DAIRY TEAM
KANSAS STATE UNIVERSITY

Dairy Research 2018

K-STATE
Research and Extension

Kansas State University Agricultural Experiment Station and Cooperative Extension Service

DAIRY RESEARCH 2018

Contents

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Foreword

Kansas State University is pleased to present the 2018 Dairy Research Report of Progress and proud to serve the growing Kansas dairy industry. During the past 5 years (2012 to 2017), total milk production in Kansas has increased by 28%, behind only Colorado and South Dakota among major dairy states. This growth is a product of a 21% increase in the number of cows as well as a 6% increase in milk per cow. At the end of 2017, 152,000 Kansas cows averaged 23,000 lb per lactation, ranking the state 16th in total milk production (3.5 billion lb). Kansas now has 290 dairy operations and averages 524 cows per herd (*Hoard's Dairyman*, March 25, 2018, pp 192-193). Adding to the growth in milk production in Kansas over the past decade is the recent surge in processing capacity in the state, resulting in a dynamic industry that is an increasingly important driver of economic activity.

Selected production traits of our Kansas State University Dairy Teaching and Research Center (DTRC) herd are shown below. The excellent functioning of our herd is largely a tribute to the dedication of our staff: Michael Scheffel (manager), Robert Feist, Alan Hubbard, Kris Frey, Eulises Jiron Corrales, Morgan Loomis, Alexandrea Eckert, Cory Sunderman, and Tony Hecht. In particular, we recognize Daniel Umscheid, who retired this year after 38 years of service to the DTRC. Special thanks are given to Haixia Liu 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) for its assistance in handling research milk samples.

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

Cows, total no.	277
Rolling herd milk, lb	31,441
Rolling herd fat, lb	1,150
Rolling herd protein, lb	918
Somatic cell count × 1,000	112
Calving interval, mo.	13.0

[1]November 6, 2018 test day (milking 2 to 3 times daily).

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

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

B.J. Bradford, Editor
2018 Dairy Research Report of Progress

Development of a High Protein Dairy Snack Based on German-Style *Quark* Cheese

Y. Ou, K.S. Babu, P. Thorakkattu, K. Getty, and J.K. Amamcharla

Summary

Current consumer trends highlight the need for developing convenient and ready-to-eat snack foods that render health benefits when consumed. Rising popularity of protein-enriched foods and beverages have led to increasing awareness among consumers of health benefits related to a dairy-rich diet. The vast majority of people have adopted this lifestyle, spotting the protein's importance in delivering vital nutrients for health and maintenance of the body, curbing hunger, sustaining a slow release of energy, and speeding the metabolism. Innovation is the key driver for the dairy snack market. The primary objective of this project was to develop an American-sourced predominantly dairy (>51%) snack which meets both the current snacking trends and REAL® Seal requirement. The research also focused on assessing the acceptance of a dairy snack ("Quick-Quark") formulated with German-style quark cheese. An acceptance test with consumers using a 9-point hedonic and a "just-about-right" scale showed that flavor, mouthfeel, and texture attributes were within the liking rate of consumers. Furthermore, the higher protein content in the Quick-Quark gives this product an added value that may have a great influence on consumers' preference.

Introduction

The World Health Organization recommends the formulation of innovative products that represent healthy and nutritious choices for consumers. Currently, consumers prefer foods with health benefits and at the same time, convenience for consumption, storage, and handling. Indeed, ready-to-use products are highly appreciated for their convenience. In general, snack sales are rising, and rising demand for dairy snacks in emerging countries coupled with the launch of new snack variants has resulted in increased demand for global dairy snacks. Dairy snacks such as yogurt and milk protein drinks increase consumers' reported energy levels. Additionally, dairy snacks may provide health benefits to the consumers and provide taste, convenience, and affordable price, the most influential factors driving demand for dairy-based snacking.

The idea of Quick-Quark was conceptualized based on Dairy Management Inc./National Dairy Council® (DMI/NDC) consumer and marketplace insights. According to the report, 56% of snacking was based on daily sustenance, long-term wellness, and health management; 49% of snacking was based on enjoyment, craving, and comfort; and 34% was based on physical and mental performance demands. Additionally, 96% of consumers chose snacks based on product taste. The new statistics of the market show a changing mindset of the consumers that diets rich in full-fat dairy are healthier than the low- and reduced-fat dairy products. Additionally, according to the Information Resources, Inc. (IRI) Snacking Report, 64% of consumers state they sometimes have a beverage as a snack. Thus, Quick-Quark could bridge the gap between meals or even replace meals for some, while providing required energy with comfort flavors as a drinkable, indulgent high protein dairy-based snack.

Product Description

Quark is a snowy white-colored and creamy unripened cheese tracing its origin to German-speaking, Slavic, northern and eastern European countries. It has a very subtle taste with a smooth, relatively light, and soft texture. Currently, there is no standard of identity of quark as per FDA regulations. Traditionally, quark is made with mesophilic bacteria and requires a lower fermentation temperature and longer fermentation time than the thermophilic yogurt bacteria. Fermentation continues until the pH reaches ~4.6, which causes coagulation of the casein proteins. Generally, quark is classified as a fresh acid-set cheese, though in some countries it is traditionally considered a distinct fermented milk product. It is soft, white, and un-aged with no added salt.

Quick-Quark™ Creamy German-Style Drinkable Quark is a REAL® Grab 'N' Go convenient and healthy dairy snack produced for the United States market, which offers today's snacking consumers a nutrient-dense indulgent moment (Figure 1). This snack is predominantly (90% of weight in the finished product) made with dairy ingredients, including whole milk, cream, milk protein concentrates (MPC), and sweetened condensed milk. Quick-Quark offers a "superfood-blend" açaí blueberry flavor and a piña colada flavor, both made with real fruits. Quick-Quark is packed in a flexible, lightweight spout pouch. Each 150-gram serving of Quick-Quark provides 200 to 220 calories and is an excellent source of dairy protein (14 grams or 28% of recommended daily value [DV]) and calcium (30% DV).

Drinkable quark meets consumers' demands for "on-the-go," "minimally processed," and "inherent nutrients," as a "clean label" REAL-Seal product with live cultures that does not contain any artificial preservatives, flavors, or colors. According to the U.S. Food and Drug Administration's (FDA) Industrial Guidance, Quick-Quark is a healthy snack, as each serving contains a good amount of vitamin D (10% DV) and calcium, both of which are nutrients of public health concern (the product also inherently contains 4.5 grams of saturated fat). Though the body of the spout-pouches are covered to reduce light exposure, the bottom of the package is transparent, which provides consumers the ability to observe the real fruit puree and attractive color of Quick-Quark. Resealable caps and slim-fit design of flexible pouches are a perfect fit for grab-and-go at convenience, grocery, foodservice, and other retail stores.

Feasibility of Production and Process Description

Industrial processing of Quick-Quark (Figure 2) is organized in three main steps: 1) preparation of dairy ingredient mixture for fermentation, which includes hydration of MPC and standardization, homogenization, heat treatment/pasteurization, and cooling of the quark mixture; 2) fermentation process using mesophilic culture; and 3) centrifugation/harvesting quark, cooling, addition of other ingredients (fruits, sugar, and sweetened condensed milk), and packaging. Each manufacturing step influences the final product quality. In order to obtain the desired mixture for fermentation, quark base preparation involves mainly fat and protein content standardization. Protein standardization aims at increasing protein content in order to improve functional properties of quark. In collaboration with a commercial manufacturer, calcium-reduced MPC is used in the process to further improve stability during storage. The cream is also added before fermentation to improve flavor and mouthfeel, and as an alternative, it can also be added post-fermentation to reduce fat loss during centrifugation.

One of the most important requirements of Quick-Quark's production is to control the temperature at every stage, so the final product is safe and enjoyable. Indeed, heat treatment at 185°F contributes to an improved quark texture by causing whey protein denaturation and its interaction with casein, resulting in decreased gel syneresis and increased gel firmness. A plate heat exchanger with a tubular holding zone is used that was designed to accurately cool the mixture to fermentation temperature (86°F).

The final pH and acidification rate are key factors to influence the quality of quark. Post-standardization, quark mixture is acidified to pH ~4.6 through long set incubation (10-12 h) using mesophilic cultures (*Streptococcus lactis* subspecies *lactis* and *cremoris*) to obtain the quark base. Fermentation is terminated by rapid cooling when the final pH reaches 4.6. Final texture and consistency of quality are critical for consumer acceptance for a drinkable product. The texture is influenced by factors such as mixture composition, starter cultures, and processing conditions. Additional ingredients including sweetened condensed milk, fruits, natural flavors, and colors are added and mixed into the quark base before aseptic filling, packaging, and labeling. All equipment used for milk storage, mixture preparation, fermentation, cooling, centrifugation, and holding of products is designed to allow clean-in-place procedures commonly used in the dairy industry. After the addition of pasteurized fruit puree and other ingredients, Quick-Quark is aseptically filled into polyethylene terephthalate-nylon-linear low-density polyethylene multi-layered flexible stand-up spout-top pouches with low-density polyethylene screw caps. Product shelf life is estimated as 30 days under refrigerated conditions. Figure 3 demonstrates several stages of making drinkable quark on the benchtop, and the photograph of the product is shown in Figure 4.

Sensory Evaluation

Sensory evaluation for Quick-Quark prototypes was tested in two stages. First, a preliminary study was conducted to test the concept of a drinkable quark snack using a consumer focus group (n = 12, 5 male and 7 female, ages 20 to 56). Each panelist evaluated three products: plain, fruit-flavored (Tropical Explosion, which included strawberry, pineapple, and mango), and fun flavor (Dulce de Leche). The focus group liked the concept of Quick-Quark. However, they suggested the flavors were not appealing. In the second stage, final formulations (both Açaí Blueberry and Piña Colada flavors) were served in a random order to a consumer panel (n = 50, 19 male and 31 female, ages 17 to 55). In both stages, demographic questions were included, in addition to 9-point hedonic scales (1 = extremely dislike and 9 = extremely like) on product attributes and packaging prototype.

Additionally, a 9-point just-about-right (JAR) scale on sweetness level and questions regarding purchasing intention were added to the second stage. Quick-Quark received very positive feedback (between 6.22 and 7.68 for all attributes) in both studies (preliminary sensory data not shown; Figure 5 shows the consumer panel results). Consumers described Quick-Quark in the open-ended questions as "smooth" and "creamy" texture and mouthfeel, "great" and "not too acidic" flavor, "very nice" aroma, and they described the packaging concept as "easy to use," "on the go," "about the right size for a snack," and "fulfills my need." Even though the label and nutritional information were not provided to the consumer panel, on average, consumers were willing to pay a premium of $2.82 per pouch against a $0.75 to $0.93 ingredient and packaging cost.

Some consumers were extremely excited after they realized the product was quark and each 150-gram pouch contain 14 grams of dairy protein along with Açaí or Piña Colada flavors. More than once we heard, "Why is this product not in the market right now," and "When will this product be available?"

Conclusions

The idea of Quick-Quark was conceptualized based on the DMI/NDC consumer and marketplace insights. While considering the market screening and the convenience as a snack, the group decided to design a high-protein Grab 'N' Go drinkable quark with a clean label using whole milk, cream, and milk protein concentrate in two flavors (Açaí Blueberry and Piña Colada). Quick-Quark is a competent snack due to its high nutrition, ease to consume, and popular flavors. The group was able to complete the development process by methodically evaluating the production process. Sensory evaluation panels deemed the product acceptable or better, suggesting Quick-Quark has potential to be a profitable dairy product.

A

Nutrition Facts

1 servings per container

Serving size **1 Pouch (150g)**

Amount Per Serving

Calories **220**

	% Daily Value*
Total Fat 8g	**10%**
Saturated Fat 4.5g	**23%**
Trans Fat 0g	
Cholesterol 30mg	**10%**
Sodium 100mg	**4%**
Total Carbohydrate 22g	**8%**
Dietary Fiber 0g	**0%**
Total Sugars 22g	
Includes 19g Added Sugars	**38%**
Protein 14g	**28%**
Vitamin D 2mcg	10%
Calcium 365mg	30%
Iron 0mg	0%
Potassium 195mg	4%

*The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

B

Nutrition Facts

1 servings per container

Serving size **1 Pouch (150g)**

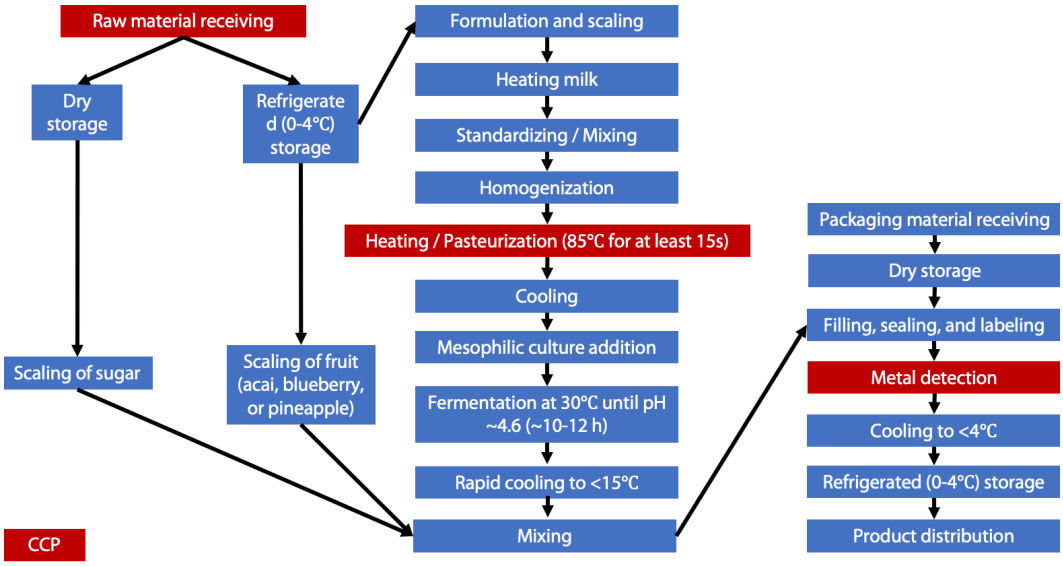
Amount Per Serving

Calories **200**

	% Daily Value*
Total Fat 8g	**10%**
Saturated Fat 4.5g	**23%**
Trans Fat 0g	
Cholesterol 30mg	**10%**
Sodium 100mg	**4%**
Total Carbohydrate 19g	**7%**
Dietary Fiber 0g	**0%**
Total Sugars 19g	
Includes 16g Added Sugars	**32%**
Protein 14g	**28%**
Vitamin D 2mcg	10%
Calcium 364mg	30%
Iron 0mg	0%
Potassium 210mg	4%

*The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Figure 1. Nutritional label for the (A) Açaí-Blueberry and (B) Piña Colada Quick-Quark.



*No Rework

Figure 2. Industrial manufacturing flow chart of Quick-Quark.

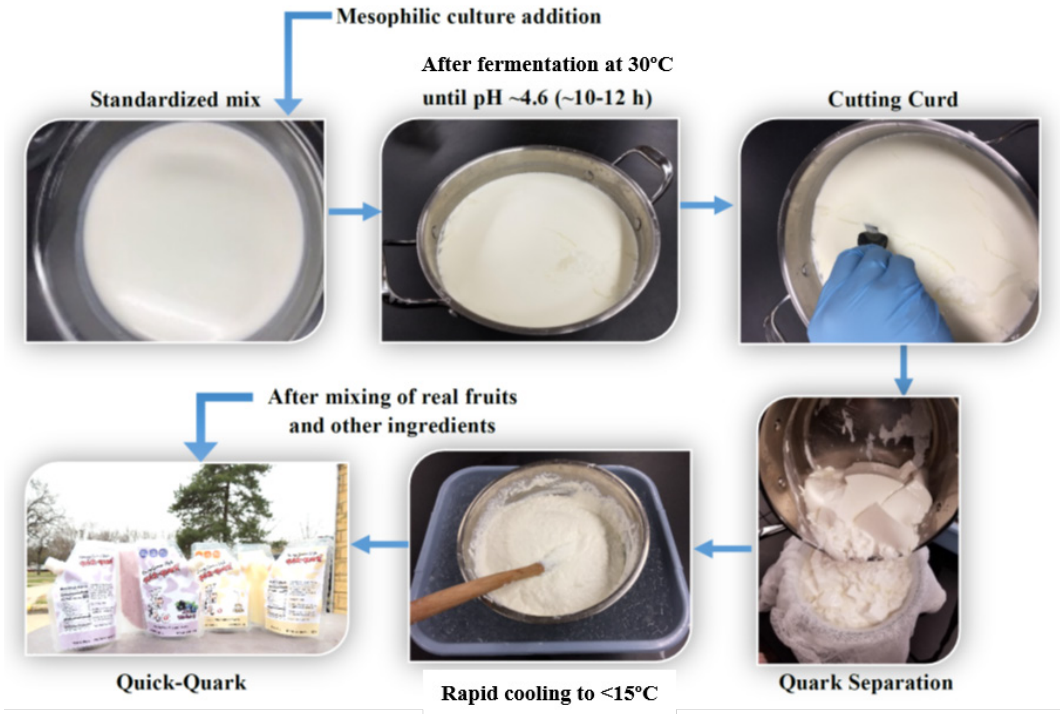


Figure 3. Preparation of quark base for the Quick-Quark.

Figure 4. Photograph of the Quick-Quark products.

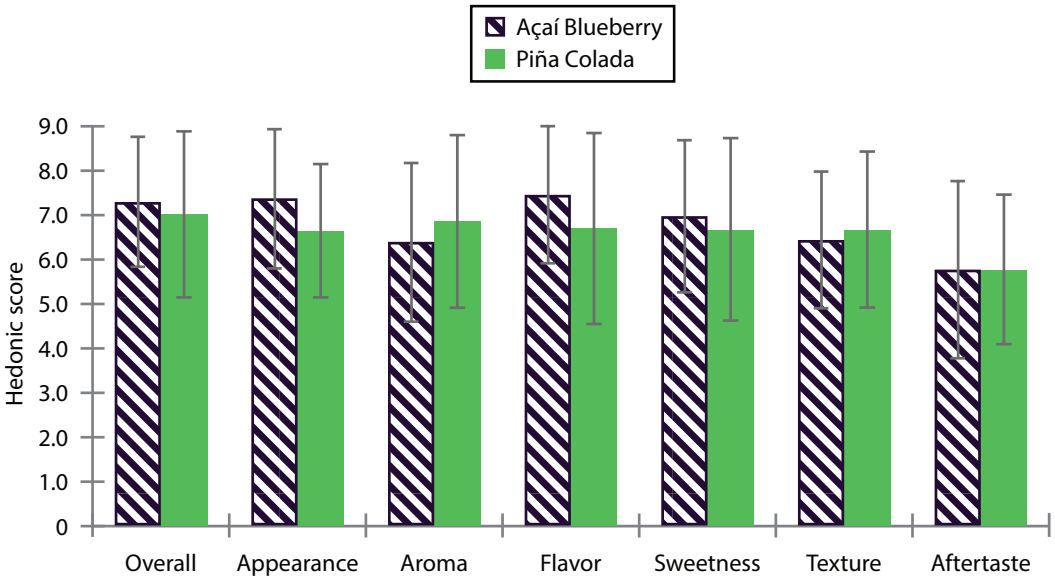


Figure 5. Sensory analysis results for the Quick-Quark products.

Effect of Draining Volume on the Yield and Enrichment Ratio During Foam Fractionation of Greek Yogurt Whey

Y.B. Ma and J.K. Amamcharla

Summary

Foam fractionation was evaluated as a cost-effective method to add value to Greek yogurt whey (GYW), a co-product of Greek yogurt manufacturing. Two separate batches of GYW were obtained from a Greek yogurt manufacturer. Whey proteins present in GYW can be concentrated and manufactured as food ingredients using a low-cost foam fractionation. The objective of this study was to apply foam fractionation with different draining volumes to GYW and evaluate its enrichment and yield of whey protein. A benchtop foam fractionation setup was built in-house, and three different foam draining volumes were used to identify the optimal processing parameters for foam fractionation of GYW. All three levels of draining volume provided enrichment of whey protein in the foamate fraction, and the 33% draining volume resulted in the highest enrichment ratio of 1.59. The yield of the foam fractionation ranged from 41 to 52%, and several improvements can be implemented to increase the yield of the process, including the use of surfactants and enzymatic hydrolysis.

Introduction

The production of Greek-style yogurt in the U.S. market has increased to 770,000 metric tons in the year 2016. Greek yogurt is generally manufactured by removing a portion of the water and water-soluble components from yogurt resulting in GYW as a co-product. Greek yogurt whey is a dilute aqueous solution containing lactose, proteins, minerals, non-protein nitrogen compounds, and organic acids. The characteristic low pH and high mineral content of GYW make it difficult to process using normal dairy unit operations. In addition, Greek yogurt whey is difficult to process using traditional processing methods since it contains a relatively high amount of lactic acid and galactose. GYW. Greek yogurt whey, unlike cottage cheese whey, is lower in value due to its lower protein and lactose contents. Current disposal methods for GYW include land application, bioreactors, and animal feed, but each of these methods has its own limitations. A low-cost value addition process for fractionating the valuable components from GYW is needed.

Recently, researchers have developed a method to enzymatically convert lactose in GYW into glucose and galactose to produce a syrup-like ingredient. The new ingredient can be used as a replacement for high-fructose corn syrup, an ingredient that is essential for the beverage industry. Also, various fermentation techniques have been examined to produce energy or health health-promoting compounds and antimicrobials, such as organic acids.

