Dairy Team
Kansas State University

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DAIRY RESEARCH 2012

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Foreword

Members of the Dairy Team at Kansas State University are pleased to present the 2012 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. At the end of 2011, Kansas ranked 12th nationally in milk yield per cow at 21,057 lb, 16th in the number of dairy cows (123,000), and 15th in total milk production (2.59 billion lb). During the past five years (2006 to 2011), total milk production in Kansas has increased by 10.4%, the number of cows has increased by 9.8%, and pounds of milk per cow have increased by 119. At the end of 2012, Kansas has just more than 300 dairy operations and approximately 332 cows per herd.

Selected production traits of our Kansas State University Dairy Teaching and Research Center (DTRC) herd are shown below. During the past few days in mid-November 2012, our cows have averaged more than 101 lb of milk per cow per day in a facility that was built and populated in 1977. The excellent functioning of the DTRC is a tribute to the dedication of our staff: Michael Scheffel (manager), Daniel Umsheid, Robert Feist, Pam Milleret, Alan Hubbard, Kris Frey, and Eulises Jiron Corrales. Special thanks are given to Colleen Hill, Cheryl Armendariz, and a host of graduate and undergraduate students for their technical assistance in our laboratories and at the DTRC. We also acknowledge the support and cooperation of the Heart of America Dairy Herd Improvement Association (DHIA) laboratory here in Manhattan, KS, for its assistance in handling research milk samples.

Kansas State University Dairy Teaching and Research Center Cows

Item	Units[1]
Cows, no.	249
Rolling herd milk, lb	29,821
Rolling herd fat, lb	1,073
Rolling herd protein, lb	905
Somatic cell count × 1,000	155
Calving interval, mo.	14.1

[1] October 9, 2012, test day (milking 3 times daily; no bST).

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.

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
2012 Dairy Research Report of Progress

Biological Variability and Chances of Error

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

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

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

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

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

Meta-Analysis of the Effects of Dietary Sugar on Intake and Productivity of Dairy Cattle

C. F. Vargas, C. D. Reinhardt, J. L. Firkins,[1] B. J. Bradford

Summary

A meta-analysis was performed to determine the possible effects of dietary sugar on feed intake and milk production in lactating dairy cattle. The database used in this analysis included 18 treatment comparisons frozm 10 studies reported from 1985 through 2011. Treatment comparisons were used only if: (1) either sucrose (9 comparisons) or molasses (9 comparisons) replaced corn grain without adding fat; and (2) sugar added by treatment ranged from 2 to 5% of dry matter. First, responses to sucrose and molasses were compared to assess whether these sugar sources could be considered together. Statistical analysis provided no evidence for different responses across sugar sources for dry matter intake (**DMI**), milk yield, energy-corrected milk (**ECM**) yield, milk fat content, or milk protein content. Different sugar sources were pooled for the remaining analyses; the combined data showed that adding sugar tended to increase DMI by 0.84 lb/day and milk fat content by 0.085%. No effects were detected for milk yield, ECM yield, or milk protein content. This analysis indicates that adding 2 to 5% dietary sugar may promote small increases in DMI and milk fat content but does not consistently increase ECM yield in lactating dairy cattle.

Key words: molasses, sucrose, lactation, dry matter intake (DMI)

Introduction

High demands for energy — especially to maintain profitable milk production levels in lactating dairy cows — are mostly covered by the dietary inclusion of highly fermentable carbohydrates. Starch has traditionally filled this role in most lactation diets, often comprising 25 to 30% of diet dry matter (**DM**). The rapid fermentation of starch can decrease ruminal pH, which can negatively affect the growth of fiber-digesting bacteria and fiber degradation. As a result, excessive starch concentrations can suppress dry matter intake (**DMI**) and lead to milk fat depression.

To lessen the negative effects of high starch concentrations and reduce costs, feedstuffs with sugar as the main soluble carbohydrate (i.e., molasses, whey, and citrus pulp) have been included in diets for dairy cattle. Sugar, like starch, is rapidly fermented in the rumen, providing large amounts of rapidly available energy for microbial protein production. Surprisingly, recent evidence indicates that sugar does not acidify the rumen like dietary starch does. One reason may be that sugar inclusion supports the growth of bacteria that utilize lactic acid, providing a population of microbes that can limit this potent acid from accumulating. By increasing ruminal pH, dietary sugar may provide a better environment for fiber-digesting bacteria, resulting in greater fiber digestibility.

Although sugar sources have been used in diets for lactating dairy cattle for many years, the value of such ingredients is still controversial. One way to address such topics is by utilizing meta-analysis. This statistical methodology allows the results of many different studies to be considered together while accounting for different sample sizes. This approach was used to

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[1] The Ohio State University.

assess how dietary sugar affects DMI, milk production, and composition responses in lactating dairy cows.

Experimental Procedures

The information used for this meta-analysis included treatments from studies reported from 1985 through 2011. Numerous databases (including CAB abstracts, Web of Science, Agricola, Agris, CRIS, PubMed, Science Direct, S-PAC, and Google Scholar) were searched for applicable studies. Treatments were included in the analysis only if the added sugar ranged between 2% and 5% of DM. Only studies that directly tested responses to molasses or sucrose replacing corn grain in lactating cows were evaluated. If the sugar source contained any additional source of fat or urea that was not matched in the control diet, the treatment was excluded from the meta-analysis to avoid possible confounding effects.

A total of 10 published studies were found with at least one treatment comparison that met the criteria. An additional two unpublished studies from Kansas State University were included in the database, providing 18 total treatment comparisons. Both published and unpublished data were included to reduce publication bias.

Cochran's Q statistic was used to test for differential responses to sucrose vs. molasses. The meta-analysis was conducted using a random effects model. In addition to determining the effects of dietary sugar on production outcomes, experimental diets were characterized to test for dietary conditions that may influence the response to sugar. The following dietary concentrations were determined for each treatment: crude fat, neutral detergent fiber (**NDF**), forage NDF (**fNDF**), starch, crude protein, rumen-undegradable protein, and rumen-degradable protein. The amount of sugar added by treatment was also included in this list of predictive variables. Effects of dietary conditions were tested using multiple regression analysis.

Results and Discussion

Because the analysis for differential responses between molasses and sucrose detected no difference in DMI, milk yield, energy-corrected milk (**ECM**) yield, milk fat content, or milk protein content, molasses and sucrose treatments were combined for all subsequent analyses.

Results of the meta-analysis are presented in Table 1. According to the combined results of the 18 treatment comparisons included in this study, supplemental sugar tended to increase DMI by 0.84 lb/day, with a 95% likelihood that the true mean response lies between a decrease of 0.08 lb/day and an increase of 1.77 lb/day. Despite the finding that DMI tends to increase with sugar supplementation, there was no evidence that dietary sugar increased milk production, protein content, or ECM production. The meta-analysis did identify a tendency for supplemental sugar to increase milk fat content by 0.085 percentage units. This finding is consistent with recent reports suggesting that sugar can help mitigate milk fat depression.

The initial analysis grouped all 18 comparisons without respect to the amount of sugar added by treatment or the basal composition of the diet used in each study. Subsequently, multiple regression techniques were used determine if responses to supplemental sugar depended on any of these predictive variables. None of the factors tested were significant predictors of milk yield, ECM yield, or milk protein content responses to dietary sugar. For DMI, two variables significantly affected the response to supplemental sugar: the fNDF content of the basal diet and the amount of sugar added to the diet. Additionally, these two factors interacted: the response to

the amount of sugar added depended on the fNDF content of the diet. This complex relationship is represented in part by Figure 1, which indicates that in a diet with low fNDF content (i.e., a low-forage diet), adding progressively more sugar (from 2% up to 5% of diet dry matter) decreases the DMI response. Conversely, adding progressively more sugar to a diet with high fNDF content increases the DMI response.

The idea that adding more sugar may increase DMI more in higher-forage diets is consistent with some of the current thinking about sugar effects in the rumen. In rations with high fNDF content, including sugar may provide more available energy to the fiber-digesting bacteria because they do not compete effectively with starch-digesting bacteria for access to energy from starch. Increasing populations of fiber-digesting bacteria should allow them to colonize the fiber substrate faster, increasing the rate of fiber degradation and freeing up space in the rumen for additional feed intake.

In summary, a combined assessment of sugar responses across 18 treatment comparisons failed to demonstrate clear production effects when sugar was added at 2 to 5% of diet DM. Interactions between fNDF content of diets and the amount of sugar added by treatments suggest that variable responses to dietary sugar may result from the wide range of diets involved in these treatments. Further research directly testing the differential effects of sugar supplementation in high-fNDF vs. low-fNDF diets may help clarify the importance of considering which diets benefit the most from additional sugar sources.

Table 1. Production responses to replacing corn grain with 2 to 5% dietary sugar as determined by meta-analysis

| Item	Change	95% confidence interval	P-value
Dry matter intake, lb/day	+0.84	-0.08, +1.77	0.08
Milk yield, lb/day	+0.24	-1.15, +1.64	0.73
Energy-corrected milk, lb/day	+0.62	-0.64, +1.87	0.33
Fat, %	+0.085	-0.005, +0.175	0.06
Protein, %	+0.009	-0.041, +0.058	0.72
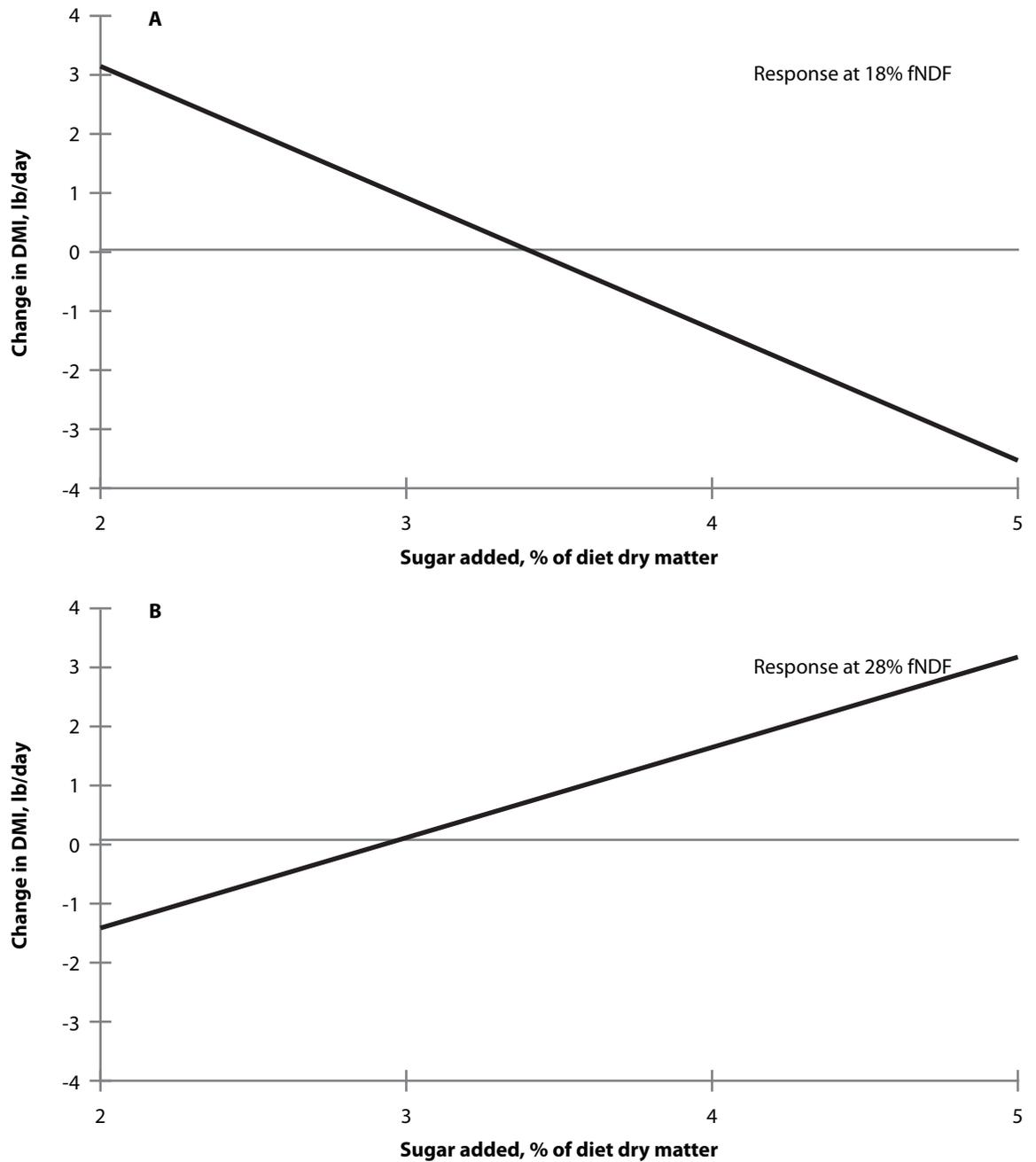
Figure 1. Interaction of added sugar and forage neutral detergent fiber (fNDF) influences dry matter intake (DMI) responses to sugar supplementation.
The interaction of factors is represented by plotting responses to the amount of added sugar at the lowest (18%) and highest (28%) fNDF levels used in diets in the database of studies.

