Kansas Agricultural Experiment Station Research Reports

Volume 5
Issue 9 *Dairy Research*

Article 11

2019

Dairy Research 2019

B. J. Bradford
Kansas State University, Manhattan, bbradfor@k-state.edu

Dairy Research 2019

Abstract

Kansas State University is pleased to present the 2019 Dairy Research Report of Progress and proud to serve the growing Kansas dairy industry.

Keywords

Dairy cattle

Cover Page Footnote

Appreciation is expressed to the following organizations for their support of dairy teaching, research, and extension at Kansas State University during 2018-2019.

DAIRY TEAM
KANSAS STATE UNIVERSITY

Dairy Research 2019

K-STATE
Research and Extension

Kansas State University Agricultural Experiment Station and Cooperative Extension Service

In November, the KSU Dairy Teaching and Research Center said goodbye to Sweety Pie 4757, shown on the front cover. A prolific and resilient producer, she gave 173,637 lb of milk over her lifetime, about 3.5 semi loads. She averaged 119 lb per day since she first calved at 24 months of age. She will be missed!

DAIRY RESEARCH 2019

Contents

Foreword

Kansas State University is pleased to present the 2019 Dairy Research Report of Progress and proud to serve the growing Kansas dairy industry. Our state maintains strong dairy growth, posting a 6% increase in total milk production in 2018, behind only Colorado and Texas for the greatest growth rate in the nation. At the end of 2018, 159,000 Kansas cows averaged 23,321 lb per lactation, ranking the state 16th in total milk production (3.7 billion lb). Kansas now has 280 dairy operations and averages 568 cows per herd, with 7 processing plants handling much of that volume.[1]

Selected production traits of our Kansas State University Dairy Teaching and Research Center (DTRC) herd are shown below. The excellent functioning of our herd is largely a tribute to the dedication of our staff: Michael Scheffel (manager), Robert Feist, Kris Frey, Ben Sims, Eulises Corrales, Alexandrea Eckert, Cory Sunderman, Tony Hecht, and Tyler Ohlde. Special thanks to the many graduate and undergraduate students for their technical assistance in our laboratories and at the DTRC. We also acknowledge the support and cooperation of the Heart of America Dairy Herd Improvement Association (DHIA) for its assistance in handling research milk samples.

Kansas State University Dairy Teaching and Research Center Herd[1]

Cows, total no.	271
Rolling herd milk, lb	31,538
Rolling herd fat, lb	1,062
Rolling herd protein, lb	916
Somatic cell count × 1,000	100
Calving interval, mo.	12.7

[1]November 7, 2019, test day (milking 2 to 3 times daily).

The sustained increases in productivity and efficiency on dairy farms in Kansas and across the U.S. are largely driven by improved technology and management decisions by dairy producers. It is our hope that the type of research presented in this report contributes to those improvements and helps to enhance the quality of dairy products to increase consumption.

Thorough, quality research is not only time-intensive and meticulous, but also expensive. Nevertheless, studies have demonstrated that each dollar spent for research yields a 30 to 50% return in practical application. Those interested in supporting dairy research are encouraged to consider participation in the Livestock and Meat Industry Council (LMIC), a philanthropic organization dedicated to furthering academic and research pursuits by the Department of Animal Sciences and Industry. Additional details about the LMIC are found at the end of this report.

B.J. Bradford, Editor
2019 Dairy Research Report of Progress

[1] *Progressive Dairyman*, https://www.progressivepublish.com/downloads/2019/general/2018-pd-stats-highres.pdf.

Does Time Interval from Thawing Multiple Straws of Semen to Insemination Affect Pregnancy Outcome of Lactating Dairy Cows?

J. Fehn and L.G.D. Mendonça

Summary

Technicians must follow guidelines when inseminating cows to avoid impacting the quality of semen and pregnancy per AI (P/AI). The interval from initiation of the thawing process of multiple semen straws until AI is an important aspect to be considered when evaluating the performance of AI technicians. Time interval from thawing to AI can be affected by the skill level of the AI technician, distance from the thawing unit to location of insemination, and labor efficiency. Because modern dairy farms are becoming larger, it is important to evaluate if the interval from thawing to AI is impacting P/AI in large herds. The objective of this study was to investigate whether time interval from handling of semen to AI affects pregnancy outcome of lactating dairy cows housed in a modern dairy farm. Most of the AI occurred within 10 minutes after initiation of the thawing process. Pregnancy outcome was not affected by sequence of AI or time interval from thawing until AI. Technicians responsible to inseminate cows in large dairy farms should strive to deposit semen in the reproductive tract within 10 minutes after starting the thawing process. If the AI technician thaws multiple straws of semen simultaneously, it is important to consider the efficiency of the technician to deposit semen after thawing, and temperature of the thawing unit.

Introduction

Reproductive programs in most U.S. dairy farms rely on artificial insemination (AI). Certain guidelines must be followed by AI technicians in order to avoid issues with sperm viability, which influences fertilization of the oocyte, and consequently, pregnancy per AI (P/AI). To ensure that fertilization capacity of the spermatozoa is optimized, frequent training sessions must be conducted with AI technicians to reinforce the importance of proper AI technique and handling of semen.

The following critical steps should be considered when evaluating procedures performed by AI technicians: handling of frozen and thawed semen straws, temperature of the thawing bath, AI gun preparation, transportation of the loaded AI gun, overall cleanliness of the equipment, and location of semen deposition in the reproductive tract. Specific recommendations for these procedures are normally used to instruct technicians. Another important aspect to be considered is the time interval from thawing multiple straws to semen deposition, which is affected by the skill level of the AI technician and distance from AI gun preparation to insemination location. In modern dairy farms, several cows are inseminated daily and some facilities require technicians to walk a considerable number of steps from AI gun preparation to insemination location. Therefore, technicians usually prepare several straws of semen at once to optimize their work, which creates another nuance when evaluating the time interval from thawing to AI.

The objective of this study was to evaluate whether time interval from handling multiple straws of semen to AI affects pregnancy outcome of lactating dairy cows housed in a modern dairy farm.

Experimental Procedures

This study was conducted during summer months in a dairy farm milking 5,100 cows in Wisconsin. Cows were housed in pens of 250 to 300 cows in a cross-ventilated free-stall barn. Estrus

detection was performed in 11 pens once daily in the morning based on tail chalk removal. The protocol of the farm consisted of one technician identifying cows in estrus in one or two pens, then preparing the AI guns outside the pens before inseminating the cows. Thawed straws were loaded in AI guns and transported in a portable AI gun warmer to maintain the temperature at 95°F. The technician was encouraged to limit the number of straws thawed simultaneously to 5 when preparing AI guns. Because in several instances more than 5 cows were in estrus in one pen, the technician had to repeat the steps described previously more than once to inseminate all cows in estrus. After inseminating all cows from one or two pens, the technician followed the same procedures in the subsequent pens.

During the study a chronometer was used once weekly for a total of 11 weeks to determine the time interval from initiation of the thawing process of multiple straws of semen until inseminations (n = 474). Initiation of the thawing process started when the first straw of semen was placed in the thawing unit. Interval from initiation of thawing until insemination was recorded for each individual cow. The thawing unit automatically kept the water temperature between 95 and 99°F. Straws of semen were kept in the thawing unit for 45 seconds after the last straw was placed in the unit.

Three AI technicians performed inseminations during the study period and a total of 18 different sires were used. In one instance, 2 technicians worked on the same day, but did not inseminate cows in the same pens. Cows were inseminated with either conventional or sex-sorted semen, which comprised 94.5% and 5.5% of the inseminations during the study period, respectively. Technicians were trained to deposit semen in the body of the uterus, immediately after passing the cervix. Pregnancy diagnosis was conducted every other week in cows with unknown pregnancy status and more than 42 days after AI. Data regarding parity (primiparous and multiparous), number of previous inseminations, days in milk at AI, and sire code were extracted from the on-farm management software. Because of the limited instances that more than 5 straws were prepared at one time, sequence of inseminations greater than 5 were grouped in one category to conduct one of the statistical analyses. In addition, services were separated into quartiles by interval from initiation of thawing to AI (quartile 1 = 2:05 to 3:38 minutes; quartile 2 = 3:39 to 4:42 minutes; quartile 3 = 4:43 to 6:01 minutes; quartile 4 = 6:02 to 11:28 minutes). Inseminations were classified as first-AI or $\geq$ second-AI. A total of 27 cows were not included in the P/AI analysis because of removal from the herd before pregnancy diagnosis, or cow-related problems on the day of AI (e.g., lameness). Pregnancy outcomes were analyzed by logistic regression using the GLIMMIX procedure in SAS v. 9.4 (SAS Inst., Cary, NC). Models used for statistical analysis included the following variables: parity, number of services, AI technician, and sequence of AI or quartiles of interval from thawing to AI. Interactions between variables and sequence of AI, or variables and quartiles of interval from thawing to AI were tested.

Results and Discussion

Time interval from initiation of thawing of multiple straws to sequence of AI is depicted in Figure 1. Technicians of this farm were encouraged to limit the number of straws thawed at one time to 5, however, there were instances that 6 to 9 straws were placed in the thawing unit. Despite the increased number of straws thawed at once, most of the inseminations occurred within 10 minutes after initiation of the thawing process. A common recommendation given to technicians is to inseminate cows within 10 minutes after thawing the straws. Although technicians of this farm were able to deposit semen in the uterus within the recommended time when greater than 5 AI guns were prepared at one time, thawing several straws simultaneously is expected to reduce the temperature of the thawing unit and may negatively impact the thawing process.

In the analyses that investigated P/AI by sequence of insemination (Figure 2), P/AI was not affected by sequence of insemination ($P = 0.30$), parity ($P = 0.90$), number of services ($P = 0.11$),

technician ($P = 0.69$), or interactions ($P > 0.28$). In the analyses that quartiles defined the interval from thawing to AI, no differences were detected ($P = 0.49$) in P/AI among quartiles (Figure 3). In addition, parity ($P = 0.98$), number of services ($P = 0.13$), technician ($P = 0.77$), and interactions ($P > 0.36$) were not associated with P/AI. The observed findings suggest that interval from initiation of thawing to AI or sequence of AI were not associated with P/AI in this specific farm when inseminations occurred within 12 minutes after starting to thaw the straws. Although P/AI was not affected by the time interval from thawing to AI, we cannot exclude the possibility that thawing multiple straws of semen at the same time can impact temperature of the thawing unit and the thawing process. Straws of semen are usually commercialized in units containing 0.5 mL or 0.25 mL of semen. Because of the increased physical size of 0.5 mL units compared with 0.25 mL units, it is possible that thawing multiple 0.5 mL straws simultaneously causes a greater reduction in the temperature of the thawing unit than thawing multiple 0.25 mL units. Therefore, if several straws of semen will be thawed simultaneously, technicians are encouraged to use 2 thawing units.

Because of the small number of services with sex-sorted semen used during the study period, it was not possible to investigate whether sex-sorted semen was more vulnerable to effects of the time interval from handling of semen to AI than conventional semen. The process to sort semen is expected to have a detrimental effect on the spermatozoa, which results in sex-sorted semen to have reduced P/AI compared with conventional semen. In addition, dose of sex-sorted semen is usually decreased compared with conventional semen. These factors may interfere with how the interval of time from handling of thawed semen until AI affect pregnancy outcomes. Another important aspect not explored in this study was the effect of sire. Several sires were used during the study period and it was unreasonable to determine whether P/AI was affected by time interval from handling of semen until AI for specific sires.

Findings from this study augment evidence that pregnancy outcomes are not impacted if AI is conducted within 10 minutes after starting the thawing process of multiple semen straws. It is important to consider that this study was conducted during summer and cows were housed in a cross-ventilated barn. Therefore, ambient conditions were controlled, which minimized the impact of weather on semen quality while being handled and transported from the thawing unit to the cow. In scenarios in which multiple straws will be thawed simultaneously, skill level of the AI technician, distance to transport the AI gun, and temperature of the thawing unit when including several straws at once should be assessed to ensure P/AI will not be affected.

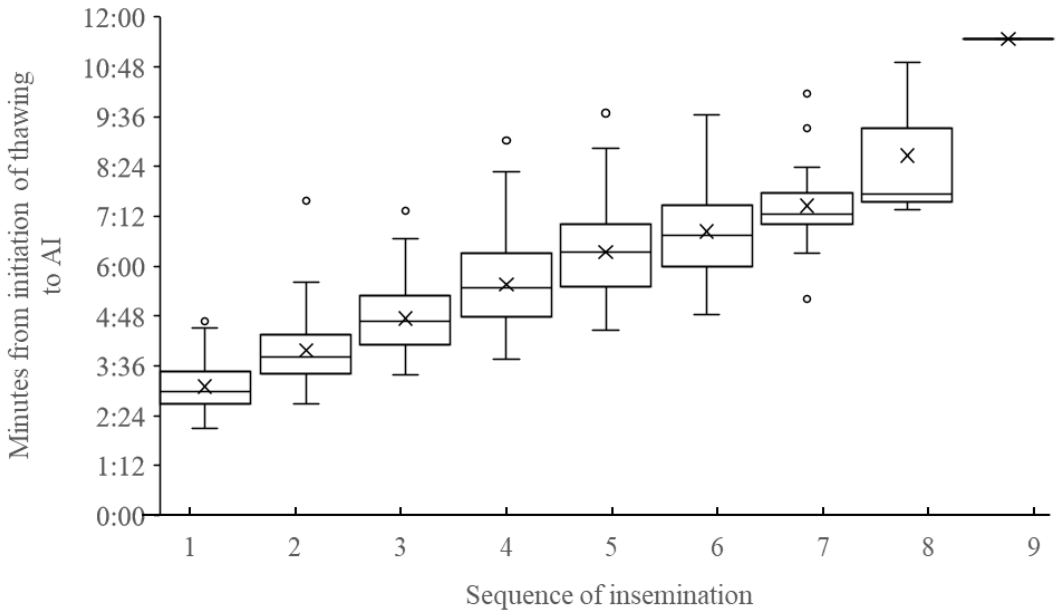

Figure 1. Minutes from initiation of thawing multiple semen straws simultaneously until AI according to sequence of insemination. Top and bottom of the boxplots represent first and third quartiles, respectively. Line through the middle of the boxplot, '×' in the box, and whiskers represent the median value, average, and outliers, respectively.

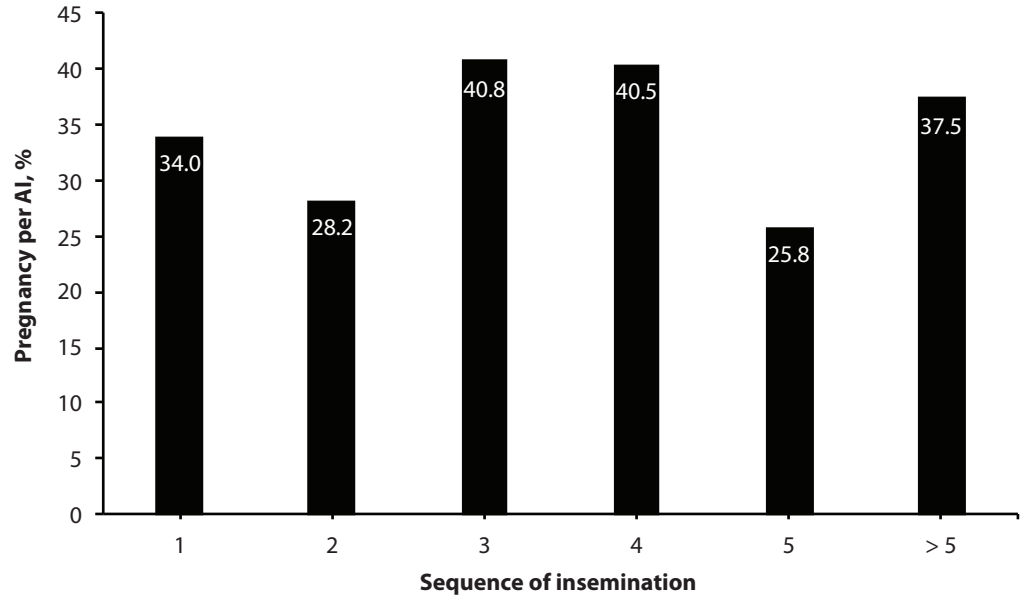

Figure 2. Pregnancy per AI according to sequence of insemination of straws of semen thawed simultaneously. Number of services for each sequence of insemination ranged from 56 to 94. Sequence of AI ($P = 0.30$), parity ($P = 0.90$), number of services ($P = 0.11$), AI technician ($P = 0.69$), and interactions between sequence of AI and forementioned variables ($P > 0.28$).

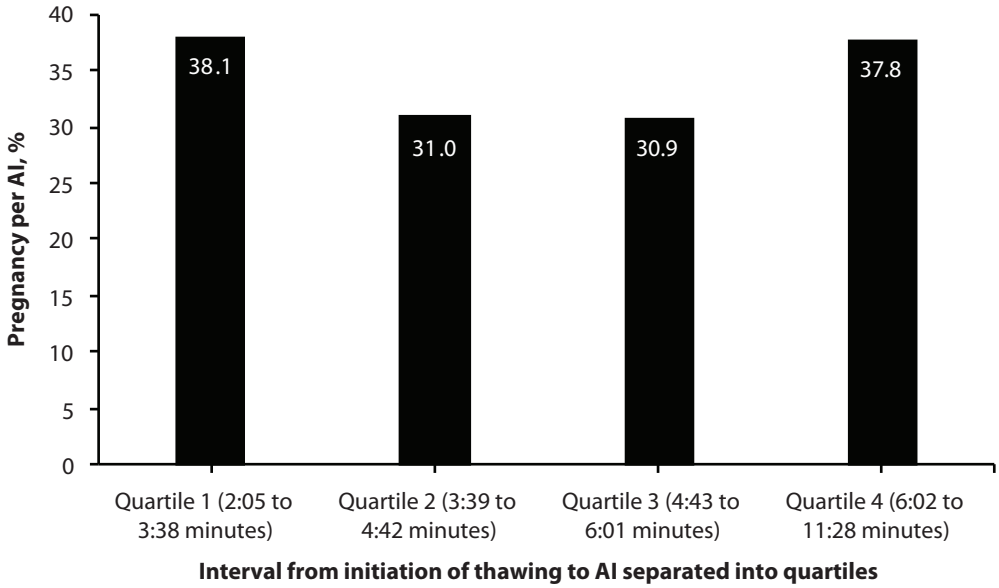

Figure 3. Pregnancy per AI according to interval from initiation of thawing until AI, separated into quartiles from multiple straws of semen thawed simultaneously. Number of services in quartile 1, quartile 2, quartile 3, and quartile 4 are 113, 113, 110, and 111, respectively. Quartiles ($P = 0.49$), parity ($P = 0.98$), number of services ($P = 0.13$), AI technician ($P = 0.77$), and interactions between quartiles and forementioned variables ($P > 0.36$).

Disease Prevalence and Its Consequences on Blood Metabolites, Physical Activities, Milk Yield, and Fertility

J.S. Stevenson and S. Banuelos

Summary

Health status of 160 lactating cows was monitored by assessing blood metabolites on days 0, 3, 7, and 14 after calving, in addition measures of physical activity during 20 days surrounding parturition. Cows with clinical disease (any with diagnosis of ketosis, metritis, mastitis, respiratory disease, or milk fever during the first 60 days in milk) were compared with outcomes in healthy cows. Expected differences were observed between health status groups for serum concentrations of free fatty acids, beta-hydroxybutyrate, haptoglobin, and calcium, but not for plasma glucose. Daily postpartum rumination and eating times were decreased in diseased cows and they spent more time resting or being inactive. Body condition scores decreased more in diseased cows, whereas body weight and milk yield were unaffected by health status. Despite early and proportionally more ovulations during the prebreeding period in healthy cows, pregnancy rate at first service and days to conception were not affected by health status, likely because of good health care of all cows having both clinical and subclinical disease.

Introduction

Approximately 50% of the dairy cows in the U.S. suffer from at least one disease event during the first 60 days in milk. Transition from pregnancy (no lactation) to lactation (not pregnant) presents the greatest risk for culling and death for a dairy cow. During this transition period, a number of metabolic and endocrine adaptations must occur to keep cows healthy.

Calving-related disorders and diseases that affect the reproductive tract are major contributors to poor fertility. In 2013, the most common clinical diseases in cows reported by dairy producers were mastitis (24.8%), any degree of lameness (16.8%), infertility (8.2%), and metritis (6.9%). Cows that have one of the aforementioned disorders were 50 to 63% less likely to resume estrous cycles by the end of the voluntary waiting period, and were 25 to 38% less likely to become pregnant after the first AI-breeding compared with healthy cows.

Relationships of health and metabolic markers, in addition to measures of resting, eating, rumination, and activity derived from CowSensor ear tags, have not been examined to determine their relationships to subsequent ovulation, estrus, pregnancy, and health status. Our objective was to characterize: (1) various metabolic (free fatty acids [FFA], beta-hydroxybutyrate [BHB], glucose, haptoglobin, and calcium) and (2) physical (body condition score, body weight, eating, rumination, and activity times) traits that affect milk yield and reproductive performance of healthy and clinically diseased lactating cows.

Experimental Procedures

Close-up nulliparous and dry Holstein cows (n = 160; Kansas State University Dairy Teaching and Research Center, Manhattan, KS) of mixed parity were enrolled between December 2017 and August 2018. Close-up cows enrolled in the study were housed in an open-front, straw-bedded maternity barn until parturition. Cows were housed in open lot free-stall barns bedded with sand after calving, and milked thrice daily. In addition to the proposed data collection, cows were monitored daily by herd personnel for body temperature and urine ketones during the first 10 days after calving to identify health disorders such as dystocia, retained placenta, mastitis, displaced abomasum, milk fever, and lameness. Any cow for which a clinical diagnosis

of ketosis, metritis, mastitis, respiratory disease, or milk fever was made during the first 60 days in milk was classified as diseased, whereas the remaining cows were classified as healthy, even though they may have manifested some other clinical or subclinical disease.

Metabolic Measures

Blood samples were collected on days 0, 3, 7, and 14 to assess serum concentrations of FFA (proxy for negative energy balance), BHB (proxy for ketosis), calcium (marker for hypocalcemia), and haptoglobin (proxy for general inflammation), and plasma concentrations of glucose (proxy for energy status; Figure 1). Body condition scores and body weights were assessed weekly beginning at calving until 73 ± 3 days in milk.

Definitions and Assessment of Disease

- Retained placenta: failure to expel fetal membranes by 24 hours after calving.
- Metritis: evidence of brown watery exudate detected by palpation per rectum at 0, 4, 7, 10, and 14 ± 3 days in milk (day 0 = day of calving).
- Ketosis: concentration of BHB in plasma greater than 10 mg/dL on day 0, 3, 7, or 14 after calving.
- Dystocia: any cow with twins or a calving difficulty score of 3 or greater. Calving difficulty scores: 1 = no problem; 2 = slight problem; 3 = use of obstetrical chains; 4 = use of a calf jack.
- Displaced abomasum: diagnosis was made on history and clinical signs in combination with auscultation findings (distinct ping identified by using a stethoscope).
- Subclinical mastitis: somatic cell count exceeding 310,000 cells/mL during the first 60 days in milk.
- Clinical mastitis: any evidence of abnormal milk observed by milkers.
- Milk fever: downer cow with blood calcium concentrations < 7.5 mg/dL.

Reproductive Traits

Blood samples also were collected weekly beginning 21 ± 3 until 73 ± 3 days in milk to determine the onset of luteal function by assessing progesterone concentration. Estrual events were recorded by CowSensor ear tags (Agis CowManager, the Netherlands) by using accelerometer technology. Beginning at 63 ± 3 days in milk, cows were enrolled in a modified Ovsynch ovulation synchronization program (GnRH — 7 days — prostaglandin F_{2a} — 24 hours — prostaglandin F_{2a} — 32 hours — GnRH — 16 hours — AI) to facilitate first postpartum AI.

Results and Discussion

Clinical Disease Prevalence

Occurrences of clinical and subclinical disease are summarized in Table 1. By definition in our study, diseased cows were those diagnosed with ketosis, metritis, mastitis, respiratory disease, or milk fever. The remaining disease conditions—calving problems (including dystocia and retained placenta), subclinical mastitis, subclinical low blood calcium (hypocalcemia), lameness, and digestive issues (including off-feed, severe diarrhea, or displaced abomasum)—also were diagnosed more ($P < 0.05$) often in diseased cows compared with healthy cows. Prevalence of the diseases in Table 1 is consistent with what has been observed in other herds and in the scientific literature.

Blood Metabolites

Blood metabolites were diagnostic of disease and consistent with expectations of unhealthy cows. Concentrations of FFA in blood serum were greater ($P < 0.05$) in diseased compared with healthy cows at calving and on days 3, 7, and 14 postpartum, suggesting greater body fat loss and greater negative energy balance in diseased cows (Figure 2; top panel).

Concentrations of BHB in blood serum, evidence of clinical and subclinical ketosis, were greater ($P < 0.05$) in diseased compared with healthy cows on days 3 and 14 postpartum, and tended ($P = 0.08$) to be greater on day 10 (Figure 2; second panel). Concentrations of haptoglobin in blood serum, an indirect measure of immune activation, were greater ($P < 0.05$) in diseased compared with healthy cows on days 0 and 3 (Figure 2; third panel).

Concentrations of calcium in blood serum were reduced ($P < 0.05$) in diseased compared with healthy cows on days 0 and 3, and tended ($P = 0.07$) to remain lower on days 10 and 14 (Figure 2; bottom panel). Rectal temperatures (°C) tended ($P = 0.09$) to be slightly greater in diseased than healthy cows on day 3 (39.0 ± 0.05 vs. 38.8 ± 0.07), but did not differ on day 7 (39.1 ± 0.04 vs. 39.0 ± 0.05). Blood plasma glucose, a proxy for energy status, was not affected by health status on days 0, 3, 7, and 14 after calving.

Physical Activity

Physical traits of rumination, resting, eating, ear skin temperature, and general activity assessed by the CowSensor ear tags were analyzed for the periods 10 days before and 10 days after calving. Cows subsequently diagnosed as diseased had no observable changes in physical traits assessed during the prepartum period. Rumination time decreased acutely from day -2 until day 2 relative to calving (Figure 3; top panel) in all cows, whereas eating time decreased gradually during the last 10 days of gestation (Figure 3; bottom panel). Resting activity during 10 days before calving was constant (Figure 3; middle panel), whereas general activity or high activity increased acutely 24 hours before calving and reached a peak between 24 and 48 hours after calving (not shown) before decreasing.

Significant changes in rumination and resting were observed shortly before and after calving and differed according to health status. Healthy cows had greater ($P < 0.01$) rumination and eating times during the first 10 days after calving and rested less or were more ($P < 0.01$) active than diseased cows. Greater resting or inactive times were consistent with a less healthy state of the cows.

Daily minimum and maximum ear skin temperatures assessed by the ear tags before and after calving did not differ between healthy and diseased cows but were highly correlated (r = 0.91 to 0.95; $P < 0.001$) with environmental temperatures.

Milk and Body Traits

Although average body weight did not differ between healthy and diseased cows during the prebreeding period (Table 2), body condition score was reduced in diseased cows (Table 2; Figure 4). Loss of body condition, which averaged 3.0 ± 0.33 at calving, was more rapid in diseased than healthy cows and remained less throughout the entire prebreeding period. Healthy cows started to increase in body condition after 7 weeks postpartum. Despite these differences, milk yield was not different between health status groups (Table 2).

Reproductive Traits

Healthy cows ovulated sooner ($P < 0.01$) after calving and tended ($P = 0.13$) to express their first estrus earlier than diseased cows (Table 3). Duration of first estrus also tended ($P = 0.12$) to be longer in healthy than diseased cows. Healthy cows had proportionally more ($P < 0.01$) ovulations and tended ($P = 0.07$) to have more estrus periods before first insemination. Pregnancy at first insemination and days to conception were not affected by health status (Table 3).

Direct effects of clinical disease on reproductive traits have been reported. Eggs collected from cows with at least one case of clinical disease had reduced fertilization rates and embryos had compromised quality as early as 5 to 6 days after AI. When conceptuses (embryos and their developing placenta) were collected on day 15 after AI from 145 lactating dairy cows in a second

study, fewer cows with clinical diseases were pregnant. When one clinical disease was diagnosed during the first 60 days in the latter study, percentage of cows pregnant on day 15 after AI decreased from 49.4 to 29.8% and uterine disease reduced the percentage of cows pregnant by more than half (49.4 to 20%).

In another study, cows that maintained or gained body condition (n = 226) between calving and the onset of the Ovsynch programs had greater pregnancy rates (41.6 vs. 32.3%) than those that lost body condition (n = 170). Cows with body condition scores ≤ 2.5 at timed AI had poorer pregnancy rates than cows with greater body condition, but the negative impact on pregnancy rate was greater for cows that lost body condition during the first 3 weeks postpartum compared with those that either maintained or gained body condition.

Conclusions

Elevated FFA, BHB, and haptoglobin, and reduced calcium were detected in cows with clinical disease, reflecting greater negative energy balance, more ketosis, altered immune function, and more hypocalcemia compared with healthy cows. Daily postpartum rumination and eating times were greater for healthy than diseased cows, which spent less time resting or inactive than diseased cows. Body condition scores were poorer in diseased cows, whereas body weight and milk yield were unaffected by health status. Despite early and proportionally more ovulations during the prebreeding period, pregnancy rate at first service and days to conception were not affected by health status, likely because of good health care of all cows having both clinical and subclinical disease.

Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.

