Kansas State University
Agricultural Experiment Station
and Cooperative Extension Service

DAIRY DAY 2004

Report of Progress 941

FOREWORD

Members of the Dairy Commodity Group of the Department of Animal Sciences and Industry are pleased to present this Report of Progress, 2004. Dairying continues to be a viable business and contributes significantly to the agricultural economy of Kansas. In 2003, dairy farms accounted for 2.8%, or $252 million, of all Kansas farm receipts, ranking 6th overall among all Kansas farm commodities. Annual milk production per cow increased 9.6% from 2001 (17,312 lb) to 2002 (18,972 lb), moving Kansas from #22 ranking in 2001 to #9 in ranking in 2002 for all the 50 of the United States. At the end of 2003, Kansas ranked #11 (19,054 lb), just 10 lb out of the #10 ranking. Wide variation exists in the productivity per cow, as indicated by the production testing program (Heart of America Dairy Herd Improvement Association [DHIA]). Almost 115,000 cows were enrolled in the DHI program from Kansas, Nebraska, Oklahoma, Arkansas, North Dakota, and South Dakota (including herds from Colorado and Missouri) beginning January 1, 2004. A comparison of Kansas DHIA cows with all those in the Heart of America DHIA program for the year 2003 is illustrated in the table below.

Comparison of Heart of America Cows with Kansas Cows - 2003

Item	HOA	KS
No. of herds	828	268
No. of cows/herd	139	139
Milk, lb	18,797	18,962
Fat, lb	695	702
Protein, lb	583	589
SCC × 1,000	395	407
Calving interval, mo.	14.4	14.6

Most of this success occurs because of better management of what is measured in monthly DHI records. Continued emphasis should be placed on furthering the DHI program and encouraging use of its records in making management decisions. In addition, use of superior, proven sires in artificial-insemination (AI) programs shows average predicted transmitting ability (PTA) for milk of all 322 Holstein AI bulls in service (August, 2004) to be +1,636 lb (range of +227 to +3,209 lb). Emphasis on use of superior genetics through more use of AI sires is warranted.

The excellent functioning of the Dairy Teaching and Research Center (DTRC) is due to the special dedication of our staff. It has served us well since 1977. Our milk production with 200 cows has improved considerably according to our last test day in September (82 lb). Our rolling herd average for milk was 29,453 lb, with 1,085 lb of fat, and 909 lb of protein.

We acknowledge our current DTRC staff for their dedication: Michael V. Scheffel (Manager), Donald L. Thiemann, Daniel J. Umsheid, William P. Jackson, Glen Farrell, Kevin Good, Allen Hubbard, Robert Fiest, and Julie Carden. Special thanks to Irene Vanderwerff and Cheryl K. Armendariz and a host of graduate and undergraduate students for their technical assistance in our laboratories and at the DTRC.

Each dollar spent for research yields a 30 to 50% return in practical application. Research is not only tedious and painstakingly slow but expensive. 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 (more details about the LMIC are found at the end of this publication).

J. S. Stevenson, Editor
2004 Dairy Day Report of Progress

CONTENTS

Dairy Day 2004

QUANTIFICATION OF VOLATILE FLAVOR COMPOUNDS IN OFF-FLAVOR AND COMMERCIAL REDUCED-FAT MILK SAMPLES

L. L. Francis, J. Lee, D. H. Chambers, I. J. Jeon, S. R. Simmons, and K. A. Schmidt

Summary

Various chemical compounds contribute to the naturally pleasant flavor of milk. Over time, however, and with unwanted chemical reactions, loss of flavor is inevitable. This study was conducted to identify and quantify volatile flavor compounds associated with off-flavored and commercial reduced-fat milk products. Fresh milk was used for the preparation of altered milk samples having off-flavors such as "light-oxidized" and "high-acid." Milk lacking freshness (i.e., milk produced two weeks before sampling and maintained at 40°F in the dark) also was compared with fresh unaltered milk and two commercial milk samples. For headspace analysis, milk samples were subjected to SPME-GC for volatile compound identification. In addition, the composition and aerobic and coliform microbial counts for all milk samples were analyzed. The milk samples did not differ in the concentrations of volatile flavor constituents. When comparing "light-oxidized" milk samples (200 lx exposure for 1 or 3 hr), 2-butanone and pentanal concentrations tended to increase as light exposure time increased. All milk samples had similar fat and total solids contents. "High-acid" milk had a greater total aerobic microbe count than the other milk samples. Fresh milk had a greater octanal concentration than the off-flavored reduced-fat milk samples did. This might indicate that octanal is an important contributor to fresh milk flavor and deserves further study.

(Key Words: Milk, Flavor, Compounds, GC Analysis.)

Introduction

Nutrition and flavor are the main reasons people consume milk and find milk acceptable. Although survey results show that milk consumption is increasing, children's milk consumption has dropped by 30% in the past 30 years. The beverages that children are preferentially consuming include soft drinks, juice, and other fruit drinks. Nutritionists indicate that, for adults, diets without milk and other dairy products generally are deficient in calcium. The flavor of milk is typically bland and, therefore, susceptible to flavor changes caused by enzymes, microbial contamination, and other catalysts. Off flavors found in commercial milk products include "high-acid," "light-oxidized," and "lacks freshness." Chemical compounds associated with these off flavors include: lactic acid for "high-acid"; heptanal, 2-heptanone, hexanal, nonanal, octanal, pentanal, and propanal for "light-oxidized"; and dimethyl sulfide, 1-butanol, 2-heptanone, hexanal, and pentanal for "lacks freshness." Hundreds of chemical compounds and their combinations are responsible for milk flavor perceived by consumers. Thus, the objectives of this study were to identify and quantify volatile flavor compounds associated with off-flavored and commercial reduced-fat milk products.

Experimental Procedures

Reduced-fat milk (3.776 L) was obtained within 2 d of production from the Kansas State University dairy plant, Manhattan, Kansas, during November and December of 2003. Milk samples were prepared to contain various off flavors, such as "light-oxidized" and "high-acid" as described in Table 1. A "lacks freshness" milk sample (Kansas State University dairy plant), two commercial milk samples (local supermarket brand purchase (Manhattan, KS), and school lunch milk packaged in a 250-mL paperboard carton) were included.

Milk samples were analyzed for headspace volatile compounds, total solids, fat content, total plate count, and apparent viscosity. For solid-phase microextraction (SPME) analysis, 75-µm Carboxen-PDMS fiber sampling at 140°F for 30 min was used to collect volatile headspace compounds from the milk. These compounds were subsequently quantified by gas chromatography, flame ion detection (GC-FID). For total solids, an atmospheric oven method was used. Fat was measured by the Babcock method and total aerobic and coliform microbial counts were done by standardized methods. Apparent viscosity was determined at a frequency of 2 Hz within a shear rate range 1 to 40 second^{-1} by using a rheometer at 40°F. Apparent viscosity values were compared at 10 second^{-1}.

An incomplete-block design was used for this experiment because sampling restrictions existed. Only four milk samples were compared per day. On one day, fresh unaltered milk, as well as the three induced off-flavored (light-oxidized at 1 hr, light-oxidized at 3 hr, and high-acid) milk samples were analyzed. On the other day, fresh unaltered milk, two commercial milk samples, and a "lacks freshness" milk sample were analyzed. The entire experiment was replicated twice during November-December, 2003. Data were subjected to analysis of variance, and significant differences among means were detected by Tukey's pair-wise comparison at a significance level of $\alpha = 0.05$.

Results and Discussion

Table 2 displays the results of the chemical and microbial analyses conducted on the seven milk samples. No significant differences were detected for fat or total-solids contents among the seven milk samples. Fat content ranged from 1.8 to 2.1%, and total solids ranged from 10.4 to 11.4%.

Total aerobic microbe counts were different ($P<0.05$) among samples. The "high-acid" milk had greater counts than the other milk samples did (Table 2). These results indicated that, despite different raw-milk sources, milk-distribution systems, and age of milk, six of the seven milk samples had similar aerobic microbe counts.

All coliform microbe counts were <1 CFU/mL for the seven milk samples. Apparent-viscosity results indicated that the various milk samples differed with respect to viscosity (range of 3.35 to 3.57 mPa·s). Mean differences are shown in Table 2; no apparent overall trend was observed.

Ten volatile flavor compounds (benzaldehyde, 2-butanone, ethyl caproate, heptanal, 2-heptanone, hexanal, octanal, 1-octen-3-ol, pentanal, and 2-pentylfuran) were quantified in all seven milk samples. Figure 1 shows the concentrations of indi-

vidual compounds in fresh unaltered milk, compared with those in the various altered milk samples. Figure 2 shows the concentrations of the individual compounds for fresh unaltered milk compared with those in the commercial and "lacks freshness" milks. Overall, mean compound concentrations ranged from 0 mg/kg (1-octen-3-ol in fresh unaltered, "light-oxidized" 1 hour and 3 hours, and commercial milk samples) to 1.962 mg/kg (2-butanone in "high-acid" milk). All ten of these compounds have been reported to contribute to milk flavor. Ranges for these compounds in the milk samples are shown in Table 3. The highest concentrations were associated with the "high-acid," commercial 1, or commercial 2 milk samples.

There are several possible reasons why no differences were observed for any of the ten compounds measured in the seven milk samples. Sampling constraints that included two commercial samples, as well as a milk sample close to its shelf-removal date, resulted in greater variation among samples, which, in turn, decreased the chances of detecting differences. Also, milk samples that were made to have "slight" to "definite" intensity changes seemed to have made fewer differences than initially hypothesized.

Stored milk has greater concentrations of heptanal, hexanal, and pentanal than fresh, non-aged milk does. We observed a similar trend in which the "lacks freshness" (14 days old) milk sample had slightly greater concentrations of heptanal, hexanal, and pentanal than the fresh unaltered milk did (2-days old; Figure 2). As a result, the rate of flavor change may not have been as quick in our study as in previously reported studies. Maintenance of a constant temperature or minimal light exposure, might have slowed the reaction rates. Greater heptanal, hexanal, and pentanal concentrations have been reported in "light-oxidized" milk compared with those in fresh, unaltered milk, but the induced light-oxidation conditions in those studies included long exposure times (18 hours) or unknown light intensities.

All seven milk samples were evaluated simultaneously by a trained descriptive panel for flavor characteristics. Panel results indicated that no differences existed for the "light-oxidized" trait among the fresh unaltered milk, milk "light-oxidized" for 1 hour, and milk "light-oxidized" for 3 hours, which agrees with our data for volatile compound concentration for these three samples. The two commercial and high-acid milk samples were rated as having some of the highest flavor-intensity scores. It is interesting to note that the greatest concentration of all of the ten compounds was associated with one of these three milk samples. This may suggest that a "good" milk flavor may be an optimum blend of specific compounds of this type.

Purposely altering milk samples did not significantly affect chemical compound concentration, nor did a trained panel detect differences among the purposely altered "light-oxidized" and the fresh, unaltered milk samples. Either the two commercial milk samples or the "high-acid" milk samples had the greatest concentration of the ten identified volatile compounds. This may suggest that high-quality milk flavor may be an optimal sum of a variety of compounds. Further work is needed to determine the importance of octanal concentration, as well as its relationship to other compounds that may contribute and influence fresh milk flavor.

Table 1. Milk Samples: Source and Preparation

Milk sample	Treatment	Holding time
Fresh unaltered[1]	None	None
High-acid[1]	Remove 100 mL reduced fat milk. Add 52 mL cultured lowfat buttermilk[2]	None
Lacks freshness[1]	None	14 d at 40°F
Light-oxidized at 1 h[1]	200 lx[3]	1 h at 40°F
Light-oxidized at 3 h[1]	200 lx[3]	3 h at 40°F
Commercial 1[4]	None	None
Commercial 2[5]	None	None

[1]Reduced-fat milk (3.776 L), plastic container (Kansas State Plastics, Inc., Hutchinson, KS).
[2]Hiland Dairy Co. (Springfield, MO).
[3]GE fluorescent light 3500K 15W, Sylvania, Inc. (Danvers, MA).
[4]Reduced-fat milk (3.776 L), plastic container, purchased at a supermarket in Manhattan, Kansas.
[5]Reduced-fat milk (250 mL), paperboard carton for school lunch program, donated by manufacturer.