Sodium Salicylate during the First 7 Days of Lactation Affects the Entire Lactation

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 if a non-steroidal anti-inflammatory drug, sodium salicylate (**SS**), benefits transition cows. At calving, 78 cows [primiparous (**1P**) n = 39; 2nd lactation (**2P**) n = 24; ≥3 lactations (**3P**) n = 15] were assigned alternately to either a control or SS treatment for 7 days and production responses were evaluated through the entire lactation. Treatment was administered via individual water bowls, delivering a mean of 123 ± 5.5 (mean ± standard deviation) grams salicylate per day during the 7 days of treatment. Cows were followed throughout the lactation by monthly milk yield and component testing, and the effects of treatment on the risk of leaving the herd and on normalized 305-day milk, fat, and protein yields were determined by Fisher's exact test and mixed model analysis, respectively. Treatment influenced both 305-day milk and fat yields differently across parities. Milk yield was increased by 17% in 3P SS cows (4,374 ± 1,549 lb greater for 3P SS cows). Primiparous SS cows tended to produce 2,155 ± 824 lb less 305-day milk than control cows; no differences were detected for 2P cows. Furthermore, 3P SS cows produced 285 ± 50 lb more 305-day milk fat and tended to produce 108 ± 40 lb more 305-day milk protein. No effects were detected in 1P or 2P cows. A treatment by parity interaction was observed for the risk of leaving the herd where 1P cows treated with SS tended to have a greater risk of leaving the herd than controls (30% vs. 6% risk). Treatment did not alter herd retention in 2P or 3P groups, and SS had no effect on the risk of leaving the herd overall. Results indicate that SS has long-term effects on lactation characteristics of aged cows, particularly on fat metabolism, but has potential negative effects for primiparous cows.

Key words: lactation, postpartum health, sodium salicylate

Introduction

The transition period is a time of metabolic problems for dairy cattle, which causes 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. It has been hypothesized that inflammation also plays a role in the development of metabolic disorders such as fatty liver and ketosis. Non-steroidal anti-inflammatory drugs (**NSAID**) 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 in metabolic disorders.

We recently conducted a study to evaluate responses to oral delivery of the NSAID sodium salicylate (**SS**; the parent compound for aspirin) during the first week of lactation. Intensive measurements were conducted during the first 21 days of lactation for this trial, during which we observed an increase in milk yield and incidence of metritis in older cows as well as an increase in energy-corrected milk and milk fat yield by week 3 across all cows treated with SS.

The purpose of this investigation was to determine if oral SS treatment during the first 7 days in milk (**DIM**) has sustained impacts through the entire lactation.

Experimental Procedures

Seventy-eight Holstein cows [primiparous (**1P**), n=39; 2nd lactation (**2P**), n=24; ≥3rd lactation cows (**3P**,) n=15] from the Kansas State University Dairy Teaching and Research Center were used in the trial. Cows were assigned to two treatments on the day of parturition. Cows that had lameness issues or milk fever before the initiation of treatment were excluded. Treatments were balanced for parity and consisted of 7-day control or SS treatments administered through individual water bowls. Cows were housed in tie-stall facilities, 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.). On the eighth day after calving, all cows were provided regular water for the remainder of lactation. Herd management data and DHIA production records were used for a retrospective analysis of whole-lactation production and risk of leaving the herd. Cows that did not remain in the herd for a subsequent calving were considered to have left the herd, and the reason entered was recorded. Predicted 305-day yields of milk, fat, and protein generated by DHIA were used for all cows for which a value was generated. Predictions were not available for 3 cows that left the herd prior to 95 DIM. To test for randomization bias in milk production, treatment effects on predicted transmitting ability (**PTA**) for milk, fat, and protein yield were tested, and no significant differences between treatments were observed (all $P < 0.50$). An additional potential confounding factor was the enrollment of 27 of the cows (14 control and 13 SS cows) in a nutrition study following the completion of the salicylate study. The study was a Latin square design with treatments that failed to influence any measured outcome. Therefore, the simple effect of enrollment in the study was included in the model. Statistical analysis of 305-day milk, fat, and protein yields were carried out using JMP (version 8.0) to estimate the fixed effects of treatment, parity, treatment by parity interaction, and subsequent study enrollment. The PTA for the variable of interest was included as a covariate to account for genetic contributions to variance.

Results and Discussion

No overall treatment effect ($P = 0.16$) was observed for the 305-day milk yield, but an interaction of treatment and parity ($P < 0.01$, Table 1) was detected. Salicylate administration increased ($P = 0.05$) 305-day milk yield in 3P cows by 4,384 ± 1,552 lb but decreased it in 1P cows by 2,155 ± 824 lb (Figure 1). Estimated 305-day milk fat yield was increased by 13% in SS cows ($P < 0.001$; Table 1). A treatment by parity interaction was observed, where 3P SS cows produced 318 ± 55 lb more fat during the entire lactation than 3P controls (34% increase, $P < 0.001$; Figure 2). The 305-day protein yield was not affected by treatment ($P > 0.50$), but a treatment by parity interaction was detected in which 3P SS cows tended ($P = 0.06$) to have greater protein yield than 3P CON (14% increase; Figure 3).

A treatment by parity interaction was observed ($P < 0.05$) for risk of leaving the herd; 1P SS cows tended ($P < 0.10$) to have a greater risk of leaving the herd than 1P controls (6% risk vs. 30% risk, Table 2). No differences were observed between treatments in 2P and 3P groups. The specific causes of removal from the herd also were statistically evaluated, but treatment did not impact any of these conditions. The most common reasons that cows left the herd were reproductive issues and chronic mastitis (Table 2).

Milk and fat yields were increased in cows treated with sodium salicylate, with the greatest differences observed in third or greater lactation cows. The divergence in milk yield observed in

3P cows at the end of the third week of lactation was confirmed to affect the entire lactation. Others who have administered aspirin to transition dairy cows reported increases in milk yield around peak lactation compared with controls. Conversely, flunixin meglumine administered for 3 days postpartum did not affect milk yield, suggesting that the type of NSAID administered is critical in determining milk production responses. Overall, SS administration during the first week of lactation seems to increase production and potentially profit in ≥3rd lactation cows, but it is not a beneficial management practice for primiparous cows, in which SS tended to decrease milk production and increase cows' risk of being removed from the herd.

It is important to note that SS is not approved for commercial use for the purposes as described in this paper. In addition, although veterinary forms of aspirin are marketed with label indications for pain, fever, and inflammation in the U.S., the FDA has not formally approved this drug; consequently, withdrawal times have not been established for food-producing animals. These results should be viewed as establishing a critical role of inflammatory pathways during early lactation, with effects that endure throughout the lactation. Future research may reveal broadly applicable techniques to put this new knowledge to work.

Table 1. Estimated 305-day yields of milk, fat, and protein

Variable	Control	Sodium salicylate	SEM	Treatment	Parity	Treatment × parity
					P-value	
Milk yield (× 1,000 lb)	26.0	27.3	6.6	0.16	<0.01	<0.01
Fat yield (lb)	901.8	1023.1	24.3	<0.01	<0.01	<0.01
Protein yield (lb)	774.0	807.0	19.9	0.23	<0.01	0.08

Table 2. Reasons cows left herd[1]

Reason	First Control	First SS[2]	Second Control	Second SS	Third or greater Control	Third or greater SS	Total
Foot or leg	....	....	....	....	1	....	1
Low production	....	1	1	....	....	....	2
Reproduction	1	3	1	2	1	1	9
Injury	....	....	....	....	....	1	1
Death	....	....	1	1	1	....	3
Mastitis	....	2	2	2	1	....	7
Udder	....	....	1	....	....	....	1
Total	1	6	6	5	4	2	24

[1]All values are numerical counts based on parity and treatment for each specific reason cows were removed from the herd.
[2] SS: sodium salicylate treatment.
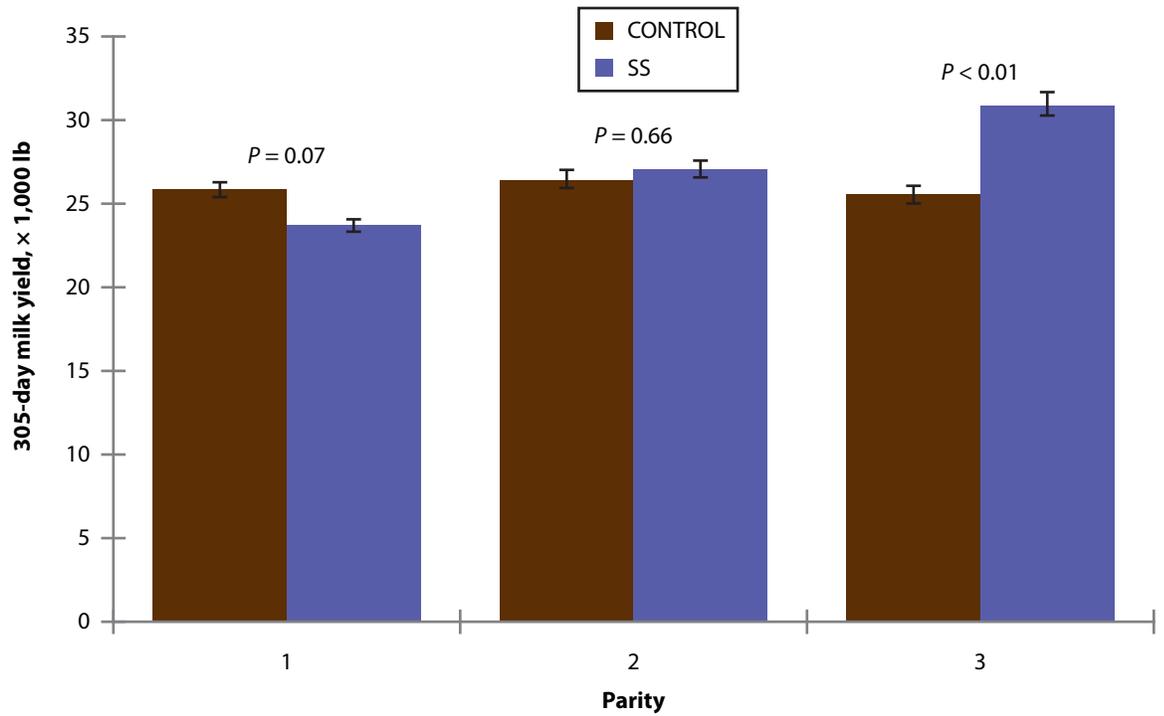
Figure 1. A treatment × parity interaction (*P* < 0.01) was detected for milk yield with third-parity sodium salicylate (SS) cows having greater milk yield and a tendency for first-parity SS cows to have a lesser milk yield. No difference was detected for second-parity cows.
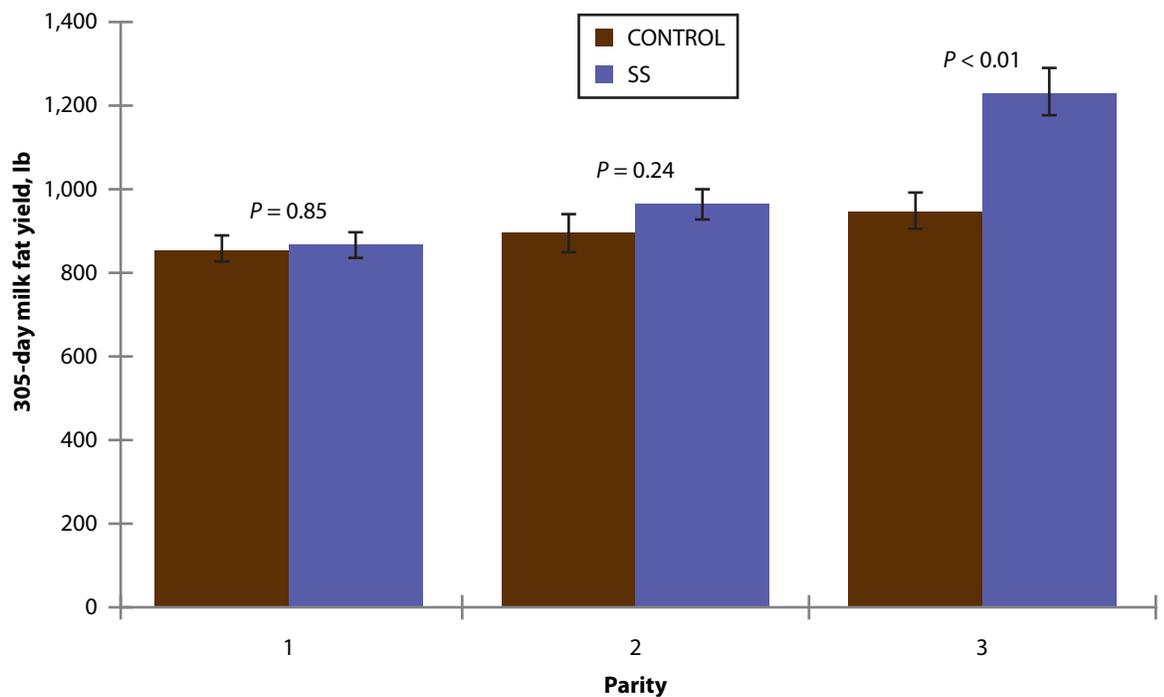

