DAIRY TEAM
KANSAS STATE UNIVERSITY

Dairy Research 2016

K-STATE
Research and Extension

Kansas State University Agricultural Experiment Station and Cooperative Extension Service

DAIRY RESEARCH 2016

Contents

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Foreword

Kansas State University is pleased to present the 2016 Dairy Research Report of Progress. The Kansas dairy industry continues to grow, ranking tenth nationally with an increase of 2,000 cows between 2014 and 2015. During the past 5 years (2010 to 2015), total milk production in Kansas has increased by 27%; the number of cows by 20%; and pounds of milk per cow by 1,256. At the end of 2015, Kansas ranked 17th nationally in milk yield per cow at 22,064 lb, 16th in the number of dairy cows (143,000), and 16th in total milk production (3.18 billion lb). Kansas now has 300 dairy operations and averages 477 cows per herd (*Hoard's Dairyman*, March 25, 2016, pp 204-205).

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), Daniel Umsheid, Robert Feist, Alan Hubbard, Kris Frey, Eulises Jiron Corrales, Morgan Taylor, Isabella Carmona, Lauren Barlow, and Cory Sunderman. Special thanks are given to Cheryl Armendariz, Wenjing Fausnett, 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.	323
Rolling herd milk, lb	31,164
Rolling herd fat, lb	1,139
Rolling herd protein, lb	914
Somatic cell count × 1,000	183
Calving interval, mo.	12.6

[1]November 8, 2016 test day (milking 2 to 3 times daily; no bST).

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

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
2016 Dairy Research Report of Progress

Biological Variability and Chances of Error

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

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

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

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

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

Assessing Training Methods to Educate Dairy Workers

L. Mendonça, B.E. Voelz, and A. Scanavez

Summary

Training employees is fundamental for dairy producers to achieve efficiency in order to increase profitability. Training videos offered online are becoming more common and recommended to train employees. The goals of this survey were to evaluate the comfort level of dairy employees in using computers and tablets, and preferred training delivery methods. A total of 71 employees from 6 dairies were interviewed. Interviews were conducted in the preferred language of the interviewee – English or Spanish. Of the respondents, 52 and 65.6% of employees consider computers and tablets easy to use, respectively. More than half of the employees reported that they do not use computers or tablets on a regular basis. The majority of employees (70%) would rather have a training session in a face-to-face format than a computer- or tablet-based format. This survey suggests that dairy employees may not be comfortable using computers in training sessions, which may limit the utility of some specific technologies to educate employees.

Key words: employee training, survey, technology

Introduction

Improved labor efficiency in dairy herds may be achieved by educating employees through training sessions. Training sessions may provide knowledge related to management practices and help employees understand the reason protocols are in place. Explaining the reasoning behind each task may reduce protocol deviation, which is key to minimize inadequate practices and achieve efficiency.

In addition to limited training courses being available for dairy employees, hiring consultants to educate workers at farms may not be feasible because of cost. Considering the widespread use of technology in the U.S., allied industry and university professionals have been creating online materials as an alternative resource to educate dairy workers. Several institutions have developed online materials in English and Spanish to enable a large audience to utilize these resources.

We conducted a survey to assess dairy employees' (1) exposure to technology, (2) comfort level in using computers and tablets, and (3) preferred training delivery methods.

Experimental Procedures

During research and extension farm visits in January and February of 2016, dairy employees (n = 71) from 6 Kansas dairy farms were randomly chosen to be interviewed by a bilingual individual. Interviews were conducted in the preferred language of the interviewee, either in English or Spanish. Before starting the interview, participants were informed that questions were being asked as part of the K-State Research and Extension

project: "Assessing training methods to educate dairy workers." In addition, participants were informed that the responses were anonymous, and questions did not have to be answered if they did not want to answer them. Questions assessed employees' access to computers, tablets and smartphones; comfort level in using technology; preferred training method; and literacy in English and Spanish.

Results and Discussion

More than half of the employees responded that they do not have a computer at home (57.7%) and never use computers (54.9%; Table 1 and 2). Approximately half of employees (52.1%) consider computers easy to use. Of the respondents, 56% of employees responded they have tablets at home. In addition, 52.1% of all employees answered that they had never used a tablet (Table 1 and 2). Ten percent of employees were not able to answer the question if tablets are easy to use because they had never used a tablet. Among the participants that answered the question, 65.6% consider tablets easy to use. These results indicate that approximately half of the employees from the dairies that participated in the survey are not using computers and tablets on a daily basis, but are exposed to digital technology because most employees have smartphones (84.5%; Table 1).

The majority (97.2%) of the interviews were conducted in Spanish because it was the preferred language of the interviewees. Furthermore, 91.5% of employees responded that could read in Spanish (Table 1), but only 19.7% of employees can read in English. This suggests that materials created for education of dairy employees must be developed in English and Spanish. Furthermore, training sessions must be offered in both languages to be certain that dairy workers will fully understand the information being provided to them.

Seventy percent of employees preferred being trained in-person instead of using a computer or a tablet (Figure 1). Because the preferred training delivery method is in-person, it is likely that participants may retain more information in courses where the delivery method is in-person, compared with online videos. Although this is just a speculation, it would be an important aspect to consider in training courses because several institutions (e.g., private companies and universities) are investing resources to create online materials to educate dairy employees.

In conclusion, despite the fact that technology is widespread in the U.S., depending on online materials alone to educate dairy employees may not fulfill immediate needs in expanding the knowledge of the current workforce, because of their limited comfort level in using computers and tablets. In addition, the finding that the majority of employees would rather be trained in-person than using a device suggests that face-to-face interactions are extremely valuable in training courses, which may result in greater engagement. Even though information may be disseminated via digital devices, face-to-face interactions may be required to educate and coach dairy employees in order to gain the greatest benefit of training sessions. It is key to keep in mind that if technology is used to educate employees, all materials should be available in English and Spanish and support must be provided to individuals with limited computer knowledge.

Acknowledgments
The authors thank the owners of the collaborating dairies.

Table 1. Access to technology, comfort level in using computers and tablets, and literacy in English and Spanish of 71 employees from 6 dairy operations

	Responses, %		
	Yes	No	More or less
Do you have a computer at home?	42.3	57.7	-
For you, are computers easy to use?	52.1	29.6	18.3
Do you have a tablet?	56.3	43.7	-
For you, are tablets easy to use?	65.6	21.9	12.5
Do you have a smartphone?	84.5	15.5	-
Can you read in English?	19.7	63.4	16.9
Can you read in Spanish?	91.5	5.6	2.8

Table 2. Frequency of dairy employees using computers and tablets

	Responses, %			
	Every day	Once a week	Less than once a week	Never
How often do you use computers?	25.4	12.7	7.0	54.9
How often do you use tablets?	21.1	19.7	7.0	52.1

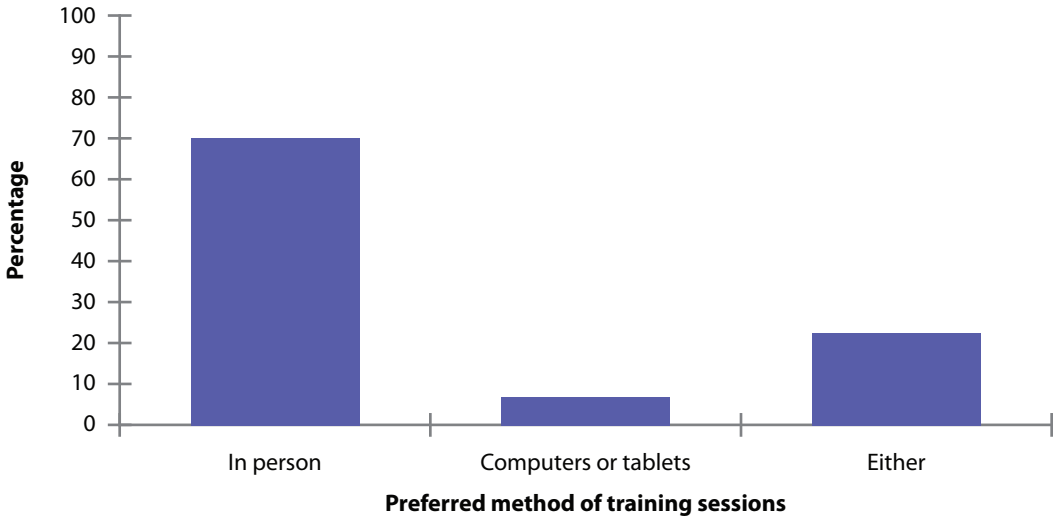

Figure 1. Preferred method of training sessions of dairy employees from 6 Kansas dairies.

Evaluation of Weather Information from On-Farm and Meteorological Stations to Assess Heat Stress in Dairy Cows in Southwest Kansas

A. Scanavez, L. Rocha, B.E. Voelz, L. Hulbert, and L. Mendonça

Summary

Heat stress represents a challenge for the dairy industry. In order for producers to implement appropriate management practices, it is crucial for researchers to assess the extent of heat stress to which cows are exposed during the summer. Temperature-humidity index (THI) may be used to determine the severity of heat stress that cows are exposed to during the summer. The objective of this study was to evaluate climate conditions by calculating THI using information from: 1) an official meteorological station, 2) loggers at the pen-level, and 3) loggers at the cow-level from a commercial dairy located in southwest Kansas. Temperature-humidity index at the cow-level was correlated with THI at the pen-level and THI from the nearest official meteorological station to the dairy. Despite the correlations, cow-level THI was 6.8 and 19.2 units greater than pen-level and station-level THI, respectively. Weather data obtained from farm-level measurements are more accurate than information collected from an official meteorological station to assess the intensity of heat stress conditions. Nonetheless, it is important to note that pen-level THI underestimates the index at the cow-level. This difference is likely to occur because of microclimates within the pen.

Key words: dairy cattle, heat stress, temperature-humidity index, heat abatement

Introduction

Heat stress causes profound changes in dairy cows' metabolism and energy partitioning. Energy required for heat dissipation mechanisms could be used for other physiological processes, which impacts milk production, reproductive efficiency, and cow health. As a result, heat stress has major implications for the profitability of dairy farms. It was estimated in 2003 that heat stress resulted in losses of approximately $900 million in the U.S. dairy industry.

In order to evaluate the severity of heat stress conditions for dairy cows, researchers use a temperature-humidity index (THI), which is a calculation that involves both ambient temperature and relative humidity. It has been previously demonstrated that THI > 68 is associated with reduced feed intake in lactating dairy cows. Nonetheless, using ambient temperature and relative humidity from official meteorological stations to calculate THI and evaluate heat stress conditions may not precisely represent climate conditions at the herd-level and cow-level.

The objective of this study was to evaluate climate conditions by calculating THI using information from: 1) an official meteorological station, 2) loggers at the pen-level, and 3) loggers at the cow-level from a commercial dairy located in southwest Kansas.

Experimental Procedures

Lactating (n = 9) and dry (n = 2) cows from a commercial dairy located in Southwest Kansas were randomly enrolled in the study in 4 replicates from June 13 to July 15, 2014. Cows were fitted with a halter that had an attached temperature logger (HOBO U23 Pro v2; Onset Computer Corp., Pocasset, MA) to assess temperature and humidity at the cow-level. Loggers were fitted inside a hose (2 inches in diameter and 3 inches in length) to avoid being removed by other cows and the hose was attached to the halter with high resistance tape. At the pen-level, ambient temperature and humidity were collected by placing temperature loggers (HOBO U23 Pro v2) approximately 10 feet above ground level. In addition, calibrated iButton temperature loggers (DS1922L, Embedded Data Systems, Lawrenceburg, KY) fitted in blank CIDR inserts were used to collect vaginal temperature. Measurements were collected in intervals of 5 minutes for 3 or 5 consecutive days. Data from the nearest meteorological station, approximately 10 miles from the dairy, were also collected. Information was recorded in intervals of 20 minutes. In order to adjust for intervals of 5 minutes, measurements were used three times. Using ambient temperature and relative humidity collected from on-farm temperature loggers and the meteorological station, THI was calculated using the equation: $T - (0.55 - (0.55 \times RH/100) \times (T-58))$; where T and RH are dry bulb temperature (°F) and relative humidity, respectively. Lactating cows were housed in two-row free-stall barns equipped with fans and with access to a dirt exercise lot. Lactating cows had access to an evaporative cooling system (fans and sprinklers) in the holding pen. Dry cows were housed in open dry lots with access to shade. Data were analyzed by ANOVA for repeated measures using the HPMIXED procedure of SAS.

Results and Discussion

Temperature-humidity indices at the cow-, pen-, and station-level are outlined in Figure 1. Average THI was greatest ($P < 0.01$) at the cow-level (cow-level = 91.9 ± 1.1; pen-level = 85.1 ± 1.1; and station-level = 72.8 ± 1.3). Despite the use of heat abatement strategies, vaginal temperature was greater ($P < 0.01$) for lactating cows compared with dry cows (102.2 ± 0.07 vs. 101.9 ± 0.07 °F). It is likely that this difference is attributed to greater metabolic heat production associated with milk production of lactating cows. There was no ($P = 0.96$) interaction between productive status and time of the day, which indicates that variations of vaginal temperature across the day were similar in lactating and dry cows (Figures 2A and 2B). Temperature-humidity indices at the cow-level gradually decreased from 18:00 to 24:00 and started increasing at approximately 08:00. Vaginal temperatures, however, did not decrease as rapid as THI. Furthermore, there was a lag for vaginal temperature to increase in relation to the increase in THI in the morning (Figures 2A and 2B).

Temperature-humidity index at the cow-level had a linear relationship with THI at the pen- and station-level (Figures 3A and 3B). Temperature-humidity index at the pen-level better explained the variability of THI at the cow-level than THI at the station-level ($r^2 = 0.82$ vs. $r^2 = 0.76$). This suggests that calculation of THI using ambient temperature and relative humidity collected at the pen-level is more accurate than calculating THI from the meteorological station.

It is possible that several factors influence THI at the cow-level. Amount of time spent under shade, relative humidity at the cow-level, and proximity with other animals

are potential aspects that may create a microclimate at the cow-level. The pattern of variation of THI across the day was independent of the location of the devices (Figure 1), however, THI at the cow-level was significantly greater, which indicates that microclimate at the cow-level is important to consider. Other studies also compared THI from measurements collected from on-farm and official meteorological stations. Findings from those studies are consistent with the work reported herein.

In conclusion, THI from measurements obtained from loggers located at the pen-level have greater correlation with the THI at the cow-level than THI from measurements collected from an official meteorological station. Nonetheless, THI at the cow-level was 7 units greater than THI at the pen-level. This information indicates that climate conditions assessed by on-farm loggers may be used to estimate the intensity of heat stress cows are being exposed to, but it is important to recognize that THI at the pen-level might be underestimated compared with THI at the cow-level.

Acknowledgments

The authors thank the owners and staff from the collaborating dairy and Filippe Scortegagna for assisting in the experiment.

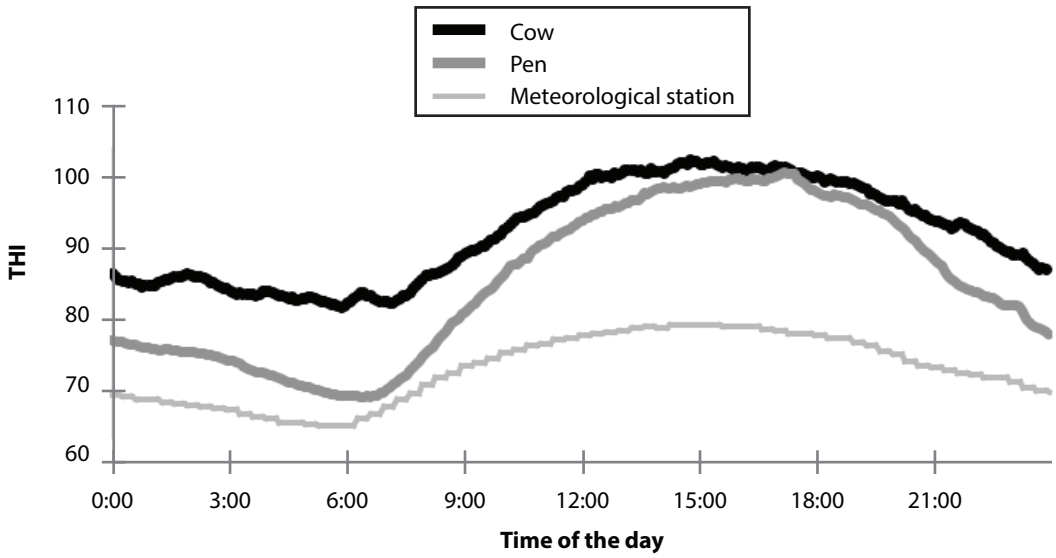

Figure 1. Temperature-humidity index (THI) during the study period at the cow-level, pen-level, and from the nearest meteorological station.

