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KANSAS STATE UNIVERSITY

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DAIRY RESEARCH 2014

Contents

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Foreword

Members of the Dairy Team at Kansas State University are pleased to present the 2014 Dairy Research Report of Progress. Dairying continues to contribute significantly to the agricultural economy of Kansas. In 2012, dairy farms accounted for 3.6%, or $541 million, of all Kansas farm receipts, ranking 7th overall among all Kansas farm commodities. The Kansas dairy industry is growing, ranking number 1 nationally with an increase of 8,000 cows from 2012 to 2013. During the past 5 years (2008 to 2013), total milk production in Kansas has increased by 21.4%; the number of cows by 14.5%; and pounds of milk per cow by 1,240. At the end of 2013, Kansas ranked 13th nationally in milk yield per cow at 21,881 lb, 16th in the number of dairy cows (134,000), and 16th in total milk production (2.93 billion lb). Kansas now has 325 dairy operations and averages 412 cows per herd (*Hoard's Dairyman*, March 25, 2014, pp. 212-213).

Selected production traits of our Kansas State University Dairy Teaching and Research Center (DTRC) herd are shown below. With the pressures of research and an aging facility, we shifted most of the herd to twice daily milking in August 2014, but we remain pleased with our production. The excellent functioning of our herd is a tribute to the dedication of our staff: Michael Scheffel (manager), Daniel Umscheid, Robert Fiest, Alan Hubbard, Kris Frey, and Eulises Jiron Corrales. Special thanks are given to Colleen Hill, Cheryl Armendariz, and a host of 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 DHIA laboratory here in Manhattan, KS, for its assistance in handling research milk samples.

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

Cows, total no.	312
Rolling herd milk, lb	31,890
Rolling herd fat, lb	1,076
Rolling herd protein, lb	941
Somatic cell count × 1,000	157
Calving interval, mo.	12.9

[1] October 8 test day (milking 2 to 3 times daily; no bST).

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 dairymen. It is our hope that the type of research presented in this report contributes to those improvements. We recognize and thank Dr. Jeffrey Stevenson for his time and expertise in editing the previous 30+ annual dairy research reports; his impact has been tremendous.

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
2014 Dairy Research Report of Progress

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.

Effects of Milk, Pasteurized Milk, and Milk Replacer on Health and Productivity of Dairy Calves

L. Hulbert, S. Trombetta, J. Noel, S. Moisá, S. Montgomery, G. Hanzlicek, and B. Bradford

Summary

Our objectives were to determine the health and blood parameters before, during, and after weaning of 114 Holstein heifers fed either accelerated milk replacer (MR; 28% CP, 18% fat) or non-saleable milk (3.59 ± 0.28% true protein; 4.12 ± 0.37% fat) that was either pasteurized (PM) or raw (RM; refrigerated and fed <24 h after collection). Calves were randomly assigned to feeding treatments at birth. Colostrum (1 L) was fed less than 14 hours after birth (MR and PM = pasteurized colostrum; RM = raw colostrum). All calves were bottle-fed 1.8 ± 0.20 L, 3 times daily; all calves were provided fresh water and grain *ad libitum* throughout the experiment. Calves began step-down weaning at age 5 weeks and completed weaning at age 6 weeks. Blood samples were collected at ages 3, 5, and 7 weeks and were analyzed for complete blood counts (CBC) using a Procyte Idexx Analyzer (IDEXX Laboratories, Inc., Westbrook, ME). Fecal scores were observed twice daily, on a 1 to 3 scale (FS1 = normal, FS2 = loose, FS3 = scours). Results showed that MR-fed calves had more ($P < 0.01$) observations (%obs) with FS > 2 than the PM- and RM-fed calves (2.3 vs. 1.6 and 1.7 ± 0.2 %obs, respectively). In addition, there were no differences in body weight or shoulder or hip height between treatments, but a treatment × week interaction ($P = 0.05$) occurred for grain consumed, with a noticeably higher increase between 6 and 7 weeks of age for MR calves. When CBC was considered, there were no differences in blood cell types, but MR-fed calves had greater mean corpuscular volume (MCV) than the other calves ($P < 0.01$), leading to higher resistance for iron deficiency anemia. In conclusion, these findings suggest that calf performance and feed intake are not affected by the administration of raw milk, pasteurized milk, or milk replacer. Moreover, CBC health parameters showed no significant changes due to administration of the different types of milk sources.

Key words: milk replacer, pasteurization, calves, hematology, fecal score

Introduction

It has become increasingly common for dairy producers to utilize non-saleable milk for feeding calves. This practice has been less common on small dairies, which sometimes struggle with variability in the supply of non-saleable milk and lack the scale to afford high-throughput pasteurization systems, but smaller-scale pasteurization systems are now available and are becoming more widely used on relatively small dairies.

Some research has been conducted to evaluate the effects of using pasteurized non-saleable milk to feed calves. Efficacy of pasteurization (i.e., reduction in bacteria load) has been tested on numerous commercial dairies, and results generally have been favorable when protocols were followed carefully. Several studies have demonstrated increased growth rates for calves fed milk compared with those fed milk replacer, even when

fat, protein, and lactose concentrations were nearly equal between the milk and milk replacer[1]. However, many calf experts now recommend "accelerated" feeding programs based on the utilization of milk replacers with more protein than fat (often 28% protein, 18 to 20% fat on a dry basis). To our knowledge, no university studies have been conducted to compare the performance of dairy heifers fed these newer-generation milk replacers compared with those fed milk.

Experimental Procedures

Heifers born at the Kansas State University Dairy Teaching and Research Center during a 12-month period were enrolled in a randomized complete block design study. Heifers with birth weights <60 lb and those born with calving scores >2 were excluded from the study; approximately 114 heifers were enrolled prior to this analysis. After receiving colostrum twice in the first 14 hours after birth (MR and PM = pasteurized colostrum; RM = raw colostrum), calves were randomly assigned to one of three treatments: raw milk (RM), pasteurized milk (PM), or milk replacer (MR). Milk used for RM and PM came from the non-saleable milk supply at the dairy, and MR was Mother's Pride (Hubbard Feeds, Mankato, MN), which contains 28% protein and 18% fat on a DM basis and was mixed per label directions to achieve 14.2% total solids. This formulation provides the same metabolizable energy per unit of volume as milk; therefore, treatments were fed on an equal-volume basis. From a macronutrient perspective, treatments differed primarily in protein supply. Calves were fed 3 times daily: 3 pints per feeding for calves <80 lb and 4 pints per feeding for calves >80 lb. Heifers were housed individually in hutches and milk/MR and starter intake was recorded daily. Heifers were weaned no earlier than 6 weeks of age and continued to receive treatment milk/MR until they consumed at least 2 lb of starter for 3 consecutive days. Intakes were recorded through weaning, although the study formally ended at 6 weeks of age. After weaning, all calves were managed uniformly.

Samples of all treatment milk/MR were collected once weekly for nutrient analysis, and pre-/post-pasteurization PM samples were collected for total bacterial counts on these days. Fecal scores were recorded twice daily on a 1 to 3 scale (FS1 = normal, FS2 = loose, FS3 = scours) to assess gastrointestinal health, and all diseases and treatments were recorded. Body weights and hip and shoulder heights were recorded weekly at birth until 24 weeks of age.

Statistical analysis was conducted using mixed effects models to assess treatment effects on blood cell profiles, the proportion of observations with abnormal fecal scores, body weight, hip height, shoulder height, milk/MR intake, and starter intake. Fixed effects were treatment, week, and treatment × week interaction. Calf nested within treatment was the random effect.

Results

Calf growth results showed only a week effect on body weight and hip and shoulder height, with no differences due to feeding raw milk, pasteurized milk, or milk replacer (Figure 1).

[1] Lee et al. 2009. Influence of equalizing the gross composition of milk replacer to that of whole milk on the performance of Holstein calves. J. Anim. Sci. 87:1129–1137.

Mean Corpuscular Volume (MCV) was the only hematological value that was affected by treatment × week interaction (Table 1). No treatment effect was detected on blood cell type percentages related to immune response (neutrophils, monocytes, lymphocytes, etc.). These blood cell types were affected by week, with a decrease in percentage for neutrophils over time and increases in the proportions of monocytes and lymphocytes (Table 1). In contrast, there was a treatment effect with higher numbers for hemoglobin (HGB), hematocrit (HCT), MCV, and Eosinophils for MR-calves and lower values for MR-calves compared with other treatments for mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), and platelets count (PLT).

Grain consumed was affected by a treatment × week interaction ($P = 0.05$), with differences mainly due to a week effect with a more dramatic increase in grain consumed from 6 to 7 weeks of age in MR calves (Figure 2).

The proportion of fecal scores higher than 2 on the 1 to 3 scale is presented in Figure 3 as percentage of total observations (twice daily). Results showed that calves that received milk replacer had more frequent occurrence of diarrhea than calves fed with pasteurized milk or raw milk ($P = 0.003$).

Discussion

Different treatments did not affect the growth performance of the calves. Calves grew at a similar rate, with no differences in height between animals fed milk or milk replacer; moreover, intake behavior increased exponentially after 6 weeks of age for all treatments. These results fail to demonstrate any advantage of the additional preweaning protein intake in the MR group.

The MCV is a measure of the average volume of red blood cells. The measure is obtained by multiplying a volume of blood by the proportion of blood that is cellular (HCT) and dividing that product by the number of erythrocytes (red blood cells) in that volume. MCV measurement allows classification of the different types of anemia. Our results showed that milk replacer-fed calves had higher MCV values compared with other treatments for all time points analyzed, with treatment affecting parameters that determine absence of anemia such as higher hemoglobin, higher hematocrit, and higher main corpuscular volume for MR-calves, suggesting that milk replacer-fed calves might be receiving higher amounts of iron through the fortified trace minerals complex of the milk replacer, which leads to an animal less prone to suffer iron deficiency type of anemia (microcytic anemia). Across species, iron is among the most limiting nutrients in milk when consumed as a complete diet.

It was previously established that feeding milk replacer may increase fecal scores in preweaned calves, particularly within the first 2 weeks of life. Presence of diarrhea might lead to dehydration that produces a falsely high hematocrit that disappears when proper fluid balance is restored. Our results were consistent with these observations (Figure 4), which might be related to the difference in protein content between milk replacer and pasteurized or raw milk.

Conclusions

These results failed to provide any evidence of differences in heifer calf growth and feed intake in response to feeding milk replacer or non-saleable milk that is raw or pasteurized. Milk replacer-fed calves might be less prone to iron deficiency anemia based on mean corpuscular volume. However, milk replacer-fed calves had a higher proportion of loose fecal scores, suggesting that the higher protein content increased the visual appearance of diarrhea.

In conclusion, if managers must choose between the use of a milk replacer or non-saleable milk based on the results of this study, they might have a hard time trying to take a decision due to the slight differences perceived in terms of calves' performance and their health status between treatments.

Table 1. Hematological values (mean) for calves that received milk replacer (MR), pasteurized milk (PM), and raw milk (RM) at weeks 3, 5, and 7

	Week 3			Week 5			Week 7				*P*-values		
	MR	PM	RM	MR	PM	RM	MR	PM	RM	SEM	Treat.	Week	T × W
WBB (%)	24.54	26.09	27.85	23.96	19.88	21.97	24.34	22.61	24.70	2.97	0.37	0.67	0.49
HGB (g/dL)	10.28	9.00	9.27	10.56	9.23	9.56	11.11	10.22	9.90	0.25	<0.01	<0.01	0.28
HCT (%)	32.28	26.72	27.72	32.99	27.06	28.17	33.51	29.66	27.81	1.01	<0.01	0.85	0.29
MCV (fL)	38.79	35.76	36.42	37.37	34.38	34.87	36.42	34.96	34.85	0.46	<0.01	<0.01	<0.01
MCH (pg)	12.47	12.37	12.33	12.06	12.11	12.09	12.22	12.33	12.23	0.24	0.54	0.23	0.40
MCHC (g/dL)	32.24	34.81	33.95	33.69	35.73	35.07	33.69	35.47	35.72	0.87	0.01	0.02	0.39
RDW (%)	40.33	40.97	41.40	40.20	41.49	41.66	40.66	42.59	42.46	0.48	0.03	<0.01	0.14
PLT (K/uL)	431	530	527	396	518	491	450	556	456	27	<0.01	0.42	0.09
Neutro (%)	43.61	42.32	43.29	40.72	39.78	37.97	37.93	34.65	37.27	1.59	0.50	<0.01	0.73
Lymph (%)	44.78	48.00	47.18	45.63	46.79	46.50	46.33	49.50	47.26	1.44	0.19	0.44	0.69
Mono (%)	10.15	9.00	8.67	12.11	12.61	11.93	15.05	15.39	15.04	0.68	0.61	<0.01	0.27
Eosin (%)	0.82	0.36	0.53	1.41	0.63	1.41	0.61	0.40	0.47	0.19	0.01	<0.01	0.28
Baso (%)	0.65	0.31	0.36	0.14	0.17	0.10	0.10	0.08	0.07	0.10	0.25	<0.01	0.12

WBB = whole blood bactericide (%); HGB = hemoglobin (g/dL); HCT = hematocrit in percentage of cells vs. plasma; MCV = mean corpuscular volume (fL); MCH = mean corpuscular hemoglobin (pg); MCHC = mean corpuscular hemoglobin concentration (g/dL); RDW = red cell distribution width calculated CV%; PLT = platelets count (K/uL); Neutro = neutrophils (%); Lymph = lymphocytes (%); Mono = monocytes (%); Eosin = eosinophils (%); Baso = basophils (%); SEM = standard error of the mean.

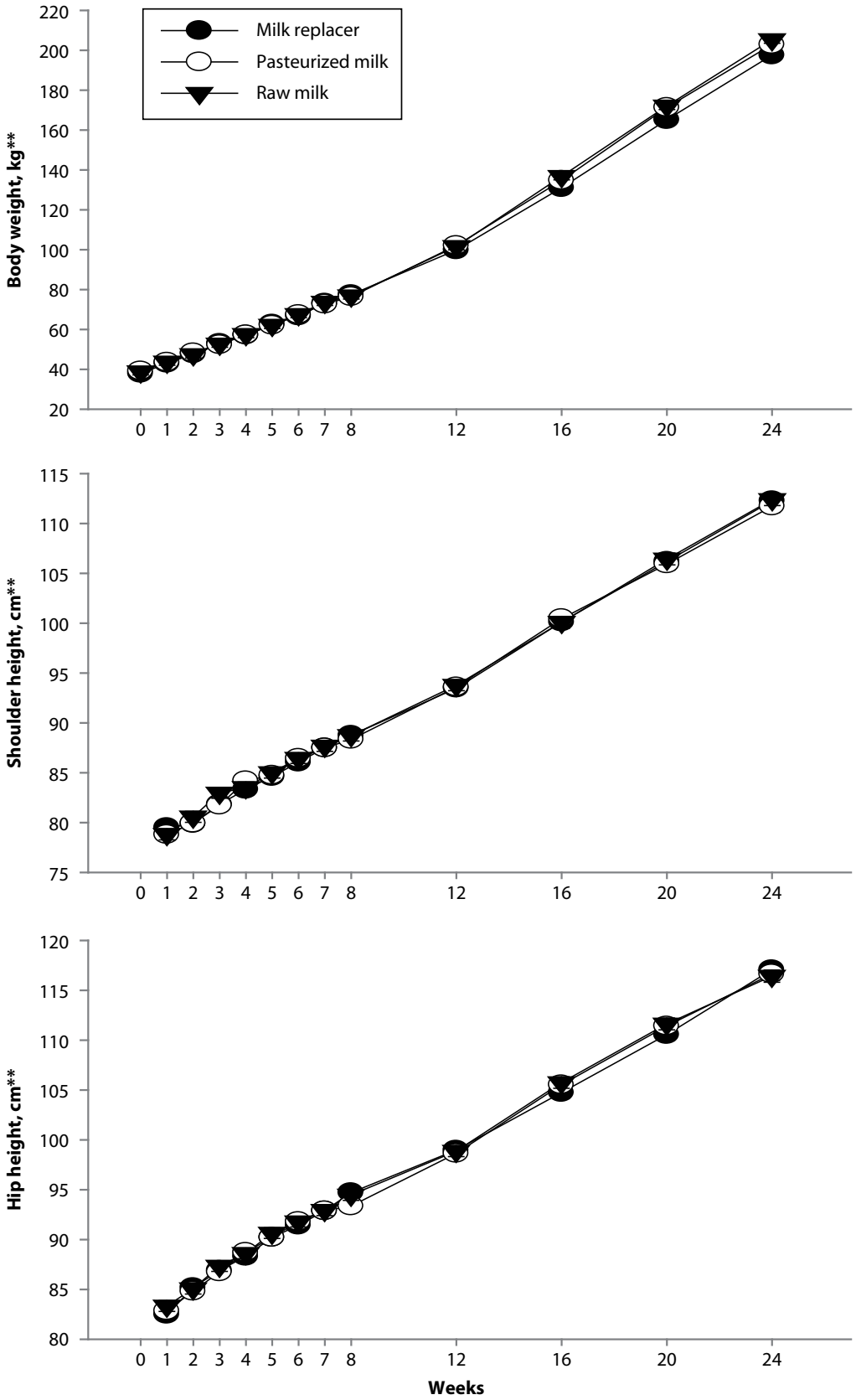

Figure 1. Body weight and shoulder and hip height for calves that received milk replacer, pasteurized milk, and raw milk. Treatments were fed from birth through weaning (6 weeks), and growth was monitored until 24 weeks of age.
P-value < 0.05: * treatment effect, ** week effect, *** treatment × week effect.
SEM = 1.91 for body weight, 0.63 for shoulder height, and 0.63 for hip height.