Table 2. Milk-fat Contents, Total Solids Contents, Total Plate Counts, and Apparent Viscosities of Reduced-fat Milk Samples from Several Sources or Induced Off-flavors

Milk	Milk fat (%)	Total solids (%)	TPC[1] (log CFU/ml)	Apparent viscosity (mPa·s)
Fresh unaltered[2]	1.95 ± 0.04^{a}	11.36 ± 0.21^{a}	0.99 ± 1.08^{b}	$3.41 \pm 0.05^{b,c}$
High-acid[3]	2.05 ± 0.06^{a}	10.98 ± 0.29^{a}	6.67 ± 1.20^{a}	$3.35 \pm 0.05^{b,c}$
Light-oxidized 1 hr[3]	1.90 ± 0.06^{a}	10.99 ± 0.29^{a}	1.00 ± 1.20^{b}	$3.44 \pm 0.05^{a,b,c}$
Light-oxidized 3 hr[3]	1.95 ± 0.06^{a}	10.97 ± 0.29^{a}	0.98 ± 1.20^{b}	$3.36 \pm 0.05^{b,c}$
Commercial 1[3]	1.90 ± 0.06^{a}	11.06 ± 0.29^{a}	1.41 ± 1.20^{b}	3.57 ± 0.05^{a}
Commercial 2[3]	1.80 ± 0.06^{a}	10.73 ± 0.29^{a}	1.60 ± 1.20^{b}	$3.50 \pm 0.05^{a,b}$
Lacks freshness[3]	2.00 ± 0.06^{a}	10.43 ± 0.29^{a}	1.39 ± 1.20^{b}	$3.51 \pm 0.05^{a,b}$

[abc]Means within column having different superscript letters differ ($P<0.05$).
[1]TPC (Total Plate Count) = total aerobic microbial count.
[2]n = 4.
[3]n = 2.

Table 3. Range in Volatile Compound Concentrations and the Associated Milk Sample

Compound	Low conc., mg/kg	Milk sample	High conc., mg/kg	Milk sample
Benzaldehyde	0.878	Lacks freshness	0.923	High-acid
2-Butanone	0.503	Commercial 2	1.962	High-acid
Ethyl caproate	0.344	Light-oxidized 1 hour	0.775	Commercial 2
Heptanal	0.727	Fresh unaltered, Light-oxidized 1 hour, Light-oxidized 3 hours	0.737	Commercial 2
2-Heptanone	0.451	Fresh unaltered, Light-oxidized 1 hour, Light-oxidized 3 hours, Commercial 2	0.458	Commercial 1
Hexanal	0.423	Commercial 1	0.476	Commercial 2
Octanal	0.445	Light-oxidized 1 hour	1.062	Commercial 2
1-Octen-3-ol	0	Fresh unaltered, Light-oxidized 1 hour, Light-oxidized 3 hours, Commercial 1, Commercial 2	0.297	High-acid
Pentanal	0.456	High-acid	0.992	Commercial 1
2-Pentylfuran	0.478	Light-oxidized 1 hour	0.998	Commercial 1

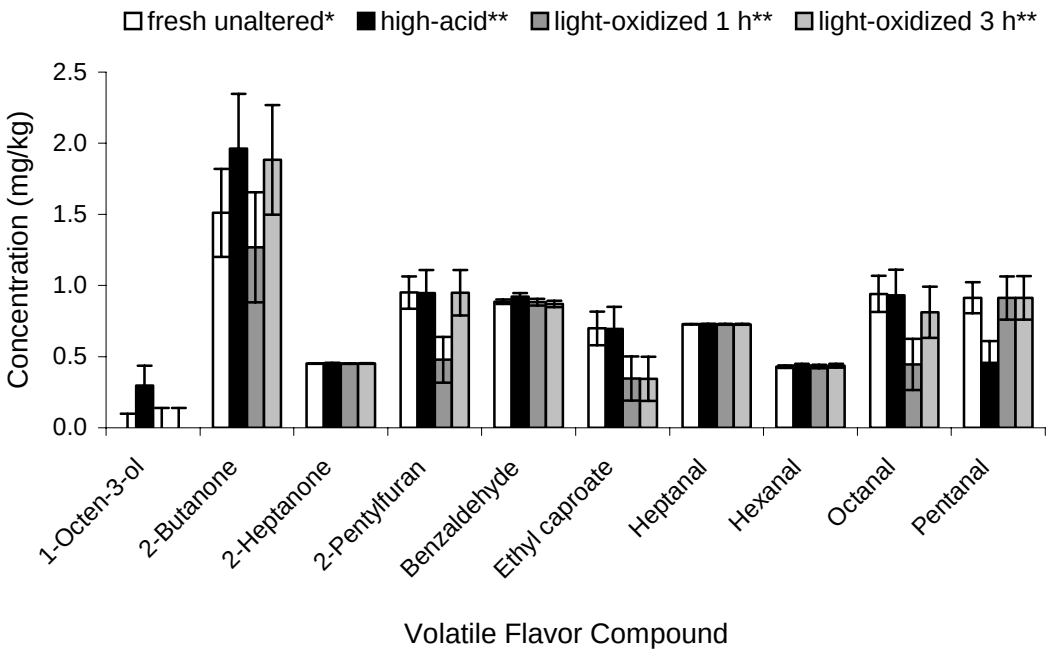

Figure 1. Mean Concentrations (mg/kg) of Volatile Flavor Compounds in Fresh Unaltered and Altered Milk Samples.

*n = 4.

**n = 2.

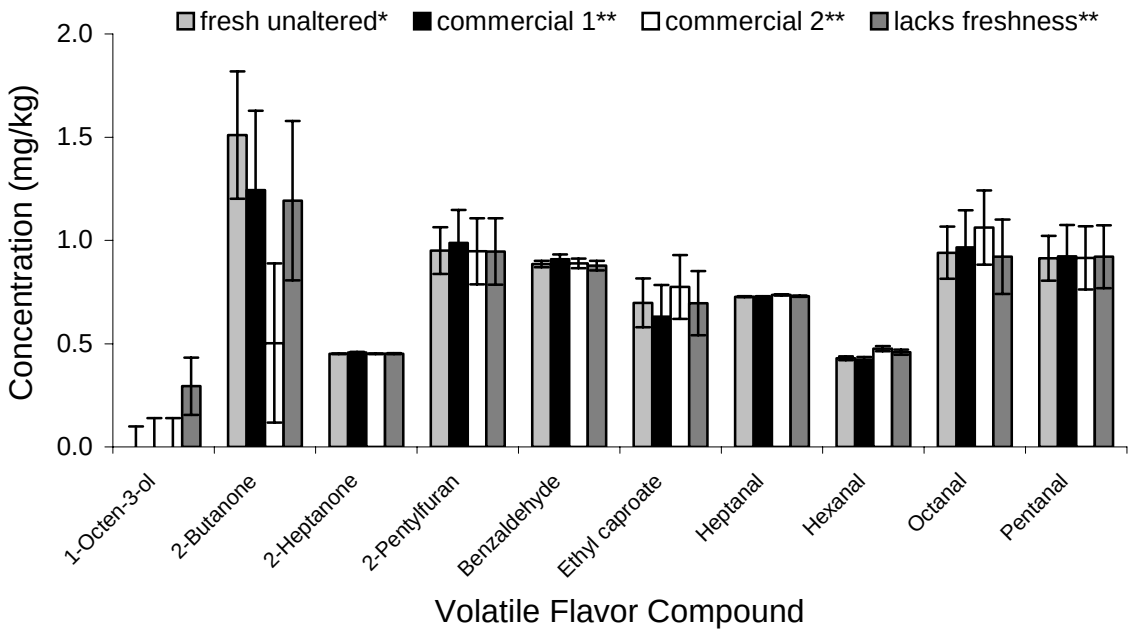

Figure 2. Mean Concentrations (mg/kg) for Volatile Compounds in Fresh Unaltered, Commercial, and Lacks Freshness Milk Samples.
*n = 4.
**n = 2.

BETA-CYCLODEXTRIN COMPLEXING TO REDUCE ANTIBIOTIC RESIDUE IN MILK

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Summary

Various percentages (1.2% to 13%) of β-cyclodextrin (β–CD) were added to water or pasteurized whole milk to study β-CD crystallization patterns. Influential factors such as crystallization time (4 to 12 h), crystallization temperature (45° vs. 72ºF), and centrifugation speed (25 to 3000 × *g*) were investigated. Optimized crystallization conditions were verified in antibiotic-tainted raw milk samples via the enzyme-linked receptor-binding assay and by solids partitioning. In water, β-CD precipitate increased significantly as β-CD concentration and crystallization time increased, but was independent of the centrifugation speed. In pasteurized whole milk, precipitate increased as β-CD concentration, crystallization time, and centrifugation speed increased. The best β-CD precipitation conditions in milk were designated as: 9.1% β-CD concentration (w/w), 4 hour crystallization time, 45ºF, and centrifugation at 25 × *g* for 10 minutes. Eleven cows were treated with cephapirin sodium or cephapirin benzathine. Milk was obtained 12 h later and treated with β-CD. As β-CD concentrations increased, precipitates increased, and the corresponding supernatants showed reduced concentrations of antibiotics when tested by enzyme-linked receptor-binding assay. Results indicated that β-CD may have the potential to reduce the residues of cephapirin sodium or cephapirin benzathine in antibiotic-tainted raw milk.

(Key Words**:** β-cyclodextrin, Cephapirin Sodium, Cephapirin Benzathine, Milk.)

Introduction

Cyclodextrins are chemically and physically stable molecules formed by the enzymatic modification of starch. β-Cyclodextrin (β-CD) is a cyclic oligosaccharide having a cavity at the center of its molecular arrangement that allows for inclusion-complexing with various compounds. Of all the cyclodextrins, β-CD has limited aqueous solubility of 1.85 g/100 mL at 77°F. Previous work has shown that β-CD can bind cholesterol from various food sources, such as milk, and thus, lower the cholesterol content in the liquid portion of the food.

Antibiotics are used to treat bacterial infections in humans and animals. Bovine mastitis is an inflammatory and highly communicable udder disease, which is often treated with the administration of antibiotics. The most common antimicrobials used in lactating cows are the group known as β-lactams (e.g., cephalosporins and penicillins). Antibiotics can enter the raw milk supply when milk from antibiotic-treated cows is inadvertently mixed with that from healthy cows. If humans sensitive to these drugs consume the antibiotic-tainted milk, they may exhibit allergic reactions that range from mild skin rashes to life-threatening anaphylaxis. Studies showed that penicillin may produce allergic reaction when its concentration is as small as 10 ppb in milk. In the United States, testing of each incoming milk shipment is mandatory; in 2002 alone, the United States disposed of 85.5 million lb of milk because of the presence of antibiotics. As of now, no tested and proven method

has been developed to remove these potentially harmful compounds from milk.

The β-Lactam drugs have structures similar to other organic compounds such as cholesterol (molecular size: Cephalosporin - 478.7; Cholesterol - 414.7). β-CD complexes may be precipitated under certain conditions, such as heating then cooling, to remove unwanted compounds from liquid solutions. The test hypothesis was to study the crystallization pattern of β-CD in water and to determine whether β-CD can complex and precipitate unwanted antibiotic residues in raw milk.

Experimental Procedures

Pharmaceutical grade β-CD (Wacker Biochem Corp. Adrian, MI), pasteurized whole milk (Kansas State University dairy plant, Manhattan, KS), and raw milk (Kansas State University Dairy Teaching and Research Center, Manhattan, KS) were obtained.

Solutions were made with 20 g of solvent (pasteurized whole milk or deionized distilled water) and a variety of concentrations (1.2, 2.4, 4.8, 9.1, or 13%) of β-CD (w/w). Solutions were stirred continuously at 500 rpm for 10 minutes at 140°F to allow for complete dissolution of β-CD.

Some solutions were held at 72 ± 2°F for 5 or 10 h to allow for precipitation. After crystallization, solutions were transferred into 50-mL centrifuge bottles and centrifuged at 25, 50, 100, 250, 500, 1000, 2000, or 3000 × *g* for 10 minutes at 22 ± 2°F. Other solutions were placed in capped centrifuge bottles immediately after mixing and heating, and were transferred to an ice bath (32 ± 2°F) to achieve a solution temperature less than or equal to 50°F within 20 minutes. After 20 minutes in the ice bath, centrifuge bottles were transferred to storage (45°F) and maintained for 4, 5, 8, or 12 h. Solutions held at refrigerated conditions were centrifuged at 25 × *g* for 10 minutes. Total solids for β-CD, raw milk, pasteurized whole milk, supernatants, and precipitates were performed as outlined in standard methods.