Table 1. Disease incidence in 160 lactating dairy cows calving during December 2017 and August 2018

	Health status		
Item	Healthy, %	Clinical disease,[1] %	*P*-value
Number of cows	104	56	
Ketosis[2]	0.0	58.5	<0.01
Metritis[3]	0.0	28.3	<0.01
Clinical mastitis[4]	0.0	42.5	<0.01
Milk fever	0.0	2.83	0.02
Calving problems[5]	1.89	8.5	<0.01
Subclinical mastitis[6]	24.1	32.1	0.04
Subclinical hypocalcemia[7]	43.4	60.4	0.04
Lameness	0.0	4.7	<0.01
Digestive	0.0	26.4	<0.01

[1]Any cow with diagnosed ketosis, metritis, mastitis, respiratory disease, or milk fever during the first 60 days in milk.
[2]Based on beta-hydroxybutyrate concentrations > 10 mg/dL on day 0, 3, 7, or 14 after calving.
[3]Diagnosed by evidence of brown watery exudate detected by transrectal palpation at 0, 3, 7, and 14 days in milk (day 0 = day of calving).
[4]Any case of abnormal milk.
[5]Any cow with twins or a calving difficulty score of 3 or greater.
[6]Based on any incidence of somatic cell counts that exceed the threshold 310,000 cells/mL during the first 60 days in milk.
[7]Based on calcium concentrations < 8.6 mg/dL on day 0, 3, 7, or 14 postpartum.

Table 2. Body score, body weight, and milk yield in healthy and diseased lactating dairy cows calving between December 2017 and August 2018

	Health status		
Item	Healthy	Clinical disease[1]	*P*-value
Body condition score[2]	2.6 ± 0.03	2.5 ± 0.03	0.02
Body weight,[2] lb	1,559 ± 9.5	1,541 ± 6.8	0.34
305-day energy-corrected milk, lb	26,893 ± 195	26,699 ± 137	0.71
Cumulative 14-week milk, lb	4,412 ± 97	4,439 ± 71	0.82
Mean 14-week daily milk, lb/day	101.6 ± 0.9	100.1 ± 0.6	0.56

[1]Any cow with diagnosed ketosis, metritis, mastitis, respiratory disease, or milk fever during the first 60 days in milk.
[2]Mean of measures from 1 week post-calving until AI at 73 ± 3 days in milk.

Table 3. Reproductive characteristics of healthy and diseased dairy cows

	Health status		
Item	Healthy	Clinical disease[1]	*P*-value
Days to first ovulation	31.6 ± 2.1	39.7 ± 1.5	0.01
Days to first estrus	40.8 ± 3.0	46.7 ± 2.5	0.13
Duration of first estrus, hours	15.8 ± 1.8	12.1 ± 1.5	0.12
Peak first estrus activity, arbitrary units	5.2 ± 0.3	4.8 ± 0.3	0.32
Cows with one or more heats before timed AI, %	72.2	57.5	0.07
Cows with one or more ovulations before timed AI, %	85.2	65.1	0.01
Days to conception	146 ± 11	151 ± 8	0.65
Pregnant to first service,[2] %	34.6	30.0	0.58

[1]Any cow with diagnosed ketosis, metritis, mastitis, respiratory disease, or milk fever during the first 60 days in milk.

[2]Ovulation was synchronized before timed AI (GnRH — 7 days — prostaglandin F_{2a} — 24 hours — prostaglandin F_{2a} — 32 hours — GnRH — 16 hours — AI).

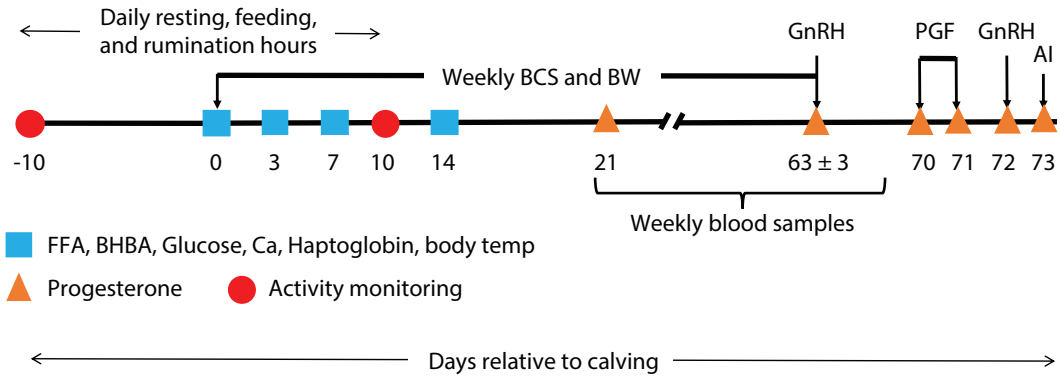

Figure 1. Experimental scheme. Body temperature and blood samples were collected on days 0, 3, 7, and 14 after calving to assess concentrations of free fatty acids (FFA), beta-hydroxybutyrate (BHBA), glucose, calcium (Ca), and haptoglobin. Weekly body scores and blood were collected weekly starting at day 21 postpartum through time AI on 73 ± 3 days in milk.

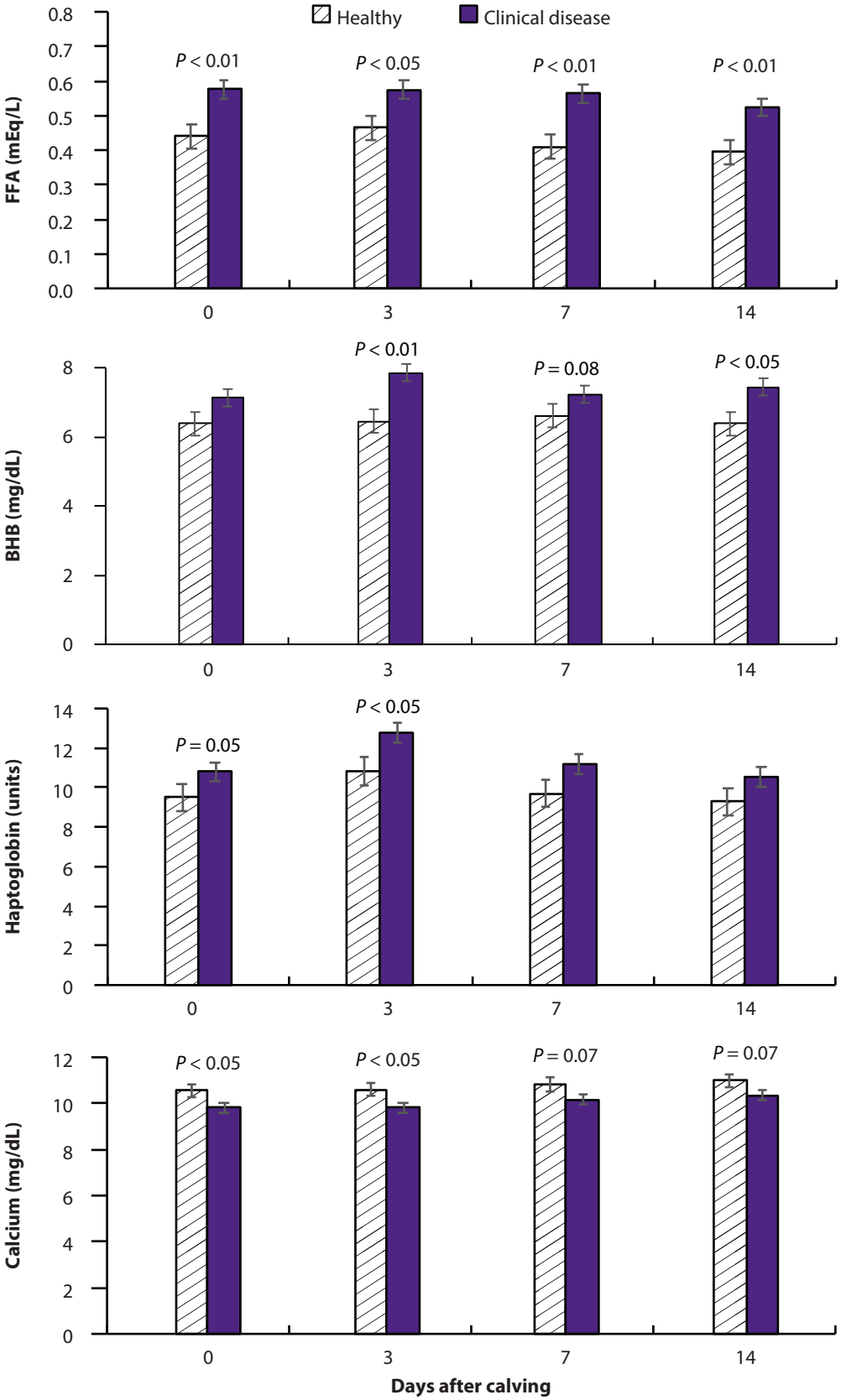

Figure 2. Plasma concentrations of free fatty acids (FFA; top panel), beta-hydroxybutyrate (BHB; second panel), haptoglobin (third panel), and calcium (bottom panel) in 160 dairy cows (calving from December 2017 through August 2018) on days 0, 3, 7, and 14 after calving in 54 healthy cows and 106 cows diagnosed with clinical disease (i.e., ketosis, metritis, mastitis, respiratory disease, or milk fever).

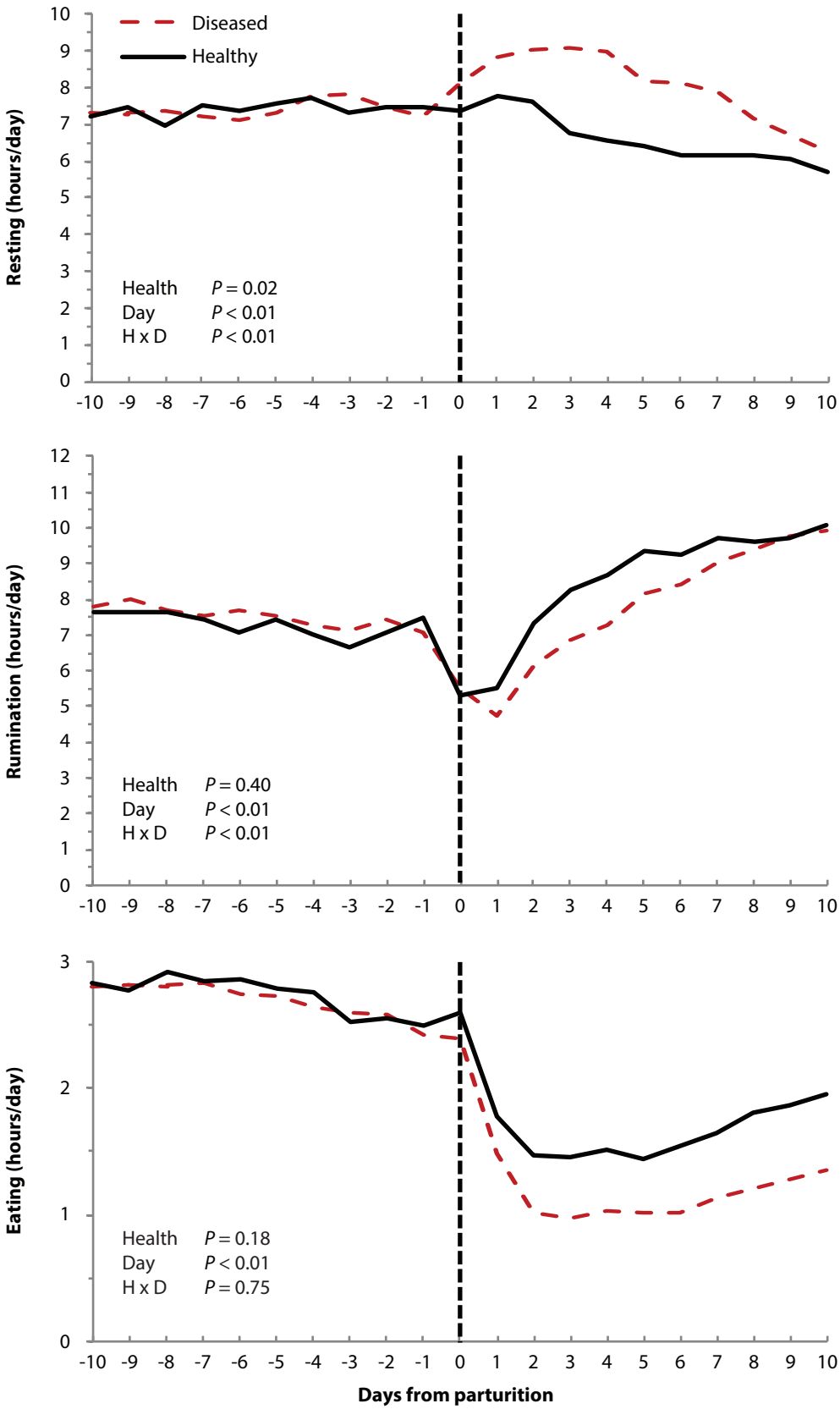

Figure 3. Resting (upper panel), rumination (middle panel), and eating (lower panel) hours per day in 160 dairy cows (calving from December 2017 through August 2018) on day -10 through day +10 from parturition in 54 healthy cows and 106 cows diagnosed with clinical disease (i.e., ketosis, metritis, mastitis, respiratory disease, or milk fever).

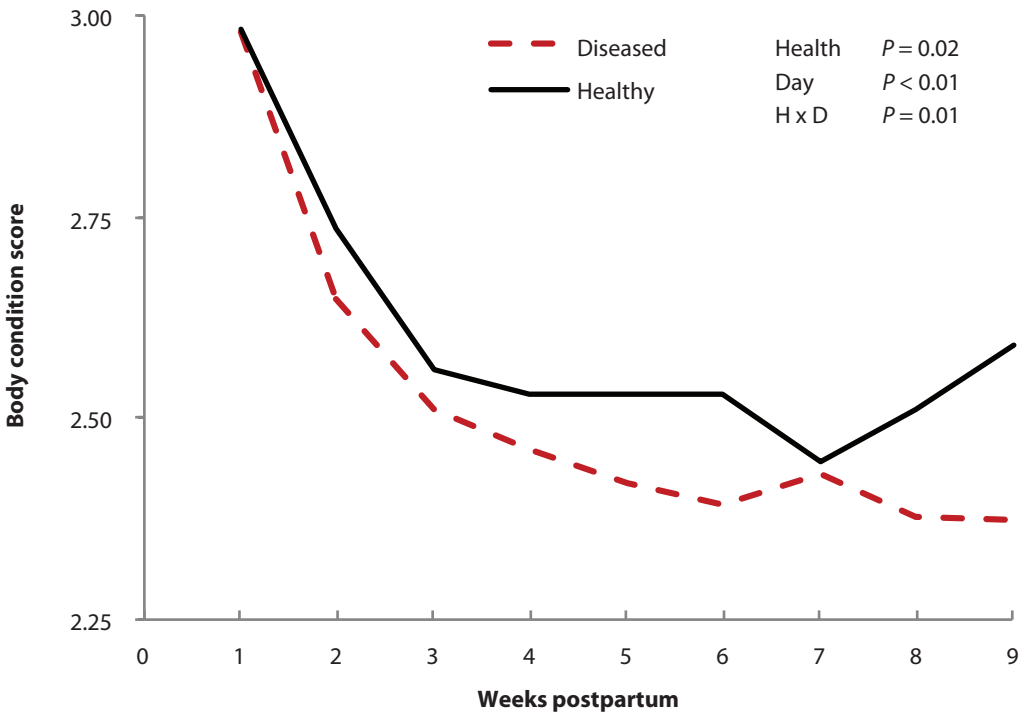

Figure 4. Changes in body condition score during the prebreeding period after calving in 54 healthy cows and 106 cows diagnosed with clinical disease (i.e., ketosis, metritis, mastitis, respiratory disease, or milk fever).

Are My Dry Cows Heat-Stressed? A Novel Approach to Assess Heat Stress of Dry Cows in Commercial Dairy Herds

A.L.A. Scanavez, C.A. Gamarra, R.S.S. de Oliveira, and L.G.D. Mendonça

Summary

Heat stress during the dry period causes major economic losses to the dairy industry. However, limited research exists regarding responses of dry cows exposed to various temperature and relative humidity gradients. In addition, no validated methods are currently available to assess heat stress in dry cows. The goals of this study were to describe core body temperature (CBT) responses of dry cows according to a variety of temperature-humidity index (THI) values, and develop and validate a practical method to assess heat stress in dry cows in commercial dairy herds. This study was comprised of 2 parts. In the first part of the study, vaginal temperature of dry cows (n = 346) with 250 to 260 days of gestation from 5 herds was assessed for 4 to 7 consecutive days in 5-minute intervals. Within dairy and parity group, cows were classified as having high (HT) or low CBT (LT). By design, CBT was greater for HT compared with LT cows (102.3 ± 0.01 vs. 101.8 ± 0.01°F). Cows classified as having HT had shorter gestation length compared with their LT counterparts (272.5 ± 0.2 vs. 275.1 ± 0.2 days). The second part of the study consisted of evaluating and validating a practical assessment method of heat stress and investigating CBT threshold values. Vaginal temperature of 1,540 dry cows with 236 to 250 days of gestation from 3 commercial dairy herds was assessed a single time using a digital thermometer. Average CBT of HT cows at each THI (data from the first part of the study) was used as a threshold value to classify cows as heat-susceptible or heat-tolerant. Cows with higher or lower CBT than the threshold defined for a given THI were classified as heat-susceptible or tolerant, respectively. Cows classified as heat-susceptible had shorter gestation length (272.5 ± 0.2 vs. 275.0 ± 0.2 days) and were more likely to have twins (11.0 vs. 3.8%) than heat-tolerant cows. In conclusion, assessment of heat stress in dry cows based on defined CBT thresholds is a useful method to identify cows expected to have shorter gestation length and more likely to have twins.

Introduction

Heat stress causes major economic losses to the dairy industry. Several studies demonstrated that exposure of lactating dairy cows to heat stress increases core body temperature (CBT) and reduces productive and reproductive performance. Recent studies indicate that the negative effects of heat stress are not limited to the lactating period. Dairy cows exposed to heat stress during the dry period have impaired milk yield in the subsequent lactation. Because of the extensive impact of heat stress on dairy cows, research has been conducted on this topic for several decades.

In order to estimate severity of heat stress conditions to dairy cows, during the 1960s researchers developed the temperature-humidity index (THI), which has been revised several times in the past decades to account for increased milk production of modern dairy cows. It is currently widely accepted that a THI $\geq$ 68 is associated with reduced performance of lactating cows. Despite the scientific evidence that a THI $\geq$ 68 is an appropriate threshold to indicate heat stress in lactating dairy cows, no studies have evaluated the THI threshold of heat stress for dry cows. In addition, the lack of data on CBT responses according to THI in dry cows is a significant limitation for determining whether conditions are predisposing a large proportion of cows to be susceptible to heat stress. Indeed, monitoring tools and screening tests to assess severity of heat stress in dry cows in commercial herds are not currently available.

Exposure to heat stress during the dry period reduces gestation length in dairy cows. Thus, gestation length is expected to be shorter in herds in which dry cows are severely affected by heat stress. Shorter gestation length has been associated with several postpartum problems, such as increased incidence of retained placenta and metritis, and reduced reproductive performance and milk yield. Therefore, there is a need to develop an effective method to assess severity of heat stress in dry cows to assist producers and consultants to determine whether heat abatement strategies should be implemented.

The primary objective of the present study was to describe CBT responses of dry cows from commercial herds according to a variety of THI values during summer. Our secondary objectives were to develop and validate a practical method to assess heat stress in dry cows in commercial dairy herds.

Experimental Procedures

The present study consists of a compilation of data from three previous experiments. Data analyses in the current study were conducted in two parts, which are referred to as "reference dataset" (part 1) and "validation" (part 2). These terms were used to define whether the data were used to develop the heat stress assessment method (e.g., reference dataset) or to evaluate its usefulness in commercial herds (e.g., validation).

Reference Dataset and Definition of CBT Threshold Values

Data used for this part of the study were from experiments conducted during the summer of 2014 (n = 2 herds) and 2017 (n = 3 herds) in Kansas dairy farms. Herds used in the study had on average 2,210 milking cows (range = 250 to 4,000). Dry cows were housed in free-stall barns (2 herds), dry-lot pens (2 herds), or a bedded pack barn (1 herd). Cows had access to shade in all herds and an evaporative cooling system was provided for cows housed in the free-stall and bedded pack barns (3 herds). Dry Holstein cows (n = 346) at 250 to 260 days of gestation and with no signs of clinical disorders were enrolled in the study in weekly cohorts. To ensure all cows were exposed to similar environmental conditions, enrollment was conducted when weather forecasts predicted maximum temperatures greater than 90°F for 7 consecutive days. Upon enrollment, a calibrated temperature logger (iButton DS1922L, Embedded Data Systems, Lawrenceburg, KY) attached to a blank CIDR was inserted into cows' vaginas. Loggers were programmed to record CBT in 5-minute intervals for 4 to 7 consecutive days. After removal of loggers, average CBT was calculated for each cow. Median values of CBT were calculated within each dairy and parity group. Cows with average CBT greater or equal to the median value were classified as having high CBT (HT; n = 176), whereas cows with average CBT below the median value were considered to have low CBT (LT; n = 170). Cows were followed until the day of calving. Data regarding date of parturition, gestation length, and pregnancy type (e.g., singleton or twins) were extracted from the on-farm management software (3 herds: DairyComp, Valley Ag Software, Tulare, CA; 2 herds: PCDart, DRMS, Raleigh, NC).

Ambient temperature and humidity were recorded in 5-minute intervals using loggers (HOBO U23 Pro v2, Onset Computer Corp., Pocasset, MA) located in the pens. Ambient THI was calculated using the equation: $THI = T - (0.55 - 0.55\ RH/100) \times (T - 58)$, where T and RH are dry-bulb temperature (°F) and relative humidity, respectively. To characterize CBT of cows across different THI values, ambient THI was identified at each time point that CBT was recorded (total of 563,673 data points). To create a CBT threshold to identify cows more susceptible to heat stress, the average of CBT of HT cows was calculated for each time point of THI available in the dataset. Therefore, based on the average CBT of HT cows for each THI time point, threshold values of CBT were established for various THI. Established threshold values of CBT according to THI were used in the second part of the study (validation of heat stress threshold values).

Validation of Heat Stress Threshold Values

Data used to validate the CBT threshold values were from an experiment conducted with dry Holstein cows in commercial herds located in Kansas (n = 2 herds) and Oklahoma (n = 1 herd) during the summer of 2018. Herds used for this part of the study had on average 4,500 milking cows (range = 4,000 to 5,500). Cows were housed in open dry-lot pens with access to shade. Once weekly, a list of cows with 236 to 250 days of gestation was generated using the on-farm management software (DairyComp, Valley Ag Software, Tulare, CA). A subset of cows (n = 50 to 70 per herd) were randomly selected from the list to be included in the study weekly. On the day of enrollment, eligible cows were evaluated by a veterinarian, and those that presented any clinical disorders (e.g., lameness) were not included in the study. Core body temperature was assessed from cows that met the inclusion criteria (n = 540, 508, and 492 for herds A, B, and C, respectively) using a high-precision thermometer (Fisherbrand Traceable Platinum Ultra-Accurate Digital Thermometer, Thermo Fisher Scientific, Waltham, MA). The one-time CBT assessments were conducted between 1900 and 2000 h. After parturition, gestation length and pregnancy type data (singleton vs. twins) were extracted from the on-farm management software.

Temperature and humidity in the pens were recorded in 5-minute intervals using loggers (HOBO U23 Pro v2, Onset Computer Corp., Pocasset, MA) fixed under the shade structures. Ambient THI at time of CBT assessment was calculated with the same formula used for the reference dataset.

Using the pre-established CBT threshold values according to THI (part 1 of study – reference dataset), cows were classified as heat-susceptible or heat-tolerant for validation of the heat stress threshold at a given ambient THI. Therefore, cows that presented CBT greater or equal to the pre-established threshold for each specific THI were considered to be heat-susceptible.

Statistical Analyses

Continuous variables were analyzed by ANOVA using the PROC GLIMMIX procedure of SAS with normal distribution and identity link. For dichotomous outcomes, the PROC GLIMMIX procedure of SAS with binary distribution and logit link was used, and incidence of events was calculated using the PROC FREQ procedure. Significance was declared at $P \leq 0.05$, and tendencies at $0.05 < P \leq 0.10$.

Results and Discussion

Study Part 1 – Reference Dataset

Core body temperature of dry cows at various THI values are depicted in Figure 1. These data add to the current knowledge of heat stress in dry cows given the large number of cows used in the study and hundreds of CBT measurements obtained in consecutive days from each cow. Furthermore, because all data were recorded in commercial dairy herds, it is likely that results reported herein accurately represent populations of cows from other commercial herds. Therefore, it is acceptable to speculate that data depicted in Figure 1 are useful for dairy producers and consultants when implementing heat abatement strategies for dry cows, such as activating fans and sprinklers based on THI values.

In the reference dataset, average CBT was ($P < 0.01$) greater for HT compared with LT cows (Figure 2; 102.3 ± 0.01 vs. 101.8 ± 0.01 °F). Although this difference occurred because of the way cows were classified into HT and LT groups, CBT values were consistently greater for HT than LT cows, regardless of THI. This indicates that the methodology used to classify dry cows into 2 groups in part 1 of the study was appropriate to identify heat-susceptible cows. Because cows were classified based on CBT within herd and, therefore, were exposed to similar nutritional and management conditions, it is possible to speculate that HT cows either had greater

metabolic heat production or impaired heat loss compared with LT cows. Differences in behavioral characteristics, such as lying time, may be another reason for discrepancies in CBT. Cows classified as HT had ($P < 0.01$) shorter gestation length compared with their LT counterparts (272.5 ± 0.2 vs. 275.1 ± 0.2 days).

Study Part 2 – Validation

The authors recognize that the strategy used to classify cows as HT or LT requires specialized equipment (e.g., temperature loggers) and is time-consuming, which likely prevents its adoption and routine use in commercial herds. Therefore, the authors focused on using the reference dataset to establish temperature threshold values that could be easily used by dairy producers and consultants to determine groups of cows with increased CBT and, therefore, more prone to have short gestation length. Average CBT, gestation length, and incidence of twining for cows susceptible and tolerant to heat stress are summarized in Table 1. Overall, heat-susceptible cows had ($P < 0.01$) shorter gestation length (272.5 ± 0.2 vs. 275.0 ± 0.2 days) and greater ($P < 0.01$) risk of twin pregnancy (11.0 vs. 3.8%) than cows classified as heat-tolerant. The interaction between dairy and CBT group was not ($P > 0.40$) associated with gestation length or risk of twinning. This lack of interaction indicates that the pre-established CBT threshold values were appropriate to identify heat-susceptible cows (e.g., present short gestation length), regardless of the dairy where the method was applied. This is an important finding because it suggests that the proposed method to assess heat stress in dry cows may be useful for commercial operations. In addition, this method may be used as a heat stress screening test for dry cows.

Figure 3 depicts proportion of cows identified as heat-susceptible weekly at each dairy. Gestation length was consistently shorter for heat-susceptible than heat-tolerant cows in the 3 dairies across all weeks of CBT assessments (Figure 3). These data further indicate the effectiveness of the method adopted to identify heat-susceptible cows. Interactions between dairy and CBT group, week of CBT assessment and CBT group, and the three-way interaction between dairy, week of assessment, and CBT group were not ($P > 0.58$) associated with gestation length.

In conclusion, the method proposed to assess heat stress in dry cows using pre-established CBT threshold values is useful to identify cows expected to have shorter gestation length. Furthermore, the method described herein was useful to identify dry cows more likely to deliver twins. Because CBT assessments were conducted more than 2 to 3 weeks before expected calving, this system allows implementation of specific management practices targeted to cows more prone to have short gestation length and twins before parturition starts. Further studies are necessary to evaluate strategies to manage heat-susceptible cows to prevent disorders associated with short gestation length or twinning, such as retained placenta and metritis.

Acknowledgments

The authors thank the owners and employees of the collaborating dairies.