While most of the current research in on the area of utilization of GYW has focused on lactose hydrolysis, the protein content in GYW can also be a component of interest due to the growing market for whey proteins. Because of the low protein concentration

and high acidity, concentrating protein from acid whey using membrane processing would lead to long processing time and high energy input, making the process unsustainable for industrial production. One alternative protein concentration technique is foam fractionation. Foam fractionation is a process in which surface-active materials are removed by air flotation to form foam. Using the density difference between the bulk liquid and the foam, the protein-enriched foam can be separated gravimetrically to produce foamate (collapsed foam) with increased protein content. The objectives of the study were to design and develop a novel foam fractionation systems and evaluate the efficiency of protein enrichment in GYW at different draining volumes.

Experimental Procedures

Two batches of GYW were procured from a commercial Greek yogurt manufacturer within the United States. The GYW was shipped frozen to Kansas State University and thawed completely before use. The foam fractionation setup was designed and fabricated in the Dairy Foods Laboratory of Kansas State University and a schematic view is shown in Figure 1. The foam fractionation setup consisted of a reservoir (1 liter), a diaphragm pump, a venturi-type air injector, a draining column, and a foamate outlet. In this study, three different draining column volumes were used to evaluate the efficiency of the foam fractionation. Three levels (low, medium, and high) of draining volume included 25% (0.25 liters), 33% (0.33 liters), and 50% (0.50 liters) of the reservoir volume . The GYW was recirculated within the reservoir using the pump via the air injector to incorporate air in the GYW as it was pumped, and consequently creating foam. As the draining column was completely filled with foam and overflowed, the draining foam was collected in a beaker. The foam fraction collected in the beaker is referred to as foamate and contains more surface-active compounds (such as protein) than the initial GYW. The foam fractionation was terminated when there was no foam coming out of the draining column for 5 min. The foamate and retentate were collected separately, weighted, and analyzed for protein content.

The total protein content of GYW, foamate, and retentate was analyzed using the Kjeldahl method. The protein concentration was calculated using the nitrogen conversion factor of 6.38. Enrichment ratio and protein yield (%) were used to evaluate the foam fractionation efficiency and calculated using equations 1 and 2, respectively.

$$\text{Yield, \%} = \frac{\text{Mass of protein in the foamate}}{\text{Mass of protein in the GYW}} \times 100 \quad [1]$$

$$\text{Enrichment ratio} = \frac{\text{Concentration of protein in the foamate}}{\text{Concentration of protein in the GYW}} \quad [2]$$

Results and Discussion

Two lots of GYW were analyzed by using the Kjeldahl method for average protein content, and results are provided in Figure 2. The GYW protein concentration served as the reference to examine foam enrichment of protein in the foamate. As shown in Figure 2, after foam fractionation, the protein concentration in foamate was greater than the retentate, showing an enrichment of protein in the foamate fraction. The results supported the hypothesis of this study that foam fractionation can enrich protein from GYW. Other studies have shown that a foam fractionation process is more efficient at

low protein concentrations, ranging from 0.01 to 0.1%. Therefore, GYW may have the advantage to better utilize foam fractionation compared to sweet whey, which contains more protein and is consequently less efficient for foam fractionation. Also, the low pH of GYW can also promote foam fractionation, as some research has shown that extracting proteins close to their isoelectric point enhances the foamability of the proteins. Greek yogurt whey contains alpha-lactalbumin and beta-lactoglobulin, and the two proteins have isoelectric points of 4.4 and 5.2, respectively, which is close to the pH of the GYW generated from Greek-yogurt production.

The protein concentration in the foamate was found to be different based on the draining volume, and both medium and high draining volume showed significant differences in protein concentration compared to the original GYW ($P < 0.05$). The greatest difference appeared in the medium draining volume, indicating it was the optimal for protein enrichment. Table 1 shows that the final foamate volume is similar among three different draining volumes, meaning that the amount of foamate generated from GYW may be related to the protein type and concentration, rather than the draining volume. However, in future studies, the amount of foamate generated from acid whey should be studied as it can impact the final yield of the protein enrichment in the foamate. Possible factors that may impact foamate generation include surfactants, protein concentration, and the pH of the acid whey.

We also observed that the medium draining volume had the greatest enrichment ratio of 1.59, followed by 1.33 from high draining volume and 1.11 from low draining volume (Table 1). The enrichment ratio is another representation of the protein concentration shown in Figure 2. It indicated that foam fractionation can increase the protein concentration in foamate by 1.59 times. Compared to other studies with acid whey, the enrichment ratio was within the reported ranges. The direct fractionation of GYW without pretreatment made the technique robust and easy to adapt in existing processing plants. For future work, lactose content in the foamate can be measured to determine the purity of the enriched whey protein. With relatively high purity whey protein, the protein enriched fraction can be further processed into usable food ingredients, such as protein powders.

The percent yield and enrichment ratio from the foam fractionation of GYW is provided in Table 1. The medium draining volume (33%) gave a yield of 52.1%. The yield from this study was not as high as some other foam fractionations of whey protein. However, use of some surfactants in the feed could potentially increase the yield. The surfactants can interact with both protein and air, and consequently can improve the fractionation yield. Several studies have used food-grade surfactants to increase the yield of foam fractionation. Another method to increase yield could be enzymatic hydrolysis. Similar to the isoelectric point approach, partial hydrolysis of the whey protein can unfold the protein and increase the structure flexibility. It has been shown that whey protein hydrolysate can have improved foamability compared to the unhydrolyzed whey protein. The modification of whey protein in GYW can potentially improve the fractionation yield.

Conclusions

In this study, GYW was foam-fractionated to enrich whey protein concentration. The foam fractionation of GYW enriched the protein content in the foamate. With different draining volumes, the medium (33%) draining volume showed the best enrichment ratio (1.59) and yield (52.1%). The technique provides a novel processing method to add value to GYW into potential food ingredients. Improvement of fractionation yield and purity of protein in the foamate should be further studied.

Table 1. Final foamate volume, enrichment ratio, and yield of protein from foam fractionation of acid whey using different draining volumes

Draining volume	Foamate volume (mL)	Enrichment ratio	Yield %
Low	326	1.11	42.8
Medium	309	1.59	52.1
High	298	1.33	40.9

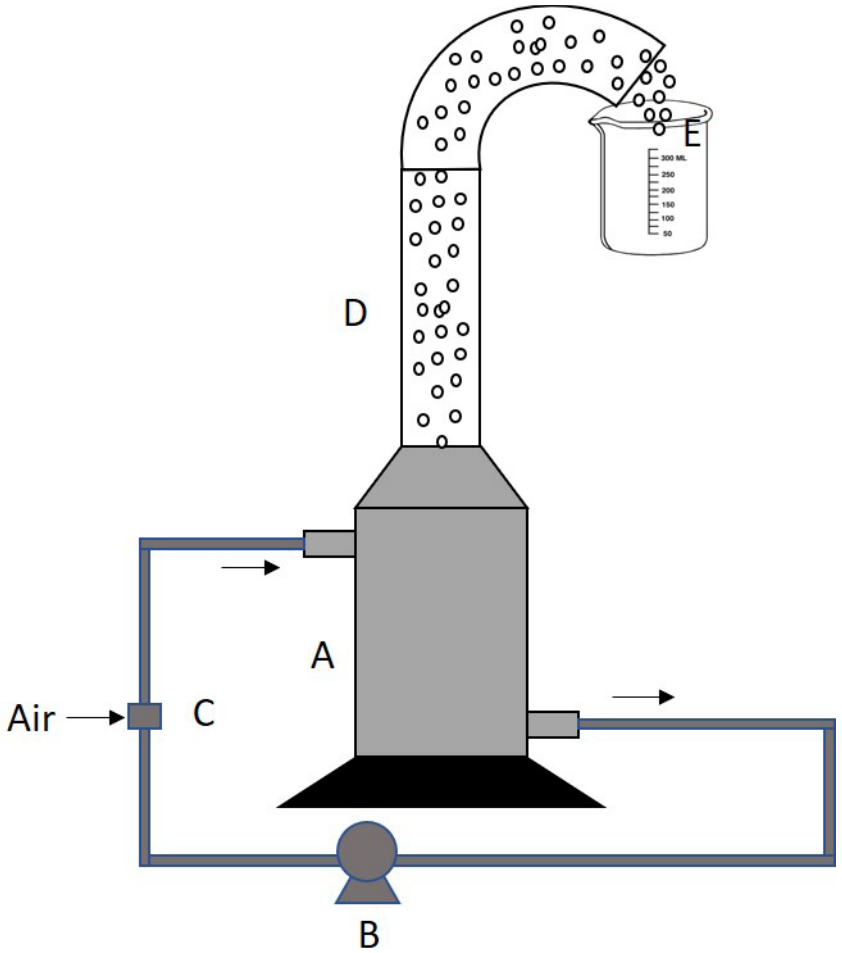


Figure 1. Schematic representation of the foam fractionation setup. A: acid whey reservoir (1 L); B: external pump; C: venturi-type injector; D: draining column; and E: foamate outlet.

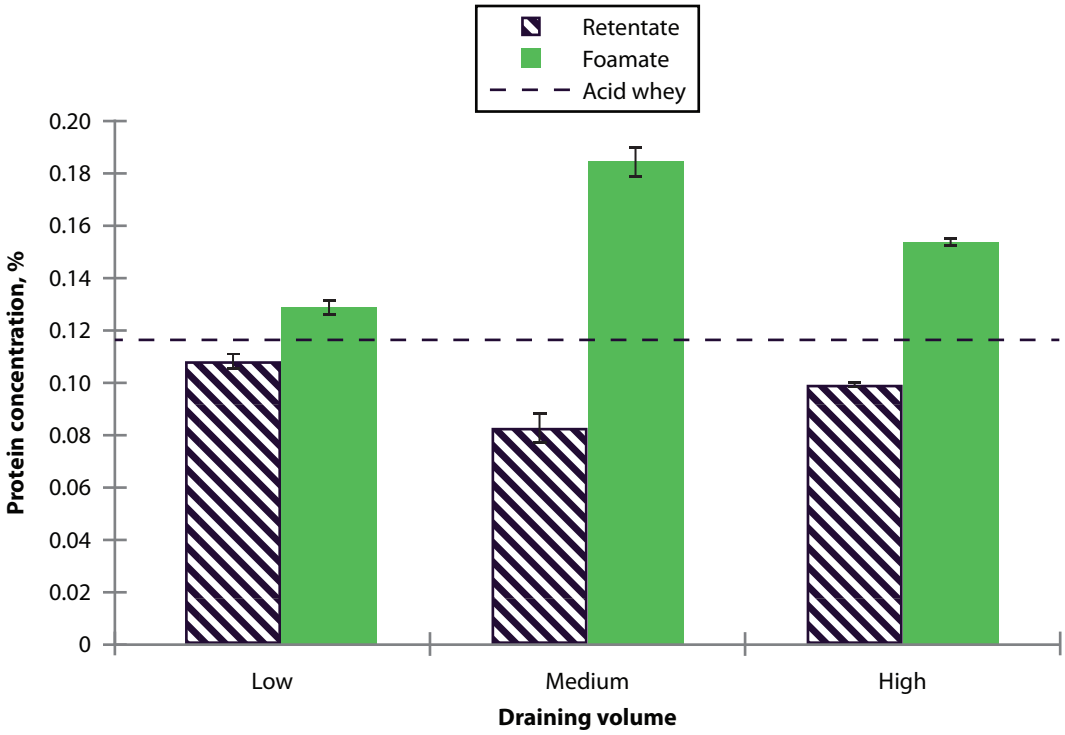


Figure 2. Protein concentration changes after foam fractionation with different draining volumes. The asterisk indicates the significant protein concentration increase in foamate within each draining volume (n = 2, $P < 0.05$).

Physiologic Responses to Feeding Rumen-Protected Glucose to Lactating Dairy Cows

J.A. Sauls-Hiesterman, S. Banuelos, B. Atanasov, B.J. Bradford, and J.S. Stevenson

Summary

Lactating Holstein cows were enrolled in a study beginning before first insemination. Cows were supplemented with a rumen-protected glucose (RPG) product to test the hypothesis that circulating progesterone concentrations could be increased by increasing blood glucose, which causes an increase in insulin, subsequently decreasing progesterone clearance by liver enzymes. Supplementation occurred at 0, 2.2, 4.4, or 8.8 lb per head per day to test a dose response. Treatment began 3 days before ovulation and continued until day 12 of the estrous cycle. Rumen-protected glucose did not impact serum concentration of glucose before or after feeding, but the change in insulin concentration (post-feeding – pre-feeding) was greater for the control cows compared with cows that received the three doses of RPG. Crude protein (CP) intake and milk urea nitrogen (MUN) increased linearly with treatment, but dry matter intake (DMI) and milk yield were unaffected by treatment. Concentrations of progesterone were unaffected by treatment, and pregnancy risk at first insemination was reduced by treatment. Rumen-protected glucose failed to increase serum insulin or progesterone concentrations.

Introduction

Progesterone is essential for maintenance of pregnancy and inhibits estrus expression in dairy cows. Peripheral concentrations of progesterone are affected by both milk yield and the rate of metabolism in the liver. Selection for increased milk yield during several decades has resulted in greater milk yield and the necessity to feed cows less roughage and higher-energy diets to support milk synthesis. High-energy diets, in turn, chronically increase liver blood flow, resulting in increased clearance of progesterone. Clearance of progesterone reduces peripheral concentrations of progesterone available to reproductive tissues, and the rate at which this occurs is a function of liver blood flow and activity of liver enzymes, particularly cytochrome P450 2C and cytochrome P450 3A. Therefore, decreasing liver enzymatic activity may increase peripheral concentrations of progesterone.

Previous research has shown that feeding a high-starch diet causes an increase in insulin production, resulting in decreased activity of liver cytochrome P450 enzymes. Insulin, a metabolic mediator between nutrition and reproduction, is secreted in response to increased concentrations of circulating glucose and functions to maintain stable concentrations of blood glucose. Previous research also indicated that insulin can effectively decrease progesterone clearance in vivo. Glucose is a key nutrient required during lactation for milk synthesis and maintenance of other body tissues, including those involved in various reproductive processes. Because most dietary carbohydrate is converted into volatile fatty acids in the rumen, a cow must synthesize glucose by gluconeogenesis in liver. The extensive demand for glucose by the mammary gland to synthesize milk may decrease the amount of glucose readily available to other body tissues, including those tissues involved in reproductive processes.

Rumen-protected glucose should facilitate more glucose being delivered to the small intestine for absorption as opposed to relying solely on its synthesis in the liver. Because circulating glucose induces secretion of insulin, a resulting increase in insulin may decrease the activity of liver enzymes involved in clearance of progesterone, and thus increase peripheral concentrations of progesterone. Increased concentrations of progesterone at estrus or during the 7 to 10 days preceding a timed insemination are associated with improved pregnancy risk in lactating dairy cows.

Therefore, the objective of the current study was to determine the effect of supplementing an RPG product on blood serum concentrations of glucose, insulin, and progesterone. We hypothesized that supplementing RPG would increase concentrations of glucose and insulin, resulting in decreased activity of liver enzymes (cytochromes P450 2C and P450 3A), thus increasing circulating concentrations of progesterone.

Materials and Methods

Sixty-one Holstein cows were enrolled in a study before first insemination at Kansas State University. Cows calved in a maternity barn on a straw-bedded pack and were subsequently housed in a sand-bedded free-stall facility. Cows were then housed in a tie-stall barn from 58 ± 3 to 72 ± 3 DIM and fed individually during the experiment. Treatments included a daily supplement of 0 (control, n = 13), 2.2 (n = 15), 4.4 (n = 15), or 8.8 lb/day (n = 16) of an RPG product (Grain States Soya, West Point, NE). Ground corn was top-dressed with the treatment supplement, so each cow received a total supplement (RPG ± ground corn) of 8.8 lb per day.

Cows were subjected to an ovulation-synchronization program to synchronize ovulation (day 0; Figure 1). Briefly, cows received injections of $PGF_{2\alpha}$ at 48 ± 3 DIM, an injection of GnRH at 51 ± 3 DIM, and $PGF_{2\alpha}$ at 58 and 59 ± 3 DIM. An injection of GnRH was administered 56 hours after $PGF_{2\alpha}$ (d -0.5) to induce ovulation of the dominant follicle. The products used were 100 µg GnRH (Factrel) and 25 mg $PGF_{2\alpha}$ (dinoprost tromethamine) from Zoetis Inc. (Kalamazoo, MI). Cows were eligible to continue in the experiment if ovulation was detected by the appearance of a new CL by day 2 and elevated progesterone (≥ 1 ng/mL) by day 4. Cows were reintroduced in the herd at 72 ± 3 DIM (day 12) and estrous cycles were resynchronized in cows for insemination (GnRH on 72 ± 3, $PGF_{2\alpha}$ on 79 ± 3 and 80 ± 3, GnRH on 81 ± 3, and timed AI on 82 ± 3 DIM).

Feed intake was recorded daily. Cows were milked thrice daily. Milk samples collected once 3 days before initiation of treatment and again on day 11 of treatment were analyzed for concentrations of fat, true protein, lactose, and somatic cells. Energy-corrected milk was calculated according to Dairy Records Management Systems as (0.327 × milk yield) + (12.95 × fat yield) + (7.65 × protein yield). Body condition score and body weight were recorded at enrollment (58 ± 3 DIM).

Blood samples were collected via coccygeal puncture on day 0, 2, and 4 to analyze concentrations of glucose and insulin. Samples were collected 1 hour before and 8 hours after the morning feeding to determine pre-feeding and post-feeding concentrations of glucose and insulin. Progesterone was measured in blood serum samples collected on day 2 and daily from day 4 through 12.

Results and Discussion

Feed Intake, Milk Production, and Milk Composition

Dry matter intake, milk yield, and milk composition are summarized in Table 2. As anticipated, starch intake decreased ($P < 0.01$) linearly with increasing dose of RPG. In contrast, intake of CP and ethanol-soluble carbohydrates (sugars) increased ($P < 0.01$) linearly with increasing dose of RPG. Neither milk nor energy-corrected milk yield were impacted by RPG dose. Dose of RPG had no effect on yields of milk fat, lactose, or SCC. In contrast, milk protein concentration tended ($P = 0.10$) to differ among treatment doses. Milk urea nitrogen concentration increased linearly ($P < 0.01$) with increasing dose of RPG.

Metabolic Analytes

The difference in post-feeding and pre-feeding concentrations of serum glucose and insulin are shown in Figure 2. The change in concentration of insulin from pre-feeding to post-feeding was greater ($P < 0.01$) for control cows compared with cows supplemented with any dose of RPG (Figure 2A). Changes in pre-feeding to post-feeding concentrations of glucose did not differ among treatments (Figure 2B).

Reproductive Traits

Concentrations of progesterone increased from day 2 to 12 of the estrous cycle but were unaffected by treatment (Figure 3). This experiment was not designed to offer sufficient power to detect differences in pregnancy risk; however, pregnancy risk (69.2%) was greater ($P < 0.01$) for control cows compared with all RPG-treated cows, with 2.2 lb/day (14.2%), 4.4 lb/day (42.9%), and 8.8 lb/day (25%) all resulting in numerically decreased pregnancy risk. Volume of the corpus luteum on day 8 of the estrous cycle averaged 9.5 ± 1.5, 10.3 ± 1.5, 9.4 ± 1.5, and 12.0 ± 1.4 cm^3, respectively, but did not differ among treatments.

Fertility continues to be a leading economic concern for the dairy industry. Measures to increase pregnancy rates and reduce early pregnancy loss would improve the efficiency of reproduction. In the current study, we focused on reducing clearance of progesterone from the peripheral circulation by supplementing RPG and found that the insulin response was diminished with RPG diets relative to the control. Supplementation with RPG caused a linear increase in CP intake and MUN concentration with increasing dose but had no impact on milk yield or DMI. Increasing MUN is associated with decreased fertility. Therefore, we conclude that RPG failed to alter insulin concentration as hypothesized and did not affect progesterone concentration.

Table 1. Ingredient and nutritional composition of the basal diet[1]

Ingredients	% Dry matter
Corn silage	22.5
Triticale silage	15.0
Alfalfa hay[2]	3.1
Alfalfa hay[3]	3.1
Corn gluten feed[4]	22.8
Whole cottonseed	4.0
Corn grain, finely ground	13.4
Concentrate mix[5]	16.1

	Basal diet	Ground corn	RPG[6]
Nutrient, % of dry matter (DM) (unless otherwise specified)			
DM, % as-fed	47.8	86.3	82.9
Crude protein (CP)	18.2	9.8	43.2
Soluble protein, % CP	18.2	9.8	43.2
Acid detergent fiber	23.9	3.9	4.9
Neutral detergent fiber	36.5	9.8	20.2
Starch	12.3	69.7	1.1
Ethanol-soluble carbohydrates (simple sugars)	8.1	5.5	38.6
NE_L, Mcal/lb	0.75	0.94	0.86

[1]Nutrient composition values presented are results of near infrared analysis of the basal diet.
[2]Lower quality alfalfa with 22.1% CP.
[3]Higher quality alfalfa with 23.9% CP.
[4]Sweet Bran (Cargill Inc., Blair, NE).
[5]Concentrate premix consisted of 59.9% expeller soybean meal (SoyBest; Grain States Soya, West Point, NE), 12.0% limestone, 10.5% sodium bicarbonate, 7.48% Ca salts of long-chain fatty acids (Megalac R; Arm & Hammer Animal Nutrition, Princeton, NJ), 2.40% magnesium oxide, 2.14% of a 1.50% stock salt, 1.50% trace mineral salt, 1.50% potassium chloride, 1.50% vitamin E (20 kIU/g), 0.94% Biotin 100 (ADM Alliance Nutrition, Quincy, IL), 0.25% selenium premix (0.06%), 0.23% 4-Plex (Zinpro Corp., Eden Prairie, MN), 0.15% vitamin A premix (30 kIU/g), 0.12% Zinpro 120 (Zinpro Corp.), 0.06% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 0.04% vitamin D premix (30,000 IU/g), 0.01% ethylenediamine dihydriodide premix (3.65% I).
[6]Rumen-protected glucose product.