Antibiotic-infused milk was obtained from K-State Dairy Teaching and Research Center 12 h after antibiotic administration. Two different β-lactam drugs (10 mL), cephapirin sodium or cepharpirin benzathine, were infused into all four quarters of 5 or 6 cows, respectively. Milks were tested by using an enzyme-linked receptor-binding assay (ELRBA) approved by the FDA for the rapid assay for presence of antibiotic in milk. Antibiotic-tainted milk was diluted with raw, bulk tank milk to obtain ratios that might occur when milk from an antibiotic-treated cow was inadvertently mixed with normal milk. Milk samples were then treated with β-CD, heated to 72°F, cooled, refrigerated, and then centrifuged for 10 minutes at 25 × *g*. Supernatants were retrieved and tested for antibiotic presence with the ELRBA test.

Three experiments were conducted. In the first experiment, the crystallization pattern of β-CD in water as a function of centrifugation speed and β-CD solution concentration was studied. A simple 2-factorial design was used with various β-CD concentrations (1.2 to 13.1%), and centrifugation speeds (1000 to 3000 × *g*). Crystallization patterns were evaluated by monitoring β-CD in the precipitate and supernatant. The second experiment focused on the effect of centrifugation speed on the precipitate recovery of β-CD in water solutions. A simple 1-factorial design comparing five centrifugation speeds (25, 50, 100, 250, and 500 × *g*) at a single β-CD concentration (9.1% w/w) was used. The same experiment was repeated with pasteurized whole milk as the diluent. In the third experiment, crystallization patterns of β-CD in water and pasteurized whole milk at 45°F as a function of holding time were determined by using a randomized block design with replication as

the blocking factor and holding time (4, 5, 8, and 12 h) as the main factor, keeping a constant concentration of 9.1% β-CD. Finally, to validate our data, antibiotic-tainted milk was obtained from 11 cows and was treated with β-CD and subsequently analyzed for the presence of antibiotics.

Results and Discussion

The main effects and interaction of β-CD and centrifugation speed were significant for total solids in the precipitate and in the supernatant. Table 1 summarizes average total solids for the precipitate and supernatant. The results illustrate the solubility characteristics of β-CD. Table 1 clearly shows that, at a β-CD concentration of 1.2% (less than the solubility limit), no solids were recovered in the precipitate. In contrast, at the greater concentration of 2.4%, a small amount of solids (11.4%) was recovered in the precipitate. When concentrations exceeded the β-CD solubility range, greater amounts of total solids were recovered in the precipitate (Table 1). For the 9.1% solution, precipitate recovery was independent of the centrifugation speed.

When considering the final objective of using β-CD to complex β-lactam antibiotic, and subsequently precipitate the complex, two conditions were thought to be important: 1) choose a β-CD concentration that is sufficient to complex with β-lactam; and 2) use conditions to maximize β-CD+β-lactam complex precipitation with minimal expenditures of energy and cost. Table 1 indicates that the greatest precipitate recovery occurred at the 13% concentration, but the difference between the 9.1% and 13% concentration was relatively small (70% vs. 75%). Therefore, a 9.1% concentration of β-CD was selected for further work to maximize complexing and recovery of the contaminant in the precipitate.

To verify whether centrifugation speed affected precipitate recovery, a range of centrifugation conditions (25 to 3000 × *g*) were selected. Results from various centrifugation speeds (Figure 1 and Table 2) illustrate that recovery of precipitate was independent of speed when water was the diluent. When milk was the diluent, amount of precipitate increased as centrifugation speed increased, indicating that the centrifugation procedure caused some of the milk solids to precipitate. To minimize milk solids in the precipitate, the slowest centrifugation speed (25 × *g*) was selected for further experiments.

To enhance the crystallization rate of β-CD and maintain the milk at refrigerated conditions, solutions of β-CD with water or raw milk were held at 45°F for 4, 5, 8, or 12 h. Results indicated that the main effects and interactions of β-CD holding time were significant for the total solids in the precipitate. In contrast, for the supernatant solids, the 9.1% solution did not show a difference. Table 3 shows the results of total solids in the supernatant and precipitate portion for raw milk or water. As the holding time increased, total solids in the precipitates of the milk also increased ($P<0.05$; Table 3). At 4 h, raw milk had minimum solids in the precipitate. Precipitate recoveries in milk exceeded the amount of added β-CD (at 12 h), indicating that milk solids were being precipitated with β-CD (Table 3). The difference of total solids in the water and milk precipitate was reported as loss of milk solids in milk precipitate. A holding time of 4 h was considered best for raw milk and was used as a standard holding time in future experiments to minimize losses of milk solids in the precipitate.

To test that hypothesis raw milk was obtained from mastitic cows 12 h after treatment with either cephapirin sodium or cephapirin benzathine. For brevity, results from two cows are presented herein. Initial results indicated that antibiotic presence was high, so various amounts of β-CD were added to the raw milk, dissolved, and precipitated, and the resultant milk supernatant was tested for the presence of antibiotics. The ELRBA values

indicated that as β-CD concentration increased, ELRBA value decreased, and some to the point of the milk being considered negative for residue (Table 4). This study provides some evidence that the β-CD may be able to complex and then precipitate unwanted β-lactam drugs in raw milk.

In water, β-CD precipitate increased as β-CD concentration increased, but the amount of precipitate was independent of centrifugation speed. In raw milk, the total solids in the precipitate increased with the increased β-CD concentration and with crystallization time. Optimal conditions for the β-CD precipitation in milk was 9.1% β-CD concentration (w/w), 4 h crystallization time at 45ºF, and centrifugation at 25 × *g* for 10 minutes. Results from ELRBA indicated that β-CD has the potential to reduce the residues of cephapirin sodium or cephapirin benzathine in antibiotic-tainted raw milk.

Table 1. Total Solids in Precipitate and Supernatant from Solutions of De-ionized Distilled Water and 1.25 to 15% (0.25 to 3 g) β-Cyclodextrin, Centrifuged at 1000, 2000, or 3000 × *g* for 10 Minutes

	Precipitate[1], g		
β-cyclodextrin	1000 × *g*	2000 × *g*	3000 × *g*
0.25 g (1.2%)	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}
0.50 g (2.4%)	0.06 ± 0.01^{f}	0.06 ± 0.02^{f}	0.05 ± 0.02^{f}
1.00 g (4.8%)	0.51 ± 0.02^{d}	0.48 ± 0.02^{e}	0.47 ± 0.03^{e}
2.00 g (9.1%)	1.39 ± 0.02^{c}	1.39 ± 0.01^{c}	1.39 ± 0.02^{c}
3.00 g (13%)	2.21 ± 0.02^{b}	2.27 ± 0.01^{a}	2.27 ± 0.01^{a}
	Supernatant[1], g		
β-cyclodextrin	1000 × *g*	2000 × *g*	3000 × *g*
0.25 g (1.2%)	0.21 ± 0.01^{k}	0.21 ± 0.01^{k}	0.22 ± 0.01^{k}
0.50 g (2.4%)	$0.38 \pm 0.02^{e,f}$	$0.37 \pm 0.02^{f,g,h}$	$0.38 \pm 0.01^{e,g}$
1.00 g (4.8%)	$0.46 \pm 0.01^{a,b,c}$	0.48 ± 0.03^{a}	$0.47 \pm 0.02^{a,b}$
2.00 g (9.1%)	$0.45 \pm 0.02^{b,c}$	$0.43 \pm 0.02^{c,d}$	$0.37 \pm 0.02^{f,g,i}$
3.00 g (13%)	$0.40 \pm 0.01^{d,e}$	$0.35 \pm 0.01^{h,i,j}$	0.34 ± 0.01^{j}

[a,b,c,d,e,f,g,h,i,j,k]Means having different superscript letters within columns and rows for either precipitate or supernatant differ ($P<0.05$).
[1]Mean and standard deviations (n = 3).

Table 2. Total Solids in Supernatant and Precipitate of De-ionized Distilled Water having 2g of β-Cyclodextrin and Centrifuged at 25, 50, 100, 250, or 500 × *g* for 10 Minutes

Force (× g)	Supernatant[1]	Precipitate[1]
25	0.40 ± 0.06[b]	1.36 ± 0.06[a]
50	0.41 ± 0.07[b]	1.36 ± 0.06[a]
100	0.35 ± 0.01[b]	1.40 ± 0.01[a]
250	0.39 ± 0.00[b]	1.35 ± 0.02[a]
500	0.39 ± 0.02[b]	1.39 ± 0.00[a]

[a,b]Means having different superscript letters within a column differ ($P<0.05$).
[1]Data represents mean ± SD (n = 3).

Table 3. Precipated Total Solids in De-ionized Distilled Water or Raw Milk Treated with 2 g of β-Cyclodextrin, Held at 45° for 4, 5, 8, or 12 Hours, and Centrifuged at 25 × g for 10 Minutes

Time	Raw milk[1]	Water[1]	Difference
4 hours	1.72 ± 0.24[b]	1.46 ± 0.03[d]	0.26
5 hours	1.98 ± 0.33[a,b]	1.48 ± 0.03[c]	0.50
8 hours	1.97 ± 0.31[a,b]	1.51 ± 0.03[b]	0.46
12 hours	2.03 ± 0.22[a]	1.53 ± 0.02[a]	0.50

[a,b,c,d]Means having different superscipts within a column differ ($P<0.05$).
[1]Mean ± SD.

Table 4. ELRBA Values of Antibiotic-tainted Raw Milk, Before and After Treatment with 0, 1, 2, or 4 g of β-Cyclodextrin

Antibiotic	ELRBA before treatment	Amount of β-Cyclodextrin, g	ELRBA after treatment
Cephapirin sodium	2.26 (positive)	0	2.19 (positive)
		1	1.35 (positive)
		2	0.93 (negative)
		4	0.89 (negative)
Cephapirin benzathine	1.26 (positive)	0	1.13 (positive)
		1	1.08 (positive)
		2	0.98 (negative)
		4	0.91 (negative)

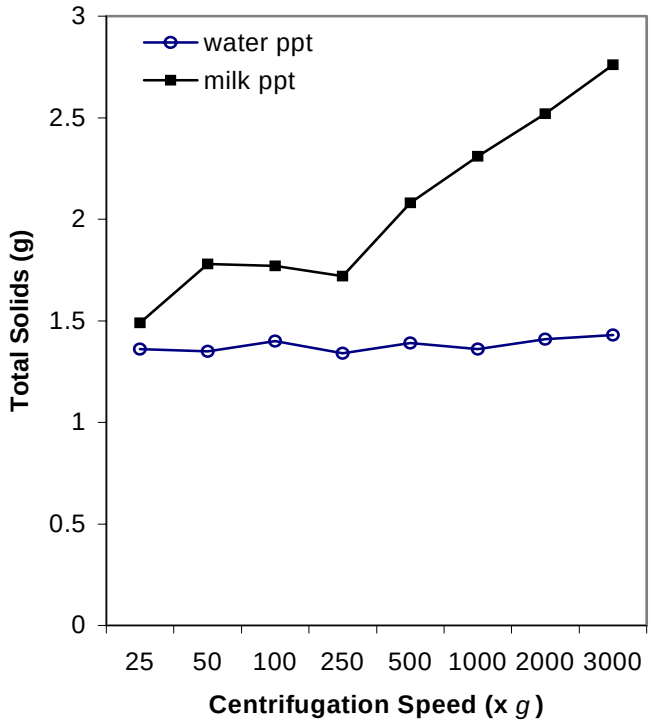

Figure 1. Total Solids in Precipitate of De-ionized Distilled Water and Milk Treated with 2 g of β-Cyclodextrin, Held at 72 ± 2°F, and Centrifuged at 25, 50, 100, 250, or 500 × g.