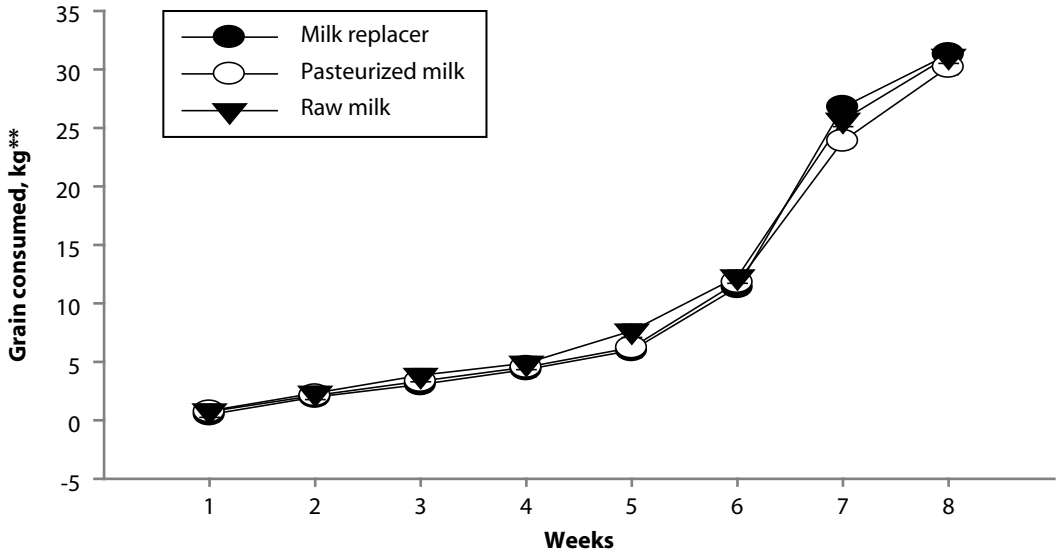

Figure 2. Grain consumed for calves that received milk replacer, pasteurized milk, and raw milk. Starter intake was recorded daily from birth until all calves were weaned (8 weeks of age).
P-value < 0.05: * treatment effect, ** week effect, *** treatment × week effect. SEM = 0.61.

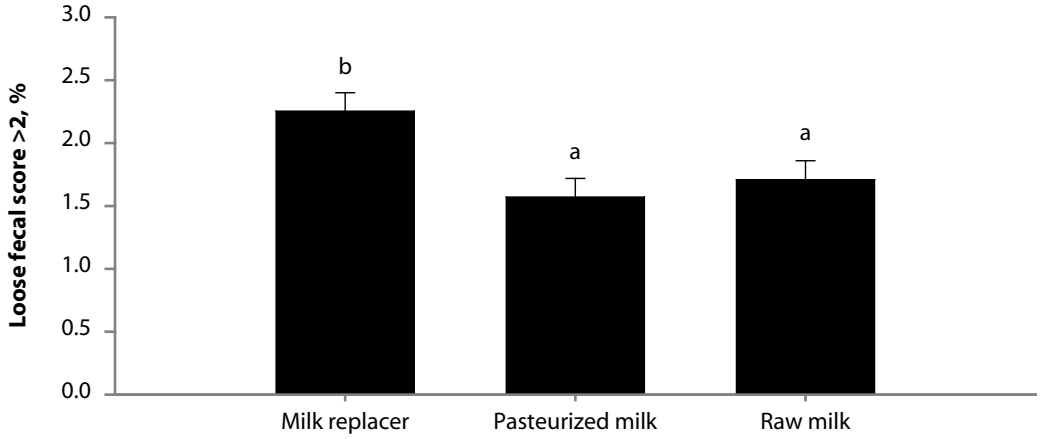

Figure 3. Percentage of observed fecal score higher than 2 on a 1 to 3 scale (FS1 = normal, FS2 = loose, FS3 = scours). Diarrhea appearance was observed twice daily in calves that received milk replacer, pasteurized milk, and raw milk.
Means denoted by different letters differed at $P < 0.05$.

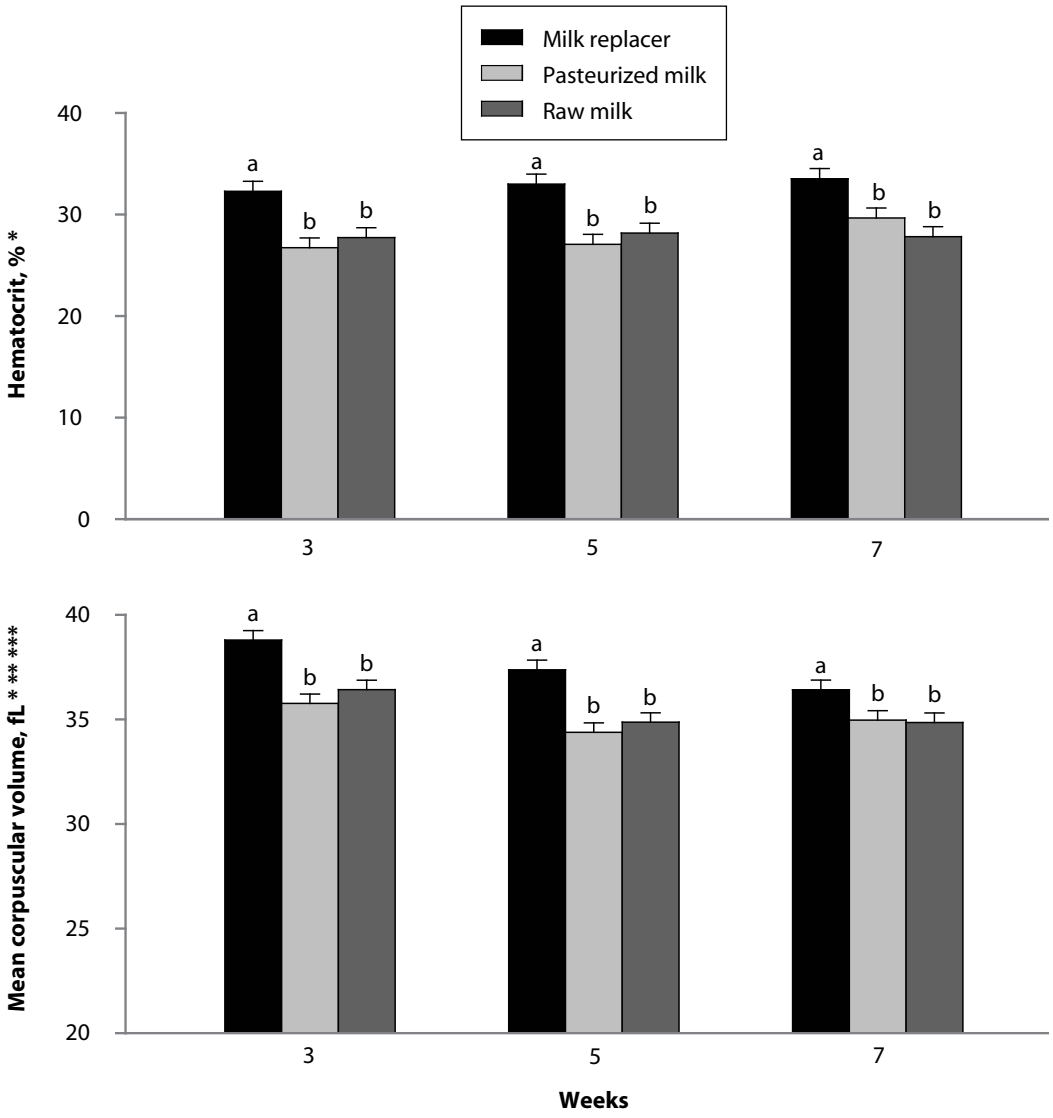

Figure 4. Percentage of hematocrit and mean corpuscular volume at 3, 5, and 7 weeks of age in calves that received milk replacer, pasteurized milk, and raw milk.
Means denoted by different letters differed at $P < 0.05$. * treatment effect, ** week effect, *** treatment × week effect.

Yeast Product Supplementation Influences Feeding Behavior and Measures of Immune Function in Transition Dairy Cows

K. Yuan, M. Muckey, L. Mendonça, L. Hulbert, and B. Bradford

Summary

Yeast supplementation has been shown to increase feed intake and production in some studies with early lactation dairy cows, but the mechanisms underlying this effect remain unknown. The objective of this study was to assess the effects of supplementing a yeast product derived from *Saccharomyces cerevisiae* on production, feeding behavior, and immune function in cows during the transition to lactation. When fed for 3 weeks before calving through 6 weeks after calving, supplementation altered feeding behavior as well as responsiveness to vaccination and gut immunoglobulin secretion. Results suggest that yeast products can modulate several aspects of immune function and promote the consumption of smaller, more frequent meals.

Key words: transition dairy cow, yeast, nutrition, immunity

Introduction

The 2 weeks after calving remain the most challenging window of time during the dairy production cycle, often accounting for more than 50% of total disease in the lactating herd. The health challenges at this time include both metabolic and infectious diseases, resulting in a complex problem, often without any obvious cause. Our current understanding of transition cows suggests that rapid weight loss caused by low feed intake as well as poor immune function during this time combine to lead to these health problems.

Relatively strong evidence indicates that dietary yeast culture can increase DMI in early lactation, and more recent evidence suggests that this can happen through alterations in meal patterns. Furthermore, many studies in model organisms and young ruminants have suggested that yeast products can alter numerous functions of the immune system. In light of the immunosuppression that occurs during the transition period, there is great interest in the use of a yeast product to enhance immune function during this time when mammary and uterine infections are such a challenge. Our objective was to determine whether supplementing a yeast product derived from *Saccharomyces cerevisiae* altered production, feeding behavior, and measures of immune function in transition cows.

Experimental Procedures

Forty multiparous Holstein cows were blocked by expected calving date and randomly assigned within block to 1 of 4 treatments (10 cows per treatment) from 21 days before expected calving to 42 days postpartum. Rations were top-dressed with yeast culture plus enzymatically hydrolyzed yeast (YC-EHY; Celmanax, Vi-COR, Mason City, IA) at the rate of 0, 30, 60, or 90 g/day throughout the experiment. The basal diets fed pre-

partum and postpartum are detailed in Table 1. Dry matter and water intake, feeding behavior, and milk production were monitored daily throughout the study.

To evaluate humoral immune function, cows were challenged (subcutaneous injection) with 1 mg of ovalbumin (OVA; Sigma-Aldrich, St. Louis, MO) diluted in vaccine adjuvant (VET-SAP, Desert King International, San Diego, CA; 0.5 mg of adjuvant dissolved in 1 mL of saline) on days -21, -7, and 14. Blood samples were collected on days -21, 14, and 21 relative to calving from the coccygeal vessels 1 hour prior to feeding for determination of antibody titer against OVA. To evaluate mucosal immune function, fecal samples were collected on days 7 and 21 of lactation for analysis of immunoglobulin A (IgA) concentration.

To evaluate the efficiency of net energy utilization for milk production, energy supplied by the diet and body condition mobilization were estimated. Energy utilization efficiency was then quantified as milk energy output / (diet energy supply + energy from body condition mobilization).

One cow in the 0 g/day treatment group was removed from the study on day 30 postpartum due to difficulty standing up in the tie-stall. Data obtained from this cow prior to removal were included in all analyses. Feeding behavior variables were calculated from logged data that included the start and end weights as well as start and end times of meals, and meals were combined if the intermeal interval was less than 12 minutes. Data were analyzed using mixed models with repeated measures over time. Models included the fixed effects of treatment, time, and their interaction, and the random effect of cow. Contrast statements were used to assess the overall effect of YC-EHY (control vs. all YC-EHY treatments) as well as the linear and quadratic effects of dose. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

Results

Pre- and postpartum DMI and water intake did not differ ($P > 0.10$) among treatments (Table 2). Quadratic dose effects ($P < 0.05$) were detected for prepartum feeding behavior, reflecting decreased meal size and increased meal frequency for cows that received 30 and 60 g/day of YC-EHY. Postpartum feeding behavior and milk yield were unaffected ($P > 0.10$) by treatments, but tendencies for increased ($P \leq 0.10$) percentages of milk fat, protein, and lactose were detected for cows receiving YC-EHY (Table 3). Furthermore, YC-EHY tended to increase the proportion of total energy supply secreted in milk nutrients ($P \leq 0.10$; Table 3).

Increasing YC-EHY dose linearly increased ($P < 0.01$) plasma anti-ovalbumin IgG levels following 3 ovalbumin challenges (Figure 1A), suggesting that treatments enhanced humoral immunity. Increasing YC-EHY dose also quadratically increased fecal IgA concentrations in early lactation ($P = 0.03$; Figure 1B), suggesting that 30 and 60 g/day doses enhanced mucosal immunity.

Discussion

These results provide some intriguing evidence that YC-EHY shifts meal patterns and results in cows eating smaller, more frequent meals. Although this was found to be significant only prepartum, numerical patterns during the postpartum period were

similar. Smaller, more frequent meals may be beneficial from the standpoint of maintaining stable ruminal conditions, because very large meals provide a lot of fermentable substrate and can result in decreased ruminal pH, at least for a short period of time. We found no evidence, however, that YC-EHY increases DMI in the critical first 6 weeks after parturition.

By accounting for energy derived from release of stored tissue (i.e., body fat), we found that YC-EHY tended to improve efficiency of energy utilization for milk production. Substantial evidence in past studies indicates that yeast products can improve diet digestibility in ruminants. Although we did not directly measure this, the tendency for increased energy efficiency with YC-EHY in our study is consistent with an increase in diet digestibility.

We found that YC-EHY increased antibody production following vaccination against OVA and, at intermediate doses, increased gastrointestinal IgA release. Humoral immunity (antibody-based protection) is thought to be suppressed during the transition to lactation, so the ability of a feed additive to enhance this capacity is encouraging. Secretion of IgA by mucosal immune cells lining the gut is one key mechanism that helps prevent attachment of gut pathogens to the epithelium, thereby minimizing gastrointestinal disease. These findings are interesting, but whether or not a feed additive limits the risk of infection or speeds recovery from infection must be tested in disease challenge studies or large-scale studies capable of assessing health risks.

Conclusions

Feeding a yeast culture product with enzymatically hydrolyzed yeast did not affect milk production or DMI during the transition to lactation but modulated feeding behavior and several aspects of immunity. In the current study, a 60 g/day dose of YC-EHY resulted in favorable changes in feeding behavior, mucosal and humoral immunity, and supported the numerically greatest energy efficiency and milk yield postpartum. Future studies with larger numbers of animals may provide more insight into production implications of these biological responses.