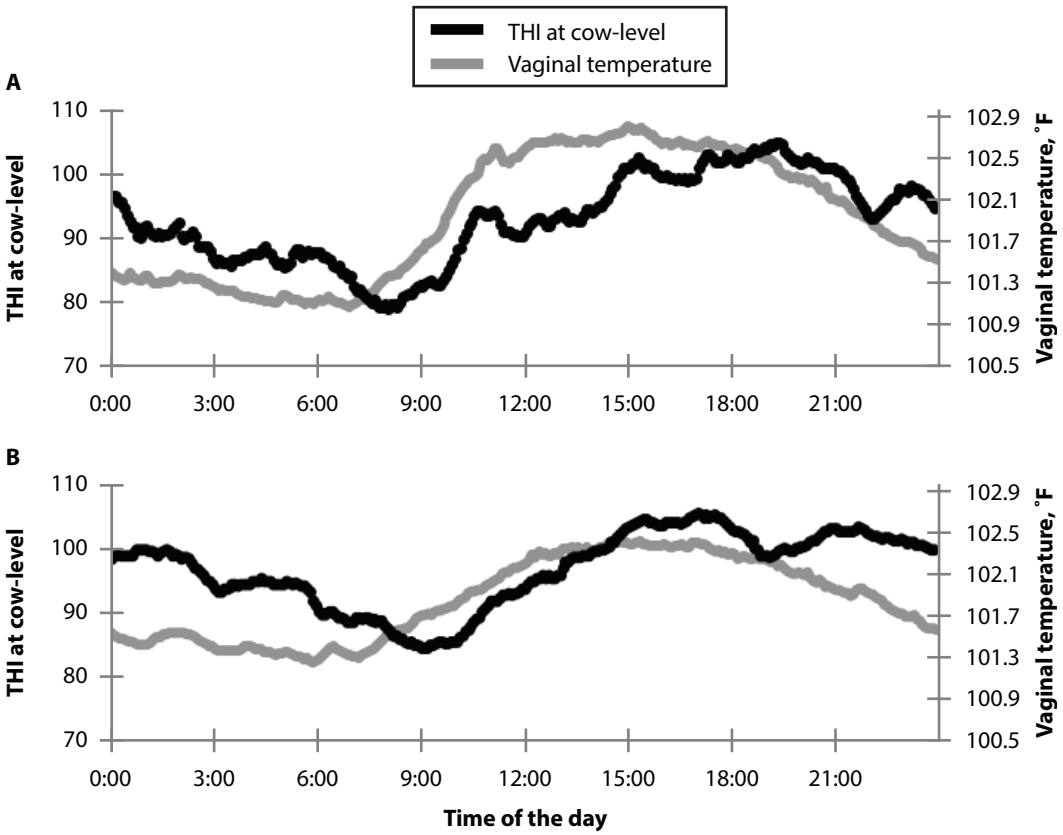

Figure 2. (A) Vaginal temperature (101.9 ± 0.07 °F) and temperature-humidity index (THI) at the cow-level (93.2 ± 1.8) of dry cows. (B) Vaginal temperature (102.1 ± 0.07 °F) and THI at the cow-level (91.6 ± 1.3) of lactating cows.

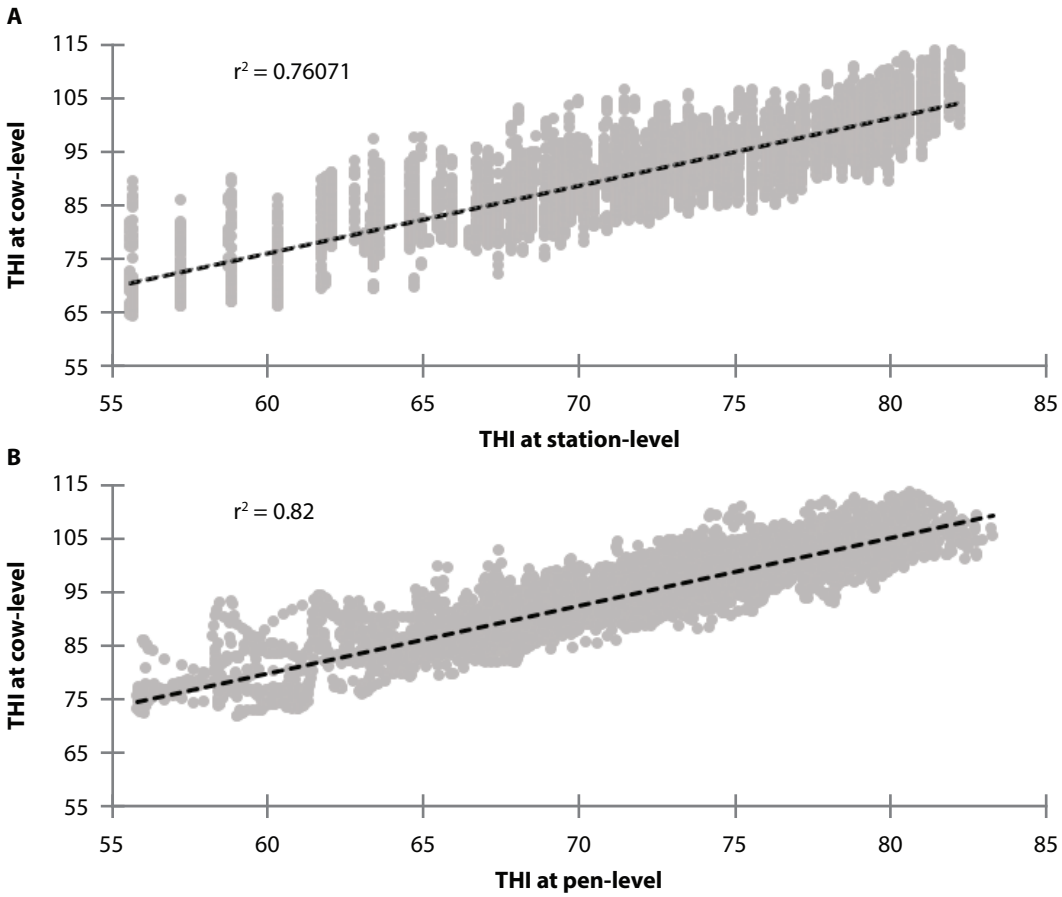

Figure 3. (A) Correlation between temperature-humidity index (THI) at the cow- and station-level ($r^2 = 0.76$). (B) Correlation between THI at the cow- and pen-level ($r^2 = 0.82$).

Increasing Estrus Expression in Lactating Dairy Cows

J.A. Sauls, B.E. Voelz, and J.S. Stevenson

Summary

This report summarizes the use of various hormones in an attempt to induce greater estrus expression of lactating dairy cows. Average detection of estrus (< 50%) in most U.S. dairy herds has been identified as a problem limiting reproductive efficiency. Detection of estrus plays an important role in reproductive management in U.S. dairy herds despite the adoption of fixed-time artificial insemination programs. When estrus was detected by an activity monitoring system or a rump-mounted device, supplementing cows with progesterone before induction of luteolysis resulted in greater intensity of estrus when compared with controls. In addition, administering estradiol cypionate at 24 h after induced luteolysis resulted in greater frequency of estrus expression compared with cows treated with testosterone propionate or controls when assessed by an activity monitor. Activity monitoring systems achieved heat-detection rates of approximately 70% (80% with estradiol) and are likely cost effective for herds achieving less than that level of heat detection.

Key words: heat, estradiol, progesterone

Introduction

Expression of estrus is dependent on several factors that include the environment, physiological factors, and hormone concentrations. Approximately 76% of large dairy herds (500 head or more) in the U.S. house dairy cows in confinement free stall barns with concrete flooring. One of the largest factors affecting expression of estrus in lactating dairy cows is the surface upon which they are observed. Cows are more likely to stand to be mounted when on a dirt surface rather than a dry grooved concrete surface.

High milk-producing dairy cows have shorter durations of estrus. A high-energy diet is fed to lactating dairy cows in order to meet their nutritional and lactation demands, and a consequence of high-energy diets is chronically increased liver blood flow, which causes increased catabolism of estradiol and progesterone (two hormones responsible for expression of estrus). Cows that ovulate an oocyte that matured in a high progesterone environment have greater chances of pregnancies per artificial insemination (P/AI). High milk-producing dairy cows often do not have sufficient concentrations of estradiol in blood circulation to cause expression of estrus, and to promote ovulation and uterine preparation for an embryo.

Several technologies on the market measure physiological changes associated with estrus. Physical activity is commonly measured for its association with estrus because of its ease of measurement and accuracy. Cows in estrus spend considerably more time walking and less time eating and resting. The experiments described in this article determined if more cows could be detected in estrus by an activity monitoring system

compared with other estrus-detection aids. Two experiments performed at the Kansas State University Dairy Teaching and Research Center assessed if cows exposed to increasing concentrations of progesterone (experiment 1) or estradiol or testosterone (experiment 2) enhanced expression of estrus.

Experimental Procedures

Experiment 1

We conducted this study with 154 cows at the Kansas State University Dairy Research and Teaching Center. Estrus was synchronized and cows were assigned randomly to receive supplemental progesterone before first postpartum insemination. Cows in the low progesterone treatment were administered 1 CIDR (Zoetis Animal Health) and did not have a functional corpus luteum (CL; No CL + CIDR). The control cows had a functional CL (CL only) but received no supplemental progesterone. Cows in the high progesterone treatment had at least one functional CL and received 2 CIDR inserts (CL + 2 CIDR). Progesterone supplementation occurred for 5 days before injecting prostaglandin $F_{2\alpha}$ (PGF) to induce estrus. An activity monitoring system (AMS; Dairymaster Moo Monitor, Kearney, Ireland) containing an accelerometer and a rump mounted pressure device (HW; HeatWatch; Chow Chips LLC, New Jersey) were fitted to cows for detection of estrus.

Experiment 2

Estrus was synchronized in 203 cows with a modified double Ovsynch protocol before first postpartum insemination. Each cow received a used CIDR for 7 days beginning on day 7 of the estrous cycle, and upon its removal, PGF was administered. Cows were assigned randomly to receive either an injection of 1 mg of estradiol cypionate (ECP), 2 mg of testosterone propionate (TP), or no injection (control) at 24 hours after PGF was administered. Estradiol is the hormone responsible for inducing expression of estrus, and testosterone is a precursor substrate to make estradiol. Cows were fitted with an AMS (Dairymaster Moo Monitor) and friction-activated patches (Patches; Estrotect heat detector patches, Rockway, Inc., Spring Valley, WI) for detection of estrus.

In both experiments, estrus was defined to have occurred if cows had at least 1 ovarian follicle ≥ 10 mm and progesterone was < 0.5 ng/mL at 72 hours after administration of PGF to induce estrus (qualifying cows).

Results and Discussion

Experiment 1

Neither occurrence of estrus nor ovulation risk for all enrolled cows differed among treatments (Table 1). As assessed by the AMS, occurrence of estrus ranged from 56 to 67%. Of all cows that expressed estrus, ovulation risk varied from 89 to 100% among treatments. As assessed by HW, occurrence of estrus varied from 45 to 61%. Of cows that expressed estrus, ovulation risk ranged from 93 to 100%. Expression of estrus occurred 1.6 to 1.8 times more ($P < 0.01$) often in primiparous than multiparous cows; however, ovulation risk did not differ between primiparous and multiparous cows.

Occurrence of estrus and ovulation for qualifying cows is also summarized in Table 1. As assessed by the AMS, occurrence of estrus among qualifying cows ranged from 62

to 79%. Of the qualifying cows that expressed estrus, ovulation risk ranged from 88 to 100% and did not differ among treatments. As determined by HW, occurrence of estrus among qualifying cows ranged from 51 to 69%. Of qualifying cows that expressed estrus, ovulation risk ranged from 93 to 100% and did not differ among treatments. Consistent with all enrolled cows, 1.5 to 1.9 times more ($P < 0.01$) qualifying primiparous than multiparous cows expressed estrus, but parity had no effect on ovulation risk.

Although peak factor (measure of the standard deviation of increase in peak activity during 3 hours), a measure of estrus intensity by the AMS, was greater ($P < 0.05$) for cows in the CL + 2 CIDR treatment compared with CL only, no other measures of estrus intensity (mean count, peak count, and mean factor) differed among treatments.

Experiment 2

Estrus expression and ovulation risk for all cows enrolled in experiment 2 are summarized in Table 2. Estrus expression determined by AMS varied from 67 to 79% among treatments, and of the cows that expressed estrus, ovulation risk ranged from 87 to 95%. More ECP cows tended ($P = 0.09$) to have activated patches compared with controls. Of the cows that expressed estrus as assessed by activated patches, ovulation risk ranged from 88 to 98% among treatments. Primiparous cows tended ($P = 0.11$; AOR = 1.93, 95% CI = 0.978 to 3.82) to be more likely to express estrus than multiparous cows.

Estrus expression and ovulation risk for qualifying cows also are summarized in Table 2. As determined by the AMS, occurrence of estrus did not differ among treatments. Of qualifying cows that expressed estrus by the AMS, ovulation risks ranged from 88 to 98%. More ($P = 0.056$) ECP cows with activated patches were detected in estrus compared with control cows. Of qualifying cows identified in estrus, ovulation risk varied from 89 to 98%. Qualifying primiparous cows were more ($P = 0.03$; AOR = 2.28; 95% CI = 1.07 to 4.85) likely to express estrus than qualifying multiparous cows.

Onset of estrus occurred earlier ($P < 0.001$) after treatment with ECP (46 ± 2 hours) and tended ($P = 0.102$) to be earlier after TP (56 ± 2 hours) compared with controls (64 ± 5 hours). Mean duration of estrus ranged from 9.5 ± 0.9 and 10.3 ± 0.6 h among treatments. Compared with controls, average peak counts suggested greater intensity of estrus after ECP ($P = 0.031$) or after TP ($P = 0.070$). Other measures of estrus intensity (mean count, mean factor, and peak factor) did not differ among treatments.

Efficiency and Accuracy of Detected Estrus

Efficiency and accuracy of the three methods employed in both experiments to detect estrus were compared in all qualifying cows. The proportions of enrolled cows meeting these criteria that were detected in estrus (efficiency), ovulated, or both (accuracy) are summarized in Table 3. Efficiency of detected estrus ranged from 61 to 75%. Ovulation risk (accuracy of detected estrus) for the cows detected by the AMS, pressure-sensitive devices, and friction-activated patches were 94, 96, and 94%, respectively. Of particular interest was the 25 to 39% of cows that did not express estrus in which ovulation occurred, ranging from 62 to 77% in the absence of estrus.

Conclusions

Only the ECP treatment successfully induced more cows in estrus, but proportions of cows detected barely exceeded 80%. Given the large proportion of cows ovulating in the absence of estrus, further research is warranted when AMS are employed to determine if more pregnancies can be achieved by inseminating cows not detected in estrus at an appropriate time after PGF-induced luteolysis. An AMS is likely an appropriate tool for herds achieving less estrus-detection risk than achieved in the current experiments (70% without estradiol). Although efficiency and accuracies of the three estrus-expression methods employed did not differ, the AMS and pressure-sensitive rump-mounted pressure detectors offer continuous monitoring of activity independent of visual assessment of friction-activated patches by herd personnel and potentially offer greater surveillance options in all cows, particularly herds in which cows are housed on concrete with less than ideal footing conditions.

Table 1. Occurrence of estrus and ovulation in all cows and qualifying cows defined to be in estrus fitted with an activity monitoring system (AMS) and a pressure-sensitive rump mounted pressure detector device (experiment 1)

	Treatment[1]			*P* - value[2]	
Item [% (no.)]	No CL + CIDR	CL only	CL + 2 CIDR	No CL vs. CL	CL vs. CL + 2 CIDR
AMS					
Estrus expression[3]					
All cows[4]	66.9 (52)	62.9 (51)	56.3 (51)	0.695	0.541
Qualifying cows[5]	70.3 (42)	79.5 (35)	61.9 (44)	0.480	0.204
Estrus and ovulation[6]					
All cows	94.1 (34)	100 (33)	89.3 (28)	0.406	0.035
Qualifying cows	92.8 (28)	100 (27)	88.5 (26)	0.367	0.047
Pressure-sensitive device					
Estrus expression					
All cows	61.2 (52)	56.2 (51)	45.2 (51)	0.637	0.306
Qualifying cows	59.5 (42)	69.3 (35)	51.3 (44)	0.902	0.216
Estrus and ovulation					
All cows	93.7 (32)	100 (30)	95.6 (23)	0.388	0.304
Qualifying cows	92.8 (28)	100 (24)	95.4 (22)	0.371	0.317

[1]Corpus luteum (CL) only; no CL + CIDR = CL + CIDR insert (d -5) for 5 d; or CL + 2 CIDR inserts (d -5) for 5 d. On d 0 CIDR inserts were removed and all cows received $PGF_{2\alpha}$.
[2]Orthogonal contrasts.
[3]Percentage of cows expressing activity assessed by AMS or pressure-sensitive patches.
[4]All cows enrolled in the experiment.
[5]Only cows with a follicle ≥ 10 mm in diameter at $PGF_{2\alpha}$ on d 0 and concentrations of progesterone ≤ 0.5 ng/mL at 72 h after $PGF_{2\alpha}$ injection. For cows in either CL treatment, progesterone > 1 ng/mL on d 0 or < 2.35 ng/mL for cows with no CL + CIDR insert.
[6]Percentage of cows that ovulated subsequent to detected estrus.

Table 2. Occurrence of estrus and ovulation in all cows and qualifying cows defined to be in estrus and fitted with an activity monitoring system (AMS) and a friction-activated patch (experiment 2)

	Treatment[1]			*P* - value[2]	
Item [% (no.)]	ECP	TP	Control	ECP vs. control	TP vs. control
AMS					
Estrus expression[3]					
All cows[4]	78.7 (68)	67.4 (68)	70.2 (67)	0.260	0.740
Qualifying cows[5]	81.2 (62)	67.5 (59)	69.4 (59)	0.138	0.827
Estrus and ovulation[6]					
All cows	94.3 (53)	87.0 (46)	95.6 (46)	0.747	0.121
Qualifying cows	96.0 (51)	87.5 (40)	97.5 (40)	0.697	0.060
Friction-activated patch					
Estrus expression					
All cows	84.3 (63)	67.7 (64)	71.4 (60)	0.092	0.667
Qualifying cows	88.0 (58)	66.1 (55)	72.7 (53)	0.056	0.487
Estrus and ovulation					
All cows	98.1 (53)	88.4 (43)	92.9 (42)	0.309	0.501
Qualifying cows	98.0 (51)	88.9 (36)	94.6 (37)	0.490	0.365

[1]Cows received an injection of $PGF_{2\alpha}$ (d 0) and assigned randomly to 3 treatments: ECP = 1 mg estradiol cypionate; TP = 2 mg testosterone propionate; Control = no treatment injection. Treatments were administered on d 1 concurrent with a second injection of $PGF_{2\alpha}$.
[2]Orthogonal contrasts.
[3]Percentage of cows expressing activity assessed by AMS or friction-activated patches.
[4]All cows enrolled in the experiment.
[5]Cows with a follicle ≥ 10 mm in diameter at $PGF_{2\alpha}$, concentrations of progesterone > 1 ng/mL before and ≤ 0.5 ng/mL at 72 h after $PGF_{2\alpha}$ injection.
[6]Percentage of cows that ovulated subsequent to detection of estrus.