Dairy Day 2004

SERVING TEMPERATURE EFFECTS ON MILK FLAVOR, MILK AFTERTASTE, AND VOLATILE-COMPOUND QUANTIFICATION IN NONFAT AND WHOLE MILK

L. L. Francis, S. H. Kong, D. H. Chambers, I. J. Jeon, S. R. Simmons, and K. A. Schmidt

Summary

Many people seem to prefer to drink milk when it is cold. Research describing flavor and aftertaste of milk, and then correlating these traits with their chemical composition, has not previously been done. The objectives of this study were to describe milk flavor and aftertaste by using a descriptive sensory panel and to quantify the headspace volatiles of nonfat and whole milk as a function of serving temperature. Headspace volatile compounds of milk samples served at 40°F and 60°F were quantified by using solid-phase microextraction (SPME) analysis, with a 75-µm Carboxen-PDMS fiber, sampling milk at 140°F for 30 minutes, and then analyzing by gas chromatography, flame ion detection (GC-FID) for quantification. Descriptive-panel results indicated that serving temperature did not affect the milk flavor. Nonfat milk flavor and texture were rated to have greater sour aromatics, and to be slightly chalky, flat, and bitter, but less sweet, than whole milk. Characterization of milk aftertaste at 15 seconds after swallowing indicated that nonfat milk had very slight sour and cooked attributes. Characterization of milk aftertaste at 90 seconds after swallowing indicated that nonfat milk had very slight cooked attributes and was less sweet than whole milk. Serving temperature did not affect concentrations of volatile compounds, but nonfat milk had a greater concentration of hexanal and lesser ($P < 0.05$) concentrations of benzaldehyde, ethyl caproate, heptanal, 2-heptanone, and nonanal than whole milk did. These data provide evidence that fat contributes to the "flavor" and aftertaste attributes of milk more than serving temperature does.

(Key Words: Milk Aftertaste, Milk Flavor, GC Analysis.)

Introduction

Milk is an excellent nutrient source for humans. But milk components are also excellent substrates for certain microorganisms and are susceptible to undesirable chemical reactions that cause the quality of fresh milk to deteriorate and cause off flavors. The taste of high-quality milk should be clean and sweet, with no odor or aftertaste. The mouthfeel of milk should be smooth and have a sensation of richness. Milk fat contributes to milk mouthfeel and flavor as a consequence of naturally present flavor compounds and by its ability to change flavor "onset and diminishment" intensities and rates. Research has shown that alcohols, carbonyl compounds, free fatty acids, and sulfur compounds all contribute to the flavor of fresh milk. Volatile compounds previously reported in raw milk include 2-butanone, ethyl caproate, heptanal, 2-heptanone, hexanal, nonanal, octanal, and pentanal.

Flavor and nutrition are the main reasons people consume milk. People who do not drink milk think that milk does not taste good, is too sour or not sweet enough, or has an unpleasant aftertaste. Sensory and chemical analyses have been conducted on milk flavor as it undergoes microbial or chemical-induced changes, but very little research has focused

on aftertaste. It has been determined that "dairy sour," "sour," "fatty mouth coating," "lingering dry mouth coating," and the time for flavors and textures to clear the mouth are attributes associated with aftertaste. One study found that high-fat milk samples had greater intensities of "fatty mouth coating," "sour," and increased time for flavor and texture to clear the mouth. These descriptors were not correlated with the chemical composition of the milk. The objectives of this study were to: 1) describe the aftertaste of nonfat and whole milk; 2) analyze chemical compositions and physical properties of nonfat and whole milks; and 3) correlate the results of descriptive analyses with those obtained from gas chromatography headspace analyses.

Experimental Procedures

Nonfat and whole milk (1.965 L) were obtained within 2 days of production from the Kansas State University dairy plant, Manhattan, Kansas, during November 2003, and were maintained in the dark at $\leq 40^{o}$F.

Five highly trained and experienced panelists from the Sensory Analysis Center at Kansas State University, Manhattan, Kansas, participated in the study. Panelists had completed 2000 hr of general sensory testing and 120 hr of training in sensory techniques and analysis. Panelists scored intensities (on a 15-point numerical scale divided into half-point increments, with 0 representing "none" and 15 representing "extremely strong") of milk texture, milk flavor, and milk aftertaste at 15 and 90 seconds after swallowing. A lexicon was developed to identify and score 3 texture, 23 flavor, and 11 aftertaste attributes of milk.

Milk samples were tempered to 40 and 60^{o}F and served to a descriptive sensory panel. Analytical testing for headspace, total solids content, fat content, total plate counts, and apparent viscosity started within 30 minutes of serving the milk samples to the panel. For headspace analysis, the milk samples were analyzed by solid phase microextraction-gas chromatography (SPME-GC) for volatile compound identification. For total solids, an atmospheric oven method was used. Fat content was measured by the Babcock method, and total aerobic counts were done by using petrifilm. Apparent viscosity was determined at a frequency of 2 Hz within a shear-rate range of 1 to 40 seconds^{-1}, with a rheometer set at 40 and 60^{o}F, and compared at 10 seconds^{-1}.

The experimental structure was a two-way factorial design with the main effects of milk type (nonfat vs. whole) and serving temperature (40 vs. 60^{o}F), with milk type by temperature as the interaction. Three replications were done. Differences among means were determined by Tukey's pair-wise comparison.

Results and Discussion

Seven major volatile compounds were present in both nonfat and whole milk: benzaldehyde, ethyl caproate, heptanal, 2-heptanone, hexanal, nonanal, and pentanal (Table 1). Whole milk had greater ($P<0.05$) concentrations of benzaldehyde, ethyl caproate, heptanal, 2-heptanone, and nonanal, and had smaller concentrations of hexanal than nonfat milk did. The pentanal concentration did not differ for the two milk types. Headspace concentrations were unaffected by serving temperature, probably because the extraction method used a heat treatment (140^{o}F for 30 minutes) to force the volatile compounds into the headspace. All concentrations of volatile compounds were greater than reported threshold values, with the exception of heptanal in nonfat milk and pentanal in whole milk (Table 1). Greater mean concentrations for heptanal, 2-heptanone, hexanal, nonanal, and pentanal were observed in our study than in previous reports.

Whole milk had greater fat and total solids contents than nonfat milk did, but had similar total plate counts (Table 2). Serving tempera-

ture did not affect the chemical contents or microbial counts.

Serving temperature and milk fat affected the apparent viscosity of the milk types. Milk served at 40°F was more viscous (3.55 mPa·s) than milk served at 60°F (2.18 mPa·s). Nonfat milk was less viscous (2.76 mPa·s) than whole milk was (2.98 mPa·s).

Results from the descriptive panel indicated that no differences were detected for the sensory attributes of milk served at either 40 or 60°F, but some texture, flavor, and aftertaste attributes were affected by milk-fat content (Figures 1 and 2). Nonfat milk texture was rated as being more chalky, less viscous, and having less "fatfeel" than whole milk. Nonfat milk was described as having less "fat," "sweet," and "sweet aromatics," but more "flat" and "sour aromatics" than whole milk. Differences in after-taste descriptors (at 15 and 90 seconds after swallowing) and their reported intensities are shown in Figure 2. At 15 seconds after swallowing, nonfat milk aftertaste was described as being less overall sweet, fat, and having a less fatty mouthfilm, but having more overall sour and cooked traits, than whole milk did. At 90 seconds after swallowing, nonfat milk aftertaste was described as being less overall sweet, fat, and having less fatty mouthfilm, but having more cooked traits than were present with whole milk. When after-taste attributes and intensities of whole and nonfat milks at 15 and 90 seconds after swallowing were compared, intensities decreased for all attributes. Our study confirms previous research in which high-fat milk samples rated higher for fatty mouthfilm aftertaste, and the nonfat milk aftertaste was rated as being more overall sour. Previous research also indicated that high-fat milk samples had greater intensities of sour taste. In contrast, the nonfat milk in our study was rated as more sour than whole milk. Others have reported that heptanal, 2-heptanone, and nonanal are compounds formed by milk fat and are often described as blue cheese, oily, and fatty flavors in milk samples. These three compounds were observed to have greater concentrations in the whole-milk sample, and the whole-milk sample was described as having a greater fat flavor and greater fat feel than the nonfat milk sample. Perhaps the best milk flavor is related to a favorable quantity of volatile flavor compounds at an optimal ratio.

Our study provides evidence that fat contributes to the flavor and aftertaste attributes of milk. Milk composition, especially fat content, affected milk aftertaste, whereas serving temperature had no effect. Whole milk had greater concentrations of benzaldehyde, ethyl caporate, heptanal, 2-heptanone, and nonanal, but had lesser concentrations of hexanal than nonfat milk did. Further work is needed to describe flavor and aftertaste differences on the basis of known off flavors present in milk.

Table 1. Mean Headspace Concentrations (mg/kg) of Volatile Flavor Compounds in Whole and Nonfat Milk

Volatile compound	Threshold value in milk (mg/kg)	Nonfat milk n = 3	Whole milk n = 3
Benzaldehyde[1]	0.0004	0.8741 ± 0.007^{b}	1.0750 ± 0.007^{a}
Ethyl caproate	0.075	0.3957 ± 0.002^{b}	0.6450 ± 0.001^{a}
Heptanal	0.12	0.0959 ± 0.002^{b}	0.9868 ± 0.002^{a}
2-Heptanone	0.70	0.9076 ± 0.002^{b}	1.0117 ± 0.002^{a}
Hexanal	0.05	0.6433 ± 0.002^{a}	0.5927 ± 0.002^{b}
Nonanal	0.22	0.9444 ± 0.03^{b}	1.1419 ± 0.03^{a}
Pentanal	0.13	0.2151 ± 0.09^{a}	0.0730 ± 0.08^{a}

[a,b]Means within row having different superscript letters differ ($P<0.05$).
[1]Value in water.

Table 2. Mean Values of Fat Total Solids, and Total Plate Counts in Nonfat and Whole Milks

Item	Nonfat milk, n = 3	Whole milk, n = 3
Fat, %	0.07 ± 0.04^{b}	3.40 ± 0.04^{a}
Total solids, %	9.97 ± 0.06^{b}	12.20 ± 0.06^{a}
TPC (log CFU/mL)	2.52 ± 2.27^{a}	1.28 ± 2.27^{a}

[a,b]Means within row having different superscript letters differ ($P<0.05$).

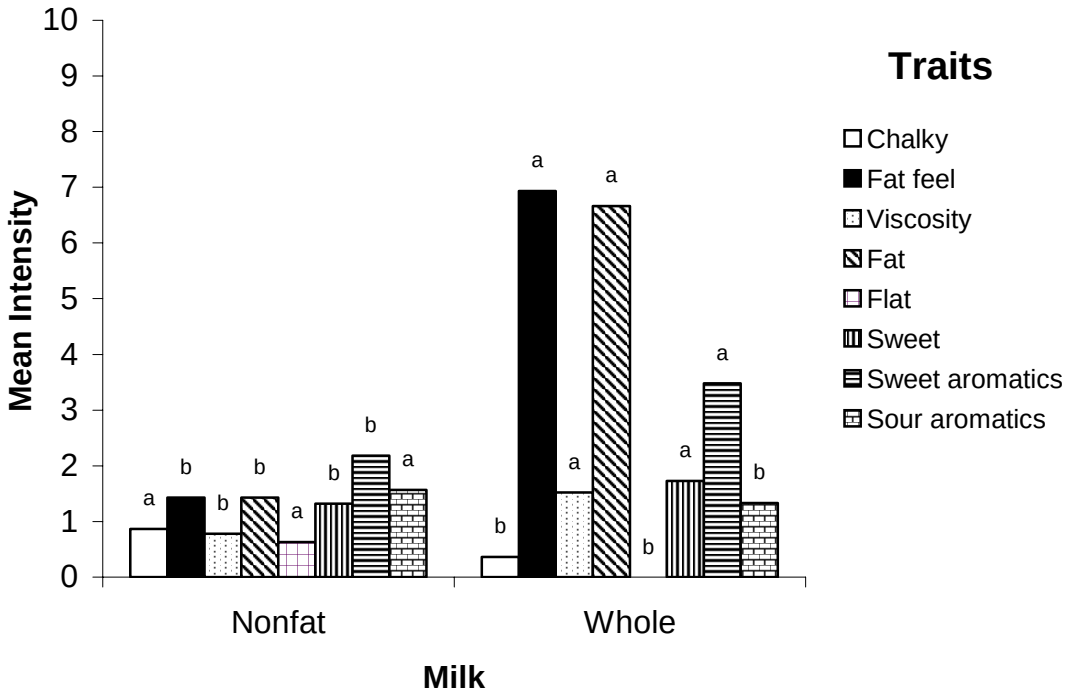

Figure 1. Mean Intensities of the Texture and Flavor Traits of Nonfat and Whole Milks. [a,b]Bars within milk type having different superscript letters differ ($P<0.05$).