	Relative humidity, %																	
TEMP, °F	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**55**	**60**	**65**	**70**	**75**	**80**	**85**	**90**	**95**
51																	101.6	101.5
52																	101.5	
53																	101.5	
54																	101.6	
55																101.4	101.7	
56																101.6	101.7	
57																101.6		
58														101.7	101.9	101.9	101.8	101.8
59													101.7	101.8	101.7	101.7	102.1	101.8
60													101.6	101.7	101.7	101.7	101.9	101.4
61													101.7	101.9	101.6	101.8	101.9	101.5
62													101.9	101.8	101.7	101.8	101.8	101.5
63												102.0	101.7	101.9	102.0	101.9	101.7	101.9
64												102.1	101.9	101.9	101.9	101.8	101.7	101.8
65											102.2	102.0	101.9	101.9	101.9	101.8	101.8	101.7
66										102.1	102.0	102.0	102.0	101.6	102.0	101.5	101.9	101.6
67										102.2	101.9	101.7	101.5	102.0	101.9	101.5	101.8	101.9
68									102.2	101.9	101.6	101.8	101.9	102.0	101.7	101.7	101.9	102.1
69									102.3	101.6	102.0	101.7	102.0	101.9	101.7	101.9	101.9	101.7
70									101.9	101.8	101.9	102.0	102.0	102.0	101.8	102.0	102.0	101.8
71								101.6	101.8	101.9	102.2	102.2	102.0	101.9	101.9	101.9	102.1	101.9
72								102.0	101.9	101.9	102.2	102.0	102.0	101.9	102.0	101.9	102.0	101.9
73							101.8	102.0	101.9	102.0	101.9	102.0	102.0	102.0	102.1	102.1	102.1	102.0
74							101.8	102.0	102.0	102.0	101.8	102.1	101.9	102.1	101.8	102.2	102.1	102.1
75							102.1	102.2	101.9	101.9	102.0	101.9	101.9	102.1	102.3	102.1	102.1	102.3
76							102.2	102.0	102.0	102.0	101.9	101.9	102.1	102.1	102.1	102.1	102.1	101.8
77						101.5	102.2	102.1	102.1	102.1	102.1	101.9	102.2	102.1	102.2	102.0	102.2	101.6
78						102.0	102.1	102.2	102.0	102.1	101.9	102.1	102.2	102.2	102.1	102.4	102.2	102.1
79					102.0	102.1	102.2	102.1	102.1	102.2	102.0	102.2	102.2	102.1	102.4	102.3	102.2	
80					102.0	102.1	102.2	102.1	102.3	101.9	102.2	102.3	102.2	102.3	102.4	102.0	101.9	
81				101.9	102.2	102.3	102.2	102.3	102.2	102.0	102.3	102.3	102.1	102.4	102.3	101.9	102.3	
82				101.8	102.2	102.3	102.3	102.3	102.2	102.2	102.3	102.3	102.2	102.5	102.2	102.3	101.8	
83				101.7	102.2	102.3	102.3	102.4	102.2	102.3	102.3	102.3	102.4	102.2	102.1	102.3	101.9	
84				101.9	102.0	102.3	102.3	102.3	102.3	102.3		102.2	102.4	101.9	102.3	101.7		
85			101.9	101.9	102.3	102.4	102.3	102.1	102.3	102.3	102.5	102.5	102.3	101.5	101.7	101.7		
86			102.0	102.1	102.4	102.4	102.2	102.1	102.3	102.3	102.3	102.1	102.1	102.0				
87			102.1	102.2	102.6	102.3	102.3	102.4	102.3	102.4	102.2	102.3	102.1	102.1				
88			102.2	102.5	102.5	102.3	102.4	102.3	102.3	102.5	102.1	102.3	102.1					
89			102.2	102.6	102.4	102.2	102.3	102.4	102.4	102.4	102.4	102.3	102.1					
90			102.3	102.5	102.3	102.4	102.3	102.4	102.4	102.4	102.2	102.3	102.3					
91				102.5	102.3	102.2	102.5	102.4	102.4	102.3	102.0							
92				102.2	102.3	102.4	102.5	102.5	102.6	102.4	102.3							
93			102.4	102.3	102.4	102.5	102.5	102.6	102.3	102.4								
94			102.4	102.4	102.5	102.5	102.5	102.5	102.3									
95			102.3	102.5	102.4	102.6	102.4	102.3	102.2									
96		102.2	102.4	102.3	102.5	102.5	102.5	102.7	102.0									
97	102.0	102.5	102.6	102.5	102.6	102.5	102.3	103.0	103.1	103.0								
98	102.0	102.3	102.4	102.5	102.6	102.5	102.2		102.9									
99	102.2	102.7	102.4	102.5	102.6	102.4												
100	102.4		102.5	102.6	102.5	102.3	102.2	102.3	102.6									
101	102.6		102.4	102.7	102.6	102.1	102.1	102.5										
102	102.6		102.4	102.7	102.5													
103			102.7	102.6														
104			102.9	102.5														
105			102.7															

Figure 1. Mean core body temperature responses of dry dairy cows (n = 346) to various combinations of ambient temperature (TEMP) and relative humidity. Core body temperature assessments were conducted every 5 minutes in dry cows between 250 and 260 days of gestation during the summer of 2014 and 2017 (study part 1 – reference dataset). Colors represent ranges of temperature-humidity index (THI), which was calculated for every time point of core body temperature. White, light gray, medium gray, and dark gray represent indexes of < 68, 68 to 72, 72 to 80, and > 80, respectively.

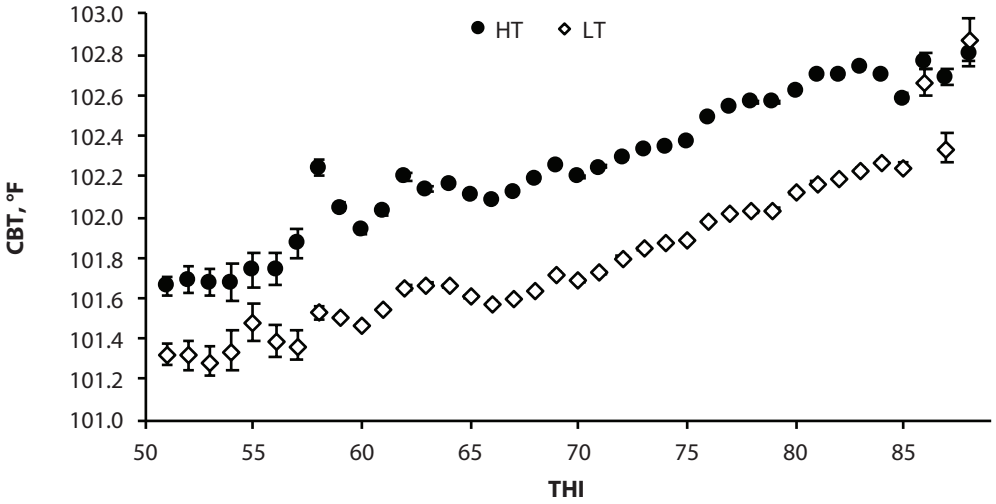

Figure 2. Average core body temperature (CBT) at various values of temperature-humidity index (THI) for dry dairy cows (n = 346) classified as having CBT higher (HT; black circles) or lower (LT; white diamonds) than the median value for their respective parity group and dairy. Assessments were conducted in cows between 250 and 260 days of gestation during the summer 2014 and 2017 (study part 1– reference dataset). Error bars represent standard error of the mean. Average CBT was greater ($P < 0.01$) for HT than LT cows (102.3 ± 0.01 vs. 101.8 ± 0.01 °F).

Table 1. Average (SEM[1]) core body temperature (CBT) in the dry period,[2] gestation length, and twinning incidence for dairy cows from three commercial dairy herds deemed as heat-susceptible and heat-tolerant based on pre-established threshold values[3,4] for validation of a method to assess heat stress of dry cows in commercial dairy herds

	CBT category[5]		
Item	Heat-susceptible	Heat-tolerant	*P*-value
Dairy A			
Number of cows	268	272	
Average core body temperature, °F	102.8 (0.03)	102.0 (0.03)	< 0.01
Gestation length, d	272.9 (0.3)	275.2 (0.3)	< 0.01
Twinning, %	11.2	4.8	0.01
Dairy B			
Number of cows	323	185	
Average core body temperature, °F	103.2 (0.02)	102.2 (0.03)	< 0.01
Gestation length, d	271.7 (0.3)	274.4 (0.4)	< 0.01
Twinning, %	9.6	1.6	< 0.01
Dairy C			
Number of cows	267	225	
Average core body temperature, °F	103.1 (0.03)	102.2 (0.03)	< 0.01
Gestation length, d	272.8 (0.3)	275.3 (0.4)	< 0.01
Twinning, %	12.4	4.4	< 0.01

[1]Standard error of the mean.
[2]Core body temperature was assessed in 1,540 cows during the summer of 2018 (study part 2 – validation of heat stress threshold values).
[3]Threshold values for classifying cows as heat-susceptible were the average CBT of cows with CBT above or equal the median value for their respective herd and parity group in the reference dataset.
[4]Reference dataset was comprised of records from 346 parous cows from 5 dairy herds that had CBT assessed between 250 and 260 days of gestation.
[5]Cows were classified as heat-susceptible or heat-tolerant based on pre-established CBT threshold values for the specific THI at CBT assessment (study part 1 – reference dataset).

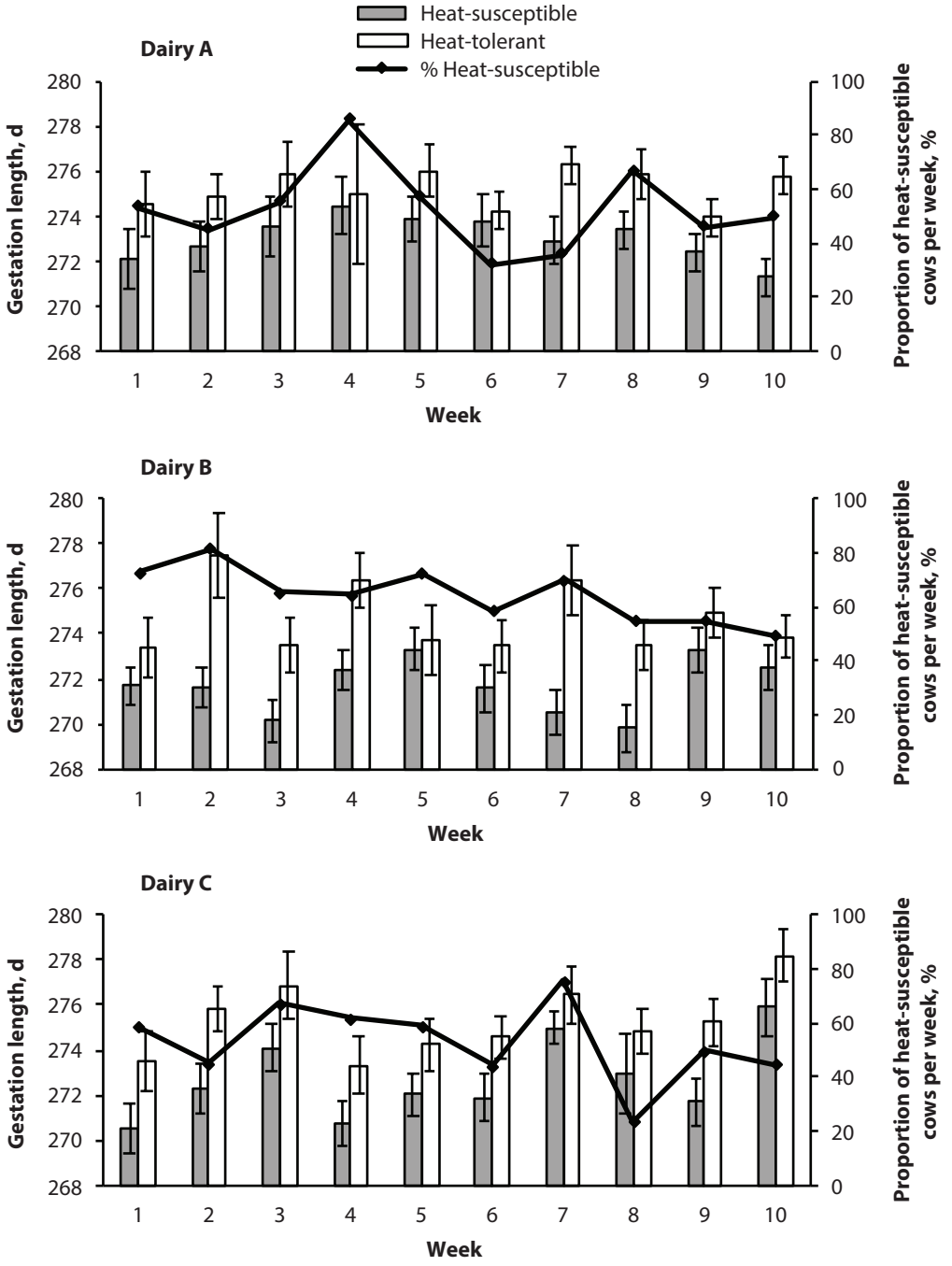

Figure 3. Left axis represents gestation length of cows from 3 commercial dairy herds identified as heat-susceptible or heat-tolerant weekly during the dry period based on core body temperature (CBT) threshold values previously defined from a reference dataset. Right axis represents the proportion of cows deemed heat-susceptible by week of temperature assessment. Error bars represent standard error of the mean.

Deliberate Exercise of Pregnant Holstein Heifers Improves Milk Composition During Lactation

J. Johnson, P. Steichen, B.J. Bradford, A.E. Rhodes, and T.G. Rozell

Summary

Exercise has substantial impacts on systemic physiology, but little research has been conducted to assess how it may influence dairy cattle in modern confined production systems. Dairy heifers were walked for up to 45 minutes, 4 days per week for 8 weeks during pregnancy to assess impacts on subsequent health and productivity. Heifers that were exercised had increased milk protein and solids-not-fat concentrations for up to 15 weeks into lactation, and increased milk fat and energy-corrected milk production at some time points during this period, as compared to sedentary contemporaries. No adverse or beneficial effects of exercise were found on locomotion, calving ease, date of parturition, or somatic cell scores. These findings point to potential impacts on lactation productivity following exercise in pregnant heifers.

Introduction

Enormous improvements have been made in the pounds of milk produced by dairy cattle through artificial selection programs as well as through improvements in nutrition. For example, an average dairy cow in the 1950s produced approximately 5,300 pounds of milk per year, while in 2019 the average cow produces approximately 23,000 pounds per year, an increase of 334% in just 70 years. Production efficiency has accounted for many of these improvements, with far more milk produced from fewer nutrients and reduced animal waste per unit of milk. Modern dairy cattle and modern production practices have allowed dairy producers to meet a greater market demand with fewer cows. Importantly, researchers at Cornell University found that the carbon footprint of the modern dairy cow is only about 37% of the dairy cow from 1944 when compared per kg of milk. Thus, improvements in efficiency of milk production have allowed for greater sustainability of dairy products as a source of human food.

Although the majority of the improvements in production efficiency in the past 60 years have involved artificial selection, it is likely that further genetic improvement may be slowed substantially by inbreeding within the Holstein breed. In a recent study, researchers found that all current Holstein bulls used for AI in North America are derived from only 2 ancestors, Hulleman and Neptune H. These investigators also calculated that the effective population size of all Holstein cattle is less than 100 individuals due to lack of genetic diversity; a similar effective population for any species in the wild would be considered an extreme risk for extinction. Thus, it is critical that researchers begin examining alternative means of improving production efficiency in Holstein cattle. One potential strategy for improving production efficiency is intentional exercise. In studies conducted in the late 1970s and early 1980s, exercise of pregnant Holstein heifers was found to improve milk composition in the subsequent lactation. In theory, exercise should improve overall fitness, body composition, and general welfare of cattle as it does in other species. Therefore, our objectives were to examine the impacts of deliberate exercise of pregnant Holstein heifers on calving, milk production, and milk composition.

Experimental Procedures

Pregnant Holstein heifers at the Kansas State University Dairy Teaching and Research Center were chosen at random to either exercise (EX; n = 12) or to remain sedentary (EC; exercise controls; n = 12). Exercised heifers underwent an 8-week endurance exercise program in an 8-panel motorized free walker 4 times per week that involved walking at a slow pace

(3.0 - 3.5 mph) for up to 45 minutes (after a 5-minute warmup at 2.5 mph and followed by a 5-minute cool-down at 2 mph). The exercise regimen was initiated approximately 11 weeks prior to parturition such that the exercise activities were stopped 3 weeks before parturition. Exercise-control heifers were taken to the exerciser but not exercised. All heifers received the same ration and access to water throughout the 8-week experimental period and were weighed weekly.

At parturition, calving ease was assessed by using a subjective scale of 1 to 5: 1 = no problems, 2 = minor problem, 3 = needed assistance, 4 = considerable force, and 5 = very difficult (e.g. C-section). In addition, "calving date accuracy" was calculated by subtracting the actual calving date from the estimated calving date based on breeding date. Milk samples were collected once weekly from all heifers beginning on day 3 of lactation (designated as week 0) through week 15 of lactation, for analysis of milk components by the Dairy Herd Improvement Association (DHIA).

All data were analyzed by ANOVA using PROC MIXED with SAS v. 9.2 (SAS Inst., Cary, NC), including body weight, milk components, and milk yield. Fixed variables included treatment, time (day, week), and time × treatment; the random variable was heifer.

Results and Discussion

Effects of Exercise on Growth and Calving Characteristics

Growth rates of heifers (based on weekly body weight) did not differ between the exercised or exercise-control groups, indicating that overall body mass was not changed by consistent exercise (Figure 1). However, body composition was not assessed in this experiment and future studies are planned to determine proportional muscle mass and fat deposition in pregnant heifers that undergo exercise. Although researchers in 1979 found that exercise improved calving ease, we found no differences in calving ease scores between exercised and sedentary heifers (Table 1). The previous researchers exercised pregnant heifers for 4 weeks prior to parturition through the day of parturition for approximately 30 minutes per day, matching our exercise conditions fairly closely except for the fact that we ceased exercise 3 weeks prior to estimated day of parturition. Thus, the calves in the previous study may have been lighter due to increased nutrient requirements by the heifers, although they did not report calf birth weights. It is also likely that genetic selection of bulls for calving ease has improved calving ease scores for all Holstein heifers since the time of the previous study.

Effects of Exercise on Subsequent Lactation

Exercise was not found to have any adverse impacts on locomotion, metabolic disorders, or observed signs of distress during the first 2 trips to the milking parlor (data not shown), while we did find that exercise had positive effects on the subsequent lactation and milk quality. Following 8 weeks of exercise that ended 3 weeks prior to parturition, both milk protein and solids-not-fat (SNF; which includes protein) content increased. Our finding that milk protein and SNF increased after exercise conflicts with some reports that have found no differences in these parameters between exercised and non-exercised multiparous cows and 2-year-old heifers. However, there does appear to be some relationship between milk protein increases after exercise and the duration of the exercise activities. When investigators increased the distance exercised from approximately 1 mile/day (where there were no differences in milk composition) to approximately 5 miles/day, milk protein was found to increase. In our experiment, heifers exercised up to approximately 3 miles per day for 4 days/week, so perhaps it is important for exercise programs to include greater distances in order to improve muscle growth to the point where greater muscle breakdown can occur during lactation to provide the necessary circulating amino acids for milk protein synthesis. Further research is necessary to determine body composition after different types of exercise programs.

Although we found that milk protein and SNF content were increased by exercise, other parameters appeared to change very slightly or not at all. Milk lactose concentration was greater in the first week of lactation, but there was no accompanying increase in total milk volume from exercised heifers at that same time, which was surprising given that lactose is generally considered to be the primary component that draws water into the mammary gland during lactation. Milk fat and energy-corrected milk appeared to be improved at certain sampling times during lactation, but there was no overall improvement in these parameters. Additionally, no differences were found between exercised and sedentary heifers for somatic cell scores, indicating that exercise had no beneficial or detrimental effect on udder health.

Conclusions

An exercise regimen that involves fairly low-speed walking (approximately 3.5 mph) for up to ~45 minutes per day appears to improve milk composition in the subsequent lactation without causing any detrimental effects on the animals. Future research will be required to examine body composition after exercise, as well as longevity of exercised animals within the milking herd.

Table 1. Averages for calving characteristics between exercised and sedentary heifers

	Treatment[1]	
Item	EX	EC
Calving ease[2]	1.8 ± 0.24	1.3 ± 0.24
Calf birth weight, lb	84 ± 2.80	86 ± 3.38
Gestation length,[3] d	285.8 ± 1.24	285.7 ± 2.02

[1]Treatment: EX = exercised heifers (n = 12); EC = exercise-control (n = 12), brought to exerciser but not exercised.
[2]Scale from 1–5, with 1 denoting no intervention and 5 denoting a cesarean section.
[3]Expected gestation length = 280 days.

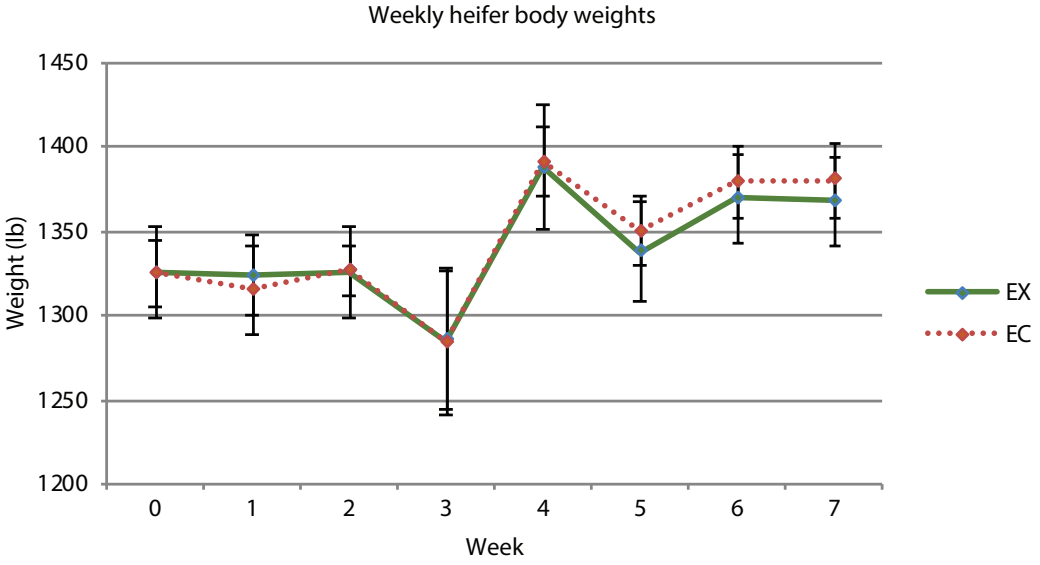

Figure 1. Average heifer weights during the 8-week exercise regimen. The exercised group of heifers (EX; n = 12) were walked at an average pace of 3 mph for an average of 30 minutes per day for 4 days/week for a total of 8 weeks. The exercise-control group of heifers (EC; n = 12) were brought to the exerciser but did not exercise. No apparent differences in growth rate were caused by activity levels during pregnancy.

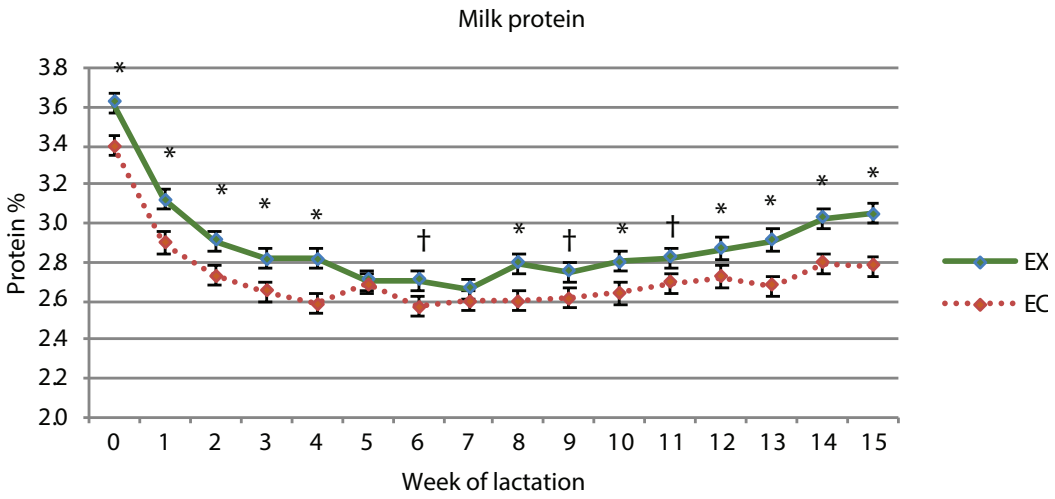

Figure 2. Average percent milk protein through 15 weeks of lactation. The exercised group of heifers (EX; n = 12) were walked at an average pace of 3 mph for an average of 30 minutes per day for 4 days/week for a total of 8 weeks. The exercise-control group of heifers (EC; n = 12) were brought to the exerciser but did not exercise. All exercise activities were stopped by 21 days prior to parturition, so the effects of exercise on improving milk protein persisted even 15 weeks into lactation (18 weeks after exercise was stopped). *$P < 0.05$; †$P < 0.10$.

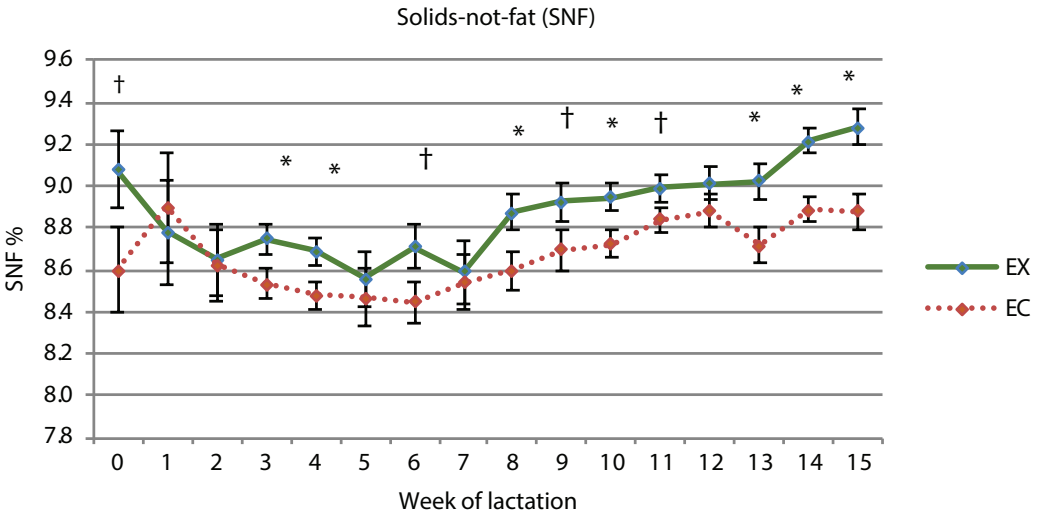

Figure 3. Average percent solids-not-fat through 15 weeks of lactation. The exercised group of heifers (EX; n = 12) were walked at an average pace of 3 mph for an average of 30 minutes per day for 4 days/week for a total of 8 weeks. The exercise-control group of heifers (EC; n = 12) were brought to the exerciser but did not exercise. All exercise activities were stopped by 21 days prior to parturition, so the effects of exercise on improving solids-not-fat persisted even 15 weeks into lactation (18 weeks after exercise was stopped). *$P < 0.05$; †$P < 0.10$.

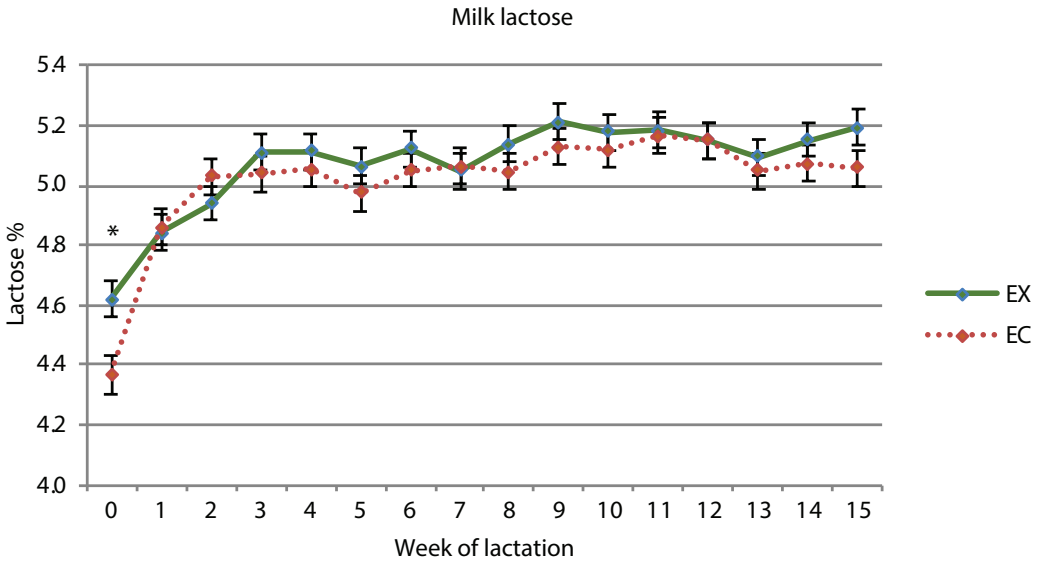

Figure 4. Average percent lactose through 15 weeks of lactation. The exercised group of heifers (EX; n = 12) were walked at an average pace of 3 mph for an average of 30 minutes per day for 4 days/week for a total of 8 weeks. The exercise-control group of heifers (EC; n = 12) were brought to the exerciser but did not exercise. All exercise activities were stopped by 21 days prior to parturition. *$P < 0.05$.

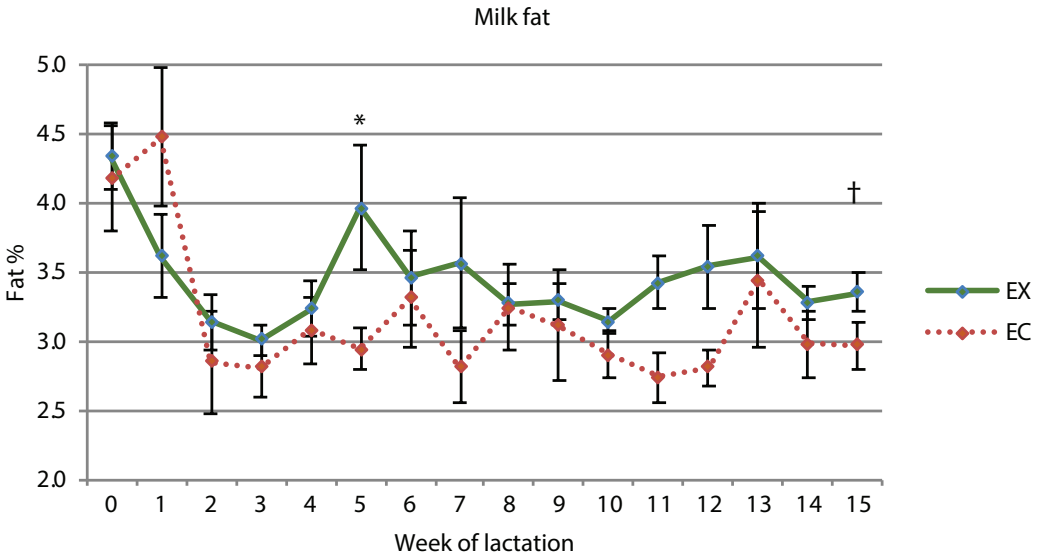

Figure 5. Average percent milk fat through 15 weeks of lactation. The exercised group of heifers (EX; n = 12) were walked at an average pace of 3 mph for an average of 30 minutes per day for 4 days/week for a total of 8 weeks. The exercise-control group of heifers (EC; n = 12) were brought to the exerciser but did not exercise. All exercise activities were stopped by 21 days prior to parturition. *$P < 0.05$; †$P < 0.10$.

