30 DIM. Data were collected from 72 commercial dairy farms and Hp and β-hydroxybutyrate (BHB) concentrations were measured in 988 Holstein cows, 0 to 12 DIM. Associations between the health disorder of interest and Hp were assessed in SAS v. 9.4, PROC GENMOD, using a Poisson distribution, log link function, p-scale option, exchangeable correlation matrix, herd as a random effect and included biologically plausible covariates and 2-way interactions. Manual backward stepwise elimination removed covariates and interactions if P > 0.10. If Hp was significant, an ROC analysis with PROC LOGISTIC was used to determine the critical threshold for predicting the health disorder by identifying the point on the curve with the maximum Youden’s index. Based on the threshold, Hp was categorized and the multivariable model was reassessed to determine risk ratios. Haptoglobin was not effective for predicting herd reported mastitis, DA, or ketosis (P > 0.10). The Hp threshold for predicting herd reported metritis (MET) was 1.52 g/L (AUC = 0.8; Se: 67%, Sp: 84%), for predicting herd removal (CULL) was 0.68 g/L (AUC = 0.7; Se: 72%, Sp: 68%), and for association with hyperketonemia (HYK; BHB ≥1.0 mmol/L) was 0.74 g/L (AUC = 0.8; Se: 67%, Sp: 84%). The Hp threshold for predicting any significant health disorder (DIS; MET, CULL, HYK) was 0.74 g/L (AUC = 0.8; Se: 45%, Sp: 72%). Risk ratios for variables remaining in the health disorder models are reported in the table. Elevated Hp was found to be associated with HYK, MET, and herd removal within 30 DIM.
Table 1 (Abstr. M50). Risk ratios (RR) for variables associated with health disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Variable</th>
<th>Threshold</th>
<th>( P )-value</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET</td>
<td>Hp (g/L)</td>
<td>1.52</td>
<td>&lt;0.01</td>
<td>8.6</td>
<td>3.0 to 24.5</td>
</tr>
<tr>
<td>HYK</td>
<td>Hp (g/L)</td>
<td>0.74</td>
<td>&lt;0.01</td>
<td>1.8</td>
<td>1.4 to 2.3</td>
</tr>
<tr>
<td></td>
<td>Parity(^1)</td>
<td>&lt;0.01</td>
<td>2.1</td>
<td>1.5 to 2.8</td>
<td></td>
</tr>
<tr>
<td>CULL</td>
<td>Hp (g/L)</td>
<td>0.68</td>
<td>&lt;0.01</td>
<td>4.9</td>
<td>2.4 to 9.9</td>
</tr>
<tr>
<td></td>
<td>Lameness(^2)</td>
<td>&lt;0.01</td>
<td>3.5</td>
<td>1.6 to 7.6</td>
<td></td>
</tr>
<tr>
<td>DIS</td>
<td>Hp (g/L)</td>
<td>0.74</td>
<td>&lt;0.01</td>
<td>1.8</td>
<td>1.5 to 2.3</td>
</tr>
<tr>
<td></td>
<td>Parity(^1)</td>
<td>&lt;0.01</td>
<td>1.6</td>
<td>1.2 to 2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lameness(^2)</td>
<td>0.04</td>
<td>1.3</td>
<td>1.0 to 1.6</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Multiparous vs. primiparous.
\(^2\)Lame vs. not lame.

Key Words: haptoglobin, metabolic health, transition cow

M51 Evaluation of reproduction and behavior of dairy cows treated with Pegbovigrastim. M. X. da Silva Oliveira\(^*\), D. D. McGeec, J. A. Brett\(^1\), and A. E. Stone\(^1\).\(^1\)Animal and Dairy Sciences, Mississippi State University, Mississippi State, MS, \(^2\)Elanco Animal Health, Greenfield, IN, \(^3\)College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.

The aim of this study was to evaluate the effects of pegbovigrastim injection (Imrestor, Elanco Animal Health, Greenfield, IN) on behavior and time to first detected heat. The study was conducted on a commercial Mississippi dairy from August 21, 2017, to March 31, 2018. Initially, a total of 270 cows were blocked by parity group (multiparous or primiparous) and randomly assigned to control (CON, \( n = 144 \)) or treatment (IMR, \( n = 126 \)). Study animals were equipped with smart leg and smart neck tags (Nedap, the Netherlands). The leg tag measured lying time, walking time, and leg activity with a 3-axis accelerometer, and the neck tag measured heat activity. At 10 ± 2 d before expected calving and again at calving, treated cows received 2.7 mL of Imrestor and CON cows received 2.7 mL of 0.9% saline. Cows were body condition (BCS), hygiene (HYG), and lameness (LAM) scored weekly. The Nedap system created heat alerts based on individual behavior changes. Weekly summations of the number of steps, time spent lying down and walking (min) then was utilized to determine weekly means of these variables since calving until 60 DIM. Unfortunately, because of malfunction in the data synch between tags and the farm software, data from only 61 cows were utilized in the analysis (CON = 43 and IMR = 18). The MIXED procedure of SAS was utilized to compare the leg activity, walking time, and lying time between the 2 treatment groups. The mixed models included fixed effects of treatments and block, and random effect of cow within block. A Cox proportional hazards regression was used to determine the relative likelihood of cows displaying estrus within their first 60 DIM. Imrestor treatment did not alter the hazard ratio of estrus occurrence during the first 60 DIM (hazard ratio = 0.99, 95% CI = 0.84 to 1.7, \( P = 0.88 \)). The median calving-to-first detected heat interval for IMR and CTR cows was 47 and 46 d, respectively (\( P = 0.45 \)). Imrestor treatment did not affect time spent lying, walking, or amount of steps given (\( P = 0.54, 0.59, \) and 0.48, respectively). In conclusion, IMR treatment did not affect the behavior or time to first detected heat in study cows.