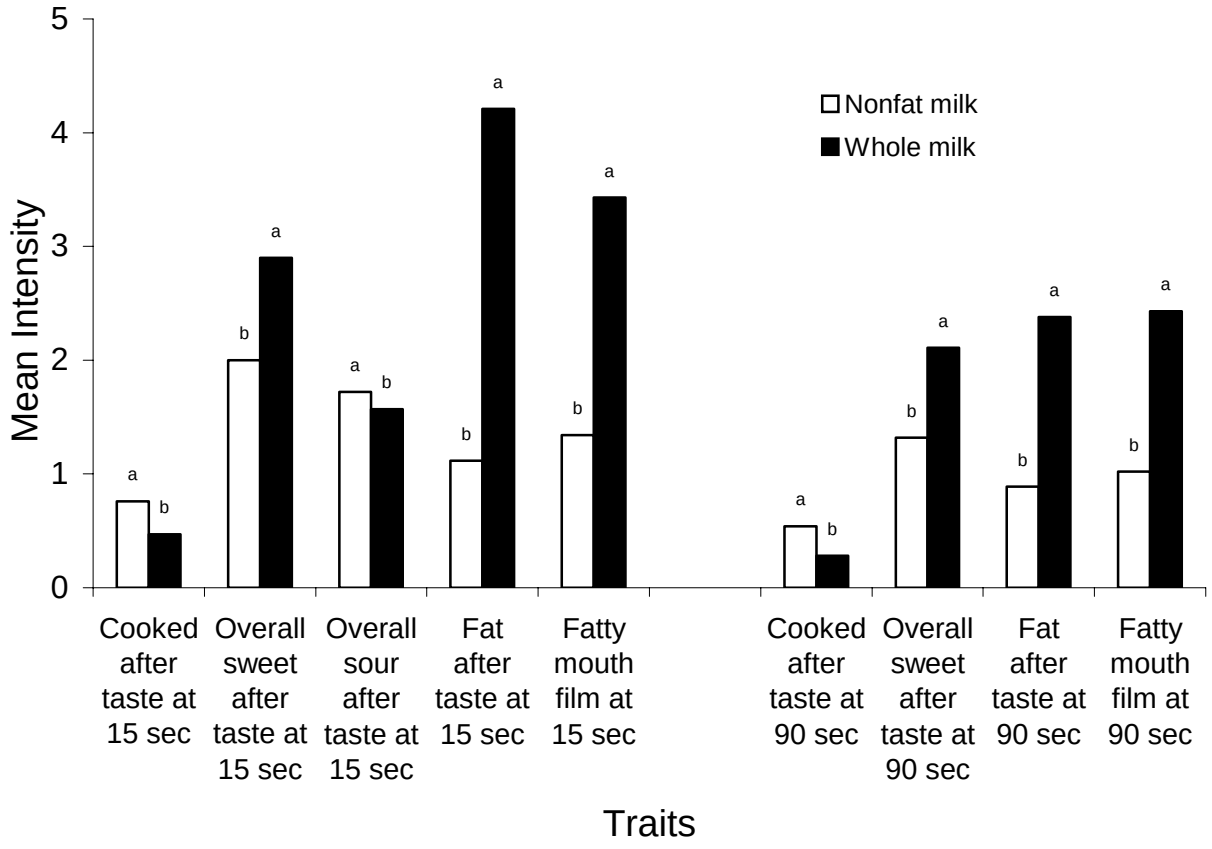

Figure 2. Mean Intensities of the Aftertaste (15 and 90 seconds After Swallowing) Traits of Nonfat and Whole Milks. [a,b]Bars between milk types having different superscript letters differ ($P<0.05$).

IMPACT OF SOAKING COWS HOUSED IN A TUNNEL-VENTILATED, EVAPORATIVE-COOLED BARN IN THAILAND

D. V. Armstrong[1], J. F. Smith, M. J. Brouk, V. Wuthironarith[2], and J. P. Harner, III[3]

Summary

Ten multiparous lactating Holstein cows were arranged in a replicated 5 × 5 Latin Square design to evaluate the effect of soaking frequency and volume of water per soaking on lactating cows housed in a tunnel-ventilated and evaporative-cooled freestall barn. Rectal temperature, respiration rate, and body surface temperatures were measured every 5 minutes. Treatments were: control (C); soaking every 5 minutes with 0.26 gallons (5L); soaking every 5 minutes with 0.53 gallons (5H); soaking every 10 minutes with 0.26 gallons (10L); or soaking every 10 minutes with 0.53 gallons (10H). Average ambient temperature and humidity were 86.5ºF and 68% outside the barn, and 80.4ºF at 86% inside the barn, respectively. Water having a temperature of 80.6ºF was applied manually from the shoulder to the tail. Treatments were applied after three initial measurements were assessed. Seventeen measurements were made during treatment application and five measurements after the treatments were stopped. Air velocity over the shoulder of the cows was 4 mph. Respiration rate and body surface temperature for all treatments were less than those of the control, except for rear udder surface temperature in the 10L treatment. Rectal temperature for 5L, 5H, and 10H were less than those of the control. Respiration rate for 5L and 5H were less than that of 10L. These data indicate that soaking can be used in combination with tunnel ventilation and evaporative pads to reduce heat stress.

(Key Words: Heat Stress, Cooling Systems, Facilities.)

Introduction

Heat stress has a major impact on milk production, reproduction, and health of dairy cows. During periods of heat stress, feed intake may decrease 6 to 16%, compared with that of cows in thermo-neutral conditions. In addition to reduced feed intake, a 30 to 50% reduction occurs in the efficiency of energy use for milk production. Two strategies have been implemented to reduce heat stress, including providing a cooler environment (cooling the air) or soaking the cow and evaporating water off her skin surface (cooling the cow). A trial was completed in Thailand to evaluate the possibility of using a combination of providing a cooler environment and cooling the cow directly by evaporating water off her skin surface.

Experimental Procedures

Lactating dairy cows used in this trial were housed in a tunnel-ventilated freestall barn

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equipped with evaporative pads. The structure, located in Thailand, was 371 ft long, 52 ft wide, and had a ceiling height of 8.5 ft. The tunnel ventilation was powered by eleven 51-inch fans, and the air was pulled through 850 ft^2 of 6-inch pads. Approximately 322 gallons of water was circulated through the evaporative pads per hour. Air velocity over the shoulder of the cows was approximately 4 mph. Average ambient temperature and humidity were 86.5ºF and 68% outside the barn and 80.4ºF and 86% inside the barn, respectively.

Ten multiparous lactating Holstein cows producing an average of 90 lb of milk were arranged in a replicated 5 × 5 Latin Square design to evaluate the effect of soaking frequency and volume of water per soaking. The experiment was carried out in 5 periods between September 6 and 11, 2003, between 1:30 and 3:30 p.m. Rectal temperature, respiration rate, and body surface temperature (shoulder, thurl, and rear udder) were measured every 5 minutes. Treatments were: control (C); soaking every 5 minutes with 0.26 gallons (5L); soaking every 5 minutes with 0.53 gallons (5H); soaking every 10 minutes with 0.26 gallons (10L); or soaking every 10 minutes with 0.53 gallons (10H). Cows were restrained in headlocks and 80.6ºF water was applied manually from the shoulder to the tail. Treatments were applied after three initial measurements were made. Seventeen measurements were made during treatment application and five measurements were made after the treatments were stopped. Data were analyzed as a completely randomized block design by using the Mixed Model of SAS (SAS Inst. Inc., Cary, NC). The difference between control and treatment was separated by using the least-significant-difference procedure.

Results and Discussion

Results are presented in Table 1. Respiration rates and body surface temperatures for all treatments were less than those of the control, except for rear-udder surface temperature of 10L. Rectal temperature for 5L, 5H, and 10H were less than that of the control. Respiration rate for 5L and 5H were less than that of 10L. The changes in respiration rate, rectal temperature, and thurl surface temperature are presented graphically in Figures 1, 2,and 3.

The results at this trial indicate that there is potential to reduce heat stress in lactating dairy cows housed in evaporative-cooled freestall barns by adding automated feedline soakers. In this trial, cows did not have a choice whether they were soaked or not soaked. In a commercial setting, dairy cows can choose when they come to the feedline to be soaked. Additional research is needed to determine how to manage feedline soakers in evaporative-cooled barns on commercial dairies.

Table 1. Impact of Soaking Cows Housed in a Tunnel-ventilated, Evaporative-cooled Barn on Respiration Rate, Rectal Temperature, and Body Surface Temperature

	Treatment[1]					
	C	5L	5H	10L	10H	SE
Respiration rate, breaths/min	72.8[a]	51.0[c]	51.6[c]	59.3[b]	55.6[bc]	2.03
Rectal temp., ºF	101.3[a]	100.7[b]	100.7[b]	100.9[ab]	100.7[b]	0.12
Body surface temp., ºF						
Thurl	93.9[a]	89.6[c]	89.2[c]	90.7[b]	91.14[bc]	0.38
Rear udder	95.5[a]	94.3[b]	94.5[b]	95.0[ab]	94.6[b]	0.25
Shoulder	93.7[a]	89.8[bc]	89.4[c]	91.2[b]	90.9[b]	0.50

[abc]Means having different superscript letters within in row differ ($P \leq 0.05$).

[1]Treatments were: control (C); soaking every 5 minutes with 0.26 gallons (5L); soaking every 5 minutes with 0.53 gallons (5H); soaking every 10 minutes with 0.26 gallons (10L); or soaking every 10 minutes with 0.53 gallons (10H).

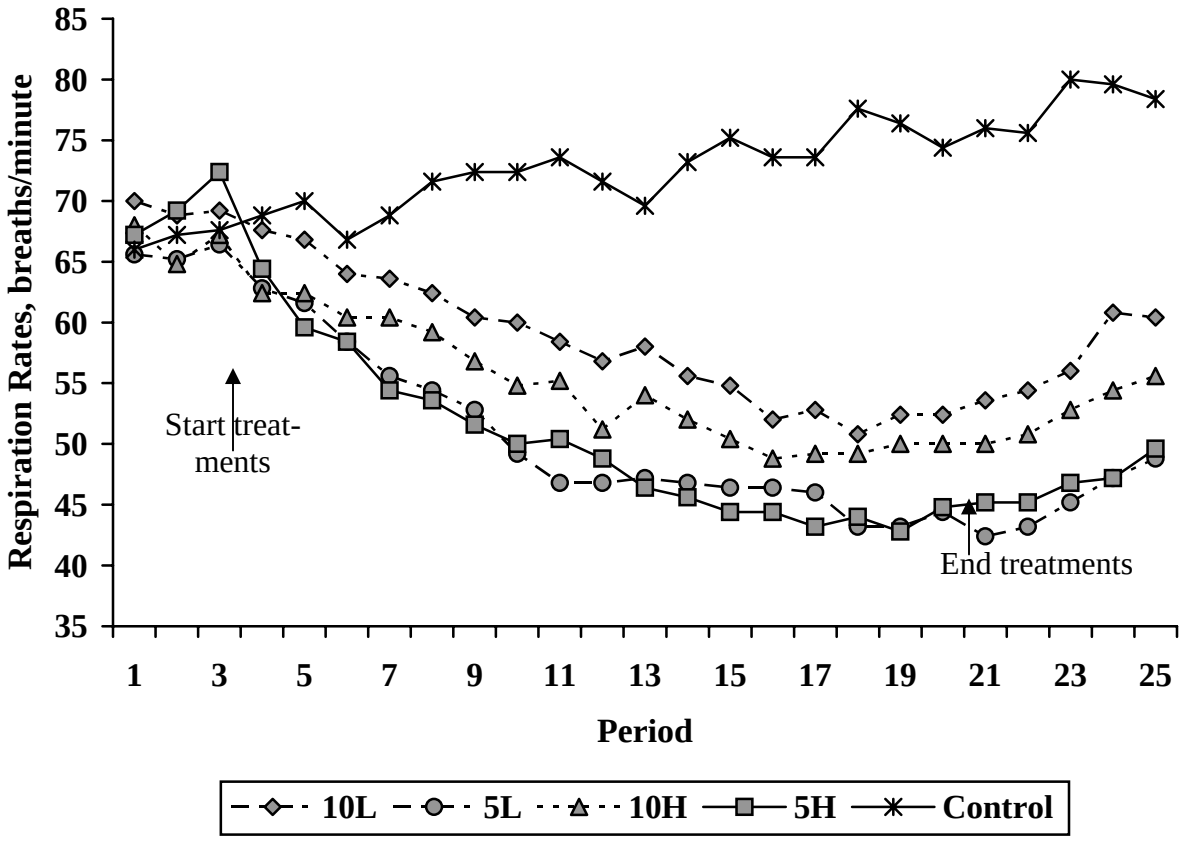


Figure 1. Impact of Soaking Frequency on the Respiration Rates of Cows Housed in Tunnel-ventilated Barns with Evaporative Pads (Thailand).