Table 2. Dry matter intake, milk yield, and milk composition in cows supplemented with varying doses of rumen-protected glucose (RPG)[1]

	Treatment,[2] lb RPG/day					*P*-value		
Item	0	2.2	4.4	8.8	SEM	RPG[3]	Linear[4]	Quadratic[4]
Dry matter intake, lb/day	53.5	54.6	52.8	56.1	1.63	0.62	0.42	0.51
Starch intake, lb/day	12.0	10.8	9.4	7.3	0.24	< 0.01	< 0.01	0.06
Crude protein intake, lb/day	11.4	12.3	12.8	15.0	0.37	< 0.01	< 0.01	0.09
Sugar intake[5] lb/day	3.9	4.7	5.3	6.9	0.13	< 0.01	< 0.01	< 0.01
Milk,[6] lb/day	109.8	111.3	107.6	111.1	2.8	0.96	0.88	0.57
Energy-corrected milk, lb/day	105.3	108.9	107.6	107.8	2.4	0.29	0.60	0.46
Fat, %	3.8	3.9	4.0	4.0	0.1	0.20	0.36	0.72
Fat, lb/day	4.0	4.6	4.2	4.6	0.2	0.29	0.28	0.65
Lactose, %	5.0	5.0	5.0	5.0	0.1	0.81	0.78	0.49
Lactose, lb/day	5.5	5.9	5.3	5.5	0.2	0.48	0.76	0.87
Milk urea nitrogen, mg/dL	15.3	16.7	17.4	20.1	0.6	< 0.01	< 0.01	0.73
Protein, %	2.7	2.6	2.7	2.6	0.1	0.10	0.15	0.74
Protein, lb/day	2.9	3.1	2.9	2.9	0.2	0.79	0.67	0.95
Somatic cell linear score	3.5	3.1	3.4	3.2	0.2	0.94	0.83	0.73

[1]Milk components and energy-correct milk (ECM) were measured in milk samples collected on day 8 of the supplemental period. Dietary nutrient components were assessed from feed samples collected weekly and composited every 2 weeks.
[2]Lactating dairy cows were supplemented with either 0 (control), 2.2, 4.4, or 8.8 lb of a rumen-protected glucose product in replacement of finely ground corn grain.
[3]*A priori* contrasts of the 0 lb (control) were compared with the combined 3 treatment means.
[4]*A priori* orthogonal contrasts for unevenly spaced treatment doses to determine linear and quadratic effects of dose.
[5]Free ethanol-soluble carbohydrates.
[6]Mean milk production from day 0 through 12. Treatment did not impact milk production but there was an effect of day ($P < 0.01$).

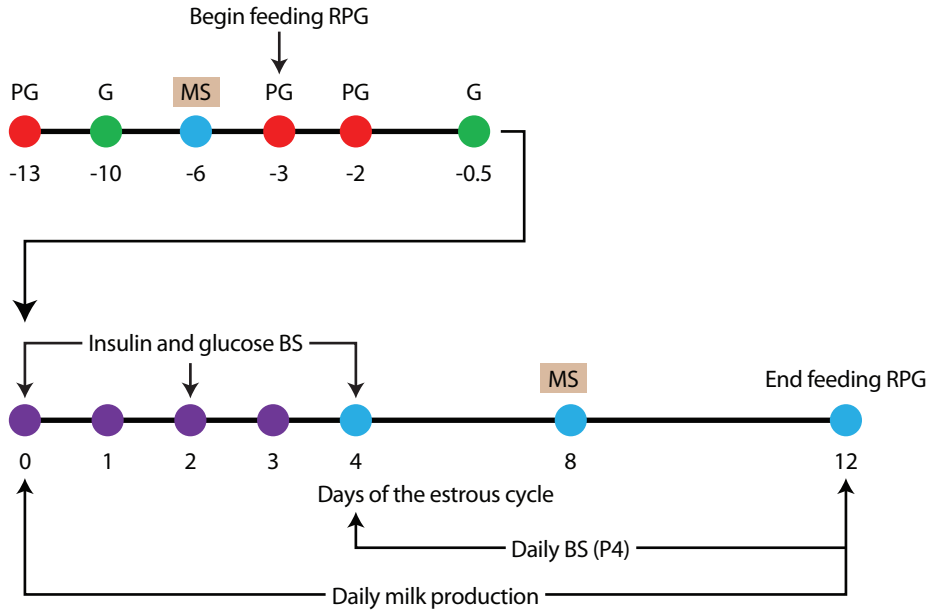


Figure 1. Illustration of the ovulation-synchronization scheme, blood collection (BS) and milk sampling (MS) schedule for supplementation of rumen-protected glucose at varying doses. Ovulation was synchronized with an injection of $PGF_{2\alpha}$ (PG) on day -13, followed by an injection of GnRH (G1) on day -10. Injections of PG were given 24 hours apart on day -3 and -2 to induce complete luteal regression. An injection GnRH (G) was given on day -0.5 to cause ovulation and begin a new estrous cycle (25 mg PG; 100 μg of GnRH). Blood samples were collected on days 0, 2, and 4 before feeding and again 8 hours after feeding to measure changes in insulin and glucose from before to after feeding. Progesterone (P4) was measured on day 2, and then daily from day 4 through 12. Corpus luteum volume was measured on day 8. Milk samples were collected on day -3 and 8 to determine milk components. Daily milk production was recorded from day 0 through 12.

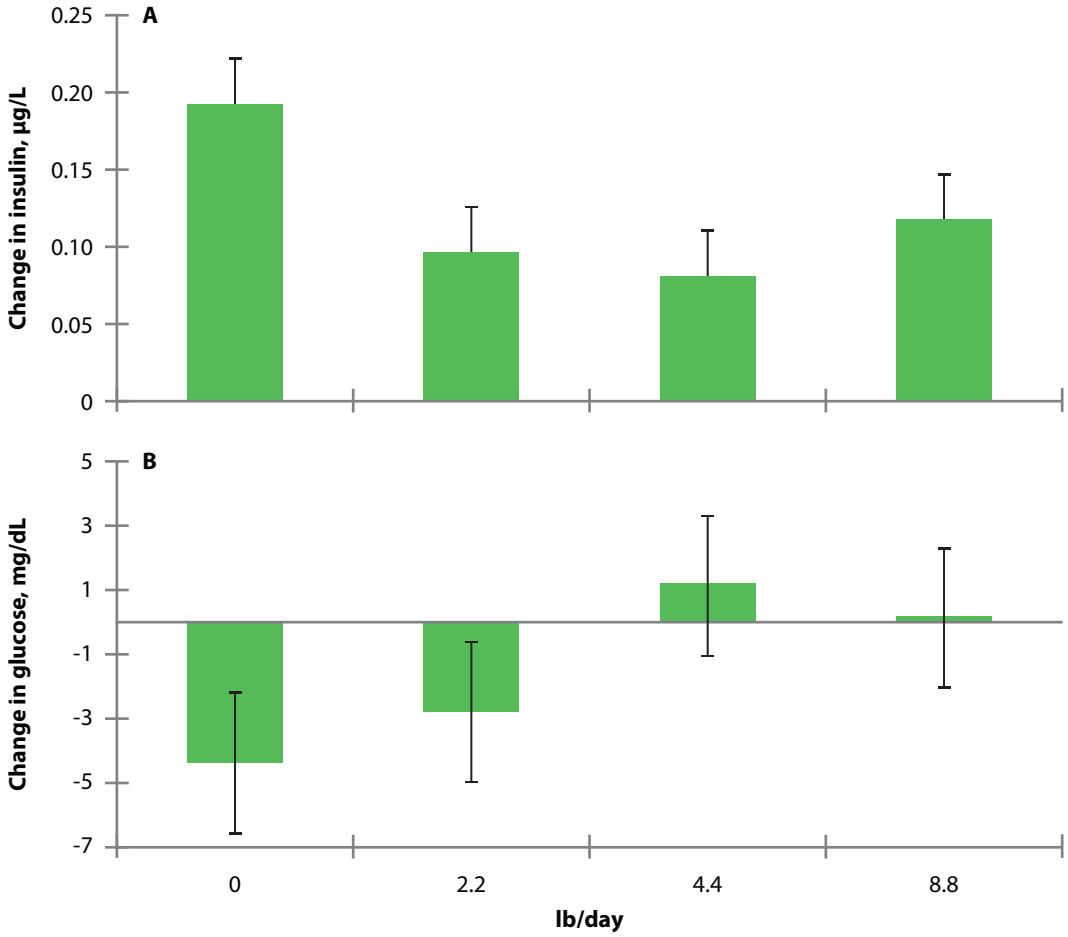


Figure 2. Composite change (post-feeding "minus" pre-feeding) in concentrations of insulin (A) and glucose (B) measured in blood samples collected on days 0, 2, and 4 pre-feeding and 8 hours later (post-feeding). Control cow receiving 8.8 lb of ground corn (0 lb RPG) had greater ($P < 0.01$) change in concentrations of insulin than the cows receiving either of the 3 doses of RPG. No differences ($P = 0.26$) in treatments were detected for the change in glucose concentration from before to after feeding.

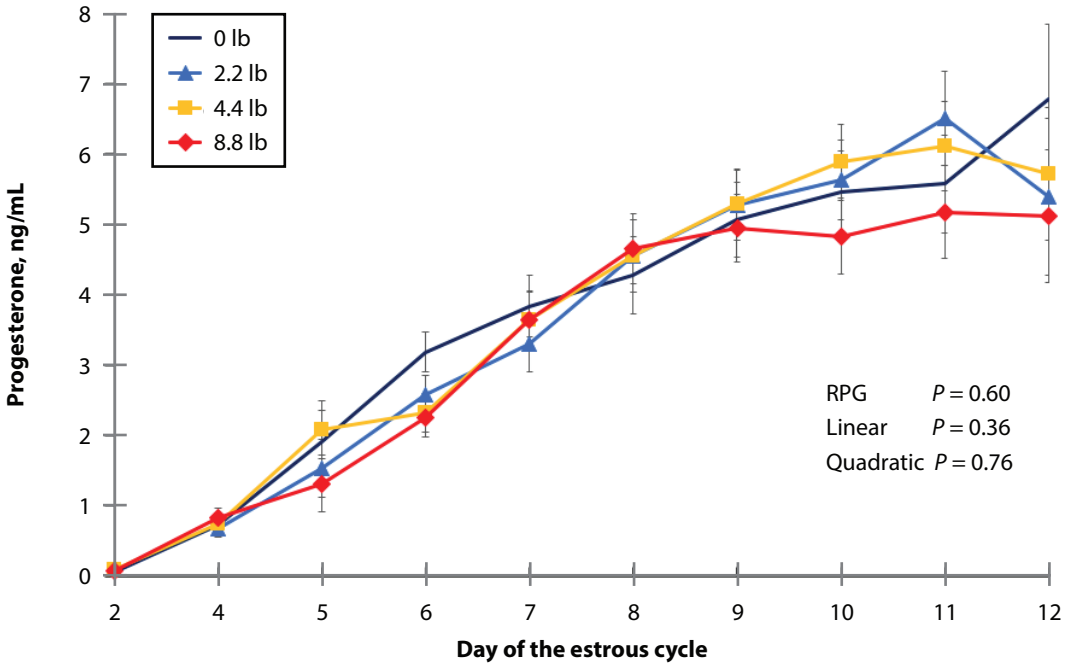


Figure 3. Daily progesterone concentrations in cows supplemented with rumen-protected glucose (RPG). Progesterone increased ($P < 0.01$) from day 2 through 12 of the estrous cycle but was not affected by dose of rumen-protected glucose treatment.

Can a "Zero Land Use" Diet Maintain Milk Production of Dairy Cows?

C.S. Takiya, C.M. Ylioja, A. Bennett, M. Davidson, and B.J. Bradford

Summary

Dairy cows can convert feeds unsuitable and unpalatable for humans into milk and play a key role in food security. Feed efficiency is usually calculated as the ratio between nutrients secreted in milk and nutrient intake, but this metric does not address concerns about human/livestock feed competition. This study aimed to evaluate whether cows fed a "zero land use" diet (diet that does not affect land used for production of human food), with or without rumen-protected amino acids, can maintain milk compared to a conventional lactation diet. Twelve second-lactation dairy cows were used in a 3×3 Latin square design experiment to evaluate 1) conventional total mixed ration (TMR) for lactating cows (CON), containing 25.7% byproduct feeds; 2) a TMR comprised of zero land use feedstuffs (ZLU); and 3) ZLU with top-dressed rumen-protected amino acids (ZLU-AA). Cows fed ZLU or ZLU-AA diets consumed less dry matter ($P < 0.01$) and decreased ($P < 0.01$) milk and energy-corrected milk yield of cows. Feed efficiency was similar between cows fed CON and ZLU but it was reduced ($P < 0.01$) when cows were fed ZLU-AA. In a scenario reflecting current food system byproduct use, cows fed ZLU diets showed greater ($P < 0.01$) human-edible metabolizable energy and protein recovery in milk than cows fed CON. Zero land use diets did not maintain milk production of late-lactation cows either with or without rumen-protected amino acids.

Introduction

To feed the growing human population, more land will need to be devoted to the cultivation of food and cash crops. Since land is a finite resource, this will result in increasing competition for production of forage and concentrate feeds for livestock. On the other hand, increased food and cash crops will generate more crop residues and agro-industrial byproducts, many of which represent valuable feed resources for ruminants. Feeding byproducts to dairy cattle can sometimes decrease feed costs and improve the environmental sustainability of milk production. Recently, we compared a diet comprised of 95% byproducts with a typical diet for lactating cows and found a slight decrease in milk production (4.5%), without altering body weight of cows producing 88 lb of milk/day. Although not explored in our previous study, formulation software highlighted a possible deficiency of metabolizable lysine and methionine in the byproduct-based diet, which could be addressed through rumen protected amino acid supplementation.

One challenge in formulating a diet that displaces no land from food production for humans is to meet the effective fiber requirement of dairy cattle. In our previous work, this was accomplished with the use of wheat straw. However, an alternative is to utilize winter cover crop forages produced opposite a food-producing crop in a dual-cropping system. The objective of this study was to evaluate whether rumen-protected amino acids can maintain milk and component yields while improving human-edible nutrient conversion rate in cows fed a "zero land use" diet (diet that does not affect land used for

production of human food) compared to a conventional lactation diet. In addition, we provide an approach to estimate the human-edible nutrient conversion rate for dairy cows.

Experimental Procedures

Twelve second-lactation dairy cows (231 ± 40 days in milk and 75.8 ± 15 lb/day milk yield at the beginning of the experiment) were assigned to a replicated 3×3 Latin square design experiment balanced for carryover effects. Adaptation to diets was allowed for 17 days, and 4 days were used for data collection and sampling in each period. Cows were blocked according to fat-corrected milk yield and days in milk, and randomly assigned to treatment sequence within block. Treatments (Table 1) were: 1) conventional TMR containing 25.7% byproduct feeds (CON); 2) TMR comprised of zero land use feedstuffs (ZLU); and 3) ZLU with top-dressed rumen-protected amino acids [ZLU-AA; 77 g/day AjiPro-L (Ajinomoto, Chicago, IL) and 145 g /day MetaSmart (Adisseo, Antony, France)]. Cows were milked and fed twice daily. All diets were formulated to meet nutrient requirements. The chemical composition of feeds is shown in Table 2.

Feed and refusals were weighed daily, targeting 10-15% refusals. During the last 4 days of each period, TMR and refusals samples were collected to assess particle size distribution and sorting index. Milk samples were collected in every milking during the last 4 days of each period, and analyzed by MQT Laboratory Services (Kansas City, MO) for solids, urea N concentration, and SCC. Fat-corrected milk yield, ECM yield, BW, and BCS were also recorded.

Maximum human-edible metabolizable energy and protein contents were estimated based on sugar, starch, true protein, and fat concentrations in corn grain (including grains in silage), corn hominy, soybean meal products, wheat middlings, and molasses. Other feedstuffs (such as spent coffee grounds) were considered unsuitable for human consumption. We calculated human-edible nutrient recovery in milk in two scenarios: one considering hominy feed and wheat middlings suitable for human consumption (thrift scenario), and the other considering them as unlikely to be consumed by humans (choice scenario).

Data were submitted to analysis of variance using the MIXED procedure of SAS 9.4 (SAS Inst., Cary, NC) including the fixed effect of diet and the random effects of period, block, and cow within block. Least square means among diets were evaluated using the Tukey test. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

Results and Discussion

Cows fed ZLU or ZLU-AA diets consumed less dry matter ($P < 0.01$) than those fed CON (Table 3). Feed sorting was not affected by treatments, except for greater sorting for feed particles of 4–8 mm length among cows fed ZLU compared to CON. The ZLU diets had a greater proportion of feed with long particles (>19 mm) in comparison with CON diet, which may have contributed to suppression of feed intake on these diets. The ZLU and ZLU-AA decreased ($P < 0.01$) milk and ECM yields substantially (Table 3). Feed efficiency was similar between cows fed CON and ZLU but it was reduced ($P < 0.01$) when cows were fed ZLU-AA. Feed efficiency in terms of ECM tended to decrease in cows fed ZLU or ZLU-AA diets. The huge negative impact on

performance of cows can likely be attributed to the forage source used in this study. Although the triticale/clover hay used in this study had considerable amounts of crude protein and neutral detergent fiber, it also had a relatively high moisture content that favored spoilage and likely impaired palatability.

The outputs of human-edible metabolizable energy and protein in milk were decreased ($P < 0.01$) by ZLU and ZLU-AA diets (Table 4). In the thrift scenario, ZLU diets (ZLU and ZLU-AA) decreased ($P < 0.01$) human-edible metabolizable energy input but increased ($P < 0.01$) human-edible protein input. Despite lesser human-edible metabolizable energy input, recovery of human-edible metabolizable energy in milk was not affected ($P = 0.55$) by treatments. Cows fed CON had greater ($P < 0.01$) milk recovery of human-edible protein in comparison with those fed ZLU diets. In the choice scenario, cows fed CON diet had greater ($P < 0.01$) human-edible metabolizable energy and protein inputs compared to those fed ZLU diets. In contrast with the thrift scenario, cows fed ZLU diets showed greater ($P < 0.01$) human-edible nutrient (metabolizable energy and protein) recovery in milk than cows fed CON in the choice scenario. These vastly different outcomes demonstrate how impactful assumptions about human edibility are in calculations regarding the efficiency of feeding livestock.

Conclusions

This study showed no evidence that a diet composed of feedstuffs that do not affect land used for production of human food can maintain milk production of late lactation cows, even if combined with rumen-protected amino acids. We suspect that the poor quality of the winter forage used in these diets was primarily to blame for the poor feed intake and resulting loss in productivity in this study. In addition, feeding ZLU diets does not necessarily improve the human-edible nutrient conversion rate in dairy cows, as this is dependent on both the ability to maintain productivity of cows and on assumptions made in calculating the value of feed ingredients for human consumption.

Table 1. Ingredient, chemical composition, and particle size distribution of diets

	Diet[1]	
Item	CON	ZLU
Ingredient, % dry matter (DM)		
Corn silage	41.9	
Alfalfa hay	12.6	
Prairie hay	1.05	
Triticale/clover hay[2]		31.6
Corn gluten feed[3]	23.1	15.1
Whole cottonseed with lint	2.62	1.38
Ground corn	11.9	
Soybean meal[4]	4.39	
Calcium salts of long-chain fatty acids[5]	0.61	
Wheat middlings		25.5
Corn hominy		12.6
Spent coffee grounds		4.36
Molasses		5.94
Minerals and vitamins	1.72	3.4
Chemical, % DM		
Dry matter, % as-fed	58.5	54.1
Crude protein	17.1	18.0
Acid detergent fiber	19.5	20.4
Neutral detergent fiber	34.4	37.3
Non-fiber carbohydrate	34.4	25.9
Ether extract	5.07	4.73
Ash	8.95	14.0
Total digestible nutrient, %	69.0	62.7
NE_L, Mcal/kg	1.61	1.46

[1]Conventional lactation diet (CON), containing 25.7% co-product feeds, and TMR composed of feedstuffs that do not affect land used for production of human food (ZLU) – water was added to achieve similar diet DM.
[2]Hay from the winter intercropping of triticale and red clover.
[3]Sweet Bran (Cargill, Blair, NE).
[4]Soy Best (Grain States Soya, Inc., West Point, NE).
[5]Megalac-R (Arm & Hammer Animal Nutrition, Trenton, NJ).
[6]Scenario considering hominy feed and wheat middlings suitable food for humans.
[7]Scenario not considering hominy feed and wheat middlings suitable food for humans.