Key Words: Imrestor, precision dairy monitoring, well being

M52 Impact of a liposome-TLR agonist stimulant on macrophage bactericidal activity against Staphylococcus aureus and on intramammary immune responses. L. Caixeta\(^*\), S. Scheu\(^2\), V. Rossod, W. Wheat\(^2\), and S. Dow\(^2,3\).\(^1\)Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN, \(^2\)Department of Clinical Sciences, Colorado State University, Fort Collins, CO, \(^3\)Laporte Therapeutics, Inc., Fort Collins, CO.

Mastitis is a major economic and welfare concern to the dairy industry and it is the most common reason for the use of antibiotics in dairy farms. The development of innovative approaches capable of enhancing udder health will contribute to the judicious use of antibiotics in dairy farms. Thus, the objective of our pilot studies was to investigate the effect of a mucosal immune stimulant (MIS; MucosImmune, Laporte Therapeutics, Fort Collins, CO) on mammary gland immune response. We hypothesized that intramammary (IMM) infusion of MIS would trigger non-specific activation of mammary gland immunity. In Experiment 1, macrophage cultures were generated from monocytes of healthy cattle (\( n = 3 \)) and incubated with MIS (or PBS) for 24h, then inoculated with a clinical mastitis isolate of S. aureus and intracellular bactericidal activity assessed 4h later. We found that MIS activation of macrophages significantly increased intracellular killing of S. aureus (from 16.7% to 44.2%; \( P = 0.004 \)), to a level comparable to that generated by IFN-g (\( P > 0.05 \)). In Experiment 2, lactating dairy cows were randomly allocated to receive IMM administration of either MIS solution (TRT; 1mL MIS and 9mL PBS; \( n = 4 \)) or 10mL of PBS (UTR; \( n = 3 \)). Milk samples for TRT and UTR cows were collected at 72h after treatment. Milk samples were incubated with S. aureus and E. coli isolated from mastitis cases for 30 min to measure levels of IgA and IgG that bound to these pathogens. Milk from cows in the TRT group had higher levels of IgA and IgG antibodies against E. coli, but lower levels of antibodies binding to S. aureus. Milk from cows in the TRT group had higher levels of IgA and IgG antibodies against E. coli, but lower levels of antibodies binding to S. aureus (Table). Our preliminary results indicated that MIS is capable of non-specific activation of intra-mammary immunity and increases milk IgA and IgG antibodies against E. coli, but not against S. aureus.

<table>
<thead>
<tr>
<th>Item</th>
<th>TRT Mean(^1)</th>
<th>SD</th>
<th>UTR Mean(^1)</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti S. aureus</td>
<td>IgA</td>
<td>55,346</td>
<td>13,833</td>
<td>100,847</td>
<td>30,595</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>19,882</td>
<td>7,739</td>
<td>102,104</td>
<td>24,773</td>
</tr>
<tr>
<td>Anti E. coli</td>
<td>IgA</td>
<td>2,388</td>
<td>1,554</td>
<td>1,275</td>
<td>848</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>2,871</td>
<td>1,742</td>
<td>1,888</td>
<td>1,456</td>
</tr>
</tbody>
</table>

\(^1\)Geometric mean fluorescence intensity (gMFI).

Key Words: mastitis, immune stimulant, bactericidal

M53 The probability of subclinical mastitis and isolated organisms in organic dairy herds varies between years. E. K. Luc\(^1\), L. G. Schneider\(^1\), V. L. Couture\(^1\), H. R. Bailey\(^1\), P. D. Krawczel\(^1\), S. R. Smith\(^1\), A. G. Ríus\(^1\), and G. M. Pighetti\(^1\).\(^1\)The University of Tennessee, Knoxville, TN, \(^2\)University of Kentucky, Lexington, KY.

