DAIRY RESEARCH 2011

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K-STATE Research and Extension

Kansas State University Agricultural Experiment Station and Cooperative Extension Service

DAIRY TEAM
KANSAS STATE UNIVERSITY

DAIRY RESEARCH 2011

Contents

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Foreword

Members of the Dairy Team at Kansas State University are pleased to present the 2011 Dairy Research Report of Progress. Dairying continues to contribute significantly to the agricultural economy of Kansas. In 2010, dairy farms accounted for 3.1%, or $430 million, of all Kansas farm receipts, ranking 7th overall among all Kansas farm commodities. In the United States, Kansas had the greatest percentage increase in milk produced between 1999 and 2004 (+57.7%) of all 50 states. At the end of 2010, Kansas ranked 11th nationally in milk yield per cow at 21,000 lb, 16th in the number of dairy cows (119,000), and 17th in total milk production (2.499 billion lb). Wide variation exists in the productivity per cow as indicated by the Heart of America Dairy Herd Improvement Association (DHIA) production testing program. At the end of 2010, 106,819 cows were enrolled in the DHI program from Kansas, Nebraska, Oklahoma, Arkansas, North Dakota, and South Dakota, including herds from Colorado (2), Missouri (5), and Texas (1). A comparison of all Kansas DHIA cows and those at the Kansas State University Dairy Teaching and Research Center (DTRC) with those in the Heart of America DHIA program for 2009 is shown in the following table.

Comparison of Heart of America (HOA) Cows with Kansas Cows and Kansas State University Cows, 2010

Item	HOA	KS	DTRC[1]
Herds, no.	517	175	...
Cows/herd, no.	208	120	236
Milk, lb	21,208	21,372	29,824
Fat, lb	785	781	1,048
Protein, lb	679	663	891
Somatic cell count × 1,000	272	323	230
Calving interval, mo.	13.8	14.0	13.8

[1] KSU Dairy Teaching and Research Center, September 12, 2011, test day (milking 3 times daily; no bovine somatotropin).

Most of this success occurs because dairy producers better manage what is measured in monthly DHI records. Continued emphasis should be placed on furthering the DHI program and encouraging use of its records in making management decisions. In addition, continued use of superior, proven sires and emphasis on use of superior genetics in artificial insemination programs is essential.

The excellent functioning of the DTRC is because of special dedication of our staff. We acknowledge our current DTRC staff for their dedication: Michael Scheffel (manager), Daniel Umsheid, Michelle Sullivan, Robert Feist, Alan Hubbard, Kris Frey, and Eulises Jiron-Corrales. Special thanks are given to Colleen Hill, Cheryl K. 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 David Sukup and the DHIA laboratory here in Manhattan, KS, for their assistance in handling research milk samples.

Thorough, quality research is not only time-intensive and meticulous, but also expensive. 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.

J. S. Stevenson, Editor
2011 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 the cattle 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 the 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 "$P < 0.05$," which 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 between 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.

Influence of Dairy Housing on Freshwater Usage on Commercial Dairies in Western Kansas

J. P. Potts and M. J. Brouk

Summary

Fresh water pumping records were obtained from 24 western Kansas dairy farms for a 10-year period from 2000 through 2009. Farms were divided by facility type: dry lot (**DL**), free stall (**FS**), or a combination (**DL+FS**). Of the facility types studied, DL averaged smaller ($P < 0.05$) demand for water at 52.6 gal/cow per day compared with FS at 61.3 gal/cow. Both DL and FS facilities had less water demand than the combination facilities of DL+FS at 71.1 gal/cow of water daily. In all cases, average freshwater pumping was less than the daily amount of 134.7 gal/cow commonly used in dairy facility design. The difference may result from water conservation efforts of the dairies and the efficiency gained from operating larger milking parlors.

Key words: free stall, dry lot, water conversation

Introduction

Water is an essential part of any dairy operation. Fresh water is needed to cool cows and milk, flush alleyways, wash udders in wash pens, water cows, and clean milking equipment. Similar to water intake requirements for cows, dairy operation water use can vary greatly depending on management practices, location, and the recycling of water on the dairy. The purpose of this study was to examine the effect of facility type on the freshwater demand of western Kansas dairy operations.

Experimental Procedures

Freshwater pumping records were obtained from the Division of Water Resources in Topeka, KS, from 24 farms for the years 2000 through 2009. The records contained a total of 189 observations during this time frame. Of the 24 farms, 12 were free stalls (**FS**), 10 were dry lots (**DL**), and 2 were combination **DL+FS** farms. Water usage was first adjusted annually by operation and then converted to a per-cow per-day basis before analysis. Procedures of SAS (SAS Institute, Cary, NC) were used to analyze the data with the MIXED procedure. Dairy types (FS, DL, or DL+FS) were the fixed effects and year was considered a random effect.

Results and Discussion

The DL dairies were the largest, with an average of 4,387 cows per farm, or 755 and 2,353 more cows per farm than the FS and DL+FS dairies, respectively (Figure 1). The average daily amount of fresh water pumped during the decade reported was 57.3 gal/cow (Figure 2), which is less than the 135 gal/cow considered the average for estimating water demand on Kansas dairy operations. Of the facility types studied, DL averaged smaller ($P < 0.05$) demand at 52.6 gal/cow per day compared with FS at 61.3 gal/cow. Both DL and FS were less than the combination facilities of DL/FS at 72.1 gal/cow daily. The variation in water usage by facility may be caused by different methods of cow cooling, pen washing, and water recycling, or by herd size. Other research has shown that positioning udder wash pens before the milking parlor can increase water usage by more than 100 gal/cow daily. Wide variation was detected when comparing the ranges of water usage on the 3 facility types on a month-to-month basis during the 10-year

period. The FS ranged from 20.1 to 193.1 gal/cow per day; DL, 15.1 to 170.1 gal/cow per day; and DL/FS, 30.9 to 90.9 gal/cow per day. Similar variation has been noted in other studies. The results indicate that annual freshwater demand by western Kansas dairy farms may be less than suggested by certain guidelines. This may occur in part because more water conservation practices or efficiencies are associated with operating larger milking parlors.

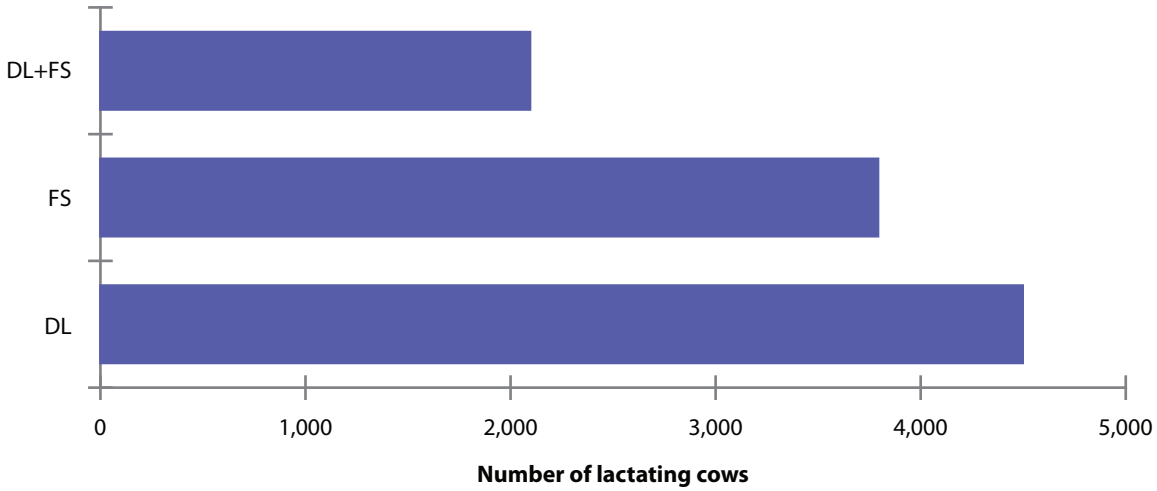

Figure 1. Average number of cows by type of dairy (DL, dry lot; FS, free stall; or DL+FS, dry lot and free stall facility).

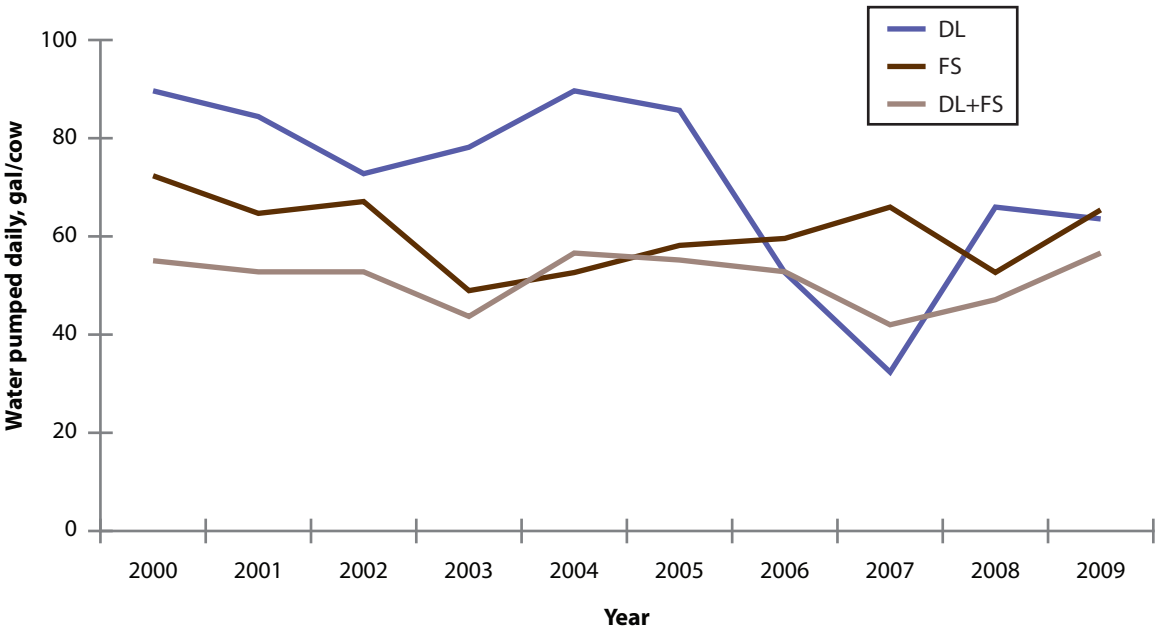

Figure 2. Ten-year comparison of fresh water pumping by different types of dairy facilities (DL, dry lot; FS, free stall; or DL+FS, dry lot and free stall facility).

Effects of Monensin on Metabolic Profile and Feeding Behavior of Transition Dairy Cows

C. R. Mullins, L. K. Mamedova, M. J. Brouk, C. E. Moore, H. B. Green, K. L. Perfield, J. F. Smith, J. P. Harner, and B. J. Bradford

Summary

Thirty-two Holstein transition cows were used to determine the effects of monensin (Rumensin, Elanco Animal Health, Greenfield, IN; 400 mg/cow daily) on metabolism and feeding behavior. Cows were assigned randomly, based on calving date, to control or monensin treatments (n = 16 per treatment) 21 days before their expected calving date, and cows remained on treatments through 21 days in milk. Feeding behavior and water intake data were collected daily. Blood samples were collected at 8 different time points during the experimental period. Monensin decreased mean and peak plasma ketone concentrations, and also decreased time between meals before and after calving. No effects of monensin supplementation were observed on milk production or other metabolic traits. Furthermore, we observed no treatment effects on disease incidence, although sample size was small for detecting such effects.

Key words: monensin, transition cow, feeding behavior

Introduction

The weeks surrounding calving are a critical time in the life cycle of a dairy cow. During this period, cows make many metabolic adjustments to support the transition from pregnancy to lactation. Feed intake can be directly linked to a successful transition. Recent research indicates a beneficial effect of monensin on postpartum feed intake, but the mechanism responsible for improved intake is not clear. Research from feedlot cattle suggests dietary monensin could modulate intake patterns, thus reducing dramatic changes in rumen pH while cattle are adapting to a higher-energy diet.

Furthermore, in recent years, monensin has been used to help mitigate the effects of a transition-related negative energy balance, which traditionally has been attributed to its ability to promote the production of glucose precursors. However, investigators across the country have uncovered evidence suggesting that the beneficial effects of monensin actually may be independent of changes in glucose production.

The primary objectives of this study were to determine the effects of monensin on transition cow feeding behavior and metabolic parameters. The secondary objectives were to assess the effects of monensin on productivity and disease incidence of transition cows.

Experimental Procedures

Thirty-two Holstein transition cows were assigned to a control or treatment group 21 days before their expected calving date (16 cows per treatment). Cows remained on their respective treatments through 21 days postpartum. Treatment cows received monensin (Rumensin, Elanco Animal Health) as a top-dress at a rate of 400 mg/cow per day. Monensin was premixed into a ground corn carrier and 2 lb of the premix was top-dressed once daily to each treatment cow. The controls received no monensin in 2 lb of the same ground corn carrier for the duration

of the study. Diets were formulated to meet or exceed National Research Council requirements (Table 1). Cows were dried off approximately 45 days before expected calving date. Monensin was excluded from the far-off dry cow ration to help ensure that no cows entering the study were influenced by prior monensin exposure.

Management of Cows and Data Collection. Cows were allowed ad libitum access to the designated treatment rations by an electronic gating system, with one cow assigned per gate. After parturition, cows were moved into a tie-stall facility where they were housed for the remainder of the study. Dry cows were fed twice daily (8:00 a.m. and 3:30 p.m.) to accommodate the capacity of the feeding system used prepartum. Lactating cows were fed once daily (3:00 p.m.) to minimize the time during which feeding behavior could not be recorded.

Cows were milked 3 times daily in a milking parlor, and milk yield was recorded at each milking. Milk samples were collected during each milking beginning at 4 days in milk and continuing through 21 days in milk. Body condition score (**BCS**; 1=thin and 5=fat) and body weight was measured 2 hours before feeding on days −21 and −7 relative to expected calving, and on days 1, 7, and 21 postpartum. Blood was collected 2 hours before feeding on these days, as well as on days -4, 4, and 14 relative to calving.

Postpartum cows (and prepartum cows with abnormalities) underwent a daily health inspection, including monitoring for urine ketones and rectal temperature. Health records were kept throughout the study to register disease incidence. If cows displayed a disorder, they were treated according to on-site standard operating procedures.

Results and Discussion

Feed Intake and Milk Production. Daily feed intake is shown in Figure 1A. As expected, average daily intake declined before calving and increased after calving in both treatments. Dry matter intake was not affected by treatment during the pre- or postpartum periods. This result was surprising because other transition cow research has shown monensin to have an effect on feed intake.

Although intakes were similar, monensin supplementation decreased ($P < 0.03$) time between meals before calving and tended ($P < 0.08$) to decrease time between meals postpartum (Table 2 and Figure 1B). These findings align with reports showing that inclusion of monensin in feedlot diets resulted in more consistent patterns of feed intake throughout the day. Even though the intermeal interval was shorter with monensin supplementation, the number of meals consumed per day and average meal duration did not differ between treatments. Meal sizes were similar overall (Table 2), but a treatment by day interaction was observed for postpartum meal size. In other words, monensin increased meal sizes on days 8, 15, 20, and 21 (Figure 1C), but not on other postpartum days. The small increase in meal frequency, coupled with similar to larger meal sizes, resulted in small, non-significant increases in dry matter intake for monensin-supplemented cows during the postpartum period. As expected, intake was noticeably different pre- and postpartum; however, the dramatic decrease in meal duration (Table 2) for postpartum cows compared with prepartum cows likely reflects differences in feeding behavior of cows in tie-stall vs. pen housing rather than a true stage of production effect. Previous studies have shown that cows in pen settings eat fewer, larger meals each day than cows housed in tie-stalls.

Daily water consumption did not differ between treatments throughout the experimental period (Table 2). Postpartum water intake started at approximately 20 gal/day, and, as expected, increased ($P < 0.001$) to 30 gal/day by day 21.

Cows receiving monensin tended ($P < 0.09$) to have smaller body weights on day 1 postpartum, but no other differences were observed between treatments for BCS or body weight (data not shown). On average, cows lost 0.6 BCS units (3.3 to 2.7) during the experiment. Milk production (85.9 vs. 86.6 ± 3.7 lb/day for control and monensin, respectively; $P = 0.92$) and concentrations of fat, protein, lactose, and solids-non-fat did not differ between dietary treatments, but milk urea nitrogen (**MUN**) was greater ($P < 0.02$) for monensin-supplemented cows (11.8 vs. 10.4 ± 0.42 mg/dL). We have no clear explanation for the observed effect on MUN; it could be related to impaired liver function in the control or a result of monensin's ruminal protein sparing effect, allowing more escape protein to reach the small intestine. A simpler explanation, however, is to consider that monensin-supplemented cows consumed, on average, an additional 0.51 lb/day of protein, with no increase in milk protein yield. If metabolizable protein supply did not limit milk protein yield, then the increase in MUN for the monensin treatment was an expected response to increased protein intake.

Metabolic and Endocrine Changes. Plasma non-esterified fatty acids (**NEFA**), ketones, glucose, and insulin concentrations are displayed in Figure 2. As expected, plasma NEFA concentrations increased dramatically before expected calving, to a peak of 878 ± 80 μM on day 1 postpartum (Figure 2A; $P < 0.001$). Monensin supplementation did not significantly alter NEFA concentrations throughout the study. This was somewhat unexpected, because previous research demonstrated that monensin could decrease NEFA concentration.

Monensin treatment decreased ($P < 0.05$) plasma ketone β-hydroxybutyrate concentration during the entire study (734 vs. 616 ± 40.9 μM). The effect of treatment day ($P < 0.001$) and a treatment by day interaction ($P < 0.01$; Figure 2B) were also significant. Most notably, monensin decreased ($P < 0.01$) plasma β-hydroxybutyrate on day 4 postpartum (777 vs. 1077 ± 71 μM). The effect on β-hydroxybutyrate is not surprising given that many other reports have demonstrated similar decreases in β-hydroxybutyrate. Smaller β-hydroxybutyrate concentrations in response to monensin are likely a result of more complete fatty acid oxidation in the liver.