Table 2. Chemical composition of feeds (% DM, unless stated)

Item[1]	DM, % as-fed	CP	ADF	NDF	NFC	EE	Ash
Corn silage	35.6	9.2	21.5	39.2	41.9	3.70	6.05
Alfalfa hay	91.5	20.3	31.8	42.8	23.4	2.30	11.3
Prairie hay	93.3	5.70	43.1	68.9	14.0	2.30	9.14
Triticale/clover hay	72.3	19.1	40.9	59.2	1.55	2.23	17.6
Cottonseed with lint	88.2	22.3	44.3	57.2	0.70	15.7	4.38
Corn gluten feed[2]	61.3	23.0	8.90	31.8	43.2	5.10	7.05
Spent coffee grounds	35.1	14.2	32.3	54.7	15.2	14.2	1.78
Molasses	70.7	5.80	-	-	-	5.70	15.4
Grain mix CON[3]	86.4	16.1	6.80	19.8	45.3	5.35	13.6
Grain mix ZLU[4]	82.5	15.4	9.7	29.1	38.9	4.45	12.2

[1]Dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF) treated with sodium sulfite and alpha-amylase, non-fiber carbohydrate (NFC), and ether extract (EE).
[2]Sweet Bran (Cargill, Blair, NE).
[3]Grain mix containing (% DM): 63.9% ground corn, 23.5% bypass soybean meal (Soy Best, Grain States Soya, Inc., West Point, NE), 3.3% bypass fat (Megalac-R, Arm & Hammer Animal Nutrition, Trenton, NJ), and 9.18% minerals and vitamins.
[4]Grain mix containing (% DM): 61.4% wheat middlings, 30.4% corn hominy, and 8.22% minerals and vitamins.

Table 3. Performance and sorting index of late-lactating cows fed a conventional lactation diet or a "zero land use" diet

	Diet[1]				
Item	CON	ZLU	ZLU-AA	SEM	*P*-value
Dry matter intake (DMI), lb/day	57.8[a]	49.4[b]	49.6[b]	1.39	< 0.01
Sorting index[2]					
>19 mm	0.84	0.89	0.89	0.040	0.47
8–19 mm	0.96	0.98	0.95	0.015	0.73
4–8 mm	0.97[b]	1.03[a]	1.01[ab]	0.014	0.04
< 4 mm	1.04	1.04	1.04	0.008	0.89
Milk yield, lb/day	70.3[a]	50.5[b]	50.3[b]	3.28	< 0.01
ECM yield[3], lb/day	70.8[a]	51.8[b]	53.4[b]	3.04	< 0.01
ECM/DMI	1.22	1.10	1.05	0.043	0.06
Fat, lb/day	2.49[a]	1.91[b]	1.96[b]	0.108	< 0.01
Protein, lb/day	2.25[a]	1.55[b]	1.59[b]	0.094	< 0.01
Lactose, lb/day	3.37[a]	2.27[b]	2.31[b]	0.073	< 0.01
Milk composition					
Fat, %	3.60[a]	3.86[b]	3.75[ab]	0.085	0.04
Protein, %	3.22	3.15	3.18	0.044	0.31
Lactose, %	4.80 [a]	4.45[b]	4.39[b]	0.049	< 0.01
Urea nitrogen, mg/dL	13.9[ab]	14.2[a]	13.7[b]	0.14	0.03
SCLS[4]	2.46[b]	3.69[a]	3.69[a]	0.25	< 0.01
Body weight change, lb/21 days	69.9	48.3	46.9	11	0.28
Body condition score change	0.04	-0.04	-0.06	0.033	0.29

[a-b]Values within rows with different superscripts differ significantly ($P < 0.05$).

[1]Conventional lactation ration (CON), containing 25.7% co-product feeds; ration composed of feedstuffs that do not affect land used for production of human food (ZLU); and ZLU with top-dressed rumen-protected amino acids [ZLU-AA; 2.72 oz/d AjiPro-L (Ajinomoto, Chicago, IL) and 1.59 oz/d MetaSmart (Addiseo, Antony, France)].

[2]Values >1.0 means that cows sorted for the specific particle size and values <1.0 means that cows sorted against the specific particle size.

[3]Energy-corrected milk (ECM).

[4]Somatic cell linear score (SCLS) = $\log_2$(somatic cell count /100) + 3.

Table 4. Human-edible (HE) nutrients conversion rate of late-lactation cows fed a conventional lactation diet or a "zero land use" diet

	Diet[1]				
Item	CON	ZLU	ZLU-AA	SEM	*P*-value
Milk metabolizable energy (ME) output, Mcal/day	22.4[a]	16.1[b]	16.2[b]	1.01	< 0.01
Milk protein output, lb/day	2.25[a]	1.55[b]	1.59[b]	0.10	< 0.01
Thrift scenario[2]					
HE ME input, Mcal/day	36.8[a]	25.6[b]	26.8[b]	1.41	< 0.01
HE protein input, lb/day	2.98[b]	3.73[a]	3.90[a]	0.099	< 0.01
Milk ME ÷ HE ME intake	0.61	0.64	0.59	0.044	0.55
Milk protein ÷ HE protein intake	0.75[a]	0.42[b]	0.40[b]	0.038	< 0.01
Choice scenario[3]					
HE ME input, Mcal/day	36.8[a]	3.92[b]	4.11[b]	1.19	< 0.01
HE protein input, lb/day	2.98[a]	0.19[b]	0.20[b]	0.15	< 0.01
Milk ME ÷ HE ME intake	0.61[b]	4.13[a]	3.84[a]	0.065	< 0.01
Milk protein ÷ HE protein intake	0.75[b]	8.21[a]	7.86[a]	0.002	< 0.01

[a-b]LSMEANS within rows with different superscripts differ significantly in LSD ($P < 0.05$).

[1]Conventional lactation TMR (CON), containing 25.7% co-product feeds; TMR composed of feedstuffs that do not affect land used for production of human food (ZLU); and ZLU with top-dressed rumen-protected amino acids [ZLU-AA; 2.72 oz/d AjiPro-L (Ajinomoto, Chicago, IL) and 1.59 oz/d MetaSmart (Addiseo, Antony, France)].

[2]Scenario considering hominy feed and wheat middlings as suitable foods for human consumption.

[3]Scenario considering that hominy feed and wheat middlings would be unlikely to be consumed by humans.

Impact of *Saccharomyces cerevisiae* Fermentation Product on Feed Intake Parameters, Lactation Performance, and Metabolism of Transition Dairy Cattle

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Summary

A *Saccharomyces cerevisiae* fermentation product (NutriTek; Diamond V, Cedar Rapids, IA) was fed from 29 ± 5 days before calving and through 42 days in milk (DIM) to evaluate the effects on feed intake parameters, milk production, and metabolism. Treatments were control (n = 30) or 18 g/d NutriTek (NT, n = 34) provided as total mixed rations. Cows were individually fed 3×/day prepartum and 2×/day postpartum. Cows were milked 2×/day with samples taken 2×/week for composition analysis. Body weight (BW) was measured at enrollment (day -29 ± 5), day 0, and day 42 relative to calving, and body condition was scored weekly. Blood samples were collected during weeks -4, -2, 1, 2, and 5 relative to calving for biomarkers of metabolism and inflammation. To evaluate adaptive immunity, cows were challenged with a subcutaneous injection of ovalbumin (egg protein) and immune response was determined by serum concentrations of anti-ovalbumin immunoglobulin G (IgG) on days 7, 21, 28, and 35 of lactation. Overall dry matter intake, BW, body condition score, and milk yield were not different between treatments. NutriTek did alter feeding behavior by increasing the number of meals consumed with less time between those meals. Milk fat concentration increased with NT during weeks 4 and 5 of lactation, which contributed to an increase in fat yield during those weeks. There were tendencies for greater milk lactose yield in control cows and greater milk urea nitrogen concentration in NT, but no treatment differences for milk protein concentration or somatic cell count. Assuming equal digestibility, energy balance was more negative for NT during weeks 4 and 5, mirroring the increase in milk fat during that time. Energy density of diets calculated from observed ECM yield and BW change did not differ by treatment. Plasma concentrations of free fatty acids, β-hydroxybutyrate (BHB), glucose, insulin, and the inflammation marker haptoglobin did not differ between treatments. NutriTek increased the incidence of subclinical ketosis (12 vs. 38%, diagnosed by urine ketones). There was no overall treatment effect for immune response to vaccination; however, a treatment × parity interaction indicated greater antibody concentration in primiparous cows supplemented with NT. A partial budget analysis accounting for milk income, feed cost, and expense associated with ketosis treatment indicated an additional $0.35 daily profit per cow for NT vs. control. In conclusion, NT supplementation during the transition period altered feeding behavior and milk fat concentration and ultimately appeared profitable in this scenario, despite the increased incidence of subclinical ketosis and a lack of response in early lactation milk yield.

[1]Diamond V, Cedar Rapids, IA.

Introduction

Saccharomyces cerevisiae fermentation products have been reported to influence the rumen environment, increasing fiber digestion, lactic acid utilization, and rumen pH. These attributes may be particularly advantageous during periods of stress, and may explain why dietary *Saccharomyces cerevisiae* fermentation product increases dry matter intake in early lactation by an average of 1.37 lb/day and energy-corrected milk by 3.64 lb/day. Beyond these proposed effects of *Saccharomyces cerevisiae* fermentation products on ruminal health, they may also affect transition cows through altered feeding behavior and immune function.

The objective of this study was to determine the effects of a new *Saccharomyces cerevisiae* fermentation product (NutriTek, Diamond V, Cedar Rapids, IA) on feed intake, feeding behavior, milk production and composition, energy balance, metabolism, and adaptive immunity during the transition period in dairy cows.

Experimental Procedures

Sixty-four prepartum Holstein cows (50 multiparous, 14 primiparous) were used in a randomized block design. Cows were blocked by parity, expected calving date, and previous 305ME yield, then randomly assigned to treatment within block. Treatments were either control (n = 30) or 18 grams NT per day (n = 34) that was incorporated into a total mixed ration (TMR). Treatments were fed from -29 ± 5 to 42 days relative to calving. Feed ingredient samples were collected once weekly, composited by 4 months, and analyzed by wet chemistry methods for dry matter, neutral detergent fiber, starch, crude protein, ether extract, and ash content (Dairy One, Ithaca, NY). Chemical analyses of individual feed ingredients were used for determination of TMR nutrient composition (Table 1).

Prepartum cows were fed treatment diets using an electronically gated feeding system (Roughage Intake System; Insentec B.V., Marknesse, the Netherlands). All cows on a given treatment diet were allowed access to 4 feed bins assigned to that treatment, and no more than 6 animals shared those 4 bins at any given time. To account for the capacity of the feed bins and potential bunk favoritism, prepartum cows were fed 3×/day. Upon calving, cows were moved to a tie-stall facility where they were fed individually twice daily. Both feeding systems electronically recorded individual feed consumption and meal patterns. As-fed feed intake was recorded on a daily basis and adjusted by TMR dry matter for determination of meal and daily dry matter intake (DMI). For feeding behavior analysis, the inter-meal interval (IMI) was defined by a gap of at least 12 minutes between distinct meals and the minimum meal weight was 0.9 lb.

Cows were milked 2×/day with milk weights recorded for each milking. Milk samples were collected 2×/week and analyzed for concentrations of fat, true protein, lactose (B-2000 Infrared Analyzer; Bentley Instruments, Chaska, MN), milk urea nitrogen (MUN; MUN spectrophotometer, Bentley Instruments), and somatic cells (SCC 500, Bentley Instruments) by MQT Laboratories (Kansas City, MO). Energy-corrected milk was calculated as (0.327 × milk yield) + (12.95 × fat yield) + (7.65 × protein yield), and fat-corrected milk (FCM) was calculated as (0.432 × milk yield) + (16.216 × fat yield).

Body condition score (BCS) was recorded weekly by 3 trained investigators. Body weight was measured at enrollment (-29 ± 5 days relative to calving), after calving, and at 42 DIM. Prepartum energy balance was calculated as net energy (NE) intake – (NE maintenance + NE pregnancy). A total of 5 jugular blood samples were taken from each cow on the following weeks relative to calving: -4, -2, 1, 2, and 5. Bloods samples were analyzed for concentrations of free fatty acids, β-hydroxybutyrate, glucose, insulin, and haptoglobin. To evaluate adaptive immunity, cows were challenged with a subcutaneous injection of ovalbumin and Quil-A adjuvant on days 7 and 21 postpartum. Immune response was measured by anti-ovalbumin IgG concentration in serum samples collected on days 7, 21, 28, and 35 postpartum.

Cow health was evaluated daily by visual inspection, rectal temperature, and urine acetoacetic acid concentration (KetoCare, TRUEplus). Cows were monitored for disorders including ketosis (urine acetoacetic acid concentration > 40 mg/dL), milk fever, displaced abomasum, retained placenta, metritis, and mastitis. All diagnosed disease and health issues were recorded.

Results and Discussion

Dry Matter Intake and Feeding Behavior

Dry matter intake exhibited the typical decrease as calving approached and increased with the progression of lactation (Figure 1A); however, DMI was not altered by NT supplementation ($P > 0.69$). Similar to DMI, all feeding behavior parameters except IMI ($P = 0.28$) were influenced by day relative to calving ($P < 0.01$; Table 2). NutriTek tended to increase prepartum meal count ($P = 0.06$; Figure 1B) and decreased the time between meals ($P = 0.03$; Figure 1C), specifically during the 10 days preceding calving. A treatment × week interaction for meal weight ($P = 0.03$) indicated control cows consumed larger meals during days -7 to -4 relative to calving. These data suggest that NT cows consumed lighter meals more often with less time between those meals leading up to calving. Interestingly, the treatment × parity interaction for meal count and IMI ($P \leq 0.03$) suggested this altered feeding behavior with NT mainly applied to primiparous cows. Meal count was greater (9.7 vs. 8.5 ± 0.4 meals/day) and IMI lesser (0.65 vs. 0.82 ± 0.04 hours) for primiparous NT cows, but both were similar for multiparous NT and control cows (8.8 vs. 8.9 ± 0.2 meals/day and 0.82 vs. 0.81 ± 0.02 hours). Postpartum, NT cows continued to consume more meals ($P = 0.03$) with a tendency for less time between meals ($P = 0.07$). Considering the lesser proportion of primiparous cows in this study, the prepartum treatment × parity interaction for meal count could partially explain why we observed differences in feeding behavior that did not translate into greater DMI. There was no treatment × parity interaction ($P > 0.20$) for postpartum meal count, therefore this potential explanation would not extend after calving.

These modulations to feeding behavior have been documented in previous transition cow studies supplementing *Saccharomyces cerevisiae* fermentation product. The more consistent meal patterns may contribute to improved, more stable rumen function in NT-fed cows.

Body Weight, Body Condition, Milk Production, and Energy Balance

Cows experienced the typical decrease in body condition and body weight during the transition to lactation ($P < 0.001$); however, there was no effect of treatment or treat-

ment × time interaction for either ($P > 0.50$). On average, cows lost 0.7 BCS units (3.6 to 2.9) and 196 lb of BW (1,519 to 1,323 lb) during the experiment.

As shown in Table 3 and Figure 2A, most milk production parameters (milk yield, energy-corrected milk, and fat-corrected milk) were not affected by treatment ($P \geq 0.32$). Milk fat concentration increased ($P = 0.01$) and milk fat yield tended to be greater ($P = 0.10$; Figure 2B) for NT cows, with differences in weeks 4 and 5. We observed no differences for milk protein yield and content, lactose yield, and milk somatic cell linear score ($P > 0.15$). Milk lactose concentration tended to be greater for control ($P = 0.06$) and MUN tended to be greater for NT ($P = 0.06$). Greater milk fat content in transition cow studies could indicate greater release of body fat; however, lack of difference in BCS and timing of the milk fat response (week 4 and 5) make that unlikely. Risk of ruminal acidosis is increased during the postpartum period. It is possible the more consistent meal patterns contributed to improved rumen function, which could decrease the risk for shifts in biohydrogenation pathways and milk fat depression. *Saccharomyces cerevisiae* fermentation product has also been documented to increase fiber-digesting bacterial populations that largely produce acetate, one of the main lipogenic precursors for de novo fatty acid synthesis. However, these potential mechanisms are unlikely to be involved only during weeks 4 and 5, making it a less compelling explanation. The exact mechanisms involved in the observed increase in milk fat yield are unclear.

Energy balance (calculated assuming equal nutrient digestibility across diets) differed by week ($P < 0.001$) and treatment ($P = 0.03$). The lesser energy balance for NT during weeks 4 and 5 aligns with the time of increased milk fat concentration. Energy balance was also less in primiparous compared to multiparous cows (-7.02 vs. -3.47 ± 0.83 Mcal/day; $P < 0.01$). To account for possible differences in nutrient digestibility, we calculated energy density of the diet as energy required (milk energy + maintenance) minus energy supplied from mobilized body reserves, divided by dry matter intake. This calculation of observed feed energy (observed diet NE_L concentration) could provide some insight into changes in digestible or metabolizable energy supply from the diet; however, no difference between diets was detected ($P = 0.18$).

Metabolic Signaling and Adaptive Immune Response

Changes over time for plasma free fatty acids, BHB, insulin, and glucose reflected the typical metabolic and endocrine changes during the transition period ($P < 0.001$). The metabolic profile was not altered by NT supplementation ($P > 0.35$; Table 4). Plasma haptoglobin concentration, a marker of inflammation, tended to differ by week ($P = 0.08$), but not by treatment ($P = 0.18$).

Potential effects of NT on the adaptive immune system were evaluated by the response to a subcutaneous injection of ovalbumin. Antibody production increased after the challenge ($P < 0.001$) as expected. There was no overall treatment effect ($P = 0.25$), but the treatment × parity interaction indicated greater anti-ovalbumin IgG concentration in primiparous cows supplemented with NT compared to control (0.36 vs. 0.28 ± 0.08 optical density; $P = 0.08$). It is possible that NT enhanced B lymphocyte activation, thus increasing antibody production in primiparous cows.

Disease Incidence

Incidences of common periparturient diseases occurring throughout the study period are outlined in Table 5. No metabolic diseases - except ketosis - differed by treatment ($P > 0.10$). Incidence of subclinical ketosis (SCK) diagnosed via urine ketones was greater in cows supplemented with NT compared to control cows (38% vs. 12%; $P = 0.02$; Figure 4A) and days of glucogenic treatment were also greater (1.7 vs. 0.4 ± 0.3 d; $P = 0.01$). To understand the observed increase in SCK incidence, despite little evidence of an overall treatment effect on plasma ketone concentrations or decreased energy balance during the window of time when ketosis was observed, a deeper investigation was carried out. The majority of ketosis diagnosis occurred between 10 and 20 days in milk ($n = 8$, NT = 6, control = 2). Because of this timing, we used week 2 plasma data to explore potential mechanisms underlying this effect. First, urine diagnosis of ketosis by urine ketones was effective, as plasma BHB concentrations were clearly greater in cows diagnosed with SCK versus those that were not ($P < 0.001$). Analysis of week 2 BHB concentrations demonstrated a parity × treatment interaction ($P = 0.02$; Figure 4B): treatment did not impact BHB in primiparous cows, but NT increased BHB concentrations in multiparous cows. The observed increase in BHB in multiparous cows fed NT occurred without any other signs of poor health – we did not observe any clinical signs, and feed intake was not impaired in this group. We suspect that the increased BHB production is a metabolic response to NT rather than a true sign of disease.

Partial Budget

A simple partial budget analysis was conducted incorporating milk income, feed costs, and the expense associated with treating ketosis (Table 6). Milk income was generated using milk fat and protein prices of $2.51 and $1.78 per lb, respectively. Individual feed ingredient costs represented those of August 2016. Prepartum and postpartum DMI and diet composition were used to calculate diet prices on a daily cow basis, and the cost of NT was incorporated at $0.13 per cow daily. Ketosis cases were treated with propylene glycol ($4/treatment) for 3 days, so ketosis incidence and treatment costs were then used to determine the ketosis expense on a daily per-cow basis for the 42-day postpartum period. This analysis revealed an additional $0.35 profit per cow daily with NT supplementation. This analysis included all numerical differences between treatments; however, since DMI and production parameters other than milk fat concentration did not statistically differ between treatments, cautious interpretation is warranted.

Conclusions

Supplementation with NT during the transition period altered prepartum and postpartum feeding behavior, with increased meals per day and decreased time between those meals. Although no effects were detected for DMI, milk yield, milk protein, or SCC, milk fat percent was increased by approximately 13% in cows receiving NT, with differences beginning after the time period in which lipid mobilization is greatest during the transition period. Body weight, BCS, energy metabolites, and an inflammatory biomarker were unaffected. Incidence of SCK was increased with NT, but the mechanism through which this occurred has yet to be elucidated. Despite the increased incidence of SCK and no observed effect in milk yield, the increased milk fat contributed to an increased marginal economic return for NT over the first 42 days of lactation.

Table 1. Ingredient and nutritional composition of the prepartum and postpartum diets of control cows and cows supplemented with a *Saccharomyces cerevisiae* fermentation product (NutriTek; NT) from -29 ± 5 days relative to calving through 42 DIM

	Prepartum		Postpartum	
	Control	NT	Control	NT
Ingredient, % of dry matter				
Alfalfa hay[1]	---		9.75	
Alfalfa hay[2]	---		9.70	
Grass hay	38.03		1.62	
Corn silage	19.83		22.32	
Wet corn gluten feed[3]	18.44		23.76	
Cotton seed	---		4.05	
Ground corn	8.28	8.23	17.92	17.87
Micronutrient premixes	15.43	15.48	10.88	10.94
Nutrient, % of dry matter (unless otherwise specified)				
Dry matter, % as-fed	63.3		59.7	
Crude protein	12.9		17.0	
Acid detergent fiber	25.0		17.8	
Neutral detergent fiber	43.1		31.3	
Nonfiber carbohydrates	30.1		37.6	
Starch	15.3		22.6	
Crude fat	5.1		6.3	
NE_L, Mcal/kg	1.42		1.66	

[1]Lower quality alfalfa with 22.1% crude protein.
[2]Higher quality alfalfa with 23.9% crude protein.
[3]Sweet Bran (Cargill Inc., Blair, NE).
NE_L = net energy for lactation.