Table 3. Percentage of qualifying cows defined to be in estrus (efficiency) during 7 d after $PGF_{2\alpha}$-induced luteolysis, ovulated, or both (accuracy) detected by an activity monitoring system (AMS), pressure-sensitive devices, or friction-activated patches in experiments 1 and 2[2]

Item [% (no./no.)]	AMS	Pressure-sensitive devices	Friction-activated patches
Experiment 1[1]			
Estrus expression	66.9 (81/121)	61.2 (74/121)	...
Ovulation	93.8 (76/81)	96.0 (71/74)	...
No ovulation	6.2 (5/81)	4.0 (3/74)	...
No estrus expression	33.1 (40/121)	38.8 (47/131)	...
Ovulation	70.0 (28/40)	76.6 (36/47)	...
No ovulation	30.0 (12/40)	23.4 (11/47)	...
Experiment 2[2]			
Estrus expression	72.2 (130/180)	...	74.7 (124/166)
Ovulation	93.8 (122/130)	...	94.3 (117/124)
No ovulation	6.2 (8/130)	...	5.7 (7/124)
No estrus expression	27.8 (50/180)	...	25.3 (42/166)
Ovulation	62.0 (31/50)	...	61.9 (26/42)
No ovulation	38.0 (19/50)	...	38.1 (16/42)

[1]Only cows with a follicle ≥ 10 mm in diameter at $PGF_{2\alpha}$ on d 0 and concentrations of progesterone ≤ 0.5 ng/mL at 72 h after $PGF_{2\alpha}$ injection. For cows in either CL treatment, progesterone > 1 ng/mL on d 0 or < 2.35 ng/mL for cows with no CL + CIDR insert.

[2]Cows with a follicle ≥ 10 mm in diameter at $PGF_{2\alpha}$, concentrations of progesterone > 1 ng/mL before, and ≤ 0.5 ng/mL at 72 h after $PGF_{2\alpha}$ injection.

Additional Small Dose of Prostaglandin $F_{2\alpha}$ at the Time of AI Fails to Improve Pregnancy Rates of Lactating Dairy Cows

J.S. Stevenson, J.A. Sauls, and L. Mendonça

Summary

In two experiments we tested the hypothesis that administering 10 mg of prostaglandin $F_{2\alpha}$ (PGF) to lactating dairy cows concurrent with timed artificial insemination would increase pregnancy outcome. In three herds with 2,135 inseminations, we failed to demonstrate a positive effect on pregnancy per AI. Although a trend was observed in experiment 1, with more cows in experiment 2, the PGF treatment failed to improve pregnancy outcomes.

Key words: prostaglandin, timed AI, pregnancy

Introduction

Treatment of domestic livestock with prostaglandin $F_{2\alpha}$ (PGF) administered intravenously, intramuscularly, or in the uterus concurrent with AI to improve conception has been a subject of recurring research since the early 1990s. Pregnancy outcomes have been improved in four studies, but not in three others. Studies in which positive pregnancy outcomes have been most consistent seem to have involved dairy cows inseminated at a timed AI near the peak of lactation in negative energy balance, in cows with less than acceptable reproductive performance (such as repeat breeders), and where stress factors negatively impact ovulation.

Apart from its primary luteolytic role to regress the corpus luteum and induce estrus, PGF has multiple actions on the female reproductive system in cattle and other farm animals. Treatment with PGF concurrent with AI could increase pregnancy rates by:

- increasing uterine contractility, thereby enhancing sperm transport;
- inducing LH release via a luteolysis-independent mechanism, thereby facilitating the ovulatory process;
- hastening luteal regression in cows that may otherwise have delayed or incomplete luteolysis at AI, thereby creating a low progesterone environment conducive for optimal gamete transport;
- inducing release of growth hormone (GH) and subsequent GH-induced insulin-like growth factor-1 secretion, known to increase fertility of lactating dairy cows during the pre- and peri-implantation periods resulting in improvements in conceptus development and a reduction in embryonic mortality;
- increasing the number of cows with a corpus luteum post-AI;
- increasing post-AI corpus luteum size and concentrations of progesterone; and (or)
- inducing secretion of oxytocin that may stimulate uterine contractions and support sperm transport because sperm transport has been improved by adding to semen or administering to females such compounds as PGF, oxytocin, estradiol, phenylephrine, or ergonovine.

Our hypothesis was that administering 10 mg of PGF (2 cc Lutalyse, dinoprost tromethamine sterile solution, Zoetis Animal Health, Whitehouse Station, NJ) concurrent with timed AI will increase pregnancy outcomes in lactating dairy cows. Our objectives were to assess ovarian characteristics and pregnancy outcomes in lactating dairy cows in response to administration of PGF.

Experimental Procedures

Experiment 1

We conducted this study with 287 lactating dairy cows in the Kansas State University Dairy Teaching and Research Center in a completely randomized design with 2 treatments. Cows diagnosed not pregnant (day 0) at 30 to 36 days after AI were body scored (1 = thin and 5 = fat), received GnRH at the open diagnosis, 25 mg of PGF on day 5 (second PGF on day 6) or on day 7, and GnRH at 56 h after the first or only PGF injection. Cows were stratified by parity (primiparous vs. multiparous) and assigned randomly to receive 10 mg PGF (n = 147) at timed AI (72 h after the first or only PGF injection) or served as untreated controls (n = 140).

Ovaries were scanned via transrectal ultrasonography 72 h before timed AI to characterize number and diameter of all ovarian follicles and re-examined 13 days after timed AI to determine incidence of ovulation, ovulation rate, and total volume of luteal tissue per CL. Blood samples were collected 3 days before timed AI (day -3), at timed AI (day 0), and on day 13 after timed AI to determine progesterone concentration. Pregnancy diagnosis was conducted at 30 to 36 days after AI.

Concentration of progesterone and volume of luteal tissue on day 13 were analyzed using procedure MIXED with the fixed effects of treatment, and with days in milk and BCS as covariates. Luteal regression was verified by changes in blood progesterone concentration between days -3 and 0. Differences in ovulation rate and progesterone were interpreted as direct effects of PGF treatment on functions of the preovulatory follicle(s) and subsequent forming CL.

Experiment 2

We conducted this study with 782 cows in one dairy operation and 1,066 cows in a second dairy. Cows were milked thrice daily. Cows with odd-numbered ear tags received no treatment (control) and even-numbered ear tag cows receiving (i.m.) 10 mg PGF concurrent with timed AI as in experiment 1. Cows were treated after first and repeat inseminations. Initial pregnancy diagnoses occurred between days 32 and 35 and were verified between days 63 and 68 after timed AI. Body condition scores were assessed biweekly so they were assigned 3 days before or 4 days after timed AI and treatment.

Outcome variables included pregnancy rates per timed AI and intervening embryo loss. Nuisance variables include lactation number, days in milk at timed AI, body condition score at timed AI, herd, and season. Binomial outcomes were modeled using logistic regression in procedure GLIMMIX in SAS. The model included the fixed effects of treatment (PGF vs. control), lactation number (1 vs. 2+), treatment × lactation number, with days in milk and BCS as covariates. Herd was treated as a random variable, with treatment differences tested by the treatment within herd variance.

Results and Discussion

Experiment 1

Percentage of cows in which luteolysis had occurred (progesterone ≤ 0.5) before treatment at timed AI did not differ between treated and control cows (93 vs. 89%). Incidence of single ovulation after timed AI exceeded 95% and did not differ between treatments (Table 1). In contrast, treatment with PGF reduced ($P < 0.05$) the proportion of cows with double ovulation compared with controls. Concentrations of progesterone at AI and volume of the luteal tissue 13 days after timed AI did not differ between treatments (Table 1). Pregnancy per AI at days 32 and 80 tended to be 10 and 18% greater for cows treated with PGF, but did not differ between treatments (Table 1).

Experiment 2

Test-day daily milk yield (92 vs. 86 lb), days in milk at treatment (114 vs. 100), and percentage of first-lactation cows enrolled in the study (38 vs. 34%) for herds 1 and 2 were similar. Pregnancy per AI at 32 to 35 days and at 63 to 68 days did not differ between treatments. Pregnancy loss also did not differ between treatments.

Despite a recent report that 10 mg of PGF increased pregnancy outcomes in 1 herd of dairy cows in Canada, we could not corroborate those results. The fact that PGF reduced double ovulation and tended to increase pregnancy outcome in experiment 1 is evidence for a potential biologic effect. Inconsistency in the pregnancy outcome, however, is consistent with other studies. In a study in which PGF at the time of AI increased pregnancy outcome, it also increased luteal tissue and progesterone concentration 13 days after treatment. Results in experiment 1 failed to verify those findings.

In conclusion, results of our study fail to demonstrate a pregnancy outcome benefit of treating cows with a small dose of PGF at AI.

Table 1. Ovarian responses and pregnancy outcomes after treatment of lactating dairy cows with 10 mg $PGF_{2\alpha}$ at AI (experiment 1)

	Treatment[1]	
Item	Control	$PGF_{2\alpha}$
Cows, no	147	140
Single ovulation, %	95.6	95.2
Multiple ovulation, %	24.2	13.8*
Progesterone,[2] ng/mL	5.8 ± 0.3	6.1 ± 0.3
Volume of CL,[2] cm^3	9.1 ± 0.5	9.3 ± 0.5
Pregnancy per AI at d 32, %	29.2	32.5
Pregnancy per AI at d 80, %	24.9	30.4

*Differs from control ($P < 0.05$).
[1]Cows were treated with 10 mg $PGF_{2\alpha}$ at AI.
[2]Day 13 after treatment.

Table 2. Herd characteristics and effects of 10 mg $PGF_{2\alpha}$ at timed AI on pregnancy outcomes (experiment 2)

	Herd	
Item	1	2
No. cows treated	1,066	782
Pregnancy per AI at 32-35 d		
Control, %	33.9 (549)	43.1 (362)
$PGF_{2\alpha}$ treatment,[1] %	30.2 (517)	44.1(401)
Pregnancy per AI at 63-68 d		
Control, %	32.2 (549)	38.1 (362)
$PGF_{2\alpha}$ treatment,[1] %	27.7 (516)	38.9 (391)
Pregnancy loss		
Control, %	4.8 (186)	11.5 (156)
$PGF_{2\alpha}$ treatment,[1] %	7.7 (155)	9.0 (167)

[1]Cows were treated with 10 mg $PGF_{2\alpha}$ at AI.

Benchmarking Reproductive Efficiency and Transition Cow Health of Kansas Dairy Herds

A. Scanavez, B.E. Voelz, and L. Mendonça

Summary

Comparing key performance indicators across dairy farms may provide insightful information to dairy producers. Differences in management philosophies, facilities, and locations of dairy farms may influence overall performance of dairy operations. An ongoing extension program aims to benchmark reproductive performance and transition cow health of dairy farms located in Kansas and adjacent states. In this report, we compiled data from 2013 to 2015 of herds enrolled in the program and divided the data in warm and cool seasons to evaluate the impact of heat stress on key performance indicators. Annual pregnancy risk and warm to cool ratio of pregnancy risk varied from 20.9 to 22.5% and 75 to 82%, respectively. Annual insemination risk varied from 63.6 to 66.4% and warm to cool ratio of insemination risk varied from 96 to 97%, which suggests that heat stress does not remarkably affect insemination risk. In contrast, conception risk is significantly affected by heat stress because conception risk in the warm season ranged from 26.7 to 29.6% and in the cool season from 34.5 to 35.4% from 2013 to 2015. Percentage of cows that were treated for mastitis within 21 d after calving was below 4% annually. Warm to cool ratio of percentage of cows treated for mastitis ranged from 139 to 170%, indicating that during summer, cows are at increased risk of being affected by early postpartum mastitis. Benchmarking key performance indicators may assist dairy producers to identify areas of opportunity for improvement.

Key words: dairy cattle, benchmarking, reproductive efficiency, cow health

Introduction

Herd reproductive efficiency influences profitability of dairy operations. Reproductive performance of dairy herds may be associated with several factors such as management strategies and facilities. Additionally, recent research has demonstrated that transition cow health greatly affects reproductive performance of dairy cows. Hence, reproduction and transition cow health are priority areas to be monitored closely in dairy farms because of their importance in efficiency and profitability.

Benchmarking reproductive performance and transition cow health of dairy herds may be useful to monitor and identify improvement opportunities in dairy operations. Considering that several factors may impact reproductive efficiency and transition cow health, comparing records across dairy farms may provide further understanding of results being achieved. Benchmarking these areas may reveal consequences of chosen management practices and impacts of facilities on key performance indicators (KPI). Staff from K-State Research and Extension developed a program to benchmark overall performance records of dairy herds on a monthly basis. The purpose of this ongoing extension program is to compare KPIs related to production, reproduction, and transi-

tion cow health of herds located in Kansas. Currently, the Dairy Records Intelligence Network (DRINK) program benchmarks 25 herds, which are located in Kansas and adjacent states and account for approximately 77,000 lactating cows. This article focuses on reproductive performance and transition cow health of participating herds in the DRINK program.

Experimental Procedures

Reproductive performance and transition cow health data were extracted from on-farm management software from herds enrolled in the DRINK program. Data pertain to 2013 - 2015. All traits were extracted at the herd level to compare monthly averages.

Reproductive performance

Pregnancy and insemination risk calculated in 21-d cycles were extracted. Pregnancy risk represented the percentage of cows that became pregnant of all cows that were eligible to become pregnant in the cycle. Insemination risk represented the proportion of cows inseminated of cows eligible to be inseminated during the cycle. Cycles were adjusted for the voluntary waiting period of the herds to calculate actual pregnancy and insemination risk. Pregnancy and insemination risk data extracted from the herds were compiled to monthly averages. Each 21-d cycle was assigned to the month in which at least 50% of the days of the cycle were within the specific month. An average of pregnancy risk was calculated for the months that had 2 cycles. Conception risk was categorized on a monthly basis and represented the percentage of cows that became pregnant among cows that were inseminated.

Transition cow health

Traits to evaluate transition cow health data were categorized on a monthly basis. Stillbirth represented the percentage of calves born dead or dead within the first 24 h after calving. Mastitis postpartum represented the percentage of cows treated for mastitis within 21 d after calving.

Months were categorized by season and averages for each season were calculated. June to August was categorized as warm season, and January to May and September to December were categorized as cool season. Data were analyzed by ANOVA for repeated measures using the MIXED procedure of SAS, or by ANOVA using the GLM procedure of SAS.

Results and Discussion

Annual pregnancy risk ranged from 20.9 to 22.5% from 2013 to 2015 (Table 1). Although annual pregnancy risk is valuable information to evaluate the end result of the reproductive program of a herd, it is more important to observe the variation across the year. For the majority of the months, average pregnancy risk is $\geq$ 21% (Table 1), however, during July and August reproductive efficiency is significantly decreased. This indicates that producers should mainly focus on achieving a pregnancy risk $\geq$ 21% during summer months. Considering that most producers desire to have a high annual pregnancy risk (e.g., 30%), the key is to emphasize improving reproductive efficiency during summer months.

Insemination risk does not vary significantly across the year (Table 1). The warm to cool ratio of insemination risk ranges from 96 to 97%, indicating that the current reproductive management practices that are in place overcome the negative effects of heat stress on estrus expression. The majority of the herds enrolled in the program use a combination of estrus detection programs with timed AI protocols. Estrus detection for most of the herds consists of observing tail paint removal once daily. Despite the fact that insemination risk is not affected during summer, conception risk is greatly affected during the warm season (Table 3). The range of warm to cool ratio of conception risk is 75 to 86% from 2013 to 2015. This indicates that poor conception risk during the summer is the main reason for low reproductive efficiency from June to August.

Annual average risk of mastitis postpartum is below 4% (Table 2). Because cows that have mastitis early postpartum have decreased reproductive performance and milk yield during lactation, it is key to monitor this indicator closely. Regardless of the year observed, mastitis postpartum during summer months is consistently > 4% (Table 2). The warm to cool ratio of mastitis postpartum ranges from 139 to 170%. The remarkable increase in percentage of cows treated for mastitis during the summer may be attributed to increased rainfall and heat stress. Postpartum cows are susceptible to health disorders because periparturient cows have decreased immune function. Thus, implementation of cooling strategies for transition cows may minimize heat stress, which may improve transition cow health. Herd-level stillbirth percentage is not negatively affected by summer. Annual stillbirth percentage varied from 4.8 to 6.0%. Herds should strive to maintain stillbirth incidence < 5% in part because it impacts the dam's survival and reproductive performance.

In conclusion, dairy producers located in Kansas that want to achieve greater annual pregnancy risk should focus on improving conception risk during the summer months. Because insemination risk is not impacted during summer, dairy producers should strive to maintain an efficient estrus detection programs independently of the season. Percentage of cows treated for mastitis is greatly increased during the summer. Benchmarking dairy herds may assist producers in identifying areas of opportunity. Lastly, maintaining accurate records of uterine diseases, mastitis, lameness, and metabolic disorders are necessary to identify potential limitations of the transition cow program.

Acknowledgments

The authors thank the owners of the collaborating dairies.