Plasma glucose concentrations decreased after parturition in both groups ($P < 0.001$), but monensin did not affect pre- or postpartum plasma glucose concentrations (Figure 2C). Previous work indicates that monensin can increase plasma glucose concentration of transition cows, but increases were not consistently reported. Furthermore, because monensin does not always affect ruminal propionate production in transition cows, we would not expect substrate-driven changes in liver glucose production, although monensin could also alter gluconeogenic enzyme capacity.

Overall, monensin treatment did not affect plasma insulin concentration (Figure 2D); however, a tendency for higher plasma insulin concentration in monensin-fed cows was detected on day 7 postpartum ($P < 0.08$). Interestingly, 5 other studies failed to observe an effect of monensin on insulin concentration in transition cows, making increased insulin concentration unlikely to be a primary mechanism by which monensin alters transition cow metabolism.

Management and Health. Incidence of health disorders is shown in Table 3. No differences were detected between treatments. Because only 32 cows were used for this study, detecting dif-

ferences in disease incidence is unlikely. A more powerful assessment of the effects of monensin on cow health is the meta-analysis of 16 different studies conducted by scientists at the University of Guelph. Overall, monensin significantly decreased the relative risk of ketosis, displaced abomasum, and mastitis. The β-hydroxybutyrate response observed in our study is consistent with the numbers reported in that meta-analysis. Therefore, monensin likely reduces the risk of diseases such as ketosis and displaced abomasa, although our study lacked statistical power to detect these differences.

In this first report of monensin's effects on feeding behavior, monensin increased meal frequency. Consistent with previous results, monensin significantly decreased plasma β-hydroxybutyrate concentrations in postpartum cows, but did not alter concentrations of plasma NEFA, glucose, or insulin during early lactation. Despite the observed beneficial effects on metabolism, no significant effects on milk production or disease incidence were detected.

Table 1. Ingredient and nutrient composition of diets

Item	Prepartum	Postpartum
Ingredient, % of dry matter		
Corn silage	30.3	34.0
WCGF[1]	19.6	21.5
Prairie hay	39.7	...
Alfalfa hay	...	16.6
Cottonseed	...	6.7
Corn grain[2]	6.5	12.4
Soybean meal 48	4.1	...
Expeller soybean meal	...	7.1
Micronutrient premix[3,4]	0.3	2.8
Nutrient, % of dry matter		
Dry matter, % as-fed	57.2	54.2
Crude protein	13.1	17.3
Acid detergent fiber	28.4	19.7
Neutral detergent fiber	49.9	36.0
Nonfiber carbohydrates	35.5	38.0
Ether extract	3.4	5.0
Ash	6.9	8.8
NE_L, Mcal/lb[5]	0.72	0.76

[1] Wet corn gluten feed (Sweet Bran, Cargill, Inc., Blair, NE).

[2] A portion of the daily corn grain (2 lb/cow) served as the top-dress carrier for 400 mg monensin (Rumensin, Elanco Animal Health, Greenfield, IN) for supplemented cows. The same amount of corn alone was top-dressed for control cows.

[3] Prepartum premix consisted of 42.6% vitamin E premix, 11.9% Se premix, 11.6% trace mineral salt, 10.8% limestone, 9.71% vitamin A premix, 6.47% 4-plex, 4.31% vitamin D premix, 2.17% magnesium oxide, and 0.53% ethylenediamine dihydroiodide.

[4] Postpartum premix consisted of 48.4% limestone, 27.3% sodium bicarbonate, 12.6% trace mineral salt, 6.04% magnesium oxide, 2.33% 4-plex, 1.51% Se premix, 1.16% vitamin E premix, 0.46% vitamin A premix, 0.21% vitamin D premix, and 0.03% ethylenediamine dihydroiodide.

[5] Estimated according to NRC (2001). Nutrient Requirements of Dairy Cattle, 7th rev. ed., National Research Council. Natl. Acad. Sci., Washington, DC.

Table 2. Feed and water intake and feeding behavior during the transition period

Item	Control	Monensin	SEM	*P*-value
Prepartum water intake, gal/day	5.4	5.0	0.4	0.48
Prepartum dry matter (DM) intake, lb/day	30.6	31.1	1.3	0.83
Intermeal interval, min.	143	126	5.0	0.03
Meal frequency, meals/day	7.57	8.05	0.36	0.35
Meal size, lb DM	4.08	4.08	0.26	0.99
Meal length, min	43.4	42.9	2.3	0.88
Postpartum water intake, gal/day	26.8	26.8	2.7	0.99
Postpartum DM intake, lb/day	40.6	43.7	0.7	0.14
Intermeal interval, min.	88.8	81.4	2.9	0.08
Meal frequency, meals/day	13.7	14.8	0.5	0.12
Meal size[1], lb DM	3.04	3.24	0.13	0.29
Meal length, min.	14.1	14.5	0.6	0.65

[1] Treatment by day interaction ($P < 0.02$).

Table 3. Incidence of health disorders during the experimental period

Disorder[1]	Control	Monensin
Retaind placenta	0	1
Body temperature (fever) > 103°F	5	7
Ketosis	5	3
Hypocalcemia	2	0
Metritis	1	1
Mastitis	7	5
Displaced abomasum	2	1
Other digestive disorder	4	3
One or more disorders	12	11
Dystocia[2]	3	5

[1] No differences were detected between treatment groups using Fisher's exact test.
[2] Dystocia was defined as a calving difficulty score > 1.

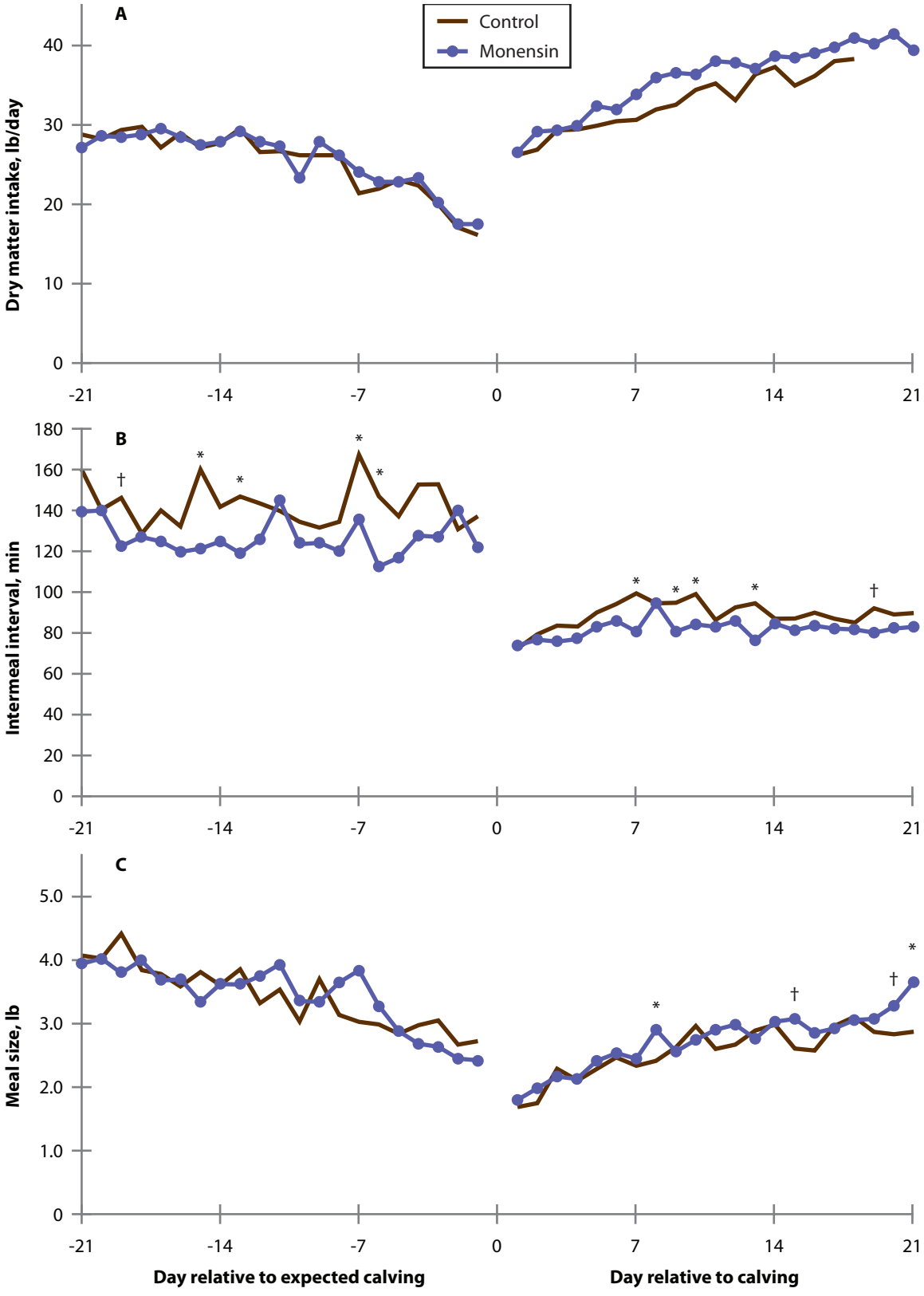

Figure 1. Dry matter intake (A), intermeal interval (B), and meal size (C) during the experimental period.

A. There was an effect ($P < 0.001$) of pre- and postpartum day (prepartum SEM = 1.94, postpartum SEM = 1.92). B. Monensin shortened ($P < 0.03$) prepartum intermeal interval and tended ($P = 0.08$) to shorten postpartum intermeal interval. An effect ($P < 0.001$) of postpartum day was detected (prepartum SEM = 10.6, postpartum SEM = 4.77; †$P < 0.10$; *$P < 0.05$). C. A postpartum treatment by day interaction was detected ($P < 0.02$; prepartum SEM = 0.4, postpartum SEM = 0.2).

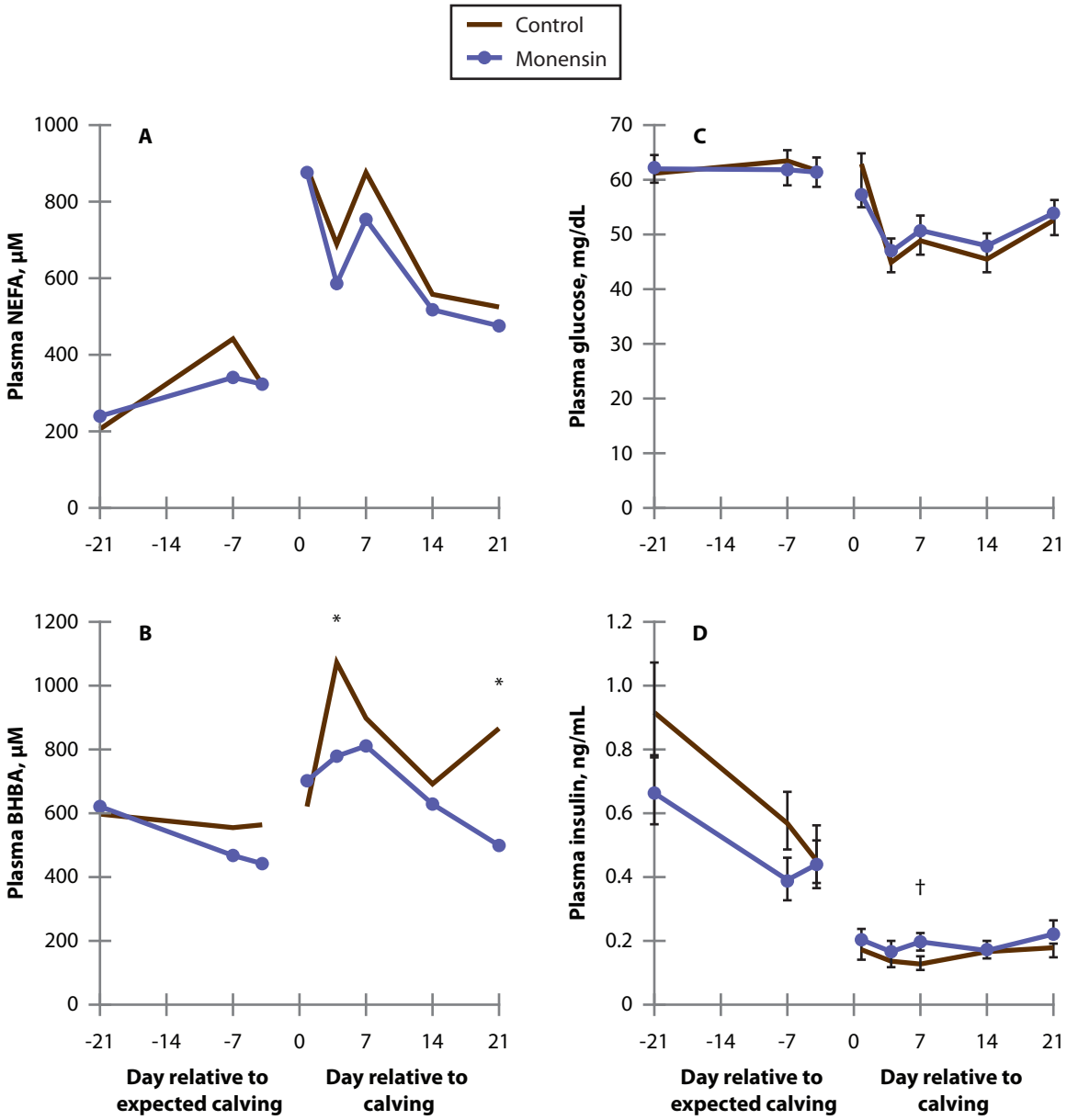

Figure 2. Plasma concentrations of nonesterified fatty acids (A), beta-hydroxybutyrate (B), glucose (C), and insulin (D) during the experimental period.

A. No treatment effects were detected, but there was a day effect ($P < 0.001$; SEM = 84.9). B. A treatment by day interaction ($P < 0.01$) was detected (SEM = 73.6; *$P < 0.05$). C. No treatment effects were detected — only a day effect ($P < 0.001$; SEM = 2.38). D. Significant effects of day ($P < 0.001$) and day by treatment interaction ($P < 0.05$) were detected. Cows receiving monensin tended ($P < 0.10$) to have greater plasma insulin concentrations on day 7 postpartum. SEM are shown in D (†$P < 0.10$).

Effects of Supplementing Methionine and Lysine in a Lactation Diet Containing High Concentrations of Corn By-products

C. R. Mullins, D. Weber, E. Block, J. F. Smith, M. J. Brouk, and B. J. Bradford

Summary

Ninety-six lactating Holstein cows were used to determine the effects of using commercial supplements to supply additional lysine and methionine in diets containing large proportions of corn by-products. Cows were assigned to 1 of 8 pens. Pens were offered rations formulated to differ in metabolizable lysine and methionine supply. The study was divided into 2 periods. During period 1, cows received similar diets, but the treatment diet supplied supplemental lysine and methionine. During period 2, the treatment diet was modified to reduce dietary crude protein. Feed intake and production were monitored daily, and milk components were analyzed 3 days per week for 4 weeks. Diet did not alter feed intake or milk production. During period 2, dietary crude protein and milk urea nitrogen (**MUN**) were decreased without sacrificing performance.

Key words: by-product, lysine, methionine, milk yield

Introduction

Cost of protein sources combined with environmental regulations demands more efficient use of dietary protein. Formulating for metabolizable protein (**MP**) has provided some progress in this area while possibly improving production. Formulating for an adequate MP supply may, however, still fail to meet the requirements of the cow if the dietary amino acid profile is not considered.

By-products from corn biofuel production are often used to provide protein and energy in lactation diets. In 2010, of the nutritionists and veterinarians who formulate rations and completed a survey, 92% used distillers grains or considered using them. Many other by-products of corn milling also are fed to dairy cattle, including corn germ meal, corn bran, corn meal, and corn gluten feed. Like corn grain, these by-products are low in lysine, so it is not surprising when nutrition models predict that diets containing large concentrations of corn by-products do not supply enough lysine to support the demands of high milk production. Many lactation diets also do not supply adequate methionine; thus, lysine and methionine are often the first-limiting amino acids in lactating cow diets.

The objective of this study was to evaluate the effects of supplementing commercial rumen-protected amino acids in a diet that was predicted to have marginally deficient lysine and methionine supply. The products used to provide the additional amino acids contained lysine embedded within calcium salts of fatty acids (Megamine-L, Arm & Hammer Animal Nutrition, Princeton, NJ) and the isopropyl ester of 2-hydroxy-4-methylthio-butanoic acid (HMBi; MetaSmart, Adisseo Inc., Antony, France), a methionine precursor.

Experimental Procedures

Ninety-six lactating Holstein cows (33 first lactation; 63 second or greater lactation) that averaged 186 days in milk were enrolled in this study. Cows were assigned to 1 of 8 identical pens with 12 free stalls in each pen. Cows were divided into pens evenly based on milk production, parity, and pregnancy status. Cows were moved into pens on May 3, 2010, and the study began on May 10, 2010, allowing for a 1-week adaptation period. During the adaption period, all pens received a common diet.

The study consisted of two 28-day treatment periods. During period 1, cows were offered either of 2 rations that were formulated to differ in metabolizable amino acid supply (Table 1). During period 2, the treatment diet was modified to decrease dietary crude protein and further increase lysine and methionine supply. Treatment diets were assigned randomly to pens. Cows were fed once daily at 110% of the expected intake. Amounts of feed delivered and refused were recorded on days 19, 20, 21, 26, 27, and 28 of each period. The total mixed rations were analyzed for dry matter (DM) on those days. Samples of all dietary ingredients were collected on days 19, 21, 26, and 28 and composited into 1 sample per period for wet chemistry analysis.