Figure 2. A treatment × parity interaction (*P* < 0.01) was observed for 305-day milk fat yield, reflecting greater milk fat yield in third-parity cows treated with sodium salicylate (SS).
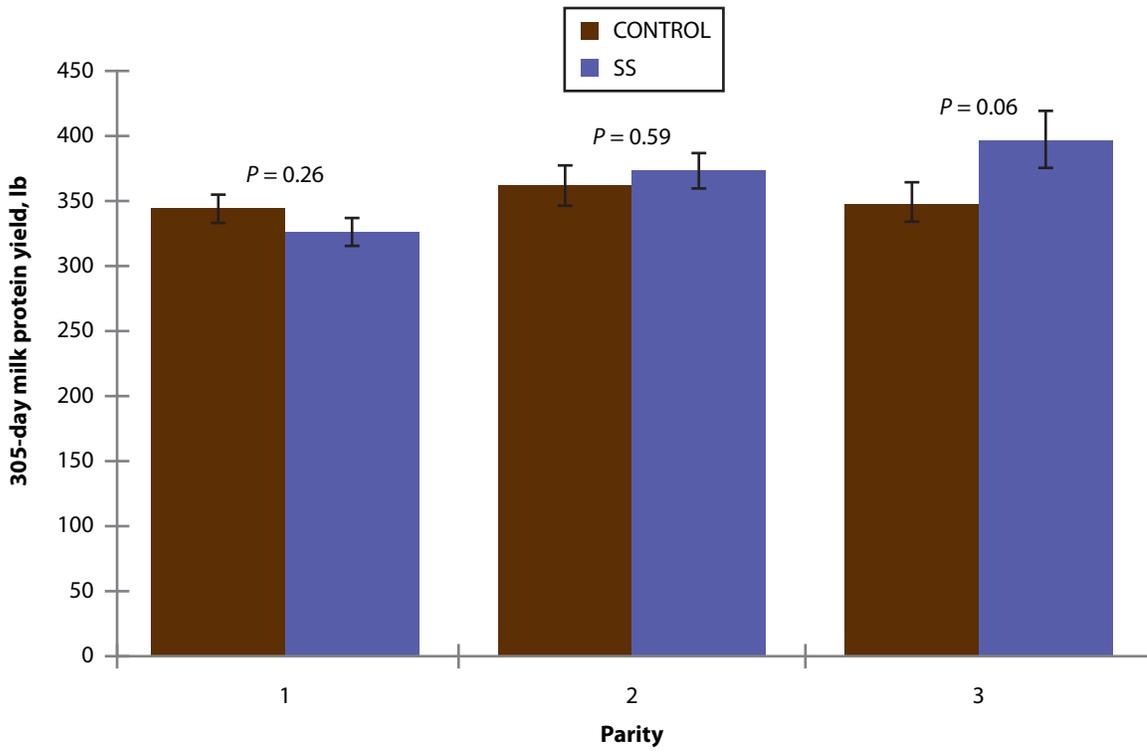
Figure 3. A tendency (*P* = 0.08) for a treatment × parity interaction was found for 305-day milk protein yield, with third-parity sodium salicylate (SS)-treated cows tending to have a greater milk protein yield through the lactation.

Hot Topic: New Research Highlights the Need for Holistic Thinking about Transition Cows

B. J. Bradford

Summary

In the past, efforts to improve the transition to lactation have focused largely on preventing infections and maximizing energy intake in transition cows, and these issues have generally been treated independently. New models, however, are emerging to explain the development of numerous transition disorders. A combination of insults, including social stress, negative energy balance, heat stress, endotoxin exposure, and oxidative stress may promote inflammation, suppress feed intake, and impair both metabolic and immune function during the transition period. These models suggest that transition cow management must be viewed holistically, because the cow's environment, nutrition, and immune function interact in many complex ways. Fortunately, a number of practical approaches can be used to improve the overall health of transition cows, which can decrease the cull rate in early lactation and improve both productivity and reproductive success.

Key words: transition cow

Physiological Interactions in the Transition Dairy Cow

Traditionally, experts on dairy cattle have focused on isolated components of dairy management: nutritionists work on diets, veterinarians respond to disease outbreaks, and others design facilities to maximize cow comfort. Today, we are learning how much nutrition, pathogens, and environmental challenges interact to influence the physiology of the cow.

One such interaction is the effect of energy balance on immune function. Nearly all transition cows experience at least 3 weeks of negative energy balance, a situation in which they require more energy for maintenance and milk production than is consumed from their diet. Recent work has demonstrated that blood metabolites that are elevated during this time, including nonesterified fatty acids (**NEFA**) and beta-hydroxybutric acid (**BHBA**), may directly impair the function of multiple types of immune cells. These effects may help to explain at least some of the decrease in immune function during negative energy balance.

Another common nutrition-related issue is the subclinical hypocalcemia that occurs in most transition cows. This issue is most commonly discussed in terms of the risk for milk fever; hypocalcemia can cause paresis because of the critical role of calcium in initiating muscle contractions and transmitting nerve signals. Calcium, however, is an important signal transducer in many other cell types, including immune cells. Monocytes from cows experiencing hypocalcemia were recently demonstrated to have low intracellular calcium stores. An inability of monocytes to utilize intracellular calcium for cell signaling is expected to dampen functional responses and the ability of these immune cells to fight pathogens. Such findings may provide a physiological basis for the long-observed links between hypocalcemia and mastitis in transition cows.

These findings are shedding light on why nutritional deficiencies and metabolic disorders can depress immune function and promote infectious disorders in the transition period. In fact, de-

creased feed intake was observed before calving in cows that ended up with subclinical ketosis or metritis after calving in several studies, suggesting that behavioral changes and nutrient imbalances can precede key transition problems by days, if not weeks. Another line of work focuses on the other side of this relationship: why biological stressors promote metabolic problems.

Stress, Sources of Stress, and the Consequences

Stress is defined as "the non-specific response of the body to any demand for change," which does not necessarily imply that stress is negative; in fact, some necessary components of the transition to lactation are certainly stressful by this definition. Although stress is difficult to define clearly and impossible to measure directly, it is worth considering because it is one way through which the intricate links between behavior, nutrition, and physiology can be understood. Common stress responses include decreased feed intake and inflammation, both of which have been implicated in most transition disorders. Social stress, infection, metabolic stress, and heat stress will be discussed as key sources of stress in the transition cow.

Social Stress

The best-studied source of social stress in transition cows is overcrowding. Competition at the feed bunk has been shown to decrease DMI of multiparous cows in the critical final week of gestation in spite of the fact that cows in this stage of production eat less than half as much dry matter as cows at peak lactation. Cows competing for access to feed also spent more time standing; standing time during the transition period has recently been documented as a key risk factor for claw horn lesions later in lactation. Feed bunk competition also results in cows consuming fewer and larger meals, which could increase the risk for ruminal acidosis, at least after the transition to a lactation ration. Although few controlled studies have been conducted to evaluate the effects of regrouping cows, anecdotal evidence suggests that repeated regrouping can induce similar stress and may likewise suppress feed intake and promote lameness.

Infection

Infectious disorders cause both specific and nonspecific responses. Among the most important stress responses to infection is inflammation. Mammary and uterine infections clearly result in both local and systemic inflammation, which can affect nearly all organs. The nonspecific inflammatory stress responses to infection promote the development of metabolic disorders by suppressing feeding behavior and directly impairing metabolic function of the liver.

Metabolic Stress

Inflammation may be a key contributor to metabolic disorders in transition cows. A retrospective study of cows on 3 commercial Italian dairies suggested that liver inflammation is associated with a problematic transition to lactation: cows with the strongest inflammatory profiles were at 8-fold greater risk for experiencing one or more transition disorders, had lower plasma calcium concentrations, took longer to re-breed, and produced less milk in the first month of lactation.

Metabolic stress can be initiated by a variety of factors, including inflammation from infection (discussed above), oxidative stress, and translocation of endotoxin from the gut. One effect of increased delivery of NEFA to the liver in early lactation is an increase in the production of reactive oxygen species, a condition commonly referred to as oxidative stress. This is especially true for cows with high body condition, likely because plasma NEFA concentrations are more elevated in these cows. Reactive oxygen species are a concern because they can damage cellular

proteins and DNA and are potent activators of inflammatory pathways. Endotoxin is a component of the cell wall of gram-negative bacteria, and detection of endotoxin by immune cells initiates a strong inflammatory response. Recent studies have demonstrated that sub-acute ruminal acidosis increases both ruminal and plasma endotoxin concentrations, which causes liver inflammation.

Metabolic inflammation can be derived from at least 3 sources: infection, oxidative stress, and endotoxin translocated from the gut. Several recent studies have shown that causing sterile inflammation in dairy cattle promotes conditions leading to fatty liver and ketosis, two of the most prevalent metabolic disorders in transition cows. In addition to promoting metabolic disorders by stimulating inflammation, oxidative stress can directly suppress immune function by damaging lipids, proteins, and DNA of immune cells. Oxidative stress may play a key role in the poor immune function observed in transition cows, a hypothesis that is supported by numerous studies demonstrating beneficial effects of supplementing antioxidants in the transition period.

Heat Stress
Another common stressor for transition cows is excessive heat load. Many operations cool lactating animals, either because these cows have the highest heat burden or because the benefits of cooling lactating cows are so easy to observe in daily milk weights during heat waves. The stress of such environments on dry cows has not received as much attention. Recent work showed that heat stress during the dry period decreased dry matter intake (**DMI**) during the week of calving by nearly 50%, decreased the function of immune cells after calving, and decreased peak milk production by more than 10 lb/day. Although the exact mechanisms linking heat stress to these long-term effects remain unclear, substantial costs are clearly associated with allowing dry cows to experience sustained heat stress.

Practical Implications
These findings suggest a number of focus areas for dairy managers aiming for a holistic management scheme to accommodate the complex nutritional, environmental, and behavioral needs of the transition dairy cow.

Housing
The clear implication of recent findings from the group at the University of British Columbia is that overcrowding dry cows is a mistake. During the financial difficulties of the past several years, numerous stories have circulated about farms decreasing stocking rates of lactating cows from 120% to 100% without losing milk in the bulk tank. Perhaps these instances are a reminder about the importance of adequate space (both in free stalls and bunk line); if anything, literature suggests that space is even more critical in the dry period. Behavioral responses to overstocking are expected to cause greater lameness and more negative energy balance and to increase the transition disorders associated with these issues. With the recent findings from the University of Florida, similar negative effects can be expected in cows that are exposed to heat stress through the dry period. Providing adequate space and keeping cows cool should be high priorities in any dry cow management plan.

Another factor worth considering is the grouping of cows. For many years, separating dry cows in far-off and close-up pens was recommended to allow different diets to be fed during these periods; however, with the information available on one-group dry cow strategies (see below), this procedure is no longer necessary. According to some, the reduced stress of not moving cows an

extra time is reason enough to make the change to a one-group dry cow system. When considering grouping strategies for dry cows, realize that subordinate cows, those who are bullied away from the feed bunk, eat less feed, and spend more time standing when overcrowded, are the most susceptible to social stress. As a result, these cows are also the most susceptible to transition disorders if not properly managed. If possible, it is wise to pen close-up heifers separately from dry cows; subordinate cows (small or simply submissive cows) can be housed with heifers if necessary. Finally, remember that pen movements affect not only the cow that is moving, but also the entire group. Even if a single pen is used to house all dry cows on a farm, the weekly influx of new cows constantly disrupts the social structure in the pen and serves as a potential source of stress. Although certainly not practical on all farms, some larger operations are experimenting with "all-in, all-out" management schemes, where a group of dry cows all enter the pen together and end up in a fresh cow pen together after all have calved. This type of system has the potential to minimize the amount of social stress for transition cows.

Nutrition

The primary goal in transition cow nutrition has been crystallized in the past decade: control body condition. No other factor that we can measure is a better predictor of a disastrous transition period than a body condition score (**BCS**) of 4 or greater. In fact, most academics who focus on transition disorders for this period advocate a target BCS of 3 or even less at calving because the consequences of high BCS have proven far more serious than those of low BCS. Cows suffering from "fat cow syndrome" experience greater decreases in feed intake than healthy cows, have greater increases in plasma NEFA, and are far more likely to have clinical cases of ketosis and even infectious disorders. The goal of controlling body condition is often best met by feeding relatively low-energy diets throughout the dry period, although a wide variety of formulations can potentially be used to accomplish this goal. The devil, of course, is in the details: preventing excessive sorting, promoting sufficient feed intake to meet energy requirements, and balancing for dietary cation-anion difference are all problems to address.

As with social stress, nutritional needs of close-up heifers can be best met by housing them separately. Because these heifers are still growing and are less susceptible to "fat cow syndrome," it is probably logical to offer them a slightly higher energy diet than multiparous cows. Likewise, anionic diets that benefit multiparous transition cows can dramatically decrease feed intake of heifers. Heifers rarely experience severe hypocalcemia, so it is best to feed them diets without added sources of anions.

Disease Prevention

The immune dysfunction that cows experience during the transition period suggests several management strategies that may help limit disease pressure and associated stress during this time. Clearly, dairies are interested in reducing pathogen loads for all cows, but if an opportunity to improve the cleanliness of certain pens arises, it would be wise to invest in fresh pens where the majority of mastitis and metritis cases occur. In addition, vaccination protocols should be designed to avoid vaccinating cows during the final 3 weeks of gestation, because the decreased function of the adaptive immune system during this period limits the effectiveness of vaccines and produces potentially harmful inflammation during a critical time.

Conclusions

Even on farms with relatively low incidence of transition cow disorders, suboptimal social settings, environmental conditions, feed intake, metabolic status, or immune function may impair

the ability of transition cows to reach their genetic potential for peak milk yield, resulting in significant economic losses throughout lactation. Although the mechanisms underlying some of these interactions remain elusive, some clear messages stand out from recent research:

- Transition cows need adequate bunk and stall space; heat stress during this period has long-term negative effects.

- Separating heifers from dry cows and minimizing group changes during the transition period encourages improved nutritional management and decreased social stress.

- Because of the numerous interactions between different physiological systems, improving feed intake after calving, improving metabolic function, or decreasing infections should positively affect the other factors, ultimately increasing health and productivity.