Table 1. Reproductive performance according to month from 2013 to 2015 of herds enrolled in the DRINK program

	Month												
Item	January	February	March	April	May	June	July	August	September	October	November	December	Annual average
Pregnancy risk, %													
2013	23.4	22.5	23.1	21.2	21.6	19.2	17.1	17.4	19.1	20.1	23.1	22.6	20.9
2014	23.7	22.9	21.9	22.1	22.4	19.6	16.5	17.4	19.6	23.2	24.3	24.6	21.5
2015	24.6	24.3	24.6	23.6	22.9	19.6	16.7	17.9	20.4	24.2	26.4	24.4	22.5
Insemination risk, %													
2013	64.1	64.4	63.6	63.9	64.8	63.4	59.2	64.0	62.2	65.0	65.6	62.9	63.6
2014	64.1	62.1	62.8	62.8	63.6	62.7	60.2	62.9	65.6	67.1	66.4	66.5	63.9
2015	66.7	64.8	66.4	65.2	65.8	62.7	63.7	66.7	69.6	67.7	69.2	68.0	66.4
Conception risk, %													
2013	36.4	33.7	36.1	36.1	32.3	31.8	29.0	28.1	30.5	31.7	36.8	36.6	33.3
2014	38.3	36.2	36.1	35.4	35.6	30.4	27.7	28.1	29.9	35.7	35.3	36.7	33.8
2015	36.4	37.1	35.6	36.1	33.5	29.9	24.4	25.9	29.6	34.5	37.1	38.7	33.2

Table 2. Transition cow health according to month from 2013 to 2015 of herds enrolled in the DRINK program

Item	January	February	March	April	May	June	July	August	September.	October	November	December	Annual average
Stillbirth, %													
2013	6.7	8.5	6.7	6.0	5.7	4.6	5.3	6.0	5.7	5.3	6.5	5.0	6.0
2014	5.6	6.4	5.4	4.7	5.4	4.9	5.8	5.1	4.9	4.8	4.6	5.5	5.3
2015	5.9	5.4	5.6	4.4	5.1	4.8	4.4	4.4	4.0	4.7	4.3	5.1	4.8
Mastitis postpartum, %													
2013	3.6	3.2	3.2	3.0	3.2	4.6	5.1	6.6	4.8	3.1	3.6	3.0	3.9
2014	3.4	3.7	2.7	3.4	2.9	5.1	5.8	5.5	3.8	3.1	2.6	3.4	3.8
2015	3.4	3.1	2.9	2.6	4.5	4.1	4.7	4.4	3.1	2.6	2.9	3.1	3.4

Table 3. Reproductive efficiency and transition cow health from 2013 to 2015 during cool and warm seasons from herds enrolled in the DRINK program

Item	Cool season[1]	Warm season[2]	Warm to cool ratio[3]
Pregnancy risk, %			
2013	21.9	17.9	82%
2014	22.8	17.9	78%
2015	23.9	18.0	75%
Insemination risk, %			
2013	64.1	62.2	97%
2014	64.6	61.9	96%
2015	67.0	64.4	96%
Conception risk, %			
2013	34.5	29.6	86%
2014	35.4	28.7	81%
2015	35.4	26.7	75%
Stillbirth, %			
2013	6.2	5.3	86%
2014	5.3	5.3	100%
2015	4.9	4.5	92%
Mastitis postpartum, %			
2013	3.4	5.4	159%
2014	3.2	5.4	170%
2015	3.1	4.4	139%

[1] Average of traits from January to May and September to December.
[2] Averages of traits from June to August.
[3] Warm season average divided by cool season average.

Development of a Berry Processing Score for Sorghum Silage

J.R. Johnson, J.P. Goeser,[1] and M.J. Brouk

Summary

This study was done in an effort to develop a berry processing score (BPS) for sorghum silage, similar to the kernel processing score (KPS) currently used for corn silage. Sorghum silage samples were collected from 3 dairies in Kansas and processed in the Grain Science & Industry grain processing laboratory at Kansas State University using one of four different roll gap settings to give four differently processed samples: unprocessed, 1.5, 1.0, or 0.5 mm. After drying, samples were placed into a Ro-Tap particle separation machine for 10 minutes until the whole sample was separated. Whole samples, as well as separated fiber and whole berry portions were analyzed for percent starch retained on each screen. As the roll gap was reduced, mean particle size (MPS) was also reduced. Percent starch passing through the 1.7 mm screen was greater at the 0.5 mm roll gap for both the whole sample and the whole berry samples, indicating successful processing of the samples. Using these data, we have determined that the appropriate screen to use in determining a BPS for sorghum silage is the 1.7 mm screen. A BPS for any sorghum silage sample can be calculated by analyzing the whole sample for the percent starch that passes through the 1.7 mm screen. This study is still ongoing and more research is needed to determine the recommended BPS in sorghum silage.

Key words: milo, sorghum, dairy cattle, feed, processing, silage

Introduction

Sorghum has become an increasingly important forage crop for dairy producers, particularly in the Midwestern and plains regions of the U.S. that routinely experience conditions of insufficient water. When compared to corn silage, sorghum silage uses ~30-50% less water, making sorghum more heat and drought tolerant. This is especially important in areas where irrigation is limited and where elevated temperatures and drought are common.

Sorghum silage has long been known to have reduced whole-plant digestibility compared to corn silage and therefore, milk yield often decreases when replacing corn silage with sorghum silage in dairy cattle diets. A primary reason for reduced digestibility is that the starch contained within the sorghum berry is extremely dense, hard, and resistant to digestion. The protein matrix binds starch more tightly in sorghum than in corn, leading to lower digestibilities and milk yield often observed with sorghum.

Kernel processing via on-board kernel processors have been used extensively in the harvest of corn silage in an effort to better expose the starch within grain (increase surface area), ultimately aiming to increase total tract starch digestibility (TTSD) for the dairy cow. Ten years ago, Wisconsin researchers established a method to determine the degree of kernel processing, or breakage, in whole plant corn harvested as silage. However, no such method has been developed for sorghum silage. Therefore, the objective of this

[1] Rock River Laboratory, Watertown, WI.

study was to develop a similar scoring system for sorghum silage. This study is still ongoing and further results will become available in the future.

Experimental Procedures

Sorghum silage samples (Croplan BMR 108, Croplan Genetics, St. Paul, MN) to be used for analysis were collected from 3 commercial dairy farms in Kansas. Eight samples were collected from each dairy resulting in a total of 24 samples. Upon returning to the lab, samples were either left unprocessed and used as the control, or run through a 9 × 6 roller mill (Ross Machine & Mill Supply, Inc., Oklahoma City, OK) using a roll gap setting of either 1.5 mm, 1.0 mm or 0.5 mm. From each dairy, 2 samples were left unprocessed and 2 samples were processed at one of the aforementioned roll gap settings. Samples were then dried in a forced-air oven at 55°C for 72 h to ensure complete removal of moisture, resulting in samples weighing ~100 g on a DM basis. Following DM determination, samples were separated using a Ro-Tap 3-dimensional separator (W. S. Tylor, Mentor, OH) fitted with screens containing square apertures of 9.50, 6.70, 4.75, 4.00, 3.35, 2.80, 2.36, 1.70, 1.18, and 0.6 mm (in addition to a pan). Samples were placed into the Ro-Tap machine for 10 min to determine mean particle size (MPS) and the percent material retained on each screen by weight was calculated. Whole sorghum berries retained on the 4.00, 3.35, 2.80, 2.36, and 1.70 mm screen were separated from the remaining sample, counted, and weighed. Preliminary research showed that all whole sorghum berries were retained below the 4.75 mm screen and above the 1.70 mm screen. Once separated, whole berry samples and the remaining fiber samples were sent to Rock River Laboratory (Watertown, WI) for DM, starch, and fiber (aNDF) analysis using wet chemistry techniques. Material retained on the 9.50, 6.70, and 4.75 mm screen were combined into a single sample prior to analysis since no whole berries were retained on those screens.

Results and Discussion

Mean particle size (Figure 1) of sorghum silage was reduced when processed with a roll gap setting of 0.5 mm. After sample separation, material retained on each screen was measured (Figure 2). While no differences were found between treatments for screen size $\geq$ 3.35 mm, there was a significant reduction in material retained on the 2.8 and 2.36 mm screens as roll gap spacing was reduced. This led to an increase in material retained on the 1.18 and 0.6 mm screens as well as the pan for more heavily processed (narrower roll gap) sorghum silage. As expected, whole berries per gram of sample weight were reduced as the roll gap setting was reduced (Figure 3). This indicates that the different roll gap settings used were effective at processing the sorghum berries. Percent starch retained by screen of the whole sample is shown in Figure 4. Starch retained below the 1.7 mm screen was greater for the 0.5 and 1.0 mm roll gap samples. After separating the whole sample into whole berry and fiber fractions, the percent starch retained on each screen was analyzed. For the fiber only portion (Figure 5), unprocessed samples had greater starch retained on the 1.7 mm screen compared to processed samples, while results were mixed for screens < 1.7 mm. For the whole berry only samples (Figure 6), all whole berries were retained above the 1.18 mm screen. There was a significant increase in starch retained on the 1.7 mm screen for samples processed at 0.5 mm compared to other treatments. This indicates that only the smallest berries were able to pass through the rollers and remain unprocessed.

Conclusions

From these data, we conclude that by measuring the amount of starch passing through the 1.7 mm screen, we can calculate a BPS for sorghum silage, similar to what is used for corn silage, which measures the percent of starch passing through the 4.75 mm screen. While digestibility issues of sorghum are still an issue, the development of a BPS for sorghum silage will give the industry a standard by which to measure the degree of processing. The next step in this process is to collect sorghum silage samples and run *in situ* rumen analysis at each of the different processing levels described above to determine the impact of reduced particle size on digestibility of the sorghum berries.

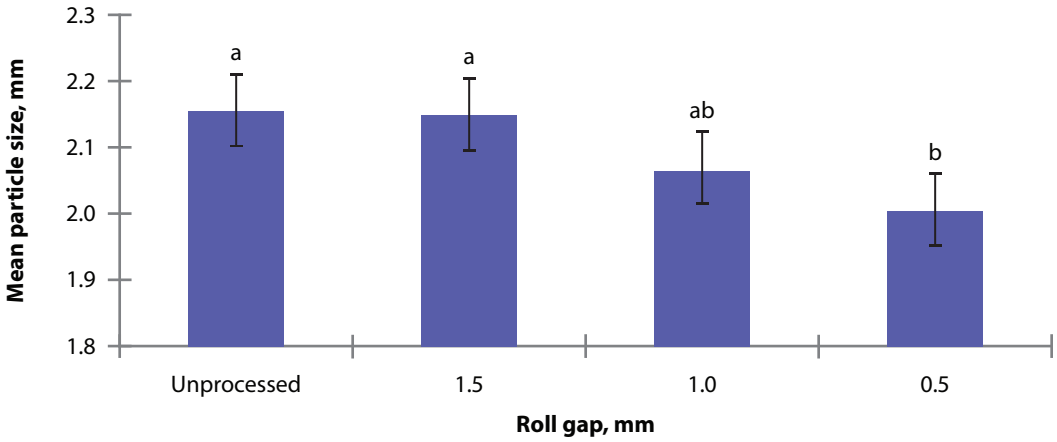

Figure 1. Least squares means for mean particle size at each roll gap setting (unprocessed, 1.5, 1.0, or 0.5 mm). Treatment effect: $P = 0.09$.
[ab] Means differ ($P < 0.05$).

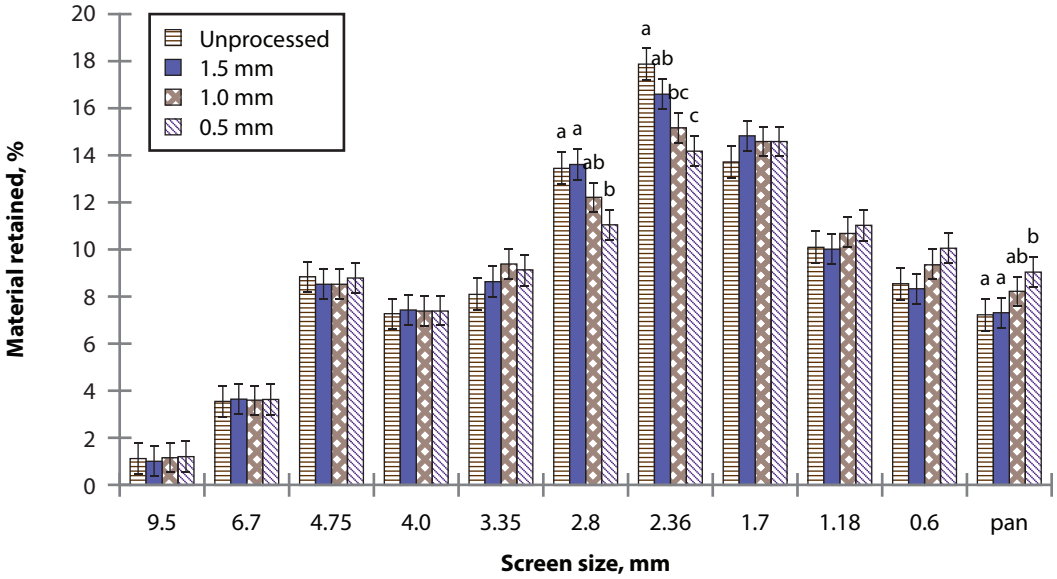

Figure 2. Percent material retained on each screen at each roll gap setting (unprocessed, 1.5, 1.0 or 0.5 mm) after complete separation using a Ro-Tap particle separator.
[abc] Means differ ($P < 0.05$).

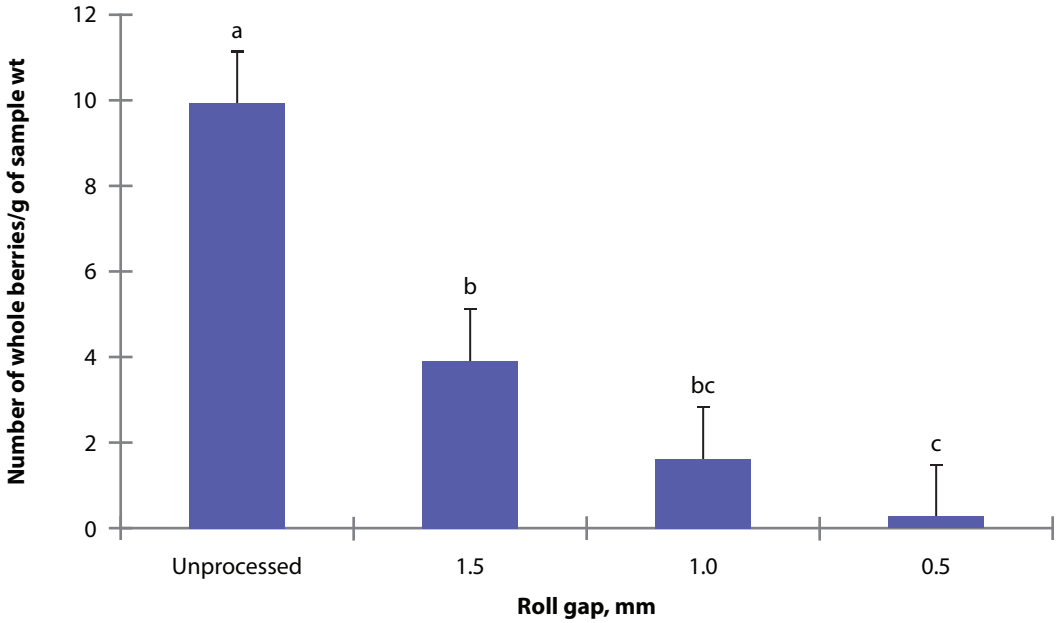

Figure 3. Whole berries per gram of sample for each roll gap setting (unprocessed, 1.5, 1.0, or 0.5 mm). Treatment effect: $P < 0.001$.
[abc] Means differ ($P < 0.05$).

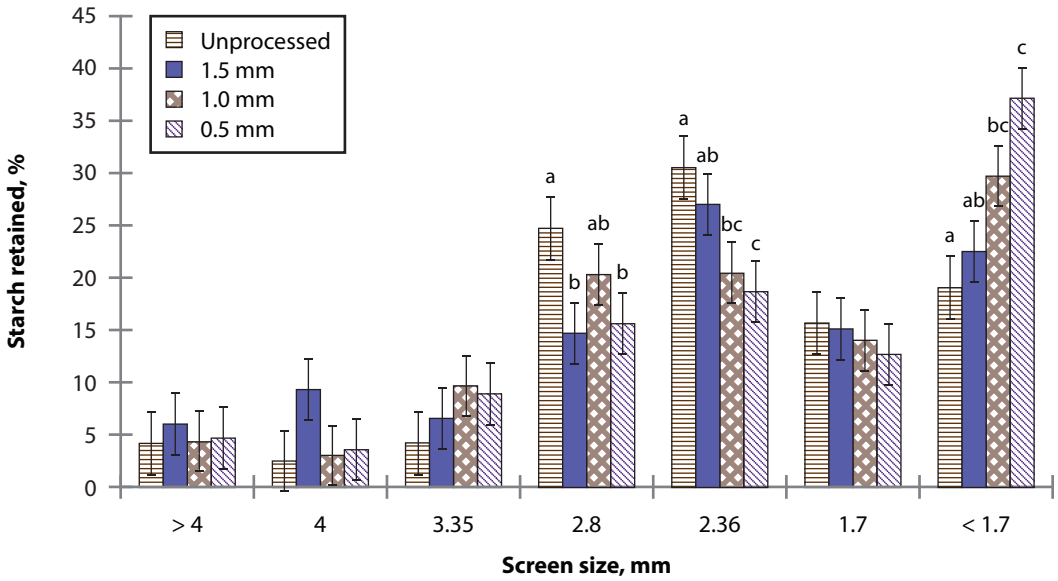

Figure 4. Percent starch retained by screen for each roll gap setting (unprocessed, 1.5, 1.0, or 0.5 mm) of whole sorghum sample after complete separation using a Ro-Tap particle separator.
[abc] Means differ ($P < 0.05$).