Table 2. Dry matter intake (DMI), water intake, and feeding behavior parameters for control cows and cows supplemented with a *Saccharomyces cerevisiae* fermentation product (NutriTek; NT) from -29 ± 5 days relative to calving through 42 days in milk

				P-values				
	Control	NT	SEM[1]	Treatment	Time[2]	Treatment × time	Parity	Treatment × parity
Prepartum measure								
DMI, lb/day	25.40	25.84	1.10	0.70	< 0.001	0.76	< 0.01	NS
Meal count/day	8.66	9.27	0.22	0.06	< 0.001	0.44	0.52	0.03
Meal weight, lb	2.89	2.84	0.13	0.75	< 0.001	0.03	< 0.01	NS
Meal length, minutes	28.28	29.49	0.94	0.28	< 0.001	0.03	0.91	NS
Inter-meal interval, hours	2.26	2.09	0.05	0.03	0.28	0.10	0.04	0.01
Postpartum measure								
Water intake, L/day	104.3	109.7	3.7	0.16	< 0.001	0.60	< 0.001	0.32
DMI, lb/day	45.38	45.62	1.15	0.84	< 0.001	0.75	< 0.001	NS
Meal count/day	11.35	12.60	0.45	0.03	< 0.001	0.70	0.52	NS
Meal weight, lb	4.39	4.45	0.24	0.83	< 0.001	0.34	0.45	NS
Meal length, minutes	23.77	25.77	1.30	0.22	< 0.001	0.62	0.05	NS
Inter-meal interval, hours	1.81	1.62	0.09	0.07	< 0.001	0.55	0.07	NS

[1]Pooled standard error of the mean.
[2]Time is by week for DMI and by day relative to calving for feeding behavior parameters.

Table 3. Lactation performance and energy balance for control cows and cows supplemented with a *Saccharomyces cerevisiae* fermentation product (NutriTek; NT) from 29 ± 5 days prepartum to 42 days in milk

				P-values		
	Control	NT	SEM[1]	Treatment	Week	Treatment × week
Milk, lb/day	91.18	88.71	2.78	0.43	< 0.001	0.24
Milk fat, %	3.96	4.32	0.11	0.01	< 0.001	< 0.05
Milk fat, lb/day	3.62	3.90	0.18	0.10	< 0.001	0.09
Milk protein, %	3.03	3.12	0.04	0.16	< 0.001	< 0.01
Milk protein, lb/day	2.73	2.67	0.09	0.48	< 0.001	0.61
Milk lactose, %	4.93	4.87	0.02	0.06	< 0.001	0.70
Milk lactose, lb/day	4.50	4.34	0.13	0.29	< 0.001	0.41
Milk urea nitrogen, mg/dL	11.51	12.42	0.38	0.06	< 0.001	0.21
Milk somatic cell linear score[2]	2.32	1.94	0.28	0.29	< 0.001	0.55
Energy-corrected milk, lb/day	96.78	99.69	3.66	0.41	< 0.001	0.09
Fat-corrected milk, lb/day	96.80	101.28	4.19	0.32	< 0.001	0.20
Energy balance, Mcal/day	-4.34	-6.15	0.74	0.03	< 0.001	0.20
Observed diet NE_L, Mcal/kg DM	1.83	1.90	0.04	0.18	< 0.001	0.19

[1]Pooled standard error of the mean.
[2]SCLS = $\log_2$(somatic cell count/100) + 3.

Table 4. Metabolic and inflammatory biomarkers in plasma of control cows and cows supplemented with a *Saccharomyces cerevisiae* fermentation product (NutriTek; NT) from 29 ± 5 days prepartum to 42 days in milk

				P-values		
	Control	NT	SEM[1]	Treatment	Week	Treatment × week
Glucose, mg/dL	64.0	62.7	2.06	0.60	< 0.001	0.14
Insulin, µg/L	0.12	0.12	0.01	0.46	< 0.001	1.00
NEFA,[2] µEq/L	420	444	21	0.36	< 0.001	0.13
BHBA,[3] µM	556	572	22	0.57	< 0.001	0.12
Haptoglobin, ng/mL	514	575	42	0.18	0.08	0.51

[1]Pooled standard error of the mean.
[2]Non-esterified fatty acids.
[3]Beta-hydroxybutyric acid.

Table 5. Disease incidence for control cows and cows supplemented with *Saccharomyces cerevisiae* fermentation product (NutriTek; NT) from 29 ± 5 days prepartum to 42 days in milk

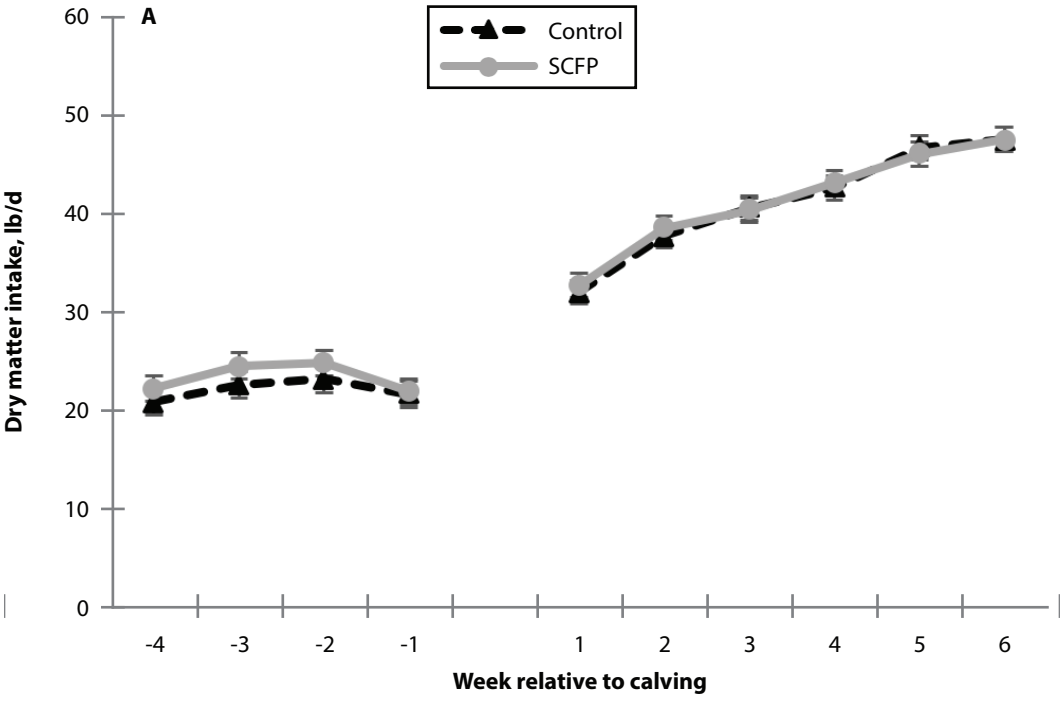
	Control	NT
At-risk[1]	33	32
Fever	9	5
Displaced abomasum	0	2
Retained placenta	2	0
Ketosis	4	12*
Mastitis	2	1
Other[2]	4	1

[1]Includes all cows that surpassed the exclusion criteria at calving. Cows excluded from analysis due to periparturient issues were included.
[2]Other includes 1 case of peritonitis resulting in death (control), 3 foot injuries (2 control, 1 NT), and 1 diarrhea/digestive upset at calving (control).
*Fisher's exact test: $P = 0.02$. No other conditions were significantly affected by treatment.

Table 6. Partial budget analysis of supplementing cows with a *Saccharomyces cerevisiae* fermentation product (NutriTek: NT) from 28 days before calving through 42 days in milk

Revenue or expense category	Control	NT
Milk income		
Fat income per cow, $2.51/lb	$9.08	$9.80
Protein income per cow, $1.78/lb	$4.87	$4.75
	$13.94	**$14.55**
Feed cost[1]		
Prepartum per cow/day	$2.67	$2.85
Postpartum per cow/day	$5.45	$5.61
	($5.70)	**(5.87)**
Expense due to ketosis (per cow daily over 42 days at risk)		
Ketosis incidence, %	*12*	*38*
Cost of treatment per case,[2] $	$12.00	$12.00
	($0.03)	**($0.11)**
Income over feed and treatment costs	$8.22	$8.57

[1]Overall feed cost divides prepartum feeds costs over 305 days plus postpartum feed costs.
[2]Ketosis treatment cost represents 3 days of propylene glycol at $4/day, including labor.



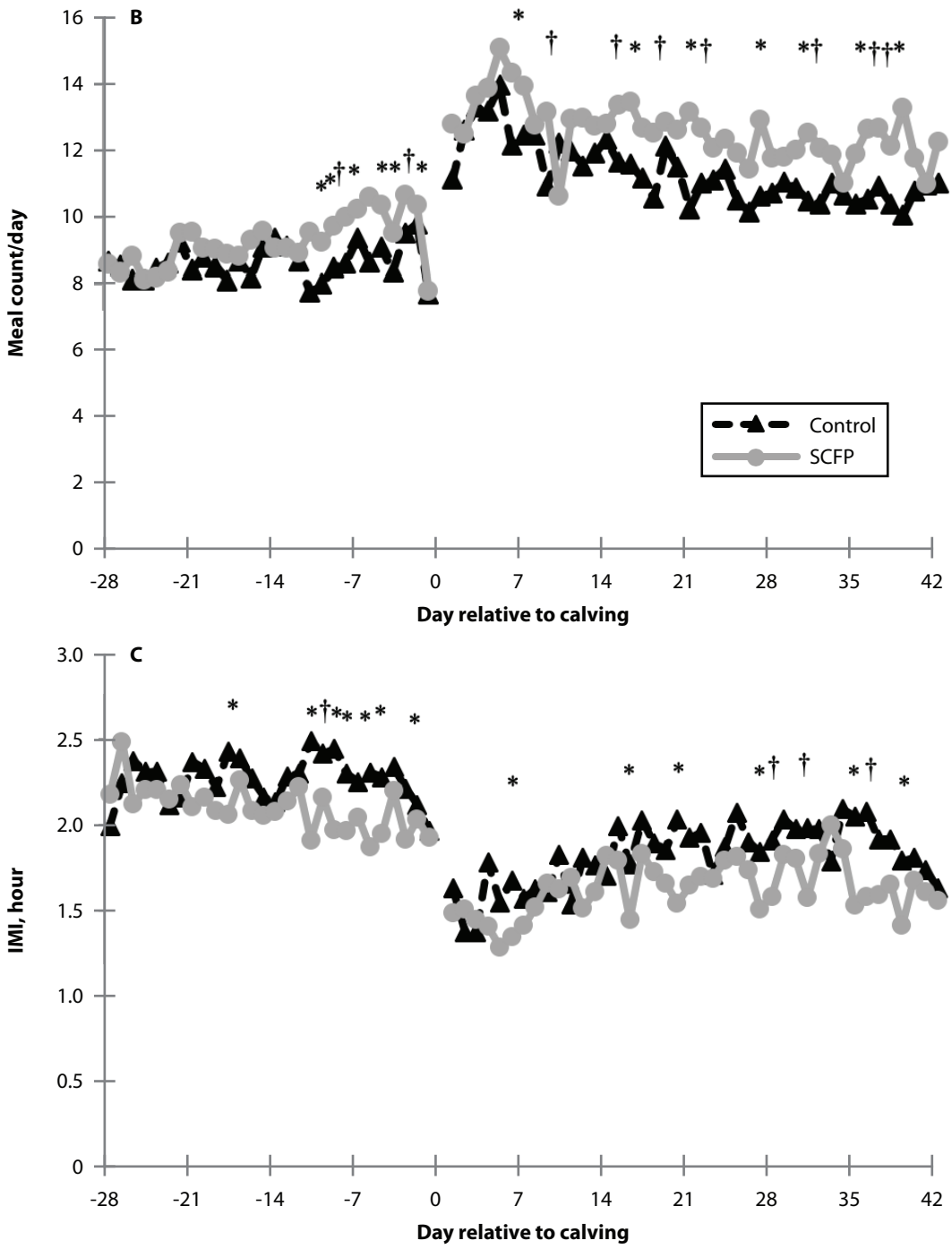
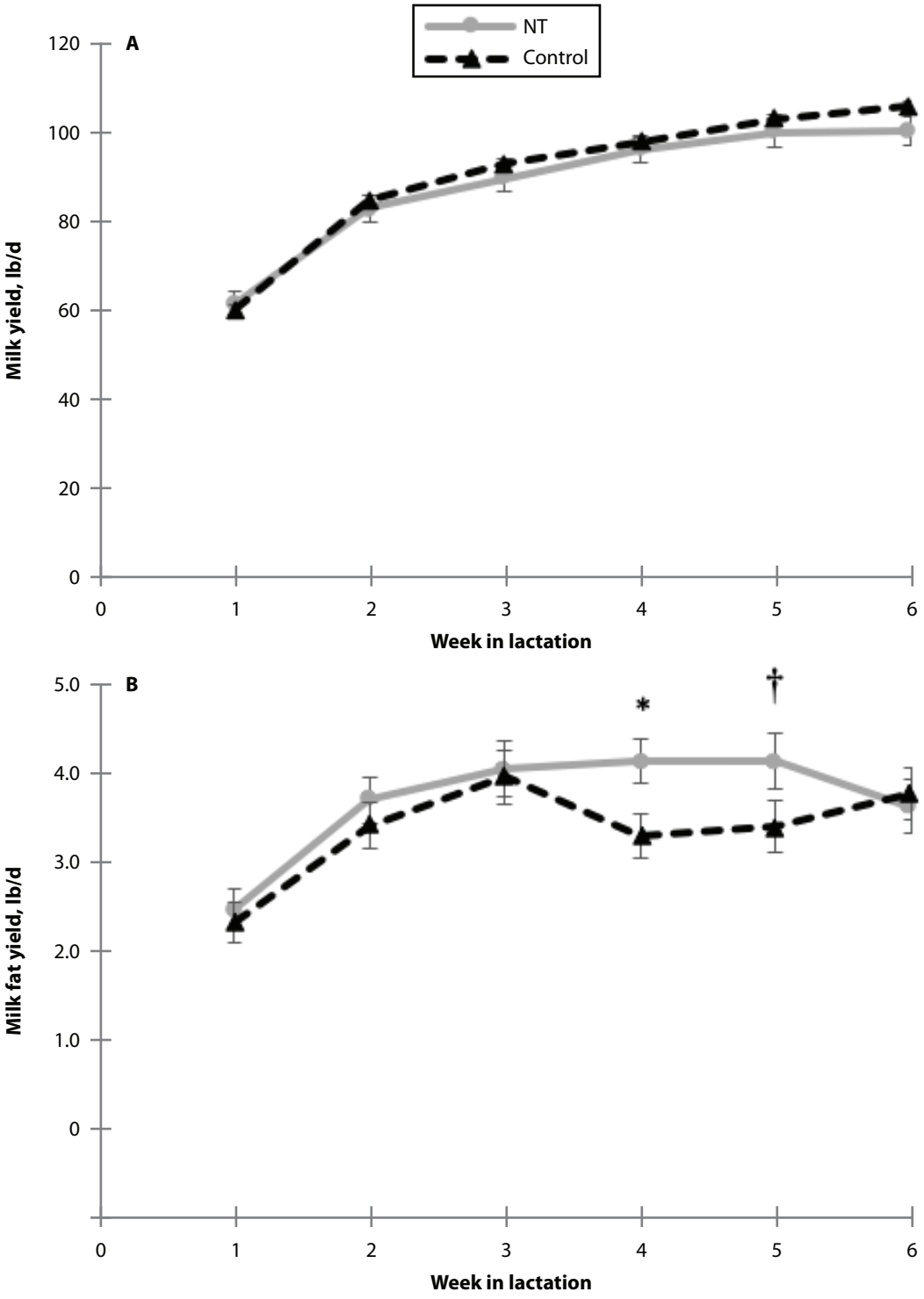


Figure 1. Dry matter intake (A), meal count (B), and inter-meal interval (IMI; C) for control cows and cows supplemented with *Saccharomyces cerevisiae* fermentation product (NutriTek, NT) from -29 ± 5 days relative to calving (DRTC) through 42 days in milk. An effect of time was present both prepartum and postpartum for all measures ($P < 0.001$). Treatment differences are indicated by *($P < 0.05$) and †($0.05 \leq P < 0.10$). A) DMI was not affected by NT ($P \geq 0.75$). B) NT cows tended to consume more meals per day prepartum ($P = 0.06$) and did increase meals per day postpartum ($P = 0.03$). Prepartum standard error of the means = 0.22, postpartum standard error of the means = 0.45. C) NT decreased time between meals prepartum ($P = 0.03$) and tended to decrease inter-meal interval postpartum ($P = 0.07$). Prepartum standard error of the means = 0.05, postpartum standard error of the means = 0.09.


NT
Control
A
Milk yield, lb/d
120
100
80
60
40
20
0
0
1
2
3
4
5
6
Week in lactation
B
Milk fat yield, lb/d
5.0
4.0
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Week in lactation

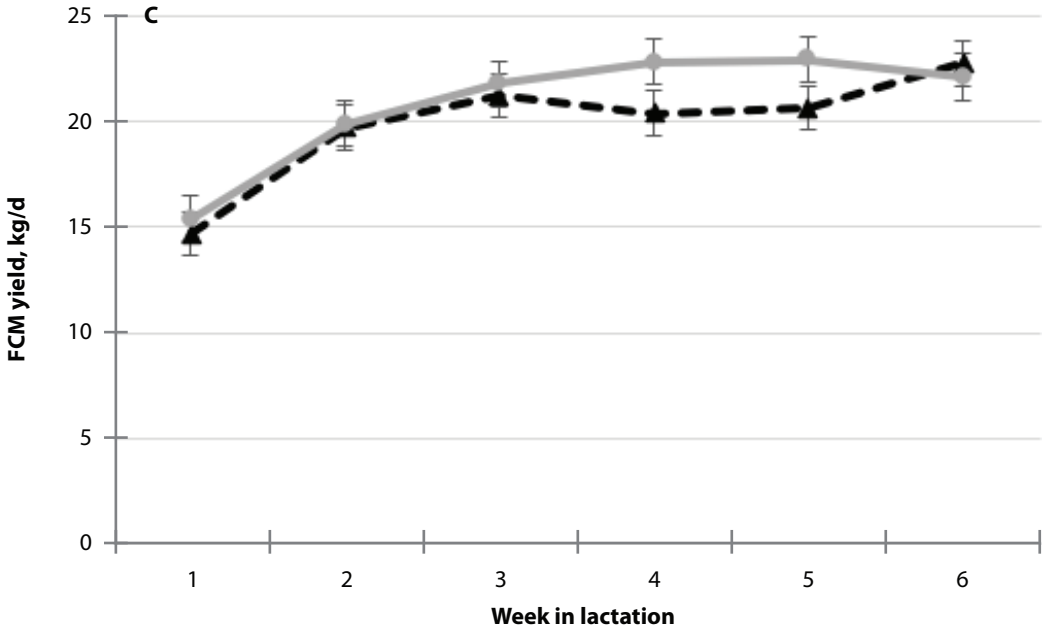

Figure 2. Milk yield, milk fat yield, and fat-corrected milk (FCM) of control cows and cows supplemented with *Saccharomyces cerevisiae* fermentation product (NutriTek, NT) from day -29 ± 5 relative to calving through 42 DIM. A) Milk yield did not differ between treatments ($P = 0.43$). There was an effect of week ($P < 0.001$), but no treatment × week interaction ($P = 0.24$). B) Weekly milk fat yield was not different for cows supplemented with NT compared to control cows ($P = 0.10$). Milk fat yield differed by week ($P < 0.001$), and there was a tendency for a treatment × week interaction ($P = 0.09$). Treatment differences are indicated by *($P < 0.05$) and †($0.05 \leq P < 0.10$). C) Fat-corrected milk did not differ between treatments ($P = 0.32$) and there was no treatment × week interaction ($P = 0.20$).