Cows were milked 3 times daily in a milking parlor, and milk yields were recorded at each milking. Milk samples were collected from every milking on each Monday, Wednesday, and Friday throughout the experiment. Milk samples were analyzed for component concentrations.

Results and Discussion

In formulating experimental diets, the strategy was to maintain large concentrations of corn by-products within diets; therefore, the control diet (Table 1) contained 26.7% wet corn gluten feed (**WCGF**) on a DM basis. The period 1 treatment diet was similar to the control; the primary differences were replacement of 190 g/cow of the calcium salts of fatty acids (Megalac-R, Arm & Hammer Animal Nutrition) with a source of calcium salts of fatty acids embedded with lysine (Megamine-L), and addition of 14 g/cow of HMBi. As expected, most nutrient concentrations were similar across treatments (Table 1); however, predicted supplies of metabolizable lysine and methionine were slightly elevated in the amino acid-supplemented group (Table 2).

Period 1 feed intake and production responses for both diets are shown in Table 3. Mean DM intake was 58.6 lb/day and mean milk yield was 88.4 lb/day, with means of 3.10% fat and 3.06% protein. No treatment effects were observed for any of the traits measured. Milk protein yield, the variable of greatest interest, was numerically smaller ($P = 0.54$) for the amino acid-supplemented diet compared with the control (2.67 vs. 2.71 ± 0.04 lb/day).

For period 2, the treatment diet was modified such that 3.6% WCGF was replaced with corn silage, expeller soybean meal was decreased from 4.9 to 2.2% of diet DM, and more Megamine-L and HMBi were added. These changes resulted in a decrease in dietary crude protein from 17.9% to 17.1%, with similar predicted lysine and methionine supply compared with the period 1 treatment diet (Table2).

Performance of cows fed the 2 diets during period 2 is shown in Table 4. Consistent with the decrease in dietary crude protein, milk urea nitrogen (**MUN**) was decreased ($P < 0.001$) in the amino acid-supplemented group (10.8 vs. 12.5 ± 0.2 mg/dL) without affecting milk production. During this period, mean DM intake was 53.8 lb/day and mean milk yield was 78.9 lb/day, with means of 3.18% fat and 3.04% protein. Beyond dietary treatment effects on MUN,

no effects were observed for any production traits measured. As in period 1, milk protein yield was numerically smaller ($P = 0.20$) for the amino acid-supplemented diet compared with the control (2.18 vs. 2.25 ± 0.04 lb/day).

Results from this study did not support the hypothesis that increasing lysine and methionine supply would increase production of cows fed a corn by-product-based diet. A number of possible explanations could explain the lack of a response. One possibility is that the products used to provide supplemental methionine and lysine did not increase post-ruminal supply of these amino acids. Past research, however, has indicated that the amino acid sources used in this study are partially protected from ruminal degradation. The efficacy of HMBi to deliver metabolizable methionine is further supported by production responses showing HMBi supplementation increases milk protein when production seems to be limited by metabolizable methionine supply.

Another possibility accounting for why lysine and methionine supplementation did not increase production is that something else was first-limiting in this scenario. Our study narrowly focused on lysine and methionine because substantial research has supported the focus on a lysine and methionine deficiency in diets similar to those fed in our study. Energy intake also could have been limiting even though all nutrition model predictions indicated a positive energy balance.

A third possibility is that the model predictions, which were used to suggest a lysine limitation in the control diet, were wrong. From these results, determining whether this was because of inaccurate predictions of metabolizable lysine supply and/or lysine requirements is impossible.

Results from this study demonstrated little response to supplementing the rumen-protected amino acids lysine and methionine. Given the results, the diet fed to control cows likely was not deficient in these amino acids, or the supplemental amino acid products that were used did not efficiently escape ruminal degradation.

Table 1. Ingredient and nutrient composition of diets fed to lactating Holstein cows

	Dietary treatment		
Item	Control	Amino acid supplemented (period 1)	Amino acid supplemented (period 2)
Ingredient, % of dry matter			
Corn silage	23.4	23.4	27.8
WCGF[1]	26.7	26.6	23.0
Alfalfa hay	18.5	18.7	19.0
Cottonseed	5.4	5.3	5.6
Ground corn grain	14.2	14.2	14.7
Ground milo	2.7	2.7	2.8
Expeller soybean meal[2]	4.9	4.9	2.2
Fish meal, Menhaden	0.3	0.3	0.3
Calcium salts of fatty acids[3]	0.7	...	...
Megamine-L[4]	...	0.7	1.2
HMBi[5]	...	0.04	0.18
Micronutrient premix[6]	3.3	3.3	3.4
Nutrient			
Dry matter, % as-fed	55.6	55.6	52.9
Crude protein	17.8	17.9	17.1
Neutral detergent fiber	33.3	32.9	30.6
Crude fat	4.7	4.5	4.7
Starch	24.0	23.5	25.6
NFC[7]	37.6	37.9	41.1
Ash	6.9	6.8	6.7
NE_L, Mcal/lb[8]	0.76	0.76	0.79
Calcium	0.83	0.79	0.94
Phosphorus	0.54	0.53	0.50
Magnesium	0.35	0.33	0.33
Potassium	1.41	1.42	1.30
Sodium	0.42	0.44	0.44

[1] Wet corn gluten feed (Sweet Bran, Cargill, Inc., Blair, NE).
[2] Soybest (Grain States Soya, Inc., West Point, NE).
[3] Megalac-R (Arm & Hammer Animal Nutrition, Princeton, NJ).
[4] Calcium salts of long-chain fatty acids plus lysine monohydrochloride (Arm & Hammer Animal Nutrition).
[5] Isopropyl ester of 2-hydroxy-4-methylthio-butanoic acid, MetaSmart (Adisseo Inc., Antony, France).
[6] The premix consisted of 41.6% limestone, 32.5% sodium bicarbonate, 6.50% Diamond V XP (Diamond V Mills, Inc.), 5.40% trace mineral salt, 5.40% magnesium oxide, 5.20% vitamin E premix, 1.66% 4-plex, 0.93% Se premix, 0.36% vitamin A premix, 0.16% vitamin D premix, 0.05% EDDI, and 0.21% Rumensin 80 (Elanco Animal Health, Greenfield, IN).
[7] Nonfiber carbohydrates; calculated as dry matter − (crude protein + NDF + crude fat + ash).
[8] Estimated according to NRC (2001). Nutrient Requirements of Dairy Cattle, 7th rev. ed., National Research Council. Natl. Acad. Sci., Washington, DC.

Table 2. Predicted metabolizable lysine and methionine supplies as a percentage of predicted metabolizable protein supply by 3 different models and predicted metabolizable lysine:methionine ratio

	Dietary treatment					
	Control		Amino acid supplemented (period 1)		Amino acid supplemented (period 2)	
Item	Lysine	Methionine	Lysine	Methionine	Lysine	Methionine
Predicted supply (g/day)						
NRC (2001)	191	65	206	67	200	69
CNCPS 5.0	181	64	197	66	197	70
CNCPS 6.1	165	62	194	67	188	68
% of metabolizable protein						
NRC (2001)	6.3	2.2	6.8	2.2	7.2	2.5
CNCPS 5.0	6.1	2.1	6.6	2.2	7.1	2.5
CNCPS 6.1	5.3	2.0	6.0	2.1	6.5	2.3
Lysine:methionine[1]						
NRC (2001)	2.92		3.07		2.90	
CNCPS 5.0	2.84		2.99		2.81	
CNCPS 6.1	2.66		2.89		2.77	

[1] Calculated based on g of predicted amino acid supply.

Table 3. Effects of supplementing lysine embedded within calcium salts of fatty acids[1] and HMBi[2] on performance of lactating Holstein cows in period 1

	Dietary treatment			
Item	Control	Amino acid supplemented (period 1)	SEM	*P*-value
Dry matter intake, lb/day	58.2	58.9	1.10	0.65
Yield, lb/day				
Milk	88.2	88.2	1.5	0.98
Milk fat	2.68	2.75	0.07	0.59
Milk protein	2.71	2.66	0.04	0.54
Milk lactose	4.29	4.31	0.09	0.96
Energy-corrected milk[3]	84.0	84.7	1.5	0.81
Milk fat, %	3.07	3.12	0.06	0.59
Milk protein, %	3.08	3.04	0.04	0.51
Milk lactose, %	4.88	4.88	0.03	0.97
MUN, mg/dL	13.9	14.3	0.2	0.25

[1] Megamine-L, Arm & Hammer Animal Nutrition, Princeton, NJ.
[2] The methionine precursor isopropyl ester of 2-hydroxy-4-methylthio-butanoic acid (MetaSmart, Adisseo Inc., Antony, France).
[3] Energy-corrected milk = (0.327 × milk yield) + (12.86 × fat yield) + (7.65 × protein yield).

Table 4. Effects of supplementing lysine embedded within calcium salts of fatty acids[1] and HMBi[2] on performance of lactating Holstein cows in period 2

	Dietary treatment			
Item	Control	Amino acid supplemented (period 2)	SEM	*P*-value
Dry matter intake, lb/day	53.9	53.5	0.9	0.91
Yield, lb/day				
Milk	79.2	78.1	1.3	0.51
Milk fat	2.29	2.33	0.07	0.65
Milk protein	2.24	2.18	0.04	0.20
Milk lactose	3.59	3.52	0.07	0.36
Energy-corrected milk[3]	84.0	84.7	1.5	0.81
Milk fat, %	3.12	3.23	0.06	0.59
Milk protein, %	3.05	3.02	0.04	0.59
Milk lactose, %	4.85	4.84	0.04	0.87
Milk urea nitrogen, mg/dL	12.5	10.8	0.2	<0.001

[1] Megamine-L, Arm & Hammer Animal Nutrition, Princeton, NJ.

[2] The methionine precursor isopropyl ester of 2-hydroxy-4-methylthio-butanoic acid (MetaSmart, Adisseo Inc., Antony, France).

[3] Energy-corrected milk = (0.327 × milk yield) + (12.86 × fat yield) + (7.65 × protein yield).

Effects of Dietary Amylase and Sucrose on Productivity of Cows Fed Low-Starch Diets

C. F. Vargas and B. Bradford

Summary

Exogenous amylase, sucrose, or a combination was used in diets with reduced starch content. The trial was performed in 48 lactating Holstein cows, and milk yield, milk composition, and dry matter intake were measured. Treatments did not affect production traits, but with slightly decreased feed intake and slightly greater milk production in amylase-fed cows, the calculated value of amylase in this study was $0.37/cow per day.

Key words: enzyme, milk production, nutrition, feed intake

Introduction

Inclusion of exogenous amylase in diets for high-producing cows is designed to enhance the utilization of carbohydrates present in feeds. In non-ruminant animals, the salivary glands secrete amylase in the saliva to begin breaking down starch as soon as food enters the mouth. In contrast, ruminants do not have salivary amylase, so starches are degraded largely by the microbial population in the rumen.

Addition of exogenous amylase has been evaluated primarily as a method to increase starch degradability. Adding exogenous amylase, however, may improve productivity of lactating cows independent of effects on total tract starch digestion. Previous studies have suggested that the primary benefit of exogenous amylase is an increase in neutral detergent fiber (**NDF**) digestibility, possibly by promoting the growth and cellulolytic activity of fiber-digesting bacteria.

The objective of this study was to evaluate dry matter (**DM**) intake, milk production, and milk components in lactating dairy cows fed amylase, 2% sucrose, or both in a low-starch diet.

Experimental Procedures

Forty-eight multiparous Holstein cows (between 70 and 130 days in milk) were blocked by parity and stage of lactation. Blocks of cows were assigned randomly to each of 4 pens (12 cows/pen). Pens were then randomly assigned to treatment sequence in a 4 x 4 Latin square design balanced for carryover effects.

Treatments were a control diet formulated for 33% NDF, 18% crude protein, 22% starch, and 4% sugar (Table 1). The 3 treatments were: (1) a control diet containing amylase (Rumistar, DSM Nutritional Products, Parsippany, NJ) added at 500 parts per million, (2) a sucrose diet with sucrose replacing corn grain at 2% of DM, and (3) a sucrose diet with amylase added at 500 parts per million. Each diet was delivered as a total mixed ration (**TMR**), and corn silage DM was determined twice weekly to adjust its inclusion rate. Cows were fed once daily for ad libitum intake and milked 3 times daily throughout the experiment. Treatment periods were 28 days, with 24 days for diet adaptation and 4 days for sample and data collection. Dry matter intake and milk yield were recorded daily.

During the final 4 days of each period, samples of orts, feed ingredients, and TMR were collected daily, composited by period, and analyzed to determine ash, neutral detergent fiber (**NDF**), crude protein, ether extract, total sugars, and starch content. Milk samples were collected at each milking during those 4 days and analyzed for concentrations of fat, true protein, lactose, urea, nitrogen, and somatic cells. Particle size was measured on 2 days and body condition scores (**BCS**; 1=thin and 5=fat) were measured at the beginning and at the end of each 28-day period.

Results and Discussion

The nutrient analyses for the treatment diets are shown in Table 2. Concentrations of NDF were relatively large for mid-lactation cows, but this was by design. The experiment was intended to assess responses to added sucrose and/or amylase in low-starch diets. Crude protein concentrations were approximately 16.5%, which was more than adequate based on observed milk urea nitrogen (**MUN**) concentrations (Table 3). Nutrient analysis indicated that the targeted replacement of 2% corn grain with sucrose was achieved. Furthermore, the diets that included amylase seemed to have greater sugar content (0.2 to 0.5%) than the treatments that lacked the enzyme, suggesting possible enzyme activity during feed storage. Table 2 also shows particle size distributions of the diets determined by using the Penn State Particle Separator. According to the guidelines for this system, the top sieve should retain between 6 and 10% of the diet, whereas in the present study the top sieve retained around 20% of DM for all the treatments, which demonstrated that the diets had large concentrations of effective fiber.

The results obtained from the milk component analysis and production of cows fed the treatment diets are detailed in Table 3. The DM intake was not altered by treatment. A tendency for an amylase by sucrose interaction was observed for milk protein content ($P = 0.06$), reflecting slightly smaller milk protein concentrations for amylase and sucrose treatments compared with control and amylase + sucrose treatments. This interaction was not observed for milk protein yield (data not shown). Solids-corrected and fat-corrected milk yield variables were not altered by treatment, although the direct effect of amylase approached significance in both cases (both $P = 0.13$), suggesting possible small increases with amylase supplementation (approximately 1.3 lb/day).

Feed efficiency for the control diet (energy-corrected milk/DM intake, or **ECM/DMI**) was 1.50; either amylase (1.57) or sucrose (1.60) treatment alone numerically increased efficiency, but the combination of the two resulted in feed efficiency identical to the control diet. Although this interaction was not significant, these results provide no evidence of synergistic benefits for the combination of amylase with high sugar content in lactation diets.

In addition to production responses to these diets, the economic impacts of the diets were modeled. Using local milk component values and estimated feed costs for Kansas in March and April 2011, both gross milk income and cost of feed for each treatment were calculated (Table 4). The two diets that contained sucrose were more expensive than the other diets because of the very high cost of this experimental ingredient, making these comparisons somewhat unrealistic. On the other hand, by adding amylase to the ration, solids-corrected milk production was slightly greater despite a decrease in DMI, resulting in an estimated increase in income over feed cost of $0.37/cow per day (if no cost is attributed to the amylase treatment). Therefore, based on these results, dairy nutritionists theoretically would be justified to incorporate amylase into diets if the added cost is less than $0.37/cow daily.

In contrast with previous studies in which exogenous amylase significantly improved feed efficiency of cows fed low-starch diets, we did not observe any significant effects of amylase, sucrose, or their interaction on intake, productivity, body condition, or feed efficiency in mid-lactation cows fed low-starch, high-fiber diets. Nevertheless, the small but economically meaningful numeric increases in feed efficiency with amylase and sucrose treatments were consistent with previously observed improvements in fiber digestibility in response to similar treatments. Based on feed efficiency responses, our results may indicate that amylase is not as advantageous in diets that are already high in sugar content. The inconsistencies between our findings and those of some previous studies highlight some unexplained interactions of amylase with animal or dietary factors.

Table 1. Ingredient composition of diets[1]

	Treatment[2]	
Ingredient	Control	Sucrose
Corn silage	38	38
Alfalfa hay	28	28
Wet corn gluten feed	10	10
Ground corn	8	6
Sucrose	-	2
Whole cottonseed	4	4
Expeller soybean meal	6	6
Soybean meal	2	2
Micronutrient premix	4	4

[1] Values are expressed as a percentage of diet dry matter.
[2] Each diet was tested with and without amylase added.