Prevention and treatment of mastitis without the use of antibiotics or synthetic products is one of the challenges organic dairies face. Greater understanding of factors affecting the probability of mastitis will aid in developing mastitis control programs. The objective was to determine if probability of subclinical mastitis and isolated organisms varied between year of 2017 and 2018 in relation to season, parity, and stage.
of lactation. Five organic dairies (n = 402 cows) were enrolled in the study. To determine the probability of subclinical mastitis, Dairy Herd Improvement (DHI) records for somatic cell score (SCS), days in milk and parity were assessed. Subclinical mastitis was characterized as cows having a SCS of >4. Aseptic quarter milk samples (n = 1,825) were collected from cows with subclinical mastitis and tested for microbiological identification. Organisms greater than 5% in prevalence were evaluated. Logistic regression (PROC GLIMMIX, SAS 9.4, Cary, NC) was used with fixed effects of season, parity, stage of lactation and year, and a random effect of cow(herd*yr) to test the association with mastitis and organism probabilities. Overall, the probability of subclinical mastitis was greater in 2017 than 2018 (P = 0.007), with cows 1.05 times more likely in the fall (OR = 1.05; 95% CI: 0.77, 1.43), 1.87 times more likely in the spring (OR = 1.87; 95% CI: 1.27, 2.76) and 2.15 times more likely in the summer (OR = 2.15; 95% CI: 1.49, 3.10) to have subclinical mastitis. 2017 also was associated with a greater probability of coagulase-negative staphylococci (CNS), Staphylococcus hyicus and Corynebacterium spp. and lower probability of Streptococcus uberis. Only S. hyicus was associated with lower probability in the spring versus the summer regardless of the year (P = 0.03; OR = 0.431; 95% CI: 0.19, 0.94). CNS was least common in the first parity cows (P < 0.04) in contrast to Staphylococcus aureus having greater probability in the first parity cows (P < 0.09) versus greater lactations. Future research targeting factors behind greater probability of S. hyicus in the summer and contrasting probabilities of S. aureus and CNS with parity will provide greater insight to developing comprehensive mastitis control programs in organic dairy systems.

Key Words: mastitis, organic

M54 Isolated microorganisms from mammary quarters milk of buffaloes in automatic milking system. D. C. Sales², H. Tonhati², J. F. Borges³, R. D. S. Gomes¹, I. L. S. Oliveira¹, H. A. P. Lopes¹, A. H. N. Rangel⁴, and J. G. B. Galvão Jr⁵, ¹Universidade Federal do Rio Grande do Norte, Macaíba, RN, Brazil, ²Instituto Federal de Educação do Rio Grande do Norte, Ipanuca, RN, Brazil, ³Universidade Estadual Paulista, Jaboticabal, SP, Brazil, ⁴Universidade Federal Rural do Semiárido, Mossoró, RN Brazil.

The aim of present study was to determine the occurrence and etiology of subclinical mastitis in buffaloes submitted to an automatic milking system. A total of 146 quarter milk samples from buffaloes without clinical mastitis were analyzed. Milk collection was performed before starting the morning milking. A single milk sample was obtained from one mammary quarter of each animal. The aseptic collection of samples for microbiological analysis was performed directly from the teat, disinfected with alcohol 70% (wt/vol). The milk was stored in a 15 mL sterile polypropylene tube, frozen and sent to the laboratory to isolate and identify bacterial organisms. The samples were incubated at 37°C for 8 h for the pre-enrichment stage, and subsequently plated on sheep blood agar, brain heart infusion agar and Sabouraud agar with chloramphenicol. After incubation, morphological characteristics of the isolated colonies and individual innocuous were evaluated, then plated in selective and differential media to observe the characteristic phenotypic aspects of the genera. A total of 140 (96%) samples were culture positive, and among these, 91 (65%) mammary quarters were infected by individual pathogens. From these, 47% were Corynebacterium spp., 25% were Staphylococcus spp., 13% Streptococcus spp., 7% Bacillus spp., 2% Klebsiella spp., 2% E. coli, 2% yeast and 1% Micrococcus spp. Corynebacterium bovis was the predominant mastitigenic organism, followed by Staphylococcus chromogenes. Among streptococci, Streptococcus agalactiae was the predominant organism. The mixed infections were detected in 35% quarters in different combinations. The most common combination was Staphylococcus spp. and Corynebacterium spp., followed by Streptococcus spp. and Corynebacterium spp., while S. aureus, S. equorum, Pseudomonas spp., Enterococcus spp. and Aerococcus viridans were present in mixed infections with low prevalence. Buffaloes managed in intensive farms may present subclinical mastitis cases. Therefore, attention is necessary in the drying management and other mastitis prevention practices, especially when the buffaloes are submitted to an automatic milking system.

Key Words: Bubalus bubalis, infection, mastitis


Clostridium are spore forming bacteria that are ubiquitous in fecal and environmental dairy samples. Toxin-producing Clostridium species such as C. perfringens, C. septicum, and C. difficile have been associated with enteric disease in ruminants. The objective of this survey was to characterize Clostridium populations in dairies across the United States to better understand the species richness, evenness and determine if there are regional differences in Clostridium populations. From November 2015 to February 2019, 14,265 fecal samples were collected from 368 farms across 26 states. Samples were transported on ice to the lab within 24 h and clostridia were enumerated on TSC agar incubated under anaerobic conditions. Counts ranged from <1.0E1 to 1.5E8 cfu/g total clostridia with a mean of 9.2E4 cfu/g and median of 6.4E2 cfu/g. Clostridia isolates were collected from a core set of over 4,000 fecal samples to identify toxin types. Over half (52.6%) of the isolates (n = 52,322) tested from cow samples were identified as C. perfringens. Calf clostridia isolates (n = 6,219) had a lower percentage identified as toxin producing C. perfringens (38.9%) than cows. In both cows and calves C. perfringens isolates were mainly Type A (99.0%). Non-C. perfringens isolates were identified using 16S PCR. The 2 most abundant species were identified as C. bifermentans group (14.4%) and C. beijerinckii group (8.6%). These species are known for their ability to produce end products such as 1,3 propanediol, butanol and acetone. Twenty-eight species of Clostridium were detected including other toxin-producing species such as C. sordelli and C. difficile. Clostridium populations were analyzed based on region. Samples collected from California, Texas, Upper Midwest, and Wisconsin had different populations (P < 0.05) compared with Idaho, Great Lakes, Mid Atlantic, Florida, I-29 Corridor and Northeast; the Northeast trended toward having a different Clostridium population (P = 0.09). This data gives a better understanding of Clostridium species in ruminants and demonstrates regional clostridia population differences across the United States.