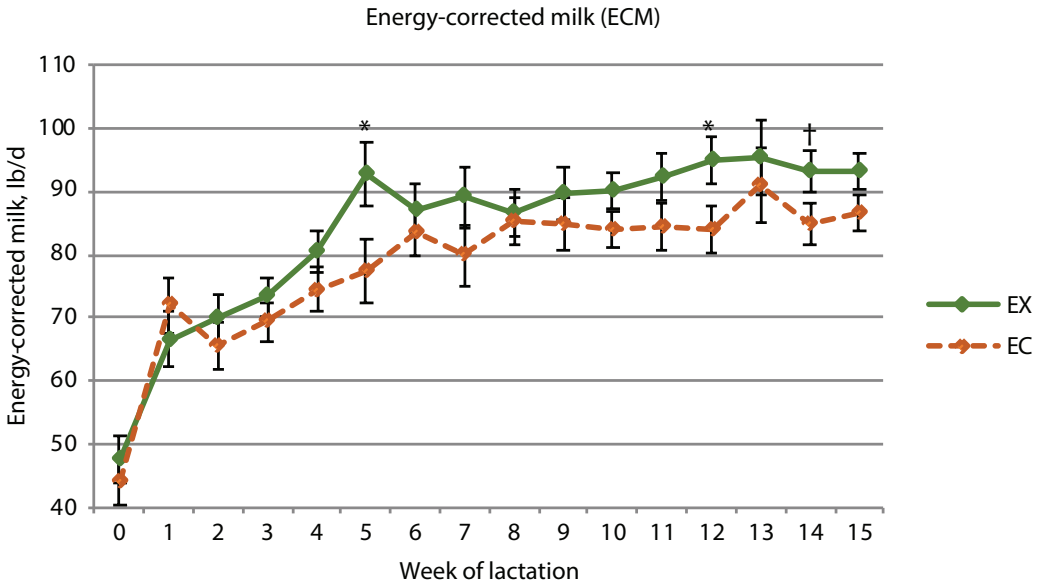

Figure 6. Energy-corrected milk production through 15 weeks of lactation. The exercised group of heifers (EX; n = 12) were walked at an average pace of 3 mph for an average of 30 minutes per day for 4 days/week for a total of 8 weeks. The exercise-control group of heifers (EC; n = 12) were brought to the exerciser but did not exercise. All exercise activities were stopped by 21 days prior to parturition. *$P < 0.05$; †$P < 0.10$.

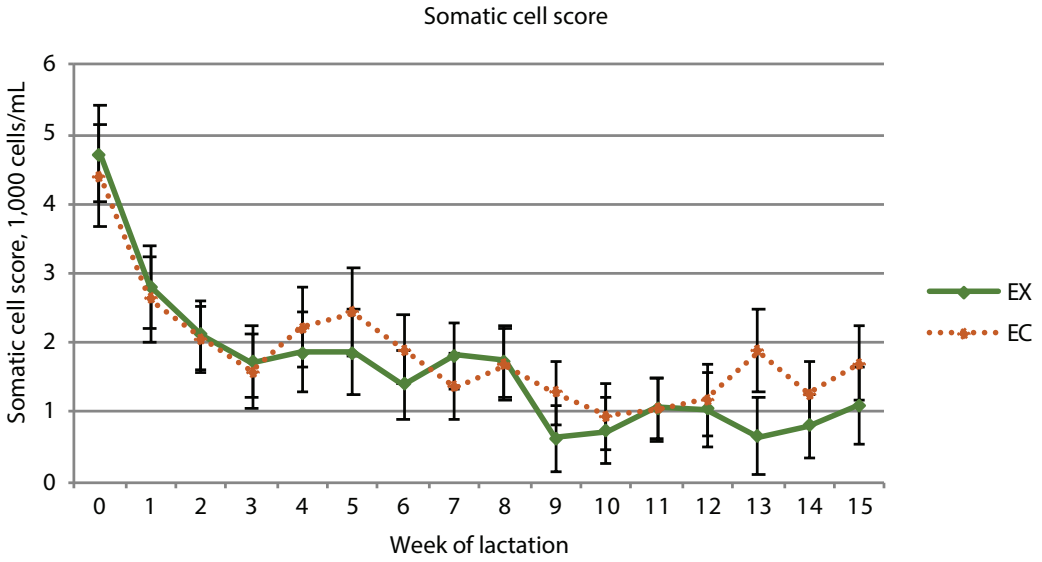

Figure 7. Somatic cell score (SCS) through 15 weeks of lactation. The exercised group of heifers (EX; n = 12) were walked at an average pace of 3 mph for an average of 30 minutes per day for 4 days/week for a total of 8 weeks. The exercise-control group of heifers (EC; n = 12) were brought to the exerciser but did not exercise. All exercise activities were stopped by 21 days prior to parturition. *$P < 0.05$; †$P < 0.10$.

Beta-Hydroxybutyrate Alters the mRNA Cytokine Profile from Mouse Macrophages Challenged with *Streptococcus uberis*

T.H. Swartz, L.K. Mamedova, and B.J. Bradford

Summary

The objective of this study was to determine if β-hydroxybutyrate (BHB) altered inflammatory responses in macrophages challenged with a common mastitis pathogen, *Streptococcus uberis*. Mouse macrophages (RAW 264.7 line) were cultured either in the presence or absence of BHB for 24 h, and then challenged or not with *S. uberis*. Relative transcript abundance of cell membrane receptors (TLR2 and GPR109a), cytokines (IL-1β, IL-10, TNFα, and TGFβ), and chemokines (CXCL2 and CCL5) were determined using quantitative real-time polymerase chain reaction (qPCR) and normalized against the geometric mean of HPRT and B2M. *Streptococcus uberis* activated the macrophages, noted by greater transcript abundance of analyzed genes. Intriguingly, *S. uberis* increased GPR109a mRNA abundance, a receptor that is activated by BHB. Consequently, BHB dose-dependently increased transcript abundance of the pro-inflammatory cytokine (IL-1β) and the anti-inflammatory cytokine (IL-10) but had no effect on TNFα or TGFβ. Moreover, BHB increased mRNA abundance of the chemokines, CXCL2 and CCL5. These data suggest a dysregulated immune response toward *S. uberis* due to BHB treatment, similar to what is seen in transition dairy cows. Future studies should be conducted in vivo to test the effect of BHB on immune function during an intramammary challenge.

Introduction

Mastitis is the most common and costly disease in the dairy industry, impairing animal welfare and decreasing milk production. The incidence of clinical mastitis is dramatically greater during the first few weeks after calving than in the rest of the lactation. At the beginning of lactation, a depression of feed intake occurs simultaneously with an increase in energy demand, resulting in metabolic stress and negative energy balance. Consequently, dairy cattle mobilize fat reserves, liberating non-esterified fatty acids (NEFA). These fatty acids are transported to the liver for energy production. However, not all NEFA are completely oxidized, resulting in the production of β-hydroxybutyrate (BHB), a major ketone body. This ketone body has long been associated with disease in early lactation, but it is important to recognize that association does not discriminate between causative disease mediators and adaptive responses that help resolve the disease. The association between ketosis and mastitis is likely due to the decreased function of host innate immune cells exposed to BHB. When neutrophils were cultured with BHB at various concentrations (0.1 to 8.0 mM), there was a stepwise reduction in extracellular killing of bacteria. Additionally, leukocytes from ketotic cows had a reduced ability to migrate toward an inflammatory response relative to those isolated from non-ketotic cows.

Streptococcus uberis is a common environmental mastitis pathogen that is responsible for a large proportion of mastitis during the first month of lactation, when negative energy balance is exacerbated. Therefore, it seems likely that BHB may be impairing immune responses toward this pathogen in early lactation dairy cattle. Hence, the objective of this experiment was to examine the effect of BHB on inflammatory mediators from macrophages during a *Streptococcus uberis* challenge. We hypothesized that BHB would attenuate inflammatory responses in a dose-dependent manner.

Experimental Procedures

Bacterial Strain and Conditions

Streptococcus uberis (kindly provided by Dr. Petersson-Wolfe, Department of Dairy Science, Virginia Tech) was originally isolated from a dairy cow with mastitis and stored in 10% skim milk at -80°C. Bacteria were streaked on an esculin blood agar plate and incubated for 24 hours. Five colonies were then cultured in Todd-Hewitt broth and incubated for 7 hours at 37°C on an orbital shaker. Bacterial suspension was pelleted with centrifugation, washed with sterile phosphate-buffered saline, and resuspended in Dulbecco's Modified Eagle Medium (DMEM) containing 1% L-glutamine and 10% heat-inactivated fetal bovine serum. Serial dilutions were used to achieve the desired concentration of colony-forming units for challenge, and challenge inoculum concentration was verified using drop plating onto esculin blood agar.

Cell Culture Conditions and Treatments

Mouse macrophages (RAW 264.7 line) were cultured in DMEM supplemented with 1% L-glutamine, 10% heat-inactivated fetal bovine serum, and 0.2% penicillin-streptomycin. Twenty-four-well plates (n = 8 wells per treatment group) were seeded with 1×10^5 cells and incubated for 24 hours at 37°C and 5% CO_2. Cells were then either treated with β-hydroxybutyrate (Sigma Aldrich) at various concentrations (0 mM, 0.6 mM, 1.2 mM, or 1.8 mM) or not for 24 hours to mimic ketosis. To maintain a neutral pH in culture media, β-hydroxybutyrate was added as sodium salt, and a treatment group with 1.8 mM added NaCl was included as an osmotic control. After the 24-hour incubation step, the medium was removed and fresh medium without antibiotics containing BHB at various concentrations (or not) and with or without 5×10^5 CFU/mL of *S. uberis* were added for 6 hours. Cells were then lysed and stored at -80°C.

RNA Isolation and qPCR

Total RNA was isolated from cell lysates using the RNeasy kit (Qiagen) and was quantified using spectroscopy (NanoDrop Technologies Inc., Wilmington, DE). One microgram of total RNA was used as template for the reverse transcriptase reaction using random primers. Quantitative real-time PCR was performed in duplicate with 200 nM gene-specific forward and reverse primers with real-time SYBR green fluorescent detection (7500 Fast Real-Time PCR System, Applied Biosystems). Primers were designed from mouse GenBank sequences and were designed to amplify an intron-spanning region of the gene. Relative mRNA abundance was quantified by the $2^{-\Delta Ct}$ method with the geometric mean of HPRT and B2M used to normalize values.

Statistical Analyses

Statistical analyses were conducted in PROC GLIMMIX SAS v. 9.4 (SAS Inst., Cary, NC). Orthogonal contrasts were performed to test the effect of *S. uberis*, overall effect of BHB within *S. uberis* challenged treatment groups, as well as linear and quadratic contrasts to test BHB dose responses. To meet the assumption of normality (PROC UNIVARIATE), all response variables required natural logarithmic transformation; least square means and standard errors were back-transformed. An outlier was defined if the observation had a studentized residual greater than 3 in absolute value, and therefore was removed from the analysis. Significance was declared at $P \leq 0.05$.

Results and Discussion

Streptococcus uberis Effects

As expected, *Streptococcus uberis* induced the immune activation in macrophages. This was evident through increased mRNA abundance of a pathogen recognition receptor, toll-like receptor 2 (TLR2, $P = 0.03$), and thus downstream increases of both pro- and anti-inflammatory cytokine mRNA abundance. In particular, *S. uberis* increased mRNA abundance (all $P < 0.01$)

of pro-inflammatory cytokines interleukin (IL)-1β and tumor necrosis factor α (TNFα), as well as anti-inflammatory cytokines IL-10 and transforming growth factor β (TGFβ). Moreover, *S. uberis* increased mRNA abundance of two chemokines (CXCL2 and CCL5), which are proteins used to attract immune cells into inflamed tissue. Lastly, *S. uberis* increased mRNA abundance of GPR109a ($P < 0.01$), a receptor for BHB that has known anti-inflammatory effects when activated. This is intriguing, as these data could imply that *S. uberis* promotes immunological tolerance, thus impairing the immune system's ability to kill this pathogen. Regardless, an increase in GPR109a should result in macrophages that are more responsive to BHB ligation.

Beta-Hydroxybutyrate Effects

Beta-Hydroxybutyrate increased mRNA abundance of both pro- and anti-inflammatory cytokines, although these data should be interpreted with caution. First, BHB dose-dependently increased mRNA abundance of the potent anti-inflammatory cytokine IL-10 (overall BHB effect, $P = 0.01$; linear BHB effect, $P < 0.01$) when compared to *S. uberis* challenged cells. Yet, BHB also dose-dependently increased mRNA abundance of the pro-inflammatory cytokine IL-1β (overall BHB effect, $P < 0.01$; linear BHB effect, $P < 0.01$). Interleukin-1β data should be interpreted cautiously; this cytokine is post-translationally regulated more so than other cytokines, so a future study must confirm if the active, secreted protein form of this cytokine was also increased by BHB. An increase in both IL-1β and IL-10 is likely due to increased abundance of the receptor that controls their expression, TLR2 (overall BHB effect, $P < 0.01$; linear BHB effect, $P = 0.01$). Lastly, BHB increased mRNA abundance of chemokines CXCL2 (overall BHB effect $P = 0.01$; linear BHB effect, $P = 0.04$) and CCL5 (overall BHB effect, $P = 0.03$; linear BHB effect, $P = 0.07$). Again, these data could imply a more robust immune response, as a greater abundance of chemokines should result in more immune cells migrating into inflamed tissue in vivo. However, in a similar study, BHB also increased chemokine mRNA abundance, yet this did not result in an increase in immune cells migrating into tissue. Thus, an increase in chemokine mRNA abundance may simply be a compensatory response in attempt to overcome a reduction in their efficacy. Future studies should examine protein abundance of these cytokines to ensure the transcriptional effects from BHB treatment indeed alter cytokine and chemokine protein profiles.

Conclusions

Streptococcus uberis is responsible for a large proportion of mastitis during the first month of lactation. In the present study, *S. uberis* increased GPR109a mRNA abundance, a receptor that is ligated by BHB. This resulted in a dose-dependent increase in mRNA abundance of both pro- and anti-inflammatory cytokines, including IL-1β and IL-10. An increase in the abundance of these cytokines could be indicative of the immune dysfunction that is typically seen in transition dairy cows. Future studies should be conducted in vivo to test the effect of BHB on immune function during an intramammary challenge.

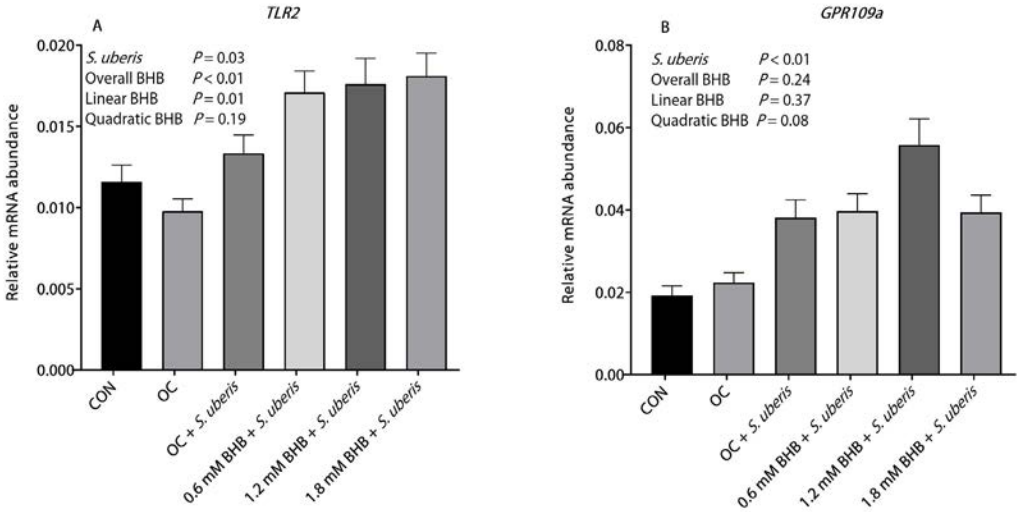

Figure 1. Effect of *S. uberis* and BHB on receptor (TLR2, A; GPR109a, B) transcript abundance in RAW 264.7 mouse macrophages. *Streptococcus uberis* challenge increased mRNA of the receptor for identification of Gram-positive pathogens, TLR2 ($P = 0.03$), as well as mRNA abundance of the receptor for BHB, GPR109a ($P < 0.01$). Beta-Hydroxybutyrate treatment linearly increased TLR2 mRNA abundance ($P = 0.01$) when compared to cells treated with just *S. uberis*. Treatment groups include: control (CON), OC (osmotic control), OC + *S. uberis*, 0.6 mM BHB + *S. uberis*, 1.2 mM BHB + *S. uberis*, and 1.8 mM BHB + *S. uberis*.

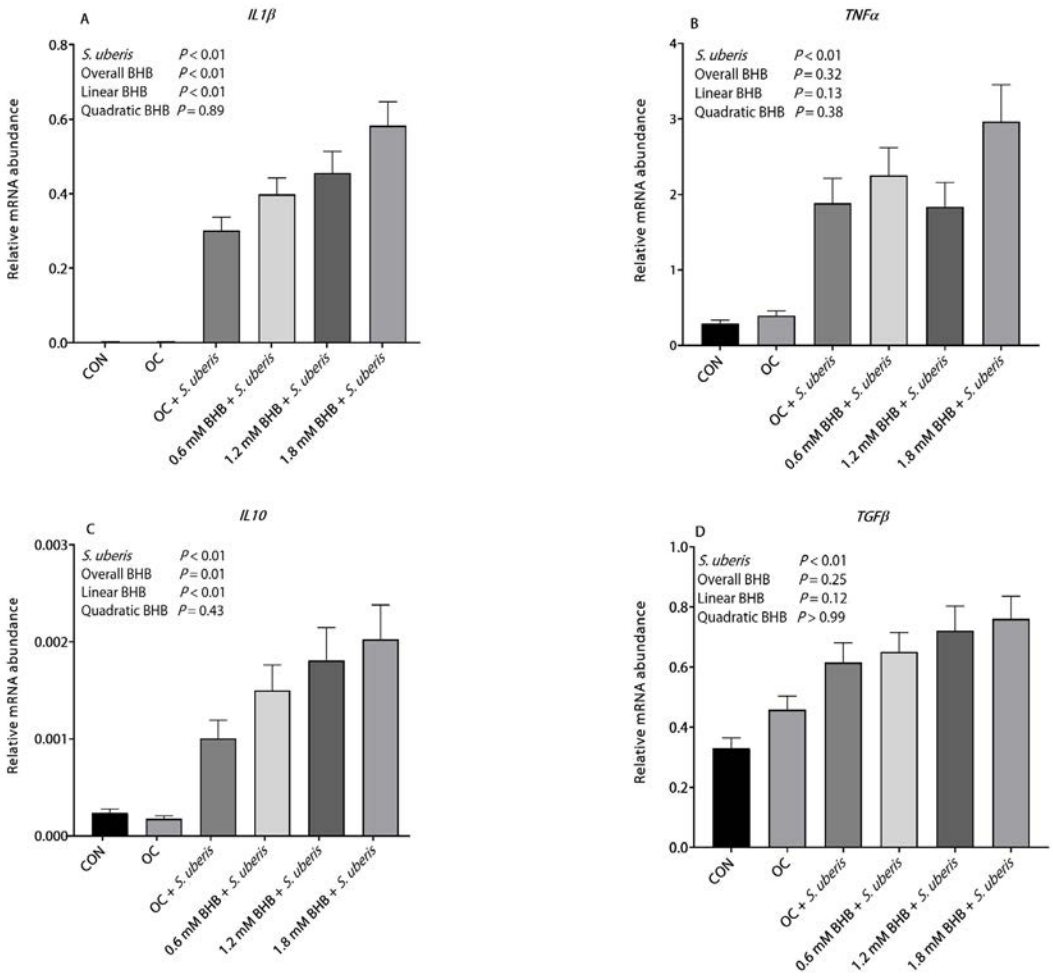

Figure 2. Effect of *S. uberis* and BHB on pro-inflammatory (IL-1β, A; TNFα, B) and anti-inflammatory (IL-10, C; TGFβ, D) cytokine transcript abundance in RAW 264.7 mouse macrophages. *Streptococcus uberis* challenge activated the macrophages, increasing both pro- and anti-inflammatory cytokine mRNA abundance (all $P < 0.01$). Beta-Hydroxybutyrate treatment linearly increased IL-1β ($P < 0.01$) and IL-10 ($P < 0.01$) mRNA abundance when compared to cells treated with just *S. uberis*, however, no effect of BHB was found on either TNFα or TGFβ. Treatment groups include: control (CON), OC (osmotic control), OC + *S. uberis*, 0.6 mM BHB + *S. uberis*, 1.2 mM BHB + *S. uberis*, and 1.8 mM BHB + *S. uberis*.

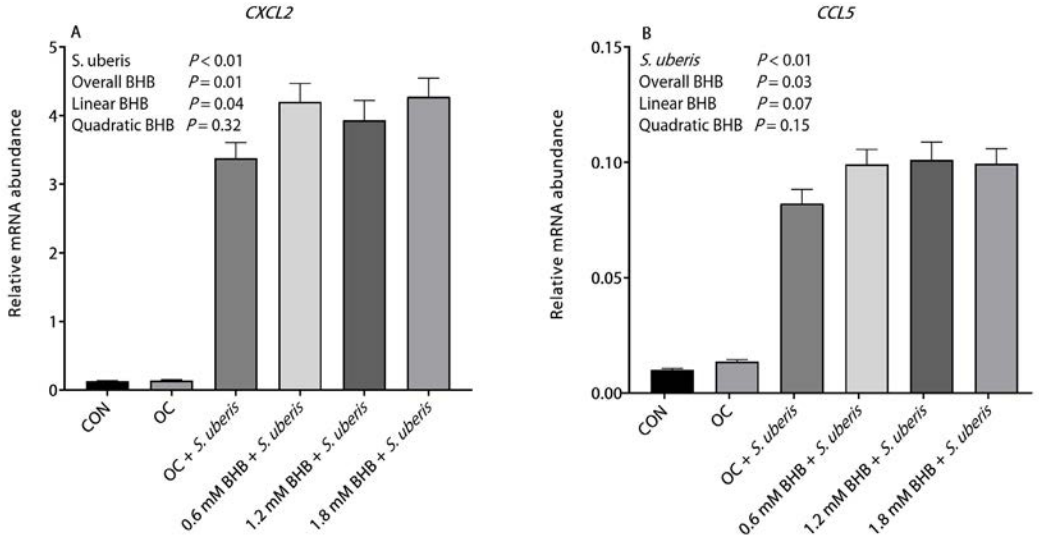

Figure 3. Effect of *S. uberis* and BHB on chemokine (CXCL2, A; CCL5, B) transcript abundance in RAW 264.7 mouse macrophages. *Streptococcus uberis* challenge activated the macrophages, increasing both CXCL2 and CCL5 mRNA abundance (both $P < 0.01$). Beta-Hydroxybutyrate treatment linearly increased CXCL2 ($P = 0.04$) as well as increased CCL5 ($P = 0.03$) mRNA abundance when compared to cells treated with just *S. uberis.* Treatment groups include: control (CON), OC (osmotic control), OC + *S. uberis*, 0.6 mM BHB + *S. uberis*, 1.2 mM BHB + *S. uberis*, and 1.8 mM BHB + *S. uberis*.

Individual Feed Intake of Transition Cows and Their Daily Activity Measures of Temperature, Eating, Rumination, Resting, and Activity Times

J.S. Stevenson, C.S. Takiya, and B.J. Bradford

Summary

Fifteen transition dairy cows bearing CowSensor® ear tags were monitored during 14 days before and after calving to assess temperature and behavior outcomes recorded by the sensors, in addition to actual individual dry matter and as-fed feed intake. The sensors—compared with reported visual observation studies—underestimated eating and resting times, but rumination time was estimated reasonably accurately. Expected changes in rumination (decreased acutely before calving and increased linearly to day 14) and general activity (increased acutely just before calving) were observed. More studies are warranted to determine how to use these activity monitors in detecting health disorders of cows that affect milk yield.

Introduction

Activity monitors can provide valuable management information about important behaviors of dairy cows that are correlated with economic traits associated with health, milk yield, and estrus detection. In the United States, more than a dozen different companies are marketing various types of activity monitors. All activity monitoring systems include three basic components: (1) sensor for each cow; (2) hardware receiver to collect the data from the sensors; and (3) computer software that outputs alerts and levels of activity. Sensors are presently in the form of either pastern-mounted pedometers, collar-mounted monitors, ear sensors, or rump-mounted transmitters. All sensors transfer data either using radio frequency or infrared technology to some configuration of a reader that transmits the data, usually in binary code, to a coordinator that translates and decodes the signal. The software is either located in an on-farm computer or a server that receives the information via web or cloud-based technology where the proprietary algorithms sort the information and determine which individual cows need attention. Website programs and apps, email alerts, text messages, and smartphone apps are available with most systems.

Measurements associated with rumination and its relationship to health was a comparatively new function in mid-2012. Some systems, such as the SCR system (Heatime®), track rumination with a tuned microphone that detects sounds of the bolus passing up and down the esophagus and has been reported as a reliable source for detection of rumination (correlations = 0.94) when compared with visual observations of rumination minutes. The CowManager CowSensor® ear tags monitor ear and jaw movements and are used for detecting rumination, eating, resting, or active classifications by a three-dimensional accelerometer. The overall kappa values for the comparison of CowSensor® and visual observation was 0.78, with kappa values of 0.85, 0.77, 0.86, and 0.47 for rumination, eating, resting, and active, respectively. Similar to correlation coefficients, kappa values can range from −1 to +1, where zero represents the amount of agreement that can be expected from random chance, and 1 represents perfect agreement between the tested methods. Pearson correlation and concordance correlation coefficients between CowSensor® and visual observations for rumination, eating, resting, and active minutes per hour were 0.93, 0.88, 0.98, and 0.73 and 0.93, 0.75, 0.97, and 0.35, respectively. Peer-reviewed publications provide strong evidence that at least these two systems (SCR and CowSensor®) can be used to monitor ruminating and resting behavior of free stall-housed dairy cattle.

Our interest was in monitoring these important behaviors in transition cows, and examining their respective relationships during the transition period. Therefore, our objective was to describe the daily variations in temperature, measures of eating, rumination, and resting times, in addition to daily measures of activity in transition dairy cows.

Experimental Procedures

Close-up cows (n = 15) enrolled in the study between July 2017 and March 2018 were housed in an open-front, straw-bedded maternity barn equipped with automatic feed stations (Insentec RIC System, Hokofarm Group, Marknesse, the Netherlands) to monitor individual feed intake. After calving, cows were housed in individual tie stalls bedded with sawdust, fed individually, and milked thrice daily. Cows were fitted with CowSensor® ear tags (Agis CowManager, the Netherlands) during midgestation to monitor temperature, daily measures of eating, rumination, and resting times, in addition to daily measures of activity.

Data were captured hourly from the ear tags, stored in a cloud (remote server system), and downloaded daily into Excel spreadsheets. We averaged the hourly data to produce a daily mean of each activity captured by the ear tag, and measured actual feed intake in the same cows. This was a preliminary effort to examine these relationships to determine how they may be used in the future to predict and monitor important production and health traits of dairy cows.

Results and Discussion

To maximize cow comfort and health, it is generally believed that a daily time budget for a lactating dairy cow should consist of: (1) eating (3 to 5 hours); (2) lying and resting (12 to 14 hours); (3) rumination (7 to 10 hours); (4) social interactions (2 to 3 hours); (5) drinking (0.5 hours); and (6) no more than 2.5 to 3 hours in the holding pen and milking parlor. Note that the total hours per day exceed 24 hours because some activities occur concurrently such as lying and resting, and ruminating. In other words, cows excel as multitaskers.

The CowSensor® software sends estrus and health alerts based on a combination of behaviors and temperature detected by the ear tag sensors that are deciphered by proprietary algorithm software. For the 15 cows studied, health alerts were transmitted for three cows and estrus alerts for seven cows during the 48 hours surrounding the calving process. Only two cows had both alerts transmitted. The average 305-day mature-equivalent milk yield of the 15 cows studied was 32,631 lb and ranged from 26,512 to 38,165 lb.

Time and Intake Outcomes

Dry matter intake, as-fed feed intake, and eating and rumination times during the prepartum and postpartum periods are illustrated in Figure 1. Rumination decreased gradually as calving approached. It decreased acutely during the last 24 hours of gestation and bottomed out by 48 hours after calving. Thereafter, rumination increased linearly to day 14 after calving. On average, relative to before calving, rumination time increased ($P < 0.001$) by 10% during the post-calving period to 7.7 hours/day (Table 1).

Eating time, dry matter intake, and as-fed intake were relatively unchanged until 24 hours before calving when feed intake increased acutely after calving, whereas eating time decreased. On average, as-fed intake increased ($P < 0.001$) 41% and dry matter intake increased 24% after calving, whereas eating time decreased ($P < 0.001$) by 44% from prepartum to postpartum periods.

Resting and high activity (data not shown) times were relatively unchanged during the prepartum and postpartum periods (Figure 2). On average, measures of resting (no activity) did not differ between prepartum and postpartum periods (Table 1). In contrast, general activity increased acutely during 24 hours before calving and remained elevated for several days before

slowly decreasing to day 14. On average, activity increased ($P < 0.001$) by 17% from the prepartum to postpartum period. Temperature increased gradually as calving approached and continued to increase only slightly to day 14. On average, temperature of cows did not differ from the prepartum to the postpartum period.