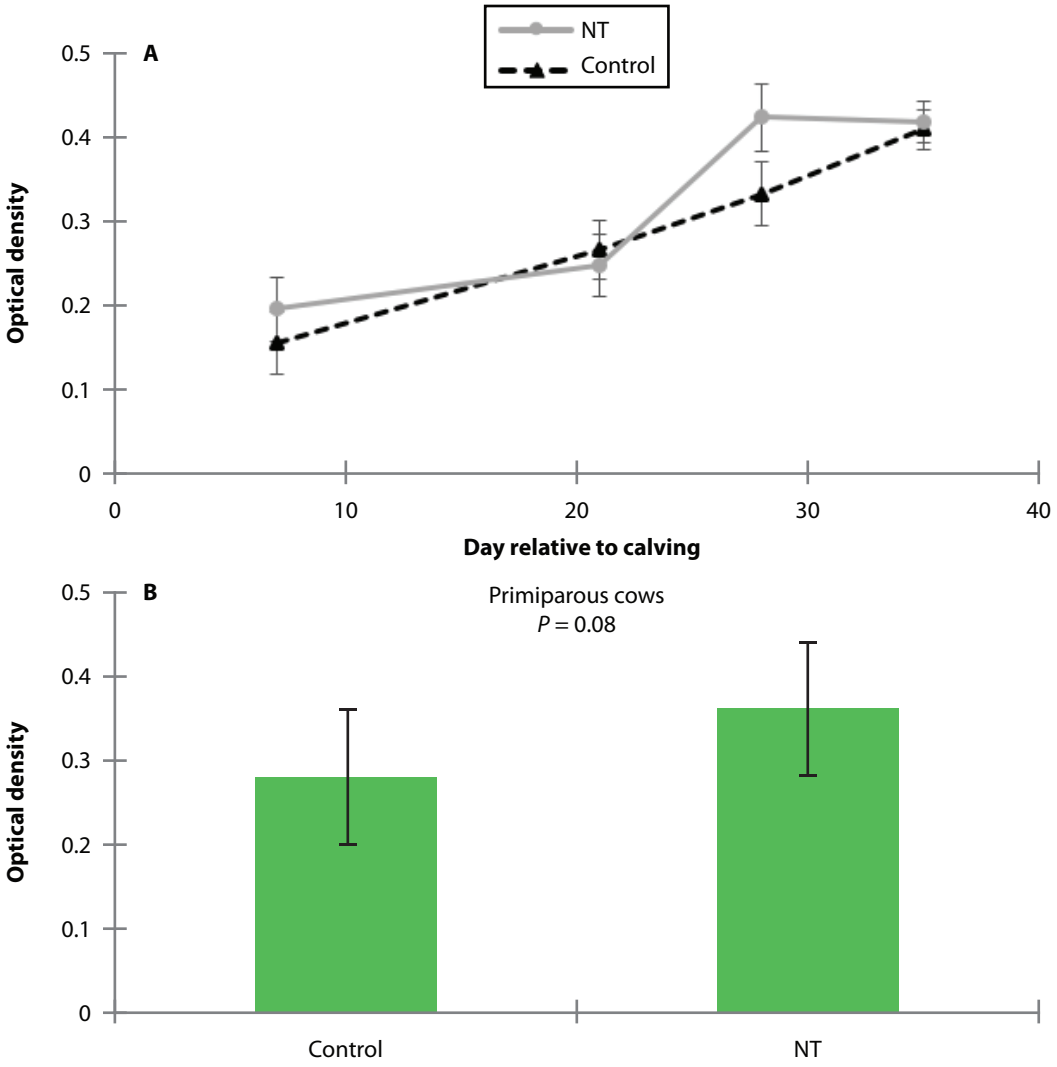

Figure 3. Serum concentrations of anti-ovalbumin-IgG in cows supplemented with *Saccharomyces cerevisiae* fermentation product (NutriTek, NT) from day -29 ± 5 relative to calving through 42 days in milk and challenged with a subcutaneous injection of ovalbumin on day 7 of lactation. A) Anti-ovalbumin IgG concentration increased with time after injection ($P < 0.001$); however, did not differ by treatment ($P = 0.25$). B) The tendency for a treatment × parity interaction ($P = 0.08$) indicated greater anti-ovalbumin IgG concentration in primiparous NT cows compared to control.

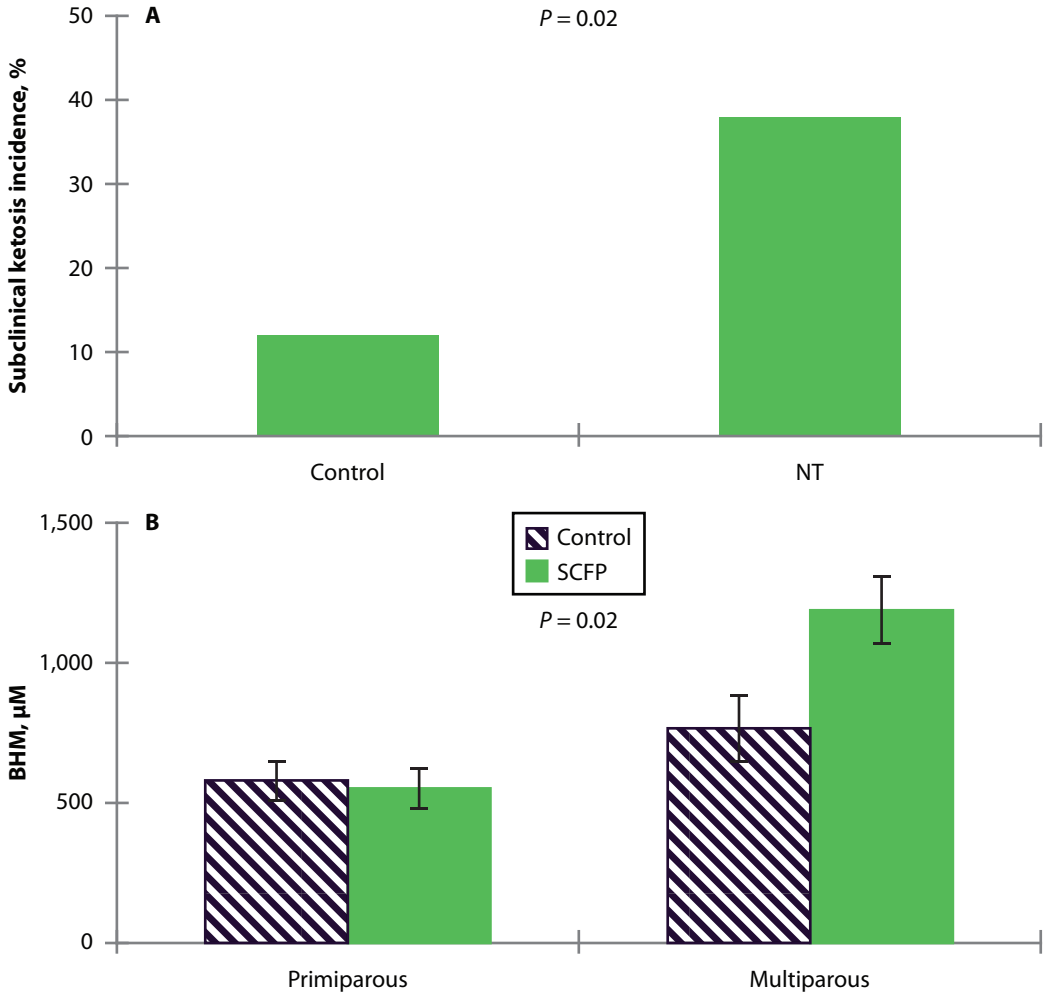

Figure 4. A) Supplementation with a *Saccharomyces cerevisiae* fermentation product (NT) from day -29 ± 5 relative to calving through 42 days in milk increased incidence of subclinical ketosis. B) When analyzed independently, week 2 β-hydroxybutyrate (BHB) concentrations demonstrated a significant parity × treatment interaction with differences for multiparous but not primiparous cows.

Dominance of Right Ovary Structures in Lactating Dairy Cows

J.S. Stevenson

Summary

The objectives were to determine the absolute and relative ovary location of dominant and preovulatory follicles and corpora lutea (CL) present in ovaries before and during hormone synchronization and their associations with CL regression, ovulation, and pregnancy rates in lactating dairy cows. Cows were exposed to presynchronization treatments of PGF_{2a}, GnRH, or both, 3 to 4 weeks before initiating a timed artificial insemination (AI) program (GnRH-1 – 7 days – $PGF_{2\alpha}$ [1 dose or 2 doses 24 hours apart] – 56 hours after first or only dose of $PGF_{2\alpha}$ – GnRH-2 – 16 hours – timed AI) in which cows were first inseminated at 72 ± 3 days in milk. Blood samples were collected to assess progesterone concentration before ovarian structures were mapped in 691 cows and before each hormone treatment (GnRH-1, $PGF_{2\alpha}$, and GnRH-2).

Follicles that ovulated and CL were detected more often in right than left ovaries. Location of dominant follicles before GnRH-1 tended to be more right than left with co-dominant follicles in both ovaries. In response to GnRH, more left-ovary follicles ovulated contralateral to CL than right-ovary follicles, but fewer left-ovary follicles ovulated ipsilateral to CL (left to left and right to right). Dominance of right-ovary CL was less than 50% because of multiple CL in both ovaries before GnRH-1 (15.8%) and before $PGF_{2\alpha}$ (35.6%), resulting from GnRH-1-induced ovulations in the latter case. Preovulatory follicles before $PGF_{2\alpha}$ were detected more often ipsilateral than contralateral to CL induced by GnRH-1, but were of equal frequency ipsilateral or contralateral to older CL present before GnRH-1.

Death of the CL in response to $PGF_{2\alpha}$ was greater for cows bearing older CL (95.3%) or older CL + younger CL (93.7%) compared with cows bearing only younger GnRH-1-induced CL (80.6%). Risk of CL death did not differ regardless of the ipsilateral or contralateral location of younger CL relative to older CL. Pregnancy rate was greater in cows having both older and younger CL (i.e., these cows had a CL at GnRH-1 and ovulated in response to GnRH-1) compared with cows having only a younger CL at $PGF_{2\alpha}$. Pregnancy rate also was greater for cows with younger CL when contralateral to older CL compared with cows bearing only younger or older CL before $PGF_{2\alpha}$.

Percentage of female calves born resulting from eggs produced by left or right ovaries did not differ . In contrast, a tendency ($P = 0.14$) existed for more heifers to be born resulting from eggs produced by right than left ovaries for cows that conceived at first service than for cows that conceived at repeat AI services.

Introduction

The two ovaries in most domestic farm animals do not function equally during the estrous cycle; one of the ovaries is often more active than the other. Ovulation occurs more frequently from right than left ovaries varying from 54 to 60% in ewes and goats

and from 60 to 65% in cows. In contrast, the left ovary in the sow is more functional, producing 55 to 60% of the oocytes, and the mare ovulates approximately 60% of the oocytes from the left ovary. Explanations for right-dominated ovulations in ruminants have included the proximity of the left ovary to the rumen and other extrinsic factors such as temperature or pressure fluctuations and mechanical contractions of the rumen. It is not clear if any intrinsic factors play a role.

Relative ovarian location of preovulatory follicles and corpora lutea (CL) resulted in shorter interovulatory intervals when follicle and CL were in the same (ipsilateral) than when in the contralateral ovary in spontaneously cycling heifers. In cattle, several studies have found positive CL-follicle intraovarian relationships during the estrous cycle, but other studies have not.

To our knowledge, no information is known about the spatial relationship (ipsilateral [same side] vs. contralateral [opposite side]) of ovarian follicles with CL and subsequent risks for ovulation, CL regression, or pregnancy during the estrous cycle when GnRH treatments are applied to accomplish hormonal synchronization before timed AI. For example, do younger GnRH-induced CL regress at different rates in presence or absence of an older CL or because of their different spatial relationship to the dominant or preovulatory follicle? Do follicles in the left or right ovary respond differently to GnRH-induced LH secretion to form CL when ipsilateral or contralateral to a younger or older CL?

Hypotheses tested included: (1) a greater proportion of ovarian structures (dominant or preovulatory follicles and older or GnRH-induced younger CL) are found in the right compared with the left ovary; (2) relative ratio of right to left ovary-bearing CL is altered after exposure to GnRH; (3) younger, GnRH-induced CL found ipsilateral to older CL have greater CL regression than those contralateral to older CL; and (4) spatial and relative location of preovulatory follicles before AI do not affect ovarian characteristics and subsequent risks of ovulation and pregnancy.

The objectives were to determine various outcomes (changes in size of dominant follicles, progesterone concentration, risks for CL regression, ovulation, pregnancy, and calf gender at birth) in lactating dairy cows exposed to hormonal synchronization that were associated with the:

- absolute and relative spatial location of ovarian structures before and after exposure to GnRH, and their spatial relation to new GnRH-induced CL or older CL;
- ovary location of preovulatory follicles and their relative location to GnRH-induced younger and older CL present before GnRH; and
- luteal environment (younger CL, older CL, or both) at the time of PGF_{2a}.

Experimental Procedures

Source of Data

Maps of ovarian structures obtained by transrectal ultrasonography constructed previously in four studies were re-examined to determine ovarian spatial locations of dominant and preovulatory follicles relative to older and younger GnRH-induced CL identified before and AI. Lactating Holstein cows were exposed to a standard 7-day timed AI program (GnRH-1 – 7 days – $PGF_{2\alpha}$ – [1 dose or 2 doses 24 hours apart]

56 hours – GnRH-2 – 16 hours – timed AI) before first postpartum AI. In one study, some cows received either one 50-mg dose of $PGF_{2\alpha}$ (dinoprost tromethamine, Zoetis Inc., Kalamazoo, MI) or two 25-mg doses of $PGF_{2\alpha}$ administered 24 hours apart; otherwise, cows received 1 standard 25-mg dose of $PGF_{2\alpha}$. In that same study, cows exposed to a 5-day Ovsynch timed AI program were not included in the present study except to examine side of ovulation and calf gender. In each of the 4 studies, estrous cycles were presynchronized with either 2 doses of $PGF_{2\alpha}$ administered 14 days apart (the last of which was administered 10 days before initiating Ovsynch) or presynchronization combinations of GnRH and $PGF_{2\alpha}$ ($PGF_{2\alpha}$ and GnRH administered 10 and 7 days before Ovsynch or GnRH and $PGF_{2\alpha}$ administered 17 and 10 days before Ovsynch).

Data Collection

Before GnRH-1, and at 0 and 48 hours after $PGF_{2\alpha}$ (first or only treatment of $PGF_{2\alpha}$), ovaries were scanned by transrectal ultrasonography (7.5-MHz linear-array transducer, Aloka 500V; Corometrics Medical Systems Inc., Wallingford, CT) and all ovarian follicles > 5 mm were sized by electronic calipers and mapped relative to location of CL. Ovulation (single or multiple) was determined by disappearance of follicles previously mapped and recorded either 6 or 7 days after GnRH treatments. Frozen images of follicles and CL were assessed using electronic calipers and measuring diameters in 2 directions perpendicular to one another. Total volume of luteal tissue was calculated ($4/3 \times r^3 \times p$, where r = radius [W/2 + H/2]/2; W = largest width and H = largest height of the structure; and p = 3.14159). When a luteal structure contained a fluid-filled cavity, volume of the cavity was subtracted from the total luteal volume. Blood samples were collected by caudal vessel puncture at GnRH-1, and 0 and 48 hours after $PGF_{2\alpha}$.

Ovary location of each dominant and preovulatory follicle was determined (right, left, or both, only when co-dominant follicles ovulated) and their spatial location (ipsilateral, contralateral, or both) relative to CL identified and present before GnRH-1 (defined as older CL that was at least 7 days old but most likely 10 to 14 days old at the time of $PGF_{2\alpha}$ treatment) or relative to new younger CL formed after treatment with GnRH-1 (approximately 5 days old at the time of $PGF_{2\alpha}$ treatment). Co-dominant follicles were verified by post-ovulatory examinations of a newly revealed CL located on the ovarian map previously occupied by dominant follicle(s). For cows that became pregnant, the side of ovulation was determined as described previously and gender of calf at birth was recorded.

Results and Discussion

Dominant Follicle Location

Frequency of finding the dominant follicle in the right ovary was greater ($P \leq 0.05$) than in the left ovary before both GnRH treatments (Table 1). A numerical shift of dominant follicle locations was observed after GnRH-1 and before GnRH-2. The proportion of dominant follicles in right ovaries did not change (57.3 vs. 55.9%), but proportions of dominant follicles in left ovaries numerically decreased after GnRH-1 (38.0 to 35.3%), whereas more multiple preovulatory follicles were found in both ovaries after GnRH-1 (4.7 to 8.8%; Table 1). This shift was not associated with any difference in ovulation risk between ovaries after GnRH-1 (Table 1). When the dominant follicle was in the right ovary before GnRH-2, frequency of ovulation after GnRH-2 tended ($P = 0.06$) to be greater (95.1 vs. 91.3%) than when the dominant follicle was

in the left ovary. Neither diameter of the dominant follicles, number of CL per cow, nor progesterone concentration differed between ovaries bearing the dominant follicle before either GnRH treatment (data not shown).

Switching ovary location of the CL dominance from before to after GnRH-1 or GnRH-2 treatments differed between ovaries. Switching of CL-ovary contralaterally from left (before GnRH) to right (after GnRH) was more ($P < 0.001$) common than right to left (Figure 1). Furthermore, when the CL-ovary dominance did not change after GnRH, it remained ipsilateral right (before GnRH) to right (after GnRH) more ($P < 0.001$) often than left to left (Figure 1). This effect did not differ in response to either GnRH-1 or GnRH-2 treatment and reinforces the greater activity of the right compared with the left ovary.

Corpus Luteum Location

Frequency of finding CL in the right ovary was greater ($P \leq 0.05$) than in the left ovary before and after GnRH-1 treatment when assessed before PGF_{2a} treatment (Table 2). The proportion of CL in the left or right ovary decreased by 13.2 or 6.5 percentage points from before to after GnRH-1, whereas the additive increase in CL detected in both ovaries doubled ($P < 0.001$) from 15.8% before GnRH-1 to 35.6% at the time of PGF_{2a} such that the frequency of CL was not different between right ovaries and both ovaries (Table 2). Ovulation risk in response to GnRH-1 tended ($P = 0.06$) to be less when CL were found in both ovaries (54.6%) compared with cows having 1 CL in the left ovary (67.3%) and none in the right ovary (60.7%). Diameter of dominant follicles did not differ between ovaries (data not shown). As expected, number of CL and progesterone concentration were greater ($P \leq 0.05$) when a CL was detected in both ovaries compared with a CL in either the right or left ovary. Subsequent ovulation risk of preovulatory follicles in response to GnRH-2 did not differ between ovaries (Table 2).

Location and Death of the Corpus Luteum

Progesterone concentrations were related to age and number of CL before $PGF_{2\alpha}$ treatment for cows having both younger CL + older CL at the time of $PGF_{2\alpha}$ treatment compared with cows having only younger or older CL (Table 3). Regression of CL was greater ($P \leq 0.05$) in cows having older CL or younger + older CL compared with only younger CL (based on progesterone cut points of < 1.0 or < 0.5 ng/mL at 48 hours after $PGF_{2\alpha}$ treatment and 24 hours before timed AI; Table 3). Cows having only older CL also had greater ($P < 0.05$) proportion of cows showing CL regression than that for cows bearing younger + older CL (cut point < 0.5 ng/mL; Table 3).

The largest preovulatory follicle tended ($P = 0.08$) to be greater in cows bearing only a new CL compared with cows bearing only older CL or younger CL + older CL. The second largest preovulatory follicle was greater ($P \leq 0.05$) in diameter for cows bearing only younger CL compared with cows having older CL and tended ($P = 0.08$) to be greater than that in cows with both CL types (Table 3). Single ovulation risk after GnRH-2 did not differ among CL age classes, but multiple ovulation risk was 2.0 and 2.9 times greater ($P \leq 0.05$) in cows bearing only younger CL after $PGF_{2\alpha}$ treatment compared with cows bearing both CL types and those bearing only older CL, respectively. Subsequent pregnancy rates at 32 days after AI tended ($P = 0.08$) to be and was greater ($P \leq 0.05$) for cows with both CL types compared with cows having older CL

or younger CL, respectively (Table 3). Pregnancy rates at 60 days after AI were greater ($P \leq 0.05$) for cows that had both CL types before $PGF_{2\alpha}$ treatment compared with cows having only older or younger CL, respectively. Cows bearing older CL before CL regression had greater ($P \leq 0.05$) pregnancy rates at 60 days after AI than cows that had only new CL. Pregnancy loss tended ($P = 0.08$) to be less for cows with both CL types compared with cows that had only older CL before timed AI (Table 3).

Trans-uterine migration of the embryo in cattle is extremely rare so the ovary from which the egg is derived determines the uterine horn in which the pregnancy will be carried (i.e., right ovulation, right-horn carried calf or left to left). In the case of twins, when ovulations occur from both ovaries, each uterine horn will bear one embryo. If double ovulation occurs from one ovary, one of the twins will eventually be found in the opposite uterine horn.

Based on single pregnancies in this study, the percentage of female calves born resulting from eggs produced by left or right ovaries did not differ for left or right uterine horns or for cows that conceived at first vs. repeat AI services (Figure 2). In contrast, a tendency ($P = 0.14$) was detected for more heifers to be born resulting from eggs produced by right than left ovaries (i.e., more females carried in right than left horns) for cows that conceived at first AI services and the opposite was observed for cows that conceived at repeat AI services (i.e., fewer females carried in the right than left horn; Figure 3).

Conclusions

We accept the first hypothesis that frequency of right ovarian structures was more prevalent in the present study, both before and after GnRH treatments. When exposed to GnRH more follicles in left ovaries ovulated contralaterally than right-ovary follicles, and the reverse was true for right ovaries; fewer left-ovary follicles ovulated ipsilaterally than right-ovary follicles. Preovulatory follicles before $PGF_{2\alpha}$ were detected more often ipsilateral than contralateral to CL induced by GnRH, but were of equal frequency ipsilateral or contralateral to older CL present before GnRH-1.

The second hypothesis is supported by more CL identified in right than left ovaries before and after GnRH. After GnRH-induction of ovulation, frequency of right-ovary CL was twice that found in left ovaries, mostly resulting from a doubling of multiple ovulation found in both ovaries.