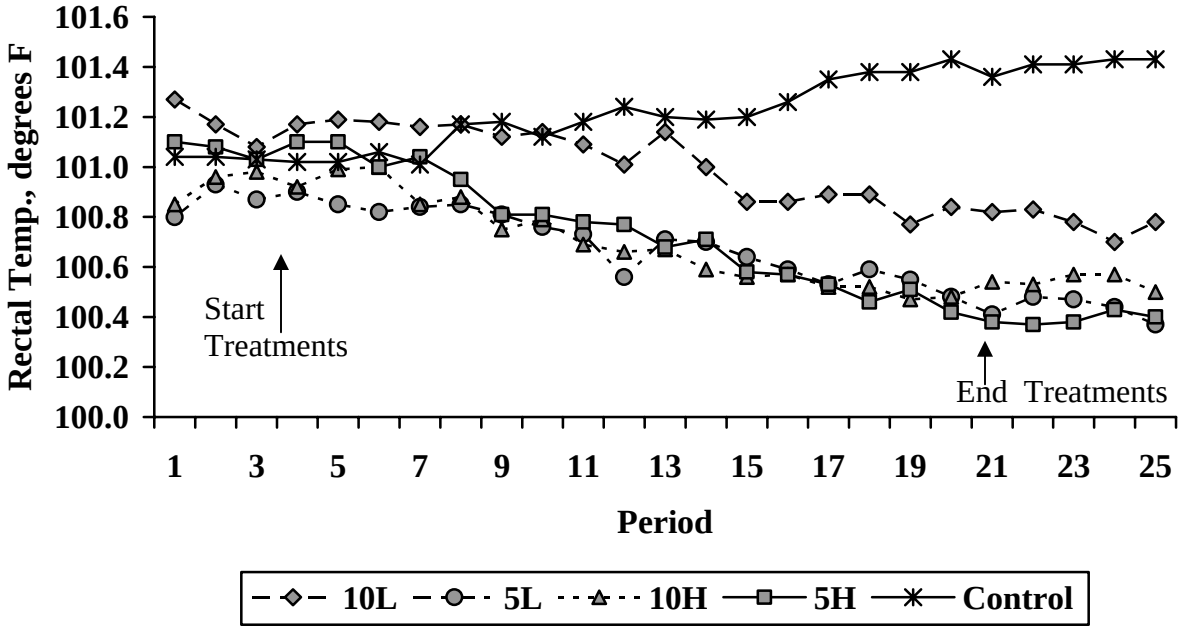


Figure 2. Impact of Soaking Frequency on the Rectal Temperatures of Cows Housed in Tunnel-ventilated Barns with Evaporative Pads (Thailand).

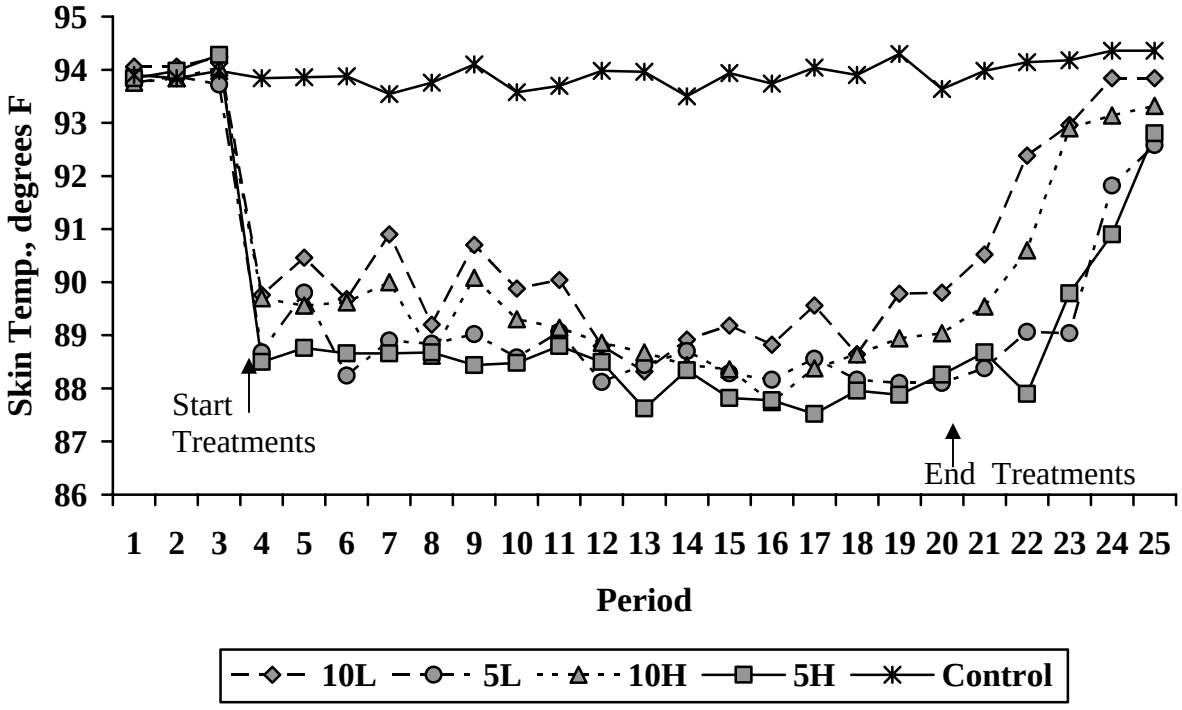


Figure 3. Impact of Soaking Frequency on Thurl Skin Temperature of Cows Housed in Tunnel-ventilated Barns with Evaporative Pads (Thailand).

Dairy Day 2004

RESPONSES OF LACTATING HOLSTEIN COWS TO LOW-PRESSURE SOAKING OR HIGH-PRESSURE MISTING DURING HEAT STRESS

M. J. Brouk, J. P. Harner[1], J. F. Smith, W. F. Miller, and B. Cvetkovic

Summary

Lactating dairy cattle were used to evaluate three different cooling systems. Eight cows were arranged in a replicated Latin-square design and assigned to each of four treatments. Treatments were control, low-pressure soaking (LPS), high-pressure misting with 1.7 gallons per minute of water (HP-1.7), or high-pressure misting with 3.4 gallons per minute of water (HP-3.4). Cows were allowed to become heat stressed in a free-stall facility, and then were moved to a tie-stall barn for 2 hours of observations during four hot and humid afternoons. Respiration rates declined when heat abatement systems were used. Respiration rates at the end of the observation period were reduced by 20, 36, and 48% for HP-1.7, HP-3.4, and LPS, respectively. Rear-udder skin surface temperature was reduced at a faster rate under the HP-4 treatment than with LPS, but the two treatments did not differ in final rear-udder skin surface temperature or vaginal temperature. The HP-3.4 treatment used the greatest amount of water during the 2-hour testing period. The result was a combination of air-cooling and soaking. Results indicated that a combination of air cooling and soaking may result in faster reduction of surface temperature. When only air cooling was used (HP-1.7), heat stress was reduced, but it was less effective than either LPS or HP-3.4. Use of a low-pressure soaking system is superior to high-pressure misting unless cattle become soaked by the high-pressure system.

(Key Words: Heat Stress Abatement, Cow Comfort, Cow Cooling.)

Introduction

Summer heat stress reduces milk production and reproductive efficiency of dairy cows. Applying water to the skin of cattle or using evaporating water to cool the air around the cow are two common methods of reducing heat stress. These two methods are commonly refereed to as low-pressure soaking and high-pressure misting. Equipment costs (ownership and maintenance) are greater for high-pressure misting systems. Low-pressure soaking removes heat via conduction and evaporation, whereas high-pressure misting only removes heat by conduction. Low-pressure soaking may more effectively remove heat because water is a better conductor than air, and because a portion of the heat energy required to evaporate water from the skin is obtained from the cow.

The objective of this study was to determine the effects of low-pressure soaking and high-pressure misting on respiration rates, rear-udder skin surface temperatures, and vaginal temperatures of heat-stressed cows.

Experimental Procedures

Eight lactating Holstein cows (4 first-lactation and 4 multiple-lactation cows) were arranged in a replicated 4 × 4 Latin-square

[1]Department of Biological and Agricultural Engineering.

design to evaluate three different heat-abatement systems. Multiple-lactation cows averaged 260 days in milk and were producing an average of 88.2 lb of milk. First-lactation cows averaged 251 days in milk and were producing 91.9 lb of milk. Cows were housed in open free stalls at the KSU Dairy Teaching and Research Unit and were milked twice daily. On four hot and humid afternoons, cattle were moved to a tie-stall barn at 2:00 p.m. during 2 hours of cooling treatments. Treatments were control (C), low-pressure soaking applied for 1 minute every 5 minutes (LPS), continuous high-pressure misting with 2 (1.7 gallons/hour) nozzles (HP-1.7), or continuous high-pressure misting with 4 (3.4 gallons/hour) nozzles (HP-3.4). All three heat-stress-abatement treatments also included axial flow fans that created 750 CFM of airflow over the cows. Respiration rates and rear-udder skin surface temperatures were measured and recorded at 5-minute intervals during the 2-hour period. Skin surface temperature was measured with an infrared thermometer. Vaginal temperature was measured and recorded every minute and subsequently averaged by 5-minute periods before data analysis. Data from the first and final 15 minutes were averaged as initial and final observations. All data were subjected to analysis of variance, with treatment as a fixed variable and period and cow as random variables. Time (5-minute interval) was used as a repeated measure within cow.

Results and Discussion

Temperature of stalls (Figure 1) declined during the experimental period. Temperatures of the control stalls were greater ($P<0.05$) than when heat abatement systems were used. The HP-3.4 treatment reduced temperature the most and temperature reduction was correlated with the amount of water applied during the treatments. Relative humidity (Figure 2) increased with the addition of water from the HP-3.4 and LPS treatments.

Respiration rates (Table 1) were reduced ($P<0.01$) by each of the heat-stress-abatement systems. The LPS treatment was more effective than the high-pressure systems in reducing final respiration rates, and HP-1.7 was not as effective as HP-3.4. The HP-1.7 system only used about half as much water as the HP-3.4 system did. Cattle treated with HP-3.4 became soaked during the course of the testing period. As a result, the HP-3.4 treatment was actually a combination of soaking and evaporative cooling. Rate of respiration-rate decline differed among treatments (Figure 3). The control did not affect respiration rate. Rate of decline was greater for LPS than for either HP-1.7 or HP-3.4. Respiration rates of cattle cooled with HP-1.7 seemed to decline at first, but reached a stable respiration rate, compared with that of cattle cooled with HP-3.4 and LPS, which resulted in a continual decline during the testing period.

Rear-udder skin surface-temperature (Table 2) was reduced ($P<0.05$) by the heat-abatement systems. Cattle treated with HP-3.4 or LPS responded similarly, with cattle treated with HP-1.7 intermediate in response. Rate of rear-udder surface temperature decline (Figure 4) differed ($P<0.05$) among treatments. Using HP-3.4 resulted in the greatest rate of decline, followed by those of LPS and HP-1.7.

Final vaginal temperature (Table 3) was least for LPS and HP-3.4 and less ($P<0.05$) than HP-1.7 and control. The LPS and HP-3.4 treatments reduce vaginal temperature 2.7°F, compared with that of control. Rate of vaginal temperature decline (Figure 5) was greatest for HP-3.4 and LPS treatments, with HP-1.7 being intermediate. Lack of heat abatement (control) resulted in increased vaginal temperature during the experimental period.

Cattle cooled with HP-3.4 became soaked during the course of the experimental period. As a result, this treatment really represented a combination of the soaking and high-pressure

misting. It is significant to note that the HP-3.4 system used the greatest amount of water, followed by that of LPS and HP-1.7. A response to the amount of water applied, as well as the method of application, was observed. Although LPS produced the lowest respiration rates, cattle from the LPS treatment did not differ from HP-3.4 in final vaginal temperature. Cooling cattle with either HP-3.4 or LPS was more effective than either the control or HP-1.7 treatment. These data indicate that there may be some advantage to the combination of reducing air temperature and soaking. Although the final vaginal temperatures of cattle treated with LPS and HP-3.4 did not differ, the rate of decline was greater for cattle treated with HP-3.4. When high-pressure misting does not soak the cow (HP-1.7), it is less effective than LPS in reducing heat stress of dairy cattle.