Pregnancy per AI after Presynchronizing Estrous Cycles with Presynch-10 or PG-3-G before Ovsynch-56 in Four Dairy Herds of Lactating Dairy Cows

J. S. Stevenson and S. L. Pulley

Summary

The objective was to determine the effect of 2 presynchronization treatments on first-service pregnancy rate in 4 dairy herds during warm and cool seasons of the year. Cows with ear tags ending with even digits at calving were enrolled in **Presynch-10** with 2, 25-mg injections of prostaglandin $F_{2\alpha}$ (i.e., **PG-1** and **PG-2**) 14 days apart. Cows with ear tags ending with odd digits were enrolled in **PG-3-G** comprising 1, 25-mg injection of PG (**Pre-PG**) 3 days before 100 μg gonadotropin-releasing hormone (**Pre-GnRH**), with the Pre-PG injection administered at the same time as PG-2 in the Presynch-10 treatment in the Presynch-10 treatment. Ten days after PG-2 or Pre-PG, all cows were enrolled in a timed artificial insemination (**TAI**) protocol (Ovsynch-56; injection of GnRH 7 days before [GnRH-1] and 56 hours after [GnRH-2] PG with AI 16 to 18 hours after GnRH-2). Median days in milk (**DIM**) at scheduled TAI were 75 days, which did not differ among herds. Cows detected in estrus before the scheduled TAI were inseminated early (early bred; **EB**). Pregnancy was diagnosed at days 32 to 38 and at days 60 to 66 after TAI by transrectal ultrasonography or transrectal palpation. Data were analyzed with herd as a random effect and with fixed effects of treatment (EB, Presynch-10, PG-3-G), parity (primiparous vs. multiparous), season (hot [June through September] vs. cool-cold [October through May]), DIM, estrus at TAI (0 vs. 1), and all 2-way interactions with treatment. The pregnancy rate at days 32 to 38 for EB (n = 472), Presynch-10 (n = 1,247), and PG-3-G (n = 1,286) were 31.4, 35.0, and 41.2%, respectively; pregnancy rate at days 60 to 66 was 29.8, 32.2, and 37.3%, respectively. Season significantly influenced pregnancy rate at days 32 to 38 and days 60 to 66, but a treatment by season interaction was not detected. The pregnancy rate for PG-3-G and Presynch-10 treatments did not differ during cool-cold weather (d 32 to 38: 46.8 vs. 44.3%; days 60 to 66: 41.6 vs. 41.1%, respectively), but PG-3-G and Presynch-10 produced a higher pregnancy rate than EB at days 32 to 38. During summer, pregnancy rate in PG-3-G was greater than in Presynch-10 (days 32 to 38: 35.9 vs. 26.7% or days 60 to 66: 33.2 vs. 24.4%, respectively), and pregnancy rate in EB cows did not differ from that of Presynch-10 cows. Although pregnancy loss did not differ for EB, Presynch-10, and PG-3-G treatments (4.0, 6.7, and 9.3%, respectively), pregnancy loss from days 32 to 38 and days 60 to 66 was 2-fold greater in thinner cows (<2.5 vs. ≥2.5; 9.0 vs. 4.4%). We concluded that presynchronizing estrous cycles with PG-3-G produced more pregnancies than inseminating cows at estrus during cooler weather and was superior to Presynch-10 during summer.

Key words: Presynch-10, PG-3-G, pregnancy rate per AI

Introduction

Timed AI (**TAI**) programs facilitate control of estrous cycles in lactating dairy cattle and provide viable options to AI programs based solely on detection of estrus. The most commonly used TAI programs are variations of the original Ovsynch protocol (injection of gonadotropin-releasing hormone or GnRH 7 days before [**GnRH-1**] and 48 hours after [**GnRH-2**] prosta-

glandin F_{2a} (**PG**) with TAI administered 16 hours after GnRH-2), which is used widely in the U.S. dairy industry.

When estrous cycles of lactating dairy cows are presynchronized to days 5 through 12 of the cycle before enrolling cows in the Ovsynch protocol, pregnancy rate per AI is further augmented. Standard presynchronization programs in which two injections of PG administered 14 days apart (**Presynch**) with the Ovsynch protocol initiated 14 days (**Presynch-14**), 12 days (**Presynch-12**), 11 days (**Presynch-11**), or 10 days later (**Presynch-10**) have been tested in lactating dairy cows. The Presynch programs generally improve pregnancy rate compared with cows randomly allocated to Ovsynch alone. Presynch programs with shorter intervals of 11 days between the second Presynch PG injection (i.e., Presynch-11) and onset of Ovsynch improved pregnancy rate compared with programs with longer intervals (Presynch-14).

Other presynchronization schemes tested included those in which PG is injected first, then GnRH is injected either 2 days (**G-6-G**) or 3 days later (**PG-3-G**), followed by enrollment in the Ovsynch protocol in 6 or 7 days, respectively, tended to improve pregnancy rate. In addition, use of an Ovsynch protocol to presynchronize cows (i.e., non-breeding Ovsynch) before the TAI Ovsynch program (**Double Ovsynch**) resulted in improved pregnancy rate compared with Presynch-12 in primiparous cows, but not in multiparous cows.

Most studies reported in the literature have excluded presynchronization and TAI treatments during summer. Dairy cows whose estrous cycles were presynchronized with Presynch-12 or a progesterone insert-GnRH combination before a TAI program and who were exposed to heat stress (temperature-humidity index [THI] > 72) were 5.8 times more likely to have a poorer pregnancy rate than those not exposed to heat stress. Furthermore, cows in that study were 7.4 times more likely to abort an established pregnancy between 28 and 56 days of gestation. Compared with cooler conditions, chronic seasonal heat stress or hyperthermia alters follicular steroidogenesis, which leads to formation of suboptimal corpus luteum (**CL**) and reduced progesterone; factors that likely reduce synchronization efficiency and subsequent fertility.

We recently demonstrated that the PG-3-G presynchronization program (PG followed in 3 days by GnRH) followed by the Ovsynch protocol 7 days after GnRH) produced more cows with CL, more CL per cow, greater progesterone, and greater ovulatory response to GnRH-1 than cows whose estrous cycles were presynchronized with Presynch-10 before applying the Ovsynch program. The objectives of the current study were to determine the effect of these 2 presynchronization treatments on first service pregnancy rate in four dairy herds during hot and cool-cold seasons of the year and to validate our preliminary report (Dairy Research 2011, Report of Progress 1057, p. 31–35) that suggested the superiority of the PG-3-G treatment for achieving greater pregnancy rate.

Experimental Procedures

Lactating dairy cows from four herds in northeast Kansas were enrolled in the study. Three herds comprised cows calving from September 2010 through September 2011, with cows from the remaining herd calving from September 2009 through September 2011. All herds included cows that were milked thrice daily and fed diets consisting of alfalfa hay, corn silage, soybean meal, whole cottonseed, corn or milo grain, corn gluten feed, vitamins, and minerals.

At calving, 3,285 dairy cows (>95% were Holsteins with the residual representing crosses of Holstein with either Jersey, Brown Swiss, or Scandinavian Red) were clustered into breeding groups on a weekly (Herds 2, 3, and 4) or biweekly (Herd 1) basis. Characteristics of herds used in the experiment are summarized in Table 1. Enrollment in the study began at a median 42 days in milk (**DIM**) (41 ± 0.1 d; mean ± SE). Cows with ear tags ending with even digits were enrolled in Presynch-10: 2, 25-mg injections of PGF$_{2\alpha}$ (i.e., **PG-1** and **PG-2**; 5 mL Lutalyse, Pfizer Animal Health, Madison, NJ) administered 14 days apart (Figure 1). Cows with ear tags ending with odd digits were enrolled in PG-3-G: 1, 25-mg injection of PG (Pre-PG; 5 mL Lutalyse, Pfizer Animal Health) 3 days before 100 µg GnRH (Pre-GnRH; 2 mL Fertagyl, Merck Animal Health, Whitehouse Station, NJ), with the Pre-PG injection administered at the same time as PG-2 in the Presynch-10 treatment (Figure 1). Cows subsequently were enrolled in a TAI protocol (Ovsynch-56; 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 PG-2 or Pre-PG injections. Treatment injections were staggered within each cluster so all cows were inseminated on the same day of the week. Cows were at a median of 75 DIM (74 ± 0.1 days) when inseminated at TAI. Treatment assignments were hand-delivered weekly to each dairy farm. At each weekly herd visit, cows scheduled for TAI that week were given body condition scores (**BCS**; 1= thin, 5 = fat).

Of the 3,285 cows originally enrolled in the study, 280 cows were dropped from the study because of culling (n = 207), death (n = 30), failure to inseminate (n = 36), or insemination after the scheduled TAI date (n = 7). Furthermore, 472 cows identified in estrus at any time after PG-1, including the day before scheduled TAI, were inseminated early before completing the entire experimental protocol (early bred; **EB**) and did not receive further scheduled injections. These 472 early inseminations occurred a median of 58 DIM (61 ± 0.5 days). The EB cows included those identified by rubbed tail chalk or tail paint, or by vaginal mucus. Final numbers of cows included in statistical analyses included 1,483 cows in the Presynch-10 treatment, 1,522 cows in the PG-3-G treatment, and 472 EB cows.

At each insemination, date, sire, technician, and breeding codes (chalk or tail paint rub, or mucus) was entered in DC305 (Herd 4) or PC-DART (Herds 1, 2, and 3) software. Full access to herd data was provided by dairy cooperators with weekly herd downloads. For purposes of determining when these EB cows were inseminated relative to treatment injections, proportions of EB cows inseminated between PG-1 and PG-2 (or Pre-PG), PG-2 (or Pre-PG) and GnRH-1, GnRH-1 and PG, and PG and GnRH-2 were determined.

Pregnancy diagnosis was conducted weekly by transrectal ultrasonography in Herds 1 and 4 and by transrectal palpation in Herds 2 and 3. Cows presented for pregnancy diagnosis were from 32 to 38 days since TAI. A second confirmatory diagnosis occurred 4 weeks later (60 to 66 days after TAI) and was performed by palpation per rectum at Herds 2, 3, and 4. The same veterinary clinic serviced Herds 2, 3, and 4, and 1 veterinary practitioner performed nearly 100% of the pregnancy diagnoses at Herds 2 and 3. A positive pregnancy outcome by ultrasonography 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. Positive pregnancy diagnosis by palpation was made by membrane slip or palpation of the amniotic vesicle.

Date of first repeat insemination was recorded for all cows after the initial AI. These cows were considered not pregnant unless a subsequent pregnancy diagnosis confirmed the pregnancy to

be established earlier at first service based on size of the fetus. Return intervals to a second AI were categorized as early (<18 d), normal (18 to 25 d), or late (>25 d) for purposes of analysis.

Results and Discussion

Early bred cows

Of the 3,005 cows that completed the study, 472 (15.7%) were inseminated early (Table 2). Proportional distribution of cows inseminated early according to their assigned treatment did not differ regardless of when AI occurred after PG-1, PG-2 (or Pre-PG), GnRH-1, or PG (before scheduled day of TAI).

Pregnancy outcomes

Factors that significantly influenced pregnancy rate at days 32 to 38 and days 60 to 66 are summarized in Table 3. No significant interactions were detected between treatment and season or treatment and parity. Although treatment only tended ($0.071 < P < 0.107$) to affect pregnancy rate at both pregnancy diagnoses, parity ($P = 0.037$), season ($P < 0.001$), and occurrence of estrus at TAI ($P < 0.001$) accounted for significant variation in pregnancy outcomes.

All cows inseminated during the cool-cold months of the year had greater ($P < 0.001$) pregnancy rates than those inseminated during summer (Table 3). Cows identified in estrus on the day of TAI had a greater ($P < 0.001$) pregnancy rate at days 32 to 38 than those not detected in estrus in both treatments: PG-3-G cows, 50.5% (n = 99) vs. 39.3% (n = 1,187), and Presynch-10 cows, 47.9% (n = 117) vs. 33.8% (n = 1,130).

Primiparous cows had greater ($P < 0.05$) pregnancy rate than multiparous cows, but only at days 32 to 38. Slightly more pregnancy loss in primiparous cows after days 32 to 38 seemed to preclude the difference in pregnancy rate for cows at days 60 to 66 (Table 3). Pregnancy loss did not differ for EB, Presynch-10, and PG-3-G treatments (5.1, 7.0, and 9.2%, respectively). Pregnancy loss between pregnancy diagnoses, however, was affected by BCS. Cows with BCS < 2.5 (n = 501) had more ($P = 0.002$) than twice as many pregnancy losses (9.0 vs. 4.4%) than cows (n = 524) with BCS ≥ 2.5.

Contrasts of PG-3-G vs. EB for pregnancy rate showed increased pregnancy rate for PG-3-G at days 32 to 38 ($P < 0.05$) and at days 60 to 66 ($P < 0.05$). During summer, pregnancy rate at days 32 to 38 was greater ($P < 0.05$) for PG-3-G than for Presynch-10 (Figure 2). Results for pregnancy rate at days 32 to 38 during cool-cold weather did not differ between PG-3-G and Presynch-10, except both PG-3-G ($P < 0.05$) and Presynch-10 ($P = 0.053$) differed from EB cows (Figure 2). Treatment differences in pregnancy rate at days 60 to 66 during summer followed the same pattern as differences at days 32 to 38 (data not shown). In contrast, during cool-cold months, only PG-3-G tended ($P = 0.115$) to have greater pregnancy rate than EB cows, and PG-3-G did not differ from Presynch-10.