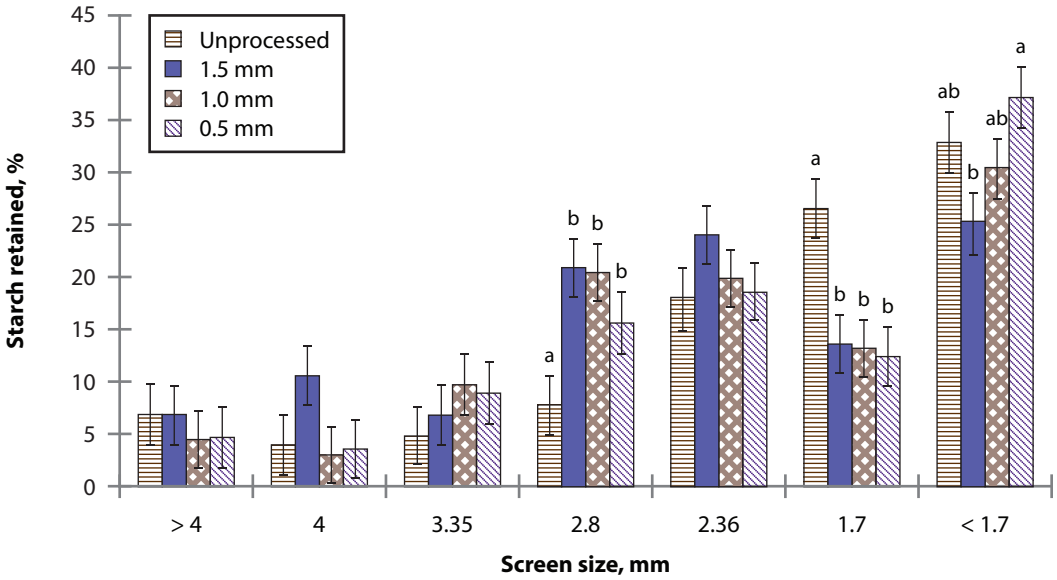

Figure 5. Percent starch retained by screen for each roll gap setting (unprocessed, 1.5, 1.0 or 0.5 mm) of fiber portion of sorghum sample.
[abc] Means differ ($P < 0.05$).

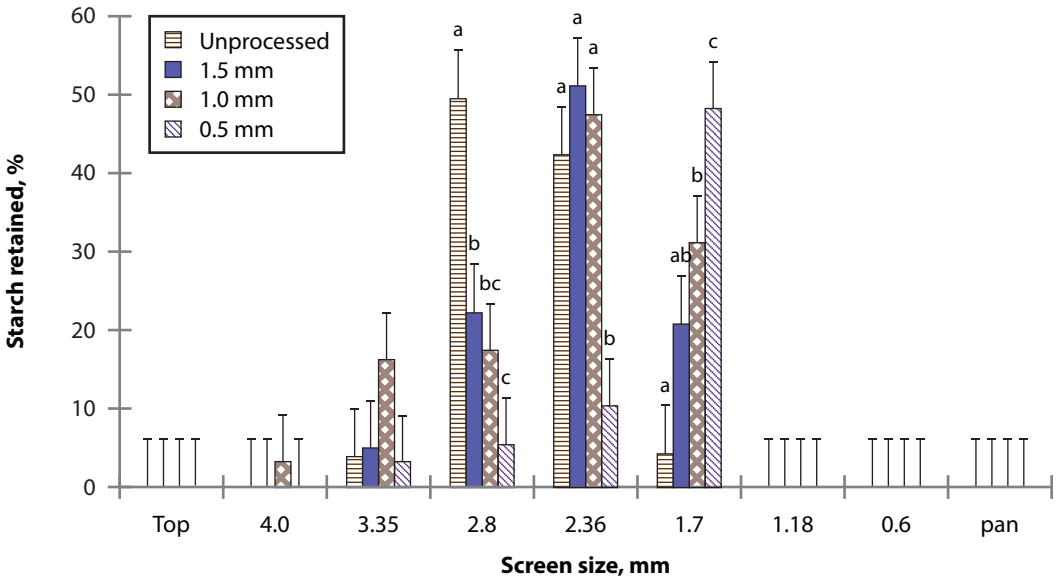

Figure 6. Percent starch retained by screen for each roll gap setting (unprocessed, 1.5 ,1.0, or 0.5 mm) of whole berry portion of sorghum sample.
[abc] Means differ ($P < 0.05$).

Effects of Dietary Zinc Source and Level on Mammary Epithelia and Dairy Food Chemistry

J. Shaffer, K. Pandalaneni, L. Mamedova, J. DeFrain,[1] J. Amamcharla, and B.J. Bradford

Summary

Twelve lactating Holstein cows (132 ± 21 days in milk) were enrolled in a Latin square experiment to explore the extent to which source and amount of supplemental dietary Zn can impact barrier function of mammary epithelial tissue. Cows received either 970 mg supplemental Zn/day as $ZnSO_4$ (LS), 1,640 mg supplemental Zn/day as $ZnSO_4$ (HS), or 1,680 mg supplemental Zn/day as a mixture of $ZnSO_4$ and Zn methionine complex (HC). Treatments lasted for 17 days followed by 4 days of sample collection. Blood and milk were collected and analyzed for markers of blood-milk leak including plasma lactose and α-lactalbumin and milk electrolytes. Total RNA was also isolated from milk cells and abundance of Zn transporter 2 (ZnT2) and clusterin, genes with potential impact on Zn-dependent apoptosis and cell survival, were measured. Finally, dairy food properties of milk (heat coagulation time, nonprotein nitrogen, and non-casein nitrogen) were also analyzed. Cows on the HS treatment tended to have higher feed intake than LS ($P = 0.06$), and milk fat percentage tended to increase for HC compared to LS ($P = 0.08$). No other effects on milk composition, yield, or production efficiency were observed. No effects were observed on markers of blood-milk leak, mRNA abundance of ZnT2 or clusterin, or dairy food chemistry properties. Concentration and source of dietary Zn did not impact mammary epithelial integrity in lactating cows during late lactation.

Key words: zinc, mammary epithelia, apoptosis

Introduction

Dietary Zn is important for immunity, reproduction, hormone activity, and the activity of many enzymes in dairy cattle. Dietary Zn availability can also impact epithelial tissues, such as mammary epithelial cells (MECs), the cells responsible for milk secretion. In basic research conducted on mice, moderate Zn deficiency impacts milk secretion, gross morphology, and apoptosis rate in the mammary gland. Research in dairy cattle nutrition has shown that feeding diets marginally deficient in Zn during early lactation can compromise mammary health. Further, health and production benefits have been shown when feeding amino acid-chelated Zn as opposed to inorganic Zn sources such as zinc sulfate or zinc oxide. In this study, we explored the extent to which these effects could be mediated by impacts on MEC structure and apoptosis, evaluated in response to feeding two levels of supplemental Zn with varying proportions provided as a Zn methionine complex. We hypothesized that cows fed higher levels or more bioavailable Zn would show fewer signs of epithelial barrier disruption in the mammary gland.

[1] Zinpro Corp., Eden Prairie, MN.

Experimental Procedures

Twelve multiparous Holstein cows in mid- to late-lactation (132 ± 21 days in milk) were enrolled in a three-period Latin square experiment. Throughout the course of the study, cows were housed in individual tie stalls equipped with automatic waterers, fed a balanced basal ration twice daily (Table 1), and milked 3 times daily. Treatments consisted of a low inorganic zinc supplement (970 mg supplemental Zn/day provided as $ZnSO_4$, LS), a high inorganic zinc supplement (1,640 mg Zn/day provided as $ZnSO_4$, HS), and a high Zn supplement partially provided in the form of an amino acid complex (1,680 mg supplemental Zn/day provided as 33% $ZnSO_4$ and 67% Zn-methionine [Zinpro, Zinpro Corp, Eden Prairie, MN] on a Zn basis, HC). Treatments were administered once daily as a gel capsule containing all supplemental trace minerals except for selenium, which was included in the grain mix (Table 2). Each cow received all three treatments and treatments were balanced for potential carryover effects across periods. Each period lasted 21 days, with 17 days of diet adaptation and 4 days of sample collection.

Milk yield, milk composition, and feed intake were recorded and averaged for each cow over each 4-d sampling period. To assess blood-milk leak, sodium and potassium concentrations were measured in milk collected during sampling periods, and blood concentrations of these electrolytes were also measured as covariates for the milk electrolyte statistical analysis. Blood plasma lactose and alpha-lactalbumin were measured as additional markers of milk-blood leak. Heat coagulation time, non-protein nitrogen, and non-casein nitrogen were measured in milk samples to assess dairy food properties of milk. Finally, mRNA abundance of ZnT2 and clusterin, genes related to Zn-mediated apoptosis and cell survival, were measured in cells collected from milk samples during sampling periods.

Data were analyzed in JMP v10.0 (SAS Institute, Cary, NC) with a mixed model using fixed effects of treatment and period, and the random effect of cow. Significance was declared at $P < 0.05$ and tendencies at $P < 0.10$. Interactions of treatment and period were also tested and removed from the model if they did not contribute significantly ($P > 0.10$).

Results and Discussion

Feed intake tended to increase for HS cows ($P = 0.06$) compared to LS, and milk fat percentage tended to increase for HC ($P = 0.08$) compared to LS. No other effects on milk composition, yield, or production efficiency were observed (Table 3). Plasma electrolyte, lactose and α-lactalbumin concentrations were also unaffected by treatment, as were milk electrolytes (Table 4). No treatment effects were observed in transcript abundance of ZnT2 or clusterin in milk cells (Table 4). Finally, no effects of treatments were observed on heat coagulation time or the proportion of nonprotein nitrogen or noncasein nitrogen in the milk (Table 5).

Results from this study indicate that supplemental zinc source and level in practical diets of mid-lactation dairy cattle have little effect on the integrity of the blood-milk barrier of the healthy mammary gland. In previous research, plasma lactose and α-lactalbumin levels have been shown to increase with disruption of the epithelial barrier separating blood from milk in the mammary gland. The lack of treatment effects

observed in this study likely indicates that epithelial integrity was not appreciably affected by our treatments, perhaps in part due to the use of healthy, non-mastitic cows. In the present study, all cattle were fed a diet which met predicted requirements for zinc intake, and treatment diets were administered for only a portion of the lactation period, in order to facilitate a Latin square experimental design.

Feeding of marginally zinc-deficient diets in early lactation has been shown to increase somatic cell counts in dairy cattle. Further, multiparous cows supplemented with an increased proportion of zinc as zinc methionine have reduced somatic cell counts and higher colostrum immunoglobulin G, indicating improved mammary health and possibly enhanced immune function. One possible explanation for these observations is that zinc supplementation leads to less apoptosis in the mammary gland, leading to a tighter blood-milk barrier. Marginally zinc deficient mice have been shown to experience dramatic increases in apoptosis rates in mammary tissue leading to differences in gross morphology of the mammary gland during lactation. If this were the case for our model, we would likely have observed differences in markers of blood-milk leak.

Another potential explanation for zinc supplementation leading to improved mammary health is a direct impact on immune responses. Dietary zinc levels impact lymphocyte numbers as well as other aspects of the immune response in mice; however, zinc supplementation in practical diets of cattle does not always impact measures of immune function. It is possible that improvements in mammary health and milk production that sometimes come with zinc supplementation are the product of improved immune response to mastitis challenge, without directly affecting apoptosis of mammary epithelial cells or integrity of the blood-milk barrier.

Transcript abundance of selected genes was determined as a further exploratory attempt to discover cellular pathways impacted by zinc supplementation in the mammary gland. Collecting RNA from an internal tissue of a living organism is typically a difficult task, requiring a biopsy procedure. In the case of mammary epithelial cells (MEC), there are alternatives to biopsy, which in turn present their own challenges. In the present study, we utilized milk somatic cells as a source of mammary tissue. Isolation of RNA from milk somatic cells has been shown to produce RNA sequencing results comparable to other more intensive sampling methods such as biopsies, laser-capture of cells from fixed tissue, and antibody-capture of milk epithelial cells. However, in a healthy cow, less than 10% of milk somatic cells are expected to be MEC, with the balance largely made up of immune cells. It is possible that transcript abundance of genes expressed preferentially in MEC could be diluted by the low proportion of MEC in milk somatic cells. Abundance was low for both ZnT2 and clusterin, which may partially explain why no differences were detected.

Conclusion

Zinc supplementation of dairy rations at 950 mg/day as opposed to 1,650 mg/day did not appear to impact the integrity of the blood-milk barrier or dairy food properties of milk, nor did the inclusion of a greater proportion of the Zn as an amino acid complex. Milk production responses to amino acid-bound Zn sources which have been documented in studies with larger sample sizes are likely due to mechanisms other than a direct impact on mammary epithelial integrity. However, it is possible that these

treatments could elicit a response in cohorts with greater mastitis pressure, with more challenges to mammary epithelial integrity.

Table 1. Formulation and composition of basal diet

Item	Value
Ingredient, % of diet DM	
Alfalfa hay	18.64
Corn silage	18.85
Wet corn gluten feed[1]	24.29
Cotton seed	4.28
Lactation grain mix[2]	33.94
DM, %	53.37
Nutrient, % of diet DM	
Crude protein	17.94
Acid detergent fiber	19.33
Neutral detergent fiber	30.40
Lignin (sulfuric acid)	4.70
Non-fiber carbohydrate	42.13
Fat (ether extract)	4.43
Zinc, ppm	58.33
Net energy for lactation,[3] Mcal/lb	0.72

[1]Sweet Bran (Cargill Inc., Blair, NE).
[2]Lactation grain mix consisted of 66.2% fine rolled corn, 20.2% expeller soybean meal (SoyBest, Grain States Soya, West Point, NE), 4.04% limestone, 0.5% stock salt, 0.5% potassium chloride, 3.53% sodium bicarbonate, 0.81% magnesium oxide, 0.13% selenium premix (0.06%), 0.05% vitamin A premix (30 kIU/g), 0.02% vitamin D premix (30 kIU/g), 0.5% vitamin E premix (20 kIU/g), 0.02% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 0.63% XP Yeast (Diamond V, Cedar Rapids, IA), 0.32% Biotin 100 (ADM Alliance Nutrition, Quincy, IL), and 2.52% Ca salts of long-chain fatty acids (Megalac R, Arm & Hammer Animal Nutrition, Princeton, NJ).
[3]Estimated according to NRC (2001).

Table 2. Composition of treatment capsules

Item[1]	30-ZS	60-ZS	60-ZC
% DM	97.97	98.28	97.74
Ca, % DM	1.55	1.33	5.23
P, % DM	0.01	0.01	0.01
Mg, % DM	0.13	0.10	0.11
K, % DM	0.23	0.21	0.26
S, % DM	15.42	15.31	10.35
Mn, % DM	12.84	9.95	9.69
Zn, % DM	7.99	11.55	10.37
Cu, % DM	1.89	1.40	1.32
Fe, % DM	0.23	0.26	0.23
Na, % DM	2.61	1.94	2.01
Cl, % DM	2.32	2.42	8.83
DCAD, mEq/100 g	909.72	935.44	801.66
Dose, grams/day			
Ca	0.19	0.19	0.85
P	0.00	0.00	0.00
Mg	0.02	0.01	0.02
K	0.03	0.03	0.04
S	1.87	2.17	1.68
Mn	1.56	1.41	1.57
Zn	0.97	1.64	1.68
Cu	0.23	0.20	0.21
Fe	0.03	0.04	0.04
Na	0.32	0.28	0.32
Cl	0.28	0.34	1.43
Met	2.71	2.71	2.69
Metabolizable Met	2.19	2.19	2.18

[1] Values are results of analysis of mineral mixes (Dairyland Laboratories, Arcadia, WI).

Table 3. Intake and milk production

	Least square means				*P* - value	
Item	30-ZS	60-ZS	60-ZM	SEM	Trt	Period
DMI, lb/day	62.1	64.0	63.7	1.4	0.06	< 0.001
Milk, lb/day	104.1	104.4	105.4	3.0	0.74	0.16
ECM, lb/day	105.9	107.8	109.3	2.6	0.36	0.23
Milk/DMI	1.68	1.63	1.66	0.04	0.13	< 0.001
ECM/DMI	1.71	1.69	1.73	0.03	0.42	< 0.001
Protein, %	3.04	3.03	3.02	0.06	0.52	< 0.001
Protein, lb/day	3.15	3.15	3.18	0.09	0.97	0.25
Fat, %	3.56	3.69	3.76	0.13	0.08	0.35
Fat, lb/day	3.68	3.84	3.92	0.13	0.15	0.17
Lactose, %	4.90	4.94	4.91	0.04	0.21	0.098
Lactose, lb/day	5.09	5.14	5.16	0.13	0.71	0.09
MUN, mg/dL	13.15	13.53	13.49	0.50	0.23	0.21

Table 4. Markers of mammary epithelial integrity

	Least square means				*P* - value	
Item	30-ZS	60-ZS	60-ZM	SEM	Trt	Period
Blood K, mM	4.35	4.59	4.36	0.09	0.12	0.11
Blood Na, mM	136.86	136.47	136.31	0.38	0.52	0.57
Blood Cl[3], mM	99.52	99.84	99.82	0.52	0.80	0.43
Blood Hb,[3] *mM	5.62	5.70	5.67	0.07	0.48	0.04
Blood Hct[3] (% PCU)	26.58	27.00	26.85	0.33	0.48	0.04
Plasma lactose, μM	21.32	20.91	19.14	2.35	0.73	0.53
Milk Na,[1] ppm	354	349	369	11	0.15	< 0.001
Milk K, ppm	1516	1492	1527	30	0.55	< 0.01
ZnT2, NTA[2]	0.59	0.39	0.35	0.15	0.25	0.054
Clusterin, NTA	2.27	0.98	1.20	0.72	0.41	< 0.01

[1]Modeled with blood Na as a covariate ($P < 0.01$).
[2]NTA = Normalized transcript abundance.
[3]Treatment × period effect significant and included in the model ($P < 0.1$).