Key Words: Clostridium, microbiology


Bovine mastitis is one of the major diseases associated with the dairy industry, causing great economic losses. Escherichia coli is the most prevalent microorganism in clinical mastitis, causing severe symptoms and even death. In Brazil, little is known about the genetic background of E. coli isolates causing mastitis, and if these isolates can cause illness
in the human host, such as a zoonotic agent. In this study, we investigated the occurrence of several virulence factor encoding genes in *E. coli* isolates from milk of cows diagnosed with clinical mastitis. This study was approved by the UNESP’s Animal Use Ethics Committee (N 0136/2017). We used 10 different dairy herds, selected by convenience, and inclusion criteria were: mastitis control programs with data storage, average milk yield >20 kg, minimum of 200 lactating cows and use of machine milking. After clinical diagnosis, milk samples were collected aseptically. The isolation and identification were performed according to National Mastitis Council. The occurrence of genes frequently used for diarrheagenic *E. coli* (DEC) pathotypes identification (*escN, bfpA, stx1, stx2, elt, est, ipaH, aatA, aggR, aaiA and ddaE*) and involved in extra-intestinal *E. coli* (ExPEC) pathogenicity (*sfaDE, fimH, iha, ecpA, papA, papC, hlyA, cnf1, sat, vat, iroN, irp2, iucD, ireA, sitA, ibe10, traT, ompT, afacBC, and cdt*) were searched by PCR in the 93 *E. coli* isolates obtained in this study. One atypical EPEC (*escN/bfpA*) and one atypical EAEC (*aaiA/aggR*) were detected. Regarding the genes associated with ExPEC pathogenicity, we observe the presence of genes encoding for: adhesins (*fimH* = 98%; *ecpA* = 48.4%), siderophores (*irp2* = 8.6%; *sitA* = 8.6%), toxins (*vat* = 3.2%; *cdt* = 2.2%; *hlyA* = 5.4%), invasin (*ibe* = 2.2%), and protecits (*ompT* = 19.4%; *traT* = 59.1%). We concluded that the majority of the isolates cannot be classified in the distinct DEC pathotypes, but possess pathogenic potential to cause extra-intestinal disease.

**Key Words:** extra-intestinal *E. coli* (ExPEC), diarrheagenic *E. coli*, virulence


*Escherichia coli* is an environmental pathogen causing clinical mastitis, a very important disease in dairy farms due to significant economic losses. This microorganism can use different mechanisms to colonize bovine mammary gland such as invasion cells, immune system evasion and biofilm production. The aim of this study was to investigate the biofilm formation by *E. coli*, isolated from milk of cows with clinical mastitis. This study was approved by the UNESP’s Animal Use Ethics Committee (N 0136/2017). We used 10 different dairy herds, selected by convenience, and study inclusion criteria were: mastitis control programs with data storage, average milk yield >20 kg, minimum of 200 lactating cows and use of machine milking. A total of 2,613 clinical mastitis milk samples was evaluated, during the period of September 2017 to September 2018. After clinical diagnosis, milk samples were collected aseptically. The isolation and identification were performed according to National Mastitis Council. *E. coli* was identified in 115 (4.4%) milk samples. For biofilm assay, we used 96-well polystyrene microplates and the 115 isolates were incubated in brain heart infusion (BHI) +1% glucose at 37°C for 18h without shaking. Under those conditions, 51 (44.3%) were not producers. Among the 54 remaining, 54 (47%) were classified as weak producers, 9 (7.8%) as moderate and only one (0.9%) as a strong producer. According to the results, it seems that biofilm formation is not a common characteristic among these isolates and does not play a key role in the establishment of mastitis in cows.

**Key Words:** antimicrobial usage, risk factors, defined daily doses


Use of antimicrobials in agriculture is controversial and knowledge of the factors that influence antimicrobial usage (AMU) are needed. The objectives of this study are to describe herd level AMU on dairy farms and investigate associations of AMU with selected risk factors. Eligible farms contained >250 lactating cows and met criteria for maintaining computerized animal health records. Data for one-year period was retrospectively collected on 40 farms and researchers visited all farms to validate case definitions and recording accuracy. Adult cows potential risk factors included culling rate, length of dry period, and association of early lactation AMU with total AMU. Preweaned calves risk factors included feeding method (esophageal; bottle; esophageal and bottle) and preventative usage of antimicrobials (yes; no). Herd level AMU was standardized as the number of defined daily doses (DDD) per cow and preweaned calf/yr, and AMU was also calculated as DDD/cow/yr and DDD/preweaned calf/yr. Linear regression was used to identify associations between AMU and continuous risk factors and t-tests were used to assess risk factors for feeding method and preventative AMU. Enrolled farms contained 52,639 cows (mean: 1,316 ± 169 SE), and 6,281 preweaned calves (mean: 180 ± 33 SE). Total herd AMU was 6.6DDD/cow and preweaned calf/yr (2.2 to 15.7). Adult cows accounted for 83% of the total herd AMU (5.5DDD). Adult cows AMU was 7.0DDD/cow/yr (2.4 to 13.2). Preweaned calves AMU was 10.4DDD/preweaned calf/yr (0.09 to 48.9). Culling rate and dry period length were not associated with AMU in adult cows (*P > 0.22*). Greater use of AMU in the first 30 DIM was associated with greater AMU in the rest of the lactation (*P < 0.001*). Dry cow therapy and AMU within the first 30 DIM represented 18% and 39% of the AMU used for adult cows respectively. Preweaned calves AMU tended to be associated with feeding method (*P = 0.08*) and were 8.6 ± 2.5DDD (esophageal feeder), 12.8 ± 5.4DDD (bottle), and 13.7 ± 3.2DDD (both methods). There was a tendency for farms that reported use of preventive antimicrobials in preweaned calves to have greater use of AMU (16.1 ± 5.3DDD vs. 9.5 ± 2.1DDD, *P = 0.10*). AMU and risk factors varied between adult cows and preweaned calves.