Table 1. Initial and Final Respiration Rates of Cattle Cooled with Different Heat-abatement Systems

Treatment*	Initial	Final	SE
	Breaths/minute		
Control	110.8	117.5[a]	4.2
HP – 1.7	108.0	94.2[b]	4.2
HP – 3.4	109.2	75.0[c]	4.2
LPS	111.2	61.8[d]	4.2

*Control = no supplemental airflow or water treatment, HP-1.7 = 750 CFM airflow and 1.7 gallons/hour continuous high-pressure misting, HP-3.4 = 750 CFM airflow and 3.4 gallons/hour continuous high-pressure misting, and LPS = 750 CFM airflow and 4 gallons/hour of water via a low-pressure soaking system.
[a,b,c,d]Means within column having different superscripts letter differ ($P<0.01$).

Table 2. Initial and Final Rear-udder Skin Surface Temperatures of Cattle Cooled with Different Heat-abatement Systems

Treatment*	Initial	Final	SE
	°F		
Control	98.1	98.1[a]	1.0
HP – 1.7	98.8	96.6[b]	1.0
HP – 3.4	99.0	94.6[c]	1.0
LPS	98.8	95.7[c,b]	1.0

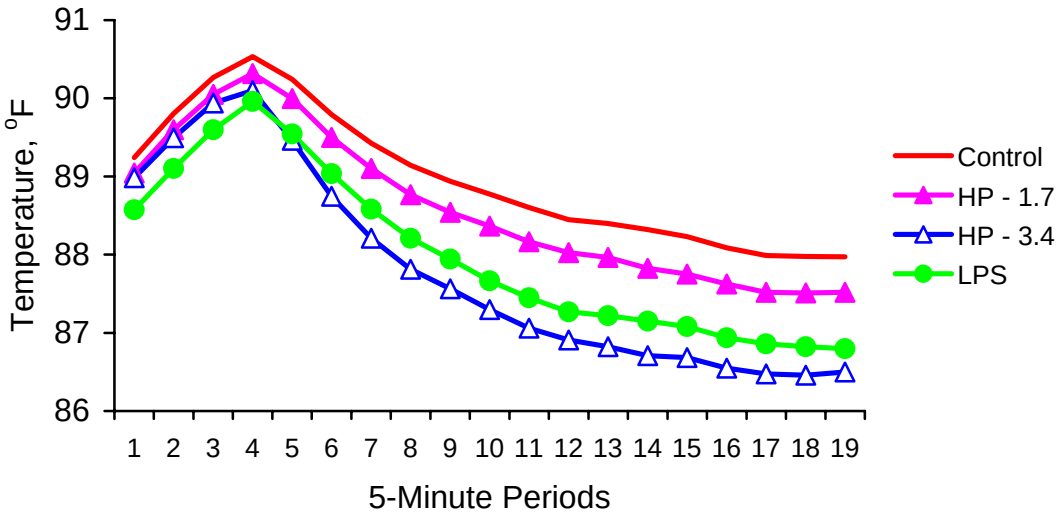
*Control = no supplemental airflow or water treatment, HP-1.7 = 750 CFM airflow and 1.7 gallons/hour continuous high-pressure misting, HP-3.4 = 750 CFM airflow and 3.4 gallons/hour continuous high-pressure misting, and LPS = 750 CFM airflow and 4 gallons/hour of water via a low-pressure soaking system.
[a,b,c,d]Means within column having different superscripts letter differ ($P<0.05$).

Table 3. Initial and Final Vaginal Temperatures of Cattle Cooled with Different Heat-abatement Systems

Treatment*	Initial	Final	SE
	°F		
Control	103.8	104.7[a]	0.4
HP – 1.7	102.9	103.3[b]	0.4
HP – 3.4	102.4	102.0[c]	0.4
LPS	102.7	102.0[c]	0.4

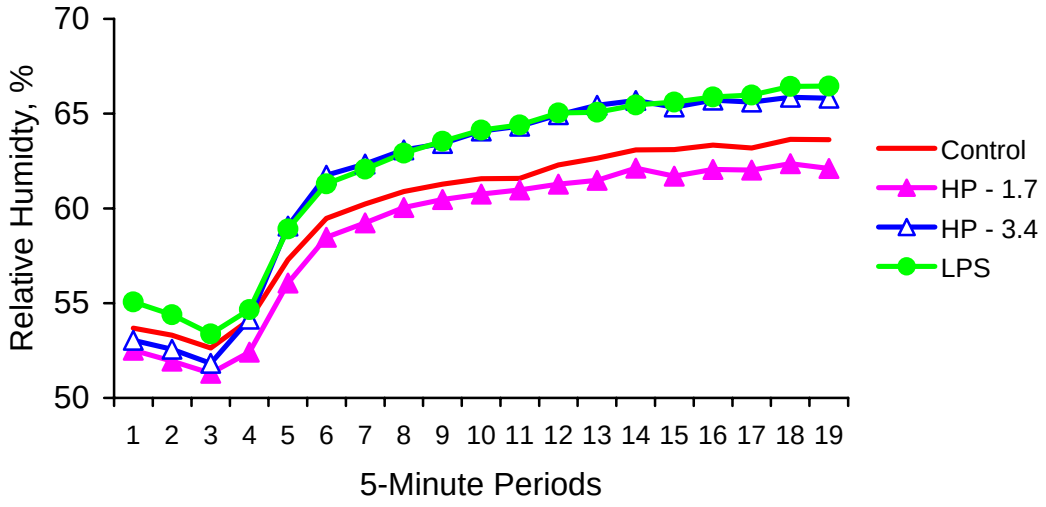
*Control = no supplemental airflow or water treatment, HP-1.7 = 750 CFM airflow and 1.7 gallons/hour continuous high-pressure misting, HP-3.4 = 750 CFM airflow and 3.4 gallons/hour continuous high-pressure misting, and LPS = 750 CFM airflow and 4 gallons/hour of water via a low-pressure soaking system.

[a,b,c,d]Means within column having different superscripts letter differ ($P<0.05$).



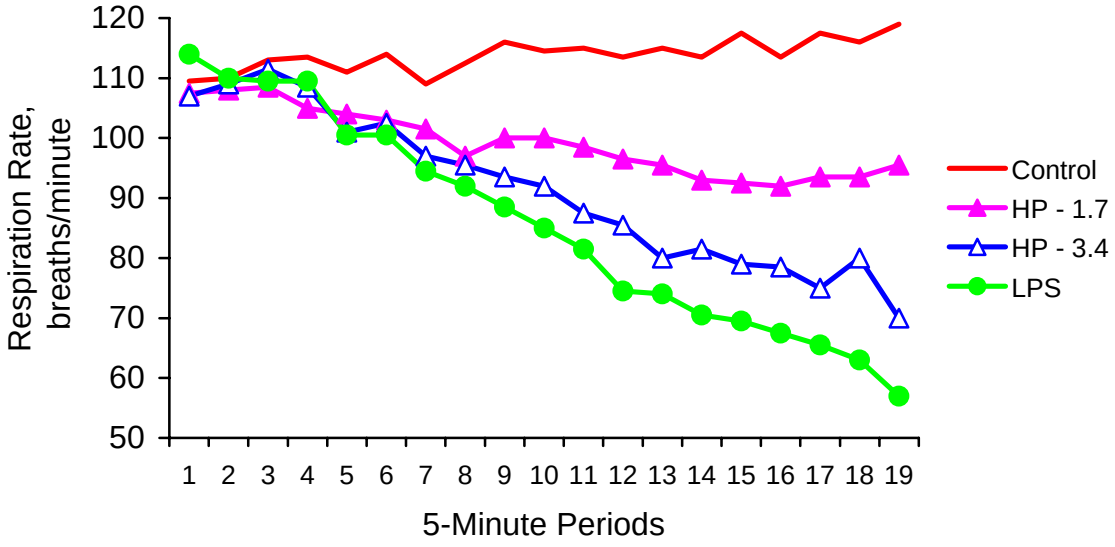
Control = no supplemental airflow or water treatment, HP-1.7 = 750 CFM airflow and 1.7 gallons/hour continuous high-pressure misting, HP-3.4 = 750 CFM airflow and 3.4 gallons/hour continuous high-pressure misting, and LPS = 750 CFM airflow and 4 gallons/hour of water via a low-pressure soaking system.

Figure 1. Temperature of Stalls Equipped with Different Heat-abatement Systems.



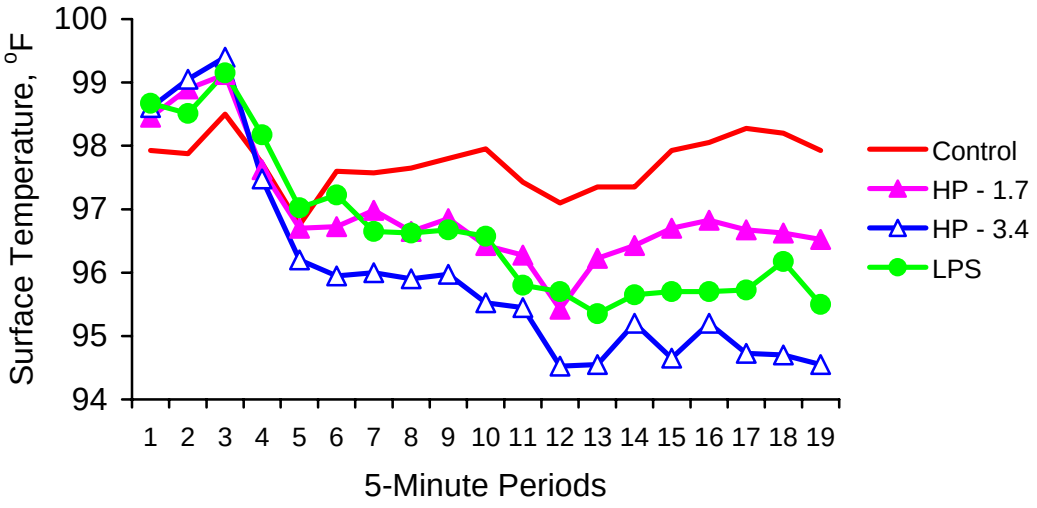
Control = no supplemental airflow or water treatment, HP-1.7 = 750 CFM airflow and 1.7 gallons/hour continuous high-pressure misting, HP-3.4 = 750 CFM airflow and 3.4 gallons/hour continuous high-pressure misting, and LPS = 750 CFM airflow and 4 gallons/hour of water via a low-pressure soaking system.

Figure 2. Relative Humidity of Stalls Equipped with Different Heat-abatement Systems.



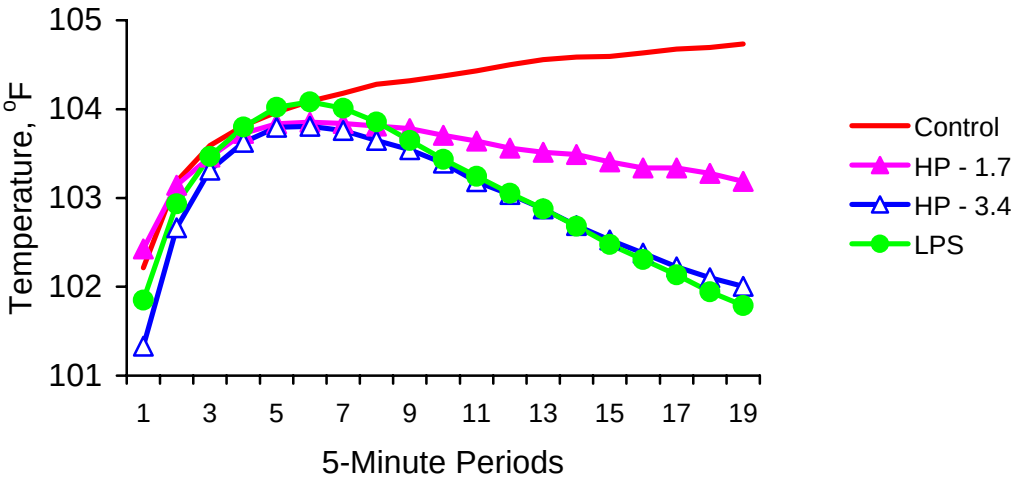
Control = no supplemental airflow or water treatment, HP-1.7 = 750 CFM airflow and 1.7 gallons/hour continuous high-pressure misting, HP-3.4 = 750 CFM airflow and 3.4 gallons/hour continuous high-pressure misting, and LPS = 750 CFM airflow and 4 gallons/hour of water via a low-pressure soaking system.

Figure 3. Respiration Rate of Cattle Cooled with Different Heat-abatement Systems.