Returns to insemination

Proportions of EB cows that returned to estrus and were reinseminated <18 days after AI were greater ($P < 0.05$) than those in PG-3-G and Presynch-10 that completed the TAI protocol (Table 4). Fewer ($P < 0.05$) Presynch-10 and PG-3-G cows returned to estrus in the normal 18- to 25-day interval compared with EB cows. More ($P < 0.05$) Presynch-10 and PG-3-G cows were reinseminated after day 25.

In summary, cows treated with the PG-3-G treatment had greater pregnancy rate per AI than those treated with Presynch-10 during summer. Based on our previous study with the same treatments, we expect that PG-3-G is a more effective presynchronization treatment than other Presynch (14, 12, 11, or 10) treatments because of the Pre-GnRH injection and its ability to induce ovulation in anovular cows, although we did not examine this hypothesis in the present study. Evidence for that hypothesis was reported in our earlier study. Furthermore, because we detected no differences in the timing or distribution of early inseminations in the EB cows, both treatments effectively induced estrus for early inseminations. Given the potential advantages to anovular cows of the Pre-GnRH injection in the PG-3-G treatment documented with the PG-3-G treatment in our earlier study and its superior pregnancy outcome response during summer, PG-3-G may be a better presynchronization treatment to employ than Presynch-10.

Table 1. Characteristics of herds in which the experiment was conducted

Item	Herd			
	1[1]	2[2]	3[2]	4[2]
Milking cows, n	235	1,528	648	1,049
Rolling herd average milk, lb	29,689	25,567	24,529	31,696
Test-day average milk, lb	93	77	77	90
Days to first service	70	77	75	67
Calving interval, months	13.8	13.3	14.5	13.1

[1] Covered, sand-bedded, 2-row free stalls with overhead sprinklers in the feed alley and shade cloth covering the feeding area and feed bunk during summer.
[2] Curtain-sided, confined 2- or 4-row barns equipped with fans (above feed lines, sand-bedded free stalls, or both), sprinklers above the feed lines, and grooved concrete floors.

Table 2. Distribution of 472 cows inseminated early according pre-assigned treatment

When early inseminated[1]	Pre-assigned treatment[1] before early artificial insemination	
	Presynch-10	PG-3-G
	------------------------- % (no.) -------------------------	
PG-1 until PG-2 (or Pre-PG)	6.2 (16)	8.0 (17)
PG-2 (or Pre-PG) until GnRH-1	56.4 (146)	53.0 (113)
GnRH-1 until PG	6.5 (17)	8.0 (17)
PG until GnRH-2	30.9 (80)	31.0 (66)
Total	100 (259)	100 (213)

[1] See Figure 1 for description of treatment programs.

Table 3. Factors included in the logistic models that significantly influenced pregnancy rate per artificial insemination (AI) at days 32 to 38 or at days 60 to 66 after AI

Factor	Cows, n	Pregnancy rate per AI, day of diagnosis	
		32 to 38	60 to 66
		---------------------- % ----------------------	
Treatment[1]			
Early bred	472	31.4[a]	29.8[a]
Presynch-10	1,247	35.0[ab]	32.2[ab]
PG-3-G	1,286	41.2[b]	37.3[b]
Parity			
1	1,185	38.1[a]	35.2[a]
2+	1,820	33.6[b]	30.9[a]
Season			
Cool-cold	2,034	42.2[a]	38.9[a]
Hot	971	29.8[b]	27.6[b]
Estrus at AI			
Yes	688	39.3[a]	35.6[a]
No	2,317	32.4[b]	30.6[a]

[a,b] Contrasts within column and factor with different superscript letters differ ($P \le 0.05$).
[1] See Figure 1 for description of treatment programs.

Table 4. Distribution of cows according to reinsemination intervals after artificial insemination (AI) at first service

Days from timed AI	Treatment[1]		
	Early bred	Presynch-10	PG-3-G
	-- % (n) --		
<18	13.2[a] (40)	9.2[b] (73)	6.9[b] (52)
18 to 25	38.3[a] (116)	28.6[b] (227)	27.2[b] (205)
>25	48.5[a] (147)	62.2[b] (494)	65.9[b] (496)

[a,b] Proportions within row having different superscript letters differ ($P < 0.05$).
[1] See Figure 1 for description of treatment programs.
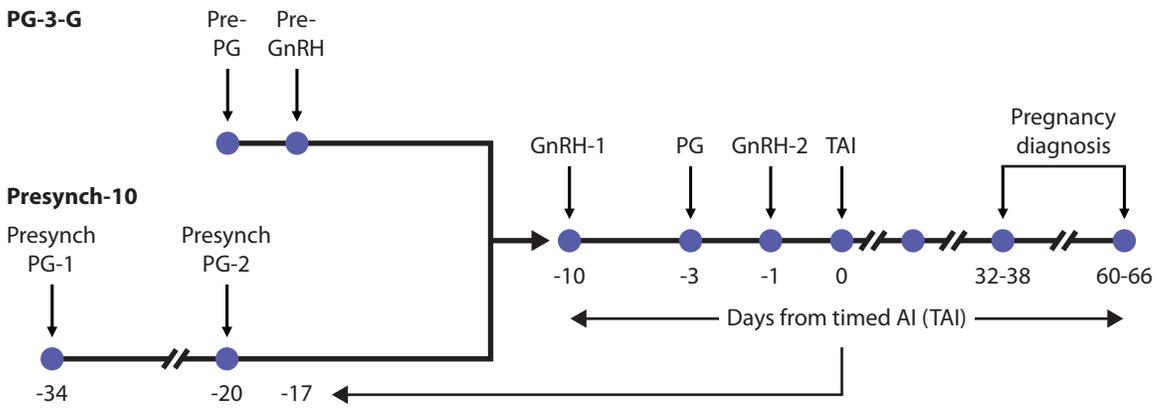
Figure 1. Experimental design. At calving, lactating dairy cows were assigned randomly to 2 treatments according to ending ear tag number (odd or even): PG-3-G or Presynch-10. Cows received 100 μg GnRH at Pre-GnRH, GnRH-1, or GnRH-2; 25 mg of $PGF_{2\alpha}$ at Pre-PG, Presynch PG-1, Presynch PG-2, and PG. Some cows were inseminated early at any time after PG-1 upon detection of estrus.
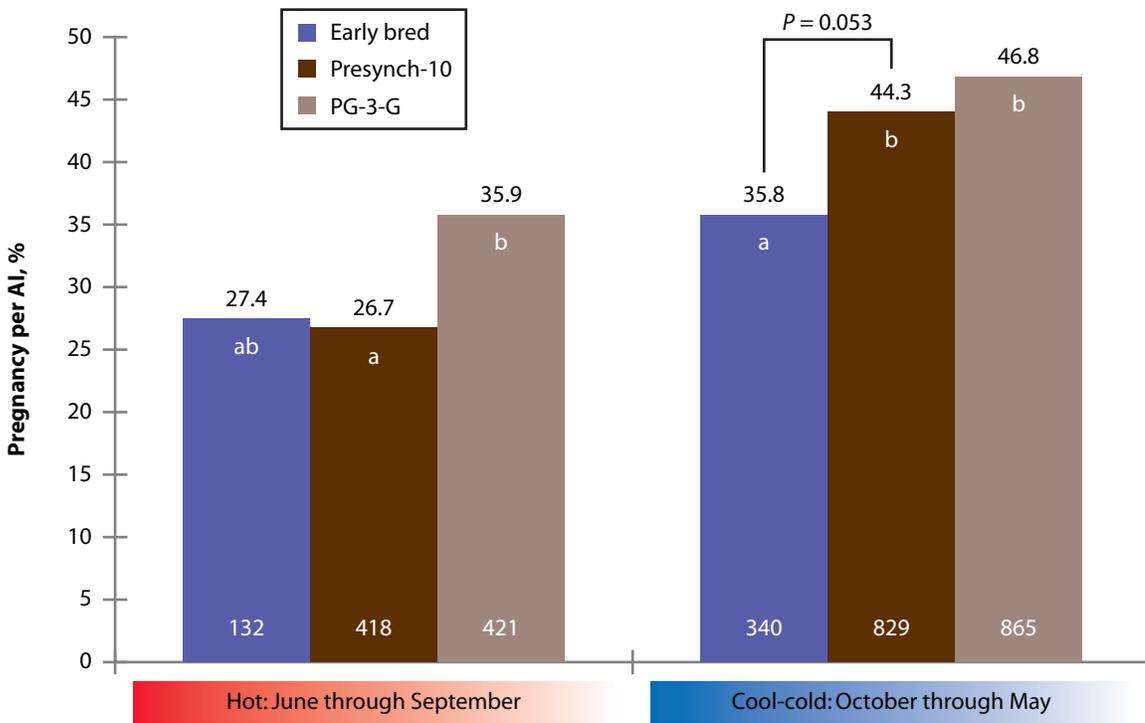
Figure 2. Pregnancy rate per artificial insemination (AI) (measured at days 32 to 38 after timed AI) for early bred, Presynch-10, and PG-3-G cows (treatments are defined in Figure 1) during June through September and October through May.

[a,b] Proportions within season having different letters differ ($P < 0.05$).

Reinsemination Intervals After Timed Artificial Insemination or Estrus-Detected Inseminations

J. S. Stevenson

Summary

The objective was to quantify the reinsemination intervals of lactating dairy cows that were either inseminated at estrus or received a timed AI (**TAI**) at first service. Cows in Experiment 1 were enrolled in a TAI program before first AI after calving. Cows detected in estrus after 50 days in milk (**DIM**) were inseminated, whereas the remainder continued in the TAI program and were inseminated as scheduled. Cows in Experiment 2 also were enrolled in a TAI program and were inseminated accordingly at first service after calving. On day 7 after TAI, cows were assigned randomly to receive either saline (control) or 1,000 IU human chorionic gonadotropin (hCG) to induce accessory luteal structures (corpora lutea) in an attempt to improve pregnancy outcome. First-repeat insemination dates were recorded for all cows after the initial AI and grouped as <18 days, 18 to 25 days, or >25 days since first AI. More cows in Experiment 1 that were inseminated at estrus returned to estrus before 25 days than TAI cows and during summer months had shorter average return intervals by 1.7 days. More cows in Experiment 2 that received saline and had no accessory luteal structures also returned to estrus before 25 days than cows receiving hCG. Equal proportions of saline and hCG-treated cows (25%) in Experiment 2 had retained at least one of their original luteal structures until day 28 after TAI, but were not pregnant at day 32. Of those nonpregnant cows that retained luteal structures, average concentrations of pregnancy-specific protein B (BioPRYN test) concentrations were slightly elevated, but failed to retain the embryos to day 32 after AI. Furthermore, progesterone concentrations of these cows that lost their embryos were compromised compared with pregnant cows by day 21 after AI. Regardless of the number of luteal structures after first insemination, 25% were retained up to 28 days after AI, indicating pregnancy had occurred but embryo loss occurred between pregnancy recognition (day 15) and days 28 to 32 after insemination. Cows receiving TAI also had longer reinsemination intervals than cows inseminated at estrus, a phenomenon that is exaggerated during summer heat stress.

Key words: human chorionic gonadotropin, estrus, reinsemination, embryo loss

Introduction

Timed AI (**TAI**) programs facilitate control of estrous cycles in lactating dairy cattle and provide viable options to AI programs solely based on detection of estrus. Although pregnancy outcomes are often similar between cows inseminated at estrus or after TAI, reinsemination intervals have not been evaluated to determine if these intervals are in any way different in terms of timing or distribution among cows. Dairy producers often report that a proportion of these cows receiving TAI are observed in estrus 10 to 12 days after AI. Furthermore, we desired to determine whether multiple-ovulating cows that failed to conceive would show differences in the onset or distribution pattern for returns to estrus after insemination.

Our objective was to determine the pattern and timing of reinsemination intervals of lactating dairy cows previously inseminated after observed estrus or TAI (Experiment 1) or after accessory luteal structures were induced by human chorionic gonadotropin (**hCG**) after insemination (Experiment 2).

Experimental Procedures

Experiment 1

Lactating dairy cows from 4 herds in northeast Kansas were enrolled in the study. Three herds comprised cows calving from September 2010 through September 2011, with cows from the remaining herd calving from September 2009 through September 2011. All herds included cows that were milked thrice daily and fed diets consisting of alfalfa hay, corn silage, soybean meal, whole cottonseed, corn or milo grain, corn gluten feed, vitamins, and minerals.

At calving, 3,285 dairy cows (>95% were Holsteins with the residual representing crosses of Holstein with either Jersey, Brown Swiss, or Scandinavian Red) were clustered into breeding groups on a weekly (Herds 2, 3, and 4) or biweekly (Herd 1) basis. Enrollment in the study began at a median 42 days in milk (**DIM**) (41 ± 0.1 d; mean $\pm$ SE). All cows were enrolled in a TAI program. Cows were at a median of 75 DIM (74 ± 0.1 days) when inseminated at TAI.

Of the original cows assigned to the TAI program, 472 cows identified in estrus were inseminated early (early bred; **EB**) before completing the entire experimental protocol and did not receive further scheduled injections. These 472 early inseminations occurred a median of 58 DIM (61 ± 0.5 days). The EB cows included those identified by rubbed tail chalk or tail paint or by vaginal mucus.

Date of first repeat insemination was recorded for all cows after the initial AI. Cows reinseminated were considered to be not pregnant unless a subsequent pregnancy diagnosis confirmed the pregnancy to be established earlier at first service based on size of the fetus. Return intervals to a second AI were categorized as early (<18 d), normal (18 to 25 d), or late (>25 d) for purposes of analysis.