Table 2. Nutrient composition of diets

	Control		Amylase	
% of dry matter (DM)	Control	Sucrose	Control	Sucrose
DM, % as-fed	57.0	55.6	54.7	56.8
Organic matter	91.5	91.6	91.3	91.4
Crude protein	16.5	16.5	16.5	16.3
Neutral detergent fiber	35.6	35.2	35.4	34.9
Starch	21.4	20.6	21.4	20.9
Sugars	6.3	8.4	6.8	8.6
Ether extract	3.2	3.0	3.2	3.0
Particle size				
Top, %	20.3	20.2	21.4	21.1
Bottom, %	27.0	27.8	28.1	27.6
Middle, %	36.3	35.3	33.5	33.9
Pan, %	16.4	16.7	17.0	17.4

Table 3. Sugar and amylase effects on productivity in low-starch diets

	Control		Amylase			*P*-value		
Item	Control	Sugar	Control	Sugar	SEM	Amylase	Sugar	Interaction
Dry matter intake (DMI), lb/day	51.8	48.9	50.5	52.7	2.9	0.42	0.89	0.11
Milk yield, lb/day	75.6	75.0	76.9	76.1	2.6	0.21	0.35	0.93
Milk fat, %	3.67	3.69	3.66	3.72	0.092	0.70	0.22	0.56
Milk protein, %	3.02	2.99	3.00	3.03	0.026	0.42	0.88	0.06
Milk lactose, %	4.78	4.77	4.78	4.77	0.028	0.90	0.19	0.95
Milk urea nitrogen, mg/dL	16.88	16.74	16.37	16.59	0.48	0.45	0.93	0.67
SCC linear score	2.08	2.01	2.34	1.97	0.27	0.53	0.21	0.38
SCM[1], lb/day	71.2	70.5	72.1	72.1	2.9	0.13	0.49	0.64
ECM[2], lb/day	77.6	76.7	78.5	78.5	2.9	0.13	0.51	0.59
Body condition score change/28 days	0.013	-0.012	-0.010	-0.116	0.045	0.17	0.18	0.37
ECM:DMI	1.50	1.60	1.57	1.50	0.12	0.82	0.77	0.19

[1] Solids-corrected milk = (12.3 × fat yield) + (6.56 × SNF yield) - (.0752 × milk yield); Tyrell and Reid (1965).
[2] Energy-corrected milk = (.327 × milk yield) + (12.86 × fat yield) + (7.65 × protein yield); Dairy Record Management Systems (2010).

Table 4. Estimated profitability of the treatments

	Control		Amylase[1]	
$/cow per day	Control	Sucrose	Control	Sucrose
Gross milk income	15.63	15.46	15.84	15.82
Feed cost	6.17	6.38	6.01	6.87
Income over feed cost	9.46	9.08	9.83	8.95

[1] Feed costs for amylase diets do not include any cost for the enzyme.

Evaluation of Methionine Availability to Dairy Cows When Added to Mechanically Extracted Soybean Meal with Soy Gums

D. W. Brake, E. C. Titgemeyer, B. J. Bradford, and C. A. Macgregor[1]

Summary

Twenty-five Holstein cows were fed 5 different diets to evaluate amounts of metabolizable methionine provided to dairy cows from a mechanically extracted soybean meal (**meSBM**) with methionine added during manufacture. The control diet was designed to be deficient in metabolizable methionine supply. Two amounts of methionine were added from either a commercially available ruminally protected product (**RPMet**) or from a meSBM with methionine added during manufacture (**meSBM-Met**). Average milk yield was 98.8 lb/day, average milk fat was 2.81%, and milk urea nitrogen (**MUN**) averaged 8.6 mg/dL. Milk protein yield was not responsive to metabolizable methionine supply, suggesting that milk protein yield was not an optimal criterion for assessing metabolizable methionine supply. Milk protein content was greater when methionine was provided as RPMet than meSBM-Met. In addition, RPMet linearly increased plasma free methionine, but meSBM-Met did not. Body condition score (**BCS**; 1=thin and 5 =fat) was increased linearly by meSBM-Met, but responses were quadratic to RPMet. Methionine added to meSBM during manufacture did not appear to be available to dairy cows, likely because of extensive ruminal degradation.

Key words: methionine, dairy cow, soybean meal

Introduction

Optimizing the efficiency with which dairy cows utilize feed protein is often a goal of dairy producers and nutritionists. Improving efficiency of protein utilization decreases the amount of ruminally undegraded protein (**RUP**) necessary to optimize lactation performance and milk protein concentration. In addition, more efficient use of feed protein would mitigate contributions of reactive nitrogen to the environment by the dairy industry.

A large portion of metabolizable protein (protein available to the dairy cow) is of microbial origin, and this is largely a result of ruminal fermentation of feed. Microbial protein is generally considered to have an adequate profile of amino acids (building blocks of proteins) to support optimum milk protein synthesis. The amino acids provided by microbial protein plus the RUP from basal dietary ingredients (i.e., forages and grains) are unfortunately not sufficient to support ideal levels of milk production. Thus, nutritionists typically include ingredients with high concentrations of RUP (e.g., heated soybean meal, blood meal) to increase the supply of metabolizable protein. Unless the RUP has an amino acid profile well suited to meet cow needs, it will yield a less than optimal response.

Methionine and lysine are the amino acids most likely to limit the efficiency of protein utilization for milk production in dairy cows fed diets based on corn, corn silage, and alfalfa. Additions of ruminally protected methionine can increase efficiency of milk protein production when lysine is not limiting. Indeed, a number of ruminally protected amino acid products are

[1] Grain States Soya, West Point, NE.

commercially available to dairy producers, but adding these products to a ration can increase complexity in generating daily rations. Improving the amino acid profile provided by mechanically extracted soybean meal (**meSBM**) and adding methionine directly to the meSBM during manufacture may be possible, particularly if the methionine was mixed with soy gums before the soy gums were applied to the meSBM.

We evaluated the availability of methionine to dairy cows when it was added to meSBM with soy gums during its manufacture. We hypothesized that the methionine would be protected from ruminal degradation and therefore provide metabolizable methionine to the dairy cows.

Experimental Procedures

Twenty-five Holstein cows were housed in tie stalls at the Kansas State University Dairy Teaching and Research Center and fed 5 different diets (Table 1) during 5 14-day experimental periods in a Latin-square design. Total mixed rations were composed primarily of corn silage, alfalfa hay, sorghum grain, soybean hulls, and meSBM (Control). Ruminally protected lysine was added to all diets to ensure that methionine was the most limiting amino acid.

Methionine was added to diets either from a commercially available ruminally protected product (**RPMet**; provided as MetiPEARL, a gift from Kemin Industries, Des Moines, IA) or from meSBM with methionine added (0.3% wt/wt on a wet-basis) during manufacture (**meSBM-Met**). The RPMet was added to provide either 2.5 or 5 g/day of metabolizable methionine based on information from the manufacturer (7.5 or 15 g/day of MetiPEARL, respectively). Methionine was provided as meSBM-Met by replacing half or all of the dietary meSBM (Soy Best, Grain States Soya, West Point, NE) with the same product manufactured with methionine added to the soy gums. The meSBM-Met treatments were designed to add either 3.8 or 7.6 g/day of total dietary methionine from meSBM-Met. Attempts were made to provide similar levels of metabolizable methionine from both meSBM-Met and RPMet; however, because the content of metabolizable methionine from meSBM-Met was unknown, inclusions of methionine were designed *a priori* based on the assumption that two-thirds of the methionine added to meSBM was resistant to ruminal degradation.

Feed samples were collected on days 9 through 13 of each period. Daily intake was calculated from feed refusals that were weighed on days 10 through 14. Total milk yields were recorded and an aliquot was collected at each of the 3 daily milkings during the final 4 days of each period. Milk was analyzed for fat, true protein, lactose, MUN, solids not fat, and somatic cells by 24 hours after collection. To estimate nitrogen balance, urine and feces were collected 8 times during days 9 through 13 of each period. Urinary output was estimated assuming cows excreted creatinine at a rate of 13 mg/lb of body weight. Fecal output was estimated using acid detergent insoluble ash as a flow marker. Whole blood was harvested from a tail vessel at 7 hours after the morning feeding on the final day of each period. Plasma was isolated from whole blood and analyzed for free amino acids. Cow body weight was recorded at the beginning and end of each period, and BCS was measured by a single trained technician at the end of each period.

Milk samples were analyzed by Heart of America Dairy Herd Improvement Association (Manhattan, KS), and energy-corrected milk (**ECM**) was calculated as: (7.2 × lb/day of protein) + (12.95 × lb/day of fat) + (0.327 × lb/day of milk).

Results and Discussion

No differences among diets were observed for dry matter intake (Table 2). Average milk yield was 98.8 lb/day, average milk fat was 2.81%, and MUN averaged 8.6 mg/dL. Excluding differences in milk true protein concentrations, no differences were observed (Table 2) in either milk yield or content. Because dry matter intake, milk yields, and milk energy component yields were not different, the efficiency of ECM production was not different among diets.

Milk true protein concentration was greater when methionine was provided as RPMet than meSBM-Met. Increasing percentage of milk protein with additions of metabolizable methionine from RPMet is in close agreement with other reports; when methionine is limiting, milk protein concentration typically increases in response to methionine supplementation. Total yield of milk protein, however, was not affected by diet. Some research indicates that additions of metabolizable methionine increase total milk protein yields in addition to milk protein content, but increases in yield are normally associated with increases in total milk yields.

No differences were observed among diets for changes in body weight or apparent digestibilities of dry matter or nitrogen (protein). Similarly, nitrogen balance was not affected by diet. Nitrogen balance is a measure of whole-body protein accretion or mobilization. Because the periods in this experiment were relatively short, assessing nitrogen balance was necessary to ensure that cows were not mobilizing tissue proteins to support lactation and thereby masking responses to the supplemental methionine. Some reports suggest that nitrogen retention is improved when metabolizable methionine replaces dietary crude protein.

Body condition score increased linearly with greater amounts of methionine provided from meSBM-Met. Responses to additions of methionine from RPMet were quadratic with the 2.5 g/day of methionine yielding body condition increases that were greater than when 0 or 5 g/day of methionine was provided by RPMet. Ruminal fermentation may have benefited from the methionine provided as meSBM-Met, which may have resulted in slight increases in dietary energy content.

Plasma concentrations of a free amino acid generally will increase when supplies of that amino acid exceed the cow's requirement. Plasma free methionine concentrations (Figure 1) increased linearly when methionine was provided from RPMet, but methionine levels were not different from the control when methionine was added from meSBM-Met. This result demonstrates that metabolizable methionine was provided by RPMet.

In conclusion, additions of metabolizable methionine from RPMet resulted in increased milk protein content and plasma free methionine concentrations, but not when methionine was provided by meSBM-Met. Methionine added to meSBM during manufacture did not seem to be available to dairy cows, likely because of extensive ruminal degradation. In vitro evaluations of the meSBM-Met product, conducted subsequent to this experiment, support this conclusion.

Table 1. Composition of diets fed to cows (% of dry matter)

	Dietary treatments				
		meSBM-Met[1]		RPMet[2]	
Item	Control	Low	High	Low	High
Ingredient					
Dry-rolled sorghum	35.3	35.3	35.3	35.3	35.3
Corn silage	25.2	25.2	25.2	25.2	25.2
Alfalfa	15.2	15.2	15.2	15.2	15.2
Soybean hulls	10.0	10.0	10.0	10.0	10.0
Soy Best[3]	9.0	4.5	---	9.0	9.0
Soy Best + methionine	---	4.5	9.0	---	---
MegaLac-R	2.0	2.0	2.0	2.0	2.0
Calcium carbonate	1.2	1.2	1.2	1.2	1.2
Sodium bicarbonate	0.8	0.8	0.8	0.8	0.8
Monocalcium phosphate	0.4	0.4	0.4	0.4	0.4
LysiPEARL[4]	0.3	0.3	0.3	0.3	0.3
Trace mineral salt	0.3	0.3	0.3	0.3	0.3
Magnesium oxide	0.2	0.2	0.2	0.2	0.2
Zinpro 4-plex	0.05	0.05	0.05	0.05	0.05
Vitamin and mineral premix[5]	0.05	0.05	0.05	0.05	0.05
Chemical composition					
Dry matter	60.8	60.8	60.7	60.8	60.8
Crude protein[6]	14.3	14.3	14.3	14.3	14.3
Acid detergent fiber[6]	21.6	21.6	21.5	21.6	21.6
Neutral detergent fiber[6]	29.5	29.6	29.7	29.5	29.5
Crude fat[6]	3.8	3.8	3.8	4.0	4.0

[1] Mechanically extracted soybean meal with methionine added during manufacture (Grain States Soya, West Point, NE). Low = 3.8 g/day of total methionine added. High = 7.6 g/day of total methionine added.
[2] Ruminally protected methionine (MetiPEARL, Kemin Industries, Des Moines, IA). Low = 2.5 g/day of metabolizable methionine provided. High = 5.0 g/day of metabolizable methionine provided.
[3] Grain States Soya.
[4] Ruminally protected lysine provided 16.2 g/day of metabolizable lysine (Kemin Industries, Des Moines, IA).
[5] Provided to diets (dry matter basis): 1,497 IU of vitamin A/lb, 1,020 IU of vitamin D/lb, 16 IU of vitamin E/lb, and 0.06 ppm Se.
[6] Percentage of total diet dry matter.

Table 2. Effect of supplemental methionine from mechanically extracted soybean meal with methionine added during processing (meSBM-Met) or from ruminally protected methionine (RPMet) on production, nitrogen status, and digestibility of lactating dairy cows

	Dietary treatments						
		meSBM-Met[1]		RPMet[2]			
Item	Control	Low	High	Low	High	SEM	*P*- value
Dry matter intake (DMI), lb/day	56.2	56.7	56.0	55.3	56.2	1.3	0.79
Milk yield, lb/day	99.2	99.9	99.0	98.5	97.7	3.1	0.65
Energy-corrected milk (ECM), lb/day	88.2	88.8	89.5	88.6	86.9	3.1	0.66
Fat, %	2.77	2.79	2.90	2.84	2.77	0.12	0.61
Fat, lb/day	2.76	2.80	2.89	2.80	2.71	0.15	0.60
Protein[3], %	2.82	2.81	2.82	2.89	2.86	0.05	0.05
Protein, lb/day	2.78	2.78	2.76	2.82	2.76	0.07	0.76
Lactose, %	4.80	4.81	4.84	4.84	4.85	0.03	0.47
Lactose, lb/day	4.76	4.81	4.78	4.76	4.72	0.15	0.91
Solids not fat, %	8.52	8.51	8.56	8.64	8.61	0.08	0.14
Solids not fat, lb/day	8.42	8.47	8.42	8.49	8.36	0.22	0.92
Milk urea nitrogen, mg/dL	8.8	8.5	8.6	8.6	8.5	0.3	0.62
ECM:DMI	1.6	1.6	1.6	1.6	1.5	0.05	0.32
Body weight change[4]	15.4	19.0	11.5	12.6	19.2	7.1	0.90
BCS change[5]	-0.06	0.06[L]	0.10[L]	0.06[Q]	-0.01[Q]	0.04	0.03
Nitrogen balance, g/day							
Intake	590	594	586	582	590	14	0.88
Urine	155	153	151	157	153	5	0.70
Fecal	233	234	219	224	236	10	0.47
Milk[6]	197	198	197	200	197	4	0.76
Retention[7]	4	9	19	1	3	7	0.30
Productive[8]	202	207	216	202	200	9	0.54
Apparent digestibility, %							
Dry matter	62.1	62.9	63.9	63.7	62.1	1.2	0.71
Nitrogen	60.8	60.9	62.8	61.8	60.0	1.2	0.49

[1] Low = 3.8 g/day of total methionine added. High = 7.6 g/day of total methionine added.
[2] Low = 2.5 g/day of metabolizable methionine provided. High = 5.0 g/day of metabolizable methionine provided.
[3] RPMet differed from meSBM-Met.
[4] Change in body weight (lb) during 14 days.
[5] Change in body condition score during 14 days.
[6] Calculated as milk crude protein ÷ 6.38.
[7] Calculated as N intake – (Urine N + Fecal N + Milk N).
[8] Calculated as N intake – (Urine N + Fecal N).
[L] Linear ($P \leq 0.05$).
[Q] Quadratic ($P \leq 0.05$).

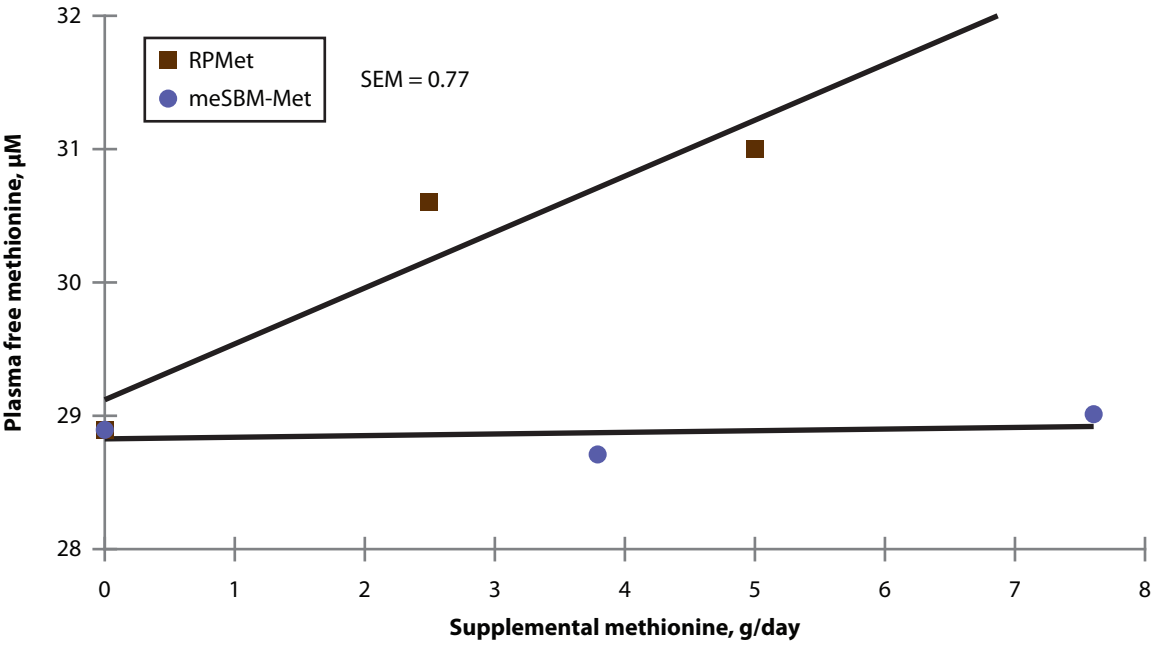

Figure 1. Effect of supplemental methionine from mechanically extracted soybean meal with methionine added during processing (meSBM-Met) or from ruminally protected methionine (RPMet) on plasma methionine concentrations of lactating dairy cows.
Supplemental methionine from meSBM-Met is listed as total methionine, whereas that from RPMet is listed as metabolizable methionine.