Table 1. Ingredient and nutrient composition of diets

Item	Prepartum	Postpartum
Ingredient, % of DM		
Corn silage	29.5	15.9
Wet corn gluten feed	21.3	34.3
Alfalfa hay	-	14.2
Wheat straw	10.9	3.3
Prairie hay	16.8	-
Cottonseed	-	5.0
Ground corn	3.4	11.2
Dry-rolled sorghum grain	3.4	6.4
Mechanically extracted soybean meal	12.3	4.8
Molasses	1.2	1.2
Ca salts of long-chain fatty acids	-	0.8
Micronutrient premix	1.3	2.9
Nutrient, % of DM		
DM, % as-fed	45.4	51.1
CP	13.0	17.7
Starch	21.1	20.2
ADF	24.4	16.9
NDF	42.5	31.0
NFC	33.8	41.1
Ether extract	3.3	4.2
Ash	6.0	8.3

Table 2. Feed and water intake and feeding behavior responses to yeast culture-enzymatically hydrolyzed yeast (YC-EHY) supplementation during the experimental period

	Treatment (YC-EHY dose/day)					*P*-value		
Item	0 g	30 g	60 g	90 g	SEM	YC-EHY vs. Con	Linear dose	Quadratic dose
Prepartum measures								
DMI, kg/day	12.1	11.9	12.6	12.1	0.51	0.86	0.77	0.76
Water intake, L/day	49.0	48.3	49.0	51.3	2.38	0.84	0.48	0.53
Meal frequency, per day	10.1	11.4	11.2	10.0	0.45	0.12	0.80	0.01
Intermeal interval, hours	2.08	1.87	1.94	2.08	0.08	0.18	0.86	0.02
Meal size, kg DM	1.23	1.06	1.16	1.24	0.06	0.27	0.68	0.04
Meal length, minutes	20.6	17.7	17.3	21.1	1.58	0.31	0.87	0.04
Postpartum measures								
DMI, kg/day	21.8	19.7	21.5	22.8	1.13	0.73	0.34	0.14
Water intake, L/day	105.6	96.1	105.6	105.2	5.15	0.59	0.72	0.40
Meal frequency, per day	12.8	13.1	13.5	12.2	0.62	0.85	0.62	0.19
Intermeal interval, hours	1.50	1.50	1.42	1.60	0.08	0.90	0.53	0.29
Meal size, kg DM	1.81	1.69	1.76	1.93	0.10	0.87	0.34	0.14
Meal length, minutes	26.4	25.0	25.3	26.7	1.11	0.56	0.80	0.19

Table 3. Milk production and composition responses to yeast culture-enzymatically hydrolyzed yeast (YC-EHY) supplementation during the experimental period

	Treatment (YC-EHY dose/day)					*P*-value		
Item	0 g	30 g	60 g	90 g	SEM	YC-EHY vs. Con	Linear dose	Quadratic dose
Milk yield, kg/day	45.3	42.6	47.8	46.7	2.53	0.90	0.39	0.72
Milk fat, %	4.11	4.38	4.33	4.17	0.13	0.20	0.80	0.09
Milk protein, %	2.99	2.89	3.04	3.12	0.07	0.76	0.08	0.17
Milk lactose, %	4.77	4.74	4.84	4.85	0.05	0.45	0.10	0.67
Milk urea nitrogen, mg/dL	13.3	14.1	13.4	13.9	0.63	0.44	0.65	0.81
Fat yield, kg/day	1.81	1.82	2.02	1.90	0.09	0.32	0.24	0.46
Protein yield, kg/day	1.34	1.21	1.42	1.42	0.08	0.86	0.17	0.42
Lactose yield, kg/day	2.16	2.03	2.32	2.26	0.13	0.78	0.30	0.79
Energy utilization efficiency[1], %	74.9	79.5	82.6	81.5	3.1	0.08	0.11	0.36

[1] Milk energy output divided by energy provided by feed and BCS mobilization.

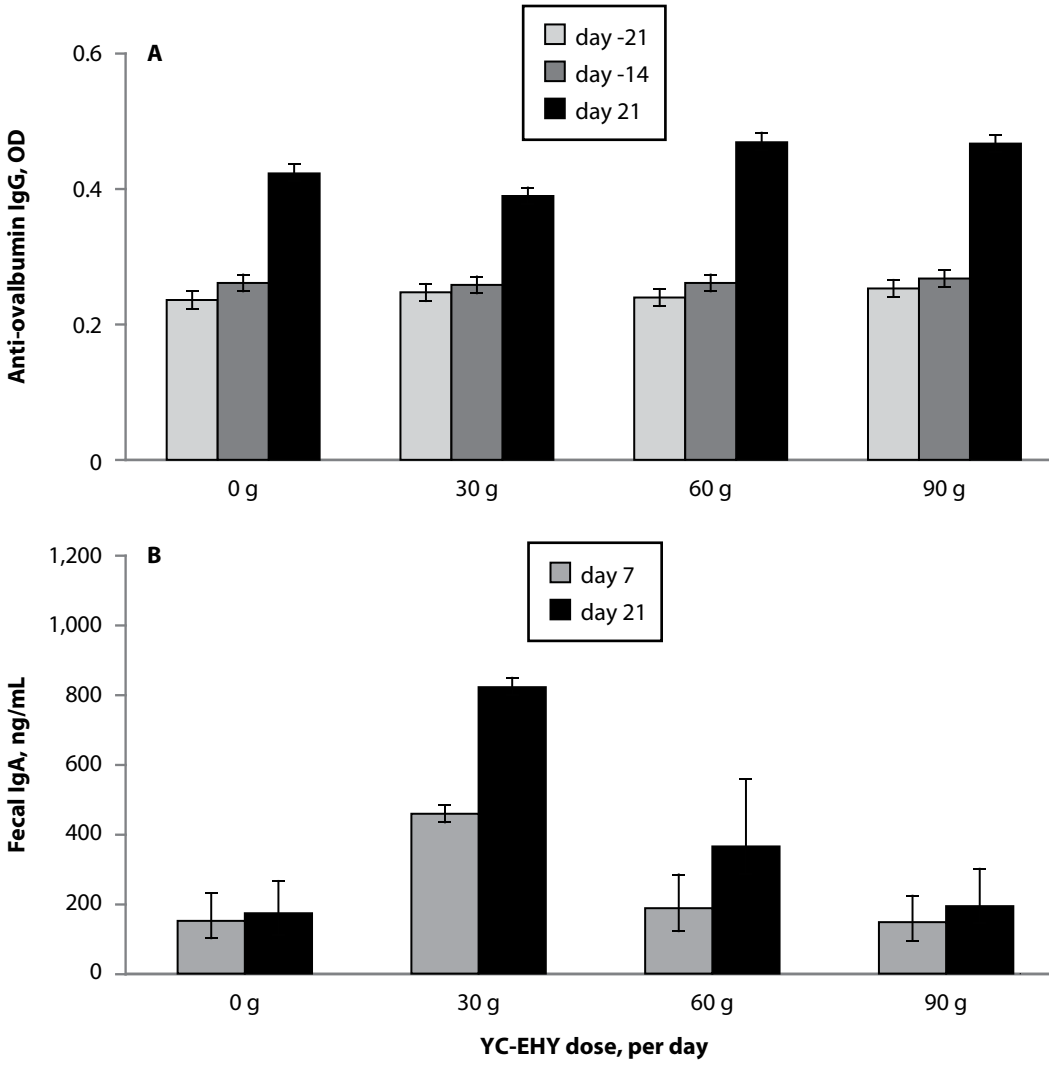

Figure 1. Antibody responses to yeast culture with enzymatically hydrolyzed yeast (YC-EHY) supplemented to dairy cows at 0, 30, 60, or 90 g/day from 21 days before expected parturition to 42 days after parturition. (A) Plasma concentrations of anti-ovalbumin IgG collected on days -21, -14, and 21 relative to calving. Cows were challenged on days -21, -7, and 14 with ovalbumin. There was a tendency for linear dose effect ($P = 0.06$) and a day effect ($P < 0.01$) but no yeast product vs. control ($P = 0.41$) or quadratic dose ($P = 0.50$) effects. A treatment × day ($P < 0.01$) effect was detected, reflecting that yeast product linearly increased ($P < 0.01$) anti-ovalbumin IgG on day 21. (B) Concentrations of IgA in fecal samples collected on days 7 and 21 relative to calving. There was a significant quadratic dose effect ($P = 0.03$), but no yeast product vs. control ($P = 0.16$), linear dose ($P = 0.73$), day ($P = 0.61$), or treatment × day ($P = 0.42$) effects.

Milking Time During Periods of Heat Stress: Part of the Solution or Part of the Problem?

L. Rocha, L. Hulbert, F. Scortegagna, B. Voelz, and L. Mendonça

Summary

Milking time may be a stressful event for lactating dairy cows during summer. Increases in body temperatures because of crowding in the milk parlor holding pen may contribute to increased heat stress. The objective of this extension project was to evaluate the effectiveness of heat stress abatement in milking facilities from two Kansas commercial dairies. Vaginal temperatures at milking were lower than vaginal temperatures before milking in one of the dairies. The lower vaginal temperatures at milking, however, were not observed in the other dairy at all milkings, likely because of differences in efficacy of heat abatement strategies. Milking facilities may be one of the factors to aggravate or alleviate heat stress in lactating dairy cows during summer.

Key words: dairy cattle, heat stress, milking parlor, cooling

Introduction

Heat stress has a tremendous economic impact in the dairy industry worldwide. A 2003 analysis estimated an annual loss of $897 million for the U.S. dairy industry due to heat stress. Decreased milk production, low reproductive efficiency, and increased mortality are the main causes of economic loss to dairy producers that have herds affected by heat stress.

Strategies to cool lactating dairy cows have been the focus of several research studies. The use of fans and sprinkler systems may effectively reduce body core temperature during summer heat stress. The use of evaporative cooling systems in the milk parlor holding pen is highly recommended because the holding pen is the location where cows are crowded, which greatly increases the risk of heat stress because of increased body temperatures. Cooling cows in the holding pen is considered a top priority when dairy producers strive to alleviate heat stress in lactating dairy cows. Although the holding pen may be perceived as a contributor to heat stress of lactating dairy cows, it also may be considered the area where good evaporative heat loss can be achieved by investing resources for an optimal ambient condition. The purpose of this extension project was to compare the effectiveness of milking facilities to achieve cow cooling in two commercial dairies.

Experimental Procedures

Two commercial dairies located in Southwest Kansas were used in this study. Cows that met the following criteria were eligible to be used in the study: 70 to 100 days in milk, non-pregnant, and average daily milk production of 70 to 140 lb. Six lactating Holstein cows from second- (2^{nd}) and third-lactation (3^{rd}) cohorts were randomly selected from each dairy, and vaginal temperature was collected every 5 minutes for 5 consecutive days (August 15 through August 19, 2014). Blank CIDR inserts and calibrated iButton temperature loggers (DS1922L, Embedded Data Systems, Lawrenceburg, KY) were used to collect vaginal temperature. During the study period, evaporative cooling systems were

used in the holding pen. At site A, cows were housed in free-stall barns equipped with fans and sprinklers, with exercise lots adjoining the free-stall barns, and at site B, cows were housed in dry lots with shade in the lounging area. Cows were milked thrice daily at site A and twice daily at site B. The time of each milking was collected from a parlor management software program (DairyPlan C21, GEA Farm Technologies, Naperville, IL). Ambient temperature and relative humidity data were collected from the meteorological station nearest to the dairies. Data were analyzed by ANOVA for repeated measures using the HPMIXED procedure of SAS, or by ANOVA using the GLM procedure of SAS.

Results and Discussion

Three cows lost the temperature loggers and were removed from the study. Days in milk and daily milk yield of cows used in the study are outlined in Table 1. Temperature, humidity, and temperature-humidity index (THI) during the study period are outlined in Figure 1. The average THI during the study period was 86.5 ± 0.54. Cows were under conditions of heat stress during the entire period of the study.

There was no ($P = 0.54$) difference in vaginal temperatures of 2nd-lactation cows from sites A and B (Figure 2A). Third lactation cows from site A had higher ($P < 0.01$) vaginal temperatures compared with 3rd-lactation cows from site B (Figure 2B). Interestingly, at site B, 2nd-lactation cows had higher ($P = 0.05$) temperatures than 3rd-lactation cows (Figure 3B). No difference was detected ($P = 0.51$) in vaginal temperatures between lactations at site A (Figure 3A). Other studies have shown differences in vaginal temperatures between primiparous and multiparous cows; however, to our knowledge, no research study has compared vaginal temperatures of 2nd- and 3rd-lactation cows. Although one could hypothesize that 3rd-lactation cows have higher body temperatures during early lactation compared with 2nd-lactation cows because of higher peak milk yield, heat dissipation of cows from the 2nd- and 3rd-lactation may be different. Body size and surface area may influence heat dissipation, and, consequently, affect body core temperature.

Vaginal temperatures of 2nd- and 3rd-lactation cows from site B were 0.7–0.9°F lower at milking time than temperatures 3 hours before milking (Table 3). At site A, this difference was not detected for all milkings (Table 2); lower temperatures at milking time were observed only during the first milking shift for 2nd-lactation cows. Third-lactation cows had lower vaginal temperatures at milking compared with 3 hours before milking in the first and second milking shift. This difference was not observed for the third milking shift. At site B, vaginal temperatures of cows 3 hours after milking were lower compared with vaginal temperatures 3 hours before milking, except for 2nd-lactation cows in the first milking shift. This result suggests that milking time for cows in site B was beneficial to alleviate heat stress. On the other hand, the same benefit was not observed for cows in site A. At site A, the lower temperature after milking, compared with before milking, was observed only in the first milking shift. In the second milking shift, cows had higher temperatures after milking. In the third shift, there was no change in temperature; cows experienced significant heat stress before, during, and after milking because vaginal temperatures were >103.5°F.

We speculate that facilities (holding pen and parlor) at site B cooled cows more efficiently than the facilities at site A. Various factors may have influenced the effectiveness of each dairy to cool cows at the holding pen and parlor. Cows at site B were housed in larger groups than cows at site A, which influenced the amount of time spent in the holding pen. In addition, parlor efficiency was different between sites; site A had a more rapid parlor turnover. Thus, time spent in the holding pen and parlor may have influenced the efficiency of the facilities to cool cows.

In conclusion, milking time during summer may positively or negatively affect vaginal temperatures of lactating cows. Dairies should evaluate the efficacy of cooling systems and ventilation in the holding pen and parlor to maximize cow cooling during milking time. Moreover, vaginal temperature loggers attached to blank CIDR inserts may be used as an assessment tool to evaluate heat stress at milking time.

Acknowledgments

The authors would like to thank the owners and staffs from both dairies.

Table 1. Milk yield and days in milk of cows used in the study (mean ± SE)

	Site A		Site B		*P*-value		
Item	2nd lactation	3rd lactation	2nd lactation	3rd lactation	Lactation	Site	Lactation × site
Milk yield (lb/day)	103.6 ± 4.14	111.5 ± 4.63	97.0 ± 3.78	113.2 ± 3.78	< 0.01	0.56	0.33
Days in milk	81.0 ± 4.09	85.5 ± 4.58	87.2 ± 3.74	88.3 ± 3.74	0.49	0.28	0.69

Table 2. Vaginal temperatures of cows from 2nd and 3rd lactation at site A (mean ± SE)

		Vaginal temperature (°F)	
Item		2nd lactation	3rd lactation
First milking shift	3 hours before milking	103.3 ± 0.15[a]	103.7 ± 0.20[a]
	Milking	102.5 ± 0.15[b]	103.1 ± 0.19[b]
	3 hours after milking	102.8 ± 0.15[b]	102.3 ± 0.19[c]
Second milking shift	3 hours before milking	102.3 ± 0.15[a]	102.3 ± 0.16[a]
	Milking	102.3 ± 0.15[a]	101.8 ± 0.15[b]
	3 hours after milking	103.3 ± 0.15[b]	102.8 ± 0.15[c]
Third milking shift	3 hours before milking	103.6 ± 0.20	103.6 ± 0.20
	Milking	103.8 ± 0.20	103.7 ± 0.20
	3 hours after milking	103.7 ± 0.20	103.7 ± 0.20

[a,b,c] Values within a column with different superscripts differ ($P < 0.01$).

Table 3. Vaginal temperatures of cows from 2nd and 3rd lactation at site B (mean ± SE)

		Vaginal temperature (°F)	
Item		2nd lactation	3rd lactation
First milking shift	3 hours before milking	101.9 ± 0.18[a]	101.9 ± 0.10[a]
	Milking	101.1 ± 0.18[b]	101.2 ± 0.10[b]
	3 hours after milking	101.8 ± 0.18[a]	101.3 ± 0.11[b]
Second milking shift	3 hours before milking	103.5 ± 0.18[a]	103.5 ± 0.15[a]
	Milking	102.6 ± 0.18[b]	102.7 ± 0.15[b]
	3 hours after milking	102.8 ± 0.18[b]	102.5 ± 0.15[b]

[a,b,c] Values within a column with different superscripts differ ($P < 0.01$).