Experiment 2

Lactating dairy cows at the Kansas State University Teaching and Research Center milked thrice daily (n = 328) and previously inseminated at first service were assigned randomly to a completely randomized design consisting of two treatments when at least 1 corpus luteum (**CL**) was detected on day 7 post-AI. Treatment consisted of 1,000 IU hCG (1 mL Chorulon, Intervet Schering Plough Animal Health, Millsboro, DE) or 1 mL of saline (control) administered i.m. Blood was collected and luteal structures were mapped and sized by transrectal ultrasonography on days 7, 14, 21, 28, and 32 after AI. Blood also was collected on d 60 from all pregnant cows.

Repeat insemination dates were recorded after the initial insemination, and return intervals to a second AI were categorized as in Experiment 1. Blood was assayed for progesterone (days 7, 14, 21, 28, and 32 after AI) and pregnancy-specific protein B (BioPRYN test; days 21, 28, and 32) concentrations to determine luteal function and evidence of a viable embryo, respectively.

Results and Discussion

Experiment 1

Proportions of EB cows that returned to estrus and were reinseminated <18 days after AI were greater ($P < 0.05$) than those cows receiving TAI (Table 1). Fewer ($P < 0.05$) TAI cows returned to estrus in the normal 18- to 25-day interval compared with EB cows. More ($P < 0.05$) TAI cows were reinseminated after day 25.

Patterns of percentage distribution of cows returning to estrus during 30 days after first AI were similar between EB cows and those receiving the TAI (Figure 1). With the exception of day 21 after AI, the proportions of reinseminations between days 20 and 25 were similar between EB and TAI cows. Mean duration of the reinsemination interval for cows returning to estrus by day 30 during the cool months of the year did not differ between EB and TAI cows, but during the summer months, TAI cows had 1.7-day longer reinsemination intervals than EB cows (Figure 1).

Experiment 2

Multiple luteal structures were induced in 70% of the cows treated with hCG regardless of pregnancy status. Of those cows responding to hCG, 75% formed one accessory luteal structure and 25% formed two or more structures. Treatment with hCG reduced ($P < 0.05$) by half the proportion of cows reinseminated between 18 and 25 days after first AI (Table 2). As a result, more ($P < 0.05$) hCG cows were reinseminated after day 25. For cows with >25 days to reinsemination, concentrations of progesterone were greater ($P < 0.001$) in hCG- than saline-treated cows on day 14 (7.1 ± 0.3 vs. 5.2 ± 0.3 ng/mL) and day 21 (5.2 ± 0.3 vs. 3.9 ± 0.3 ng/mL), respectively, but concentrations were similar (2.5 ± 0.4 ng/mL) between treatments by day 28. Mean duration of the reinsemination interval for cows returning to estrus by day 30 was nearly 4 days longer for cows treated with hCG than with saline (Figure 2).

Retention of at least one post-AI luteal structure in cows treated with hCG or saline through day 28 in nonpregnant cows was observed. In fact, by day 28, this proportion was 25% and did not differ between treatments. We further studied the retention of luteal structures by examining concentrations of progesterone and pregnancy-specific protein B (**PSPB**; BioPRYN test, BioTracking, Moscow, ID) in nonpregnant (nonpregnant–retained CL) and pregnant (pregnant–retained CL) cows that retained at least 1 luteal structure to day 28 post-AI as well as in cows in which all structures regressed (nonpregnant–no CL) before day 28. Pretreatment concentrations of progesterone on day 7 did not differ among groups before treatment with hCG or saline (Figure 3). On day 14, however, only saline-treated nonpregnant–no CL cows had lesser ($P < 0.05$) concentrations of progesterone, indicating that either hCG, pregnancy status, or both increased progesterone secretion. By d 21, cows that retained their luteal structures and were pregnant or retained their luteal structures and were not pregnant on day 28 or 32 had greater ($P < 0.05$) concentrations of progesterone than cows whose luteal structures regressed before day 28. In addition, on day 21, hCG further increased ($P < 0.05$) progesterone in pregnant cows compared with all other groups. By day 28, the 3 groups of cows differed ($P < 0.05$) from one another within treatment (Figure 3) and pregnant, hCG-treated cows had the largest concentrations compared with all other groups.

This potential for pregnancy in the nonpregnant–CL retained group that resulted in subsequent early embryonic loss is supported by elevated concentrations of PSPB in cows in the 3 groups. Although concentrations did not differ on day 21, 28, or 32 between the 2 groups with retained CL for which a full complement of samples were available for all 3 sampling days (days 21, 28, and 32), when all samples were included in the analysis, the nonpregnant–no CL group tended ($P = 0.08$) to differ from the nonpregnant–retained CL group (55.4 vs. 134.0 pg/mL) on d 32 (Figure 4). Only 6 of 31 cows in the nonpregnant–retained CL group (for which samples for PSPB were available on d 21, 28, and 32) had elevated PSPB (>100 pg/mL) on day 32. Furthermore, only 2 of these 6 cows had elevated PSPB on both days 28 and 32. A lack of

any significant PSPB elevation on day 28 in nonpregnant–retained CL cows may be a dilution effect of the remaining cows having concentrations of PSPB at or near the sensitivity of the assay.

Why differences occurred in return to insemination intervals (Table 1) for EB cows and cows that completed both TAI programs is unclear. Fewer shorter returns (<18 d) in TAI cows are consistent with our observations that less than 5% of cows returned to estrus during this interval in Experiment 2 after a TAI at first service. In Experiment 2, approximately one-third returned to estrus in the normal 18- to 25-day period, which is consistent with Experiment 1. Furthermore, 25% of cows that retained their original luteal structures to d 28 after TAI at first service were diagnosed as not pregnant at d 32 in Experiment 2. These differences in return intervals could exist partly because EB cows are nearly "100% synchronized" compared with a lesser percentage of TAI cows and are a result of pregnancy failure in any cow after pregnancy recognition but before first pregnancy diagnosis.

The fact that 25% of cows in Experiment 2 retained at least 1 luteal structure to day 28 after TAI indicates that some of the previously observed long estrous cycles were associated with early embryo loss after pregnancy recognition. Only 6 of 31 had elevated PSPB on day 32 and only 2 of 6 had elevated PSPB on days 28 and 32, which may indicate that these embryos were late-developing. Although these embryos may have been sufficiently viable to prevent luteolysis by secreting adequate amounts of interferon-tau and survived variously beyond day 21, they lacked the ability to produce adequate concentrations of PSPB by the trophectoderm binucleate cells. Suggestion of early embryo loss also is supported by our observation that these nonpregnant cows with retained CL had progesterone concentrations intermediate between those in nonpregnant cows that returned to estrus by day 21 and those in cows diagnosed as pregnant on day 28 or 32 (Figure 3). Evidence that some of these nonpregnant retained-CL cows were pregnant also was supported by elevated concentrations of PSPB on day 32 (data not shown).

Many pregnancy losses occur before pregnancy recognition, but in high-producing lactating dairy cattle, substantial losses continue to occur up to 42 to 56 days after insemination. Several factors affect pregnancy losses in cattle, such as compromised oocytes, resulting in poorly developed embryos incapable of cross-talking with the endometrial epithelial cells, to inadequate uterine environment and infectious agents resulting in death of the embryo from undernourishment. Other studies indicated anovulation or anestrus, metabolic status of the cow, some dietary ingredients, as well as occurrence of diseases, predispose the cow to experience embryonic and fetal death. Although some insemination protocols might affect embryo survival, when TAI has been implemented properly, it has not influenced embryonic or fetal death in cattle.

Reinsemination intervals clearly differ between cows inseminated at estrus and those inseminated at TAI. Some of these differences may stem from pregnancies that have occurred after TAI, thus accounting for longer average reinsemination intervals. It is also clear that when cows have multiple luteal structures, such as induced by hCG in Experiment 2, reinsemination intervals are longer, but despite that, the proportion of cows with retained luteal structures are similar at 25% at day 28, even though all of these cows are nonpregnant at day 32.

Table 1. Distribution of cows according to reinsemination intervals after artificial insemination (AI) at first service (Experiment 1)

Days from timed AI	Early bred	Timed AI
	----------------------------- % (n) -----------------------------	
<18	13.2[a] (5/40)	6.8[b] (5/125)
18 to 25	38.3[a] (44/116)	27.9[b] (78/432)
>25	48.5[a] (71/147)	64.0[b] (634/990)

[a,b] Proportions within row having different superscript letters differ ($P < 0.05$).
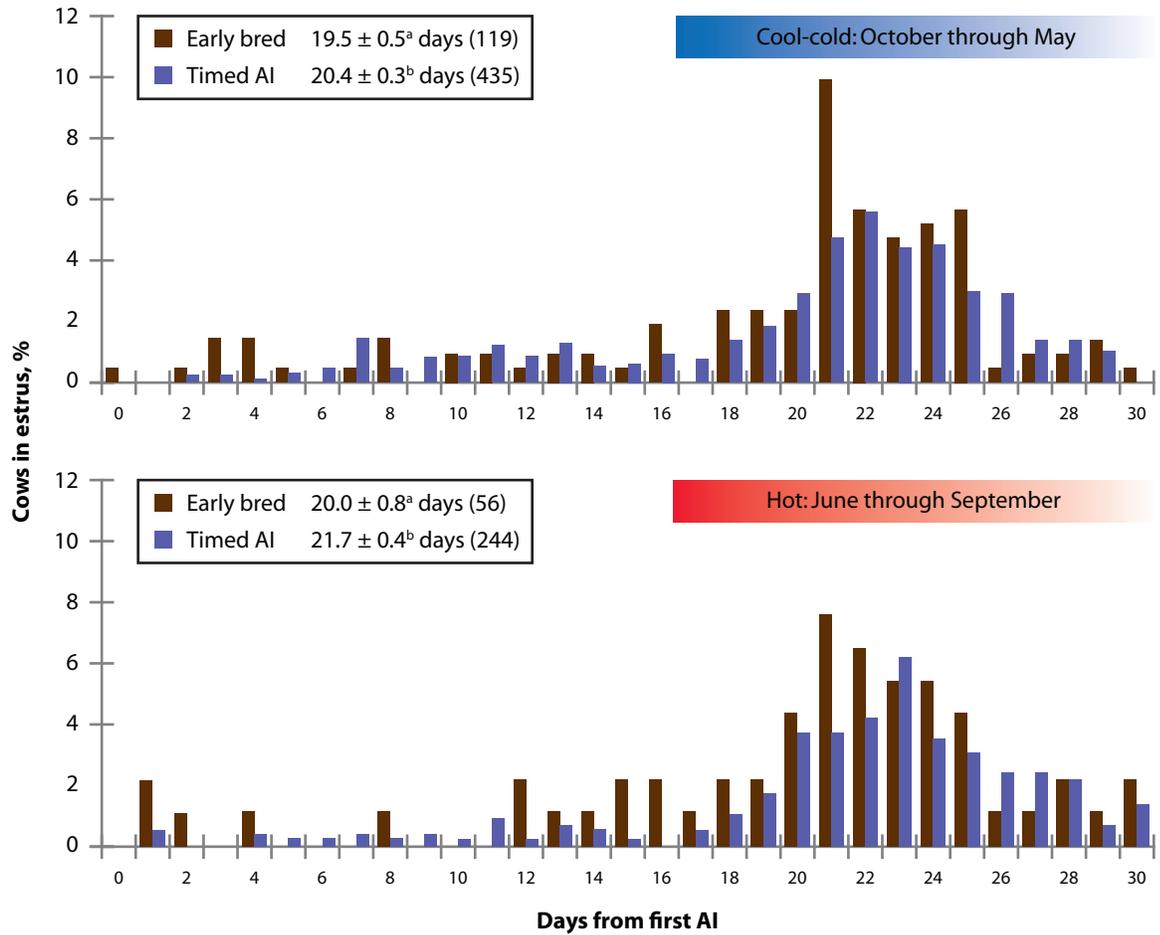
Figure 1. Proportion of cows in estrus after first service after having been first inseminated at estrus or timed inseminated. Duration of cycles is the average reinsemination interval for cows reinseminated up to 30 days after first service.