Effects of Sodium Salicylate on Productivity of Postpartum Dairy Cows

J. K. Farney, L. K. Mamedova, J. E. Minton, J. F. Coetzee, L.C. Hollis, and B. J. Bradford

Summary

Inflammation has been proposed as a contributor to metabolic disorders in transition dairy cows. The purpose of this experiment was to determine whether a non-steroidal anti-inflammatory drug, sodium salicylate (**SS**), benefits transition cows. At calving, 78 cows (primiparous, n = 39; second lactation, n = 28; ≥3 lactations, n = 11) were assigned alternately to either a control (**CON**) or SS treatment for 7 days and remained on study until 21 days postpartum. Treatment was administered via individual water bowls at a concentration of 2.5 g/L, delivering a mean of 183 ± 8.5 g/day SS during the 7 days of treatment. Milk yields were collected daily and milk samples were collected twice weekly. Data were analyzed using mixed models with repeated measures over time. No treatment effects were detected for daily feed or water intake. Milk yield for third or greater lactation cows tended to increase ($P < 0.10$) with SS at the end of the trial (days 19 to 20). Milk protein content increased ($P < 0.05$) with SS in first- and second-lactation cows during week 1 and milk urea nitrogen (**MUN**) decreased ($P < 0.01$) with SS. Milk fat content increased ($P < 0.05$) with SS in weeks 2 and 3 postpartum. A 10% increase ($P < 0.05$) in energy-corrected milk (**ECM**) was observed for SS cows during week 3. Metritis incidence increased ($P < 0.01$) with SS in third or greater lactation cows, but no other effects on disease incidence were detected. In contrast to our hypothesis that SS treatment would decrease transition disorder incidences, SS treatment seemed to promote increased milk fat content and milk energy output during early lactation with no effect on total disorder incidence.

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

Introduction

The transition period is a time of metabolic problems for dairy cattle, and these problems cause substantial costs for producers. A majority of the metabolic issues stem from the negative energy balance associated with the increase in nutrients needed for the mammary gland. In addition to negative energy balance contributing to transition disorders, inflammation has been hypothesized to play a role. Specifically, one study utilizing 3 commercial Italian dairies showed that cows exhibiting the strongest inflammatory profiles were 8 times more likely to experience transitional disorders, had lower plasma calcium concentrations, took longer to re-breed, and produced less milk during the first month of lactation. Another study at Kansas State University showed a disruption in normal metabolism in dairy cows subject to a low-level, short-term induced inflammation. These findings support the hypothesis that inflammation is a contributing factor to transition disorders.

Non-steroidal anti-inflammatory drugs (**NSAIDs**) are used to combat the effects of inflammation (i.e., pain, redness, and swelling). These types of drugs have been used in dairy research as a component of treatment protocols for bacterial infections (primarily mastitis); however, a few NSAIDs have been used to treat metabolic disorders. The purpose of this experiment was to determine if an oral dose of sodium salicylate (**SS**) during the first 7 days after calving can minimize transition disorders while maintaining milk production.

Experimental Procedures

Seventy-eight Holstein cows (primiparous, n = 39; second lactation, n = 28; ≥3 lactations, n = 11) from the Kansas State University Dairy Research Center were enrolled in the experiment. Cows were assigned alternately to either of 2 treatments on the day of parturition. Cows that had lameness issues or milk fever were not enrolled in the experiment. Treatments were balanced among parities and consisted of 7-day CON or SS treatments administered through individual water bowls. Cows were housed in a tie-stall facility, fed ad libitum at 6:00 a.m. and 6:00 p.m., and milked thrice daily (2:00 a.m., 10:00 a.m., and 6:00 p.m.). Sodium salicylate in a molasses carrier was administered at 2.5 g/L and the CON cows received the same concentration of molasses in the water as the SS cows during the treatment period. On the eighth day after calving, all cows were placed on regular water during the remainder of the experiment. The cows were monitored daily for signs of illness, feed intake, and water intake. Milk yield was recorded during the entire 21 days and milk samples were collected and milk components were measured twice weekly. Body condition score (**BCS**; 1= thin and 5=fat) was evaluated by three trained personnel on days 1, 7, 14, and 21. All common disorders were diagnosed according to clearly established criteria. Data were analyzed using mixed models with repeated measures over time. Traits of interest included treatment, parity, time (week or day as the unit measure of time), and interactions of treatment with time and parity.

Results and Discussion

No treatment effects were detected for daily feed or water intake. Water and feed intake followed the same pattern as milk production, increasing during the 3-week experiment. All feed and water intakes were similar to our predicted values, indicating that our treatment did not negatively affect an already stressed transition dairy cow.

Treatment did not affect milk yield; however, older cows (second lactation and greater) produced ($P < 0.001$) more milk than first-lactation cows. In addition, a treatment by parity by day effect for milk yield was detected where third-lactation and older cows treated with SS tended ($P < 0.10$) to produce more milk on days 19 to 20 (Figure 1). Furthermore, a 10% increase in ECM was detected ($P < 0.05$) for SS cows during week 3 (Figure 2). As expected, third-lactation and older cows produced the most ($P < 0.05$) ECM, with first-lactation cows the smallest and second-lactation cows intermediate. Milk protein content was increased ($P < 0.05$) by SS treatment in first and second-lactation cows during week 1, and this effect held true for first-lactation cows through weeks 2 and 3. Milk urea nitrogen (**MUN**) was decreased ($P < 0.05$) by SS treatment (12.9 vs. 11.5 ± 0.4 mg/dL). Milk fat was increased ($P < 0.05$) by SS treatment during weeks 2 and 3 (Figure 3). Milk fat content also decreased ($P < 0.01$) over the 3-week period. No treatment effects were observed for milk lactose content or somatic cell count.

Metritis incidence was increased ($P < 0.01$) by SS in third-lactation and older cows, but no other effects on diseases were detected (Table 1). The incidence of metritis should be carefully interpreted because of the small number of cows in this treatment (n = 11). A greater number of control cows had to be treated during the first week of the experiment, whereas more SS cows showed their first signs of a disorder during the second week of the experiment (Figure 4).

The milk results from this study are similar to those in another study conducted in Italy in which researchers gave transition dairy cows daily injections of aspirin for 5 days postpartum. The authors observed an improved milk yield during the first 2 months of lactation and increased first-service conception rates. The Italian group observed an apparent increase in me-

tritis incidence (30.4% aspirin-treated vs. 13.6% control), but a decrease in ketosis incidence (4.4 vs. 22.7%). In contrast, we did not detect any overall differences in disease incidence in our study using a different NSAID administered orally. Nevertheless, the increase in mastitis in older cows treated with SS is at least consistent with the negative effects reported by the Italian group. In general, caution is warranted in the use of NSAID therapy in transition cows; 2 recent studies also reported increased risk of metritis and decreased feed intake when transition cows were treated with flunixin.

Sodium salicylate seems to alter some metabolic pathways leading to measurable changes in the transition dairy cow. The exact pathways that are affected are still being investigated, but given that SS has potent anti-inflammatory effects, at least some of the metabolic responses to SS are likely mediated by decreases in inflammatory signals. These results, then, suggest that the effects of inflammation on the transition dairy cow warrant further exploration.

Table 1. Disorder occurrences

Disorder	Incidence (%)[1]	CON (n)[2]	SS (n)[3]	*P*-value[4]
Ketosis	32.1	12	13	NS
Lameness	5.1	3	1	NS
Temperature[5]	28.2	11	11	NS
Metritis	23.1	8	10	NS[8]
Displaced abomasum	7.7	2	4	NS
Diarrhea	7.7	4	2	NS
Retained placenta	10.3	4	4	NS
Mastitis	19.2	7	8	NS
Other[6]	11.5	4	6	NS
Multiple[7]	44.9	16	19	NS

[1] Total diagnosed and treated incidence of each specific disorder out of all 78 cows.
[2] Count of control cows diagnosed with the specific disorder.
[3] Count of salicylate treated cows diagnosed with the specific disorder.
[4] Not significant ($P > 0.10$).
[5] Cows had a fever >104°F but no other symptoms.
[6] Cows that were diagnosed with something other than that listed above (i.e., hardware disease).
[7] Cows that were diagnosed and treated as having more than one disorder during the trial.
[8] Third-parity cows were the only cows that showed a significant increase ($P < 0.05$).

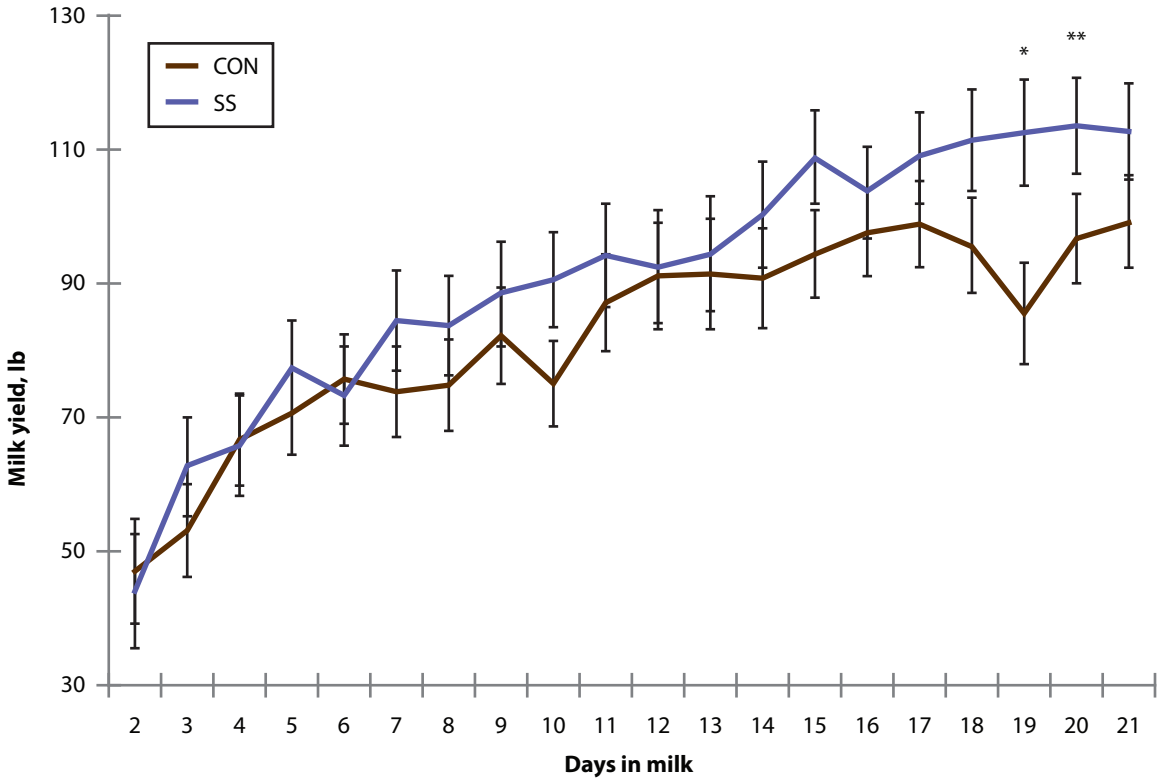

Figure 1. Milk yield for third-lactation or greater cows (n = 11) shows a treatment by parity by time interaction ($P < 0.05$) for milk yield in which sodium salicylate (SS)-treated cows had greater milk yield than the control (CON) on day 19 and tended to be greater on day 20.* $P < 0.05$; ** $P < 0.10$.

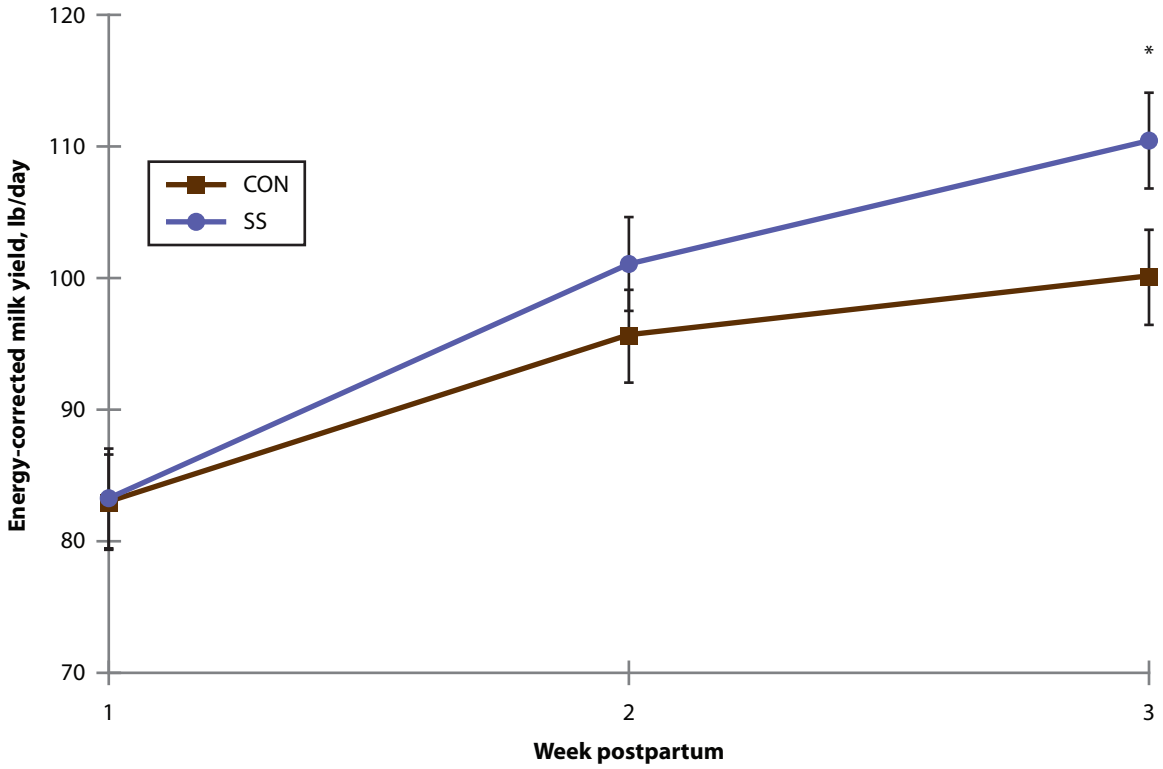

Figure 2. Energy-corrected milk yield shows a treatment by week interaction ($P = 0.11$) with a clear increase ($P < 0.05$) during week 3 for sodium salicylate (SS)-treated cows compared with the control (CON). * $P < 0.05$.

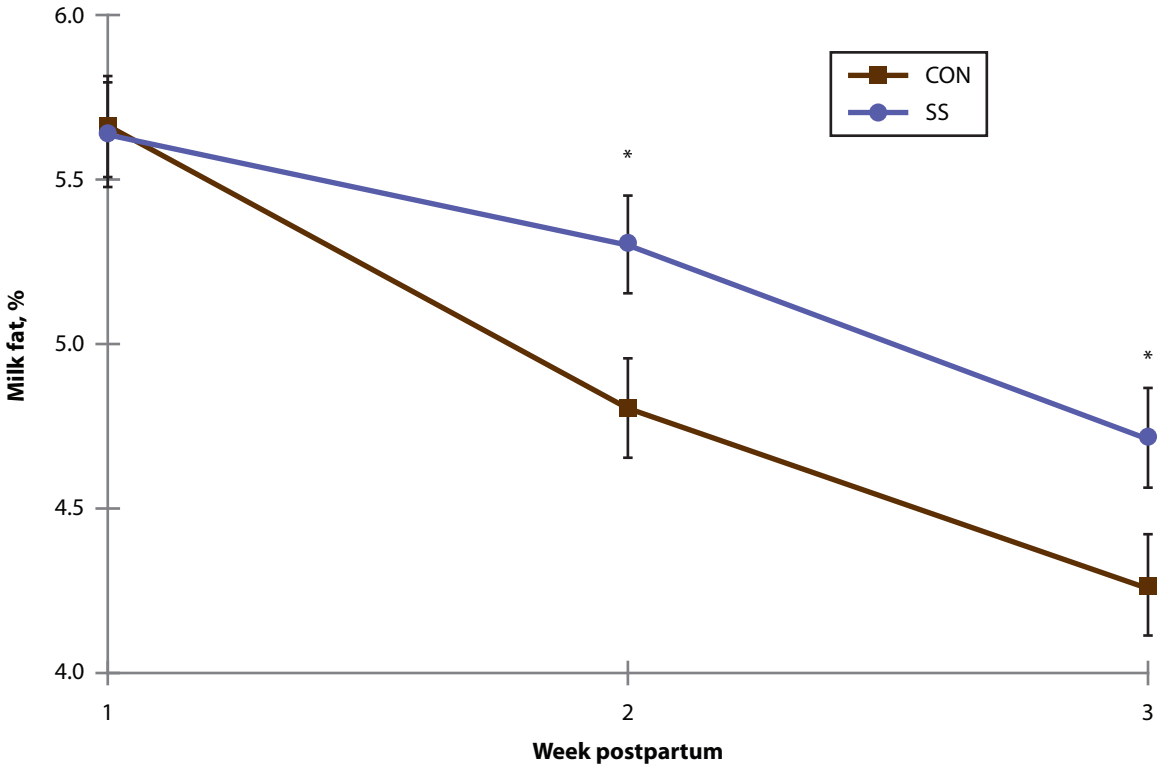

Figure 3. Milk fat percentage by week shows that milk fat was greater ($P < 0.05$) for sodium salicylate (SS)-treated cows than the control (CON) during weeks 2 and 3.* $P < 0.05$.