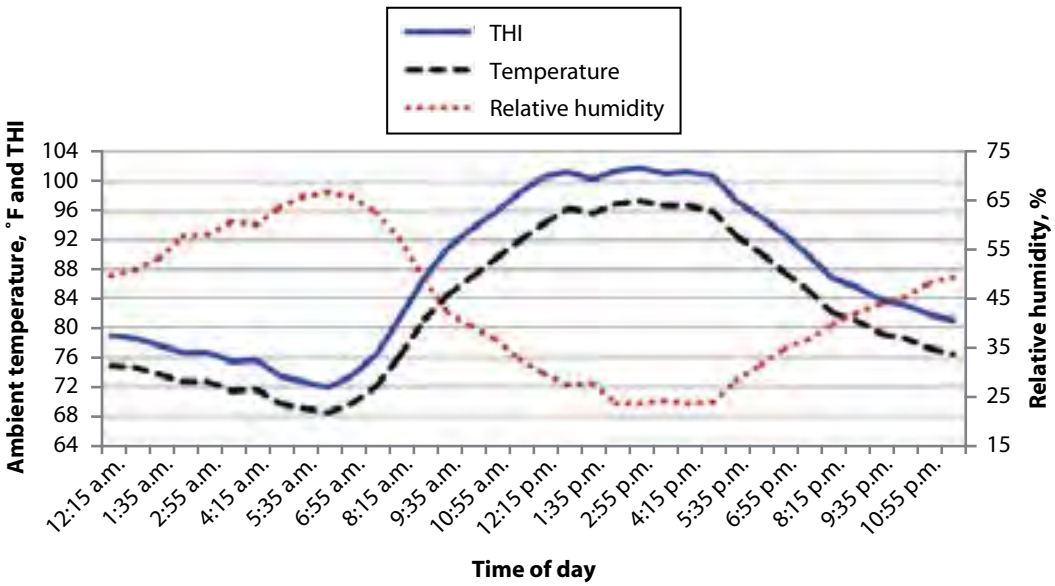

Figure 1. Temperature, relative humidity, and temperature-relative humidity index (THI) during the study period.

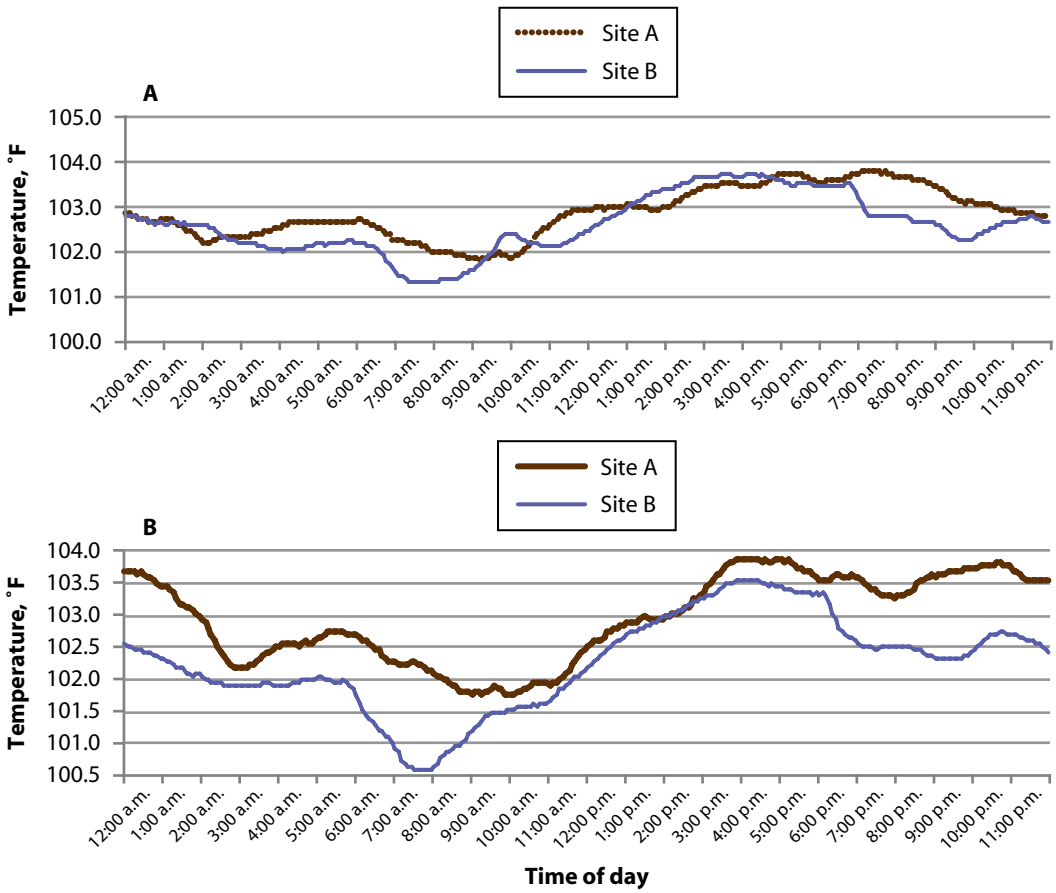

Figure 2. (A) Vaginal temperatures of 2nd-lactation cows from sites A and B. Site was not associated with temperature ($P = 0.54$), but the interaction between site and time of the day ($P < 0.01$) was associated with vaginal temperature; SEM = 0.19. Cows were milked at site A at approximately 1:30 a.m., 10:00 a.m., and 6:00 p.m. Cows were milked at site B at approximately 7:45 a.m. and 7:45 p.m. (B) Vaginal temperatures of 3rd-lactation cows from sites A and B. Site and the interaction between site and time of the day were ($P < 0.01$) associated with vaginal temperature; SEM = 0.10. Cows were milked at site A at approximately 1:30 a.m., 10:00 a.m., and 6:00 p.m. Cows were milked at site B at approximately 6:25 a.m. and 6:25 p.m.

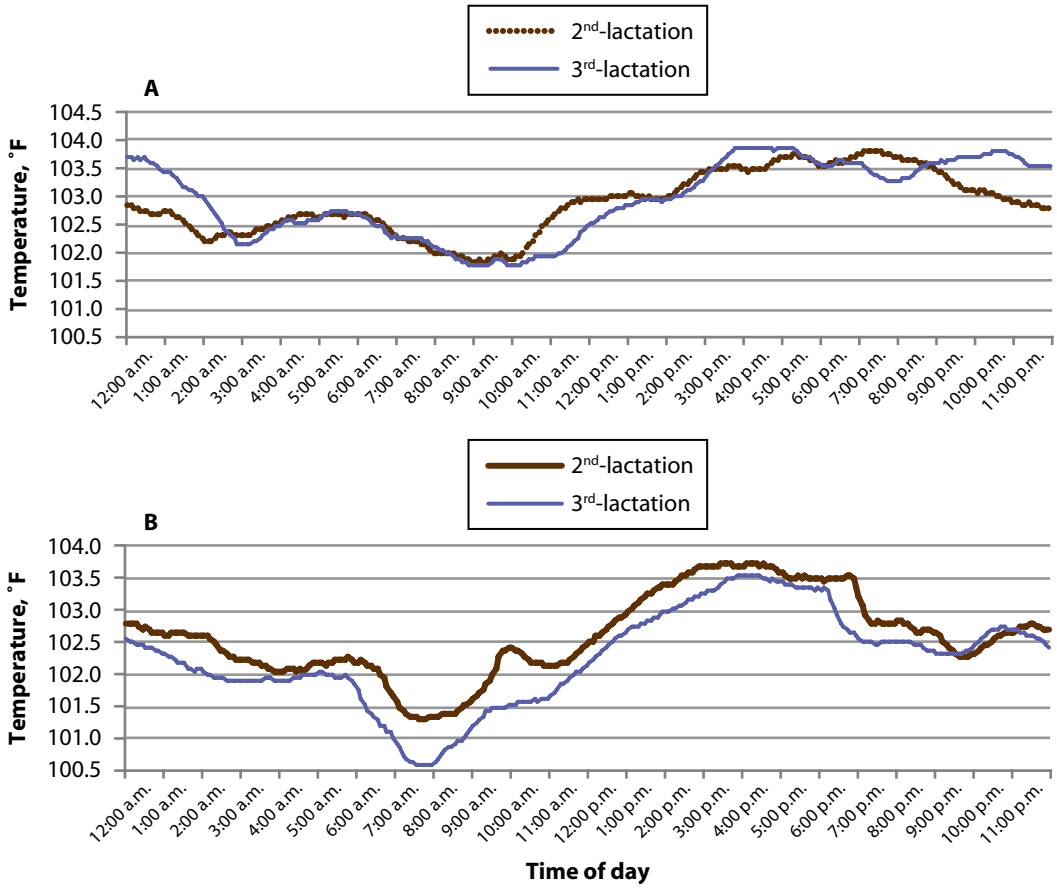

Figure 3. (A) Association between lactation number and vaginal temperature at site A. Lactation number was not ($P = 0.51$) associated with vaginal temperature, but the interaction between lactation number and time of the day was ($P < 0.01$) associated with vaginal temperature; SEM = 0.15. (B) Association between lactation number and vaginal temperature at site B. Lactation number and the interaction between lactation number and time of the day were ($P \leq 0.05$) associated with vaginal temperature; SEM = 0.16.

Luteolysis and Pregnancy Outcomes after Change in Dose Delivery of Prostaglandin $F_{2\alpha}$ in a 5-day Timed Artificial Insemination Program in Dairy Cows

J. S. Stevenson, S. L. Pulley, and S. L. Hill

Summary

Three experiments were conducted to determine if a larger dose of prostaglandin $F_{2\alpha}$ (PG) administered on day 6 of a 5-day Ovsynch timed artificial insemination (AI) program would induce regression of the corpus luteum to facilitate AI and pregnancy outcomes similar to a traditional 5-day program with two doses of PG. When applying a 5-day program, cows that ovulate in response to the first GnRH injection have a new corpus luteum (CL) that is 2 days younger when PG is administered in a 5- versus 7-day program. To regress successfully the younger CL, a second injection of PG must be given 24 hours after the first PG injection to prevent reduced pregnancy rate after the timed AI. These experiments demonstrated that administering 50 mg PG (10 mL Lutalyse) on day 6 produced luteolysis as efficiently as 25 mg PG (5 mL Lutalyse) administered on days 5 and 6 when the cut point for progesterone was 1 ng/mL 72 hours after the first PG injection or 48 hours after the larger PG dose. In contrast, when the cut point was 0.5 ng/mL, the larger dose of PG was less effective. Pregnancy outcomes in cows did not differ between treatment doses except in one herd (Exp. 3). Although pregnancy outcomes were reduced only in one herd with the larger PG dose, this difference may be confounded with the earlier injection of the second GnRH injection 16 hours before timed AI, rather than failure of luteolysis in response to the larger dose of PG. Delaying the timing of AI, injection of the second GnRH, or both may be warranted to allow sufficient time for progesterone to decrease to basal concentrations in response to a larger dose of PG on day 6 to prevent a reduction in fertility.

Key words: luteal tissue, luteolysis, ovulation, progesterone, pregnancy rate

Introduction

Most timed AI (TAI) programs apply a combination of GnRH and prostaglandin $F_{2\alpha}$ (PG) to control follicular wave initiation, ovulation, and corpus luteum (CL) regression (luteolysis) in dairy herds before first or repeat AI. These programs generally consist of injecting GnRH (day 0), a standard 25-mg dose of PG on days 5 and 6 (5-day program) or a single dose of PG on day 7 (7-day program), GnRH at 56 hours (Ovsynch-56) or 72 hours (CO-Synch-72) after PG with TAI administered on day 8 (5-day program) or day 10 (7-day program). Unless PG is administered on days 5 and 6 in a 5-day program, luteolysis fails to occur in a proportion of cows that formed a new CL after GnRH administration on day 0.

A recent report in nonlactating cows modified the 5-day program by applying a larger dose (250% of normal) of a PG analog on day 6 and compared that with standard doses of PG on days 5 and 6. Results indicated that the larger dose of PG on day 6 (which required less animal handling) produced luteolytic outcomes and final preovulatory fol-

licle diameter similar to the standard doses of PG on days 5 and 6. In a study consisting of 2 experiments conducted in lactating dairy cows, estrous cycles were presynchronized before a 5-d Ovsynch program to test whether a single large dose (200% of control) of either Estrumate or cloprostenol (1 mg) or Lutalyse or dinoprost (50 mg) administered on day 5 would produce acceptable rates of luteolysis, pregnancy, or both compared with two standard split doses (0.5 or 25 mg, respectively) administered on days 5 and 6. In the first experiment, cows were treated with 0.5 mg cloprostenol on days 5 and 6 (d 0 = GnRH-1 as in Figure 1) compared with 1 mg cloprostenol (double dose) on day 5. More luteolytic failures occurred, and pregnancy outcomes were reduced by as much as one-third in cows receiving the 1-mg dose of cloprostenol in the first experiment. In the second experiment of similar design, a 50-mg dose of dinoprost (10 mL Lutalyse) administered on day 5 resulted in reduced pregnancy outcomes compared with the standard 25-mg doses administered on days 5 and 6. In contrast, increasing the dose of cloprostenol from 0.5 to 0.75 mg (2 to 3 mL) on day 7 of a 7-day program increased luteal regression in multiparous, but not in primiparous cows, resulting in improved pregnancy outcomes at 39 days after AI.

We hypothesized that administering 50 mg of PG to lactating dairy cows on day 6 would produce similar rates of luteolysis as measured by decreased CL tissue area and serum progesterone without compromising pregnancy outcomes. Our objectives were to: (1) determine the effect of the standard control dose of PG on days 5 and 6 with a single larger (200% of control) dose of PG on day 6 in lactating dairy cows before first postpartum AI (Experiment 1) on luteal tissue area and progesterone concentrations and before repeat services on progesterone concentrations (Experiment 2); and (2) assess luteolysis (one herd) and pregnancy outcomes in two separate herds (Experiment 3).

Materials and Methods

Experiment 1

Estrous cycles were presynchronized (GnRH [2 mL Factrel, Pfizer Animal Health, Madison, NJ] 7 days before administration of 25 mg of PG [5 mL Lutalyse, Pfizer Animal Health]) in 61 lactating Holstein cows (18 primiparous and 43 multiparous). Eleven days later, cows were enrolled randomly within parity in a 5-day Ovsynch-72 program (62 to 71 DIM) and treatments were administered as illustrated in Figure 1 (control cows [25-mg dose of PG on days 5 and 6; n = 31] and treated cows [single 50-mg dose of PG on day 6; n = 30]).

On day 0, follicles and original CL were mapped and measured by transrectal ultrasonography (5.0 MHz linear-array transducer, Aloka 500V, Corometrics Medical Systems, Inc., Wallingford, CT). On days 5 through 9, ovarian follicles, new GnRH-induced CL, and original CL were measured. The largest ovarian follicle on day 8 was traced back to its first appearance to determine the putative preovulatory follicle diameter. Spherical cavity-free area of luteal structures was calculated. Luteolysis was defined to occur when concentrations of progesterone were ≥1 ng/mL on day 5 and <1 ng/mL on day 8. Blood serum was assayed by radioimmunoassay for progesterone in both experiments. Assay sensitivity was 1.9 ± 0.5 pg/mL. Inter- and intra-assay coefficients of variation for 4 assays were 6.5 and 7.9%, respectively.

Pregnancy was diagnosed by transrectal ultrasonography on day 32 after TAI. A positive pregnancy outcome required presence of anechoic uterine fluid and a CL ≥ 25 mm in diameter or anechoic uterine fluid and presence of an embryo with a heartbeat.

Experiment 2

Cows diagnosed not pregnant to a previous AI were treated with GnRH on day 0 and assigned randomly to the same two treatments as described in Exp. 1 (Figure 1). Blood was collected on days 0, 5, 6, and 8. Only data from 63 cows having serum progesterone ≥1 ng/mL on day 5 were analyzed. Concentrations of progesterone and occurrence of luteolysis were analyzed as in Exp. 1.

Experiment 3

Weekly clusters of lactating dairy cows were enrolled in two treatments (Figure 2) during an entire calendar year as part of a 5-day timed AI Resynch-Ovsynch program (GnRH 5 days before [day 0; GnRH-1] and 56 [p.m. on day 7; GnRH-2] or 72 hours [day 8; GnRH-2] after PG with timed AI on day 8). Enrollment occurred on the same day (day 0) as a negative pregnancy diagnosis (30 to 36 days after last AI in herd 1 or days 34 to 40 in herd 2). Control cows received a 25-mg dose of PG (5 mL Lutalyse) on days 5 and 6 (2 × 25), and treated cows received one single 50-mg dose of PG on day 6 (1 × 50; 10 mL Lutalyse). Cows in herd 1 were blocked by parity and assigned randomly to treatments: 2 × 25 (n = 142) or 1 × 50 (n = 140). In herd 2, even-tagged cows received the 2 × 25 (n = 422) treatment, and odd-tagged cows received 1 × 50 (n = 450) treatment. Body condition scores were assessed (1 = thin and 5 = obese) either weekly in herd 1 or monthly in herd 2.