Table 2. Proportion of cows reinseminated at various intervals after first insemination

Days to reinsemination	Treatment[1]	
	Saline	hCG
	----------------------------- % (n/n) -----------------------------	
<18	3.6 (3/83)	1.1 (1/95)
18 to 25	25.3[a] (21/83)	12.6[b] (11/95)
>25	71.1[a] (59/83)	86.3[b] (60/95)

[a,b] Means differ ($P < 0.05$) between treatments.
[1] Cows were treated with either hCG or saline on day 7 post-artificial insemination.
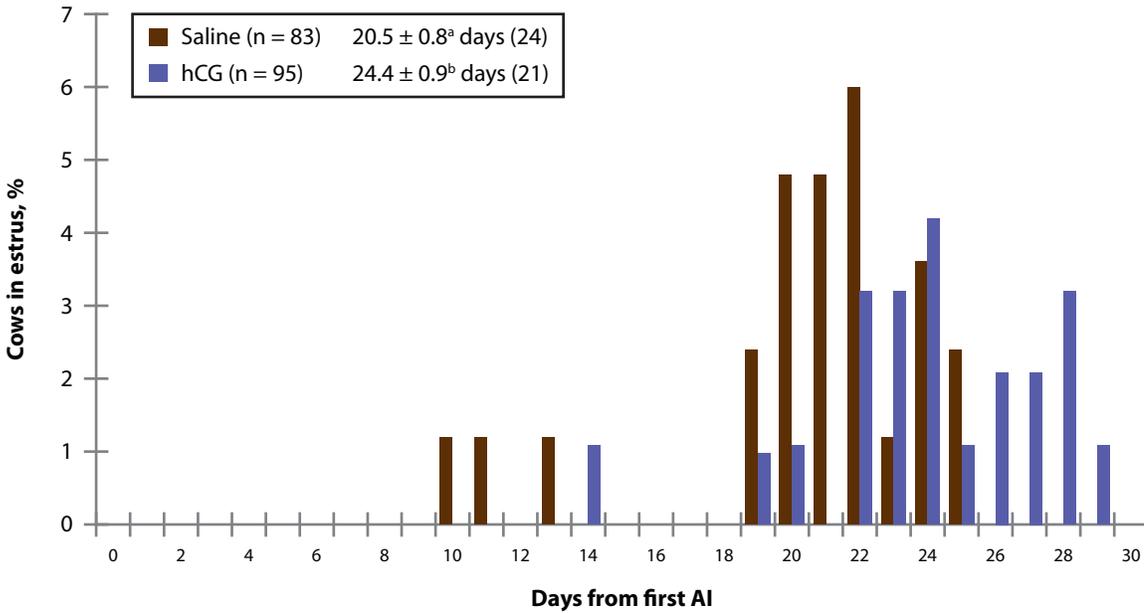
Figure 2. Proportion of cows in estrus after first service after having been timed artificially inseminated (TAI) at first service and received with saline or human chorionic gonadotropin (hCG) on day 7 after the TAI. Duration of cycles is the average reinsemination interval for cows reinseminated up to 30 days after first service.
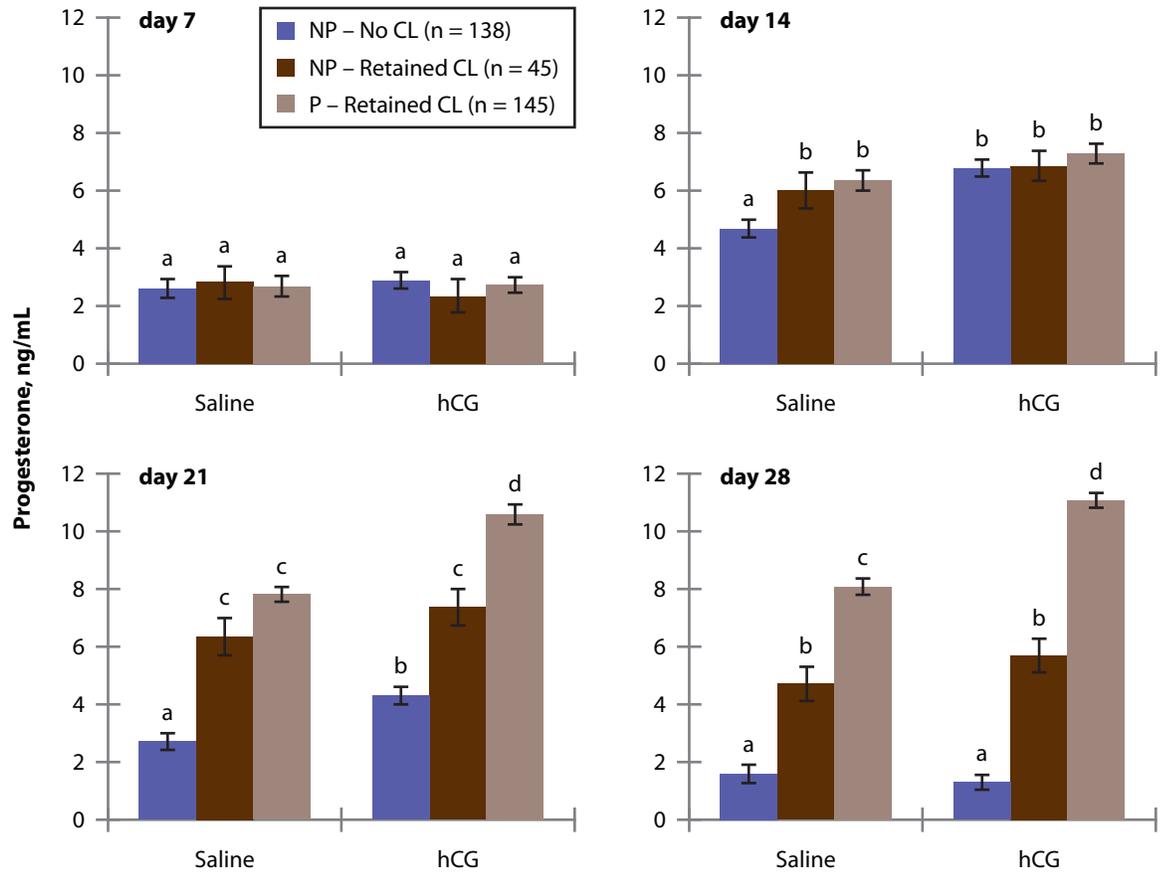
Figure 3. Concentrations of progesterone on days 7, 14, 21, and 28 post-GnRH-AI in cows whose: (1) original corpora lutea (CL) regressed before day 28 after first artificial insemination and were not pregnant (NP) on day 32 (NP – no CL), (2) original CL were retained until day 28 but were not pregnant on day 32 (NP – retained CL), and (3) original CL were retained until day 28 and were pregnant (P) on day 32. Bars with different superscript letters within day differ ($P \leq 0.05$).

Evaluation of Yogurt with Enhanced Cysteine Content

S. Bala and K. A. Schmidt

Summary

Amino acids are the building blocks of protein and assist with metabolism in the body. In the human body, the amino acid cysteine can be synthesized from methionine by the enzyme Υ-cystathionase. Because certain human subpopulations such as those prone to cataracts have decreased Υ-cystathionase activity, dietary cysteine may be beneficial. Nutritionally, yogurt mix is one of the best dairy food sources of methionine and cysteine, but the heat treatment used in manufacturing yogurt decreases the dietary availability of cysteine. Last year, it was shown that supplementing yogurt mixes with whey protein isolate (**WPI**) (>90% protein) and processing yogurt mixes at a lower temperature produced yogurts with increased cysteine. Because the quality or cysteine content of the yogurt during the expected storage life is unknown, this study was conducted to determine if a combination of WPI addition and non-optimal process conditions could produce a yogurt with higher cysteine content and an acceptable shelf life. In this study, control yogurt mixes were made with nonfat dry milk (**NDM**) and processed at 90°C for 7 minutes, whereas the experimental yogurt mixes were made with NDM and WPI and processed at 70°C for 20 minutes. Both mixes were cooled, inoculated, fermented into yogurt, stored at 4°C, and evaluated periodically over a 60-day period. The experimental yogurts had ~2X more cysteine than the control yogurt; this trend was present throughout storage. After 60 days of storage, the water-holding capacity (**WHC**) and firmness was greater and the syneresis was less for the experimental yogurt than the control yogurt. These results show that yogurt supplemented with WPI and processed at less optimal conditions may be a good source of the conditional amino acid cysteine during storage.

Key words: yogurt, whey protein isolate, cysteine

Introduction

In 2011, the USDA reported an 85% increase in yogurt popularity and sales since 2002. This increased popularity may be attributed to the nutritional and health benefits of yogurt, such as improved digestibility and lactose utilization and antagonism toward enteric pathogens. When pasteurized at high temperatures, yogurt mixes have greater whey protein denaturation (**WPD**) but less dietary availability of cysteine. In milk, cysteine is a component of whey proteins. Heat causes whey proteins to unfold, which causes whey and casein proteins to aggregate. This aggregation contributes to high-quality yogurt, which is defined as exhibiting a firm gel, expressing minimal syneresis, and maximizing water-holding capacity (**WHC**). Yogurt firmness and syneresis are therefore related to protein content and WPD, because these properties are functions of the number and strength of the whey protein-casein interactions.

According to the Centers for Disease Control and Prevention (CDC, 2012), the current estimate is that nearly 20.5 million (17.2%) Americans who are 40 years and older have a cataract in one or both eyes; 30.1 million Americans are predicted to have cataracts by 2020. Studies of the occurrences and causes of cataracts have shown that elderly rats (24 to 26 months) had less or no Υ-cystathionase (an enzyme that converts methionine to cysteine) in their eye lenses compared with young rats (5 to 6 months). Other researchers have reported that increased cataract formation was associated with decreased glutathione (**GSH**) contents in human eye lenses.

One of the substrates for GSH synthesis is cysteine (Figure 1); hence, the decreased activity of Υ-cystathionase has been one of the focus in cataract research. These data suggest that people prone to cataracts may benefit from consuming dietary cysteine because it is a precursor for GSH synthesis.

Most commercial yogurts contain 9 to 14% milk solids, which are derived from the milk base and additional milk solids. Previous researchers have reported on the impact of various supplements (nonfat dry milk [**NDM**], whey protein concentrates [**WPC**], or whey protein isolate [**WPI**] in yogurt mixes. Typically, as WPC concentrations increased, yogurt had significantly greater firmness and reduced syneresis. Yogurts containing WPI had an even greater reduction in syneresis and increase in WHC. In our previous research, we formulated yogurts with various levels of WPI and observed greater firmness (1.4X) and cysteine contents (~4.5X), but we used non-optimal process conditions. Although the yogurt was of good quality on day 1, we did not know whether the cysteine or gel quality would be sustained during the expected yogurt shelf life. Thus, this research project was undertaken to: (1) compare protein and cysteine contents in an experiment yogurt mix containing WPI and processed at less optimal conditions to a control yogurt, and (2) assess cysteine contents and gel quality of these yogurts during storage (day 1, 15, 30, 45, and 60).

Experimental Procedures

Low-heat NDM, WPI, and yogurt cultures were obtained from commercial suppliers and maintained at −2 or −10°C (culture) until usage. Two formulations were made: a control (**C**) mix consisting of 12.5% NDM and an experimental (**E**) mix consisting of 10% NDM and 2.5% WPI. Dried dairy powders were rehydrated in deionized distilled water at 22 to 24°C for 30 minutes. The C mix was processed at 90°C for 7 minutes to ensure ~90% WPD, whereas the E mix was processed at 70°C for 20 minutes to minimize WPD (and preserve cysteine). Both mixes were then cooled to 43°C, inoculated with *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus,* packaged into sterile containers, incubated until pH 4.6, and then placed in storage (4 ± 1°C) for up to 60 days.

Standardized, published methods were followed for all analyses, and three replications were performed. Mixes were made and analyzed for protein contents, WPD, and cysteine contents using a randomized complete block design. Stored yogurts were evaluated for cysteine contents, firmness, syneresis, and WHC on days 1, 15, 30, 45, and 60 using a split-plot design. All data were analyzed using SAS (SAS Institute, Cary, NC), and significant ($P < 0.05$) means and interactions were differentiated using Fisher's LSD tests.

Results and Discussion

The C yogurt was formulated and processed like a set-style commercial product. To ensure that the mixes were of different protein composition, mixes were analyzed for protein (true protein and whey) and WPD (Table 1). The E mixes had 32% and 189% more true protein and whey protein, respectively, than the C mixes. Mixes did not significantly differ in casein content (~3.34%). WPD was ~19X less for E mixes than C mixes due to the processing difference. Table 1 also displays the effects of the manufacturing steps (formulation [unheated], processed [heat treatment], and fermentation) on cysteine. The heating step (process) dramatically affected cysteine, with the C mix exhibiting a 65% loss vs. the E mix exhibiting only a 21% loss compared with their respective unheated mixes. Interestingly, fermentation did not significant-

ly affect the cysteine content (the cysteine losses during fermentation were ~3% in both yogurt samples), but the E yogurt required 30 minutes more fermentation time than the C yogurt.

To make an effective delivery vehicle for a compound such as cysteine, the product needs to be acceptable to the consumer not only on day 1, but also throughout storage. On day 1, all yogurts had similar total solids content (12.35%), pH (4.42), syneresis (6.51%), and WHC (22.83%), but the E yogurt was ~2.1X more firm and had ~190% more cysteine than the control yogurt. Compared with a commercial, set-style yogurt (128 g) purchased at a local grocery store, the E yogurt had similar firmness (133 g).

With time, yogurt exhibits syneresis and shrinks away from the package. These two qualities, which are directly related to the whey protein–casein interactions, are considered defects by consumers; hence, the storage stability of this yogurt was evaluated. Results showed significant interactions for gel quality. Overall, firmness of the E yogurt was ~2.1X greater than the C yogurt (Figure 2), and both yogurts exhibited a significant increase in firmness (25%) from day 1 to day 15. The firmness of C yogurt was constant throughout the remaining storage period but the firmness of the E yogurt decreased from day 30 to day 45. The E yogurt exhibited 76% less syneresis than the C yogurt on day 1, and both yogurts decreased in syneresis from day 1 to day 15 (Figure 3), but yogurt syneresis remained constant thereafter. On day 1, the E yogurt had greater WHC than the C yogurt. The WHC of the E yogurt remained constant throughout storage, but the C yogurt increased in WHC (21%) from day 1 to day 15 and eventually decreased to its initial value at day 60 (Figure 4). More importantly, the E yogurt had greater cysteine content (398.4 mg/L) than the C yogurt (135.7 mg/L), and the cysteine content was not affected by storage time, which suggests that greater cysteine content would be stable and available throughout the storage life. These results indicate that WPI supplementation of yogurt mix combined with less optimal process conditions may produce a yogurt that effectively delivers cysteine.

Conclusions

In yogurt, cysteine content is a function of the type and amount of milk protein and the heat treatment of the mix. A yogurt made from mix with WPI and processed at less optimal conditions was shown to have greater cysteine content and acceptable gel quality. Further research involving sensory properties of this yogurt and investigation of how enhanced cysteine in yogurt affects GSH production in tissue cultures should be pursued.