The third hypothesis was rejected because, although CL regression was greater for cows bearing older CL or older CL + younger CL compared with cows bearing only younger CL, CL regression was unaltered regardless of ipsilateral or contralateral location of younger CL relative to older CL. Regression of CL was compromised in cows bearing younger CL, which was associated with greater-size ovulatory follicles that developed in a reduced progesterone environment leading to 2 to 3 times more multiple ovulation, and lesser subsequent pregnancy rates than in cows with at least 1 older CL before $PGF_{2\alpha}$ treatment.

Not only is the right ovary more active in producing follicles and CL in dairy cows based on present evidence, but more fertilized oocytes produced from right than left ovaries cleave and form blastocysts based on another study in the scientific literature.

A tendency for more right-ovary derived heifer calves occurred when conceptions occurred at first AI service and the opposite tended to occur when conceptions occurred at repeat AI services.

Table 1. Ovary location of the dominant follicle (DF) and subsequent ovulation risk to GnRH treatments during the Ovsynch timed AI program

Ovary	Cows, no.	DF location, %	Ovulation risk, %
	Before GnRH-1 (onset of Ovsynch)		
Left	253	38.0[a]	67.2[a]
Right	381	57.3[b]	66.7[a]
Both	31	4.7[c]	100[b]
	Before GnRH-2 (end of Ovsynch)		
Left	244	35.3[a]	91.3[aA]
Right	386	55.9[b]	95.1[abB]
Both	61	8.8[c]	98.4[b]

[a,b]Means within column and GnRH treatment differ ($P \leq 0.05$).
[A,B]Means within column and GnRH treatment tended to differ ($P = 0.06$).

Table 2. Location of corpora lutea (CL) at the time of GnRH-1 and PGF_{2a} treatments during the Ovsynch with number of CL per cow (± standard error), progesterone concentration before treatments, and subsequent ovulation risk after GnRH and PGF_{2a} treatments

Ovary	Cows, no.	CL location, %	CL per cow, no.	Progesterone, ng/mL	Ovulation risk, %
		Before GnRH-1 (onset of Ovsynch)			
Left	214	34.8[a]	1.2 ± 0.03[a]	3.7 ± 0.2[a]	67.3[aA]
Right	303	49.3[b]	1.2 ± 0.02[a]	3.7 ± 0.1[a]	60.7[ab]
Both	97	15.8[c]	2.2 ± 0.05[b]	5.0 ± 0.2[b]	54.6[bB]
		After GnRH-1 treatment (time of PGF_{2a})			
Left	150	21.6[a]	1.5 ± 0.06[a]	5.8 ± 0.3[a]	94.7[a]
Right	297	42.8[b]	1.5 ± 0.04[a]	5.3 ± 0.2[a]	92.6[a]
Both	247	35.6[b]	2.4 ± 0.05[b]	6.8 ± 0.2[b]	95.5[a]

[a,b]Means within column and GnRH treatment differ ($P < 0.05$).
[A,B]Means within column and GnRH treatment tended ($P = 0.06$) to differ.

Table 3. Characteristics of corpora lutea (CL) at the time of $PGF_{2\alpha}$ treatment and concentrations of progesterone at 0 and 48 hours thereafter including preovulatory follicle diameters (± standard error), luteolytic risk, and subsequent ovulation and pregnancy risks

	CL age class		
Item	Younger CL	Older CL	Younger CL+ older CL
Cows, no.	72	215	368
CL/cow, no.	1.2 ± 0.08[a]	1.4 ± 0.05[b]	2.3 ± 0.03[c]
Progesterone, ng/mL			
0 hours	3.4 ± 0.3[a]	6.2 ± 0.2[b]	7.0 ± 0.1[c]
48 hours	0.8 ± 0.11[a]	0.3 ± 0.06[b]	0.4 ± 0.05[b]
Proportion of cows,[1] %			
Progesterone < 1.0 ng/mL at 48 hours	80.6[a]	95.3[b]	93.7[b]
Progesterone < 0.5 ng/mL at 48 hours	65.3[a]	86.5[b]	79.6[c]
Largest preovulatory follicle (mm)	14.7 ± 0.3[A]	13.8 ± 0.2[B]	13.8 ± 0.2[B]
Second preovulatory follicle (mm)	12.7 ± 0.4[aA]	11.3 ± 0.4[b]	11.9 ± 0.2[bB]
Single ovulation risk after GnRH, %	91.7[a]	93.4[a]	94.3[a]
Multiple ovulation risk after GnRH, %	39.4[a]	13.4[b]	19.6[b]
Pregnancy rate at 32 days after AI,[2] %	26.4[a] (72)	39.4[bA] (213)	46.8[bB] (363)
Pregnancy rate at 60 days after AI,[2] %	23.6[a] (72)	33.3[a] (213)	43.5[b] (363)
Pregnancy loss 32-60 days after AI, %	10.5[AB] (19)	15.5[A] (84)	7.1[B] (170)

[a,b,c]Means within row differ ($P < 0.05$).
[A,B]Means within row tended to differ ($P = 0.08$).
[1]Hours after PGF_{2a} treatment.
[2]Number of cows differed from total because of culling before pregnancy diagnosis occurred.

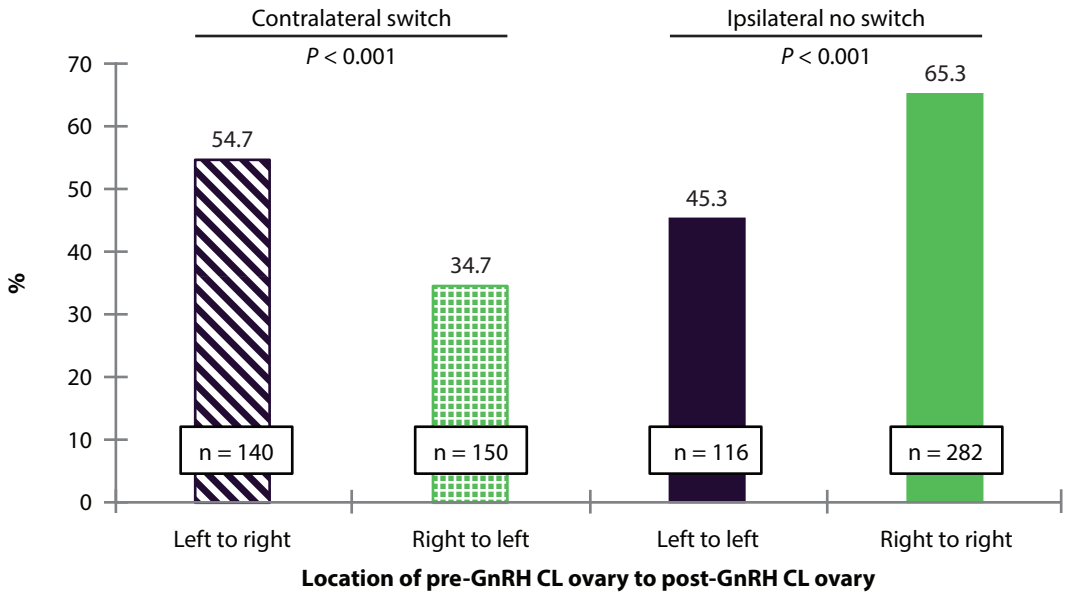

Figure 1. Ovary location of the GnRH-induced corpus luteum (CL) relative to CL present before GnRH treatment in lactating dairy cows. Results include cows exposed to GnRH-1 at the onset of Ovsynch and those exposed to GnRH-2 administered 56 hours after PGF_{2a}. No differences were detected for relative locations after either GnRH treatment so data were combined. Switching of successive CL contralaterally from left (before GnRH) to right (after GnRH) ovaries was more ($P < 0.001$) common than right to left. The new CL on right ovaries were detected more ($P < 0.001$) frequently ipsilaterally right (before GnRH) to right (after GnRH) than for left to left.

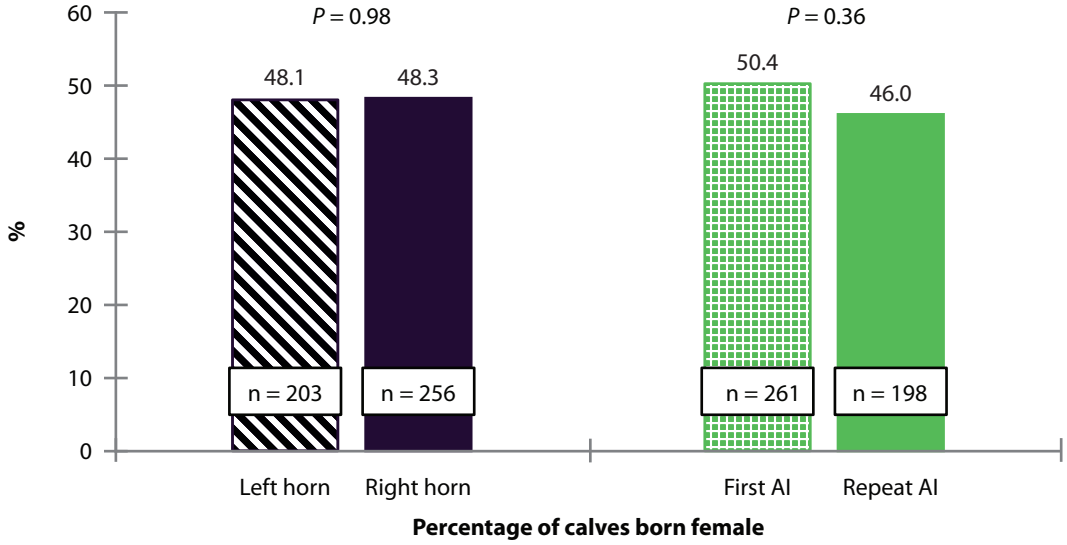

Figure 2. Percentage of female calves born that were carried in either the right or left uterine horn and percentage of female calves born resulting from first or repeat AI services.

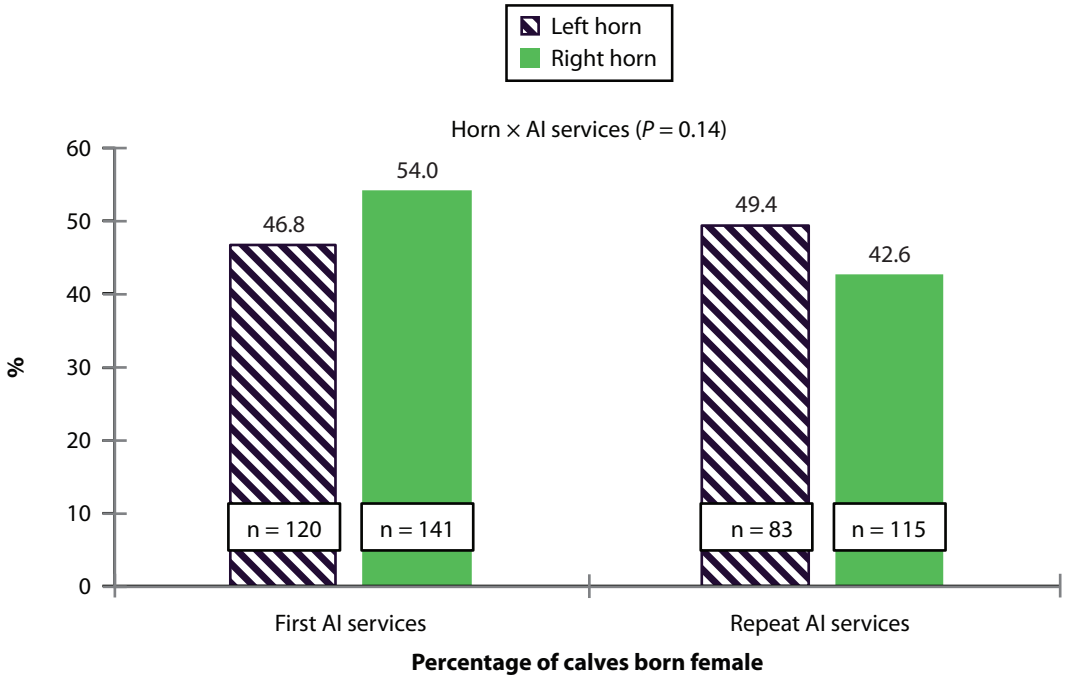

Figure 3. A tendency for more female calves born resulting from right-ovary derived eggs at first services (carried in the right than left horns) compared with fewer right than left pregnancies for cows conceiving at repeat AI services.

Gestation Length and Overall Performance in the Subsequent Lactation of Dairy Cows Conceiving to Holstein, Jersey, or Angus Semen: An Observational Study

A.L.A. Scanavez and L.G.D. Mendonça

Summary

Breeding strategies adopted by commercial dairy herds have evolved in recent years by incorporating the use of several breeds, including beef sires. Results of such strategies on offspring performance have been studied but reports on the effects on dam's overall performance after calving are still lacking. The goal of this observational study was to investigate the associations between sire breed of previous conception, gestation length, and postpartum performance of dairy cows. Records from Holstein and crossbred cows from a Kansas commercial herd were extracted. Data pertaining to cows that conceived from Holstein, Jersey, or Angus sires and initiated second lactation or greater from June 2017 to May 2018 were used in this study. Gestation length was shorter for cows that conceived from Holstein (274.9 ± 0.6 days) compared with Angus sires (276.5 ± 0.6 days). Cows that conceived from Jersey sires had the longest gestation length (278.0 ± 0.4 days). For Holstein cows, milk yield in the first 60 days after calving was influenced by sire breed used on the previous lactation. Holstein cows that became pregnant with Holstein sires had the lowest milk yield compared with other permutations of dam and conceptus breeds. Interestingly, sire breed of previous conception did not influence milk yield in the subsequent lactation of crossbred cows. Cows that became pregnant with Angus sires had greatest incidence of postpartum disorders compared with cows conceiving from Jersey or Holstein sires (28.0, 11.5, and 9.4%, respectively). Nonetheless, sire breed of previous conception did not affect probability of culling in the first 60 days after calving. This study presents evidence that breed of conceptus influences gestation length and milk yield of the dam in the subsequent lactation.

Introduction

Breeding programs in dairy herds have historically been designed to generate replacement heifers of superior quality to promote genetic progress according to the long-term objectives of each operation. With recent advances in reproductive performance, many dairy herds are producing a surplus of heifers, which can negatively impact profitability when feed prices are high, or the market value of replacement heifers is low. In order to optimize profitability, several dairy producers are striving to generate only the necessary number of dairy replacement heifers to maintain the lactating herd. Therefore, dairy semen is utilized in a limited number of inseminations and beef sires are used in the remaining services. On Kansas dairy farms, this strategy is currently being adopted by herds milking both Holstein and crossbred cows. Even though it is well-documented that breed of sire influences performance of the offspring, the effects of sire breed of previous conception on postpartum health, productive, and reproductive performance in the subsequent lactation of the dam are unknown.

Objectives of the present study were to investigate the association between sire breed of previous conception and gestation length, health, productive, and reproductive performance in the subsequent lactation of dairy cows from one commercial herd in Kansas.

Experimental Procedures

Records of Holstein (n = 749) and crossbred (Holstein × Jersey; n = 360) cows from a large commercial dairy herd in Kansas were used for this observational study. Data of cows initiating their second lactation or greater from June 2017 to May 2018 were extracted from the on-farm management software. In the previous lactation, cows were inseminated with Holstein or Jersey semen for the first 3 services. After the third insemination, cows were inseminated with Holstein, Jersey, or Angus sires until conception. Records of cows that became pregnant at first insemination were not included in the analyses.

For each cow, gestation length was calculated by subtracting date of calving from date of previous conception. Cows that aborted or had gestation length shorter than 256 days or longer than 297 days were not included in the study. Data for offspring sex, twinning, and stillbirths were also extracted from the farm software.

Postpartum information regarding occurrence of uterine diseases (retained placenta and metritis), non-uterine diseases (mastitis, displaced abomasum, pneumonia, and lameness), and culling within the first 60 days in milk (DIM) were evaluated. Health or culling events occurring after 60 DIM were not included in the analyses.

Monthly milk tests were conducted in the herd and estimated weekly milk yield was calculated by the on-farm software. Weekly milk yield for the first 8 weeks of lactation was extracted. Records of cows culled before 60 DIM were used until the last complete week preceding culling.

Cows were eligible to be inseminated starting at 50 DIM. Days in milk at first service was calculated as the difference between date of first insemination and date of calving. In addition, pregnancy per artificial insemination (P/AI) at first service was calculated by dividing the number of cows that became pregnant at first AI by the number of cows inseminated.

A linear regression model was used to investigate the association of sire breed of previous conception and gestation length. In addition to sire breed, variables explored in the initial model included cow breed (Holstein vs. crossbred), lactation number, projected milk yield, DIM at conception, season of the year when calving occurred, twinning, offspring sex, and the interaction between sire breed of previous conception and cow breed.

Association of milk yield in the first 8 weeks and sire breed of previous conception were explored using a mixed model statistical approach (MIXED procedure of SAS; Version 9.4, SAS Inst. Inc., Cary, NC). In addition to the variables described in the initial model, gestation length, duration of dry period, days spent in close-up pen, occurrence of stillbirth, week of lactation, and the interactions between sire breed and week, cow breed and week, sire and cow breeds, and week and sire and cow breeds were tested.

Logistic regressions were used to evaluate the association between sire breed of previous conception and occurrence of diseases, culling, and P/AI at first service, and a linear regression was used to explore the association between sire breed of previous conception and DIM at first service in the subsequent lactation.

Given the breeding strategy of the herd, DIM at conception in the previous lactation was forced to remain in all models because it was considered a confounder variable. All linear and logistic regressions were conducted using the GLIMMIX procedure of SAS.

Results and Discussion

Days in milk at conception was considerably greater ($P < 0.01$) for cows that became pregnant with Angus sires than cows pregnant with Jersey or Holstein sires (221.1 ± 43.7, 126.0 ± 44.8, and 122.8 ± 54.5 DIM, respectively). This occurred because of the breeding strategy used by the herd, which did not use Angus semen in the first 3 services. Angus-bred cows also had greatest projected 305-day mature equivalent milk yield (Table 1). Because cows with greater milk yield are less likely to be culled, it is possible that the decision not to use Angus semen in the first 3 services resulted in a biased projected milk yield, favoring cows inseminated with beef semen. Further descriptive information about cows used in the study is summarized in Table 1.

Cows inseminated with Holstein semen had the shortest ($P < 0.01$) gestation length (274.9 ± 0.6 days) and conception with a Jersey sire resulted in the longest gestation length (278.0 ± 0.4 days; Table 2). We speculate that the reduced size of Jersey- and Angus-crossbred calves delayed the initiation of the calving process of cows pregnant with Jersey and Angus sires, and ultimately, extended gestation length of the dams. Further variables and their respective associations with gestation length are presented in Table 2.

Incidence of uterine or non-uterine diseases and mortality within 60 DIM were not affected ($P > 0.16$) by breed of cows or by breed of sire of previous conception (Table 3). When uterine and non-uterine diseases were combined, there was a tendency ($P = 0.07$) for cows conceiving with Angus sires to have greater ($P < 0.05$) disease incidence within 60 DIM than cows conceiving with Jersey or Holstein sires. High body condition score (BCS) at dry-off has been demonstrated to be a risk factor for several metabolic and infectious diseases early in the subsequent lactation. Even though BCS was not assessed in the present study, we speculate that Angus-bred cows had greater BCS at dry-off because they became pregnant in later stages of lactation compared with cows inseminated with Holstein or Jersey sires. High BCS may have contributed to the increased risk of health disorders observed in this group of cows. Nonetheless, sire breed of previous conception was not associated ($P > 0.77$) with proportion of cows sold or culled within 60 DIM. Dam breed impacted culling in the first 60 DIM because a greater proportion ($P < 0.01$) of Holstein cows were sold or culled within 60 DIM than their crossbred counterparts.

For Holstein cows, sire breed of previous conception was associated ($P = 0.02$) with milk yield (Figure 1). Holstein cows that conceived to Holstein sires had reduced ($P \leq 0.09$) milk yield in the first 60 DIM compared with other permutations of dam breed and sire breed of previous conception. Milk yield for crossbred cows was not

affected by sire breed of previous conception. It is possible that conceptus breed influenced size of calves at birth. The potentially larger size of Holstein calves and lack of heterosis of Holstein dams may have negatively impacted calving difficulty, and consequently, decreased milk production in early lactation.

Days in milk at first service did not differ ($P = 0.28$) among cows that conceived from Holstein, Angus, or Jersey sires in the previous lactation (62.2 ± 0.3 d). For P/AI at first service, an interaction occurred between dam breed and sire breed of previous conception. Holstein cows inseminated with Holstein sires had greater ($P = 0.05$) P/AI at first service than the other combinations of dam and sire breeds explored in the study (48 vs. 29%). It is important to note that to investigate differences in P/AI, a large number of services must be evaluated. Therefore, limited conclusions related to reproductive performance can be drawn from this observational study. Days in milk or P/AI at first service did not differ between crossbred and Holstein cows.

In conclusion, the current study presents evidence that gestation length is shorter for dairy cows conceiving with Holstein semen compared with Jersey or Angus sires. In addition, Holstein cows that become pregnant with Holstein sires have reduced milk yield compared with cows pregnant with Jersey or Angus sires, but sire breed does not seem to affect subsequent milk yield of crossbred dams. Further research is necessary to confirm these findings and to evaluate the economic implications of using beef or dairy semen in Holstein and crossbred cows in order to help dairy producers to make profitable decisions when planning breeding programs.