Correlations

Correlations among prepartum outcomes are shown in Table 2. Dry matter intake was correlated negatively with the estimated eating time and resting time, but correlated positively with rumination and temperature data collected via ear tags. Eating time and rumination were associated negatively with resting and activity. Rumination was positively correlated with temperature. As might be expected, resting was associated negatively with activity.

Correlations among postpartum outcomes are shown in Table 3. Dry matter intake was associated negatively with temperature, whereas eating time was correlated negatively with resting and activity. As with prepartum rumination times, rumination after calving was associated negatively with resting and activity. Postpartum activity was related positively with ear tag temperature.

General Discussion

Eating time was underestimated by the CowSensor® compared with other known visual observations of 3 to 5 hours per day. This underestimation in eating time is partly explained by the difference in energy density of diets fed before and after calving. Resting times of 7 to 8 hours per day were underestimated by the CowSensor® compared with visual observations of 12 to 14 hours per day. In contrast, rumination time was quite accurate and was in the range of 7 to 10 hours based on visual observations (Table 1). Rumination time is an important part of the system's ability to provide health alerts that also are tied to no activity or resting time and eating time.

Although the absolute hour measures of this system to estimate various behaviors are not consistent with accepted time budget results, their relative measures to identify outliers for health and estrus alerts are good. We have found in our general herd management that the CowSensor® does a great job of identifying cows with digestive issues hours before we visually identify these cows. The system showed that rumination time plummeted while inactive or resting status increased in these cows. Furthermore, rumination times also decrease acutely during 24 to 48 hours before cows come into estrus.

Conclusions

These preliminary results are promising for identifying behaviors that are associated with health and production while demonstrating their ability to measure behaviors typically observed visually in transition dairy cows.

Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.

Table 1. Average prepartum (days -14 to -1; day 0 = calving) and postpartum (days 0 to 14) daily feed intake and daily activity monitor characteristics in 15 transition dairy cows

Item	Prepartum	Postpartum	*P*-value
Feed intake (lb/day)	41.8 ± 0.9	58.8 ± 0.8	<0.001
Dry matter intake (lb/day)	26.4 ± 0.4	32.7 ± 0.4	<0.001
Eating time (hours/day)	1.8 ± 0.04	1.0 ± 0.05	<0.001
Rumination (hours/day)	8.3 ± 0.1	8.6 ± 0.1	0.06
Resting (hours/day)	8.1 ± 0.1	8.1 ± 0.1	0.71
Activity (hours/day)	2.9 ± 0.04	3.4 ± 0.04	<0.001
Temperature[1] (°C)	27.8 ± 0.1	28.1 ± 0.1	0.13

[1]Ear tag measures the temperature around the ear, which is slightly warmer than the environmental temperature.

Table 2. Simple correlations among prepartum dry matter intake, cow temperature, and behavioral traits (days -14 to -1; day 0 = calving)

	Eating	Rumination	Resting	Activity	Temperature[1]
Dry matter intake (lb/day)	-0.22**	0.43**	-0.30**	0.20*	0.40**
Eating time (hours/day)		0.17*	-0.37**	-0.40**	-0.26**
Rumination (hours/day)			-0.73	-0.14	0.29**
Resting (hours/day)				-0.25**	-0.12
Activity (hours/day)					0.04

**$P < 0.01$.
*$P < 0.05$.
[1]Ear tag measures the temperature around the ear, which is slightly warmer than the environmental temperature.

Table 3. Simple correlations among postpartum dry matter intake, cow temperature, and behavioral traits (days 0 to 14 after calving)

	Eating	Rumination	Resting	Activity	Temperature[1]
Dry matter intake (lb/day)	-0.10	0.51**	-0.42**	-0.02	0.02
Eating time (hours/day)		0.03	-0.47**	-0.22**	0.05
Rumination (hours/day)			-0.60**	-0.42**	0.03
Resting (hours/day)				-0.11	-0.13
Activity (hours/day)					0.32**

**P < 0.01.
*P < 0.05.
[1]Ear tag measures the temperature around the ear that is slightly warmer than the environmental temperature.

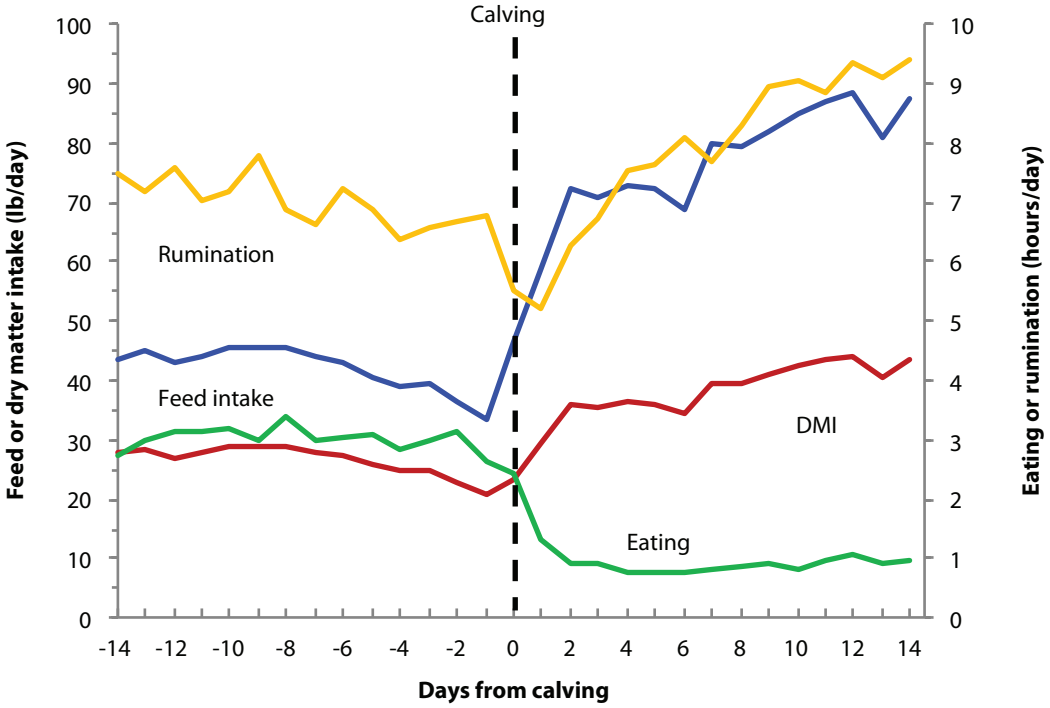

Figure 1. Daily dry matter intake (DMI; red), daily as-fed intake (blue), eating time (green), and rumination time (yellow) in 15 transition dairy cows during 14 days before and after calving.

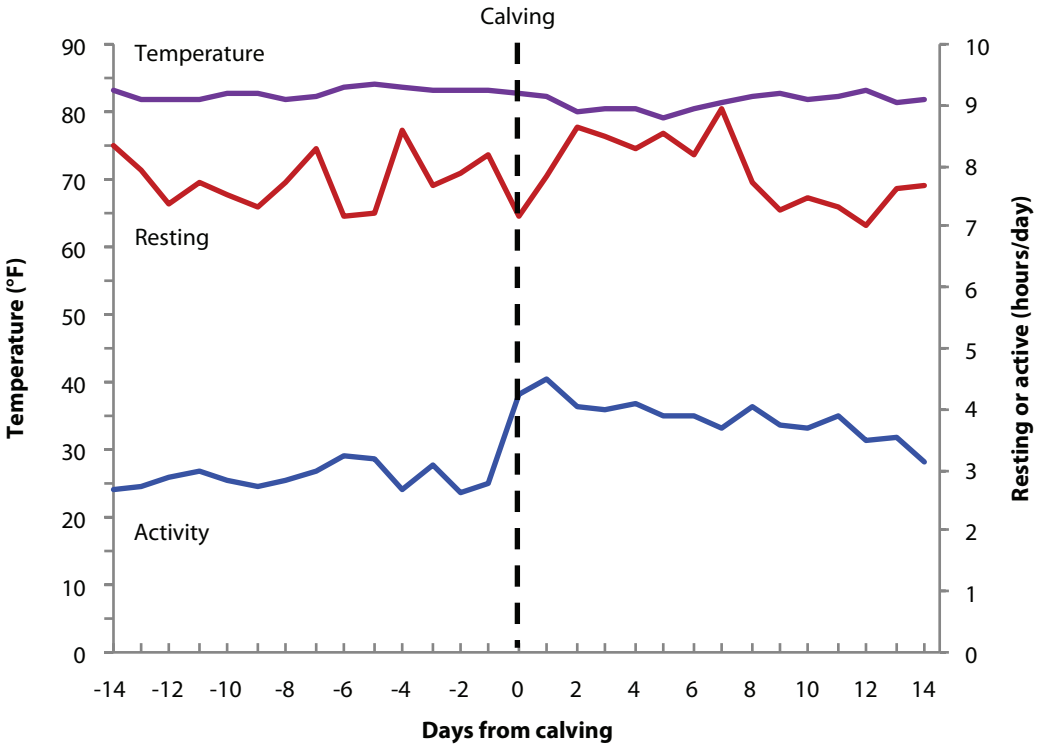

Figure 2. Daily ear tag temperature (purple), resting time (red), and activity time (blue) in 15 transition dairy cows during 14 days before and after calving.

Effects of a High-Protein Corn Product on Nutrient Digestibility and Production Responses in Mid-Lactation Dairy Cows

W.E. Brown and B.J. Bradford

Summary

An experiment was conducted to assess the effects of a high-protein corn product (56% crude protein; CP) relative to other sources of protein on the lactation performance of dairy cows. Twenty-four Holstein cows (1,367 ± 105 lb of body weight, 111 ± 34 days in milk, 2.28 ± 0.46 lactations; mean ± standard deviation) were randomly assigned to treatment sequence in a replicated 4 × 4 Latin square design balanced for carryover effects. Cows were individually fed one of four diets with a different protein concentrate source during each 28-day period, including: soybean meal (SBM), high-protein corn product (HPCP), soybean meal with rumen-bypass soy protein (SBMBP), and canola meal with rumen-bypass soy protein (CANBP). Diets were formulated for equal concentrations of CP and balanced to meet lysine and methionine requirements. The SBM diet was formulated to provide 5.7% rumen-undegradable protein (RUP), while SBMBP and CANBP diets were formulated for 6.8% RUP to match HPCP. The CANBP diet increased dry matter intake compared with SBM and HPCP. Treatment affected milk yield, as SBMBP and CANBP increased yield compared with SBM, but HPCP decreased milk yield compared to all treatments. HPCP reduced CP intake as a percent of total intake and increased the CP content of feed refusals, indicative of selection against HPCP. HPCP decreased apparent total tract CP digestibility, leading to less urine nitrogen excretion and greater fecal nitrogen output. SBMBP and CANBP performed equally in nearly every variable measured, except SBMBP increased milk urea nitrogen concentration. In conclusion, the HPCP diet reduced milk yield, milk component yields, urine nitrogen excretion, and increased fecal nitrogen excretion due to lesser total tract CP digestibility.

Introduction

Corn grain is a useful feed ingredient in lactating dairy cow diets, serving as a readily available energy substrate; however, it is low in crude protein (approximately 7%). Through several methods of corn processing to produce value-added products such as ethanol or corn sweeteners, the composition of the resulting co-products possess elevated concentrations of protein, which have beneficial use to livestock as a protein source.

Dry-milling is the most common process used for ethanol production and has resulted in large quantities of distillers grains with solubles (DGS) available for livestock feed in the United States. Since most of the starch has been removed from the grain kernel after fermentation, DGS has an elevated composition of crude protein compared with the whole kernel at approximately 27 to 31%. Removal of bran and germ components before fermentation with wet- or dry-milling enhances the efficiency of ethanol production while further increasing the protein concentration of the resulting distillers grains from approximately 25% to more than 40%. Removal of the fat portion of the DGS increases protein concentration marginally to approximately 34%. Distillers grains with solubles have been fed to beef and dairy cattle successfully as a protein source, and the inclusion has become more common as ethanol production grows.

More nascent technologies are being evaluated to further add value to DGS. One concept includes using sieving and sedimentation to separate particles based upon size and density, which can increase protein concentration up to 40%. Distillers grains with solubles are a potentially attractive feedstock for second-generation, fiber-based ethanol production because of the fiber

content and ability to streamline production processes. Considering the protein concentration of DGS and that second-generation ethanol production only utilizes fiber and residual starch, a co-product high in crude protein is produced (34 to 50%). The ability to cost-effectively integrate various technologies into existing production processes will ultimately determine their viability in the future, and the feeding value of these co-products is yet to be determined.

Not all protein sources are created equally because of differences in amino acid (AA) profiles. The two most limiting AA in the lactating dairy cow are generally lysine and methionine. Lysine concentration as a percentage of CP is greatest in soybean meal and least in corn products, while methionine concentration is greatest in canola meal and least in soybean meal. When diets are formulated for similar CP concentration, the lack of methionine or lysine in certain protein concentrates may inhibit performance if there are insufficient essential AA provided in other forms. Therefore, the objective of this experiment was to assess the productivity responses of high-producing Holstein cows by replacement of common protein sources with a proprietary high-protein corn product, while maintaining consistent concentrations of methionine and lysine across diets, and to assess nitrogen digestibility and retention.

Experimental Procedures

Twenty-four multiparous Holstein cows near peak lactation (111 ± 34 DIM, 2.3 ± 0.46 lactations, mean ± SD) from the Kansas State University Dairy Teaching and Research Center (Manhattan, KS) were randomly assigned to treatment in a replicated 4 × 4 Latin square design balanced for carryover effects. Cows were housed in a tie-stall barn. Treatment periods were 28 d, with 25 d for diet adaptation and 3 d for sample collection. Cows were fed twice daily and milked thrice daily.

Cows were individually fed 1 of 4 diets *ad libitum* with a different protein concentrate source during each 28-d period, including: soybean meal (SBM), high-protein corn product (HPCP), soybean meal with bypass protein (SBMBP) and canola meal with bypass protein (CANBP). A base total mixed ration was delivered to each cow with treatment top-dressed and mixed by hand at each feeding. Diets were formulated for equal concentrations of CP and balanced to meet lysine and methionine requirements. The SBM diet was formulated to provide 5.7% rumen-undegradable protein (RUP), while SBMBP and CANBP diets were formulated for 6.8% RUP to match HPCP. The diets also were formulated to provide equal concentrations of forage NDF and starch. Nutrient composition and ingredients are shown in Table 1.

Feed offered and refused for each cow was recorded daily during the final 3 days of each treatment period. Water intake was recorded daily. Milk samples were collected at every milking during the final three days of each treatment period and analyzed for composition by MQT Labs (Kansas City, MO). Fecal grab samples were collected for determination of total tract digestibility, and urine samples were collected. Total daily urinary N excretion was calculated using the estimated volume of urine excretion from creatinine analysis.

Results were analyzed using mixed models to account for the fixed effects of treatment, period, treatment × period interaction, and the random effect of cow. Differences were declared significant when $P < 0.05$ according to Tukey's honestly significant difference.

Results and Discussion

Dry matter intake and water intake responses to treatment are shown in Table 2. The CANBP increased the dry matter intake compared with SBM and HPCP ($P < 0.01$). Cows appeared to selectively refuse the HPCP, resulting in a higher concentration of CP in the refusals and decreased total nitrogen intake for this treatment ($P < 0.001$). The HPCP decreased milk yield compared with all other treatments, but SBMBP and CANBP increased milk yield compared

with other treatments ($P < 0.001$; Table 3), which likely drove the increased intake response for CANBP. The HPCP decreased milk protein concentration, milk urea nitrogen concentration ($P < 0.01$), and yields of fat, protein, and energy-corrected milk ($P < 0.001$).

As shown in Table 4, HPCP decreased urine nitrogen excretion compared with SMB and CANBP ($P < 0.02$), and it increased fecal nitrogen excretion compared with all other treatments ($P < 0.001$). The pattern of nitrogen flow in this scenario aligns with decreased protein digestibility typical of over-heated protein products. Heating feed products is a process used to reduce moisture concentration for long-term stability or to reduce ruminal digestibility to increase nitrogen available for intestinal absorption (RUP), compared with bacterial degradation for use by ruminal microbes. However, too much heating renders the protein indigestible by either microbial or mammalian enzymes, leading to passage of the nitrogen through the feces. With less protein digested in the intestinal tract, there was less nitrogen absorbed to be excreted in the urine and milk.

The reduction in milk production by HPCP could be the result of two issues: 1) less consumption of total CP from cows sorting against the HPCP, and 2) a reduction in total tract nitrogen digestibility. Lactating dairy cows often sort against (preferentially avoid) longer particles to achieve a more energy dense diet. However, the increased CP concentration of feed refusals for cows fed HPCP suggests that the cows did not prefer the HPCP, despite it being a nutrient-dense product. Analysis of the concentrate top-dresses including the protein supplements revealed up to an 80-fold increase in acid-detergent insoluble crude protein (ADICP; Table 5). The ADICP is a measurement of heat treatment of feedstuffs and Maillard product formation. Maillard product formation decreases feed digestibility and can be seen visually as a darker color imparted to the product with increased heating. The HPCP was very dark in color, supporting the speculation that the product was overheated during the drying process.

The diets in this study were formulated to achieve equal concentration of CP and were balanced to meet lysine and methionine requirements of the cows, partially negating differences in AA profiles between corn, soybean, and canola products. The increase in milk production in SBMBP and CANBP compared with SBM, despite meeting lysine and methionine requirements, demonstrates that increasing RUP supply to the animal (beyond meeting limiting essential AA requirements) may still have positive effects on milk production. This is counter to the thinking that meeting the requirements for the most limiting essential amino acids, methionine and lysine, is all that is needed to enhance milk production.

In other studies, canola meal has been a successful substitute for soybean meal in lactating dairy cow diets, either maintaining or increasing milk production. In the present study, canola meal performed similarly to soybean meal when bypass protein was provided and lysine and methionine were supplemented to meet the cows' requirements. Canola meal can serve as a useful substitute for soybean meal in dairy rations, assuming market conditions are favorable for its use.

Conclusions

In summary, cows fed the HPCP diet produced less milk and milk components. Cows on HPCP consumed less CP and had lower apparent total tract CP digestibility, suggesting that the HPCP production process may have resulted in Maillard production formation, consistent with the increased ADICP measured in this treatment mix. The SBMBP and CANBP diets performed similarly when balanced for methionine and lysine, supporting previous data demonstrating canola meal as a successful substitute for soybean meal. Further efforts to maximize protein quality from high-protein corn product production processes are warranted.

Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.

Table 1. Ingredients and nutrient composition of experimental diets

	Diet[1]			
Item, % dry matter	SBM	HPCP	SBMBP	CANBP
Dietary ingredients				
Corn silage	34.5	34.5	34.5	34.5
Alfalfa hay	19.5	19.5	19.5	19.5
Soybean meal, 47.5% solvent extracted	10.7	...	3.9	...
High protein corn product[2]	...	9.4	...	...
Canola meal, 37% solvent extracted	...	...	...	8.9
Expeller soybean meal[3]	...	...	8.0	4.7
Ground corn grain	22.6	22.6	22.6	22.6
Soybean hulls	7.8	8.8	6.8	5.0
Ca salts of long chain fatty acids[4]	0.75	0.75	0.75	0.75
Lysine hydrochloride[5]	0.32	0.68	0.11	0.26
HMBi[6]	0.24	0.17	0.17	0.15
Micronutrient premix[7]	3.6	3.7	3.6	3.6
Diet nutrient composition				
Dry matter	52.8	52.6	52.9	52.9
Crude protein	16.8	16.8	16.8	16.8
aNDFom[8]	30.2	30.3	30.0	30.3
Starch	22.5	22.7	22.6	22.7
Ether extract	4.3	4.9	4.5	4.7
Ash	8.2	8.1	8.2	8.3

[1]SBM = soybean meal. HPCP = high-protein corn product. SBMBP = soybean meal + bypass protein. CANBP = canola meal + bypass protein.
[2]Proprietary high-protein corn product.
[3]SoyPlus (Landus Cooperative, Ames, IA).
[4]Megalac-R (Arm & Hammer Animal Nutrition, Princeton, NJ).
[5]Lysine hydrochloride, lecithin, and hydrogenated vegetable oil, AjiPro-L (Ajinimoto Animal Nutrition North America, Chicago, IL).
[6]Isopropyl ester of 2-hydroxy-4-methylthio butanoic acid, MetaSmart (Adisseo Inc., Antony, France).
[7]The premix consists of 30.5% limestone, 20.8% sodium bicarbonate, 30.5% Kruse lactation premix, 4.40% trace mineral salt, 4.42% white salt, 7.08% magnesium oxide, 4.42% vitamin E premix, 0.69% Zinpro 4-plex, 0.35% Zinpro 120, and 0.19% Rumensin 90.
[8]Amylase-treated neutral detergent fiber, ash-free.

Table 2. Dry matter and water intake

	Diet[1]					
Item	SBM	HPCP	SBMBP	CANBP	SEM	*P*-value
DMI, lb/d	60.8[b]	60.2[b]	61.9[ab]	63.7[a]	1.00	<0.01
Feed refusal CP, %	15.5[b]	18.8[a]	14.6[b]	15.0[b]	0.37	<0.001
Nitrogen intake, g/d	753[ab]	722[b]	762[a]	783[a]	12.7	<0.001
CP intake, % of total DMI	17.0[a]	16.5[b]	17.0[a]	16.9[a]	0.04	<0.001
Water intake, gal/d	28.7[ab]	29.2[a]	29.6[a]	27.2[b]	0.79	<0.001

[1]DMI = dry matter intake. CP = crude protein. SBM = soybean meal. HPCP = high-protein corn product. SBMBP = soybean meal + bypass protein. CANBP = canola meal + bypass protein.
[abc]Means with different superscripts within a row are significantly different by Tukey's HSD ($P < 0.05$).

Table 3. Production of milk, milk components, and milk nitrogen excretion

	Diet[1]					
Item	SBM	HPCP	SBMBP	CANBP	SEM	*P*-value
Milk, lb/d	87.9[b]	82.5[c]	92.6[a]	93.5[a]	3.94	<0.001
Fat, %	3.86	3.91	3.88	3.87	0.10	0.86
Protein, %	3.08[a]	3.00[b]	3.03[ab]	3.04[ab]	0.04	<0.01
Lactose, %	4.81	4.84	4.84	4.84	0.03	0.59
Solids non-fat, %	8.57	8.53	8.54	8.55	0.06	0.59
Milk urea nitrogen, mg/dL	11.7[a]	10.0[c]	11.6[a]	10.8[b]	0.34	<0.01
Somatic cells, 1,000 cells/mL	102	61	72	105	37.0	0.41
Fat, lb/d	3.31[bc]	3.17[c]	3.48[ab]	3.55[a]	0.11	<0.001
Protein, lb/d	2.67[a]	2.47[b]	2.80[a]	2.82[a]	0.11	<0.001
Lactose, lb/d	4.23[b]	3.97[c]	4.50[a]	4.54[a]	0.18	<0.001
Solids non-fat, lb/d	7.52[b]	6.99[c]	7.89[ab]	7.98[a]	0.33	<0.001
Fat-corrected milk, lb/d	91.5[b]	86.9[b]	96.6[a]	97.9[a]	3.31	<0.001
Energy-corrected milk, lb/d	91.9[b]	86.6[c]	96.8[ab]	97.9[a]	3.40	<0.001
Feed efficiency, lb FCM/lb DMI	1.52[ab]	1.49[b]	1.56[a]	1.52[ab]	0.03	0.06
Milk nitrogen secretion, g/d	198[a]	180[b]	204[a]	207[a]	8.2	<0.001

[1]SBM = soybean meal. HPCP = high-protein corn product. SBMBP = soybean meal + bypass protein. CANBP = canola meal + bypass protein.
[abc]Means with different superscripts within a row are significantly different by Tukey's HSD ($P < 0.05$).

Table 4. Urine and feces nitrogen excretion and apparent total tract crude protein digestibility

	Diet[1]					
Item	SBM	HPCP	SBMBP	CANBP	SEM	*P*-value
Urine nitrogen excretion, g/d	348[a]	255[b]	308[ab]	338[a]	23.9	0.02
Fecal nitrogen excretion, g/d	264[c]	313[a]	270[bc]	287[b]	6.8	<0.001
Apparent total-tract nitrogen digestibility, %	65.1[a]	56.5[c]	64.6[ab]	63.4[b]	0.47	<0.001

[1]SBM = soybean meal. HPCP = high-protein corn product. SBMBP = soybean meal + bypass protein. CANBP = canola meal + bypass protein.
[abc]Means with different superscripts within a row are significantly different by Tukey's HSD ($P < 0.05$).

Table 5. Post-hoc nutrient analysis of treatment top-dresses

	Diet[1]			
Item, % dry matter	SBM	HPCP	SBMBP	CANBP
Top-dress nutrient composition				
Dry matter	91.7	87.5	92.4	92.1
Crude protein	36.1	35.8	35.6	35.7
aNDFom[2]	16.5	17.1	15.7	17.1
ADICP[3]	0.1	7.9	0.1	1.0
Starch	12.8	13.6	13.5	13.9
Ether extract	7.7	10.9	8.9	9.7
Ash	6.8	6.1	6.5	7.4

[1]SBM = soybean meal. HPCP = high-protein corn product. SBMBP = soybean meal + bypass protein. CANBP = canola meal + bypass protein.
[2]Amylase-treated neutral detergent fiber, ash-free.
[3]Acid detergent insoluble crude protein, a measure of Maillard products.

Identifying a Milk-Replacer and Weaning Strategy for Holstein Calves Using Automated Behavioral Measures of Lying and Environmental Enrichment Device Use

E.M. Bortoluzzi, T.M. Hill,[1] L.L. Deikun,[1] L.A. Ruiz, M.J. Goering, T.S. Dennis,[1] F.X. Suarez-Mena,[1] J.D. Quigley,[1] R.L. Schlotterbeck,[1] M.V. Alonso,[2] R. Mazloom,[3] M. Jaberi-Douraki,[3] and L.E. Hulbert

Summary

In dairy production, "weaning readiness" is often based on solid feed intake. The goal of this study was to determine weaning readiness using feed-intake, lying-behaviors, and the use of an environmental enrichment device (EED) in calves that underwent 1 of 4 milk-replacer and weaning protocols. Twenty-eight male Holstein calves (95 ± 2.6 lb BW at 1 d of age) were housed in individual pens and initially fed one type of milk replacer (25% crude protein (CP), 17% fat, 1.45 lb of dry matter (DM)) via nipple-buckets twice a day (AM and PM), and one type of textured calf starter (*ad libitum*; 20% CP and 37% starch). At age 3 days, calves were randomly assigned to one of the four nutrition-weaning strategies:

1. MOD-STEP - 1.46 lb per day of milk replacer; 2-step weaned, initiated at age 6 weeks, completed 3 days later;
2. HI-STEP - 2.4 lb per day of milk replacer; 2-step weaned, initiated at age 5 weeks and completed 1 week later;
3. HI-LATE - 2.4 lb per day of milk replacer; 2-step weaned, initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD - 2.4 lb per day of milk replacer; 5-step weaned, initiated at age 6 week and completed 2 weeks later.

Each calf's pen had an EED, which included a dummy-nipple attached to a bottle and holder. A sensor and automated logger tracked each event (1 Hz) that the calf manipulated the EED (25 Hz sensitivity). Each calf was fitted with an accelerometer on the back leg to automatically measure lying behaviors. The device collected the y-axis (lie vs. stand) and z-axis (right or left percent during lying) of the calf every minute. For this experiment, 3-day sample periods were analyzed before and after weaning was initiated. In addition, the 3 days following weaning-completion were sampled.

Feed intake among MOD-STEP calves increased by 1.0 ± 0.19 lb after the first bottle was removed ($P \leq 0.05$), and then by 1.5 ± 0.19 SE lb after completion of weaning ($P \leq 0.05$). The use of EED did not change among MOD-STEP calves ($P > 0.05$), but after weaning, they increased their lying time, especially on their left side ($P \leq 0.05$). These changes in lying-behaviors may indicate increased comfort and maturity of the rumen. On the contrary, calves in the HI-STEP treatment ate the least amount of feed overall ($P < 0.05$), and they used the EED the most ($P > 0.05$). Calves in the HI-STEP treatment showed reduced lying bouts after weaning ($P \leq 0.05$), but no other lying-measures changed ($P > 0.05$).

The HI-LATE calves had similar feed intake and EED use compared to MOD-STEP calves. These findings suggest that weaning age needs to be more than 8 weeks for calves fed 2.4 lb of milk replacer per day. Gradual weaning may also improve feed intake and reduce EED use.

[1] Nutrition and Research Center, Provimi, Brookville, OH.
[2] Department of Animal Production, University of Leon, Leon, Spain.
[3] Institute of Computational Comparative Medicine, https://iccm.k-state.edu/. Kansas State University, Manhattan, KS.

When calves were gradually weaned starting at age 6 weeks and completed at age 8 weeks, they had the same amount of solid feed intake as HI-LATE calves. More research is needed to determine if increased feed intake and reduced EED use are also indicators that cross-sucking is less likely to occur when calves are grouped after weaning.

Introduction

Solid feed intake is commonly used by calf raisers to determine if a calf is ready to be weaned from milk or milk replacer. Before 2010, milk or milk replacer was fed at a minimal plane of nutrition (e.g., 20% CP, 20% fat, 1 lb of dry matter per day). This low plane of nutrition combined with a 2-step weaning process motivated calves to eat solid feed at the earliest age possible. The 2-step weaning strategy involved reducing the milk or milk-replacer by 1/2 for a few days, and once the calf ate a significant amount of feed, the second half of the liquid diet was removed. On average, most U.S. calves were weaned at weaning initiated at 6 weeks of age.