**Key Words:** antimicrobial usage, risk factors, defined daily doses
M59 Associations between serum total protein and genomic predicted transmitting abilities for production, fitness, and conformation in organic Holstein calves. I. Haagen1,2, L. Han1, L. Hardie1, B. Heins2, and C. Dechow1, 1The Pennsylvania State University, University Park, PA, 2University of Minnesota, Morris, MN.

The objective of this study was to evaluate associations between serum total protein concentration and genomic predicted transmitting abilities (gPTA) for production, health and fertility in organic Holstein calves. Calves (n = 159) were born between January 2015 and May 2018 on 2 organic dairies. Serum total protein levels were recorded on farm and extracted from farm management software. Observations were restricted to the first 3 d following birth. Calves were genotyped (~150K markers) and received official genomic evaluations through the Council on Dairy Cattle Breeding and Holstein Association USA. Serum total protein was regressed on age (0, 1, 2, or 3 d) and the linear effect of a single gPTA; the gestation length (GL) regression also included a quadratic term. The mean serum total protein concentration for all calves was 6.54 g/dL.

Production (milk, fat, and protein yields) and conformation (e.g., type, stature, strength, body depth, dairy form) gPTA were not significantly associated with serum total protein. Among fitness traits, lower gPTA for somatic cell score and higher gPTA for mastitis resistance tended (P < 0.10) to be associated with higher serum total protein whereas milk fever resistance was unfavorably associated (P < 0.05) with serum total protein. Lower daughter stillbirth (DSB) was associated (P < 0.05) with higher serum total protein. Linear and quadratic effects for GL were significantly (P < 0.05) associated with serum total protein; calves with high GL had lower serum total protein with an intermediate optimum for GL gPTA of −1. These results suggest that genetics may play a role in serum total protein concentrations in organic Holstein calves. Significant effects of traits observed near birth such as GL and DSB suggest that factors such as calf size or stress from the birthing process could influence serum total protein.

Key Words: calf health, organic, serum total protein

M60 Genetic polymorphism of κ-casein on Coalho cheese yield from Zebu cows. I. L. S. Oliveira1, R. C. Madruga2, R. D. S. Gomes3, E. P. E. Silva1, E. G. S. O. Silva1, J. S. Bezerra1, M. F. Bezerra1, D. C. Sales4, L. H. F. Borba1, J. G. B. Galvão Jr.5, and A. H. N. Rangel*1, 1Universidade Federal do Rio Grande do Norte, Macaíba, RN, Brazil, 2Associação Brasileira dos Criadores de Zebu, Parnamirim, RN, Brazil, 3Universidade Federal Rural de Pernambuco, Recife, PE, Brazil, 4Universidade do Estado de São Paulo, Jaboticabal, SP, Brazil, 5Instituto Federal de Educação do Rio Grande do Norte, Ipanguaçu, RN, Brazil.

The objective of this study was to evaluate the influence of the genetic polymorphism of κ-casein on the yield of Coalho cheese made from Zebu cow milk. The raw milk for producing the Coalho cheese came from Guzerá (n = 3), Gir (n = 7) and Sindi (n = 10) breeds. Genomic DNA was extracted from the capillary bulb of the 20 animals and submitted to PCR (polymerase chain reaction) analysis to determine the genetic polymorphism of κ-casein. The amplified products were run in capillary electrophoresis to verify the quality and concentration of the fragments. Band reading was performed using GeneMapper Software. Finally, allele and genotype frequencies were obtained for the 3 evaluated breeds. The data were submitted to ANOVA using SAS software (version 9.0) and the differences between the means were tested by the Tukey test at 5% significance. The Sindi breed obtained higher (P < 0.05) performance for the Coalho cheese yield (93.66 g of total solids per liter, TS/L) in relation to the Guzerá (82.25 g TS/L) and Gir (83.33 g TS/L) breeds. The allele frequencies found for the κ-casein genetic polymorphism were 0.75 (allele A) and 0.25 (allele B) for the Sindi breed; 1.0 (allele A) and 0.0 (allele B) for the Gir breed; and 0.83 (allele A) and 0.17 (allele B) for the Guzerá breed. The obtained genotypes for the genotype frequency were AA and AB, being AA (0.50) and AB (0.50) for the Sindi breed; AA (0.66) and AB (0.33) for Guzerá; and AA (1.0) and AB (0.0) for Gir. Thus, the Sindi breed obtained the highest frequency of the B allele (25%) when compared with the other breeds, enabling a higher percentage of total solids in the cheese, and therefore implying in a positively higher yield (g TS/L) of Coalho cheese for the breed. Therefore, the higher frequency of the κ-casein B allele has a positive influence on Coalho cheese yield.