In herd 1, ovaries were scanned by transrectal ultrasonography to determine the number of CL and number of ovarian follicles ≥10 mm in diameter on day 0. Subsequent to treatment and timed AI, pregnancy was diagnosed by transrectal ultrasonography 30 to 36 days after AI. In herd 2, pregnancy was determined by palpation per rectum of the uterus and its contents on days 34 to 40 after AI. In both herds, a second pregnancy confirmation was conducted between 60 and 70 days post-AI.

In herd 1, blood was collected on days 0, 5, 6, and 8. Blood serum was assayed for progesterone by radioimmunoassay. Assay sensitivity was 1.3 ± 0.5 pg/mL. Inter- and intra-assay coefficients of variation for 5 assays were 8.2 and 4.8%, respectively.

Results and Discussion

Experiment 1

On day 0, 51 of 61 cows had at least 1 CL and 15 had 2 or more CL, whereas 10 cows (5 cows per treatment) had no CL. On day 5, 34 of 61 cows had least 1 new CL, and 5 cows had 2 or more new CL. Therefore, the ovulation response to GnRH on day 0 was 31 of 61 (50.8%). Numbers of cows with 1, 2, or ≥ 3 total CL (original plus new CL) on day 5 were as follows: 1 CL: 12 vs. 13; 2 CL: 13 vs. 14; and ≥3 or more CL: 6 vs. 3 for control and 50-mg cows, respectively.

Original luteal tissue area was similar on day 5 but differed between treatments on day 6 ($P = 0.001$) and day 7 ($P = 0.009$) and tended ($P = 0.068$) to be less on day 8 for the

control. In contrast, no differences were detected between treatments for GnRH-induced luteal tissue area.

Concentrations of progesterone differed ($P = 0.001$) only on day 6 between treatments (Figure 3; upper panel). Luteolysis occurred in all 31 controls but failed to occur in 2 of 30 (6.7%) 50-mg cows in which no CL were present on day 0, but 1 or 3 new GnRH-induced CL were present on day 5 in the 2 cows with luteolytic failure.

Pregnancy outcomes were 12 of 30 (40%) for control cows and 15 of 30 (50%) for 50-mg cows. One control cow was culled before pregnancy was determined.

Experiment 2

Concentrations of progesterone differed between treatments only on day 6 (Figure 3; lower panel). Luteolysis occurred in all 29, 50-mg cows but failed to occur in 2 of 34 (5.9%) controls. Pregnancy outcomes at day 32 after TAI were 17 of 33 (52%) for control cows and 13 of 29 (45%) for 50-mg cows. One control cow was culled before pregnancy diagnosis.

Experiment 3

Progesterone. Concentrations of progesterone differed ($P < 0.01$) between treatments on day 6 and 8. More ($P < 0.05$) 1 × 50 than 2 × 25 cows had concentrations of progesterone ≥1 ng/mL on day 6, but similar proportions of cows in each treatment had low (<1 ng/mL) concentrations by day 8 (Figure 4). Progesterone also differed among cycle statuses on day 0 and 5, but not between treatments, which had not yet been administered. Both anestrous and new-CL cows in both treatments had low concentrations on day 0 and differed ($P < 0.05$) from those of early- and late-cycle cows, which differed ($P < 0.05$) from one another.

On day 5, concentrations of progesterone were near baseline in late-cycle cows, suggesting early spontaneous luteolysis before treatment on days 5 or 6, whereas concentrations in new CL cows increased ($P < 0.01$) by 7.4 to 10.3 times from day 0 to 5, indicating ovulation occurred after GnRH-1 on day 0. Concentrations of progesterone in early-cycle cows were elevated on both days 0 and 5 (Figure 4). Relative differences among cycle statuses for cows in both treatments on day 6 were consistent with what was observed on day 5, except on day 6, new-CL and early-cycle cows no longer differed from one another. By day 8, concentrations of progesterone did not differ among cycle statuses for cows in either treatment.

Luteolysis. Cows treated with 50 mg PG on day 6 were more ($P = 0.003$) likely to have incomplete luteolysis when the cut point on day 8 was <0.5 ng/mL. In contrast, when the cut point was <1 ng/mL, no difference was detected between treatments (Table 1). Luteolysis in cows most likely to fail to respond to PG (early-cycle and new-CL cows) did not differ between early-cycle and new-CL cows (86.5 vs. 83.0%) for the <0.5 ng/mL cut point or for the <1 ng/mL cut point (97.4 vs. 100%), respectively.

Cycle status within treatment reflected the overall treatment effects for luteolysis. Although treatment differences were not detected for early-cycle cows between treatments, luteolysis in the 2 × 25 vs. 1 × 50 cows reflected the overall treatment differences

in Table 1 at the <0.5 ng/mL cut point (94.8 vs. 78.2%; $P = 0.495$) and at the <1 ng/mL cut point (87.5 vs. 79.3%; $P = 0.191$), respectively. Defined luteolysis was not affected by either BCS or number of follicles >10 mm assessed on day 0.

In response to treatment on day 5, concentrations of progesterone on day 6 decreased in 2 × 25 cows in response to the first of two 25-mg injections and differed ($P = 0.001$) from those in the 50-mg treatment for early-cycle and new-CL cows only, whereas no treatment differences were detected in late-cycle and anestrous cows (Figure 4). Although concentrations of progesterone had decreased further by day 8, difference ($P = 0.001$) between treatments existed only for early-cycle cows, indicating that early-cycle cows also may have had additional new luteal tissue in the form of a new CL that was resistant to the luteolytic effects of PG.

Pregnancy Outcomes. Pregnancy per AI at the first and second diagnosis period was reduced in anestrous and late-cycle cows compared with early-cycle and new-CL cows in both treatments for cows in herd 1. Pregnancy per AI (30 to 36 days post-AI) for cows in herd 1 with luteolysis defined at the cut point of <0.5 ng/mL did not differ between 2 × 25 vs. 1 × 50 treatments (48.5% [n = 101] vs. 37.5% [n = 80]) or at the cut point of <1 ng/mL (46.3% [n = 108] vs. 41.0% [n = 100]), respectively. Neither parity ($P > 0.50$) nor BCS had any effect ($P > 0.31$) on pregnancy per AI in herd 1.

Pregnancy outcomes at 30 to 40 day differed ($P < 0.001$) among herds, but the 1 × 50 cows tended ($P = 0.071$) to have lesser fertility only in herd 2 (Table 2). This treatment difference in herd 2 was confirmed ($P = 0.036$) at the later pregnancy diagnosis in herd 2 (Table 2).

We conclude that one large dose (50 mg PG) administered on day 6 is luteolytic (regressed the CL) using a cut point of 1 ng/mL, which is consistent with earlier reports in non-lactating and lactating cows (Exp. 1 and 2), but not so when a more conservative cut point of 0.5 ng/mL was applied (Exp. 3). Although progesterone may eventually decrease to sufficient concentrations (complete functional luteolysis) with time, it may not achieve sufficiently basal concentrations in some cows to prevent reduced pregnancy outcomes, particularly those defined as early-cycle, having a new CL on day 0, or both. Although pregnancy outcomes were reduced only in herd 2 with 1 × 50 treatment compared with herd 1, this difference may be confounded with the earlier injection of GnRH-2 16 hours before timed AI in herd 2 rather than failure of luteolysis to the larger dose of PG. Delaying the timing of AI, injection of GnRH-2, or both, may be warranted to allow sufficient time for progesterone to decrease to basal concentrations in response to a larger dose of PG on day 6 to prevent a reduction in conception.

Table 1. Luteolysis at 2 different cut points for cows treated with 2 × 25 or 1 × 50 mg of $PGF_{2\alpha}$ in herd 1 (Exp. 3)[1]

Treatment[2]	Luteolysis (%)	Odds ratio	95% confidence interval	*P*-value
---------- < 0.5 ng/mL ----------				
2 x 25	93.3	Referent		
1 x 50	78.5	0.264	0.108 – 0.645	0.003
---------- < 1 ng/mL ----------				
2 x 25	100.0	Referent		
1 x 50	96.3	< 0.1	. . .	0.947

[1] Progesterone ≥1 ng/mL on day 5 and either <0.5 or <1 ng/mL 72 hours later on day 8.
[2] Cows received either 25 mg PGF_{2a} (PG) on days 5 and 6 or a 50 mg PG on day 6 as part of a 5-day Resynch-Ovsynch program (d 0 = GnRH-1).

Table 2. Pregnancy per AI in both herds at 30 to 40 and 60 to 70 days post-AI (Exp. 3)

	Treatment[1]		
Stage of pregnancy, days	2 × 25	1 × 50	*P*-value
30 to 40	% (n)		
Herd 1	37.2 (139)	33.3 (134)	0.517
Herd 2	24.7 (422)	19.5 (450)	0.071
60 to 70			
Herd 1	31.3 (139)	28.2 (134)	0.608
Herd 2	22.7 (422)	16.9 (450)	0.036

[1] Cows received either 25 mg PGF_{2a} (PG) on days 5 and 6 or a 50 mg PG on day 6 as part of a 5-day Resynch-Ovsynch program (d 0 = GnRH-1).

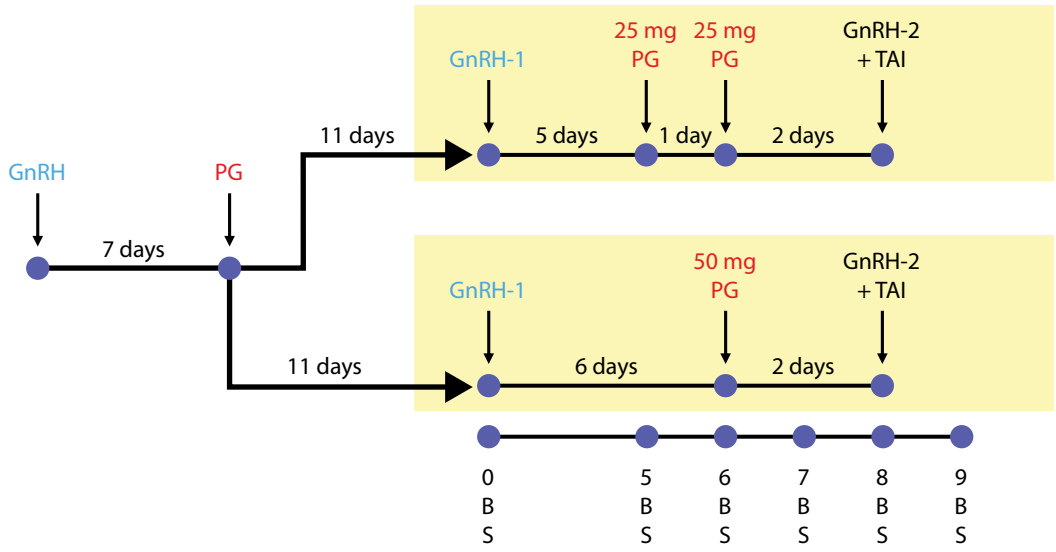

Figure 1. Scheme of treatments and measurements for Experiments 1 and 2. In Experiment 1, estrous cycles were presynchronized by injecting 100 μg GnRH and 25 mg of PG beginning between 62 and 71 days in milk. Cows were assigned randomly to receive either 25-mg doses of PG on days 5 and 6 or a 50-mg dose of PG on day 6. Ovarian structures were measured by transrectal ultrasonography (S) and mapped on day 0 and days 5 through 9. Both original CL on day 0 and GnRH-induced CL identified on day 5 were measured and monitored for diameter and luteal area (CL cavity area was deducted from total luteal area). Blood samples (B) were collected before each ovarian scan.

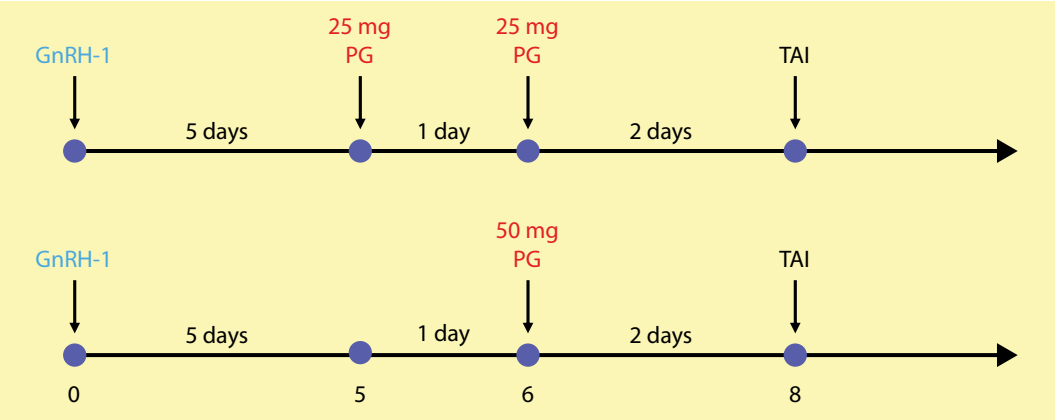

Figure 2. Experiment 3 treatment schemes in two herds. In herd 1, cows were assigned randomly to receive either 25 mg PGF_{2a} (PG) on days 5 and 6 or a 50-mg dose of PG on day 6. Blood samples were collected on days 0, 5, 6, and 8 to measure progesterone. In herd 2, even-tagged cows received 25 mg PG on days 5 and 6, and odd-tagged cows received 50 mg PG on day 6. The second GnRH (GnRH-2) injection was administered on the afternoon of day 7, and timed AI (TAI) occurred on the morning of day 8 in herd 2, but TAI occurred in herd 1 when GnRH-2 was administered on day 8 (72 hours after the first 25-mg PG treatment).

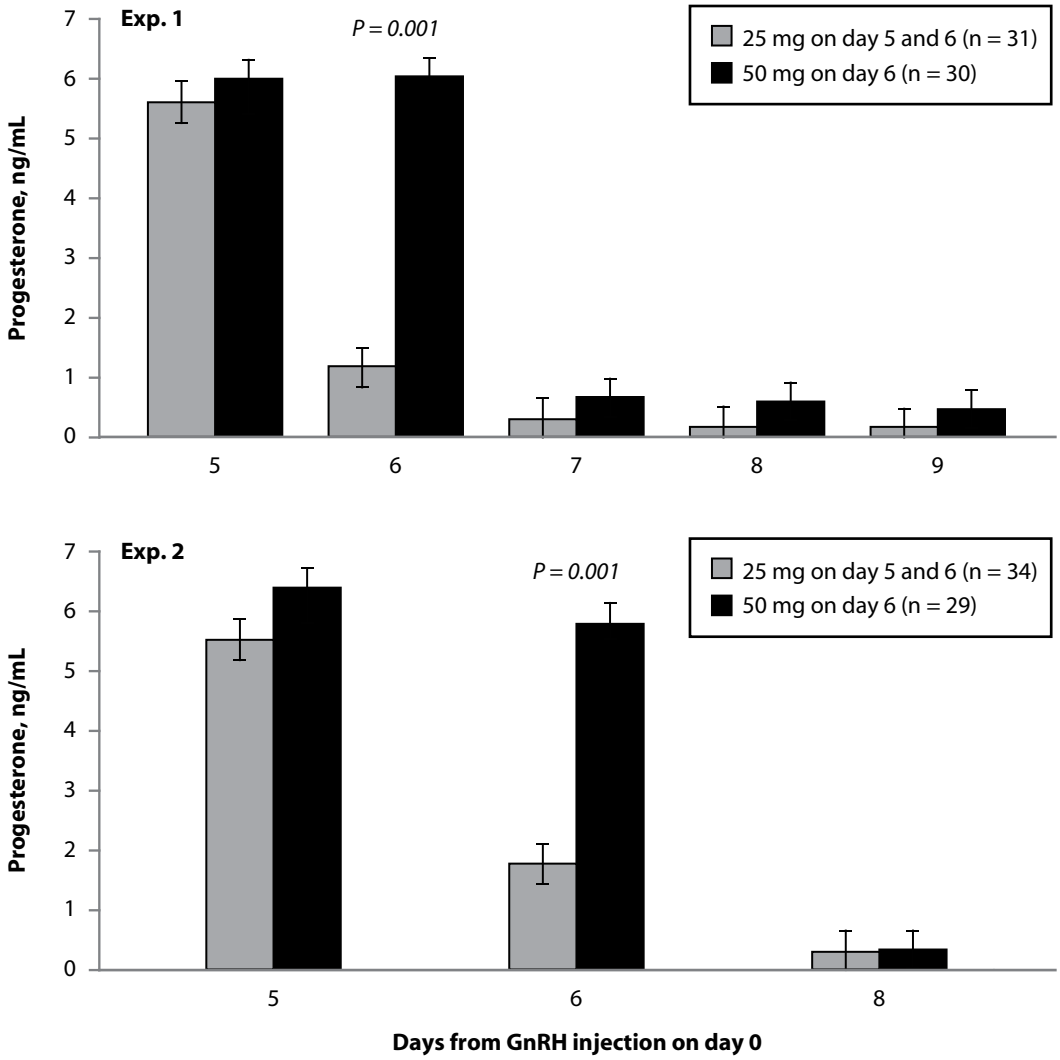

Figure 3. Concentrations of progesterone in control (25-mg doses of PG on days 5 and 6) and treated cows (50 mg of PG only on day 6) for cows in Exp. 1 (upper panel) and Exp. 2 (lower panel). Concentrations differed ($P = 0.001$) between treatments only on day 6 in both experiments.