Table 1. True protein and whey protein contents, whey protein denaturation (%) of yogurt mixes, and cysteine contents as a function of process treatments (means ± standard error)

	Composition	Control[1]	Experimental[2]
Mix	True protein %	4.17[b]±0.07	5.51[a]±0.02
	Whey %	0.77[b]±0.01	2.23[a]±0.02
	WPD[3,4] %	70.28[a]±0.73	3.45[b]±0.88
Manufacturing step	Cysteine (mg/L)		
	Unprocessed mix	306.98[C]±1.65	504.96[A]±2.59
	Processed mix[4]	138.52[D]±4.21	400.15[B]±12.08
	Fermented	135.74[D]±4.64	398.32[B]±19.79

[a,b] Means (n=3) within rows with different lower case superscripts differ ($P < 0.05$).
[A-D] Means (n=3) with different upper case superscripts differ ($P < 0.05$).
[1] Nonfat dry milk (NDM) (12.5%).
[2] NDM (10%) + whey protein isolate (WPI) (2.5%).
[3] Whey protein denaturation.
[4] Control processed at 90°C for 7 minutes and experimental processed at 70°C for 20 minutes.
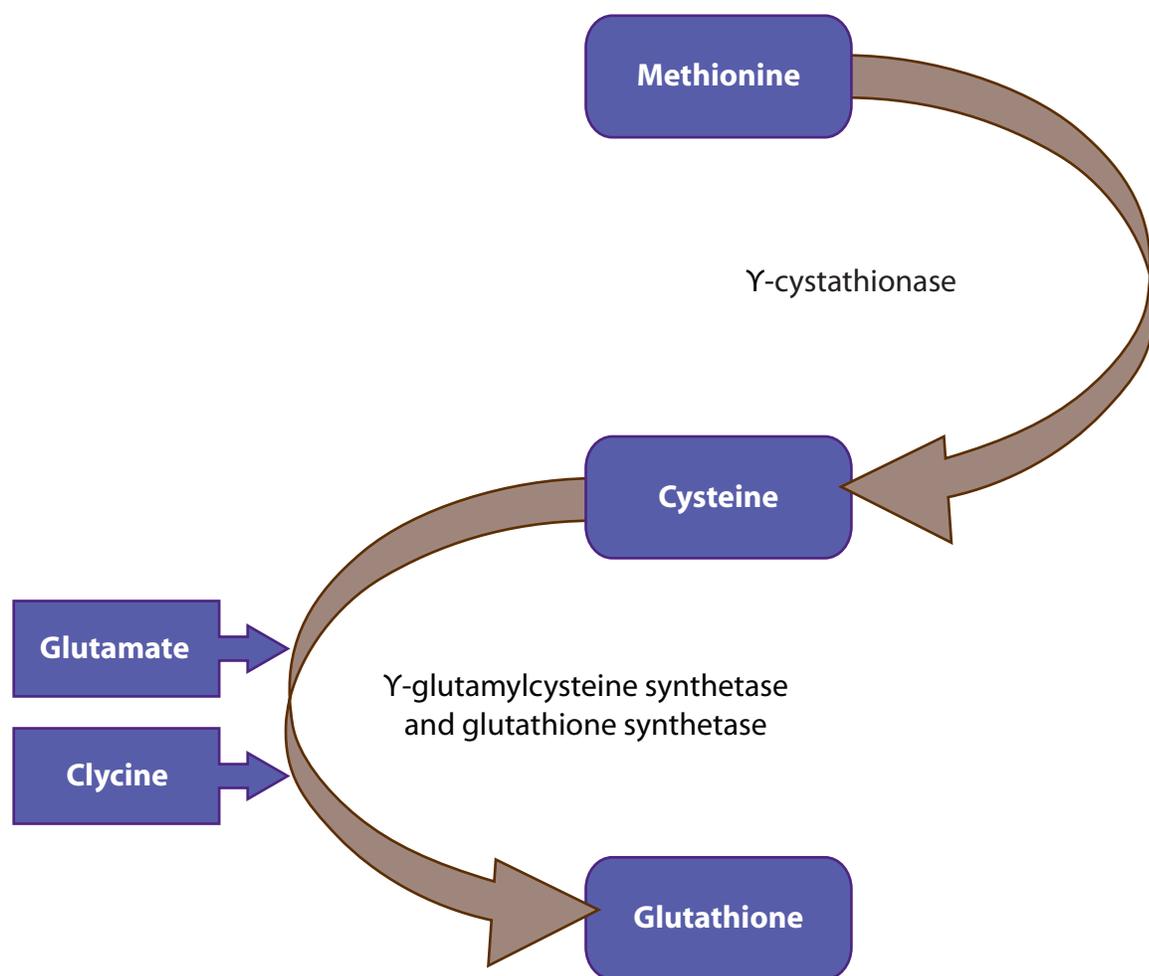
Figure 1. Glutathione synthesis.
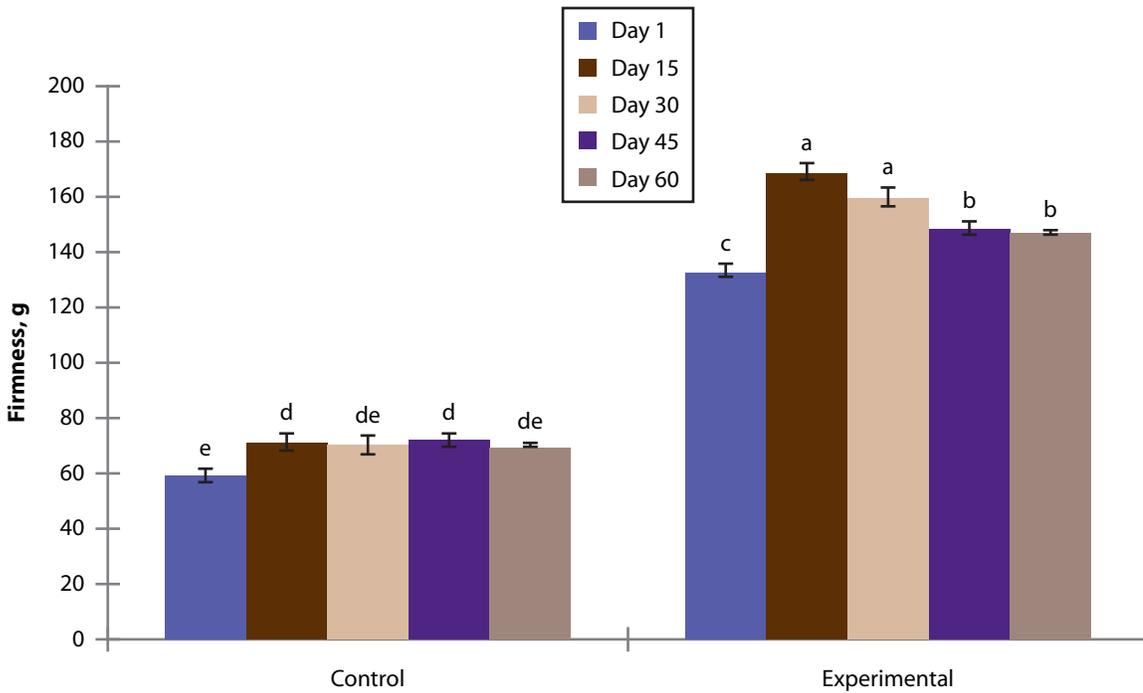
Figure 2. Yogurt firmness during 60 days of storage. Control: nonfat dry milk (NDM) (12.5%) processed at 90ºC for 7 minutes. Experimental: NDM (10%) + whey protein isolate (2.5%) processed at 70ºC for 20 minutes.

[a-e] Bars with different superscripts differ ($P < 0.05$).
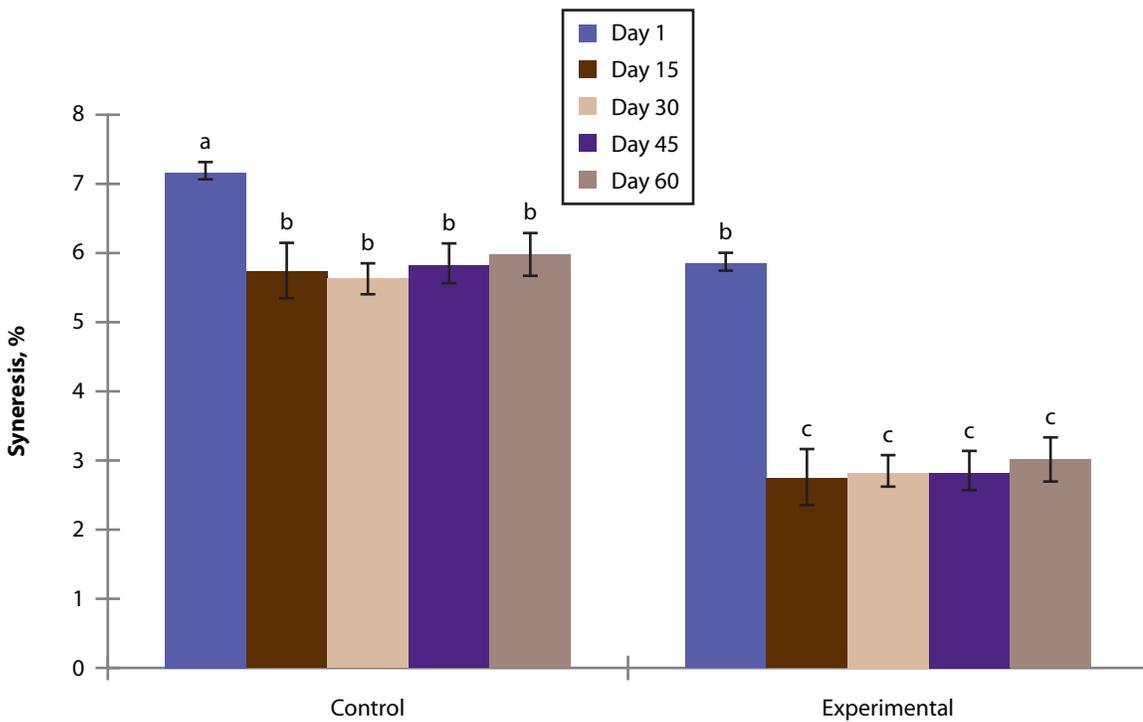
Figure 3. Yogurt syneresis during 60 days of storage. Control: nonfat dry milk (NDM) (12.5%) processed at 90ºC for 7 minutes. Experimental: NDM (10%) + whey protein isolate (2.5%) processed at 70ºC for 20 minutes.

[a-c] Bars with different superscripts differ ($P < 0.05$).
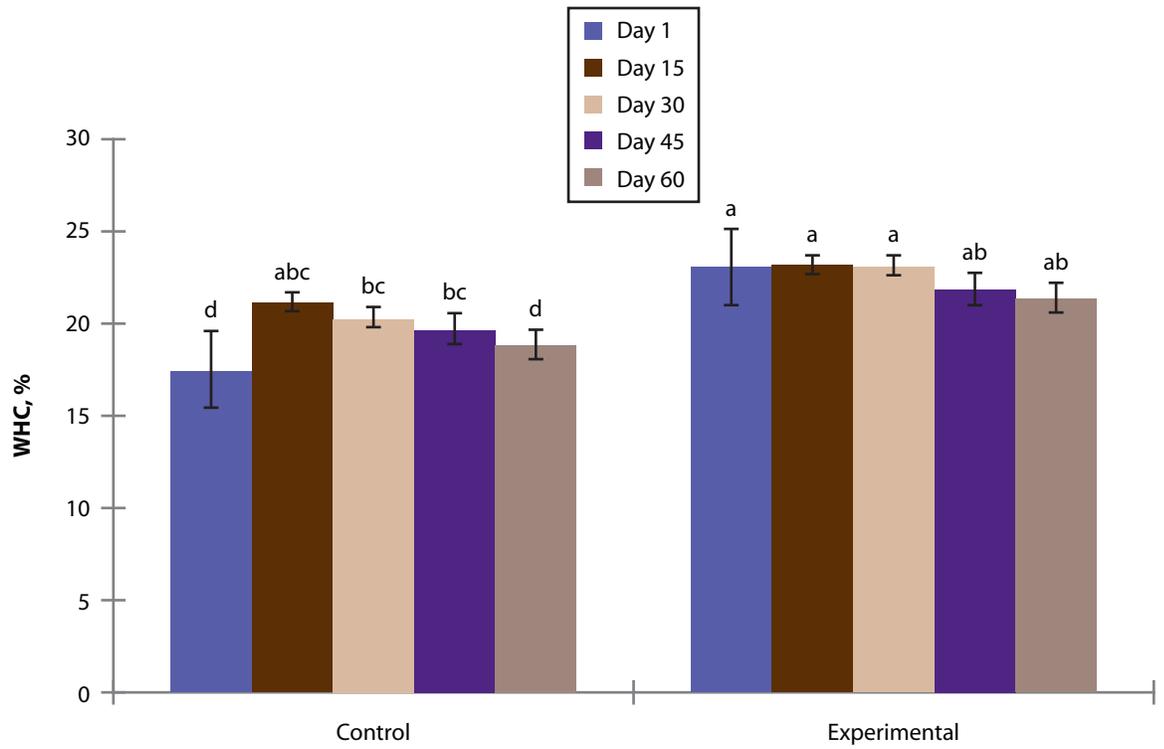
Figure 4. Yogurt water-holding capacity (WHC) during 60 days of storage. Control: nonfat dry milk (NDM) (12.5%) processed at 90ºC for 7 minutes. Experimental: NDM (10%) + whey protein isolate (2.5%) processed at 70ºC for 20 minutes.

[a–c] Bars with different superscripts differ ($P < 0.05$).

Acknowledgments

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