After 2010, calf raisers were encouraged by researchers to feed greater amounts of milk or milk replacer. For example, 2.4 lb per day of a formula with greater protein (e.g. 25% CP, 17% fat of DM) was considered a high plane of nutrition. Although accelerated preweaning body weight gain improved health after calves were group housed, researchers and early adopters of this strategy reported that the same 2-step weaning method did not motivate calves to consume solid feed before or during the two-step weaning strategy. One solution was to feed a "moderate" nutritional strategy with the same weaning protocol. An example of a moderate can include milk replacer that also has 25% CP and 17% fat, but lesser amount is offered (e.g. 1.5 lb/d of DM). Other solutions included feeding the high plane of nutrition and: 1) apply the 2-step weaning at a later age; or 2) apply a more gradual weaning method.

In addition to monitoring solid-feed intake, our lab proposed that two behavioral toolsets could be added to determine weaning readiness. Our laboratories and previous researchers used lying-behavior measures to monitor cattle comfort. As calves mature the number of lying bouts (i.e. naps) should decrease if they are comfortable with their environment but their overall time spent resting increases for rumination. In addition, as cattle age, the laterality of lying shifts to the left side. Some researchers suggest this lying-posture is used to make room for an active rumen.

Another major behavioral change among mature calves is decreased non-nutritive sucking (NNS). Early in life, NNS is important among most mammals for the neonate to develop the fine motor skills required for solid-food intake. However, as a ruminant grows, it should trade-off NNS behaviors for solid-feed intake. Our lab invented an environmental enrichment device (EED) that automatically measures how much a calf manipulates a dummy-nipple, which included NNS. Therefore, we proposed to use these three behavioral toolsets to determine weaning readiness of calves that underwent four different nutritional and weaning strategies.

Experimental Procedures

Holstein bull calves (age 3–4 days) were purchased from 1 dairy, transported for 3.5 h, and received at the Nurture Research Center nursery (Provimi, Brookville, OH). All calves were under the approval of that institution's animal care and use committee and cared for according to the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (FASS, 2010). Calves were housed in a barn with natural ventilation in individual pens (3.9 × 7.9 feet) with deep-bedded straw. Twenty-eight calves were randomly chosen out of a 96-animal project for this study. During their first 3 days of age, calves were fed 1.5 lb of one type of milk replacer (25% CP, 17% fat, DM) split into two feedings (6:00 AM and 3:30 PM). Water and a textured calf starter (20% CP and 37% starch) were provided *ad libitum* throughout the study. Solid

feed (textured calf starter) intake was measured by weighing back the remaining solids at the end of each day.

Calves were randomly assigned to one of four nutrition/weaning protocols at 3 days of age (Figure 1). Calves in MOD-STEP treatment (Figure 1) were fed the same amount of milk replacer that they started with, then at age 6 weeks, a 2-step weaning was initiated by removing the PM feeding and was completed 3 days later by removing the AM milk feeding. A high plane of milk replacer nutrition (HI) was fed to all other calves. At age 4 days, these calves were stepped up to 2.4 lb of the same milk-replacer that they started with (Figure 1). At age 5 weeks, weaning was initiated for HI-STEP calves by removing the PM feeding and was completed one week later. At age 7 weeks, weaning was initiated for HI-LATE calves by removing PM feeding, and was completed one week later. Starting at age 7 weeks, both feedings of the HI-GRAD calves were reduced by three 20% increments every four days, then the PM feeding was withdrawn for three days until weaning was completed at 8 weeks by removing the AM feeding.

From age 6 days until 1 week after weaning was completed, calves were provided an EED, which consisted of a dummy nipple and bottle with sensor that logged events every time it was manipulated by the calf (20 Hz sensitivity of movement; 1 Hz collection-rate, HOBO State Data Logger UX90–001M). In addition, a 3-axis accelerometer (Onset Computer Corp., Bourne, MA) was attached medially to a hind leg of each calf for the same time period. These accelerometers recorded standing and lying (y-axis) and lateral lying percent (z-axis) behaviors in 1-minute intervals.

For the analysis, the behavior and starter intake data were sampled for 3 days prior to weaning initiation (pre), the first 3 days after weaning initiation (during), and first 3 days after weaning completion (after). For these data, a linear mixed model with fixed effects of time, treatment and treatment × time was fitted and analyzed by restricted likelihood ANOVA using the MIXED procedure of SAS (v. 9.2, SAS Inst., Cary, NC). Treatment, time, and interaction differences of $P < 0.05$ were considered significant at $P \leq 0.05$.

Results and Discussion

Solid Feed Intake

Solid feed intake and automated behavioral data are presented in Table 1. The MOD-STEP calves increased their feed intake after weaning was initiated and again after weaning was complete (Figure 2; $P \leq 0.05$). This behavior was similar to calves fed a low plane of nutrition. Therefore, calf raisers could use feed intake alone as a behavioral measure for calves with this nutrition-weaning strategy.

In the three days following weaning initiation, HI-STEP calves increased their solid feed intake compared to their pre-weaning measures, and again once weaning was finalized (Figure 2; $P \leq 0.05$). However, overall, HI-STEP calves consumed the least amount of feed (Table 1; $P \leq 0.05$). This finding replicated what other researchers and calf raisers had reported when they initiated weaning at age 5 weeks of age and completed weaning on or before a week later. Pre-weaning feed intakes were very low, which indicates that HI-STEP calves need to have more than just feed intake monitored to determine weaning readiness.

Calves in HI-LATE and HI-GRAD treatments did not significantly increase feed intake after weaning was initiated (Figure 2; $P > 0.05$), but once weaning was completed for both groups, calves consumed the same amount of feed as MOD-STEP calves after weaning (Figure 2; $P \leq 0.05$).

Lying Behaviors

Total lying duration, left-sided lying, and number of lying bouts (lying for longer than 120 consecutive seconds) increased among MOD-STEP calves after weaning completion (Figure 3; $P \leq 0.05$). These are all indications that the weaning method and feeding strategy influenced maturity in activity among MOD-STEP calves.

On the contrary, the only lying-behavior measure that changed among HI-STEP calves was a decrease in lying bouts significantly after weaning was completed (Figure 4; $P \leq 0.05$). Calves in HI-LATE and HI-GRAD treatments did not change lying behaviors during the sample periods (Figures 3 and 4; $P > 0.05$). Additionally, HI-GRAD calves had a slight, but not significant, reduction in lying time during weaning; no changes were observed in lying on their left or on number of lying bouts (Figures 3 and 4; $P > 0.05$).

Environmental Enrichment Devices

Calves in MOD-STEP did not change their EED use after weaning was initiated or completed (Figure 5; $P > 0.05$). Over the entire experiment, calves in MOD-STEP treatment accounted for just 14.3% of the frequent EED users, which was similar to what was expected in the analysis (Table 2; $\chi^2 = 63.59$, $P \leq 0.001$). These data suggest that MOD-STEP calves may have traded their NNS behaviors for solid feed intake at an earlier age than calves in the other treatments. A similar finding was observed in research comparing EED-use among low plane of nutrition fed calves vs. high plane of nutrition fed calves in a cohort of Holstein bull calves.

Calves in the HI-STEP treatment used the EED twice as much as calves in the MOD-STEP treatment (Table 1; $P \leq 0.05$). They also made up 42.9% of the frequent-EED users (Table 2; $\chi^2 = 63.59$, $P \leq 0.001$). These findings suggest that HI-STEP calves did not trade off their NNS for increased feed intake.

Calves in HI-LATE used the EED as much as the MOD-STEP calves (Table 1). This finding contrasts with our previous research involving HI-LATE calves and EED-use. In this previous experiment, HI-LATE calves did not use the EED very often until weaning was initiated. Then, these HI-LATE calves used the EED three times the amount than the HI-LATE calves in the current experiment. In the current study none of the HI-LATE calves in this treatment could be classified as EED-frequent users (Table 2; $\chi^2 = 63.59$, $P \leq 0.001$). Furthermore, all calves in the high plane of nutrition treatments were treated the same until weaning initiation. For this experiment, we may have randomly selected a group of calves that have homogenous NNS temperament for the HI-LATE cohort. More research is needed with a greater number of calves per treatment to determine if EED-use is related to other toolsets for identifying temperament.

Although HI-GRAD calves did not change their EED-usage after weaning was initiated (Figure 5), overall, they used the EED 1.6 times more than the MOD-STEP and HI-LATE calves (Table 1; $P \leq 0.05$), and they made up 33.3% of the EED-frequent users (Table 2; $\chi^2 = 63.59$, $P < 0.001$). This finding is an indicator that the gradual weaning method may not help calves reduce NNS when fed a high plane of milk replacer.

Conclusions

The strategy for milk replacer and weaning strategy needs careful consideration because early-life behavioral development sets the foundation for a stress- and immune-resilient adult. The MOD-STEP nutrition-weaning strategy may help calves extinguish NNS and adopt more nutritive oral behaviors. The HI-LATE strategy may also be considered, especially for calves housed in colder environments. However, calves in this program need to be weaned at a later age, which means they will consume overall more milk replacer. Therefore, the housing strategy must also be considered when adopting a new nutrition-weaning strategy. Calves placed in

groups before they extinguish NNS are likely to cross-suck. Some preweaning housing types are built for Holstein calves that are less than 5 weeks of age, therefore late-aged weaning impedes space allowance. Previous research indicated that space allowance also influences starter intake and immune resilience. Solid feed intake served as a reliable and traditional indicator for determining weaning readiness. The lying-loggers are helpful for researchers, but must be worn by the calf, which is not practical devices for large calf raising operations. The EED fastens to the pen rather than the calf. The EED enhances the calves' environment and simultaneously provides a quantitative measure of weaning readiness.

Table 1. Solid-feed intake[1] and automated behaviors of calves fed four different milk replacer programs before weaning, after weaning initiation, and after weaning completion

	Treatment[2]					*P*-values		
	MOD-STEP	HI-STEP	HI-LATE	HI-GRAD	SEM[3]	TRT	Time	TRT × time
Number of calves	7	7	7	7	—	—	—	—
Solid feed, lb/day	2.4[a]	1.2[b]	2.3[a]	2.1[a]	0.19	<0.001	<0.001	<0.001
Lying time, min/day	1130	1079	1126	1072	18.7	0.067	0.017	0.001
Lie left,[4] %	591.2	552.7	571	534.9	20.64	0.261	0.156	0.005
Lying bouts,[5] no./day	24.5	23.1	25.1	23.9	1.66	0.845	0.036	<0.001
EED use, sec/day	153[c]	307[a]	102[d]	253[b]	21.8	0.036	0.101	<0.001
EED bouts,[6] no./day	3.2[c]	11.1[b]	7.3[b,c]	22.0[a]	3.55	0.006	<0.001	<0.001

[a,b,c,d]LS-means differ ($P < 0.05$; Tukey-Kramer adjustment).

[1]Three-day sample periods were collected before (Pre) and after the first step of weaning (During), as well as after the last step of weaning (After).

[2]Treatments were:

1. MOD-STEP (1.46 lb per day of milk replacer); 2-step weaned; initiated at age 6 weeks, completed 3 days later;
2. HI-STEP (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 5 weeks and completed 1 week later;
3. HI-LATE (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD (2.4 lb per day of milk replacer); 5-step weaning initiated at age 6 weeks; both feedings of the HI-GRAD calves were reduced in three 20% increments every four days, then the PM feeding was withdrawn for three additional days. Weaning was completed at age 8 weeks, by removing the AM feeding.

[3]Largest standard error of the mean (SEM).

[4]While in the lying position, the time in min per d a calf leaned to the left.

[5]The number of times per day calves napped for 2 or more minutes.

[6]The number of times a calf moved from not touching to touching the environmental enrichment device (EED) for at least 2 sec with 1 sec interval.

Table 2. Percent of calves classified as normal or frequent[1] environmental enrichment device (EED) users for each treatment during the sample periods[2]

	Normal				Frequent			
Treatment[3]	n[1]	%[4]	Expected[5]	Residuals	n	%	Expected[5]	Residuals
MOD-STEP	6	85.7	90	2.35	1	14.3	10	-2.35
HI-STEP	4	57.1	90	-5.66	3	42.9	10	5.66
HI-LATE	7	100.0	90	6.22	0	0.0	10	-6.22
HI-GRAD	5	66.7	90	-2.90	2	33.3	10	2.90

[1]Total number of calves that were categorized as normal (< 400 × per day) or frequent (> 400 × per day) EED users.
[2]Three-day sample periods were collected before (Pre) and after the first step of weaning (During), as well as after the last step of weaning (After).
[3]Treatments were:

1. MOD-STEP (1.46 lb per day of milk replacer); 2-step weaned; initiated at age 6 weeks, completed 3 days later;
2. HI-STEP (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 5 weeks and completed 1 week later;
3. HI-LATE (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD (2.4 lb per day of milk replacer); 5-step weaning initiated at age 6 weeks; both feedings of the HI-GRAD calves were reduced in three 20% increments every four days, then the PM feeding was withdrawn for three additional days. Weaning was completed at age 8 weeks, by removing the AM feeding.

[4]Percent of calves in each treatment that were categorized as normal or frequent EED users.
[5]Expected percentage of calves for each group (normal and frequent) according to chi-square analysis ($\chi^2 = 63.59$, $P < 0.001$).

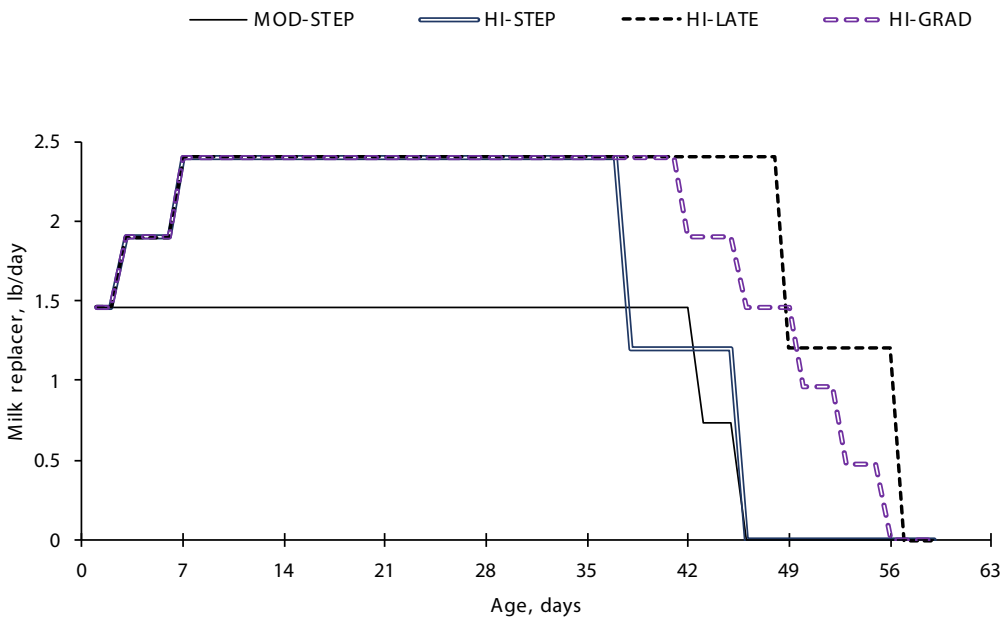

Figure 1. Milk replacer timeline for Holstein bull calves (n = 28) that were placed into 4 nutrition-weaning treatments:

1. MOD-STEP (1.46 lb per day of milk replacer); 2-step weaned; initiated at age 6 weeks, completed 3 days later;
2. HI-STEP (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 5 weeks and completed 1 week later;
3. HI-LATE (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD (2.4 lb per day of milk replacer); 5-step weaning initiated at age 6 weeks; both feedings of the HI-GRAD calves were reduced in three 20% increments every four days, then the PM feeding was withdrawn for three additional days. Weaning was completed at age 8 weeks, by removing the AM feeding.

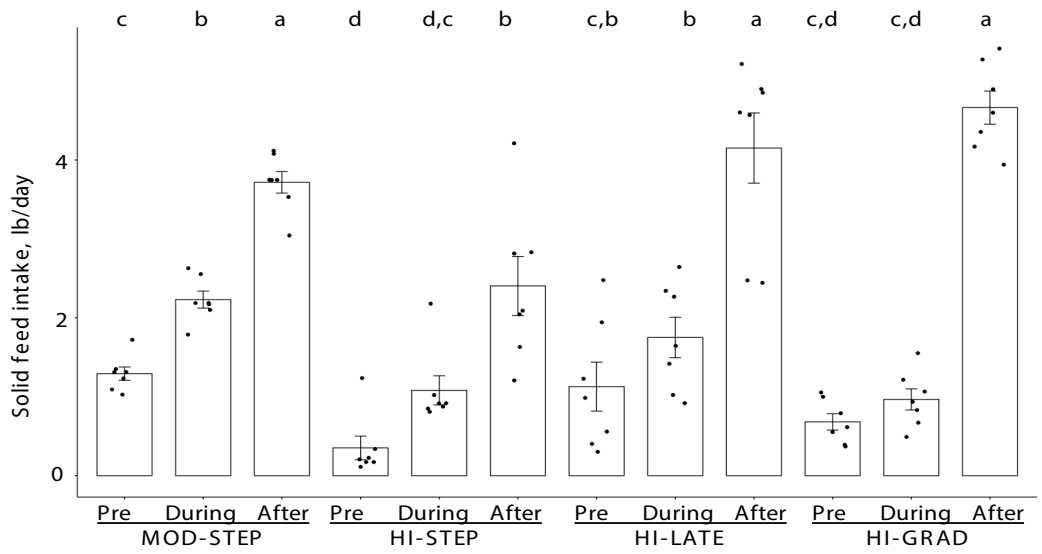

Figure 2. Solid feed intake for Holstein bull calves (n = 28) that were placed into 4 nutrition-weaning treatments:

1. MOD-STEP (1.46 lb per day of milk replacer); 2-step weaned; initiated at age 6 weeks, completed 3 days later;
2. HI-STEP (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 5 weeks and completed 1 week later;
3. HI-LATE (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD (2.4 lb per day of milk replacer); 5-step weaning initiated at age 6 weeks; both feedings of the HI-GRAD calves were reduced in three 20% increments every four days, then the PM feeding was withdrawn for three additional days. Weaning was completed at age 8 weeks, by removing the AM feeding.

For this experiment, 3-day sample periods were collected before (Pre) and after the first step of weaning (During), as well as after the last step of weaning (After). *P*-values for TRT, TIME, and TRT × TIME were < 0.01.

[a,b,c,d]LS Means differ ($P < 0.05$; Tukey-Kramer adjustment). For each plot, the box represents the average for each treatment group during each time period, the lines represent the standard error of the mean, and the dots represent the distribution of individual calves.

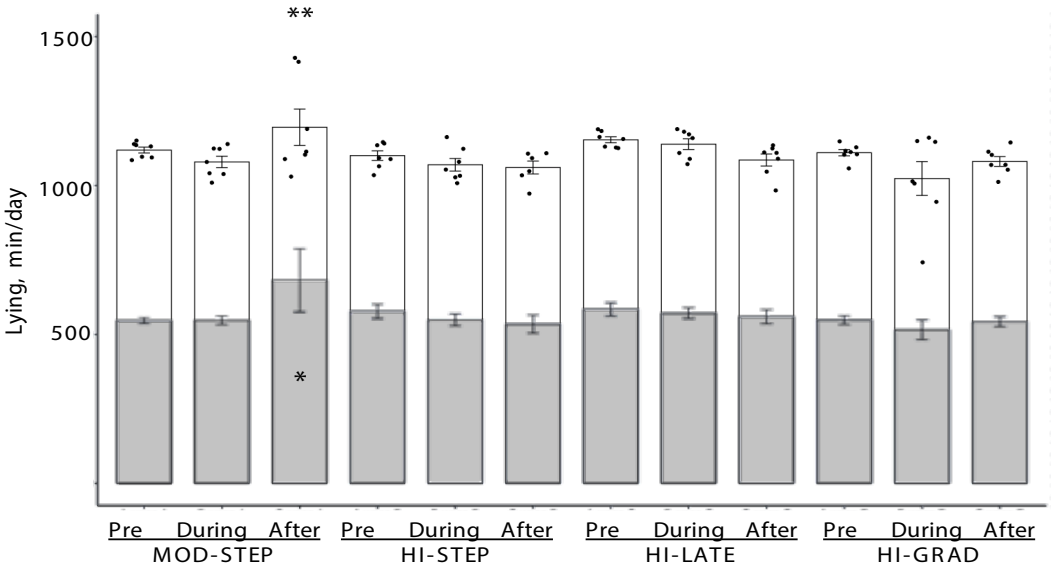

Figure 3. Lying duration for Holstein bull calves (n = 28) that were placed into 4 nutrition-weaning treatments:

1. MOD-STEP (1.46 lb per day of milk replacer); 2-step weaned; initiated at age 6 weeks, completed 3 days later;
2. HI-STEP (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 5 weeks and completed 1 week later;
3. HI-LATE (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD (2.4 lb per day of milk replacer); 5-step weaning initiated at age 6 weeks; both feedings of the HI-GRAD calves were reduced in three 20% increments every four days, then the PM feeding was withdrawn for three additional days. Weaning was completed at age 8 weeks, by removing the AM feeding.

For this experiment, 3-day sample periods were collected before (Pre) and after the first step of weaning (During), as well as after the last step of weaning (After). *P*-values for TRT, TIME, and TRT × TIME were < 0.01.

**Total Lying duration differs ($P < 0.05$; Tukey-Kramer adjustment); *Lying percent on the left side differs ($P < 0.05$; Tukey-Kramer adjustment). For each plot, the whole box represents the average lying time for each treatment group during each time period; the gray box represents the percent of time spent lying more to the left than the right. The lines represent the standard error of the means. The dots represent the distribution of individual calves for total lying duration.

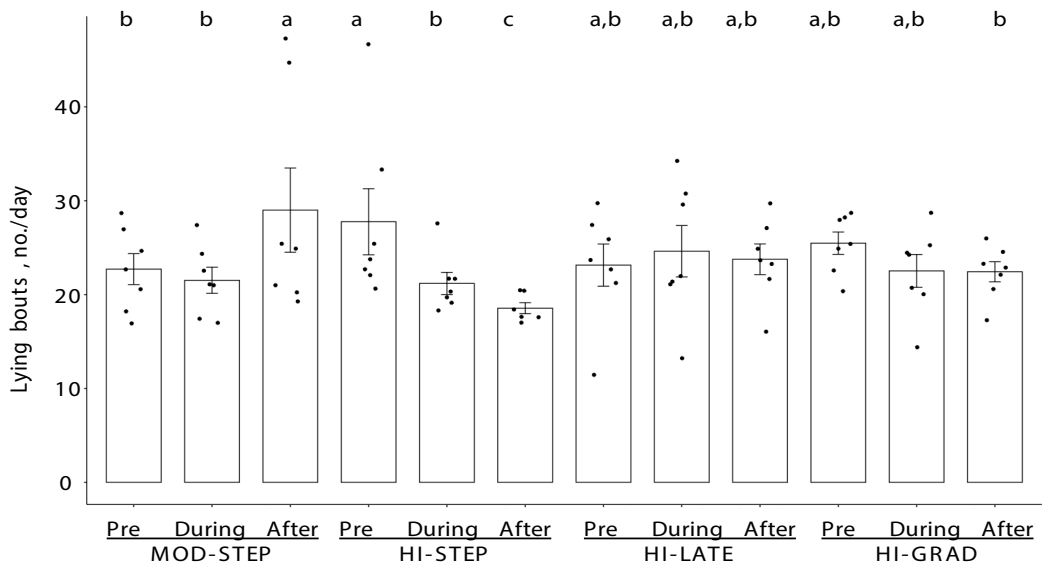

Figure 4. Number of lying bouts for Holstein bull calves (n = 28) that were placed into 4 nutrition-weaning treatments:

1. MOD-STEP (1.46 lb per day of milk replacer); 2-step weaned; initiated at age 6 weeks, completed 3 days later;
2. HI-STEP (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 5 weeks and completed 1 week later;
3. HI-LATE (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD (2.4 lb per day of milk replacer); 5-step weaning initiated at age 6 weeks; both feedings of the HI-GRAD calves were reduced in three 20% increments every four days, then the PM feeding was withdrawn for three additional days. Weaning was completed at age 8 weeks, by removing the AM feeding.

For this experiment, 3-day sample periods were collected before (Pre) and after the first step of weaning (During), as well as after the last step of weaning (After). *P*-values for TRT, TIME, TRT × TIME were < 0.01.

[a,b,c]LS Means differ ($P < 0.05$; Tukey-Kramer adjustment). For each plot, the box represents the average for each treatment group during each time period, the lines represent the standard error of the mean, and the dots represent the distribution of individual calves.

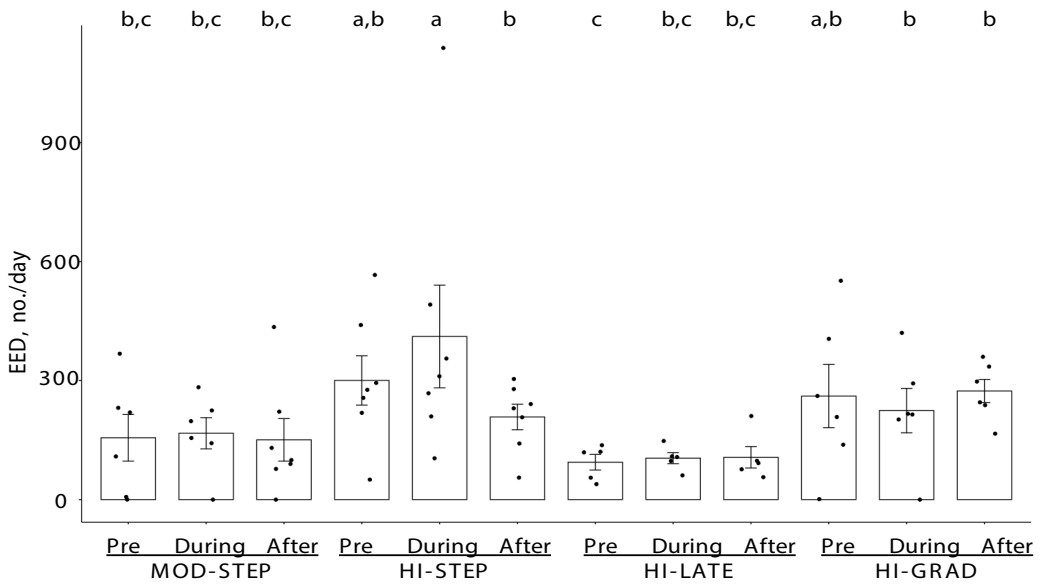

Figure 5. Environment Enrichment Device (EED) use for Holstein bull calves (n = 28) that were placed into 4 nutrition-weaning treatments:

1. MOD-STEP (1.46 lb per day of milk replacer); 2-step weaned; initiated at age 6 weeks, completed 3 days later;
2. HI-STEP (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 5 weeks and completed 1 week later;
3. HI-LATE (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD (2.4 lb per day of milk replacer); 5-step weaning initiated at age 6 weeks; both feedings of the HI-GRAD calves were reduced in three 20% increments every four days, then the PM feeding was withdrawn for three additional days. Weaning was completed at age 8 weeks, by removing the AM feeding.

For this experiment, 3-day sample periods were collected before (Pre) and after the first step of weaning (During), as well as after the last step of weaning (After). *P*-values for TRT, TIME, and TRT × TIME were < 0.01.

[a,b,c]LS Means differ ($P < 0.05$; Tukey-Kramer adjustment). For each plot, the box represents the average for each treatment group during each time period, the lines represent the standard error of the mean, and the dots represent the distribution of individual calves.

Effects of Two Commercial Supplemental Fat Products on Body Condition Score and Cow- and Herd-Level Milk Yield and Composition in a Commercial Dairy Herd in Kansas

A.L.A. Scanavez, C.A. Gamarra, R.S.S. de Oliveira, and L.G.D. Mendonça

Summary

Feeding fat supplements to lactating dairy cows is an effective strategy to increase energy density of rations and increase milk yield. However, it is not clear whether supplementing a specific fat supplement for the entire lactating herd provides better results than others in commercial dairy herds. The objective of this study was to compare the effects of fat supplementation with two commercial products on changes in body condition score (BCS) and cow- and herd-level milk production and composition in a large commercial dairy herd. The study was conducted in a herd milking approximately 1,500 Holstein cows. One of two treatments was assigned to the herd in a single-subject crossover design with 4 periods. Treatments were inclusion of 0.24 lb/cow per day of a supplement rich in saturated fats (Propel; Propel Energy Plus) or 0.22 lb/cow per day of a supplement containing calcium salts of long-chain fatty acids (Control). Treatments were applied to all lactating cows during four consecutive weeks. Milk yield recorded during the last week of each period was used for statistical analyses. In addition, milk samples were collected in the last week of each period to determine test-day milk protein, fat, somatic cell count, and urea nitrogen concentrations. At the beginning and at the end of each experimental period, BCS was assessed from a subset of cows to evaluate BCS change. Herd-level milk fat, protein, and somatic cell count were recorded daily by the milk cooperative. Bulk tank milk fat and protein contents on the fourth week of fat supplementation were similar between Control and Propel treatments. Average milk yield during the fourth week of fat supplementation (yield recorded daily in the last week of the experimental period) was greater for Control than Propel supplementation (83.4 vs. 82.1 ± 1.7 lb/day). In the analyses that used test-day data, milk yield did not differ between Control and Propel treatments. Supplementation with Propel resulted in greater milk fat (4.50 vs. 4.29 ± 0.12%) and reduced milk protein content (3.12 vs. 3.14 ± 0.03%) compared with Control. In addition, milk urea nitrogen was reduced for Control vs. Propel cows (12.5 vs. 13.1 ± 0.04 mg/dL). Supplementation with Propel increased energy-corrected milk (93.9 vs. 91.7 ± 3.1 lb/day) and fat-corrected milk (96.3 vs. 93.5 ± 3.3 lb/day) yields compared with Control supplementation. Proportion of cows that lost BCS during the fat supplementation periods did not differ between treatments; however, BCS change tended to be more pronounced during supplementation with Propel than Control treatment (-0.03 vs. 0.02 ± 0.04). In conclusion, fat supplementation using the Propel treatment resulted in greater milk fat content, energy-corrected milk, and fat-corrected milk compared with fat supplementation with Control. Our findings suggest that the type of market to which milk is sold should be considered in the choice of fat supplements.