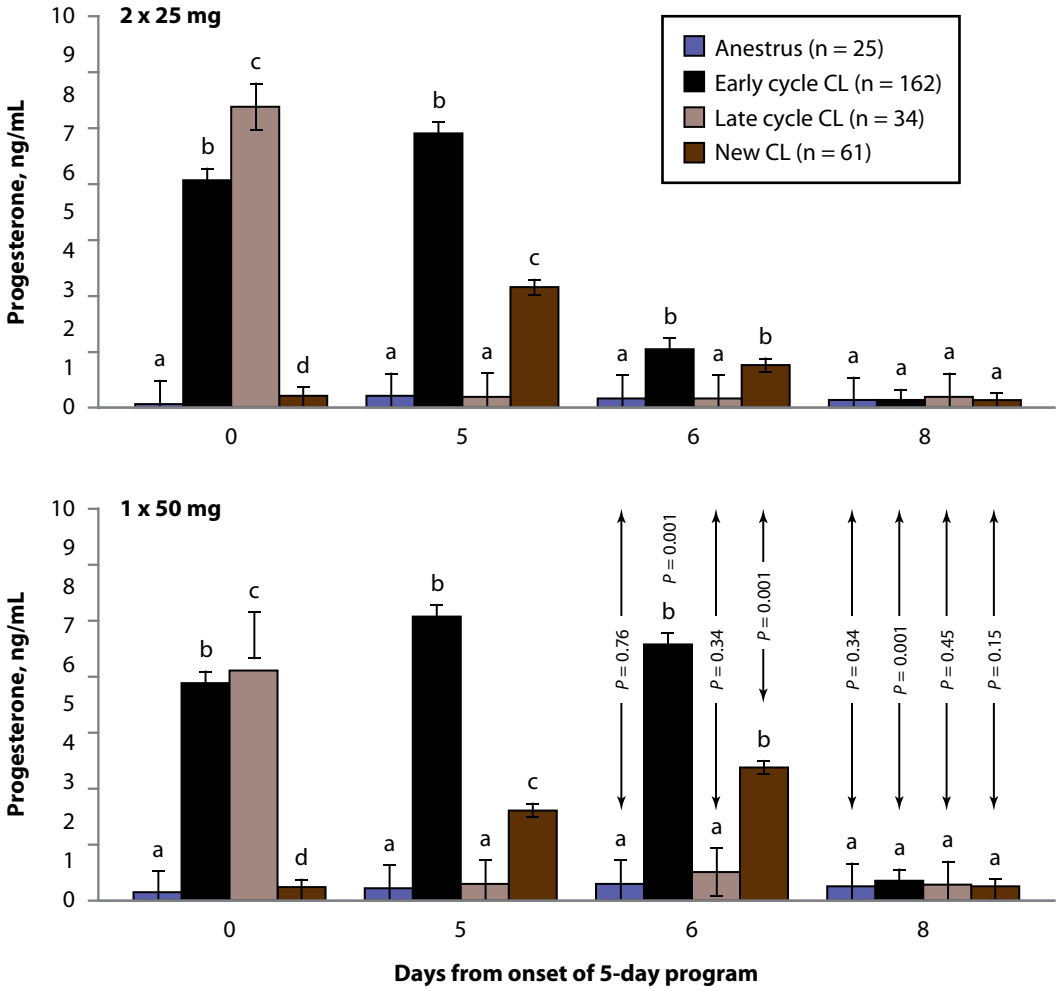

Figure 4. Concentrations of progesterone on days 0, 5, 6, and 8 for cows treated with either 25 mg $PGF_{2\alpha}$ (PG) on days 5 and 6 (2 × 25) or 50 mg PG on day 6 (1 × 50) in Exp. 3. Cows were classified by concentrations of progesterone on day 0 and 5: (1) anestrus (<1 ng/mL on both days); (2) early cycle (≥1 ng/mL on both days); (3) late cycle (≥1 ng/mL on day 0 and <1 ng/mL on day 50; and (4) new CL (<1 ng/mL on day 0 and ≥1 ng/mL on day 5). [a, b] Means within treatment differed ($P < 0.05$) among cycle statuses.

Effects of Postpartum Treatment with Non-Steroidal Anti-Inflammatory Drugs on Milk Production and Culling Risk in Dairy Cattle

A. Carpenter, C. Ylioja, C. Vargas, L. Mamedova, L. Mendonça, J. Coetzee[1], *L. Hollis, R. Gehring, B. Bradford*

Summary

Inflammation during early lactation is common in dairy cattle, and a high degree of inflammation during this time has recently been associated with both lower productivity and greater risk of disease during that lactation. Early lactation treatments with two non-steroidal anti-inflammatory drugs were compared with a placebo treatment to evaluate effects on whole-lactation productivity and retention in the herd. Both meloxicam and sodium salicylate increased whole-lactation milk and milk protein yields by 6 to 9%, despite being administered for only 1 or 3 days in early lactation, respectively. In addition, meloxicam treatment tended to decrease the risk of cows leaving the herd during the lactation. These results indicate that postpartum inflammatory signals have long-lasting effects on lactation in dairy cattle.

Key words: transition dairy cow, inflammation, non-steroidal anti-inflammatory drug

Introduction

A growing body of research indicates that systemic metabolic inflammation is elevated in dairy cows at parturition and that this inflammation may play a role in the development of metabolic disorders during the transition period. Furthermore, inflammation has been linked to negative production outcomes. In one study, authors reported that cows in the highest quartile of inflammation had decreased milk production compared with their counterparts (30.9 kg/day vs. 24.4 kg/day in cows with low inflammation). In previous work at Kansas State, we administered dairy cattle with the non-steroidal anti-inflammatory drug (NSAID) sodium salicylate (SS) via drinking water in the week following calving in an attempt to prevent inflammation. Despite the fact that transition disorders were rare in either group, treatment with SS was associated with elevated whole-lactation milk production in older cows. Cows in their third or later lactation that received SS produced 21% more milk over a 305-day lactation.

Meloxicam is another drug in the NSAID class. Previous research in lactating cattle has focused on its use during clinical mastitis or following assisted calving, but meloxicam's effects on milk production in normal postpartum dairy cattle have not yet been investigated. Considering the effects of SS on production, it is likely that meloxicam may also have beneficial effects on lactation. Therefore, the objective of this study was to determine if SS or meloxicam would have similar effects on whole-lactation productivity of dairy cows on a commercial dairy farm.

[1] Iowa State University, Ames, IA.

Experimental Procedures

Multiparous cows (n = 51 per treatment) from a commercial dairy were enrolled in the study 12 to 36 hours after calving. Cows receiving SS treatment (SS) received a placebo bolus on day 1 of treatment and an oral drench containing 125/g/day of SS in 375 mL of water for 3 consecutive days beginning on day 1 of treatment. Meloxicam-treated cows (M) received 675 mg of meloxicam as a bolus on day 1 of treatment in combination with 3 consecutive daily drenches of 375 mL of water. Control animals (CON) received a placebo bolus on day 1 and water drenches. Treatments were blocked by mastitis at parturition (CON = 1, M = 2, SS = 2), breed (CON = 6, M = 6, SS = 4 crossbreds; all other cows were Holsteins), dystocia (CON = 5, M = 5, SS = 6), and twin births (CON = 4, M = 4, SS = 3). Dystocia was defined as a calving difficulty score of 3 or greater.

Whole-lactation milk yield data were analyzed with a covariate of predicted transmitting ability for milk production, the fixed effects of block, parity, treatment, week, and treatment × week interaction, and the random effect of cow. The model accounted for repeated measures over time with an autoregressive covariance structure. Treatment contrasts were evaluated using the Tukey test with significance declared at $P < 0.05$. Removal rate from the herd and pregnancy rate were evaluated by Cox regression proportional hazard analysis, and disease incidences were tested by Fisher's exact test.

Results

Milk production responses to treatment were evaluated using two different data sources. Adjusted 305-day mature equivalent yields of milk, fat, and protein through DHIA testing revealed significant whole-lactation milk and protein responses to both M and SS treatments, representing 6 to 9% advantages for the NSAID treatments (Table 1). Numerical differences in fat yield were of similar magnitude (5 to 6%) but were not statistically significant. In addition, daily milk yield data from the farm management system were analyzed to assess treatment responses over time. The overall treatment effect on daily milk production was again significant for both NSAID treatments ($P < 0.05$, Figure 1), with a slightly larger mean response of 10 to 12%. As we observed in a previous study with SS, milk yields did not diverge until the second month of lactation, but differences in productivity then remained through the end of lactation. Despite the increase in productivity, body condition score monitoring throughout lactation revealed no differences between treatments (Table 1).

Treatments did not alter pregnancy rate on first service (mean: 23%) and had no impact on risk of pregnancy throughout the lactation. However, analysis of retention within the herd did reveal a tendency for M to improve retention compared with CON ($P = 0.06$), with SS intermediate (Figure 2). By the end of the lactation, 41% of CON cows had left the herd, compared with 31% for SS and only 25% for M. The only culling reason that differed between M and C was the code "other disease," which accounted for 8 culls in CON and only 2 in M ($P = 0.09$); this code was used for a variety of conditions, including key transition problems such as ketosis and respiratory disease.

Discussion

These results represent the fourth study demonstrating that short-term early lactation treatment with SS can enhance peak milk yield and the first to demonstrate similar re-

sults with M. More importantly, this study is the first such finding with a relatively large sample size and with daily milk yield data to allow for accurate analysis of the lactation curve following treatment.

The fact that early lactation treatment (for as little as 1 day) with an anti-inflammatory agent can influence milk production for at least 10 months is fascinating and is not easily explainable. Our group is currently conducting research to explore the possibility that interrupting early lactation inflammation programs the mammary gland to allow for increased expression of milk synthesis genes as lactation proceeds.

Equally exciting is the possibility that M treatment can improve health in early lactation to limit the culling rate. Our sample size for this outcome was minimal, however, and our findings, which were of marginal significance, should be considered preliminary. In previous work, we carefully evaluated the effects of SS on metabolic health and found no evidence of improvement, but the current results seem to hint at a different response to M treatment, particularly in the critical first 60 days of lactation. Larger studies should help clarify whether these findings are meaningful or not.

Conclusions

Both M and SS increased 305-day milk and protein yields compared with CON with no effect on 305-day milk fat. These responses were primarily due to increased peak milk yield and sustained differences through late lactation and did not appear until the second month of lactation. Furthermore, neither treatment affected body condition score, and M tended to improve retention in the herd compared with CON. The long-term benefits of early lactation NSAID use are surprising and will require further research to understand the underlying mechanisms. Although using these approaches commercially is not currently legal, ongoing research may allow for nutritional or pharmaceutical approaches to take advantage of these findings in the future.

Table 1. Whole-lactation (305-day) mature equivalent milk yield and body condition score (BCS) responses to early lactation treatment with anti-inflammatory drugs

	Treatment				
Item	CON	Meloxicam	Sodium salicylate	SE	*P*-value
Milk yield (lb)	23,091[b]	24,707[a]	25,161[a]	1,072	0.02
Fat yield (lb)	869	919	913	40	0.13
Protein yield (lb)	739[b]	785[a]	789[a]	31	0.03
BCS[1]	3.24	3.30	3.20	0.12	0.52

[1] 1 to 5 scale; 1 = thin and 5 = fat.

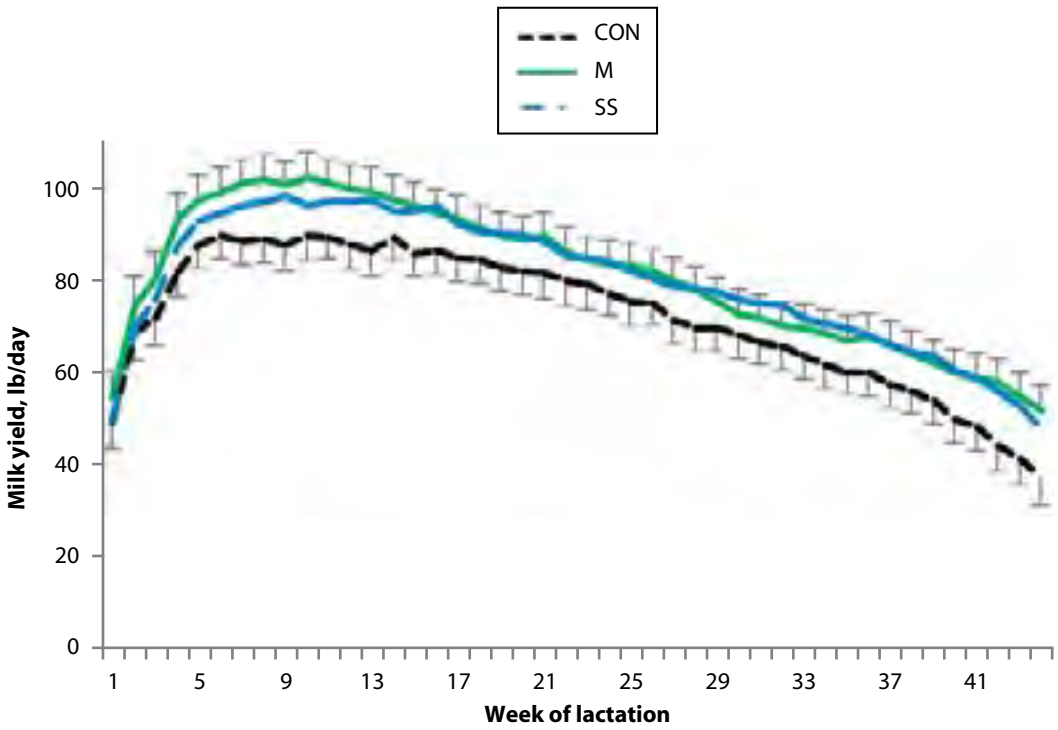

Figure 1. Whole-lactation milk yield responses to early lactation treatments. Daily milk yield data were summarized by week and evaluated for the entire 44-week lactation. Both meloxicam (M) and sodium salicylate (SS) significantly increased daily milk yield by an average of 8.7 and 7.5 lb/day, respectively ($P < 0.05$), compared with the control. Treatment differences were not significant until week 7 of lactation, then remained significant or marginally significant ($P < 0.10$) for most weeks through the end of lactation.