Key Words: Zebu cattle, genotype frequencies, capillary electrophoresis.


The sex of the offspring can be predetermined by flow cytometric sorting of highly purified X- or Y-chromosome-bearing sperm subpopulations. The technology has undergone several improvements in the past 10 years and it is now widely used by dairy and beef cattle, small ruminants, deer, equine and porcine industries. To ensure the quality of the sorted product, Sexing Technologies analyzes various sperm parameters at multiple checkpoints during the sex sorting process. One of these quality parameters is sperm sex purity, which is assessed using a Genesis-I high resolution Analyzer, a modified MoFlo SX sperm sorter, developed for sperm analysis using ST proprietary technologies. Each Analyzer is calibrated using certified sperm nuclei as a standard, and has to meet predetermined specifications of number of oriented cells for the Side Fluorescence detector path and quality of resolution on DNA content on the Forward Fluorescence detector path before running any purity analysis. This confirms the instrument is well aligned and meeting the required orientation and resolution criteria to perform accurate assessment of sex sorted sperm purity. High performance sperm sorting systems known as Genesis-III have advanced digital processing, multiple heads and automation built in. These systems are verified multiple times a day to confirm that the setup is accurate for the desired sex ratio, while optimizing and maintaining productivity. Estimation of the percent of X- and Y-chromosome-bearing sperm is also performed on each batch of sex-sorted sperm produced. Typically, 3,000–5,000 cells are analyzed per sample depending on the target purity. The implementation of these technological advancements provides high confidence in the quality assessment of Sexing Technologies’ sex-sorted products, which reflects in the most recent field results. Field data collected in the years of 2016–2018 confirmed that an in vitro analyzed X-chromosome-bearing sperm purity of 91.1 ± 0.18% resulted in an in vivo female calf percent of 90.3 ± 0.18%, based on 9,447 calvings.

Key Words: sex sorting, sperm quality, sex purity
Cows have been genotyped and used to increase the size of the training population in multiple-step genomic evaluations of dairy cattle with limited number of proven bulls. However, some of the genotyped cows are daughters of progeny tested bulls, which have their estimated breeding values (EBVs) predicted based on the parent average (PA) and the phenotypic information from their daughters, which would cause double count of information. The objective of this study was to investigate the impact of accounting for PA and genotyped daughters’ average (GDA; i.e., the contribution of genotyped daughters to the bull’s EBV) in the estimation of deregressed EBVs (dEBVs) used as pseudo-phenotypes in genomic evaluations. In addition, an alternative deregression method was proposed (NEW). A simulated dairy cattle data set was used to compare 8 scenarios defined based on the number of bulls, genotyped bull’s daughters, and genotyped cows not sired by the genotyped bulls. For all these scenarios, Genomic EBVs (GEBVs) were predicted using dEBVs estimated based on 4 methods: VR, that includes PA and GDA; VRpa, that excludes PA; and JA and NEW, which exclude PA and GDA from the dEBVs using either all information available in the complete pedigree or only information from parents and genotyped daughters, respectively. The dEBVs estimated by the VR and NEW showed the lowest (0.24 to 0.36) and highest (0.33 to 0.50) validation reliabilities across scenarios, respectively. The VRpa and NEW methods produced the least biased GEBVs (inflation/deflation) and showed the most consistent bias estimates (regression coefficient) across scenarios (1.08 to 1.17). Among all methods, the JA method displayed the largest variability in bias (1.00 to 1.75) across scenarios. Therefore, it was shown that removing PA and GDA information from dEBVs can increase the reliability of genomic predictions for populations with limited number of proven bulls. In addition, the proposed NEW deregression method addresses the double counting of information and it is a feasible alternative to generate dEBVs used in multiple-step genomic evaluations.

**Key Words:** double-counting, genomic BLUP (GBLUP), training population

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**M64** Approximate generalized least squares method for large-scale genome-wide association study. L. Ma\(^1\), J. Jiang\(^2\), D. Prakapenka\(^3\), J. Cole\(^4\), and Y. Da*\(^5\), \(^1\)University of Maryland, College Park, MD, \(^2\)University of Minnesota, Saint Paul, MN, \(^3\)USDA/ARS, Beltsville, MD.