Table 5. Dairy food properties of milk

	Least square means				*P* - value	
Item	30-ZS	60-ZS	60-ZC	SEM	Trt	Period
Milk NPN[1], ppm	286	306	296	12	0.51	< 0.01
Milk non-casein N,[2] ppm	791	816	809	42	0.92	0.052
Native HCT[2], min	11.29	11.35	10.89	1.66	0.78	0.19
6.4 HCT, min	0.90	1.35	2.80	0.27	0.40	0.09
6.6 HCT, min	12.31	12.46	11.20	0.77	0.39	0.87
6.8 HCT, min	12.28	12.98	13.03	1.68	0.89	0.22
7.0 HCT, min	12.12	10.17	10.41	1.68	0.59	0.33

[1]NPN = non-protein nitrogen; HCT = heat coagulation time.
[2]Treatment × period effect significant and included in the model ($P < 0.1$).

Effectiveness of Two Ruminally Protected Methionine Sources for Lactating Dairy Cows

M. Ardalan, C.F. Vargas-Rodriguez, G.I. Zanton,[1] M. Vázquez-Añón,[1] E.C. Titgemeyer, and B.J. Bradford

Summary
Two sources of ruminally protected methionine were tested for their ability to provide available methionine to lactating dairy cattle. Based on milk protein yield and milk protein percent, NTP-1401 (an unreleased product from Novus International, Inc., St. Charles, MO) and Smartamine (Adisseo, Alpharetta, GA) provided similar amounts of available methionine to the cows. These two products led to different methionine-related compounds appearing in blood plasma, suggesting that they contained different methionine precursors.

Key words: availability, ruminally protected methionine

Introduction
Feeding diets with excessively high crude protein concentrations to meet dairy cow requirements for metabolizable protein leads to excretion of excess nitrogen in manure. One approach to reducing dietary crude protein content is supplementing specific limiting amino acids to the cow, but these supplemental amino acids need to be protected from ruminal degradation to be effective nutrient sources for cattle. To effectively use these ruminally protected amino acids in diet formulation, it is critical to know the extent to which the amino acids are available to the cow, that is, they must be protected from ruminal degradation, yet available for intestinal digestion.

In the past several decades there has been a great deal of interest in investigating the role of methionine on the yield of milk components in lactating cows. Methionine is typically the most limiting amino acid for milk protein synthesis and optimal dairy production. As a consequence of the beneficial effects of methionine supplementation, such as increased milk protein content and yield, various ruminally protected methionine products are commercially available.

The objective of this experiment was to evaluate the relative effectiveness of two different ruminally protected methionine sources for supporting milk production in dairy cows. One of the sources is a product that has been extensively evaluated (Smartamine), whereas the other is a newly developed product that is not yet on the market (as of November 2016).

Experimental Procedures
All procedures involving animals were approved by the Kansas State University Institutional Animal Care and Use Committee.

[1] Novus International, Inc., St. Charles, MO.

Twenty-one Holstein dairy cows between 80 and 140 days in milk (11 primiparous and 10 multiparous) were housed at the Kansas State University Dairy Teaching and Research Center in tie-stalls with rubber mats and wood shavings. Cows were used in 4 replicated 5 × 5 Latin squares with treatment sequences balanced for carryover effects as best possible; 2 squares contained only primiparous cows and 2 squares contained multiparous cows. The extra animal was provided treatments in a sequence that was identical to another primiparous cow. Treatments were: a) control (no methionine supplement); b) NTP-1401, a methionine-providing product from Novus International Inc., supplemented at 7.5 grams of product daily; c) NTP-1401 supplemented at 15 grams of product daily; d) Smartamine-M (Adisseo, Alpharetta, GA), a ruminally protected methionine product, supplemented at 7.5 grams of product daily; and e) Smartamine-M supplemented at 15 grams of product daily. Smartamine is methionine coated with poly (2-vinylpyridine-co-styrene) sensitive to acidic pH in the abomasum, and contains 75% DL-methionine. The diet (Table 1) was fed as a total mixed ration once daily and added to bunks twice daily. Cows were individually fed at 6:00 a.m. and 4:00 p.m. for ad libitum intake with free access to water. Treatments were top-dressed on the total mixed ration and hand mixed with top third of diet at the time of feeding. Total daily feed was adjusted to allow for approximately 10% refusals. The diet was evaluated by the Cornell Net Carbohydrate and Protein System (version 4.0) and found to meet the metabolizable protein and energy requirements when DMI was 25.58 kg/d for a lactating Holstein cow producing 45 kg/d of milk with 3.5% milk fat and 3.00% milk true protein. Diets were formulated to have a moderate level of crude protein (16%), a predicted deficiency of metabolizable methionine (1.85% of metabolizable protein), and a sufficient provision of lysine (6.8% of metabolizable protein). Experimental periods were each 14 days long and included 10 days for adaptation to treatments and 4 days for sample and data collection.

Cows were milked 3 times daily in a milking parlor at 7:00 a.m., 3:00 p.m., and 11:00 p.m. Milk yields were recorded at each milking. Feed intake and milk production were measured over the final 4 days of each period. Milk samples were analyzed at the Heart of America DHIA, Manhattan, KS. Blood samples for plasma amino acid analysis were collected from the tail vein from each cow at 4 hours after the afternoon feeding on the final day of each period.

Results and Discussion

Diets were formulated to be deficient in metabolizable methionine, but to provide sufficient lysine. Because our diets were deficient in methionine, we expected increases in milk protein percentage and yield if the products were effective in providing bioavailable methionine to the cows. Previous work has shown that milk protein is a sensitive indicator of methionine supply and a useful response for determining the effectiveness of ruminally protected methionine sources. In our study, milk protein was the primary response criteria.

Dry matter intake and milk production and composition are shown in Table 2. Dry matter intake was not affected by methionine supplementation ($P \geq 0.14$). Although supplementation with methionine can sometimes increase milk production in dairy cows, milk yield in our study averaged 46.2 kg/day and was not affected by the methio-

nine supplements. Similarly, milk fat yield and percentage (3.49%) were not affected by methionine supplementation.

Milk protein percentage and milk protein yield increased linearly with methionine supplementation ($P < 0.01$), indicating that methionine increased protein synthesis in the mammary gland. There were no differences between the two methionine sources for milk protein percentage or yield, suggesting that they provided similar amounts of available methionine to the cows. Linear regressions of milk protein yield (Figure 1) and milk protein percentage (Figure 2) against supplement amount within source led to slope ratios (NTP-1401/Smartamine) of 95% for protein percentage (not different from 100%, $P = 0.65$) and 84% for protein yield (not different from 100%, $P = 0.60$), further suggesting no differences between sources for increasing milk protein.

In addition to measuring production responses of the cows, we also measured plasma amino acid concentrations to further characterize the effectiveness of the products. For protein synthesis, cattle require that the natural isomer of methionine (L-methionine) be available within the cell. However, methionine supplements are typically synthesized chemically, and these products therefore contain other forms of methionine that the cow subsequently must convert to L-methionine within the body. Smartamine-M contains DL-methionine, which is a 50:50 mixture of the natural L-methionine and the unnatural D-methionine. It has been previously demonstrated that cattle are able to convert D-methionine to L-methionine, and thus D-methionine can be an available source of methionine for cattle. Methionine hydroxy analog (MHA; 2-hydroxy-4-methylthio-butyric acid) is also a synthetic methionine source. Although MHA is not a true amino acid, it is metabolized by the cow to produce L-methionine after it is absorbed from the gut.

The effects of NTP-1401 and Smartamine on plasma concentrations of L-methionine, D-methionine, the hydroxy analog of methionine (MHA; 2-hydroxy-4-methylthio-butyric acid), and total methionine equivalents (the sum of L-methionine, D-methionine, and MHA) are shown in Figures 3 through 6. Plasma L-methionine (the natural form of methionine) was linearly increased ($P < 0.01$) when either Smartamine or NTP-1401 was included in the diet, and there were no differences between the two methionine sources ($P = 0.61$). Supplementation with Smartamine significantly increased plasma D-methionine, whereas NTP-1401 did not increase plasma D-methionine. Because D-methionine is an unnatural isomer of methionine, plasma concentrations were below detection limits for cows fed the control diet. Smartamine contains D-methionine, so its ability to increase plasma D-methionine is not surprising. The inability of NTP-1401 to increase plasma D-methionine suggests that this product did not contain D-methionine. Cows receiving NTP-1401 demonstrated increases in plasma MHA, whereas those fed Smartamine did not show any increase. The lack of response for Smartamine was expected because Smartamine does not contain MHA. The increase in plasma MHA in response to NTP-1401 supplementation suggests that the NTP-1401 contains MHA that is protected from ruminal degradation.

Because the two methionine products provided different methionine precursors to the cows, we added the concentrations of the different methionine-related compounds to derive an estimate of total availability based on the blood data. Plasma concentrations of total methionine equivalents increased linearly when either methionine source was

supplemented, and there were no differences between the two sources (Figure 6). This would be suggestive of similar bioavailable methionine provision by the two products, which agrees with the observations for milk protein concentration and amount.

In summary, we compared two ruminally protected methionine sources for lactating cows. Smartamine, a product extensively used in the dairy industry, and NTP-1401, a new product that is not yet commercially available, led to similar increases in milk protein as well as in concentrations of total methionine equivalents in plasma, suggesting that the products provided similar amounts of available methionine to the cows.

Table 1. Ingredient composition of the diet

Ingredient	% of DM
Corn silage	29.9
Alfalfa	23.9
Whole cottonseed	3.9
Finely rolled corn	20.1
Solvent soybean meal	3.4
SoyBest[1]	1.8
Soybean hulls	8.1
Dried molasses	2.6
Blood meal	1.5
Energy Booster 100[2]	2.1
Limestone	0.47
Dicalcium phosphate	0.55
Salt	0.39
Trace-mineralized salt[3]	0.18
Sodium bicarbonate	0.59
Magnesium oxide	0.20
Copper sulfate	0.0053
Zinc oxide	0.0089
Selenium premix, 600 mg Se/kg	0.030
Vitamin A premix, 30,000 IU/g	0.018
Vitamin D premix, 20,000 IU/g	0.0053
Vitamin E premix, 44 IU/g	0.18
Ethylenediamine dihydriodide, 4.4%	0.0008
Rumensin 90[4]	0.0075
Yeast[5]	0.055
Biotin premix, 220 mg biotin/kg	0.11

[1] Mechanically extracted soybean meal with soy lecithins added during manufacture (Grain States Soya, West Point, NE).
[2] Rumen bypass fat with 98% fatty acids (Milk Specialties, Eden Prairie, MN).
[3] Composition: > 95.5% NaCl, 0.24% Mn, 0.24% Fe, 0.05% Mg, 0.032% Cu, 0.032% Zn, 0.007% I, and 0.004% Co.
[4] Provided 15 mg monensin/kg diet DM (Elanco Animal Health, Indianapolis, IN).
[5] Diamond V XPC Yeast (Diamond V Mills, Inc., Cedar Rapids, IA).

Table 2. Effect of supplemental methionine from the NTP-1401 or from Smartamine on milk production

	Dietary treatment						*P* - value[1]		
		NTP-1401 (g/d)		Smartamine (g/d)			Effect of methionine			Source ×
Item	Control	7.5	15	7.5	15	SEM	Linear	Quadratic	Source	level
No. of observations	20	21	21	21	21					
DM intake, kg/day	27.2	27.0	26.8	27.0	27.2	0.43	0.59	0.74	0.54	0.39
Milk yield, kg/day	46.4	46.4	46.0	46.1	46.4	1.1	0.72	0.89	0.99	0.29
Milk:DM intake	1.71	1.72	1.72	1.71	1.71	0.029	0.67	0.80	0.38	0.82
Protein, %	2.77	2.82	2.86	2.81	2.87	0.041	<0.001	0.94	0.99	0.10
Protein yield, kg/day	1.28	1.30	1.31	1.29	1.33	0.026	0.004	0.84	0.92	0.14
Fat, %	3.46	3.48	3.51	3.50	3.50	0.094	0.35	0.84	0.94	0.71
Fat yield, kg/day	1.59	1.60	1.61	1.61	1.61	0.041	0.53	0.99	0.77	0.99

[1] Probability that effects are due to random chance; values smaller than 0.05 are considered significant. Effects of methionine are for averages across both methionine sources. Source is a comparison of NTP-1401 to Smartamine averaged across both levels. Source × level determines if the effects of the two methionine sources are similar across both levels of supplementation.

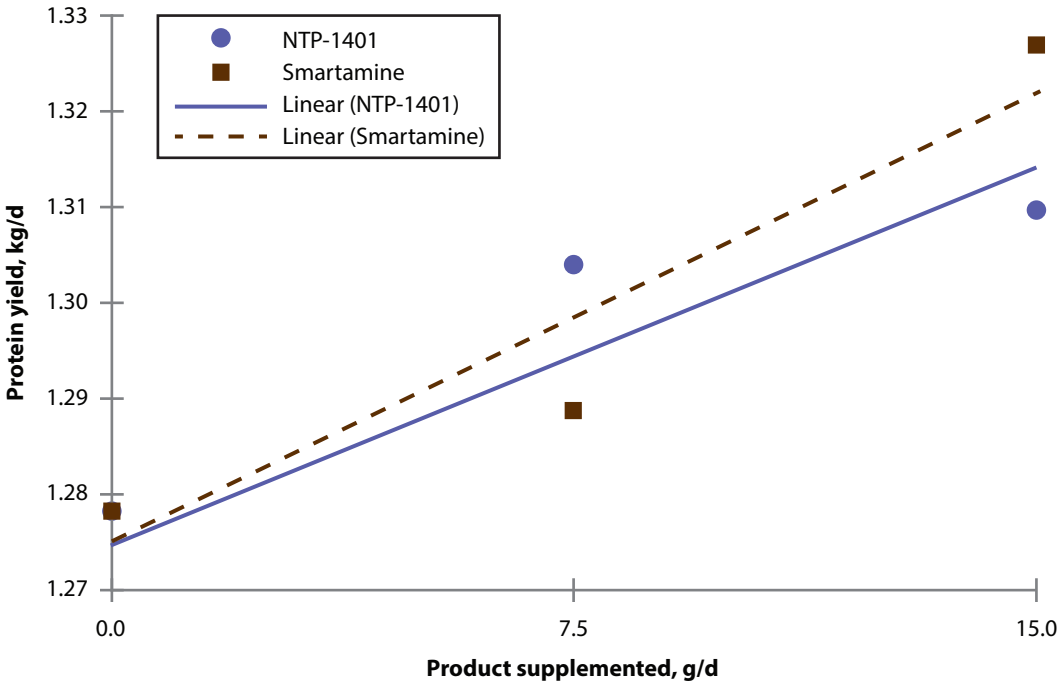

Figure 1. Linear regression of milk protein yield against supplemental methionine from NTP-1401 or Smartamine. Milk protein yield = 1.275 + 0.0026 (± 0.0010) × NTP-1401 + 0.0031 (± 0.0010) × Smartamine. The slopes of the different methionine sources were not significantly different ($P = 0.60$).

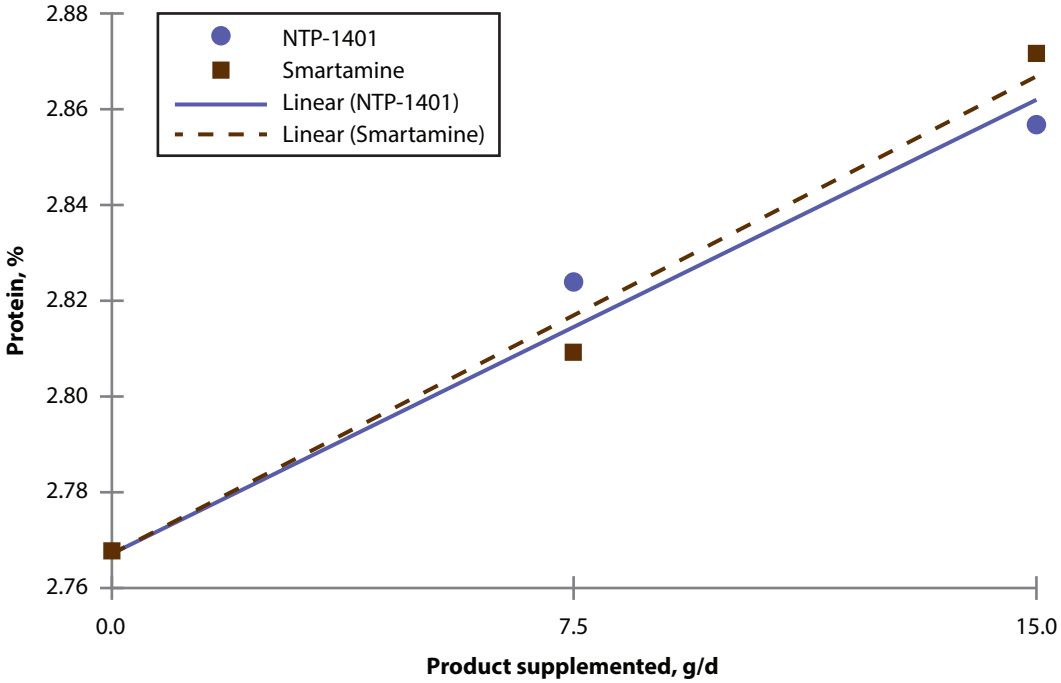

Figure 2. Linear regression of milk protein percentage against supplemental methionine from NTP-1401 or Smartamine. Milk protein percentage = 2.77 + 0.0064 (± 0.0008) × NTP-1401 + 0.0067 (± 0.0008) × Smartamine. The slopes of the different methionine sources were not significantly different ($P = 0.65$).