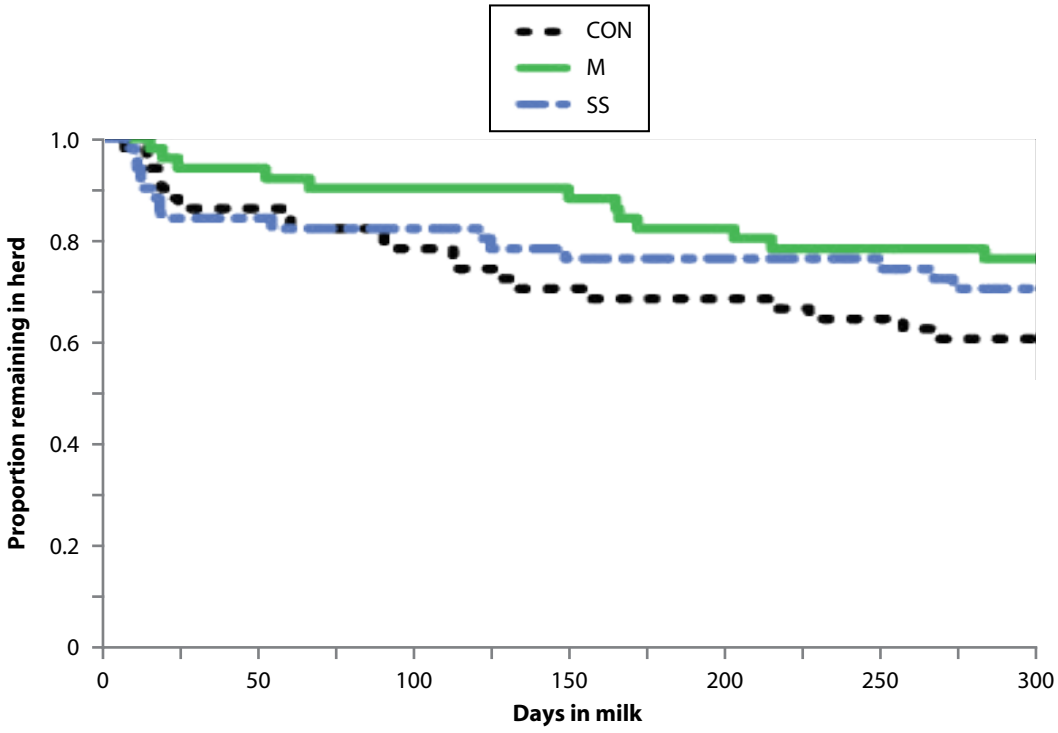

Figure 2. Survival analysis of retention in the herd after early lactation treatments. Meloxicam (M) treatment tended to increase retention in the herd compared with the control (CON), as assessed by the Wilcoxon Chi-squared test ($P = 0.06$). Sodium salicylate (SS) did not differ from other treatments.

Preliminary Studies on *In Situ* Monitoring of Lactose Crystallization Using Focused Beam Reflectance Measurement

K. Pandalaneni, J.K. Amamcharla

Summary

Isothermal crystallization of lactose was studied at supersaturated concentrations (w/w) of 50%, 55%, and 60% at temperatures 20°C and 30°C using an *in situ* system, focused beam reflectance measurement (FBRM), and a refractometer. The FBRM data were compared with Brix readings taken over time using a refractometer during isothermal crystallization. Chord length distribution obtained from FBRM in the ranges of <50 μm (fine crystals) and 50 to 300 μm (coarse crystals) were observed and evaluated in relation to the extent of crystallization and rate constant results deduced from the refractometer measurements. The measured fine crystal counts increased with supersaturated concentration and temperature during isothermal crystallization. On the other hand, coarse counts were observed to increase with decreasing supersaturated concentration and temperature. The total crystal counts (coarse + fine crystals) obtained from FBRM increased as the temperature increased at all concentrations. The robustness of FBRM in understanding isothermal lactose crystallization at various concentrations and temperatures was successfully evaluated in the study.

Key words: lactose crystallization, focused beam reflectance measurement

Introduction

Lactose is the most abundant carbohydrate present in milk. It is found in concentrations of 4.4 to 5.2% and is one of the major constituents in infant formulations, dried milk, and whey products. Commercial production of lactose involves concentration of whey or whey permeate by evaporation followed by batch crystallization. During the process, α-lactose crystallizes as tomahawk-shaped crystals. Crystal size distribution and lactose yield are the most important criteria to monitor during industrial crystallization of lactose and are influenced by the degree of supersaturation, rate of cooling, agitator speed, presence of impurities, and viscosity of supersaturated feed material.

The dairy industry currently is depending on refractometer measurements to follow lactose crystallization, but this approach provides no information on crystal size distribution during crystallization. *In situ* monitoring of lactose crystallization to meet the special requirements of crystal size distribution (CSD) is needed. Focused beam reflectance measurement (FBRM) could be used to monitor the CSD and chord length distribution (CLD) *in situ* from supersaturated lactose solution.

The FBRM uses a monochromatic laser (785 nm) rotating at a constant speed of 2 meters per second. As particles pass in front of the probe window, light is backscattered from the particles to the sapphire window. The duration of the backscatter is measured and particle chord length is obtained. Schematic representation of working principle of

FBRM is shown in Figure 1. The objective of the present study was to evaluate the applicability of FBRM for *in situ* monitoring of the isothermal crystallization of lactose.

Experimental Procedures

Experimental Design

A 2 × 3 factorial design was used in the study, with temperature and concentration as independent variables. Concentrations (50%, 55%, and 60%) w/w were randomly assigned to temperatures (20°C and 30°C) and resulted in 6 measurements. Experiments were conducted randomly and in two replications.

Preparation of Supersaturated Lactose Solution

Lactose solutions of desired supersaturation were prepared using 99.7% pure alpha lactose (Davisco Foods International, Inc., Le Seuer, MN). Desired concentrations of lactose (w/w) were obtained by dissolving 250, 275, and 300 g of α-lactose in 250, 225, and 200 g of distilled water to prepare 50%, 55%, and 60% (w/w) supersaturated solutions, respectively. The lactose and water mixture was heated to 87±3°C under continuous stirring to dissolve all the crystals. A lid was placed on the beaker to avoid moisture loss during heating. After ensuring the dissolution of all the crystals, the supersaturated lactose solution was rapidly cooled to the desired experimental temperature (20°C or 30°C) without agitation.

Isothermal Crystallization of Lactose

Isothermal lactose crystallization studies were carried out in a batch crystallizer specially designed for this work. The FBRM probe (Particle Track E25, Mettler-Toledo AutoChem, Inc., Columbus, OH) was immersed in a purpose-built batch crystallizer for *in situ* monitoring of the crystallization process as shown in Figure 2. The batch crystallizer was placed in a temperature-controlled water bath that could maintain a constant temperature. An overhead stirrer with a four-bladed propeller (Caframo, Georgian Bluffs, Ontario, Canada) was placed in the crystallizer to facilitate stirring. As shown in Figure 2, the propeller was maintained at 2.5 cm above the bottom of the beaker containing the supersaturated sample. The FBRM *in-situ* probe was fixed at a height of 5 cm from the bottom of the beaker and at an angle of 30 ± 5° to the vertical axis of the stirrer for all the crystallization experiments.

Crystallization Monitoring Using an FBRM Probe

Before the start of each experiment, the FBRM probe was cleaned thoroughly with distilled water to avoid interference from unwanted particles, as suggested by the manufacturer. The data from the FBRM probe were acquired using iC FBRM (version 4.3.391, Mettler-Toledo) every 3 minutes for the first 60 minutes and every 30 minutes thereafter during isothermal crystallization. Three categories of crystal chord length ranges, 0.5–50 μm, 50–300 μm, and 300–2,000 μm, were monitored and are designated fine, coarse, and large, respectively.

Determination of Extent of Crystallization and Rate Constant

At regular intervals, an approximately 1 ml of the crystal suspension was removed from the crystallizer using a dropper to measure the refractive index of the suspension. The refractive index, expressed in terms of °Brix, of lactose solution was measured using a

digital refractometer (Reichert Technologies, Depew, NY). A calibration curve was used to convert the °Brix reading to actual lactose concentration solution, and refractometer readings were subsequently used to calculate the mass of crystals and extent of crystallization at any time *t* during isothermal crystallization. Mass of crystals at any time *t* was calculated from the initial lactose concentration C(0) and lactose concentration C(*t*) at time *t* using Equation 1. Water was assumed to represent 5% of the total mass of lactose crystal.

$$M_{Crystal}(t) = M_{H_2O}(0)\ \frac{C(0) - C(t)}{95 - 0.050C(t)} \quad (1)$$

where $M_{Crystal}(t)$ is mass of crystals at given time *t*, $M_{H_2O}(0)$ represents 5% of the total mass of lactose crystal, C(0) is initial concentration, and C(*t*) is concentration at time *t*.

The extent of crystallization at time *t* was calculated using Equation 2 from the mass of crystals at time *t* obtained from Equation 1 and saturation concentration of lactose at temperature 20°C and 30°C.

$$\%Y(t) = \frac{M_{Crystal}(t)}{M_{Crystal}(t \to \infty)}\ X100 \quad (2)$$

where Y(*t*) is the extent of crystallization at time *t*, $M_{Crystal}(t)$ is the mass of crystals obtained from Equation 1, and $M_{Crystal}(t \to \infty)$ is the mass of crystals at lactose solubility at experimental temperature.

From the concentration difference ΔC, plotted against time *t,* it was observed that the curve shows best the first-order decay fit. The rate constant can be deduced from plotting Equation 3, the first-order decay equation, where $[A_0]$ is the initial concentration of lactose solution before crystallization and *k* is the rate constant. $[A]$ is the concentration difference ΔC, at a given time *t,* where ΔC = C(*t*) – C(t ∞), with C(t ∞) as lactose solubility value at the temperature of interest.

$$ln\left(\frac{[A]}{[A_0]}\right) = -kt \quad (3)$$

Results and Discussion

Determining Extent of Crystallization and the Rate Constant from Brix Values

The extent of crystallization was determined during the isothermal crystallization of lactose for 50%, 55%, and 60% concentrations at 20°C and 30°C. Figure 3 shows the extent of crystallization during isothermal crystallization of lactose for 50%, 55%, and 60% concentrations at 20°C and 30°C, respectively. Figure 3 shows that the extent of crystallization was higher at 30°C than at 20°C for all concentrations of lactose. The time required for isothermal crystallization to reach 90% was 300, 360, and 420 minutes for 60%, 55%, and 50% lactose solutions, respectively. On the other hand, the extent of crystallization at 20°C did not reach 90% even at 630 minutes for all the lactose concentrations studied. The extents of crystallization at 30°C were calculated to be 93%, 95%, and 96% for 50%, 55%, and 60% solutions, respectively. The extents of crystallization at 20°C were calculated to be 80%, 83%, and 86% for 50%, 55%, and 60% solutions, respectively.

Extent of crystallization increased with lactose concentration at a given experimental temperature. A maximum extent of crystallization was observed for the 60% supersaturated solution concentration followed by 55% and 50% lactose concentrations at 30°C. A similar trend was observed at 20°C.

Rate constants for the isothermal crystallization of lactose at 20°C and 30°C were calculated using Equation 3. For calculation purposes, it was assumed that the rate of mutarotation proceeded at a higher rate than crystallization of α-lactose. This assumption is based on the fact that mutarotation is not a limiting factor during crystallization of lactose. The rate constants obtained at different concentrations and temperatures are shown in Figure 4, which shows that the rate constants were higher at 30°C than at 20°C for the three lactose concentrations studied.

Overall, refractometry is a suitable technique to calculate crystal mass throughout lactose crystallization. Another advantage of this technique is that it can be implemented easily regardless of crystallizer design; however, refractometer readings do not provide information on crystal size distribution.

Evaluation of FBRM Data

Plots of fine crystal counts (<50μm) obtained from FBRM during the isothermal crystallization of lactose at various temperatures and concentrations against time are shown in Figure 5. A steep increase in the fine crystal count was observed during the initial phase of crystallization (first 15 minutes) and can be attributed to primary nucleation of lactose crystals. These findings were in agreement with the extent of crystallization and the rate constant as shown in Figures 3 and 4, respectively. A further increase in fine crystal counts after the initial rapid increase was due to secondary nucleation and disintegration of lactose crystals. The disintegration of lactose crystals was caused by attrition and collisions between crystals and the crystallizer walls and impeller.

The plot of coarse crystal counts (50–300 μm) obtained from FBRM during isothermal crystallization of lactose at various temperatures and concentrations is shown in Figure 6, which shows a substantial difference between coarse crystal counts of lactose at 30°C and 20°C. The growth of crystals at 20°C was also relatively higher than that of the lactose crystals obtained at 30°C. The number of coarse crystals increased as temperature and supersaturation decreased; in other words, isothermal crystallization at 20°C and a concentration of 50% were favored to produce the largest mean-squared crystals in the present study. In contrast, the count of fine crystals increased as temperature and supersaturation increased. These results suggest that growth of crystals was favorable as temperature decreased, whereas an increase in temperature favored nucleation and formation of finer crystals.

Counts of larger particles (300–1,200 μm) were found to be negligible and were not included in the analysis.

Evaluation of Chord Length Distribution

Chord length distributions obtained from FBRM at various time intervals during isothermal crystallization of lactose for all the treatments are shown in Figure 7. Crystal counts at 30 minutes at 30°C were clearly higher than at 20°C for all supersatu-

rated concentrations, which supports observations from Figure 5. Total crystal counts increased with time for the first 6 to 8 hours and decreased during the last few hours in 55% and 60% concentrations. At 50% concentration, however, counts were reported to increase more at 20°C than at 30°C throughout the experiment, which is also apparent in Figure 6. A decrease in total crystal counts can be explained as a combination of the breakage of crystals and the interference of smaller crystals. A prominent decrease in the number of coarse crystals at 30°C can be explained by the fact that, as the growth and density of crystals increases, the probability of the probe detecting particle width rather than particle length is high. Impeller speed, apart from enabling active crystallization by uniform supersaturation and mass transfer, also causes breakages, which could be an additional explanation for a decrease in coarse crystal count as time proceeds and was easy to track using FBRM.

Conclusion

The efficiency of FBRM in studying lactose crystallization with respect to operation parameters such as concentration and temperature was evaluated. FBRM is a powerful tool, and it can be used to follow secondary nucleation as a result of attrition and breakage apart from chord length distribution and crystal size. The results of this study imply that changes in concentration and temperature were well understood in terms of crystal size and counts over time using FBRM. The data obtained from FBRM supplements and strengthens refractive index data.

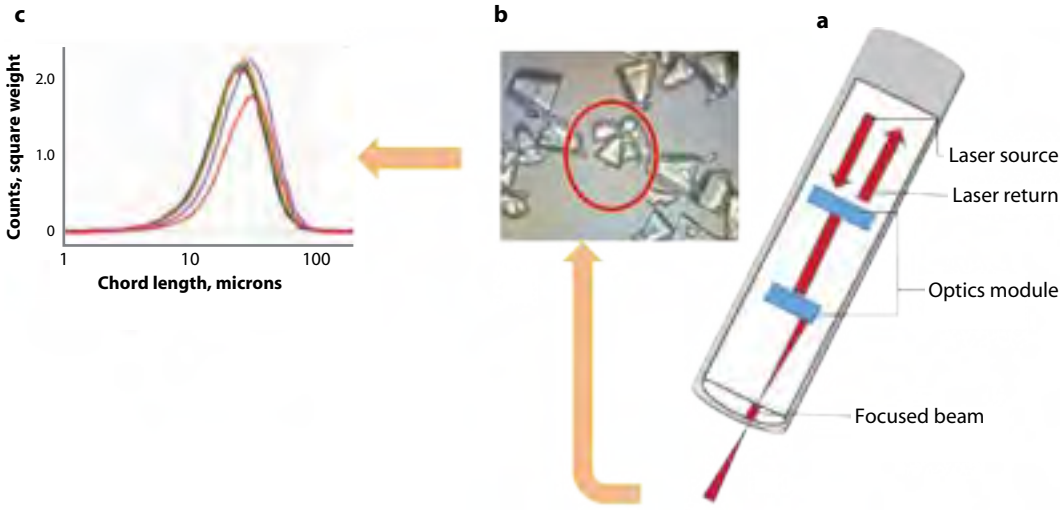

Figure 1. (a) Focused beam reflectance measurement (FBRM) probe design, (b) detection of particles by probe using a laser moving at constant velocity, and (c) chord length distribution graph obtained from crystal distribution.