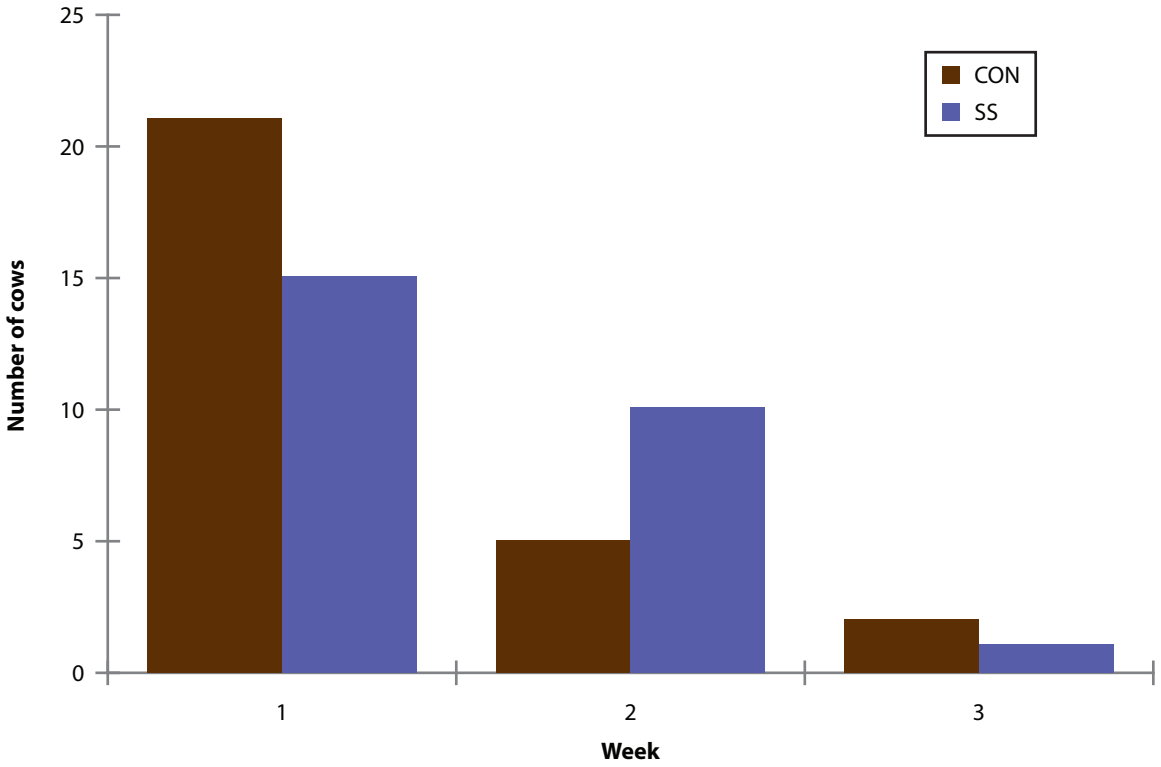

Figure 4. Distribution of cows with diagnosed disorders for treated (SS) and control (CON) cows.

Prostaglandin $F_{2\alpha}$ and GnRH Administration Improved Progesterone Status, Luteal Number, and Proportion of Ovular and Anovular Dairy Cows with Corpora Lutea Before a Timed Artificial Insemination Program

J. S. Stevenson, S. L. Pulley, and H. I. Mellieon, Jr.

Summary

The objective of this research was to increase the proportion of cows with at least 1 functional corpus luteum (**CL**) and elevated progesterone at the onset of the timed artificial insemination (**TAI**) program. Postpartum Holstein cows in 1 herd were stratified by lactation number at calving (September 2009 through August 2010) and assigned randomly to 1 of 2 treatments: (1) Presynch-10 (n = 105): two 25-mg injections of prostaglandin $F_{2\alpha}$ (**PG**) 14 days apart (Presynch); and (2) PG-3-G (n = 105): one 25-mg injection of PG 3 days before 100 μg gonadotropin-releasing hormone (**GnRH**; Pre-GnRH), with the PG injection administered at the same time as the second PG in the Presynch-10 treatment. Cows were enrolled in a TAI protocol (Ovsynch; injection of GnRH 7 days before [GnRH-1] and 56 hours after [GnRH-2] PG with AI 16 to 18 hours after GnRH-2) 10 days after the second or only PG injection. Blood samples for progesterone or estradiol analyses were collected on median days in milk (**DIM**): 36, 39, 50, 53 (Pre-GnRH), 60 (GnRH-1), 67 (PG), 69 (GnRH-2), and 70 (TAI). Ovarian structures were measured by ultrasonography on median DIM 53, 60, 67, 69, and 6 days post-TAI to determine follicle diameters, ovulation response to GnRH, or both. Although progesterone concentration did not differ between treatments before Pre-GnRH injection, the proportion of cows with at least 1 CL tended to be greater for PG-3-G than Presynch-10 cows, and more PG-3-G cows ovulated after Pre-GnRH than ovulated spontaneously in Presynch-10. Furthermore, diameter of follicles that ovulated tended to be smaller in PG-3-G than Presynch-10 cows after Pre-GnRH. At GnRH-1, the proportion of cows with progesterone ≥1 ng/mL, the number of CL per cow, and the proportion of cows with at least 1 CL were greater for PG-3-G than Presynch-10. Neither follicle diameter nor percentage of cows ovulating after GnRH-1 differed between treatments. At PG injection during the week of TAI, progesterone concentration and the proportion of cows with progesterone ≥ 1 ng/mL tended to be greater for PG-3-G than Presynch-10, and PG-3-G had more CL per cow than Presynch-10. No ovarian characteristics differed between treatments after GnRH-2, including progesterone concentration, number of CL per cow, and total luteal volume 7 days after GnRH-2.

Many of the previous ovarian traits were improved in both ovular and anovular cows after PG-3-G compared with Presynch-10. Pregnancies per AI at days 32 and 60 were only numerically greater for PG-3-G vs. Presynch-10 cows, largely because of differences detected during months without heat stress. We concluded that the PG-3-G treatment increased ovulation rate and luteal function 7 days before the onset of Ovsynch, resulting in improved follicular synchrony and predisposing potentially greater pregnancies per AI in lactating dairy cows.

Key words: luteal function, ovulation, presynchronization, progesterone, timed artificial insemination (TAI)

Introduction

Timed artificial insemination (**TAI**) programs facilitate control of reproductive cycles in lactating dairy cattle and provide viable options to AI programs solely based on detection of estrus. Preliminary studies formed the foundation for developing a TAI program when gonadotropin-releasing hormone (**GnRH**) administered to control follicle waves was followed in 6 or 7 days by prostaglandin $F_{2\alpha}$ (**PG**) to regress functional luteal tissue in a coordinated fashion. According to a survey of 103 Alta Genetics progeny-test herds, development of the Ovsynch protocol (injection of GnRH 7 days before [**GnRH-1**] and 48 hours after [**GnRH-2**] PG with TAI administered 16 hours after GnRH-2) led to its adoption in more than 85% of these large (> 500 cows per herd) U.S. dairy herds; nationally, the mean percentage of 231,288 cows inseminated after a TAI protocol was reported to be 43.4% and differed among 4 regions of the U.S.

Lactating dairy cows treated with the Ovsynch program beginning from days 5 through 12 of the estrous cycle had greater incidences of ovulation and pregnancies per AI (**P/AI**) than cows treated at other stages of the cycle. On the basis of the hypothesis that fertility after a TAI program was related to the stage of the estrous cycle (or stage of the first follicular wave), presynchronization of estrous cycles was attempted before the Ovsynch program by using 2 injections of PG administered 14 days apart (**Presynch**). The second Presynch injection given 12 d before the onset of the TAI program resulted in a larger proportion of cows in diestrus at the onset of the TAI program. These cows had greater P/AI than cows initiating the TAI programs at random stages of the estrous cycle, as did cows in subsequent experiments in which estrous cycles were presynchronized after administration of 1 or 2 presynchronizing injections of PG.

Therefore, the interval between the standard second Presynch PG injection and the onset of Ovsynch is important to the stage of estrous cycle or stage of follicular wave in which cows are found at the time of GnRH-1. Assuming that luteolysis occurs from 0 to 5 days after the second Presynch PG injection, intervals of 14 (Presynch-14), 12 (Presynch-12), 11 (Presynch-11), and 10 days (Presynch-10) would synchronize a majority of the cows to days 9 through 14, days 7 through 12, days 6 through 11, or days 5 through 10 of the cycle. On the basis of studies in which GnRH was given at various stages of the first follicular wave, intervals of 10 or 11 days (days 5 through 11 of the cycle) should facilitate greater ovulatory responses to GnRH-1. Moreover, Presynch-14 was concluded to decrease ovulatory responses to the first and second GnRH injections and to result in lesser P/AI compared with an 11-day interval.

The objectives of the present study were to test which of 2 presynchronization methods administered before a TAI program produced the greatest percentage of cows having a functional CL and elevated progesterone concentrations before enrollment in a TAI program to increase subsequent P/AI. The first treatment selected in which both $PGF_{2\alpha}$ and GnRH were administered before applying the Ovsynch protocol (Peters and Pursley, 2002) is similar to Double Ovsynch, but it excludes the initial GnRH injection of the presynchronization portion of Double Ovsynch. The second treatment represents what should be the best standard Presynch protocol in which the interval from the second Presynch $PGF_{2\alpha}$ injection to the onset of the Ovsynch protocol was reduced from 14 to 10 days. This treatment (Presynch-10) should result in more cows that are earlier in their estrous cycle and first follicular wave (days 5 to 10) at the onset of the Ovsynch protocol than a Presynch-14 program in which most cows would be later (days 9 to 14) in their estrous cycle at the first GnRH (GnRH-1) injection of the TAI program.

Experimental Procedures

Lactating Holstein cows were enrolled at calving from September 2009 through August 2010 at the Kansas State University Dairy Teaching and Research Center in Manhattan. Cows were housed in covered free stalls and fed twice or thrice (summer) daily a total mixed ration calculated to meet nutrient requirements for lactating dairy cows producing 110 lb of 3.5% milk (NRC, 2001). The diet consisted of alfalfa hay, corn silage, soybean meal, whole cottonseed, corn or milo grain, corn gluten feed, vitamins, and minerals. Cows were milked every 8 hours in a double 6 Herringbone milking parlor.

At calving, 210 cows enrolled in the study were stratified by lactation number (1 vs. 2+) and assigned randomly to receive 1 of 2 presynchronization treatments (Figure 1). The first treatment (**PG-3-G**) consisted of a 25-mg i.m. injection of PG (Pre-PG; 5 mL Lutalyse, Pfizer Animal Health, Madison, NJ) 3 days before a 100-μg i.m. injection of GnRH (Pre-GnRH; 2 mL Fertagyl, Merck Animal Health, Whitehouse Station, NJ). The second treatment (**Presynch-10**) was timed so the second of two 25-mg i.m. injections of PG (5 mL Lutalyse, Pfizer Animal Health) was administered on the same day as the Pre-PG injection in the PG-3-G treatment (Figure 1). The Ovsynch TAI program was initiated 10 days after either the Pre-PG or Presynch PG-2 injection. Treatment injections were staggered within cluster so all cows were inseminated on the same day every 2 weeks (Figure 1).

At calving, a new breeding cluster of cows was initiated every 2 weeks. Body condition scores (**BCS**; 1 = thin, 5 = fat) were assigned and pregnancies were diagnosed as illustrated in Figure 1. The monthly Dairy Herd Improvement test day energy-corrected milk (**ECM**) yield after 60 DIM near the onset of treatment was recorded for cows enrolled in the study. Three technicians performed inseminations, with 1 technician conducting more than 85%. Multiple sires were used. Pregnancy diagnosis was conducted by transrectal ultrasonography (5.0 MHz linear-array transducer, Aloka 500V, Corometrics Medical Systems, Inc., Wallingford, CT) on days 32 and 60 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.

Blood samples were collected by puncture of caudal vessels into evacuated tubes as indicated in Figure 1 to later assess concentrations of progesterone in blood serum. Ovarian scans were conducted by transrectal ultrasonography (Figure 1) to measure all ovarian follicles and determine when ovulation occurred after GnRH injections.

Results and Discussion

More ($P < 0.05$) PG-3-G cows ovulated after the Pre-GnRH injection than spontaneously ovulated in the Presynch-10 treatment (Table 1). As a result of this ovulatory response to the Pre-GnRH injection, a number of further differences were detected between treatments 7 days later when the Ovsynch protocol was initiated with the GnRH-1 injection (Table 1). More PG-3-G cows had at least 1 CL, more PG-3-G cows had elevated progesterone concentrations, and the average CL per cow was greater in the PG-3-G cows than in Presynch-10 cows (Table 1). The ovulation response to GnRH-1 did not differ between treatments, but more PG-3-G than Presynch-10 cows had multiple ovulations (Table 1).

Before the Ovsynch PG injection was administered, more PG-3-G than Presynch-10 cows had elevated progesterone concentrations, and average progesterone concentration tended to be greater (Table 1). In addition, the average number of CL per cows was greater for PG-3-G

cows. No other differences were detected at the time of GnRH-2 or in response to GnRH-2. Incidences of CL regression after PG and percentages of cows ovulating after GnRH-2 did not differ between treatments.

In this preliminary study, pregnancies per AI were numerically greater for PG-3-G than Presynch-10 cows at days 32 and 60, particularly during the moderate to cold weather (October to May) months of the study. Fertility was very poor during the summer of 2010 (Figure 2), as evidenced by poor P/AI observed when the heat index exceeded 72.

Anovular cows, those which had not initiated estrous cycles before the treatments were applied, had numerous positive responses to the PG-3-G compared with the Presynch-10 treatment. These included greater ovulation response to the Pre-GnRH injection, more cows with at least 1 CL, greater progesterone concentration, and more CL per cow before the GnRH-1 injection of Ovsynch. The results indicate that anovular cows may be better candidates for the PG-3-G treatment even if fertility may not be improved. Furthermore, pregnancy results for both treatments during moderate to cold months were promising at days 32 and 60 of gestation.

Table 1. Selected outcomes after treatment

	Treatment[1]	
Item	PG-3-G	Presynch-10
Cows, no.	105	105
Ovulation after Pre-GnRH, %	80.0[a]	53.3[b]
GnRH-1		
Cows with corpora lutea (CL), no.	94.3[a]	76.2[b]
CL per cow, no.	1.2 ± 0.1[a]	0.9 ± 0.1[b]
Cows with progesterone ≥ 1 ng/mL, %	90.5[a]	76.2[b]
Ovulation, %	79.0[a.]	69.5[a]
Multiple ovulation, %	21.7[a]	8.2[b]
Ovsynch PG		
Cows with progesterone ≥ 1 ng/mL, %	93.3[c]	85.7[d]
Progesterone, ng/mL	5.9 ± 0.3[c]	5.0 ± 0.4[d]
CL per cow, no.	2.0 ± 0.1[a]	1.5 ± 0.1[b]
Pregnancies per artificial insemination (AI) (day 32), %	40.0[a]	33.3[a]
Moderate to cold	59.1	45.1
Summer	7.7	8.8
Pregnancies per AI (day 60), %	35.9[a]	30.5[a]
Moderate to cold	54.7	42.3
Summer	5.1	5.9
Pregnancy loss, %	7.5[a]	8.6[a]

[a-b] Treatments differ ($P \leq 0.05$).
[c-d] Treatments tend to differ ($P < 0.10$).
[1] See Figure 1 for treatment descriptions.

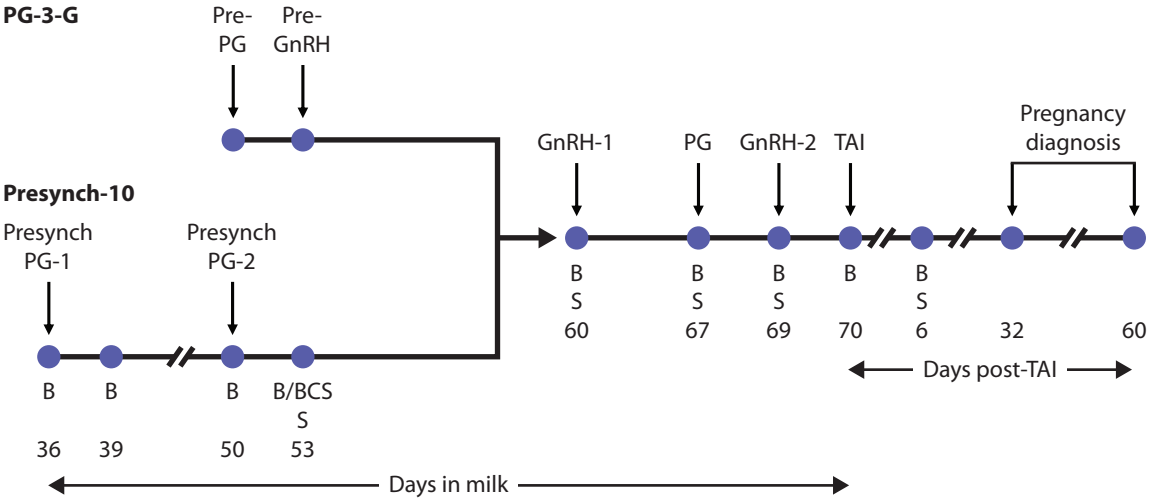

Figure 1. Experimental design of treatments and measurements.
At calving, lactating dairy cows were assigned randomly to 2 treatments, PG-3-G or Presynch-10. Cows received 100 µg GnRH at Pre-GnRH, GnRH-1, or GnRH-2; 25 mg of $PGF_{2\alpha}$ at Pre-PG, Presynch PG-1, Presynch PG-2, and PG. Blood (B) samples were collected by puncture of a caudal blood vessel and ovaries were scanned (S) by using transrectal ultrasonography. Positive pregnancy diagnoses required the presence of anechoic uterine fluid and a large corpus luteum or anechoic uterine fluid and presence of a viable embryo. BCS = body condition score; TAI = timed artificial insemination.