Introduction

Feeding fat to lactating dairy cows is a strategy commonly used to increase energy density and adjust levels of specific fatty acids in rations. Despite the high fat content in feeds such as cottonseed and full-fat soybeans, rations formulated for high-producing dairy cows usually require supplemental fat. Lactating dairy cows supplemented with fat increase milk yield by approximately 2.3 lb/cow per day and have greater BCS compared with non-supplemented cows. In spite of its consistent benefit to milk yield, fat supplementation usually reduces dry matter intake, and milk fat and protein contents. However, these results are highly heterogenous in

the scientific literature. Variability in productive responses resulting from fat supplementation is partially explained by the source and biochemical profile of the fat supplement. In fact, it has been suggested that strategic feeding of a combination of a fat supplement rich in linoleic acid during the transition period followed by supplementation with a fish-oil-based fat supplement improves both reproductive and productive performance of dairy cows compared with other fat supplementation strategies. Nonetheless, this approach has not been widely adopted, given that most herds prefer to rely on a single product as source of supplemental fatty acids. In addition, it is not clear whether supplementing a specific fat for the entire lactating herd provides better results than others. Therefore, decisions on which product to use in dairy operations is frequently made arbitrarily.

Most studies that evaluated the effects of various sources of fat supplementation on milk yield and composition were conducted in facilities (e.g., tie-stall barns) that are not comparable to systems used by large commercial herds. Furthermore, to our knowledge, very limited data are available comparing herd-level indicators such as bulk tank fat and protein content in herds using different sources of supplemental fat. Comparing productive outcomes by supplementing the herd with different fat sources in a commercial setting may aid producers to make more reliable and profitable decisions for their operations.

The objective of the present study was to compare the effects of fat supplementation with two commercial products on changes of body condition score and cow- and herd-level milk production and composition in a large commercial dairy herd located in Kansas.

Experimental Procedures

This study was conducted in a commercial dairy herd located in Kansas from April to July 2019. Approximately 1,500 lactating Holstein cows were housed in two-row free-stall barns bedded with sand and were milked three times daily. Primiparous and multiparous cows were kept in the same pen during the first 2 weeks of lactation. After this period, primiparous and multiparous cows were housed separately until the end of the lactating period. Cooling systems comprised of sprinklers and fans were present in all pens and in the holding area. Lactating cows were fed twice daily with a total mixed ration formulated by a nutritionist. The herd was assigned to one of two treatments in a single-subject crossover design with 4 periods. Treatments were inclusion of 0.24 lb/cow per day of a supplement rich in saturated fats (Propel Energy Plus, Purina Animal Nutrition) or 0.22 lb/cow per day of a supplement containing calcium salts of long chain fatty acids (Control; Megalac, Church and Dwight Co. Inc.). Treatments were designed to result in isoenergetic and isonitrogenous diets (Table 1). Treatment was applied to all lactating cows during four consecutive weeks. After this period, the treatment was changed for all lactating cows. Each treatment was applied during two alternated 4-week periods, in a total of 4 experimental periods.

Milk yield from each cow was automatically recorded daily, and data recorded during the last week of each period were used for statistical analyses. Milk samples were collected by the Heart of America Dairy Herd Improvement Association (Kansas City, MO) from all cows in the last week of each period to determine milk protein, fat, somatic cell count, and urea nitrogen concentrations. Energy-corrected milk and 3.5% fat-corrected milk were calculated using the following formulas: energy-corrected milk = (0.327 × lb of milk yield) + (12.95 × lb of fat) + (7.2 × lb of protein) and fat-corrected milk = (0.432 × lb of milk yield) + (16.216 × lb of fat). At the beginning of each experimental period, a list was generated with all lactating cows and their respective days in milk (DIM). Cows were classified into the following stages of lactation: fresh (15 to 30 DIM), early (31 to 100 DIM), middle (101 to 180 DIM) or late (181 to 230 DIM). Within each stratum, BCS was assessed using a 5-point scale from a subset of 40 cows at the beginning and end of each experimental period to evaluate BCS change. Cows with less than 15 or more than 230 DIM were not included in the BCS analyses. Ambient

temperature and relative humidity of 2 pens were recorded every 5 minutes using loggers to calculate temperature-humidity index (THI). Herd-level milk fat, protein, and somatic cell count were recorded daily by the milk cooperative (Dairy Farmers of America). During the study, data regarding lactation number, days in milk, and reproductive status of all lactating cows were extracted daily from the on-farm management software (PCDart, DRMS, Raleigh, NC). Data were analyzed by ANOVA using the PROC GLIMMIX procedure of SAS v. 9.4 (SAS Inst., Cary, NC). For the test-day data and average milk yield during the fourth week of fat supplementation, treatment (Propel and Control), parity (primiparous and multiparous), and days in milk were included as fixed variables, and period as a random variable. Significance was declared at $P \leq 0.05$, and tendencies at $0.05 < P \leq 0.10$.

Results and Discussion

Diets used for lactating dairy cows in the current experiment are presented in Table 1. Increased values of ambient temperature, average THI, and maximum THI were observed in the last 2 supplementation periods (e.g., June and July), which coincided with reduced milk fat and protein contents (Table 2). Despite changes in milk components during periods of increased THI, bulk tank fat and protein contents were comparable between treatments. Further descriptive data are depicted in Table 2. Average milk yield during the fourth week of fat supplementation (yield recorded daily in the last week of the experimental period) was ($P < 0.01$) greater for Control than Propel supplementation (83.4 vs. 82.1 ± 1.7 lb/day, data not shown in tables). In addition, milk yield during the fourth week of fat supplementation was ($P < 0.01$) negatively associated with DIM and was ($P < 0.01$) greater for multiparous than primiparous cows (87.2 vs. 77.8 ± 1.7 lb/day).

Individual milk components were only available at the end of the fourth week of fat supplementation (test-day data). In the analyses that used test-day data, milk yield did not ($P = 0.70$) differ between Control and Propel treatments (Table 3). However, milk fat content was ($P < 0.01$) greater in the Propel than Control treatment (4.50 ± 0.12 vs. 4.29 ± 0.12%). These results contrast with bulk tank milk fat on the fourth week of fat supplementation, which was similar between Control and Propel treatments (Table 2). Even though we observed these contrasting findings (test-day vs. bulk tank data), it should be noted that the analyses using test-day data were adjusted for parity and DIM, therefore, conclusions should be drawn from these specific analyses. The increased milk fat content for Propel compared with Control was likely caused by distinct concentration of unsaturated fats in the supplements. Supplementation with Control resulted in greater ($P = 0.03$) milk protein content and decreased ($P < 0.01$) milk urea nitrogen compared with supplementation with Propel (Table 3). The authors speculate that during supplementation with Propel, cows had greater uptake of fatty acids by the mammary gland to produce milk fat, which likely resulted in less energy available to convert amino acids from the diet into milk protein. However, the magnitude of the difference in milk protein (0.02%) between Control and Propel treatments is likely not economically meaningful for commercial dairy herds. Supplementation with Propel increased ($P < 0.01$) energy-corrected milk and fat-corrected milk by approximately 2.5 lb/cow compared with Control supplementation (Table 3).

Body condition score at the beginning of the fat supplementation period tended ($P = 0.08$) to be lesser in the Control compared with the Propel treatment (2.93 vs. 2.98 ± 0.06; Table 4). No differences ($P > 0.61$) were detected in BCS at the end of supplementation periods, or in DIM at which BCS were assessed in the Control and Propel treatments (Table 4). In addition, the proportion of cows that lost BCS during the fat supplementation periods did not ($P = 0.32$) differ between Control and Propel treatments. Body condition score change tended ($P = 0.10$) to be more pronounced during supplementation with Propel than Control treatment (Table 4). It is possible, given that more energy was necessary for milk fat synthesis during supplementation with Propel, less energy was available to support body fat reserves, resulting in greater

BCS change for cows supplemented with the Propel treatment. Even though BCS loss observed during supplementation with Propel was modest, it is possible that greater losses could occur if supplementation was carried out for a longer period. Long-term effects of fat supplementation were not the focus of the current study, but it should be evaluated in future trials.

In conclusion, under the conditions described in the current study, fat supplementation using the Propel treatment resulted in greater milk fat content, energy-corrected milk, and fat-corrected milk than fat supplementation with Control. Because of contrasting findings in fluid milk yield (test-day data vs. yield recorded daily in the fourth week of supplementation) for Control and Propel treatments, the type of market to which milk is sold by the herd is a factor that may be considered in the decision to choose fat supplements. Nonetheless, further studies on the effects of supplementing various sources of fat in commercial settings are necessary to provide dairy producers and consultants with reliable information to support profitable decisions.

Acknowledgments

The authors thank Dr. Troy Wistuba and Michael Thomas from Purina Animal Nutrition (Gray Summit, MO), and the manager, nutritionist, and employees of the collaborating dairy for support during the project.

Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.

Table 1. Ingredients and chemical composition of diets with Control or Propel fat supplementation

	Fat supplementation[1]	
Item	Control	Propel
Ingredient, % of dry matter		
Corn silage	26.9	26.9
Alfalfa hay	12.1	12.1
Wheat straw	2.6	2.6
OneTrak[2]	31.7	31.7
Corn, ground	18.7	18.7
Cottonseed, whole	5.9	5.9
Mineral mix[3]	1.8	1.8
Control fat supplement	0.37	---
Propel fat supplement	---	0.40
Water	0.005	0.005
Chemical composition		
Crude protein, %	16.0	16.0
Acid detergent fiber, %	18.5	18.5
Neutral detergent fiber, %	29.0	29.0
Starch, %	25.6	25.6
Sugar, %	6.0	6.0
Non-fiber carbohydrates, %	40.0	40.0
Net energy$_l$, Mcal/lb	0.78	0.78

[1]Fat supplementation consisted of either of 0.24 lb/cow per day of a supplement rich in saturated fats (Propel Energy Plus, Purina Animal Nutrition) or 0.22 lb/cow per day of a supplement containing calcium salts of long chain fatty acids (Control; Megalac, Church and Dwight Co. Inc.).
[2]Pre-blended concentrate (Cargill Corn Milling, Blair, NE).
[3]Mineral premix consisted of 39.9% sodium bicarbonate, 24.7% soybean meal, 9.9% magnesium oxide, 8.2% bentonite adsorbent (AB 20, Phibro Animal Health Corporation, Teanek, NJ), 7.0% limestone, 5.3% potassium chloride, and 4.9% DCAD Plus (Arm & Hammer Animal Nutrition, Princeton, NJ).

Table 2. Descriptive information (mean ± SD[1]) of the fourth week of fat supplementation of each period

	Fat supplementation[2]			
Item	Control	Propel	Control	Propel
Month of supplementation	April	May	June	July
Bulk tank milk fat,[3] %	3.92 ± 0.09	3.89 ± 0.04	3.81 ± 0.02	3.81 ± 0.04
Bulk tank milk protein,[3] %	3.16 ± 0.02	3.17 ± 0.01	3.08 ± 0.02	3.10 ± 0.03
Bulk tank SCC,[3] cells/mL × 1,000	196 ± 7	188 ± 20	185 ± 8	199 ± 9
First lactation cows in the herd,[4] %	37.5 ± 0.2	35.0 ± 0.1	41.3 ± 0.1	40.1 ± 0.1
Days in milk of lactating cows[4]	192 ± 0.9	199 ± 0.7	192 ± 0.7	199 ± 0.9
Temperature,[5] °F	56.7 ± 4.2	66.8 ± 5.8	75.9 ± 6.0	75.2 ± 4.3
Relative humidity,[5] %	65.9 ± 10.0	81.1 ± 6.0	73.0 ± 8.1	63.9 ± 6.5
Temperature-humidity index (THI)[5]	56.4 ± 3.3	65.7 ± 5.3	72.7 ± 4.8	71.2 ± 3.0
Maximum THI[5]	63.9 ± 3.2	73.1 ± 4.6	79.5 ± 3.9	77.4 ± 3.1

[1]SD = standard deviation.
[2]Fat supplementation consisted of either of 0.24 lb/cow per day of a supplement rich in saturated fats (Propel Energy Plus, Purina Animal Nutrition) or 0.22 lb/cow per day of a supplement containing calcium salts of long chain fatty acids (Control; Megalac, Church and Dwight Co. Inc.).
[3]Data extracted from the Dairy Farmers of America website (http://www.dfamilk.com/).
[4]Data extracted from the on-farm management software.
[5]Information obtained from loggers installed in 2 pens at the farm to obtain temperature and relative humidity.
SCC = somatic cell count.

Table 3. Test-day traits of cows supplemented with Propel or Control for 4 weeks

	Fat supplementation (FS)[1]		*P* – value		
Item	Propel	Control	FS	Parity	DIM
Milk yield, lb/d	83.1 ± 1.4	82.9 ± 1.4	0.70	<0.01	<0.01
Energy-corrected milk yield,[2] lb/d	93.9 ± 3.1	91.7 ± 3.1	<0.01	<0.01	<0.01
Fat-corrected milk yield,[3] lb/d	96.3 ± 3.3	93.5 ± 3.3	<0.01	<0.01	<0.01
Fat, %	4.50 ± 0.12	4.29 ± 0.12	<0.01	<0.01	<0.01
Protein, %	3.12 ± 0.03	3.14 ± 0.03	0.03	<0.01	<0.01
Milk urea nitrogen, mg/dL	13.1 ± 0.04	12.5 ± 0.04	<0.01	<0.01	<0.01
Somatic cell count, cells/mL × 1,000	207 ± 11	165 ± 11	<0.01	0.03	<0.01

[1]Fat supplementation consisted of either of 0.24 lb/cow per day of a supplement rich in saturated fats (Propel Energy Plus, Purina Animal Nutrition) or 0.22 lb/cow per day of a supplement containing calcium salts of long chain fatty acids (Control; Megalac, Church and Dwight Co. Inc.).
[2]Energy corrected milk yield = (0.327 × lb of milk yield) + (12.95 × lb of fat yield) + (7.2 × lb of protein yield).
[3]Fat-corrected milk yield = (0.432 × lb of milk yield) + (16.216 × lb of fat yield).
DIM = days in milk.

Table 4. Body condition score (BCS)[2] in the beginning and end of the supplementation period of 2 different fat sources

	Fat supplementation (FS)[1]		*P* - value		
Item	Propel	Control	FS	Parity	DIM
Initial BCS	2.98 ± 0.06	2.93 ± 0.06	0.08	<0.01	<0.01
Days in milk at initial BCS	105 ± 4	108 ± 4	0.64	0.40	---
Final BCS	2.96 ± 0.03	2.94 ± 0.03	0.62	<0.01	<0.01
Days in milk at final BCS	137 ± 4	136 ± 4	0.85	0.41	---
BCS change from final to initial[3]	-0.03 ± 0.04	0.02 ± 0.04	0.10	0.73	<0.01
Proportion of cows that lost BCS,[3] %	53.0	47.0	0.32	0.94	0.06

[1]Fat supplementation consisted of either of 0.24 lb/cow per day of a supplement rich in saturated fats (Propel Energy Plus, Purina Animal Nutrition) or 0.22 lb/cow per day of a supplement containing calcium salts of long chain fatty acids (Control; Megalac, Church and Dwight Co. Inc.).
[2]Body condition score was assessed on a scale of 1 (severe underconditioned) to 5 (obese) with 0.25-point increments.
[3]BCS change from initial to final assessment and proportion of cows that lost BCS during each supplementation period was calculated by subtracting final BCS from initial BCS.
DIM = days in milk.

Combined Risk Factors and Digestive Disorders in Mid-Lactation Holstein Cows: A Case Study

S.T. Quanz, L.K. Mamedova, M.J. Brouk, P. Gott,[1] and B.J. Bradford

Summary

Digestive disorders can be a significant cause of disease on dairies and are frustrating because of their unpredictability. Diets that may support excellent health in most cases may nonetheless result in significant gastrointestinal disease, even leading to deadly conditions such as hemorrhagic bowel syndrome. To our knowledge, there is limited research on these conditions, as many risk factors fail to reproduce disease when experimentally administered to cows, leading many to conclude that these disorders are generally multifactorial in nature and difficult to replicate. In this case study, we document the outbreak and resolution of digestive disorders among 15 control cows enrolled in a larger production study. Over 14 weeks, cows were individually fed, with milk yield and composition, blood variables, and health observations recorded. The diet included drought-stressed corn silage that introduced difficulties including low energy density, high dry matter content (making it unstable at feedout), and mycotoxin contamination. By weeks 4–5 on the study, sporadic diarrhea began to appear and milk fat content had dropped from 3.7% to 3.4%, on average. Coincident with the onset of environmental heat stress, three cows developed severe digestive disorders, resulting in a displaced abomasum in one cow. At that point, the diet was changed to replace some corn silage with wheat straw, a direct-fed microbial was added to the diet, and organic acid treatment of the silage face was initiated. Within a month after these changes were implemented, essentially all signs of digestive problems resolved, including milk fat content, fecal consistency, and blood plasma concentrations of haptoglobin and D-lactate. This case study points to multiple factors that likely combined to lead to microbial and gastrointestinal disruptions resulting in clinical disease in a subset of cows.

Introduction

Digestive disorders, ranging from mild diarrhea to hemorrhagic bowel syndrome, occur among lactating cows on many farms. These challenges can happen sporadically, often leaving producers and their advisors frustrated by the lack of any obvious cause of the problem. A wide variety of factors are thought to contribute to these digestive challenges, including the presence of opportunistic gastrointestinal pathogens, excessive flow of fermentable carbohydrate or protein to the hind gut, and the presence of mycotoxins in the diet. These conditions are thought to contribute to a disruption of the gut barrier (i.e., "leaky gut"), which in turn leads to bacterial invasion and dramatic inflammation of the tissue. However, research progress on this topic has been slow, as challenge studies that have introduced individual risk factors have generally failed to replicate the digestive disorders.

On-farm outbreaks of gastrointestinal disease probably differ from controlled studies in that multiple risk factors occur simultaneously. If these factors could be documented over the course of such an outbreak, it could help to improve our understanding of the disease process. Unfortunately, commercial farms almost never have relevant samples collected prior to this kind of outbreak, preventing analysis of factors contributing to onset of the disease.

In the process of conducting a study, we recently dealt with an outbreak of gastrointestinal disease. Fortunately, we collected samples for the purpose of the primary study that allowed us to document some relevant changes that likely contributed to disease progression and resolution

[1] Biomin America Inc., Overland Park, KS.

over the course of 14 weeks. Our objective in this case study is to describe our observations and evaluate plausible explanations for the disease process.

Experimental Procedures

Fifteen multiparous Holstein dairy cows (10 in lactation 2, 4 in lactation 3, and 1 in lactation 4) between 94–197 days in milk were housed at the Kansas State University Dairy Teaching and Research Center (Manhattan, KS) in tie-stalls with rubber mats and wood shavings. These cows were part of a larger production study; here we are reporting responses in control cows only. Cows were fed a total mixed ration (TMR; Table 1) twice daily, consisting of corn silage, alfalfa hay, corn milling product (Sweet Bran, Cargill, Blair, NE), whole cottonseed, and a grain mix. The corn silage was from 2018 and was drought-stressed—as a result, it had low starch content, which required us to add more concentrate to meet energy requirements. Feed and feed refusal samples were collected every week, combined into bi-weekly composites, and analyzed for chemical composition.

Cows were milked 3 times daily at 04:00, 11:00, and 16:00 hours. Milk yield was recorded at each milking and milk samples were collected for 6 consecutive milkings on Thursday and Friday every week for component analysis. In addition, blood samples were collected from the tail vein once every other week between the second and third milkings. Plasma was collected and later analyzed for intestinal health biomarkers.

Data were analyzed to assess the effects of study week, parity, and their interaction, while accounting for the random effect of cow in a repeated-measures statistical model.

Results and Discussion

Over the course of the study, 5 of the 15 cows were removed from the study over health concerns. Some loose manure was noted relatively early in the study, particularly from week 4 on; however, for most cows and most days, manure consistency was within the normal range.

The first cow to be removed from study was in her fourth lactation and developed hock inflammation in week 4. The next three cows were removed from the study for digestive disorders. During week 5, the first serious summer heat stress window occurred, with mean weekly environmental temperature-humidity index climbing past 70 (Figure 1). At the end of week 5, a second-lactation cow suddenly (within 24 hours) went off feed and stopped producing milk. Physical examination revealed extremely high rumen motility, but her body temperature and water intake were normal. Within 48 hours, she was diagnosed with a displaced abomasum. In week 7, two more second-lactation cows were removed from the study within 48 hours of each other after 4 days of declining feed intake. Digestive tract abnormalities (high motility, diarrhea) were again observed, and one of these cows also showed some apparent neurological issues.

At this point in the study, samples were sent for initial mycotoxin analysis and the diet was adjusted to partially replace an excessively dry corn silage (>40% dry matter) with a more typical corn silage and some straw to enhance effective fiber content of the diet. Furthermore, we began treating the silage faces with organic acids (Ultra-Curb, Kemin, Des Moines, IA) to limit mold growth at feed-out and added an anti-mycotoxin product (Biofix Plus Pro; Biomin America, Overland Park, KS) to the grain mix at 0.10% of the ration (dry matter basis). All of these changes were in place by the end of week 8. One additional cow (third lactation) was removed from the study during week 13 due to clinical mastitis.

Dry matter intake (Figure 2) was greater for cows in parity 3+ vs. parity 2 ($P = 0.01$), and it varied by week ($P < 0.01$), with weeks 2 and 4 differing from week 1 ($P < 0.05$). The uptick in

feed consumption in week 2 likely reflects adaptation to the new diet, whereas shifts up and down after that time likely reflect a combination of responses to heat stress and declining milk yield with advancing days in milk.

Milk yield is shown in Figure 3. Not surprisingly, parity 3+ cows produced more milk than parity 2 cows ($P = 0.05$). In addition, milk yield varied by week ($P < 0.01$) with lower production in weeks 2, 3, 9, 10, 11, 12, 13, and 14 relative to week 1 ($P < 0.05$). Although a progressive decrease in milk yield is expected in this group of cows past peak lactation, the more dramatic decline in milk yield from weeks 9 through 14 was likely a response to the change in diet, particularly the inclusion of dietary straw to increase effective fiber content. Previous research has shown that adding slowly-fermenting fiber to lactation diets typically decreases fluid milk yield.

Parity groups did not differ in milk fat content ($P = 0.47$, Figure 4); however, there was week-to-week variation ($P = 0.01$). Fat content was decreased in weeks 2, 3, 4, 5, 7, 9, and 10 relative to week 1 ($P < 0.05$) before recovering in weeks 11–14. Second lactation cows tended to have greater milk protein content compared with older cows ($P = 0.098$, Figure 5). In addition, weeks 2, 4, 7, 8, 10, 11, 13, and 14 all differed from week 1 ($P < 0.05$). Energy-corrected milk yield was greater in parity 3+ cows vs. second lactation cows ($P = 0.02$; Figure 6), and weeks 2, 9, 10, 11, 12, 13, and 14 were different from week 1 ($P < 0.05$). Feed efficiency (defined as ECM yield /dry matter intake) is shown in Figure 7. Cows in lactation 3+ lactations group had greater FE than younger cows ($P = 0.01$), and efficiency was greater in weeks 2 and 4 vs. week 1 ($P < 0.05$).

Blood samples collected biweekly throughout the study were analyzed for haptoglobin, an acute phase protein that is elevated during systemic inflammation. Plasma haptoglobin concentrations (Figure 8) tended to be greater in second lactation cows vs. parity 3+ cows ($P = 0.08$), and week 1 differed from weeks 3, 5, 9, and 11 ($P < 0.05$), reflecting a rise above baseline in the middle of the study and a significant decrease by week 11. This temporal pattern aligned with visual observations of digestive function (e.g., gut motility, fecal consistency) and suggested that changes put in place in weeks 7–8 likely had a positive impact on the inflammatory status of cows by week 11. D-lactate concentrations (Figure 9) in plasma tended ($P = 0.051$) to be greater in second lactation cows compared to parity 3+ cows, and weeks 7, 11, and 13 differed from week 1 ($P < 0.05$). Like haptoglobin, D-lactate rose gradually from week 1, peaking in week 7 and then declining to concentrations less than baseline by weeks 11–13. As D-lactate is produced primarily by microbial metabolism, its increased concentration in plasma by week 7 likely indicates a decline in gut barrier function (leaky gut) and/or excessive hind-gut fermentation, with an apparent return to a more normal status by the end of the study. It is also notable that all 3 of the cows that left the study due to digestive disorders were second-lactation cows.

Twice during the study (weeks 9 and 14), feed and fecal samples were collected to enumerate viable clostridia bacteria and those from the species *C. perfringens* specifically. The drought-stressed dry corn silage had clostridia concentrations in week 14 almost 50× greater than samples collected in week 9, and the concentration of clostridia in the TMR increased from weeks 9 to 14 (Table 2). In contrast, fecal samples showed decreased total clostridia as well as *C. perfringens* from weeks 9 to 14, suggesting a shift in the gut microbial ecosystem that inhibited this population. This disparity could be the result of the diet composition change, or potentially a change in mycotoxin-microbe-gut interactions following the incorporation of mitigation strategies.

Mycotoxin concentrations detectable in the ration throughout the study are represented in Figure 10. There is relatively little evidence available to establish concentrations of mycotoxins that are of concern for ruminants. There is some information regarding responses to aflatoxins, but we did not detect that class in any ration samples analyzed. Instead, we found that cows

were constantly exposed to type B trichothecenes at diet concentrations between 843 and 1,069 parts per billion (ppb, as-fed). *Fusarium* fungi produce trichothecenes including nivalenol and deoxynivalenol (DON, also known as vomitoxin), the two compounds detected in our samples. Zearalenone is also produced by *Fusarium* fungi, but is an estrogenic metabolite. During the study, zearalenone concentrations in ration samples hovered around the detection limit of 51.7 ppb (as-fed), with a peak concentration of 109.8 ppb detected. On their own, these mycotoxins present challenges to producers, but previous research has shown that exposure to mixtures of mycotoxins can have a more acute impact.

As a case study, interpretation of these observations must be carried out cautiously. Multiple factors were changing simultaneously during the course of the study, including weather conditions, microbial and mycotoxin contaminants, forage sources, and mitigation strategies. Furthermore, we must acknowledge that the removal of the most susceptible cows in the middle of the study likely contributed to several measurements returning to normal ranges by the end of the study.

With those caveats in mind, we propose that the gastrointestinal health challenges observed during this study emerged as a result of "stacked stressors." The diet formulation likely introduced one risk factor; although not extreme, the diet was marginal in terms of supply of physically-effective fiber, which made disturbance of the gut microbial ecosystem more likely. Secondly, the consistent exposure to mycotoxins likely contributed to disruption of both the microbial populations and the gut itself. Finally, the onset of summer heat stress seemed to tip some of the cows over the edge into clinical disease.

Assuming that the proposed etiology is correct, what could have been done to avoid these problems? Because we did not see a drop in the measurable mycotoxins during the study (Figure 10) and heat stress continued to challenge the cows (Figure 1), the resolution of disease biomarkers and clinical signs of digestive problems by week 11–12 suggest that at least some of our changes were likely effective. Increasing dietary forage has a protective role in microbial stability in the gut, and directly contributes to slowing passage of feed through the gastrointestinal tract. Organic acid treatment of the silage face (particularly for an excessively dry silage) can help to limit fungal growth at feed-out, and mycotoxin binding products are effective at binding at least some mycotoxins, helping to wash them out of the gut with less impact. Unfortunately, due to more extreme weather patterns in the Midwest, mycotoxins are becoming more prevalent in animal feeding programs and need to be addressed.

In summary, we documented a clear outbreak of gastrointestinal disruption in mid-lactation cows, occurring with a mild milk fat depression and a significant increase in markers of systemic inflammation and gut microbial disruption. These issues resolved following dietary changes, although complete resolution of the problem took approximately a month. This case study provides insights into the disease process and ideas for dealing with similar problems on dairy farms.

Table 1. Ingredient and chemical composition of total mixed ration (% dry matter (DM))

	Diet	
	Weeks 1–8	Weeks 9–14
Ingredients		
Corn silage	16.66	12.57
Alfalfa hay	20.78	20.93
Corn milling product[1]	17.36	17.48
Cottonseed	2.79	2.81
Corn grain	24.79	24.96
Expeller soybean meal[2]	12.15	12.23
Limestone	1.25	1.26
Sodium bicarbonate	0.85	0.86
Calcium salts of long-chain fatty acids[3]	0.83	0.84
Micronutrient pre-mix	2.54	2.44
Wheat straw	-	3.51
Direct-fed microbial[4]	-	0.11
Nutrients		
DM, %	61.7	59.9
Net energy for lactation, Mcal/kg DM	1.69	1.63
Crude protein	18.5	19.4
Ether extract	5.03	5.01
Neutral detergent fiber	31.4	31.3
Acid detergent fiber	20.3	20.9
Ash	8.46	9.72
Ca	1.00	1.25
P	0.49	0.50

[1]Sweet Bran, Cargill, Blair, NE.
[2]Soy Plus, Landus Cooperative, Ames, IA.
[3]Essentiom, Arm & Hammer Animal Nutrition, Princeton, NJ.
[4]Biofix Plus Pro; Biomin America, Overland Park, KS.