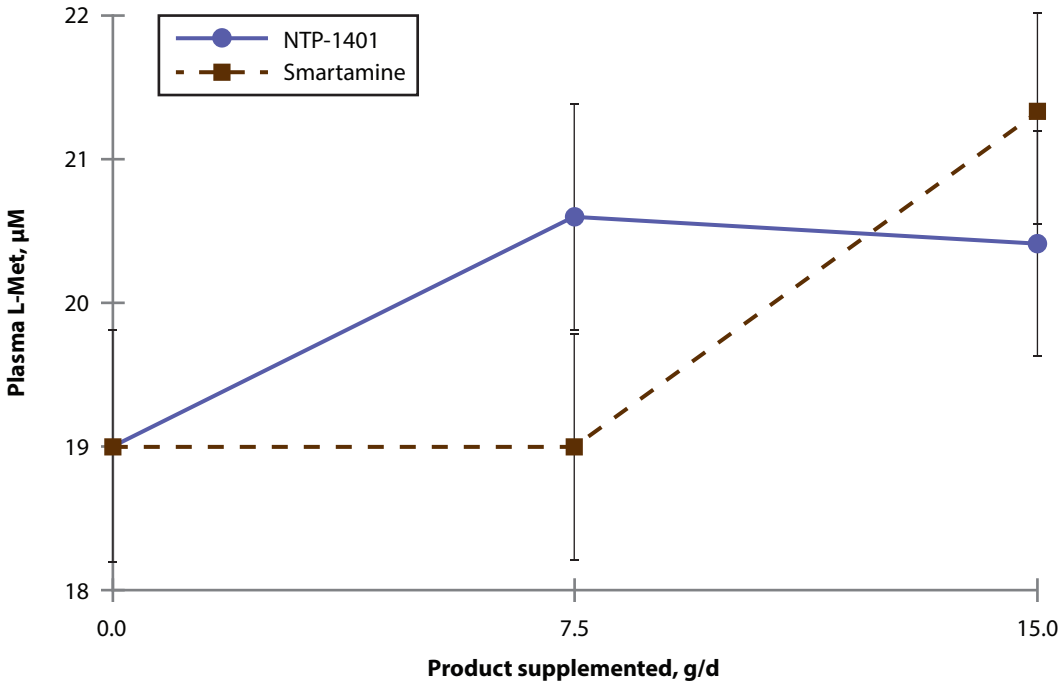

Figure 3. Effects of supplemental NTP-1401 and Smartamine on plasma concentrations of L-methionine. Linear effect of methionine supplementation ($P = 0.04$); quadratic effect of methionine supplementation ($P = 0.84$); differences between sources ($P = 0.61$); and source × level interaction ($P = 0.08$).

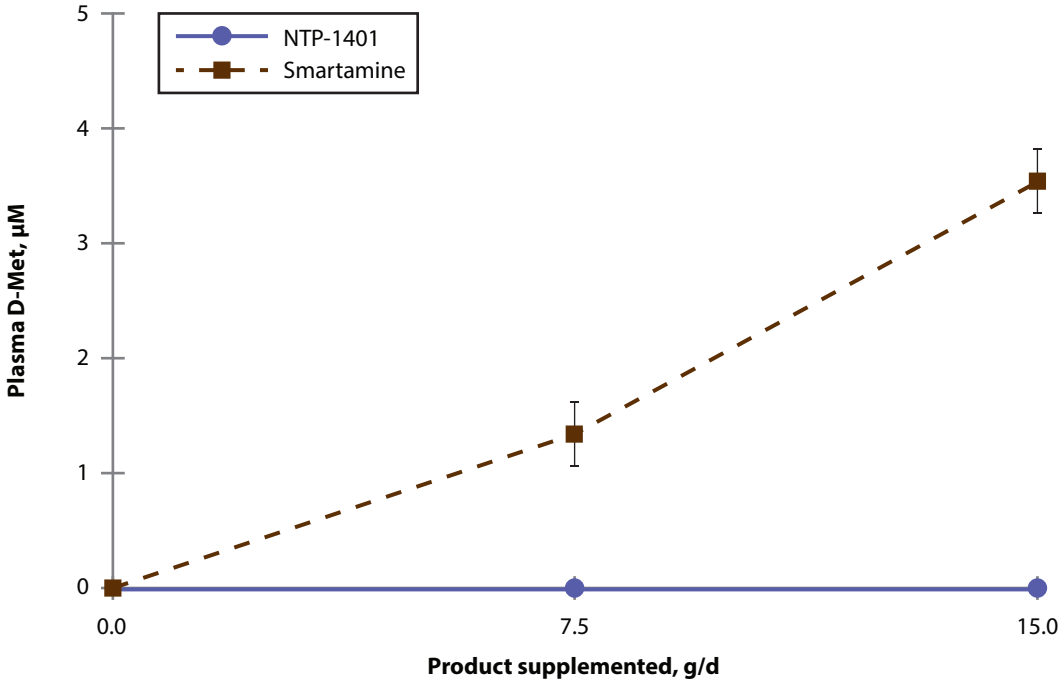

Figure 4. Effects of supplemental NTP-1401 and Smartamine on plasma concentrations of D-methionine. Linear effect of methionine supplementation ($P < 0.0001$); quadratic effect of methionine supplementation ($P = 0.44$); differences between sources ($P < 0.0001$); and source × level interaction ($P = 0.0001$).

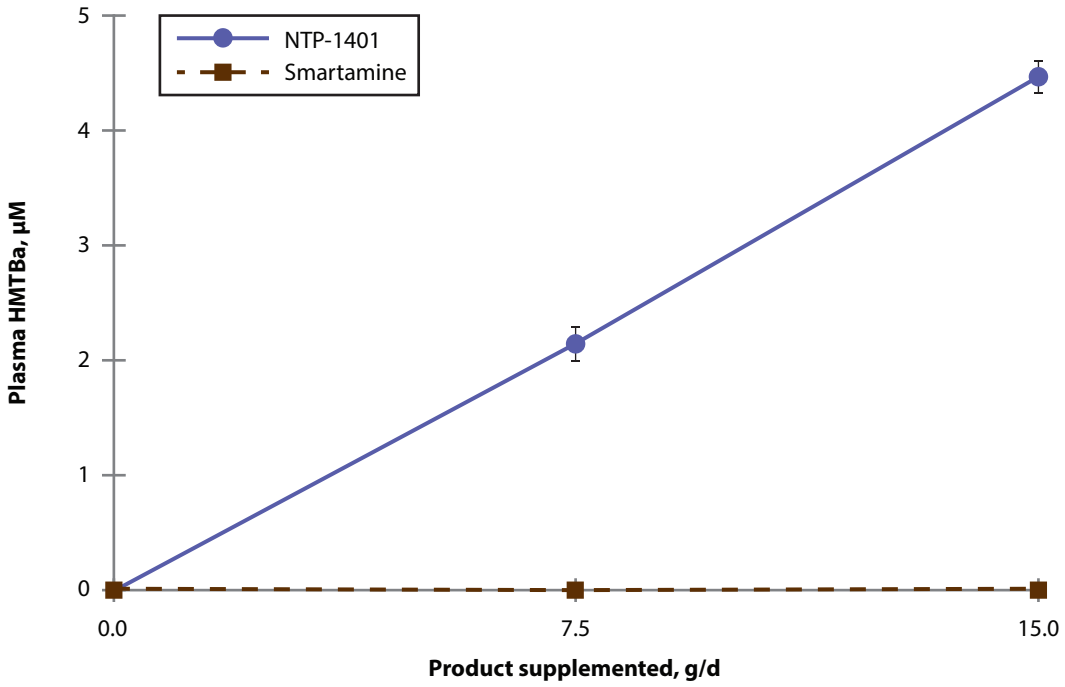

Figure 5. Effects of supplemental NTP-1401 and Smartamine on plasma concentrations of methionine hydroxy analog. Linear effect of methionine supplementation ($P < 0.0001$); quadratic effect of methionine supplementation ($P = 0.44$); differences between sources ($P < 0.0001$); and source × level interaction ($P < 0.0001$).

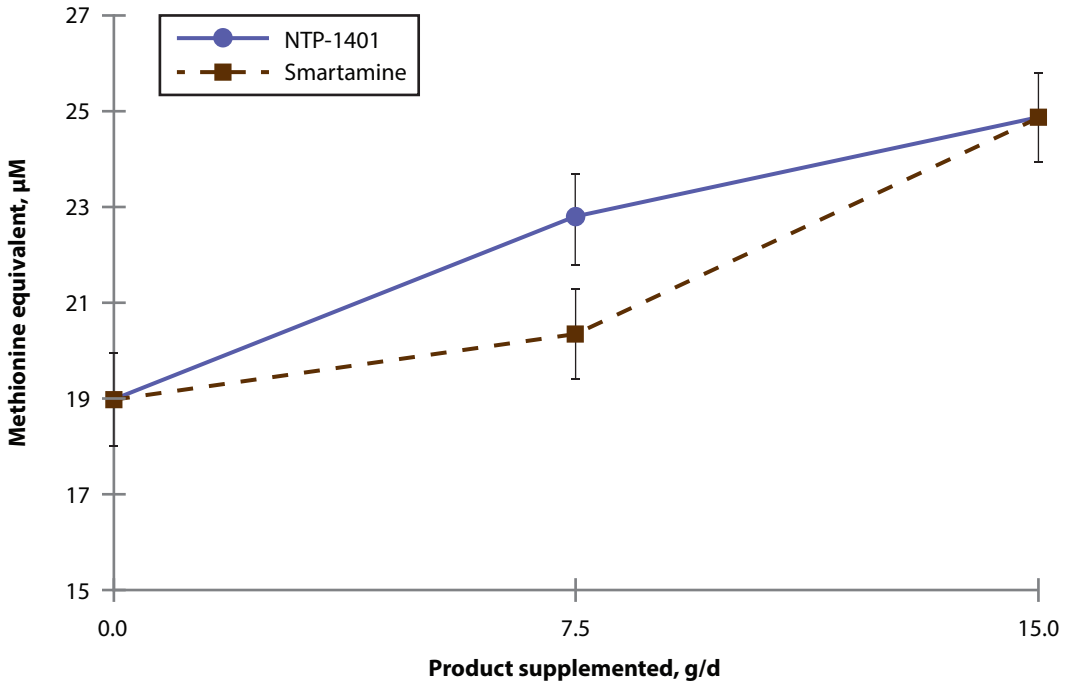

Figure 6. Effects of supplemental NTP-1401 and Smartamine on plasma concentrations of total methionine equivalents (sum of L-methionine, D-methionine, and methionine hydroxy analog). Linear effect of methionine supplementation ($P < 0.0001$); quadratic effect of methionine supplementation ($P = 0.63$); differences between sources ($P = 0.13$); and source × level interaction ($P = 0.14$).

Bioavailability of Ruminally or Abomasally Infused L-carnitine in Holstein Heifers

K. Olagaray, C. Armendariz, A. Bellamine,[1] S. Jacobs,[1] E. Titgemeyer, and B.J. Bradford

Summary

This study evaluated the relative bioavailability of carnitine delivered by different methods in dairy cattle. Four Holstein heifers were used in a split-plot design to compare ruminally or abomasally infused L-carnitine. The study included 2 main-plot periods, with infusion routes allocated in a crossover design. Within main-plot periods, each of 3 subplot periods consisted of 4-d infusions separated with 4-d rest periods. Subplot treatments were infusion of 1, 3, and 6 g L-carnitine daily. Doses were increased within a period to minimize carryover. Treatments were delivered in two 10-h infusions daily. Blood was collected before the start of infusions and on day 4 of each infusion to obtain baseline and treatment carnitine concentrations. There was a dose × route interaction ($P < 0.05$) and route effect ($P < 0.01$) for increases in plasma carnitine above baseline, with increases above baseline being greater across all dose levels when infused abomasally compared to ruminally. Results demonstrated superior bioavailability of carnitine when ruminal exposure was physically bypassed.

Key words: L-carnitine, bioavailability, dairy cow

Introduction

Fatty liver is a metabolic disease that commonly affects postpartum dairy cows. In response to negative energy balance that typically occurs in early lactation when feed intake is insufficient to meet the high energy demand of lactation, fatty acids are released from adipose tissue stores as an energy source. However, this lipid mobilization can deliver fatty acids to the liver at a rate that exceeds the organ's oxidative capacity, resulting in accumulation of liver lipids which is associated with decreased metabolic function. L-carnitine plays an essential role in the transport of long chain fatty acids from the cytosol into the mitochondria of hepatocytes. Increased transport of these fatty acids can potentially stimulate hepatic long chain fatty acid oxidation, thereby limiting lipid accumulation.

It has been clearly demonstrated that carnitine can be degraded by ruminal microbes, but the extent of ruminal degradation is unknown. Abomasal and ruminal infusions of carnitine have previously been equally effective at increasing plasma carnitine concentrations, suggesting some carnitine might escape ruminal degradation and be available for intestinal absorption. It has been suggested that degradation rate may be dependent on diet composition and the length of time animals are fed supplemental carnitine, as ruminal microbes seem to adapt to carnitine supplementation by increasing degradation rate. Previous studies have assumed up to 80% of supplemental carnitine is ruminally degraded in lactating dairy cows. The objective of this study was to evaluate the

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relative bioavailability of carnitine when administered at different sites in the rumen gastrointestinal tract at varying rates.

Experimental Procedures

Four Holstein heifers previously fitted with ruminal cannulas were used in a split-plot design to assess the relative bioavailability of ruminally or abomasally administered L-carnitine. However, one heifer was removed just prior to the end of the first treatment period due to an intestinal blockage requiring surgery. A second heifer was removed due to an infection during phase 2 of period 2, and the first heifer removed from the study replaced her at that time. The study was therefore an incomplete design. Heifers were housed in a tie-stall facility and fed a dairy ration once daily. The diet met estimated requirements for all nutrients and was supplemented with niacin (7.8 g/day niacin in the form of 12 g/day Niashure, Balchem Corp., New Hampton, NY).

The study was conducted in 2 periods, both preceded by 2 weeks without treatment to obtain baseline samples and for washout between periods. Each period had 3 phases, each consisting of 4 days of infusions at a different dose of carnitine, with 4 days between phases. The treatments were 1) ruminal infusion of carnitine at 1, 3, and 6 g carnitine/day and 2) abomasal infusion of 1, 3, and 6 g carnitine/day. Each carnitine treatment was dissolved in water and also included 6 g/day of larch arabinogalactan, and total volume infused was 4 L/day across treatments. The dosage used in each phase escalated, with phase 1 at 1 g/day, phase 2 at 3 g/day, and phase 3 at 6 g/day. The site of infusion was randomized; 2 heifers received ruminal infusions in period 1, followed by abomasal infusions in period 2, and the other heifer was treated in the opposite sequence. Daily infusions (throughout each 4-day infusion) were split into 2 equal aliquots, each infused during 10-hour infusion periods, allowing 2 hours between infusions.

Throughout the study, feed and water intake were recorded daily with the final three days of each infusion phase used for analysis. Total mixed ration samples were collected every two weeks and composited for nutrient analysis by Dairy One Forage Laboratory (Ithaca, NY; Table 1). Health was monitored daily.

Prior to the start of infusions and at 1.5 hours after initiation of the first daily infusion on day 4 of each phase, blood samples (coccygeal vein) were collected to obtain baseline and treatment carnitine concentrations. Concentrations of total carnitine in plasma were determined by an enzymatic radioisotope method.

Statistical analysis was performed using JMP (version 12, SAS Institute, Cary, NC). Dependent variables (feed intake, water intake, and change in plasma carnitine concentration) were analyzed to determine the fixed effects of route of administration, dose of carnitine, and their interaction along with the random effects of heifer and phase within period. Contrast statements were used to statistically test linear regression coefficients with increasing doses for ruminal vs. abomasal infusions, and least square means were regressed against dose for the 2 infusion routes to assess relative bioavailability. Significance was declared at $P < 0.05$ and tendencies were declared at $0.05 \leq P \leq 0.10$.

Results and Discussion

Water intake was not affected by carnitine infusion across dose or route (all $P > 0.40$; Table 2). Although not affected by infusion route ($P = 0.13$), dry matter intake (DMI) did tend to increase quadratically with carnitine dose ($P = 0.07$), being highest for the 3 g/day carnitine. The tendency for a DMI effect is likely the result of our small sample size and was largely driven by data from one heifer. Previous studies have not documented DMI responses when carnitine was infused abomasally or ruminally up to 12 g/d. When carnitine was abomasally infused at a high rate (100 g/d), DMI was decreased during the first two weeks of lactation.

Plasma carnitine concentrations are reported as the difference between baseline and treatment concentrations in Table 2. A dose × route interaction was observed ($P = 0.045$), which can largely be attributed to the linear increase in plasma carnitine concentrations with increased dose for abomasal infusion, without a significant effect for ruminal infusions. A route response was observed ($P = 0.005$) with carnitine being more bioavailable across all dose levels when infused abomasally compared to ruminally. Interestingly, increases in plasma carnitine concentrations in response to ruminal infusion appeared to plateau at 3 g/d; this could be impacted by the sequence of treatments, given that adaptation of ruminal microbes may enhance carnitine degradation after a longer period of exposure. It is also possible that L-carnitine transport from the gut reaches an upper limit at these doses. To further characterize the relative bioavailability of carnitine via these 2 routes of administration, a dose-response analysis was conducted (Figure 1). This assessment suggests that the relative bioavailability of carnitine is greater when supplied to the abomasum vs. the rumen. It should be noted that this assumes that increases in plasma concentration are directly related to the amount of carnitine absorbed.

Conclusion

Carnitine is likely degraded in the rumen, and although the extent of degradation remains unknown, our findings clearly indicate abomasal administration of carnitine results in superior bioavailability. Dietary supplementation with rumen encapsulation may be most effective to maximize carnitine delivery and absorption in the small intestine.