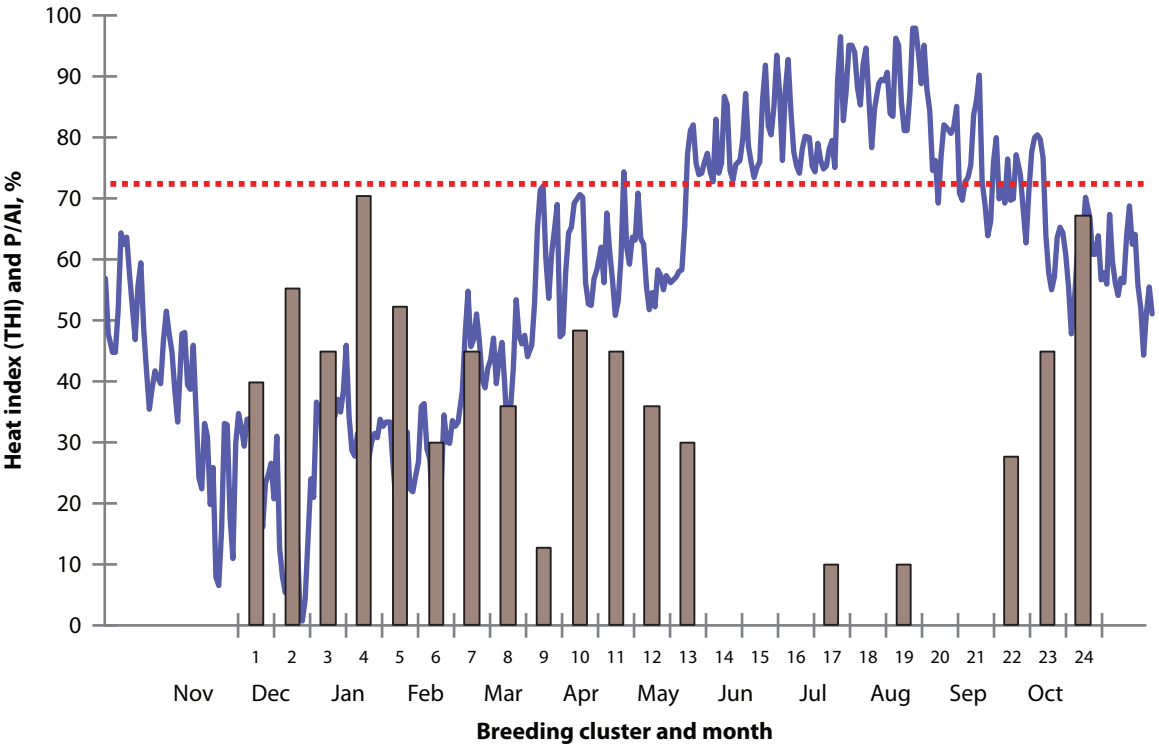

Figure 2. Pregnancies per artificial insemination (P/AI; bars).
P/AI assessed at day 32 post-timed AI in the 24 breeding clusters of cows inseminated during the experiment are superimposed over the average heat index (temperature-humidity index; continuous line) during the same period. The broken line (THI = 72) indicates where mild heat stress begins (mild = 72 to 79; moderate = 80 to 89; severe = 90 to 98; and danger > 98).

Evaluation of the 5- vs. 7-day CIDR Program in Dairy Heifers Before Timed Artificial Insemination

H. I. Mellieon, Jr., S. L. Pulley, G. C. Lamb[1], J. E. Larson[2], and J. S. Stevenson

Summary

Our objectives were to determine: (1) the effectiveness of an injection of $PGF_{2\alpha}$ to regress the corpus luteum before initiating an timed artificial insemination (**TAI**) program, (2) ovulation response to gonadotropin-releasing hormone (**GnRH**), and (3) pregnancy outcomes in dairy heifers inseminated with conventional and gender-biased semen. Heifers (n = 545) from 3 locations (Florida, Kansas, and Mississippi) were assigned randomly to 1 of 2 treatments: (1) 25-mg prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) injection and controlled internal drug release (**CIDR**) insert on day −7 followed by 100 μg of GnRH administered on day −5, and a 25-mg $PGF_{2\alpha}$ injection at CIDR insert removal (**7D**) on day 0; or (2) 100 μg of GnRH and insertion of previously used autoclaved CIDR on day −5 and a 25-mg $PGF_{2\alpha}$ injection at CIDR removal (**5D**) on day 0. Artificial insemination occurred after detected estrus from days 0 to 3. Those heifers not detected in estrus were inseminated on day 3 (72 hours after $PGF_{2\alpha}$) and given a second 100-μg dose of GnRH (72 hours after CIDR removal). Blood collected on days −7 and −5 was assayed to determine concentrations of progesterone and presence of a CL (progesterone ≥1 ng/mL) on d −7. Blood progesterone concentrations on days 0 and 3 were used to determine if luteolysis occurred in all heifers. Pregnancy was determined on days 32 and 60 and intervening pregnancy loss was calculated. Of those heifers in the 7D treatment having progesterone ≥1 ng/mL on day −7, the proportion having progesterone <1 ng/mL 2 days later (luteolysis) was greater ($P < 0.05$) than that in the 5D treatment (43.0 vs. 22.9%), respectively. A treatment by location interaction was detected for pregnancies per AI. The Kansas location had no detectable treatment differences. In contrast, the 7D treatment produced more ($P < 0.05$) pregnancies in the first replicate of the Florida location and at the Mississippi location. We concluded that the 5D protocol was not more effective in producing acceptable luteolysis, pregnancy, and ovulation rates compared with the modified 7D protocol.

Key words: CIDR, progesterone, timed artificial insemination (TAI)

Introduction

Since 1997, it has been known that dairy heifers do not respond as well as lactating dairy cows to gonadotropin-releasing hormone + prostaglandin $F_{2\alpha}$ (**GnRH + $PGF_{2\alpha}$**) protocols to synchronize estrus, ovulation, or both. For example, a multi-site study demonstrated that heifers treated after Ovsynch (GnRH injection 7 days before and 48 hours after $PGF_{2\alpha}$ with timed AI administered 72 hours after $PGF_{2\alpha}$) averaged 35% conception compared with non-treated heifers inseminated after estrus (74%).

A recent study in suckled beef cows was the first to reduce the interval from GnRH to $PGF_{2\alpha}$ from 7 to 5 days. The authors hypothesized that reducing the interval between injections would allow the maturing ovulatory follicle to develop during a longer proestrus in an environment with decreased progesterone. Pregnancies per AI (**P/AI**) in the 7-day program were 59% and increased to 70% in the 5-day program. Furthermore, differences between the 7- and 5-day

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programs in beef heifers in another study showed a tendency for greater P/AI in the 5-day treatment (62.5%) compared with the 7-day program (52%).

When GnRH induced ovulation of follicles in a 5-day program, the resulting corpus luteum (**CL**) may not undergo luteolysis when an injection of $PGF_{2\alpha}$ is administered 5 days after GnRH. When a similar 5-day controlled internal drug release (**CIDR**) program was administered in dairy heifers, however, 1 injection of $PGF_{2\alpha}$ yielded similar P/AI (46.4%) when compared with 2 injections of $PGF_{2\alpha}$ given 12 hours apart (48.6%).

The hypothesis for the current study was that the 5-day CO-Synch + CIDR protocol would increase P/AI in treated dairy heifers and synchronization of estrus and ovulation would serve as a useful management tool to facilitate timed artificial insemination (**TAI**) in heifers. The objectives were to: (1) gauge the effectiveness of an injection of $PGF_{2\alpha}$ to regress the corpus luteum before initiating a TAI program, (2) gauge ovulation response to GnRH, (3) determine the ovulation response to the first injection of GnRH in both treatments, and (4) assess pregnancy outcomes.

Experimental Procedures

This experiment was conducted at 3 locations: (1) Kansas State University Dairy Teaching and Research Center in Manhattan, (2) a commercial dairy farm in Marianna, FL, and (3) Mississippi State University Bearden Dairy Research Center in Starkville. Heifers no less than 355 days of age were assigned randomly to receive either a 5- or 7-day synchronization program, both of which incorporated an intravaginal CIDR (Pfizer Animal Health, New York, NY) insert (Figure 1). The CIDR inserts were used once previously for 7 days and were then cleaned and autoclaved.

On day −7 of the experiment, heifers in the 7-day treatment (**7D**) received an injection of 25 mg $PGF_{2\alpha}$ i.m. (5 mL Lutalyse, Pfizer Animal Health). On day −5, heifers received a 100-μg injection of GnRH i.m. (2 mL Factrel, Pfizer Animal Health). In the 5-day treatment (**5D**), heifers received the first GnRH injection at the time of CIDR insertion on day −5. The CIDR insert was removed from heifers in both treatments on day 0 concurrent with a 25-mg injection of $PGF_{2\alpha}$. Blood samples were collected from all heifers via coccygeal venipuncture before administration of injections on days −7, −5, 0, and 3 to measure concentration of progesterone.

Heifers were inseminated artificially with frozen-thawed semen based on either standing estrus from days 0 to 3 or at 72 hours post-CIDR removal (TAI). Heifers receiving the TAI also received injection of GnRH. Heifers inseminated based on standing estrus did not receive a second GnRH injection. Pregnancy was diagnosed 32 days later by transrectal ultrasonography based on the presence of uterine fluid or presence of a viable embryo. Pregnancy was reconfirmed approximately 4 weeks later.

Holstein heifers in Kansas enrolled in the experiment averaged 402 ± 18 days of age and 942 ± 82 lb body weight at enrollment. Heifers were fed a total mixed ration consisting of prairie hay, corn, soybean meal, corn silage, minerals, and vitamins, with water provided ad libitum. Feed was delivered to feed bunks twice daily and heifers were housed in dirt lots with a concrete apron next to the feed bunk. The experiment was conducted in 19 replicates (n = approximately 10 heifers/replicate) from October 2009 through January 2011. Some heifers detected as not

pregnant were re-enrolled in the same treatment up to 2 additional times. Heifers at this location were inseminated with gender-biased semen.

Jersey, Holstein, or Jersey × Holstein heifers in Florida were enrolled in the experiment. At enrollment, body condition scores (**BCS**; 1=thin, 5=fat) of heifers averaged 3.0 ± 0.3. The experiment was conducted in 3 replicates from January through April 2010. Heifers at this location were randomly assigned to receive either conventional or gender-biased semen. An additional group of Jersey, Holstein, or Jersey × Holstein heifers were treated in 3 replicates from December 2010 through March 2011. Heifers at this location were inseminated using conventional semen.

Holstein and Jersey heifers in Mississippi enrolled in the experiment averaged 476 ± 60 days of age and 746 ± 91 lb body weight. The experiment was conducted in 3 replicates from December 2010 through January 2011. Heifers at this location were inseminated using gender-biased semen.

Results and Discussion

Concentrations of serum progesterone on d –7, –5, 0, and 3 are represented in Figure 2. At the onset of treatments, progesterone concentrations did not differ on days –7 or –5. Of those heifers in the 7D treatment having progesterone ≥1 ng/mL on day –7, the proportion having progesterone <1 ng/mL 2 days later (luteolysis) was greater ($P < 0.05$) than that in the 5D treatment (43.0 vs. 22.9%, respectively).

The largest follicle detected in heifers on day –5 did not differ between the 7D and 5D treatments (12.7 ± 0.3 vs. 12.1 ± 0.3 mm), respectively, but more ($P < 0.001$) of the largest follicles ovulated in response to GnRH on day –5 in the 7D than the 5D treatment (47.2 vs. 27.6%). The second-largest follicle did not differ in diameter between the 7D and 5D treatments (10.0 ± 0.3 vs. 9.8 ± 0.3 mm), respectively, or in its ovulatory response to GnRH (16.1 vs. 10.9%). In contrast, total ovulatory response of the 2 largest follicles was greater ($P < 0.001$) in the 7D vs. 5D heifers (51.1 vs. 30.4%).

The 7D treatment had decreased ($P < 0.05$) progesterone concentrations on day 0 compared with the 5D treatment, but this difference did not persist through day 3 at TAI. Luteolysis in response to $PGF_{2\alpha}$ on day 0 indicated that luteolysis occurred in 90.1% of heifers in the 7D treatment and did not differ from that of 88.6% of heifers in the 5D treatment.

Pregnancies per AI determined 32 days post-AI are illustrated for the 3 locations in Figure 3. The Kansas location had no detectable treatment differences. In contrast, the 7D treatment produced greater ($P < 0.05$) pregnancy rates in the first replicate of the Florida location and at the Mississippi location (Figure 3).

With estrus detection being performed from d 0 until 72 hours after CIDR removal, 166 heifers (30.6%) were inseminated before TAI, and 39.2% of early-inseminated heifers became pregnant. The remainder of the 376 heifers submitted to TAI at 72 hours after CIDR removal and 26.2% became pregnant. The P/AI for heifers inseminated at estrus and for those receiving TAI differed ($P = 0.006$).

Pregnancies per AI in heifers having elevated (≥ 1 ng/mL) progesterone on day –7 were 36% (n = 345) and differed ($P < 0.05$) from those having decreased progesterone (< 1 ng/mL; 20%, n = 194). This difference also existed for heifers having elevated progesterone on day –5 (33%, n = 41 vs. 20%, n = 105). For heifers having decreased vs. increased progesterone at TAI, P/AI was increased ($P = 0.006$) from 12% (n = 51) to 32% (n = 460). Pregnancy loss calculated between 32 and 60 days post-AI was minimal (between 2.7 and 4.4%) and did not differ between treatments, although insufficient numbers of observations precluded detection of any differences.

We concluded that treatment of dairy heifers with the 5D Co-Synch + CIDR protocol failed to increase P/AI compared with the modified 7D protocol used in the current study. The P/AI were similar in one Florida and the Kansas locations, but favored the 7D treatment in the second Florida and Mississippi locations. With the majority of semen used in the study being gender-biased, P/AI were expected decrease as reported in other studies. The combination of synchronization and use of gender-biased semen are the probable cause of the reduced P/AI, because previous studies used conventional semen at TAI. The potential for increased pregnancy rates with the use of the 5D CIDR program has been shown in previous studies, but various protocols have produced mixed results. This variability indicates that further studies are required to identify a reliable TAI program for dairy heifers.

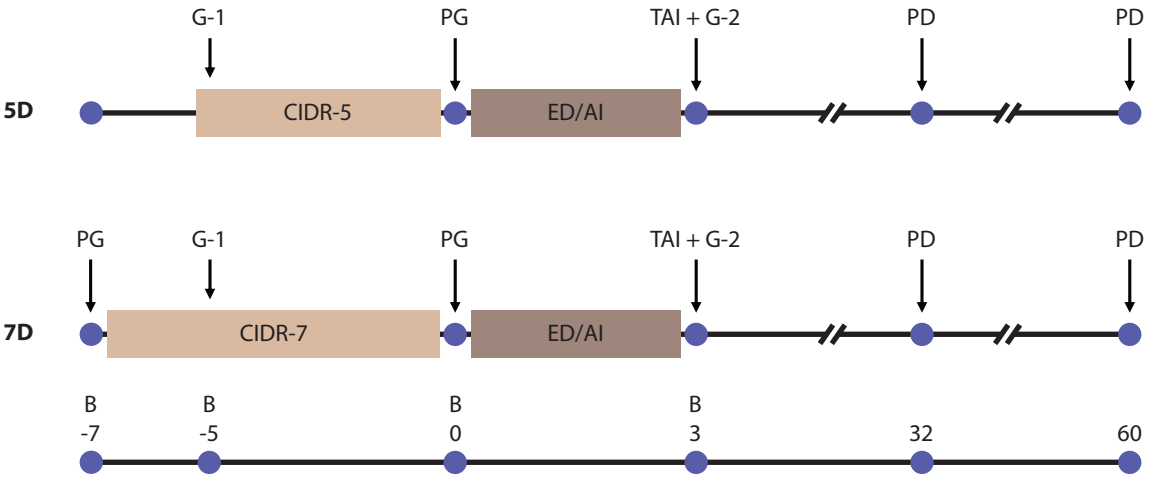

Figure 1. Experimental design of treatments.
Both treatments consisted of a gonadotroin-releasing hormone (GnRH; G1) injection at day -5 with $PGF_{2\alpha}$ injection on day 0. The 7D treatment also included a controlled internal drug release (CIDR) insert on day -7 concurrent with a prostaglandin $F_{2\alpha}$ (PG) injection, whereas the 5D treatment included a CIDR on day -5 at GnRH-1. Estrus detection (ED) and AI at detected estrus occurred from days 0 to 3. Those heifers not detected in estrus were time-inseminated (TAI) on day 3 (72 hours after PG) and concurrent with the second GnRH (GnRH-2) injection. Pregnancy was diagnosed on day 32 by ultrasound and heifers detected as not pregnant were re-treated up to 2 times on the same treatment in some cases. A second pregnancy diagnosis was conducted 4 weeks later to calculate pregnancy loss since the first positive pregnancy diagnosis. GnRH = 100 μg of GnRH, PG = 25 mg of $PGF_{2\alpha}$, US = transrectal ultrasonography, and B = blood collection.