Table 2. Average clostridia and *C. perfringens* enumerated in feed and fecal samples

	Clostridia CFU[1]/g		*C. Perfringens* CFU/g	
	Week 9	Week 14	Week 9	Week 14
Feed samples				
Total mixed ration	1,280	8,700	100	50
Dry corn silage	440	21,850	20	50
Wet corn silage	20	<10	<10	<10
Fecal samples	34,000	5,000	31,000	4,100

[1]Colony forming units; an approximation of viable bacteria.

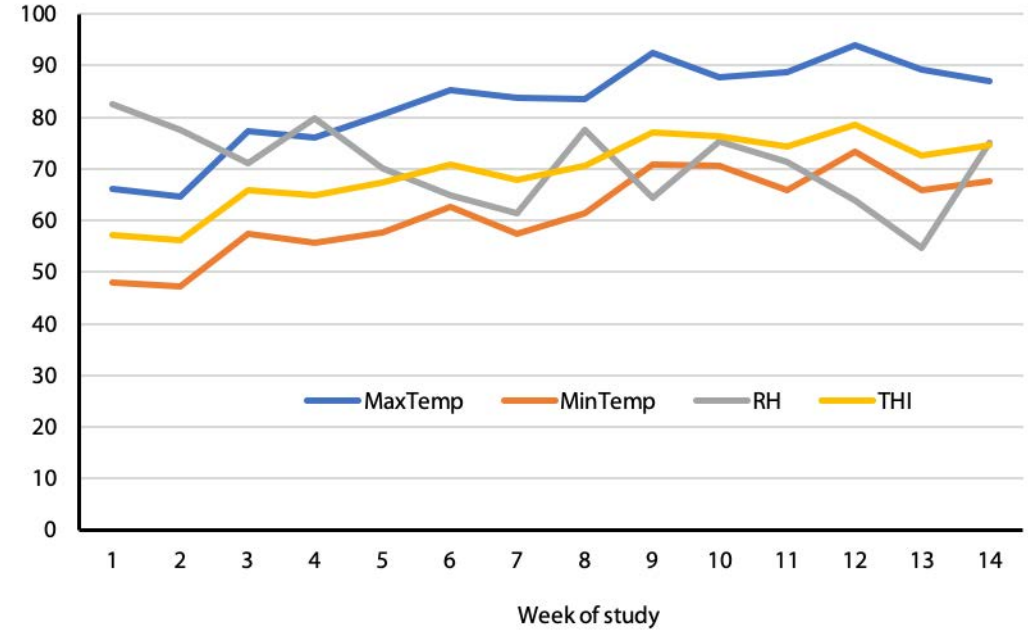

Figure 1. Averages for external maximum daily temperature (MaxTemp, °F), minimum daily temperature (MinTemp, °F), relative humidity (RH, %), and temperature-humidity index (THI) by week of the study.

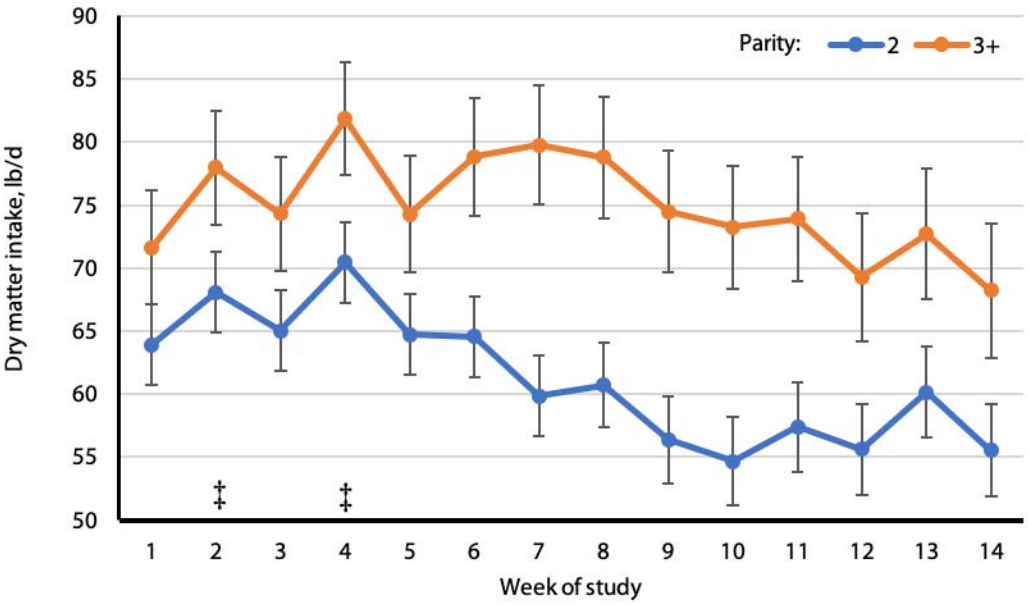

Figure 2. Dry matter intake of cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
‡ $P < 0.05$ vs. week 1.

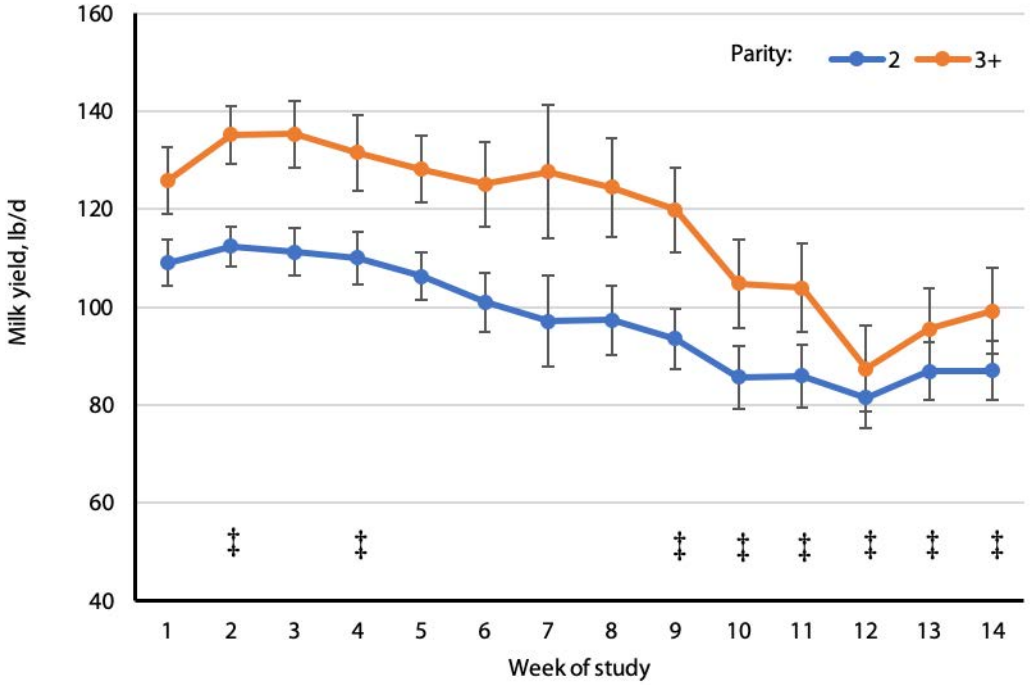

Figure 3. Milk yield of cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
‡ $P < 0.05$ vs. week 1.

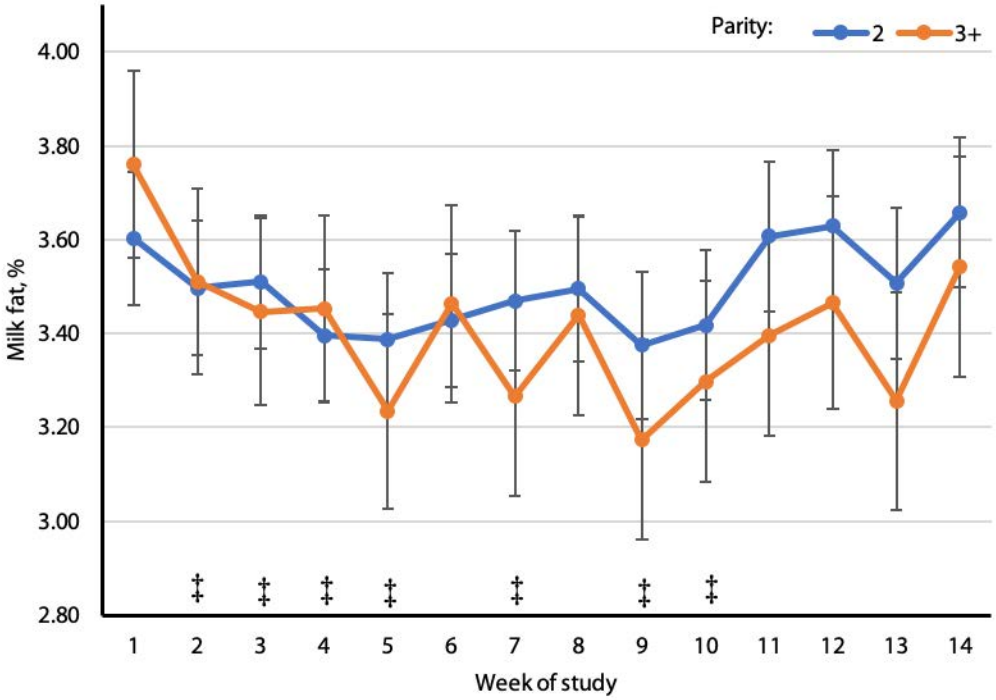

Figure 4. Fat content of milk from cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
‡ $P < 0.05$ vs. week 1.

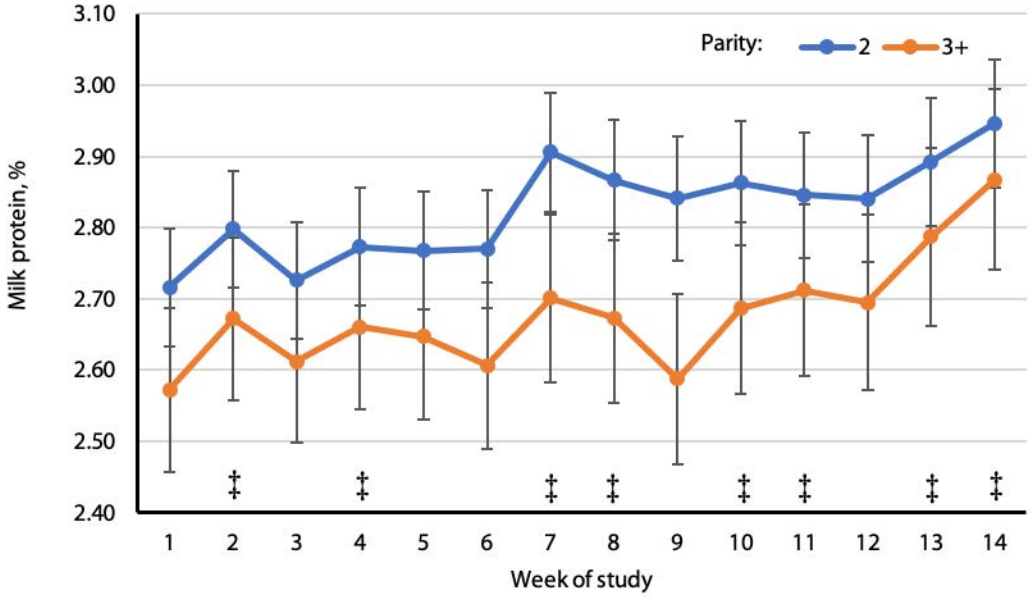

Figure 5. Protein content of milk from cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
‡ $P < 0.05$ vs. week 1.

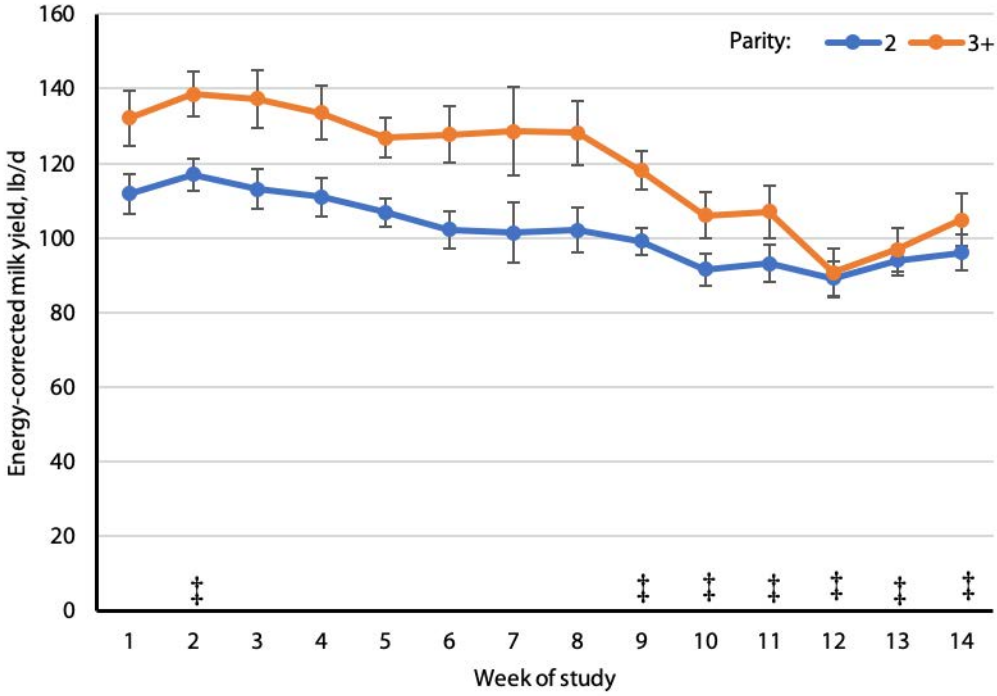

Figure 6. Energy-corrected milk yield of cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
‡ $P < 0.05$ vs. week 1.

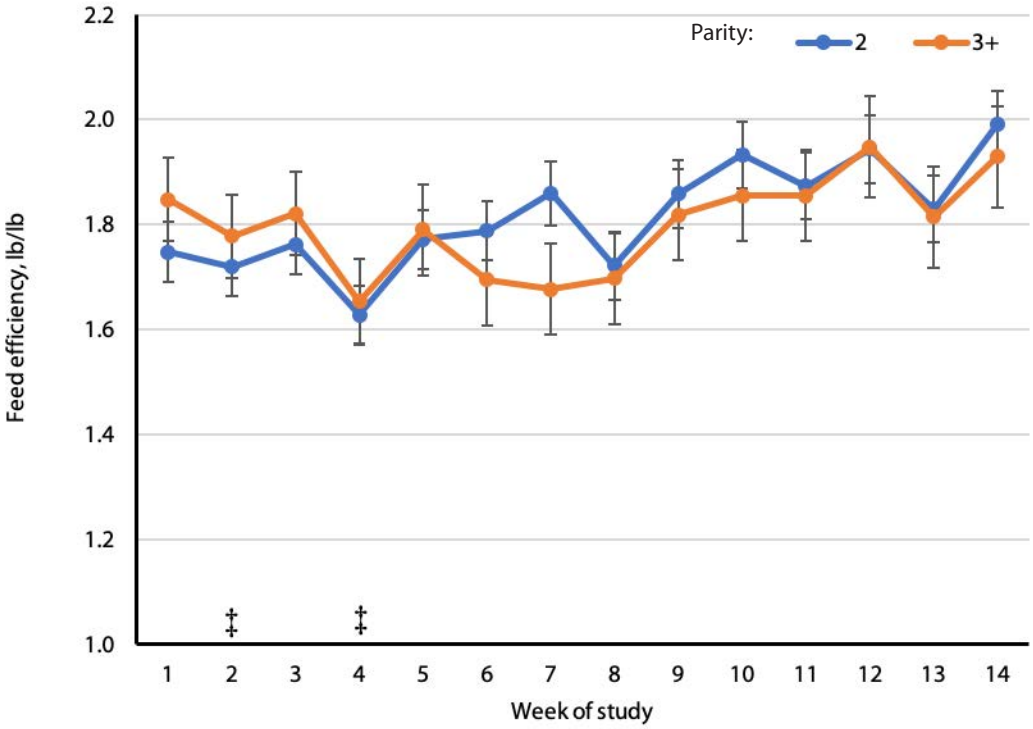

Figure 7. Feed efficiency of cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
‡ $P < 0.05$ vs. week 1.

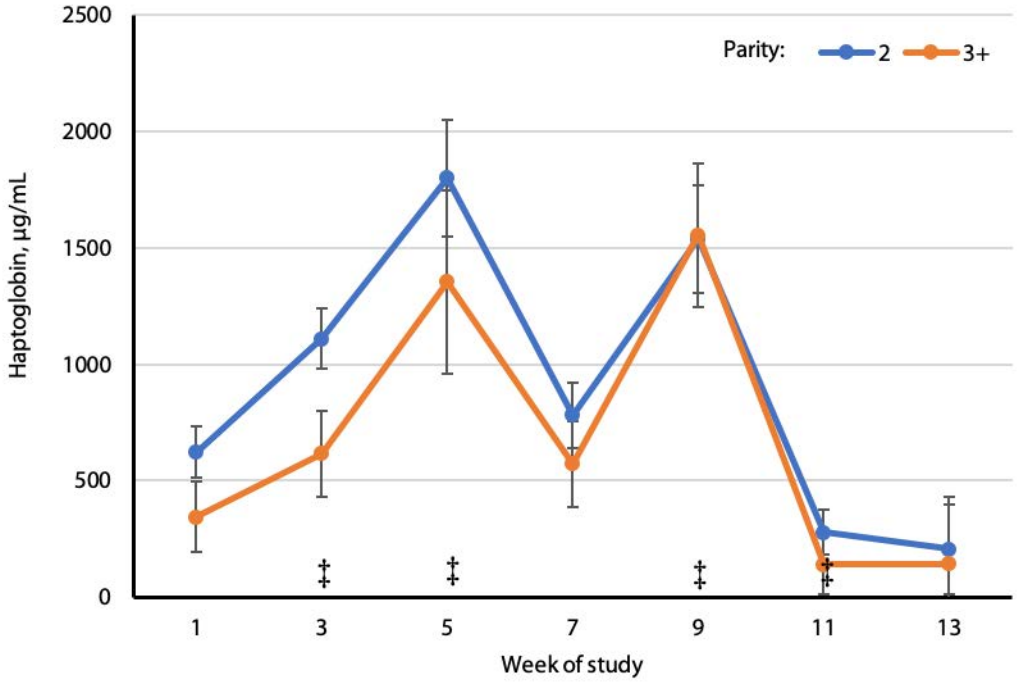

Figure 8. Blood plasma haptoglobin concentrations of cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
‡ $P < 0.05$ vs. week 1.

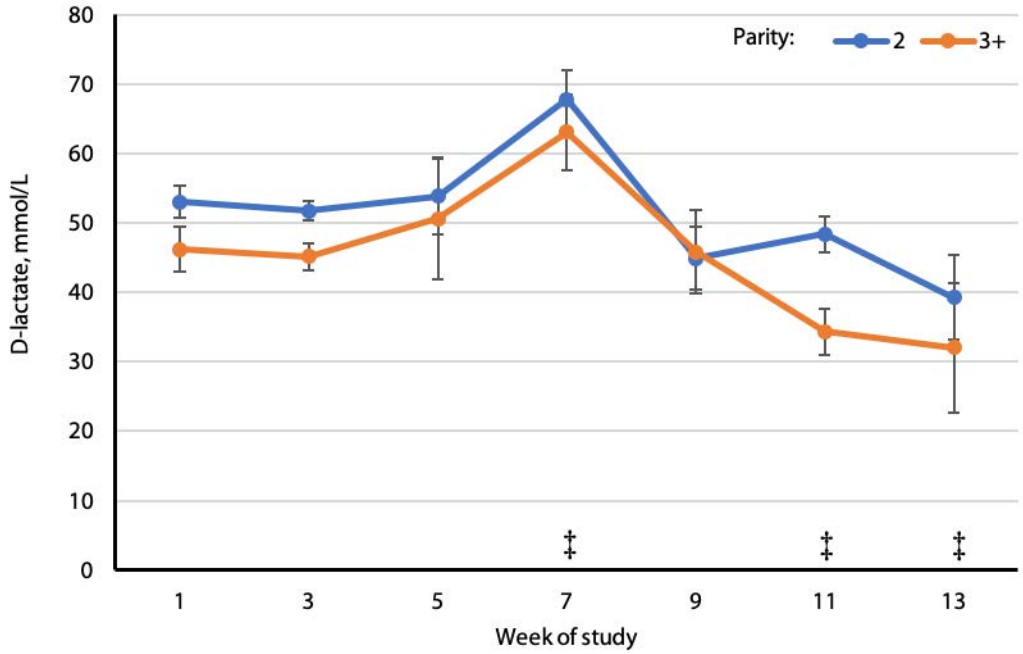

Figure 9. Blood plasma D-lactate concentrations of cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
‡ $P < 0.05$ vs. week 1.

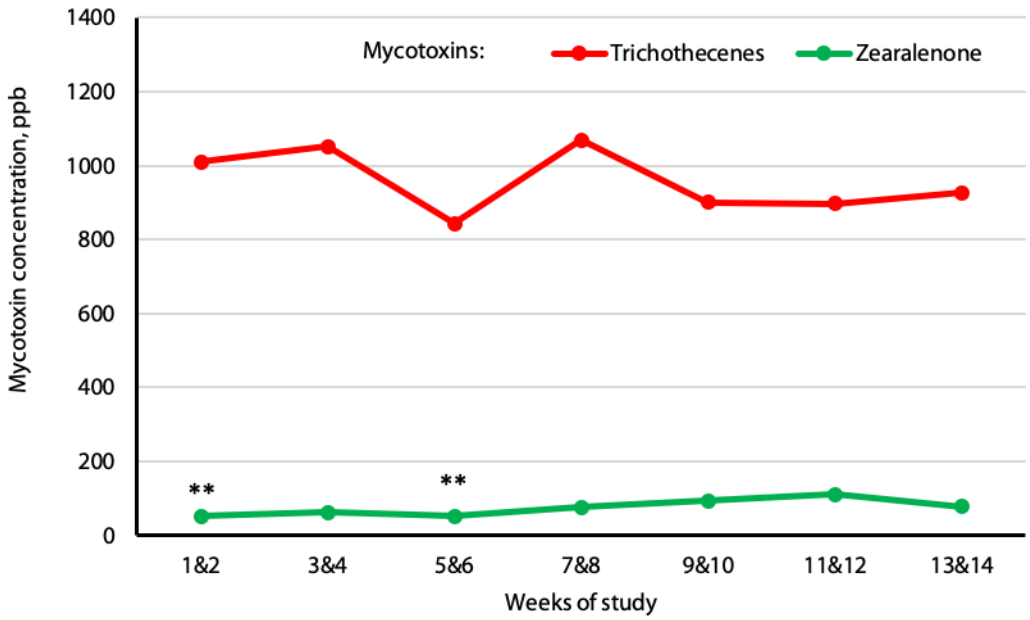

Figure 10. Mycotoxin concentration of composite ration samples (as-fed basis). Values are means, with trichothecenes being the summation of nivalenol and deoxynivalenol concentrations.
**Weeks where zearalenone concentrations were below detectable limit of 51.7 ppb.

Acknowledgments

Appreciation is expressed to the following organizations for their support of dairy teaching, research, and extension at Kansas State University during 2018-2019.

Absolute Innovations, Osceola, IN
Aerotech, Mason, MI
AgTech, Inc., Manhattan, KS
Alta Genetics, Watertown, WI
Arm & Hammer Animal Nutrition, Princeton, NJ
Balchem Corporation, New Hampton, NY
Bayer Animal Health, Shawnee Mission, KS
Biomin America, Overland Park, KS
BouMatic, Madison, WI
Built So-Well, Manhattan, KS
Cargill, Inc., Wayzata, MN
CTI John Deere, Clay Center, KS
Dairy Farmers of America, Kansas City, KS
Dairymaster, County Kerry, Ireland
Dekalb Asgrow, St. Louis, MO
DeLaval, Kansas City, MO
Diamond V Mills, Cedar Rapids, IA
Dupont Pioneer, Johnston, IA
Edlong Dairy Technologies, Elk Grove Village, IL
Evonik Industries AG, Essen, Germany
Grain States Soya, West Point, NE
Heart of America Dairy Herd Improvement Association, Manhattan, KS
High Plains Dairy Management Conference
Hubbard Feeds, Mankato, MN
Idaho Milk Products, Jerome, ID
Idexx Laboratories, Westbrook, ME
Iowa Limestone, Des Moines, IA
K-State Research and Extension, Manhattan, KS
KanEquip, Wamego, KS
Kansas Dairy Commission, Hays, KS
Kansas Department of Agriculture, Manhattan, KS
Kansas Department of Health and Environment, Topeka, KS
Kemin Industries, Des Moines, IA
Land O'Lakes Animal Milk Products, Shoreview, MN
Landus Cooperative, Ames, IA (Tim Brown, Westmoreland, KS)
Livestock and Meat Industry Council, Manhattan, KS
Merck Animal Health, Whitehouse Station, NJ
Mid Kansas Cooperative, Manhattan, KS
NutriQuest, Mason City, IA
Parnell Pharmaceutical, Overland Park, KS
Phibro Animal Health, Teaneck, NJ
Poet Nutrition, Sioux Falls, SD
Provimi North America, Brookville, OH
Purina Animal Nutrition, Gray Summit, MO
Rota-Mix, Dodge City, KS
Select Sires, Plain City, OH
Sweet Bran, Blair, NE
Tastepoint by IFF, Philadelphia, PA
Trouw Nutrition USA, Highland, IL
USDA National Institute of Food and Agriculture, Washington, D.C.
White Star, Manhattan, KS
Whorton, Inc., Horton, KS
Zoetis Animal Health, Florham Park, NJ

The Department of Biological and Agricultural Engineering and the College of Veterinary Medicine at Kansas State University are recognized for their cooperation and contributions to our dairy research and teaching program.

Biological Variability and Chances of Error

Variability among individual animals in an experiment leads to problems in interpreting the results. Although cows on treatment X may have produced more milk than those on treatment Y, variability within treatments may indicate that the differences in production between X and Y were not the direct result of treatment alone. Statistical analysis allows us to calculate the probability that such differences occur because of the treatment applied rather than from chance.

In some of the articles herein, you will see the notation "$P < 0.05$." That means the probability of treatment differences resulting from chance is less than 5%. If two averages are reported to be "significantly different," the probability is less than 5% that the difference is from chance, or the probability exceeds 95% that the difference resulted from the treatment applied.

Some papers report correlations or measures of the relationship among traits. The relationship may be positive (both traits tend to get larger or smaller together) or negative (as one trait gets larger, the other gets smaller). A perfect correlation is one (+1 or -1). If there is no relationship, the correlation is zero.

In other papers, you may see an average given as 2.5 ± 0.1. The 2.5 is the average, 0.1 is the "standard error." The standard error is calculated to be 68% certain that the real average (with an unlimited number of animals) would fall within one standard error from the average, in this case between 2.4 and 2.6.

Using many animals per treatment, replicating treatments several times, and using uniform animals increase the probability of finding real differences when they exist. Statistical analysis allows more valid interpretation of the results, regardless of the number of animals in the experiment. In all the research reported herein, statistical analyses are included to increase the confidence you can place in the results.

The Livestock and Meat Industry Council, Inc.

The Livestock and Meat Industry Council, Inc. (LMIC) is a nonprofit charitable organization supporting animal agriculture research, teaching, and education. This is accomplished through the support of individuals and businesses that make the LMIC a part of their charitable giving.

Tax-deductible contributions can be made through gifts of cash, appreciated securities, real estate, life insurance, charitable remainder trusts, and bequests as well as many other forms of planned giving. The LMIC can also receive gifts of livestock, machinery, or equipment. These types of gifts, known as gifts-in-kind, allow the donor to be eligible for a tax benefit based on the appraised value of the gift.

Since its inception in 1970, the LMIC has provided student scholarships, research assistance, capital improvements, land, buildings, and equipment to support students, faculty, and the industry of animal agriculture. If you would like to be a part of this mission or would like additional information, please contact the Livestock and Meat Industry Council/Animal Sciences and Industry, Weber Hall, Manhattan, KS, 66506 or call 785-532-1259.

LMIC Board Members

Gene Barrett
David Clawson
Doug Deets
Mark Gardiner
Craig Good
Mark Gratny
Ken Grecian
Kim Harms
Frank Harper
Roy Henry
Patsy Houghton
Virgil Huseman
Justin Janssen
Jerry Kuckelman
Debbie Lyons-Blythe
Steve Mangan
Bill Miller
Lisa Moser
Stanton O'Neil
Rich Porter
Jim Riemann
Randall Spare
Tom Toll
Mark Young

Royal Board Members

Dell Allen
Kyle Bauer
Jerry Bohn
Galen Fink
Randy Fisher
Lyle Gray
Sam Hands
Bernie Hansen
Greg Henderson
Steven Hunt
Steve Irsik
Larry Jones
Kenny Knight
Mark Knight
Pat Koons
Kelly Lechtenberg
Jan Lyons
Gina Miller
Andrew Murphy
Tom Perrier
Harland Priddle
Lee Reeve
Don Smith
Ken Stielow
Mikel Stout
Kathleen Strunk
Duane Walker
Warren Weibert

DAIRY RESEARCH 2019

Publications from Kansas State University are available at: ***www.ksre.ksu.edu***

KANSAS STATE UNIVERSITY AGRICULTURAL EXPERIMENT STATION AND COOPERATIVE EXTENSION SERVICE

K-State Research and Extension is an equal opportunity provider and employer.

January 2020