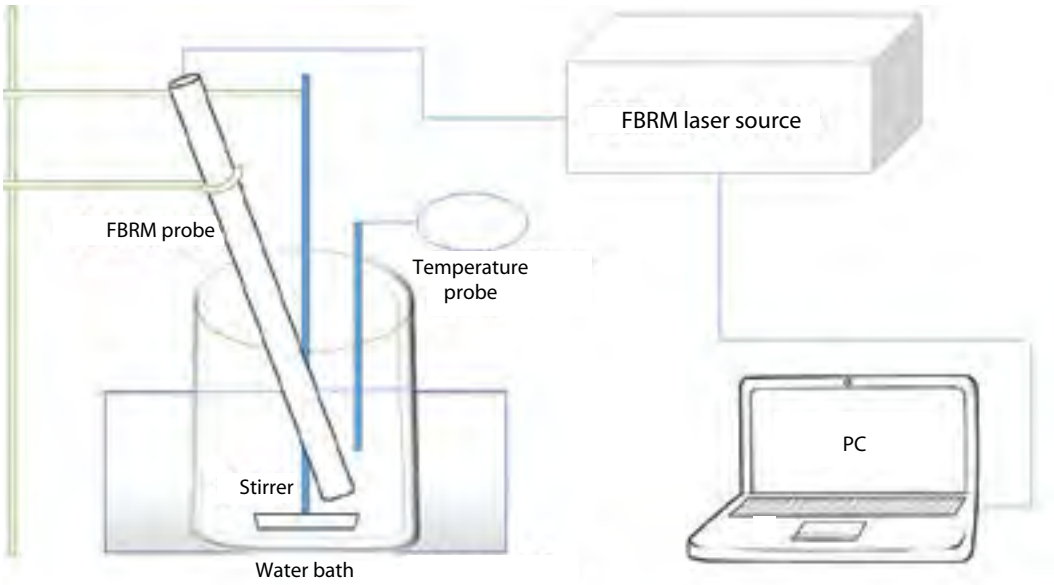

Figure 2. Experimental setup for evaluation of lactose crystallization using focused beam reflectance measurement (FBRM).

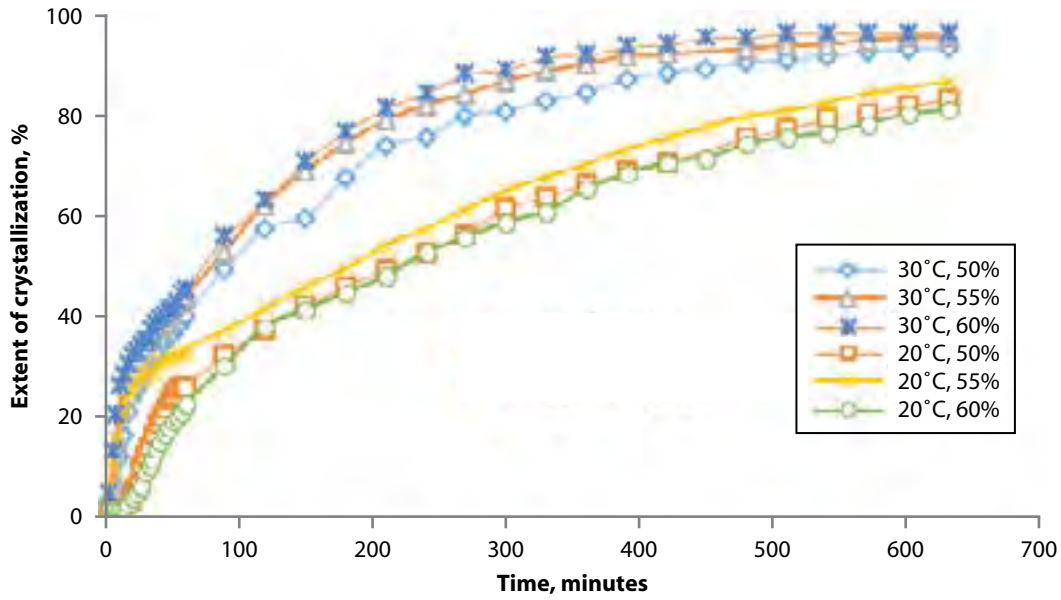

Figure 3. Extent of crystallization for 50%, 55%, and 60% at 20°C and 30°C.

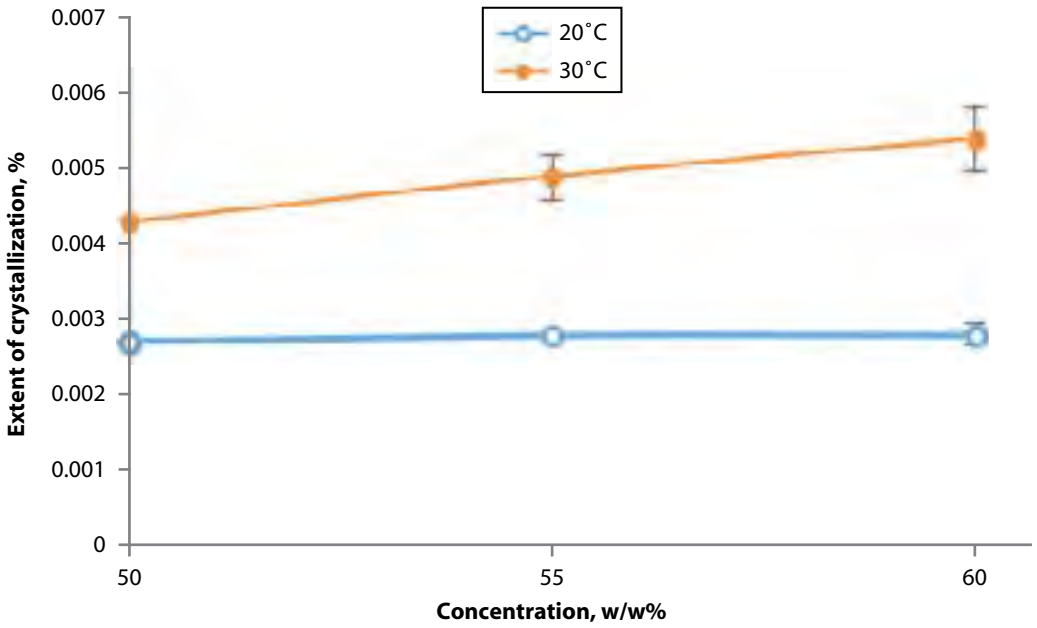

Figure 4. Rate constants of different concentrations at 20°C and 30°C.

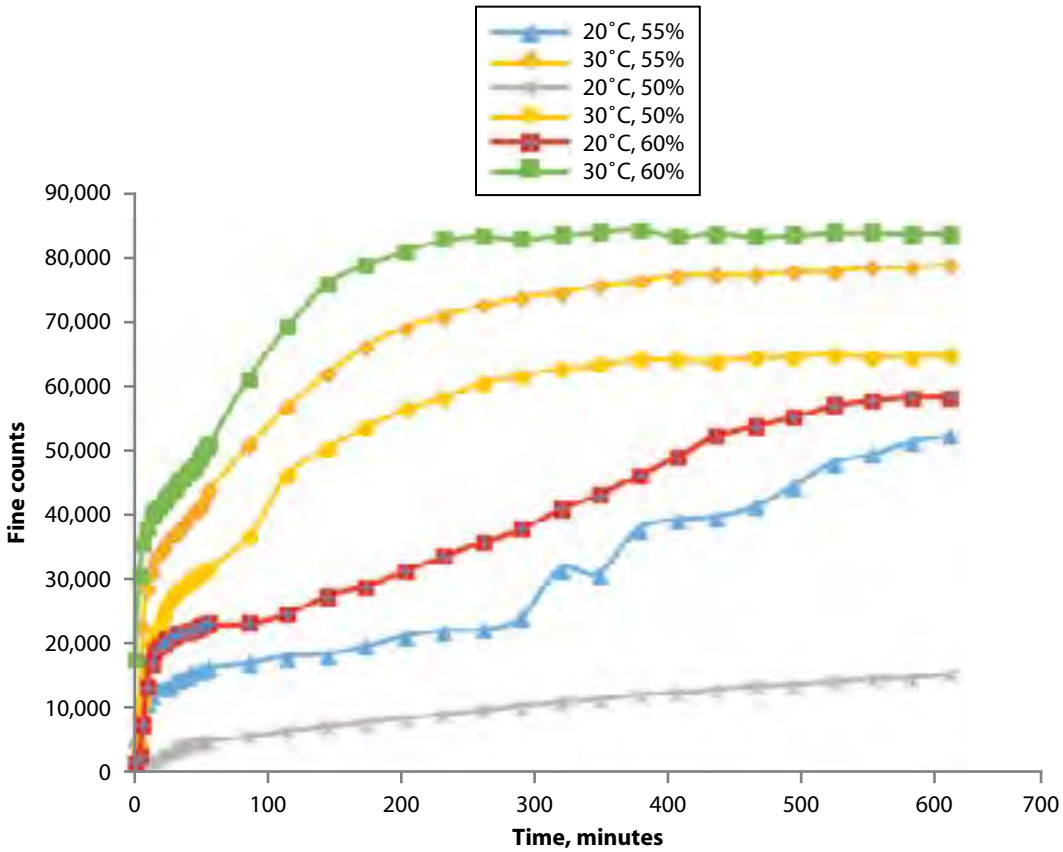

Figure 5. Count of fine crystals (<50μm) for 50%, 55%, and 60% concentrations at 20°C and 30°C.

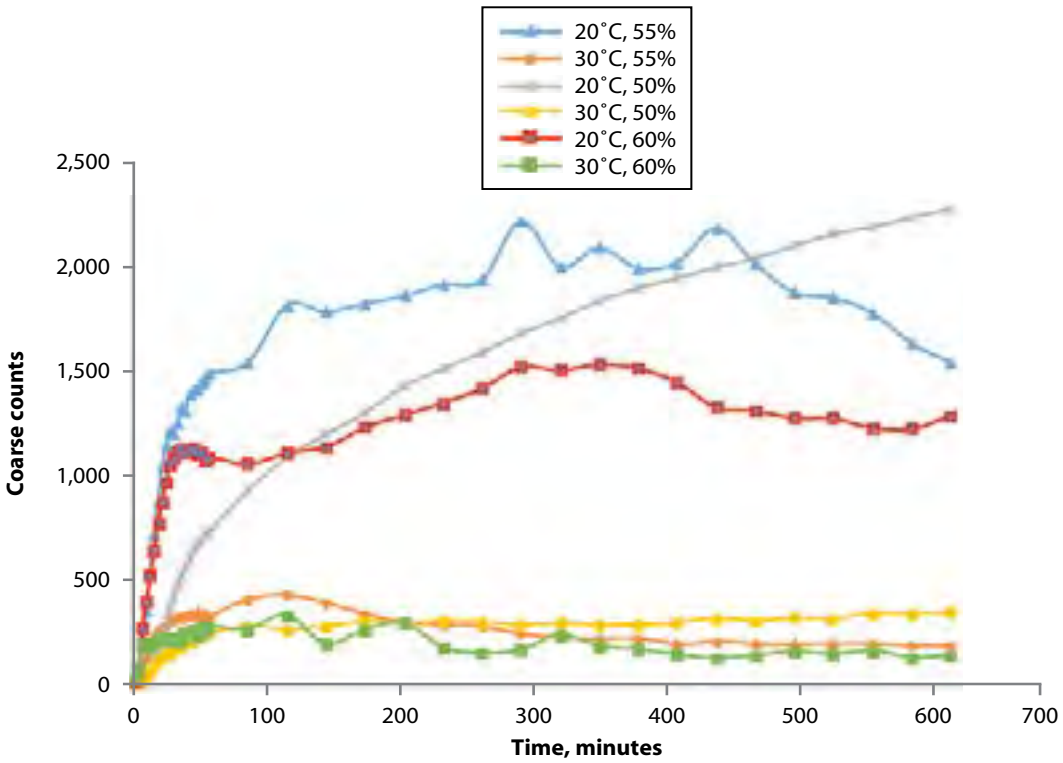

Figure 6. Count of coarse crystals (50–300μm) for 50%, 55%, and 60% concentrations at 20°C and 30°C.

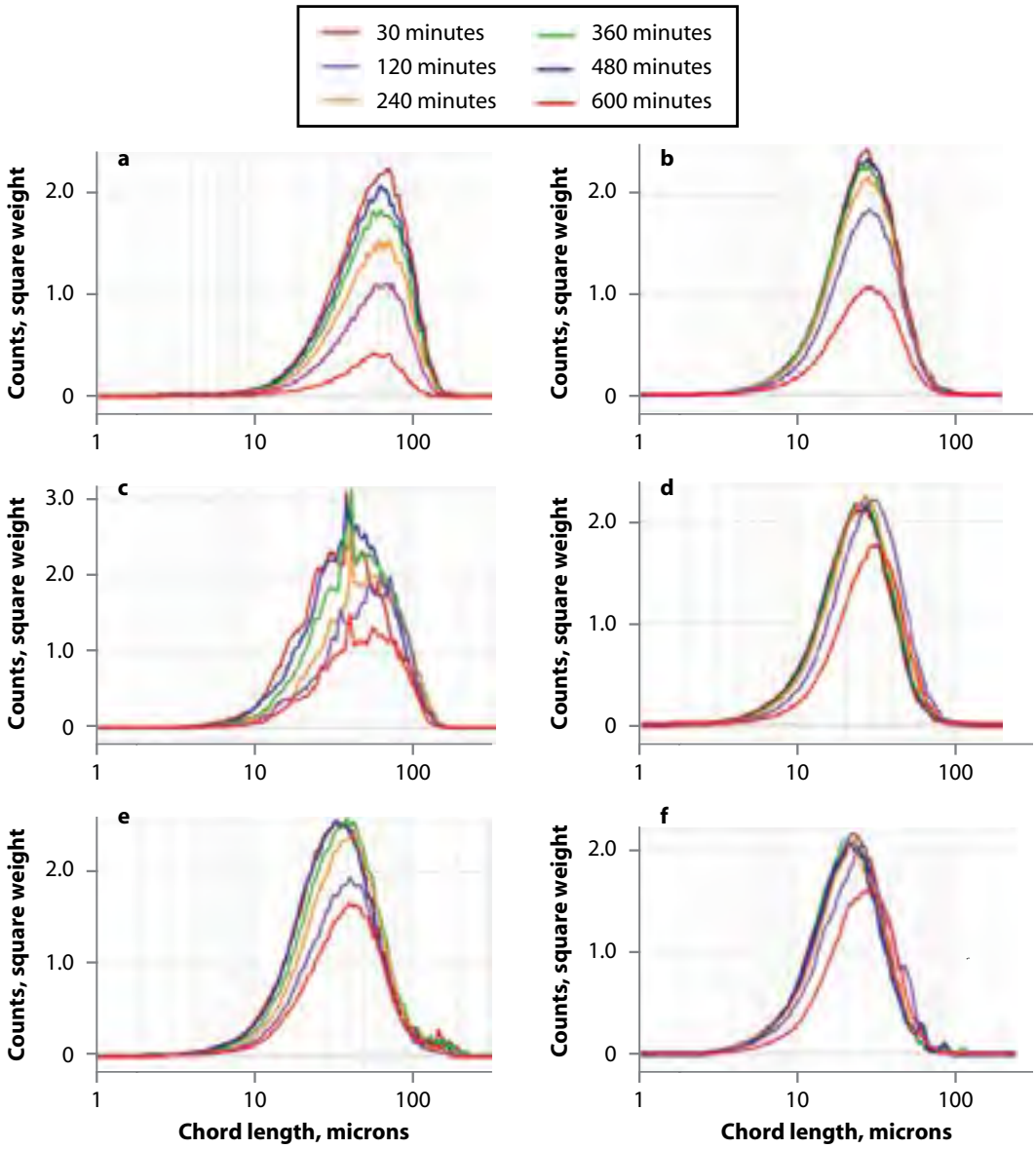

Figure 7. Chord length distributions of crystals obtained from focused beam reflectance measurement data at different time periods. (A) 20°C, 50%; (b) 30°C, 50%; (c)20°C, 55%; (d) 30°C, 55%; (e) 20°C, 60%; (f) 30°C, 60%.

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BouMatic, Madison, WI
Cargill, Inc., Wayzata, MN
Dairymaster Co., Kerry, Ireland
Dekalb Asgrow, St. Louis, MO
DeLaval, Kansas City, MO
Diamond V Mills, Cedar Rapids, IA
DuPont Pioneer, Johnston, IA
Grain States Soya, West Point, NE
Heart of America Dairy Herd Improvement Association, Manhattan, KS
High Plains Dairy Management Conference
Hubbard Feeds, Mankato, MN
Iowa Limestone, Des Moines, IA
Kansas Agricultural Experiment Station, Manhattan, KS
Kansas Dairy Commission, Victoria, KS
Kansas Farm Management Association, Manhattan, KS
Kansas Health and Environment, Topeka, KS
Kemin Industries, Des Moines, IA
Livestock and Meat Industry Council, Manhattan, KS
Merck Animal Health, Whitehouse Station, NJ
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Rota-Mix, Dodge City, KS
Select Sires, Plain City, OH
Sweet Bran, Dalhart, TX
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Varied Industries Corporation, Mason City, IA
Zinpro Corp., Eden Prairie, WI
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