The use of genomic relationships among individuals is an effective approach for population stratification correction in the analysis of genome-wide association study (GWAS), but the matrix inversion required for the statistical testing of SNP effects limits the sample size that can be analyzed by GWAS methods using relationship matrices. We propose an approximate generalized least squares (AGLS) method for GWAS using large samples. The AGLS utilizes the mixed model result that the least squares (LS) solution to fixed SNP effects is the GLS solution or best linear unbiased estimation if the best linear unbiased prediction of polygenic effects is removed from the phenotypic observations. Since the LS method is computationally efficient, no sample size limitation for this method is expected for the foreseeable future even though dairy genomic and phenotypic data are growing at a fast pace. Combined with a previous method and computing tool for epistasis testing, the AGLS method offers capability for testing and estimating additive, dominance and epistasis effects as well as estimating allelic and genotypic effects in large-scale GWAS. AGLS was compared with BOLT-LMM that is capable of large-scale GWAS for testing additive effects. The results showed that AGLS and BOLT-LMM identified the same significant additive effects with only minor differences in a sample of 294,079 cows. For the same sample analyzed by AGLS and BOLT-LMM, the GWAS without polygenic correction lacked sensitivity, i.e., different chromosomes and different SNP within each chromosome had similar effects, except for SNP in and around the *DGAT1* gene on chromosome 14. These results showed that polygenic correction is necessary for large-scale GWAS and that AGLS is an efficient and versatile method for large-scale GWAS analysis, especially in dairy cattle where the polygenic animal effect is routinely estimated.

**Key Words:** genome-wide association study (GWAS), SNP, generalized least squares
haplotype analysis for genomic prediction and estimation, we developed a computing pipeline to implement haplotype analysis. The pipeline includes 3 components, preparation of input data for haplotype analysis, genomic prediction and estimation using GVCHAP, and analysis of GVCHAP results. The input preparation starts with formatting SNP data for 2 imputing programs. A utility program with options to define haplotype blocks by a fixed number of SNPs or a fixed distance in base pairs per block then divides the haplotypes from either imputing program into haplotype blocks where each block is treated as a multi-allelic locus and is formatted as haplotype genotypes where each haplotype genotype contains 2 haplotypes. The haplotype genotypes are used as an input file for running GVCHAP. Another utility program fills in most of the parameter file required by GVCHAP as an input file. The data preparation step also contains utility programs for defining validation samples by random assignment of individuals to each validation sample or by a user-provided list of individuals for assigning to validation samples. GVCHAP is the main program for genomic prediction and estimation providing GREML estimates and GBLUP for additive and dominance effects of haplotypes and single SNPs. To reduce the computing time in cross validations due to calculation of genomic relationships, GVCHAP has a 2-step strategy to save the genomic relationship matrix during the first fold of validation and read in the genomic relationships for the remaining folds of validations. This 2-step strategy is helpful for k-fold validations and for multiple traits. The last component of the computing pipeline calculates observed prediction accuracies and produce input file for graphical analysis of haplotype and SNPheritabilities.

Key Words: genomic selection, haplotype, SNP

M66 Changes of genomic predictions with the algorithm of proven and young (APY) using different core animals in dairy cattle. S. Tsutara1, D. A. L. Lourenco1, Y. Masuda1, I. Misztal1, and T. J. Lawlor2, 1University of Georgia, Athens, GA, 2Holstein Association USA, Brattleboro, VT.

The algorithm of proven and young (APY) can be used to predict genomic (G)EBV with a single-step genomic BLUP when the number of genotyped animals is large. Since the APY approach uses an approximation of the inverse of the genomic relationship matrix, GEBV could change when different genotyped animals are chosen as core animals. The objective of this study was to investigate what creates substantial changes in GEBV with APY when different core animals are used. Two data sets were simulated: 1) 60K genotyped animals, 70K phenotypes, 100K animals in pedigree and 2) 120K genotyped animals, 140K phenotypes, 200K animals in pedigree. In both cases, phenotypes in 12 generations and genotypes in the last 6 generations were simulated, assuming the heritability 0.5 and the additive genetic standard deviation (SD(a)) 7.1. Changes in GEBV using different core animals were compared. In addition, for udder depth (1 of 18 linear type traits), 570K genotyped animals in addition to 9M phenotypes and 10M animals in pedigree were used to investigate changes in GEBV by different core animals. Maximum Difference was selected as a potential measure of model performance among individual elite animals. Intentionally excluding close family members from the core animals needs to be taken into consideration. In the simulation study, GEBV changed the most when the status of genotyped animals moved between core and non-core when using the smallest core size (10K in this simulation). Maximum differences in GEBV were smaller for 60K genotyped animals than those for 120K genotyped animals. Reliabilities (squared correlations between GEBV and true breeding values) were similar in any core sizes but lower than that for GEBV calculated without APY. The results from udder depth were in agreement with those from simulation, and GEBV changes decreased from 73% to 16% of SD(a) for non-core genotyped animals by increasing the core size from 10K to 60K. The extremely large change in GEBV was not found. Reliabilities were similar for any core size. For simulation and udder depth, GEBV changes asymptotically decreased by increasing the core size.