Table 1. Descriptive data (mean ± SD[1]) of multiparous cows that conceived in the previous lactation after artificial insemination using Holstein, Jersey, or Angus sires after two or more services at a commercial dairy farm in Kansas

	Sire breed of previous conception		
Item	Holstein	Jersey	Angus
Number of cows	107	895	107
Lactation number[2]	3.4[a] (1.4)	2.9[b] (1.0)	3.0[b] (1.1)
Previous projected 305-day mature equivalent milk yield, lb	26,434[a] (4354)	27,944[b] (4965)	29,301[c] (4750)
Days in milk at conception	122.8[a] (54.5)	126.0[a] (44.8)	221.1[b] (43.7)
Dry period length, days	56.4[a] (6.1)	60.8[b] (6.8)	62.1[b] (7.6)
Days spent in close-up pen	26.4[a] (5.7)	30.2[b] (5.9)	30.2[b] (5.1)
Stillbirth, %	0.9[a]	2.4[a]	7.6[b]
Twinning, %	1.9	3.7	9.6

[1]Standard deviation.
[2]Lactation number when cows conceived after insemination with Holstein, Jersey, or Angus sires.
[abc]For each item, values within a row with different superscripts differ ($P \leq 0.05$).

Table 2. Gestation length (mean ± SEM[1]) of cows that conceived after artificial insemination using Holstein, Jersey, or Angus sires after two or more services at a commercial dairy farm in Kansas

Item	Gestation length, d	SEM[1]	*P*-value
Sire breed of previous conception			< 0.01
Holstein	274.9[a]	0.6	
Jersey	278.0[b]	0.4	
Angus	276.5[c]	0.6	
Lactation number[2]			< 0.01
1	275.4[a]	0.5	
2	276.5[b]	0.5	
3	277.2[b]	0.5	
≥ 4	276.8[b]	0.6	
Season of calving			0.01
Summer	275.7[a]	0.5	
Fall	277.0[b]	0.5	
Winter	276.6[b]	0.5	
Spring	276.5[b]	0.5	
Twin calving			< 0.01
Yes	274.1[a]	1.2	
No	278.9[b]	0.7	
Offspring sex			< 0.01
Female	275.5[a]	0.9	
Male	277.3[b]	0.9	

[1]Standard error of the mean.
[2]Lactation number when cows conceived after insemination with Holstein, Jersey, or Angus sires.
[ab]For each item, values within a column with different superscripts differ ($P \leq 0.05$).

Table 3. Incidences of uterine, non-uterine, or combinations of uterine and non-uterine diseases within 60 days in milk (DIM) and proportion of cows that died, were sold, and were culled within 60 DIM for multiparous Holstein or crossbred cows that conceived in the previous lactation after artificial insemination using Holstein, Jersey, or Angus sires after two or more services at a commercial dairy farm in Kansas

	Cow's breed		Sire's breed			*P*-value	
Item	Holstein	Crossbred	Holstein	Jersey	Angus	Cow's breed	Sire's breed
Uterine diseases,[1] %	6.1	4.7	3.7	4.6	16.8	0.55	0.45
Non-uterine diseases,[2] %	8.0	5.6	5.6	6.9	11.2	0.17	0.22
Diseases within 60 DIM,[3] %	14.2	10.3	9.4[a]	11.5[a]	28.0[b]	0.13	0.07
Died within 60 DIM, %	1.3	0.3	0.0	1.1	0.9	0.21	0.60
Sold within 60 DIM, %	6.0[a]	1.7[b]	1.9	4.0	12.2	< 0.01	0.90
Culled within 60 DIM, %	7.3[a]	1.9[b]	1.9	5.1	13.1	< 0.01	0.78

[1]Retained placenta or metritis.
[2]Mastitis, displaced abomasum, pneumonia, or lameness occurring within 60 DIM.
[3]Retained placenta, metritis, mastitis, displaced abomasum, pneumonia, or lameness within 60 DIM.
[ab]For each item, values within a row with different superscripts differ ($P \leq 0.05$).

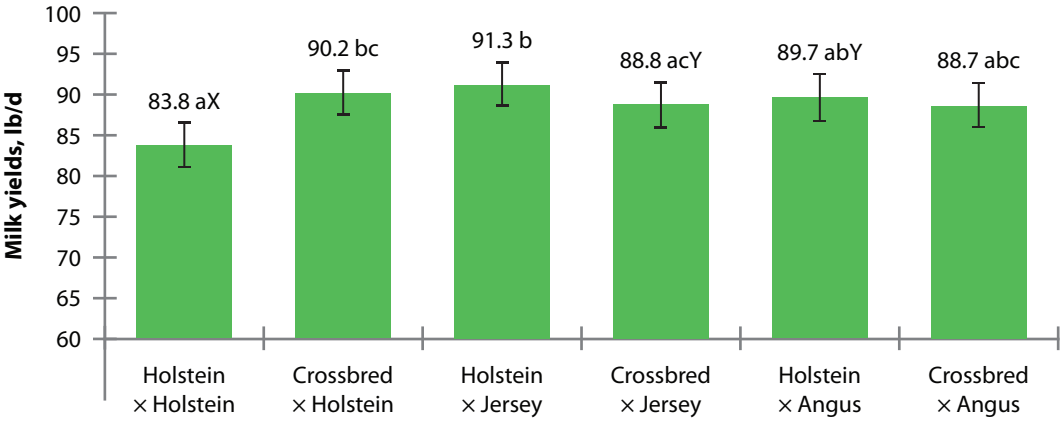

Figure 1. Average milk yield in the first 8 weeks of lactation of multiparous Holstein or crossbred cows that conceived in the previous lactation after artificial insemination using Holstein, Jersey, or Angus sires at a commercial dairy farm in Kansas. Cow breed, $P = 0.53$; sire breed of previous conception, $P = 0.25$; interaction between cow and sire breed, $P = 0.02$. Differences and tendencies are represented by different letters (a, b, c: $P \leq 0.05$; X, Y: $P \leq 0.10$).

Relationship Between Body Condition Score Change During the Prepartum Period and Week Four Milk Yield of Dairy Cows

C.A. Gamarra, A.L.A. Scanavez, and L.G.D. Mendonça

Summary

The objectives of the study were to (1) evaluate the association between body condition score (BCS) change in the prepartum period and week four milk yield and (2) explore whether average week four milk yield can be used as an indicator of the percentage of cows having BCS loss before calving at the herd level. Cows that had excessive BCS loss had decreased milk yield in early lactation relative to cows that did not change BCS or had moderate BCS loss in the dry period. Nonetheless, monitoring average week four milk yield does not appear to be a reliable indicator of the percentage of cows that underwent excessive BCS loss in the prepartum period. In conclusion, despite its association with BCS change during the dry period, week four milk yield cannot be used as a reliable parameter to monitor whether cows experience excessive BCS loss before calving.

Introduction

Evaluation of postpartum performance is critical to monitor transition cow programs of dairy herds. Poor transitions from late gestation to early lactation can affect health, production, reproduction, and lifetime profit of dairy cows. Events such as pen moves and diet changes during the transition period may impair postpartum performance. In addition, management practices in the prepartum pen can affect dry matter intake of cows, mobilization of body fat before calving, and, consequently, overall performance in the postpartum period. Therefore, monitoring herd parameters related to postpartum performance can assist in evaluating the transition cow program of dairy herds.

It has been suggested that week four milk yield can be used as an indicator of transition cow performance and a predictor of future production of dairy cows. It is possible that reduced week four milk yield of the herd may indicate limited feed intake in the pre- and postpartum period, substantial changes of body condition score (BCS) during the transition period, and increased proportion of cows with postpartum diseases. Nonetheless, to our knowledge, no published studies have evaluated the validity of week four milk yield for monitoring transition cow programs at the herd level. The objectives of this study were to evaluate the association between BCS change in the prepartum period and week four milk yield and explore the possibility of using week four milk yield as an indicator of percentage of cows that had excessive BCS loss before calving.

Experimental Procedures

Body condition score was assessed for 1,244 dry Holstein cows from 3 commercial dairy herds (Dairy A = 448; Dairy B = 433; Dairy C = 363 cows). Assessment consisted of evaluating cows (lactation ≥ 1) on a scale of 1 to 5 (1 = severe underconditioned; 5 = obese) at 242 days of gestation and in the first week after calving. Cows were classi-

fied according to the difference of BCS from the prepartum period to the first week of lactation: excessive loss (E-loss: ≤ -0.75), moderate loss (M-loss: -0.5 and -0.25), and no loss (No-loss: ≥ 0). Cows were housed in dry-lot corrals with shades during prepartum period. In the postpartum period, cows from one of the herds (Dairy C) were housed in freestall barns with access to a dirt exercise lot. Dairies A and B housed postpartum cows in dry-lot corrals. Weekly milk yield from weeks 1 to 4 were extracted from the on-farm management software of the herds. Occurrence of twinning and uterine diseases in the first three weeks of lactation were also extracted from the same software.

Cows calved during a 72-d period and were grouped biweekly to evaluate whether average week four milk yield could be used as an indicator of the proportion of cows that experienced excessive BCS loss in the prepartum period.

Data were analyzed statistically by using SAS (version 9.4, SAS Inst. Inc., Cary, NC). Week four milk yield was analyzed using a mixed-effects statistical model. Body condition score change, dairy, twinning, and dairy × BCS change were included as fixed effects. Milk yield in the first four weeks of lactation was analyzed using BCS change, dairy, twinning, week, dairy × BCS change, dairy × week, week × BCS change, and dairy × week × BCS change as fixed effects.

Results and Discussion

The negative impact of BCS loss in the prepartum period was previously demonstrated in a large observational study that evaluated 16,104 lactations. Cows that had excessive BCS loss from dry-off to calving had reduced 3.5% fat-corrected milk in the subsequent lactation compared with cows that did not have excessive BCS loss. Similar findings were observed in the current study, in which BCS change in the prepartum period tended ($P = 0.07$) to be associated with week four milk yield (Figure 1). Cows with excessive BCS loss had decreased ($P \leq 0.04$) week four milk yield compared with cows with moderate or no BCS loss. In addition, milk yield in the first four weeks of lactation decreased ($P < 0.01$) for cows that had excessive BCS loss vs. cows that had moderate or no BCS loss (Figure 2; E-loss = 76.4 ± 3.9; M-loss = 82.5 ± 3.7; No-loss = 83.3 ± 3.8 lb/day). In these analyses, there were no interactions between dairy and BCS change. These studies demonstrate strong evidence of the impact of prepartum BCS change on milk production after calving. In the current study, the three herds had milk meters, which allowed us to evaluate precisely the association between BCS change and milk production in early lactation because of daily milk yield. The dairies utilized in the previous study did not have milk meters, but cows were tested monthly, therefore, milk yield and components were evaluated for the entire lactation (305 days).

In the current study, the percentage of cows having excessive, moderate, or no BCS loss were 16%, 49%, and 35%, respectively. In the previous report, 10% of cows had excessive BCS loss, whereas 40% and 60% had moderate loss and no BCS loss, respectively. The current study was conducted during the summer, which may be the reason for a greater proportion of cows having excessive BCS loss. Heat stress is associated with reduced feed intake and may result in a greater percentage of cows mobilizing body energy reserves.

Although there is an association between prepartum BCS loss and milk production after calving, week four milk yield is not a good indicator to estimate previous BCS change in the dry period (Figures 3 and 4). Week four milk yield has been used anecdotally to assess transition cow performance at dairy farms. Indeed, in DairyComp, on-farm software widely adopted by U.S. dairy producers, week four milk yield is calculated for each cow and it is utilized in reports to screen for transition cow problems and estimate 305-day mature equivalent milk yield. To assess whether week four milk yield could be used as an indicator of BCS change in the dry period, average week four milk yield and percentage of cows not having excessive BCS loss was calculated for cohorts of cows that calved within a 14-day period (biweekly timeframe). The rationale for grouping cows that calved in a 14-day period was to investigate the value of evaluating average week four milk yield on a frequent basis at dairy farms. Results from the current study suggest that average week four milk yield calculated from cohorts of cows does not indicate whether a large proportion of those cows lost excessive BCS in the prepartum period. In dairy A, for example, the percentage of cows that did not lose excessive BCS prepartum varied from 76 to 91% in the biweekly cohorts, whereas there was a small variation in average week four milk yield, ranging from 98 to 102 lb/day (Figure 3). Similar findings were observed when we also accounted for proportion of cows having postpartum uterine diseases in the first 3 weeks after calving (Figure 4), presenting evidence that it is unclear if week four milk yield can be used as a parameter to evaluate performance of transition cows in dairy herds.

It is likely that several factors impact week four milk yield, not only prepartum BCS change or postpartum uterine disease. Even though week four milk yield is associated with BCS change before calving, week four milk yield does not seem to be a reliable parameter to evaluate BCS change during the dry period in dairy herds.

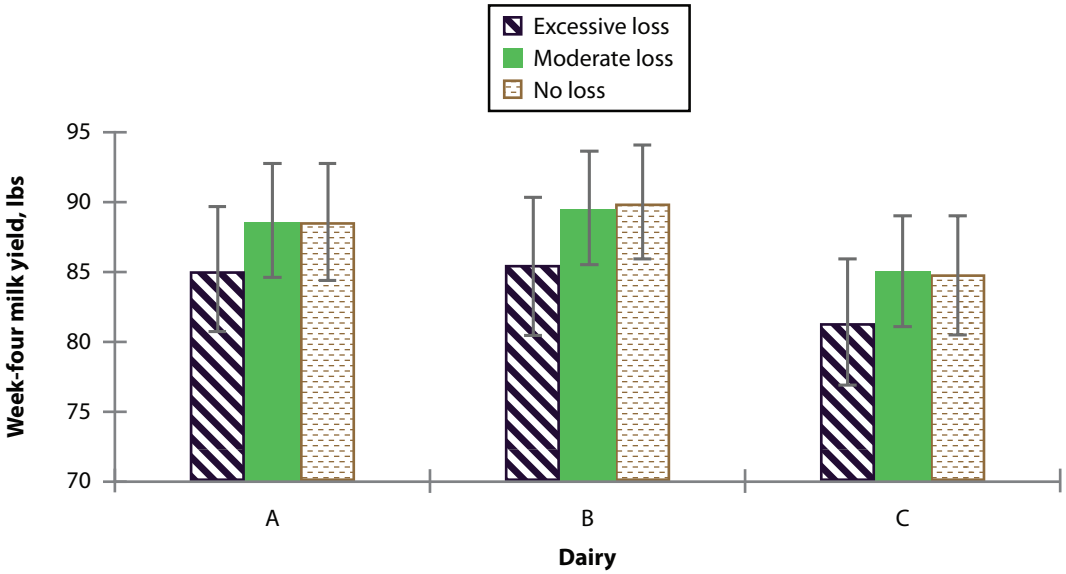

Figure 1. Week four milk yield of cows that had excessive loss (≤ -0.75), moderate loss (-0.5 and -0.25), or no loss (≥ 0) in body condition score in the prepartum period from three dairy herds (excessive loss = 84.0 ± 4.1; moderate loss = 87.8 ± 3.8; no loss = 87.8 ± 3.8 lb/day). Body condition score change: *P* = 0.07; dairy: *P* = 0.01; and body condition score change × dairy: *P* = 0.99.

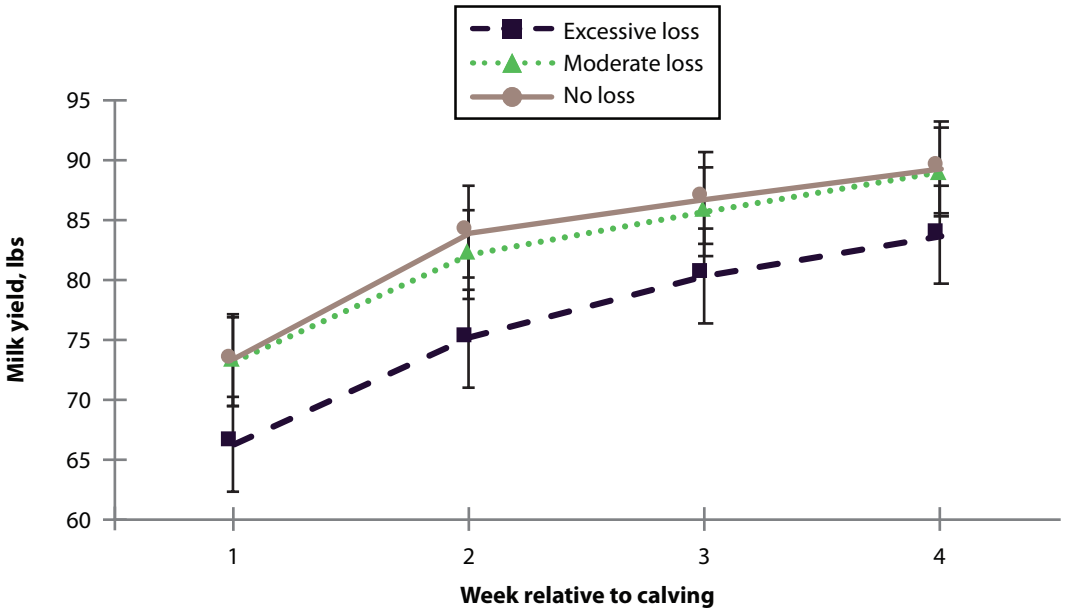

Figure 2. Average milk yield in the first four weeks after calving of cows that had excessive loss (≤ -0.75), moderate loss (-0.5 and -0.25), or no loss (≥ 0) in body condition score in the prepartum period. Body condition score change: *P* < 0.01; week: *P* < 0.01; dairy: *P* = 0.55; body condition score change × dairy: *P* = 0.97; body condition score change × week: *P* = 0.08; and body condition score change × dairy × week: *P* = 0.18.

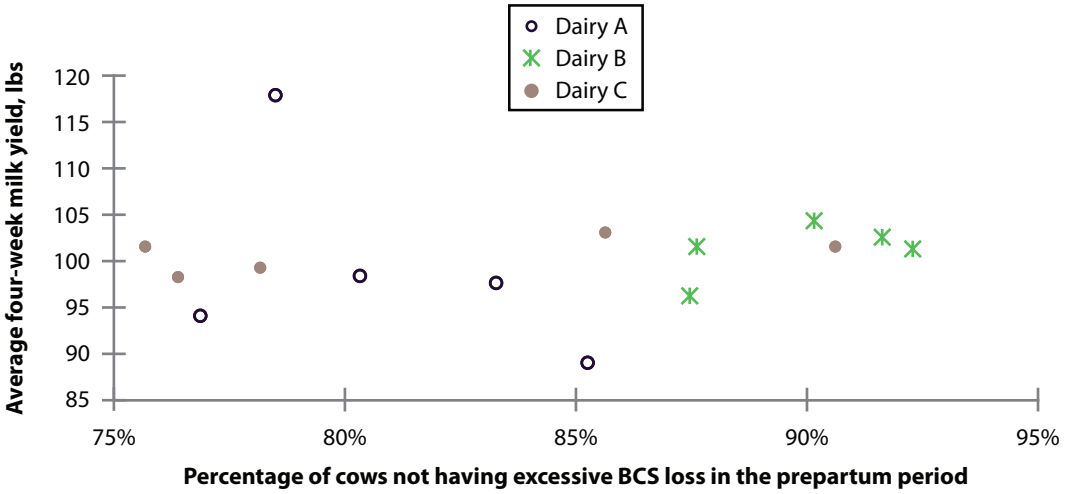

Figure 3. Average week four milk yield and percentage of cows not having excessive body condition score (BCS) loss. Cows calved during a 72-day period. Five groups of cows calved in biweekly periods in each of 3 herds.

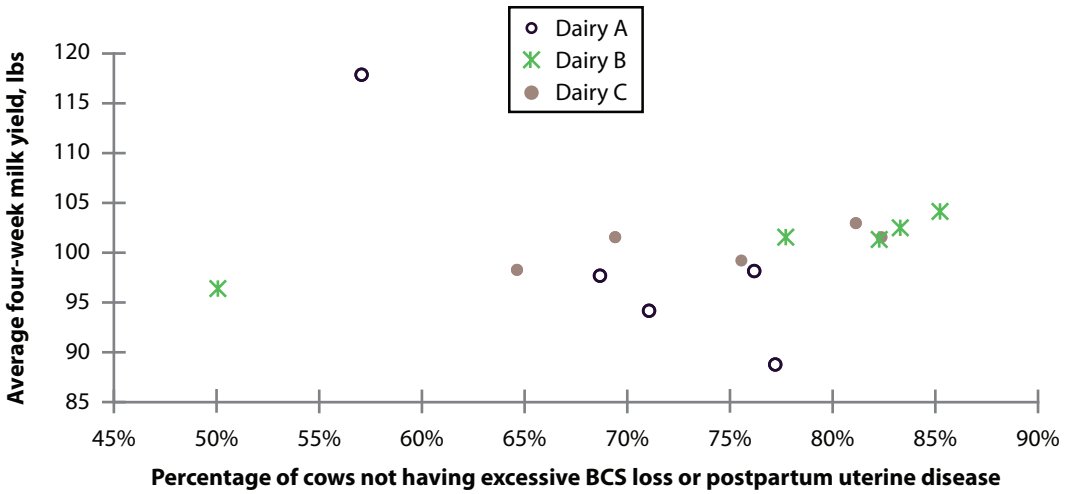

Figure 4. Average week four milk yield and percentage of cows not having excessive body condition score (BCS) loss or postpartum uterine disorders. Cows calved during a 72-day period. Five groups of cows calved in biweekly periods in each of 3 herds.

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

Biological Variability and Chances of Error

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

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

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

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

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

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