Table 1. Ingredient and nutritional composition of the basal diet

Item	Value
Ingredient, % of dry matter	
Alfalfa hay	21.0
Grass hay	1.7
Corn silage	16.1
Wet corn gluten feed[1]	25.7
Cotton seed	4.4
Fine rolled corn	20.4
Micronutrient premix[2]	10.7
Nutrient, % of dry matter (unless otherwise specified)	
Dry matter, % as-fed	53.5
Crude protein	17.9
Acid detergent fiber	24.75
Neutral detergent fiber	43.8
Lignin	4.55
Non-fiber carbohydrate	26.75
Starch	17.9
Crude fat	4.75
Net energy for lactation,[3] Mcal/lb	0.73

[1]Sweet Bran (Cargill Inc., Blair, NE).
[2]Premix consisted of 58.8% expeller soybean meal (SoyBest, Grain States Soya, West Point, NE), 11.8% limestone, 1.47% stock salt, 1.47% trace mineral salt, 1.47% potassium chloride, 10.3% sodium bicarbonate, 2.35% magnesium oxide. 0.23% 4-Plex (Zinpro Corp., Eden Prairie, MN), 0.12% Zinpro 100 (Zinpro Corp.), 0.25% selenium premix (0.06%), 0.15% vitamin A premix (30 kIU/g), 0.04% vitamin D premix (30 kIU/g), 1.47% vitamin E premix (48 kIU/g), 0.01% ethylenediamine dihydriodide premix, 0.06% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 1.84% XP Yeast (Diamond V, Cedar Rapids, IA), 0.92% Biotin 100 (ADM Alliance Nutrition, Quincy, IL), and 7.35% Ca salts of long-chain fatty acids (Megalac R, Arm & Hammer Animal Nutrition, Princeton, NJ).

Table 2: Effect of carnitine infusion on intake performance and plasma carnitine concentration

	Ruminal infusion (/day)			Abomasal infusion (/day)				*P* - value		
Item	1 g	3 g	6 g	1 g	3 g	6 g	pSEM[1]	Dose	Route	Dose × route
DMI, kg/d	18.01	18.76	18.87	16.84	19.01	17.43	0.97	0.07	0.13	0.35
Water intake, L/d	7.71	8.04	8.60	8.27	9.24	7.88	0.69	0.66	0.58	0.41
Plasma,[2] μM	-0.57	12.33	9.04	4.54	20.47	35.90	4.82	0.099	< 0.01	0.045

[1]Reported SEM is pooled across route and dose levels.
[2]Plasma concentrations reported are the difference between baseline and treatment concentrations.

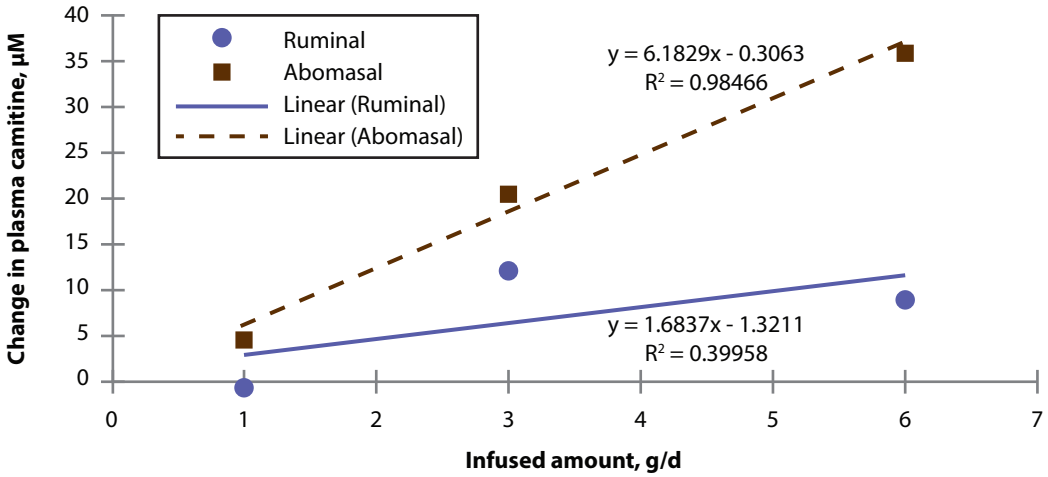

Figure 1. Marginal plasma carnitine responses to carnitine infusion differ by infusion route. Differences in plasma carnitine concentrations (post minus pre-infusion concentrations) are plotted against infusion amount. The slopes differ between infusion routes (*P* = 0.02), reflecting greater apparent bioavailability for abomasally-delivered carnitine compared to ruminal infusion.

Bioavailability of Rumen-Protected Carnitine in Lactating Dairy Cows

K. Olagaray, J. Shaffer, C. Armendariz, A. Bellamine,[1] S. Jacobs,[1] E. Titgemeyer, and B.J. Bradford

Summary

For this study, 56 lactating Holstein cows (143 ± 72 days in milk) were used in a randomized complete block design to evaluate 2 rumen-protected products compared to crystalline carnitine. Treatments were a) control, b) 3 grams/day crystalline L-carnitine (raw), c) 6 grams/day raw, d) 5 grams/day 40COAT (40% coating, 60% L-carnitine), e) 10 grams/day 40COAT, f) 7.5 grams/day 60COAT (60% coating, 40% L-carnitine), and g) 15 grams/day 60COAT. Treatments were top-dressed to diets twice daily. The 14-day experiment included a 6-day baseline-measurement period with the final 2 days used for data and sample collection and an 8-day treatment period with the final 2 days used for data and sample collection. Plasma, urine, and milk samples were analyzed for L-carnitine. Crystalline ($P < 0.001$) and 40COAT ($P = 0.01$) linearly increased plasma L-carnitine, and 60COAT tended to linearly increase plasma L-carnitine ($P = 0.08$). Total daily excretion (milk + urine) of L-carnitine averaged 1.52 ± 0.04 grams in controls, increased linearly with crystalline and 40COAT, and increased quadratically with 60COAT (all $P < 0.05$). Crystalline increased plasma L-carnitine and milk + urine L-carnitine more than 40COAT and 60COAT (all $P < 0.05$). Carnitine supplementation increased carnitine concentrations in plasma, milk, and urine; however, the rumen protection did not provide additional increases in concentration.

Key words: L-carnitine, bioavailability, dairy cow

Introduction

Fatty liver is a common metabolic disease that affects postpartum dairy cows. Depressed feed intake and increased energy demands of lactation lead to negative energy balance that stimulates fat mobilization, often in excess of the liver's oxidation capacity, causing liver lipid accumulation. L-carnitine stimulates hepatic fatty acid oxidation through increased transport of long chain fatty acids from the cytosol to mitochondria, and has been observed to decrease liver triglyceride accumulation during the transition period. L-carnitine is commonly degraded in the rumen, thus affecting its intestinal availability. An *in vitro* study estimated that 80% of dietary carnitine was degraded in rumen fluid after microbial adaptation. Studies implementing abomasal infusions have observed linear increases in urine, milk, plasma, and liver concentrations in response to infusions up to 6 grams/day. Rumen-protected products are intended to prevent degradation in the rumen and increase the amount reaching the small intestine for absorption. In this experiment, two rumen-protected carnitine products were supplemented in the diets of mid-lactation Holstein cows and the L-carnitine concentrations in milk, urine, and plasma were determined to assess their relative bioavailability. Production responses including milk yield, milk components, and feed intake were also determined.

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Experimental Procedures

For this study, 56 mid-lactation Holstein cows (143 ± 72 days in milk) from the Kansas State University Dairy Teaching and Research Center were used in a randomized complete block design to determine the relative bioavailability of 2 rumen-protected carnitine products compared to crystalline (raw) carnitine. Cows were blocked by parity and level of milk production and then randomly assigned to 1 of 7 treatments within the block (8 cows per treatment). Cows were housed in a tie-stall facility and adapted for 4 days prior to 2 days of sample collection for baseline values. Following the 6-day baseline period, treatments were applied for a total of 8 days, with the final 2 days used for data and sample collection. The study was performed in 2 cohorts of cows.

Cows were milked 3 times daily at 0400, 1000, and 1800 h. The basal diet met estimated requirements for all nutrients and was fed as a total mixed ration twice daily (0600 and 1800 h). Animals had ad libitum access to feed in individual mangers and feed offered was adjusted daily to achieve 12-20% refusals. During the treatment period, the basal diet was top-dressed twice daily with the following treatments: a) control (no supplement); b) 3 grams raw carnitine; c) 6 grams raw carnitine; d) 5 grams carnitine with protection 1 (40% coating, 60% L-carnitine content); e) 10 grams carnitine with protection 1 (40% coating); f) 7.5 grams carnitine with protection 2 (60% coating, 40% L-carnitine coating); and g) 15 grams carnitine with protection 2 (60% coating). Supplementation rates were designed to provide 3 or 5 grams/day carnitine, regardless of the protection method.

During the 2-day collection periods, feed and water intake as well as milk yield were recorded. Ration samples were collected on each day of both baseline and treatment collection periods and composited for nutrient analysis by Dairy One Forage Laboratory (Ithaca, NY; Table 1). Health was monitored daily and one cow (7.5 grams of 60% coating) was removed from the study due to illness detected by a rapid decline in dry matter intake (DMI).

Over the course of the 48-hour collection period, urine and blood samples (coccygeal vein) were collected immediately prior to the initial feeding (2000 h), and 6, 12, and 18 hours after feeding. Blood samples were collected into K_3EDTA tubes and immediately placed on ice. Plasma was separated by centrifugation (1,500 × *g* for 15 minutes) and stored in microcentrifuge tubes at -20°C. Throughout the collection period, urine samples were composited by equal volumes into microcentrifuge tubes and frozen at -20°C until analysis of total carnitine and creatinine. Urine creatinine concentration and expected creatinine excretion of 29 mg/kg of BW daily was used to estimate daily urine volume. Two milk samples were collected at all 6 milkings during the 2-day collection periods, one used for milk component analysis by Heart of America Dairy Herd Improvement Association (Manhattan, KS) and the other frozen until carnitine analysis. Prior to analyses, the milk samples were composited in equal volumes by collection period. Concentrations of total carnitine in plasma, milk, and urine were determined by an enzymatic radioisotope method.

Statistical analysis was performed using SAS (version 9.3, SAS Inst., Cary, NC). The mixed procedure was used to model treatment response variables using the covariate for the same variable from the basal period, the fixed effects of treatment and parity, and

the random effect of block. Responses were assessed with 7 contrasts that assessed the linear and quadratic responses to raw, 40% coating, and 60% coating carnitine treatments as well as overall contrasts between raw and 40% coating, raw and 60% coating, and 40% vs. 60% coating treatments. Significance was declared at $P < 0.05$ and tendencies were declared at $0.05 \leq P \leq 0.10$.

Results and Discussion

Production responses are summarized in Table 2. The 60% coating product linearly decreased DMI ($P = 0.02$) and raw carnitine tended to linearly decrease DMI ($P = 0.07$). The 60% coating product had a quadratic effect on milk fat percent ($P = 0.04$) and a tendency for a quadratic effect on milk yield ($P = 0.07$). Supplementation of raw carnitine had a quadratic effect on milk protein percent ($P = 0.04$). The 60% coating product tended to decrease milk protein percent ($P = 0.10$) and increase milk lactose percent ($P = 0.08$) compared to the 40% coating product. There were no treatment effects on milk yield, milk urea nitrogen, or yields of milk fat, protein, and lactose. This lack of response on milk yield and composition is consistent with the 6 g/day of abomasally infused raw carnitine found in a previous study.

Overall, there were no parity effects on plasma, milk, and urine carnitine concentrations, and only a tendency for a parity effect on total daily carnitine excretion ($P = 0.10$). Plasma samples were collected at 0, 6, 12, and 18 hours after feeding to assess diurnal variation; however, there was no effect of time on plasma carnitine concentrations ($P = 0.23$). Supplementation with raw carnitine or the 40% coating product increased plasma carnitine concentrations linearly ($P < 0.001$ and $P = 0.01$, respectively) whereas supplementation with the 60% coating product tended to linearly increase plasma carnitine ($P = 0.08$). Raw carnitine increased plasma carnitine compared to both the 40% coating product ($P = 0.03$) and the 60% coating product ($P < 0.001$). Urine carnitine concentrations also increased linearly with the raw ($P = 0.03$) and 40% coating products ($P = 0.02$). Effects on milk carnitine concentrations were more numerous, with linear effects across all sources, a quadratic tendency for the 40% coating product ($P = 0.08$). Raw carnitine increased milk carnitine concentration compared to the 60% coating product ($P < 0.01$) and tended to increased milk carnitine compared to the 40% coating product ($P = 0.08$). These effects were mirrored in daily milk carnitine output, with linear effects seen for all forms of supplementation, a significant difference between the 60% coating product and raw carnitine ($P < 0.01$), and a tendency for a difference between the 40% coating product and the raw carnitine ($P = 0.08$).

Conclusion

Carnitine supplementation in the forms of raw carnitine and the 40% coating product were effective in linearly increasing carnitine concentrations. The subtle responses seen for the 60% coating product, which were significantly lower than that for raw carnitine in several metrics, may have been due to over-encapsulation that hindered liberation of the carnitine and its absorption in the small intestine. Effective ruminal protection of L-carnitine while maintaining intestinal availability needs further investigation.

Table 1. Ingredient and nutritional composition of the basal diet

Item	Value
Ingredient, % of dry matter	
Corn silage	35.0
Alfalfa hay	14.2
Wet corn gluten feed[1]	27.3
Cotton seed	2.7
Fine-rolled corn	13.7
Micronutrient premix[2]	7.0
Nutrient, % of dry matter (unless otherwise specified)	
Dry matter, % as-fed	49.9
Crude protein	17.5
Acid detergent fiber	23.3
Neutral detergent fiber	36.3
Lignin	4.1
Non-fiber carbohydrate	33.0
Starch	16.2
Crude fat	4.9
Net energy for lactation, Mcal/lb	0.75

[1]Sweet Bran (Cargill Inc., Blair, NE).

[2]Premix consisted of 54.6% expeller soybean meal (SoyBest, Grain States Soya, West Point, NE), 14.8% limestone, 2.34% stock salt, 1.56 trace mineral salt, 0.16% potassium chloride, 10.9% sodium bicarbonate, 2.49% magnesium oxide, 0.24% 4-Plex (Zinpro Corp., Eden Prairie, MN), 0.12% Zinpro 100 (Zinpro Corp.), 0.26% selenium premix (0.06%), 0.16% vitamin A premix (30 kIU/g), 0.05% vitamin D premix (30 kIU/g), 1.56% vitamin E premix (48 kIU/g), 0.01% ethylenediamine dihydriodide premix, 0.07% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 1.95% XP Yeast (Diamond V, Cedar Rapids, IA), 0.97% Biotin 100 (ADM Alliance Nutrition, Quincy, IL), and 7.8% Ca salts of long-chain fatty acids (Megalac R, Arm & Hammer Animal Nutrition, Princeton, NJ).

Table 2. Effect of carnitine supplementation on performance and milk production parameters

		Raw		40 coat		60 coat		
Item	Control	3g	6g	5g	10g	7.5g	15g	SEM[1]
DMI,[2] lb/day	60.0	59.4	57.7	59.2	58.1	57.9	56.8	2.0
Water intake,[3] liters/day	128.2	131.4	128.4	136.8	120.6	125.8	125.9	5.6
Milk, lb/day	98.8	99.2	98.6	98.6	99.4	94.8	97.5	2.4
Milk fat,[4] %	3.66	3.53	3.47	3.50	3.47	3.31	3.48	0.11
Milk protein,[5] %	2.86	2.91	2.84	2.90	2.88	2.86	2.85	0.04
Milk lactose, %	4.92	4.94	4.92	4.92	4.89	4.95	4.95	0.03
Milk somatic cell linear score	1.61	1.46	1.96	1.22	1.53	2.01	1.21	0.48
Milk urea nitrogen, mg/dL	13.34	13.41	13.33	13.04	13.10	13.10	13.35	0.30
Milk fat, lb/day	3.62	3.42	3.44	3.51	3.35	3.15	3.42	0.15
Milk protein, lb/day	2.82	2.89	2.78	2.87	2.87	2.71	2.78	0.09
Milk lactose, lb/day	4.85	4.90	4.85	4.85	4.85	4.67	4.83	0.13

[1]Reported SEM is pooled across treatment groups.
[2]Linear effect of 60% coating product ($P < 0.05$).
[3]Quadratic effect of 40% coating product ($P < 0.05$).
[4]Quadratic effect of 60% coating product ($P < 0.05$).
[5]Quadratic effect of raw carnitine ($P < 0.05$).

Table 3. Least squares means for concentrations of L-carnitine in plasma, milk, and urine from mid-lactation Holstein cows fed different amounts and sources of L-carnitine

		Raw		40 coat		60 coat		
Item	Control	3g	6g	5g	10g	7.5g	15g	SEM[1]
Plasma, μM[2,3,6,7]	8.59	9.80	12.17	9.36	10.46	8.62	9.77	0.47
Milk								
μM[2,3,4,7]	137.5	166.4	174.3	145.6	176.1	143.2	161.8	5.31
g/day[2,3,4,7]	0.97	1.17	1.22	1.05	1.22	0.99	1.15	0.03
Urine								
μM[2,3]	9.63	10.37	11.47	10.02	11.63	9.93	10.74	0.62
g/day[2,3,4,7]	0.557	0.617	0.701	0.587	0.644	0.557	0.629	0.03
Total excreted carnitine,[8] g/day[2-7]	1.52	1.78	1.92	1.62	1.87	1.54	1.79	0.04

[1] Reported SEM is pooled across treatment groups.
[2]Linear effect of raw carnitine ($P < 0.05$).
[3]Linear effect of 40% coating product ($P < 0.05$).
[4]Linear effect of 60% coating product ($P < 0.05$).
[5]Quadratic effect of 60% coating product ($P < 0.05$).
[6]40% coating product vs. raw carnitine ($P < 0.05$).
[7]60% coating product vs. raw carnitine ($P < 0.05$).
[8]Milk plus urine carnitine.

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