Key Words: single-step genomic BLUP, linear type traits, US Holsteins


Cholesterol deficiency (CD) in Holstein cattle results from an insertion in the APOB gene which traces to the bull Maughlin Storm. Calves that are homozygous for CD cannot transport cholesterol and perish before 6 mo of age. The mutation may have a partially dominant effect with lowered cholesterol in heterozygous animals. Our aims were to evaluate plasma cholesterol in heterozygous animals and to determine if CD carrier status is associated with show ring success. Blood was collected from 26 genotyped Holsteins in the Penn State dairy herd and included 12 CD carriers and 14 non-carriers. Plasma samples were analyzed for cholesterol concentration (mg/dL) using mixed models that included linear and quadratic effects of DIM. Additionally, CD carrier status of cows placing in the top 5 and top 10 in cow classes at World Dairy Expo that were sired by known CD carriers was compiled. From 2006 to 2018 there were 199 instances of 119 cows with genotypic data available and sired by a CD carrier sire placing in the top 10; of those, 114 were CD carriers and 85 were non-carriers. There were 117 instances from 75 cows for top 5 placings. Chi-squared tests for equal proportions were evaluated to determine if the following were associated with CD status: placing in the top 10 with cows allowed to contribute multiple years, placing in the top 5 with cows allowed to contribute multiple years, top 10 cows only considering individuals once, and top 5 cows only considering individuals once. There was a trend (P = 0.09) for non-carriers to have higher plasma cholesterol (155 mg/dL) than CD carriers (137 mg/dL). Further, there were significantly (P < 0.05) more CD carriers in the top 5 and 10 of classes at World Dairy Expo when cows were considered over multiple years; there was a trend (P = 0.08) for placement in the top 5 cows when individuals were only considered once. This data indicates that plasma cholesterol levels may be reduced in CD carriers but should be validated with a larger sample size. Moreover, reasons for an apparent advantage of CD carriers over non-carrier siblings at an elite cattle show warrants further investigation.

Key Words: cholesterol deficiency, show, cholesterol

M68 The lactation curve of the Italian river buffalo. M. Fioletti1, A. Cesariani2, R. Negri1, and N. Macciotta*2, 1Associazione Italiana Allevatori, Rome, Italy, 2Università di Sassari, Dipartimento di Agraria, Sassari, Italy.

The river buffalo (Bubalus bubalis bubalis) is currently farmed in Italy for milk production. The dairy buffalo industry is currently increasing due to the high market value of the most famous product, the mozzarella cheese. A breeding program based on AI and progeny test exist even if its impact is constrained by technical and logistic problems. Aim of this work is the study of the lactation curve of the Italian river buffalo during a 3-year period to look for possible modification and to analyze its modifications during the last 30 years. Data were test day records of 221,350 lactations of 124,015 buffalo cows, born in the period 1980–2011 recorded by the Association of Buffalo breeders (ANASB).
Lactations with at least 7 records between 6 and 300 d in milk were considered. Individual curves were fitted with 4 mathematical functions: the Wood incomplete gamma function (WD), the Wilmink function (WIL), a fourth-order Legendre orthogonal polynomials (LEG4), and a quadratic spline with one knot (QSPL). As expected, individual variability resulted in a huge variation of goodness of fit: only 25% of lactation showed an adjusted R² for the LEG4 larger than 0.83. According to the sign of the b parameter of WD, curves were classified as standard (88%) or atypical (12%); that is, without the lactation peak. Looking at the main parameters of the lactation curve shape, they exhibited a small variation during the considered period, even though with a quite irregular trend. The peak production showed an initial increase, from about kg 4.9 in 1980 to 5.11 in 1995, followed by a slow decrease that led to a value of 4.98 in 2011. The time at peak occurrence and the persistency of lactation did not show relevant variability in the 1980–2011 decades.

**Key Words:** lactation curve, river buffalo, milk yield

**M69**  
**Haplotype-based methods to select animals to sequence for later accurate imputation.** A. M. Butty¹, M. Sargolzaei¹², F. Miglior¹, P. Stothard³, F. S. Schenkel¹, B. Gredler-Grandl⁴⁵, and C. F. Baes*¹⁶, ¹University of Guelph, Guelph, ON, Canada, ²Select Sires Inc., Plain City, OH, ³University of Alberta, Edmonton, AB, Canada, ⁴Qualitas AG, Zug, Switzerland, ⁵Wageningen University, Wageningen, the Netherlands, ⁶University of Bern, Bern, Switzerland.

The availability of array genotypes in dairy cattle has increased steadily in the last decade, and imputation to whole-genome sequence (WGS) has been widely studied. Although variants with minor allele frequency (MAF) below 0.05 usually represent more than half of all WGS variants identified, they are commonly excluded in most studies. Imputation of such variants is often inaccurate, impeding the use of such rare variants in further analyses. Furthermore, reference population selection also has a large impact on the accuracy of imputation. In this study, we present 2 novel methods of selection that rely on haplotype information and evaluate them in comparison with 2 previously described methods. The Genetic Diversity Index method optimizes the number of unique haplotype alleles present in the future selected group of animals, whereas the Highly Segregating Haplotype method aims to capture the most haplotype alleles possible, starting with alleles of high frequency in the population. We first simulated whole-genome sequence data of a dairy cattle population, mimicking the MAF distribution and the linkage disequilibrium pattern found in the North-American Holstein population. Reference populations of 50 to 1,200 animals were created using the 4 different selection methods. Finally, a group of target animals with simulated high-density genotypes was imputed. Accuracy of imputation was measured and compared for allelic r² between true and imputed genotypes for variants of different MAF. Imputation accuracy for common variants was between 0.85 and 0.99, whereas imputation accuracy of rare variants varied between 0.40 and 0.91. In general, methods based on selecting animals for their genetic diversity led to better imputation accuracy of variants with a MAF below 0.05. Methods targeting animals carrying common haplotype alleles led to higher imputation accuracies of variants with higher MAF. Therefore, the intended use of the imputed WGS must be accounted for at the time of selecting the animals comprising the future reference population.

**Key Words:** sequencing, imputation, haplotype