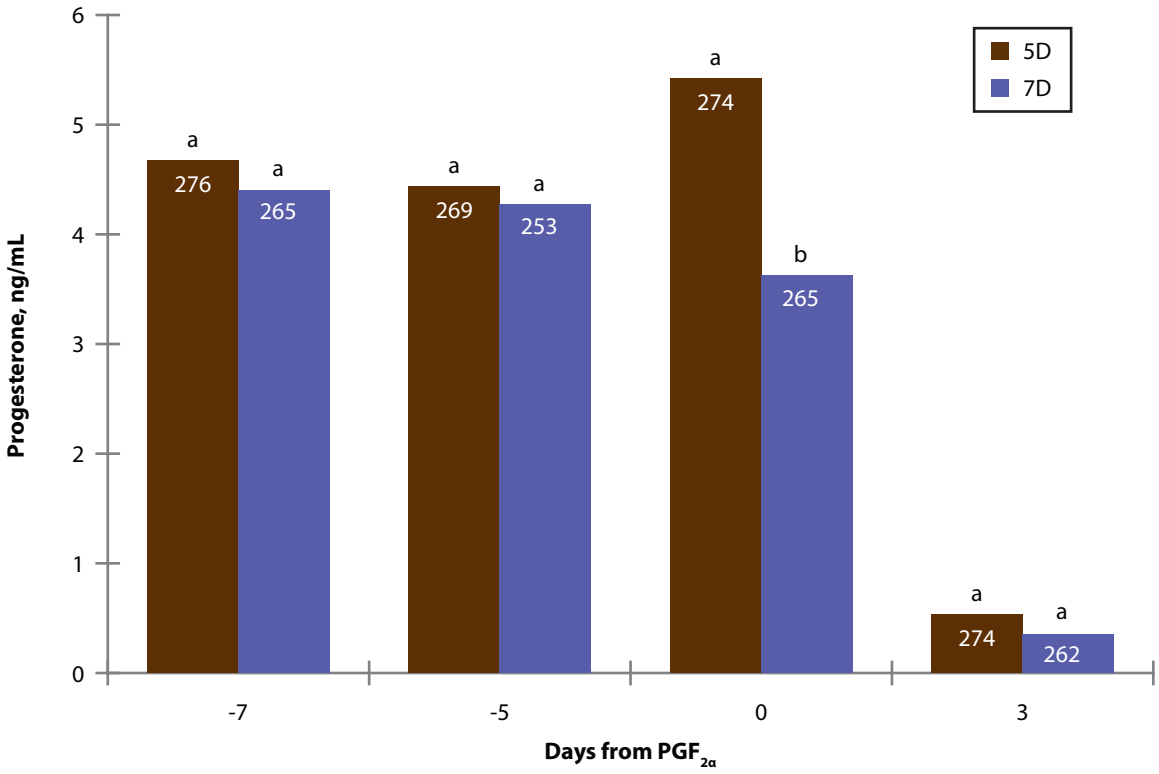

Figure 2. Concentrations of serum progesterone in heifers from day of common $PGF_{2\alpha}$ treatment (day 0) in the 5D and 7D treatments.

[a-b] Means within experimental day having different letters differ ($P < 0.05$).

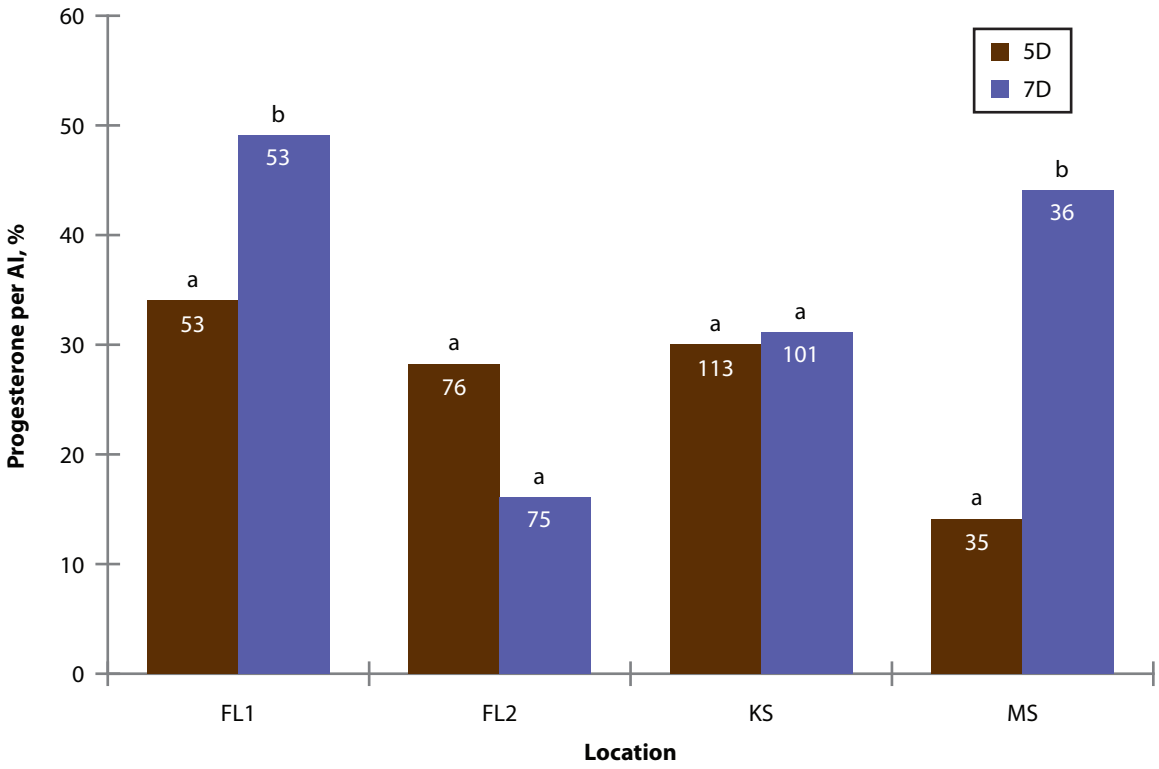

Figure 3. Pregnancy rates of heifers at 32 days post-AI.

[a-b] Means within location having different letters differ ($P < 0.05$).

Enhancing Cysteine Content in Yogurt

S. Bala and K. A. Schmidt

Summary

Cysteine is considered a conditional amino acid for certain subpopulations. For example, in elderly people, cysteine has been associated with diverse functional properties as a general antioxidant as well as a specific role linked to cataract reduction or prevention. Yogurt is an excellent source of protein, the sulfur-containing amino acids methionine and cysteine in particular. Heat, however, can denature these amino acids and affect their bioavailability. A yogurt mix supplemented with whey proteins (an abundant source of cystiene) coupled with minimal pasteurization of yogurt mixes may increase the availability of cysteine in the final product.

In this study, yogurt mixes were supplemented with nonfat dry milk (**NDM**) or whey protein isolate (**WPI**; 90% protein), processed at 90°C for 7 minutes or 70°C for 20 minutes, then fermented into yogurt following a conventional procedure. Supplementing yogurt mix with WPI vs. NDM increased cysteine content by 140%. In contrast, overall cysteine contents decreased by 17% in mixes treated at 70°C for 20 minutes and 35% in mixes treated at 90°C for 7 minutes. Mixes supplemented with WPI and treated at 70°C for 20 minutes produced yogurts that had greater cysteine contents and slightly greater firmness and water-holding capacity, but the yogurts exhibited less syneresis compared with those made from mixes supplemented with NDM and treated at 90°C for 7 minutes. These results indicate that yogurt may be an excellent delivery vehicle for the conditional amino acid, cysteine.

Key words: yogurt, cysteine, heat treatment

Introduction

In 2009, USDA reported that yogurt popularity and sales had increased by 79% since 2000. Yogurt is an excellent source of whey proteins, but typical heat treatment of commercial yogurt mixes denatures about 90% of the whey proteins, resulting in decreased dietary availability of sulfur-containing amino acids, cysteine and methionine. Specifically, heat causes whey proteins to unfold, exposing free sulphydryl groups that initiate disulfide bonds with other sulfur groups. A high-quality yogurt can be described as firm, exhibiting low syneresis (release of a liquid from the gel that typically collects on the surface), and having a pleasant acid taste. The firmness and wheying off are directly related to protein content as well as whey protein denaturation. In addition, acid can denature milk proteins, especially the pH change that occurs during yogurt fermentation (6.4 to 4.6) and yogurt storage (4.6 to 4.2), but an amount of whey protein denaturation has not been reported.

The importance of dietary cysteine has been studied in rats and humans. Elderly rats (24 to 26 months) had less or no gamma-cystathinase, an enzyme in their eye lenses, compared with young rats (5 to 6 months). The decreased gamma-cystathinase activity was associated with a 50% decrease in cysteine and glutathione contents. In cells, cysteine is one of the components used to synthesize glutathione. Decreased glutathione content in human eye lenses has been associated with cataract formation; hence, if the substrate cysteine is decreased or depleted, the production of glutathione is decreased, too. According to the Centers for Disease Control and Prevention, an estimated 20.5 million (17.2%) U.S. citizens who are 40 years old or older have

a cataract in 1 or both eyes, and the total number of people who will have cataracts by 2020 are estimated at 30.1 million. For humans, these data indicate that certain subpopulations may benefit from increased cysteine in their diets.

When manufacturing yogurt, a common practice is to boost nonfat milk solids (from 9 to 14%) by adding nonfat milk solids, typically nonfat dry milk (**NDM**), which in turn increases protein content from 3.24 to 5.22 g/100 mL. To produce a high-quality product (minimal syneresis, firm gel, and maximum water-holding capacity), most yogurt processors manufacture mixes to induce approximately 90% whey protein denaturation by heating the mix to approximately 90°C for 2 minutes or more. Researchers previously showed that yogurt supplemented with whey protein concentrate (**WPC**) rather than NDM had increased whey protein content (0.75 to 2.07 g), firmness (15.10 to 32.44 g), protein network hydration (2.44 vs. 2.47 g water/g solids), and water retention (72.7 vs. 88.4%), but cysteine contents were not reported. When contrasting whey protein isolate (**WPI**) with WPC, WPI has about 3 times more protein, allowing WPI to be an excellent source of the sulfur-containing amino acids. Thus, a research strategy combining WPI supplementation with low process treatments may result in a yogurt with enhanced cysteine content and acceptable quality. The specific objectives were to: (1) determine cysteine contents in yogurt made with different supplements (WPI vs. NDM) and (2) assess changes in cysteine content as functions of heat treatment and fermentation and evaluate resultant yogurt quality.

Experimental Procedures

Low-heat NDM, WPI, and yogurt cultures were obtained from commercial suppliers and maintained at −2 or −10°C (culture) until usage. To simulate a typical commercial yogurt (based on milk solids), mixes were formulated as shown in Table 1 and the calculated cysteine content was based on NDM and WPI protein content. Dairy powders were rehydrated in deionized, distilled water at 22 to 24°C for 30 minutes, then subdivided and treated at 70°C for 20 minutes or 90°C for 7 minutes, cooled to 43°C, inoculated with *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus,* packaged into sterile 120-ml cups, and incubated until pH 4.6. Cups were removed from the incubator and cooled by placing in storage (4 ± 1°C) until the following day.

Two replications were made, data were analyzed, and significant means and interactions were differentiated. Cysteine contents and pH were assessed before heat treatment, after heat treatment, and after 1 day of yogurt storage. Cysteine content was analyzed using Ellman's reagent. Yogurt mixes and/or samples were analyzed for total solids, pH, syneresis, water-holding capacity, and firmness using standard published methods.

Results and Discussion

Table 2 shows the cysteine content in the mixes and yogurt samples as functions of heat treatment and fermentation. Supplementation with WPI vs. NDM resulted in a 142% increase in cysteine content (comparing the non-heated mixes), and these values agreed with the predicted calculated values reported in Table 1. As expected, the statistical analyses showed that heat treatment significantly affected cysteine content. On average, mixes treated at 70°C for 20 minutes had a 17% loss in cysteine, whereas those treated at 90°C for 7 minutes had a 37% loss. Supplementation also affected the cysteine content, and the mixes containing WPI had greater cysteine contents compared with mixes without WPI. More surprising, however, was that cysteine contents were not affected by the fermentation process (in either mix or heat treatment)

because the decrease in cysteine contents from heated mix to fermented yogurt was on average approximately 1.5 mg cysteine/L. These data indicate that if cysteine is available at the start of fermentation, it will be available 1 day after storage.

Overall, milk proteins are good buffering agents. The WPI is an abundant source of protein, but a poor source of lactose, which is necessary for the fermentation process. Because the mixes containing WPI have greater protein and less lactose (data not shown), mix pH, mix total solids, and fermentation times also were monitored. All mixes had similar total solids content, approximately 12.4%, which indicates consistency in formulation. When analyzing the pH data, an interaction was observed between the heat treatment and formulation. As shown in Table 3, the pH of mixes containing WPI was greater than mixes without WPI, but the pH of yogurts containing WPI was less than yogurts without WPI. On the other hand, fermentation time was a function of heat treatment because mixes treated at 70°C for 20 minutes required 30 minutes more to ferment than mixes treated at 90°C for 7 minutes, which needed approximately 5.5 hours. The greater heat treatment may not only initiate some component degradation, facilitating culture growth, but also should reduce microbial counts and destroy enzymes from the initial milk sources that might compete or interfere with the yogurt culture.

To make an effective delivery vehicle for a compound such as cysteine, the product needs to be acceptable to the consumer. To evaluate quality, 3 physical properties were measured — water-holding capacity, firmness, and syneresis — all of which affect consumer preference and are directly related to protein content and whey protein denaturation. In this study, mixes without WPI treated at 90°C for 7 minutes and resultant yogurts were most similar to a commercial plain, set-style yogurt; hence, it was considered a control sample. All these properties had significant interactions and the means are shown in Table 4. Mixes containing WPI treated at 90°C for 7 minutes produced yogurts that were approximately 8 times more firm, held approximately 4 times more water, and exhibited almost no syneresis compared with the control yogurt. But mixes containing WPI treated at 70°C for 20 minutes produced yogurts that were 1.4 times as firm and exhibited slightly greater water-holding capacity and less syneresis than the control yogurt. More critically, the cysteine content of the yogurt containing WPI was 4.5 times greater than that of the control. These results indicate that WPI supplementation of yogurt mix, combined with a more minimal heat treatment may result in a yogurt that is an effective delivery vehicle for cysteine.

Conclusions

In yogurt, cysteine content is a function of type and amount of milk protein and heat treatment. Mixes supplemented with WPI and treated at 70°C for 20 minutes produced yogurts with the greatest cysteine content, more water-holding capacity and firmness, and less syneresis compared with the control yogurt. Syneresis and firmness are related to the number of whey protein-casein interactions, which are induced by denaturation. In this experiment, the yogurt made from mixes supplemented with WPI and treated at 90°C for 7 minutes had almost no syneresis and great firmness compared with the other yogurts, perhaps to the point that consumers would question whether the product is a yogurt. To pursue this concept, further research monitoring whey protein denaturation and shelf life is needed.

Table 1. Yogurt mix formulations, protein content, and predicted cysteine content

	Formula		Composition	
Mix	Nonfat dry milk (%)	Whey protein isolate (%)	Protein (g/1000 mL)	Predicted cysteine content (mg/1000 mL)
Nonfat dry milk solids	12.5	0.0	45.0	350
Nonfat dry milk solids + whey protein isolate	9.0	3.5	70.6	750

Table 2. Mean cysteine contents of yogurt mixes, unheated or heated to 70°C for 20 minutes or 90°C for 7 minutes and yogurt (1 day old)

	Mix		
Item	NDM solids[1]	NDM + WPI[2]	Average
	Cysteine content (mg/1000 mL)		
Unheated	301.3 ± 5.6	729.8 ± 11.8	515.3[a] ± 8.1
70°C for 20 min	240.1 ± 5.0	631.8 ± 14.6	435.8[b] ±9.8
90°C for 7 min	141.4 ± 10.2	546.8 ± 16.8	343.8[c] ± 13.5
Yogurts 1 day old			
70°C for 20 min	236.0 ± 4.4	630.7 ± 13.9	433.1[b] ± 9.1
90°C for 7 min	140.4 ± 9.1	545.2 ± 17.5	342.5[c] ± 13.3
Average	211.6[b] ±6.76	616.6[a] ± 14.9	

[a-c] Means within item or between mixes with different superscript letters differ ($P < 0.05$).
[1] Nonfat dry milk (12.5%).
[2] Nonfat dry milk (9%) and whey protein isolate (3.5%).

Table 3. Mean pH of yogurt mixes, unheated or heated to 70°C for 20 minutes or 90°C for 7 minutes and yogurt (1 day old)

	Mix	
Item	NDM[1]	NDM solids + WPI[2]
	pH	
Unheated	6.57[bc] ± 0.02	6.61[a] ± 0.01
70°C for 20 min	6.56[c] ± 0.02	6.61[a] ± 0.02
90°C for 7 min	6.55[c] ± 0.01	6.56[c] ± 0.02
Yogurts 1 day old		
70°C for 20 min	4.55[d] ± 0.01	4.49[e] ± 0.02
90°C for 7 min	4.53[d] ± 0.01	4.46[e] ± 0.01

[a-e] Means with different superscript letters differ ($P < 0.05$).
[1] Nonfat dry milk (12.5%).
[2] Nonfat dry milk (9%) and whey protein isolate (3.5%).

Table 4. Mean physical properties of yogurt with different formulation and heat treatment

	Mix			
	NDM solids[1]		NDM solids + WPI[2]	
Properties	70°C for 20 minutes	90°C for 7 minutes	70°C for 20 minutes	90°C for 7 minutes
Syneresis (%wt/wt)	$8.10^{a}\pm0.12$	$2.17^{b}\pm0.03$	$1.66^{c}\pm0.20$	$0.47^{d}\pm0.06$
Firmness (g)	$20.55^{d}\pm0.63$	$51.42^{c}\pm0.97$	$70.86^{b}\pm0.65$	$413.80^{a}\pm8.0$
WHC[3] (%wt/wt)	$16.95^{d}\pm0.25$	$20.52^{c}\pm0.66$	$23.84^{b}\pm0.23$	$62.71^{a}\pm0.56$
pH	$4.55^{a}\pm0.01$	$4.53^{a}\pm0.01$	$4.49^{b}\pm0.02$	$4.46^{b}\pm0.01$

[a,b,c,d] Means within rows with a different superscript letter differ ($P < 0.05$).
[1] Nonfat dry milk (12.5%).
[2] Nonfat dry milk (9%) and whey protein isolate (3.5%).
[3] Water-holding capacity.

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