Control = no supplemental airflow or water treatment, HP-1.7 = 750 CFM airflow and 1.7 gallons/hour continuous high-pressure misting, HP-3.4 = 750 CFM airflow and 3.4 gallons/hour continuous high-pressure misting, and LPS = 750 CFM airflow and 4 gallons/hour of water via a low-pressure soaking system.

Figure 4. Rear-udder Skin Surface Temperature of Cattle Cooled with Different Heat-abatement Systems.



Control = no supplemental airflow or water treatment, HP-1.7 = 750 CFM airflow and 1.7 gallons/hour continuous high-pressure misting, HP-3.4 = 750 CFM airflow and 3.4 gallons/hour continuous high-pressure misting, and LPS = 750 CFM airflow and 4 gallons/hour of water via a low-pressure soaking system.

Figure 5. Vaginal Temperature of Cattle Cooled with Different Heat-abatement Systems.

RESPONSES OF LACTATING HOLSTEIN COWS TO DIFFERING LEVELS AND DIRECTION OF SUPPLEMENTAL AIRFLOW

M. J. Brouk, J. P. Harner[1], J. F. Smith, W. F. Miller, and B. Cvetkovic

Summary

Seven heat-stressed, lactating Holstein cows were exposed to six different cooling systems to evaluate the effects of air velocity and direction of airflow. Cows were arranged in a 7 × 7 Latin-square design. Six cooling treatments were compared with a control. Supplemental airflow was provided by axial flow at one of three velocities: 500, 750, or 900 cubic feet per minute (CFM). Airflow was either from the front to rear (FRT) or from the right side (SIDE) of the cow. Combined cooling treatments were FRT-500, FRT-750, FRT-900, SIDE-500, SIDE-750, or SIDE-900. All cooling systems used a low-pressure soaking system that operated 1 minute every 5 minutes. Respiration rates, rear-udder skin surface temperature, and vaginal temperature were measured and recorded during 2 hours of treatment during seven hot and humid afternoons. Cooling systems reduced respiration rate, rear-udder skin surface temperature, and vaginal temperature. When airflow was 750 or 900 CFM, no differences were observed among treatments. When airflow was 500 CFM, rate of decline of rear-udder skin surface temperature and vaginal temperature were reduced, compared with those of other treatments. These results indicate that there was no advantage to increasing airflow more than 750 CFM when using a low-pressure soaking system that wets the cattle every 5 minutes. Differences due to airflow direction were only observed when airflow was reduced to 500 CFM. At 500 CFM, airflow from head to tail was not as effective as from the side. Current recommendations of 750 CFM of airflow directed at the side of the cow are effective in reducing heat stress of lactating dairy cattle.

(Key Words: Heat Stress Abatement, Cow Comfort, Cow Cooling.)

Introduction

Heat stress is a major concern for Kansas dairy producers. Many different heat-stress-abatement systems reduce the negative effects of heat stress and improve summertime performance and subsequent production throughout the rest of the lactation. Improvement in reproductive performance also may impact the following lactation. These systems generally increase removal of body heat by transferring heat directly or indirectly to water and increasing air velocity over the cows. Previous studies and recommendations demonstrated that increasing soaking frequency and providing 600 to 700 cubic feet per minute (CFM) of supplemental airflow reduced respiration rates and rectal temperatures. In these experiments, airflow direction was from the head to the tail. But the most common direction of supplemental airflow used on commercial dairy farms is directed to the side of the cow when standing at the feedline or resting in free stalls.

Department of Biological and Agricultural Engineering.

The objective of this study was to determine the effects of differing amounts of airflow directed to either the side or head of lactating dairy cattle.

Experimental Procedures

Seven heat-stressed, mid-lactation Holstein cows averaging 250 days in milk and producing an average of 84.5 pounds of milk were arranged in a 7 × 7 Latin-square design. Cows were housed in a free-stall barn, fed for ad libitum intake, and milked twice daily. Cows were allowed to become heat stressed and then were moved to a tie-stall barn for a period of 2 hours at 2:00 p.m. of each afternoon of testing. Seven different combinations of airflow velocity and direction of flow were evaluated on seven hot and humid afternoons. Treatments were control (C) or one of six heat-stress-abatement combinations. All heat-stress-abatement treatments included a low-pressure soaker system that wet the cattle for 1 minute at 5-minute intervals. Supplemental airflow was provided by axial flow at one of three velocities: 500, 750, or 900 CFM. Airflow was either from the front to rear (FRT) or from the right side (SIDE) of the cow. Combined cooling treatments were FRT-500, FRT-750, FRT-900, SIDE-500, SIDE-750, or SIDE-900.

Operation of the heat-abatement systems began after 15 minutes of initial observation. Respiration rates were observed and recorded every 5 minutes throughout the 2-hour testing period. Body surface temperature was measured with a digital infrared thermometer and recorded every 5 minutes during the testing period. Body temperature was measured and recorded every minute with a data logger and vaginal probe and subsequently was averaged by 5-minute intervals before analysis. Data from the first 15 minutes and final 15 minutes were averaged as initial and final observations. All data were subjected to analysis of variance, with treatment as a fixed effect and period and cow as random variables. Time (5-minute interval) was utilized as a repeated measure within cow.

Results and Discussion

Cooled-stall temperatures averaged 2ºF less, with 10% greater relative humidity compared with that of controls (92 vs. 94 ºF). Final respiration rates of treated cattle were less ($P < 0.01$) than those in controls (Table 1). Although treated and control cows did not differ during the initial measurements, a 32% reduction in respiration rates was observed during the final 15 minutes of the study. No differences were observed in final respiration rates among cooling treatments. Respiration rates did not vary for control cows over time (Figure 1). When cows were treated with heat-abatement systems, however, respiration rates declined in a similar manner for all treatments.

Rear-udder skin surface temperature did not differ initially among treatments, but was reduced ($P<0.05$) by cooling treatments, compared with that of the control (Table 2). Cooling-system treatment differences were observed between FRT-500 and SIDE-900. Little variation was observed in rear-udder skin temperature during the treatment period (Figure 2). When airflow was reduced to 500 CFM, the rate of temperature reduction was reduced, compared with that of the other treatments.

Final vaginal temperatures were less ($P<0.05$) in all cooling treatments than in controls (Table 3). The only difference among treatments was between FRT-500 and FRT-900. Control cows had similar vaginal temperatures during the entire period of the experiment (Figure 3), and the decrease in vaginal temperature resulting from FRT-500 was less than that of the other treatments.

When cows were treated with heat-abatement systems, declines in respiration rate, rear-udder skin surface temperature, and

vaginal temperature were observed. This agrees with several studies. Minimal differences occurred in the responses to treatments. When airflow was reduced to 500 CFM, there was a reduction in the rate of decline of vaginal temperature and rear-udder skin temperature. This response was more pronounced when the direction of airflow was head to tail. When airflow was 750 or 900 CFM, the responses were similar. Airflow of less than 750 CFM may not adequately reduce heat stress in dairy cattle. These data also indicate that there is no advantage to increasing the airflow more than 750 CFM, when using a low-pressure soaker system in conjunction with supplemental airflow.

Table 1. Average Initial and Final Respiration Rates of Cows Treated with Different Cooling Systems

	Initial	Final	
Treatment*	Breaths/minute		SE
Control	109.0	113.0[a]	7.0
FRT-500	110.5	79.6[b]	7.0
FRT-750	107.4	78.7[b]	7.0
FRT-900	110.1	73.9[b]	7.0
SIDE-500	111.8	79.4[b]	7.0
SIDE-750	114.3	75.0[b]	7.0
SIDE-900	106.3	71.8[b]	7.0

*Control = no cooling system, FRT-500 = head to tail airflow at 500 CFM, FRT-750 = head to tail airflow at 750 CFM, FRT-900 = head to tail airflow at 900 CFM, SIDE-500 = right side airflow at 500 CFM, SIDE-750 = right side airflow at 750 CFM, and SIDE-900 = right side airflow at 900 CFM.

[a,b]Means within column having different superscripts letters differ ($P<0.01$).

Table 2. Average and Final Rear-udder Skin Surface Temperatures of Cows Treated with Different Cooling Systems

	Initial	Final	
Treatment*	°F		SE
Control	99.3	99.3[a]	1.0
FRT-500	99.7	98.2[a,b]	1.0
FRT-750	99.0	97.2[b,c]	1.0
FRT-900	99.0	97.0[b,c]	1.0
SIDE-500	99.3	96.8[b,c]	1.0
SIDE-750	99.3	96.6[b,c]	1.0
SIDE-900	99.7	96.1[c]	1.0

*Control = no cooling system, FRT-500 = head to tail airflow at 500 CFM, FRT-750 = head to tail airflow at 750 CFM, FRT-900 = head to tail airflow at 900 CFM, SIDE-500 = right side airflow at 500 CFM, SIDE-750 = right side airflow at 750 CFM, and SIDE-900 = right side airflow at 900 CFM.

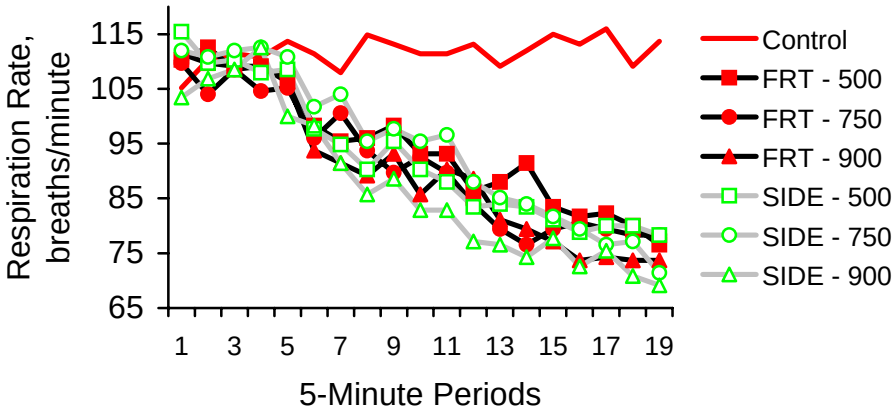
[a,b,c]Means within column having different superscripts letters differ ($P<0.05$).

Table 3. Average and Final Vaginal Temperatures of Cows Treated with Different Cooling Systems

	Initial	Final	
Treatment*	°F		SE
Control	103.5	104.4[a]	0.6
FRT-500	104.0	103.3[b]	0.6
FRT-750	103.5	102.6[b,c]	0.6
FRT-900	103.5	102.2[c]	0.6
SIDE-500	103.8	102.6[b,c]	0.6
SIDE-750	103.5	102.4[b,c]	0.6
SIDE-900	103.8	102.6[b,c]	0.6

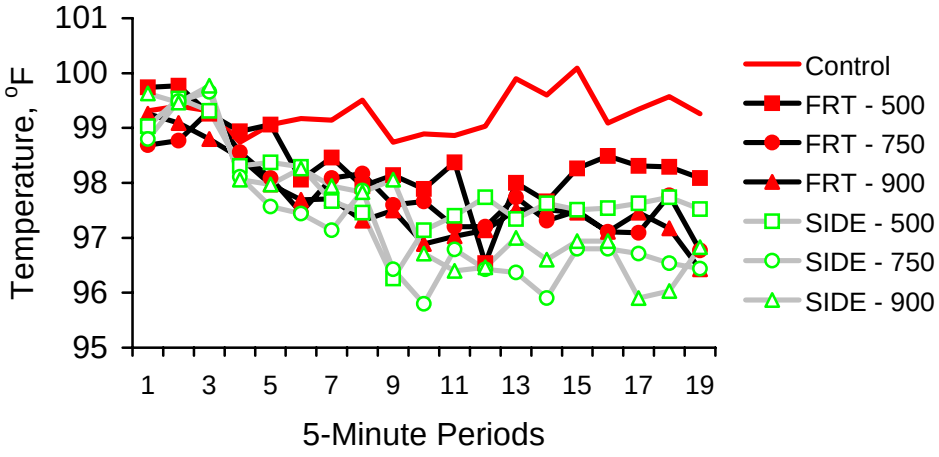
*Control = no cooling system, FRT-500 = head to tail airflow at 500 CFM, FRT-750 = head to tail airflow at 750 CFM, FRT-900 = head to tail airflow at 900 CFM, SIDE-500 = right side airflow at 500 CFM, SIDE-750 = right side airflow at 750 CFM, and SIDE-900 = right side airflow at 900 CFM.

[a,b,c]Means within column having different superscripts letters differ ($P<0.05$).



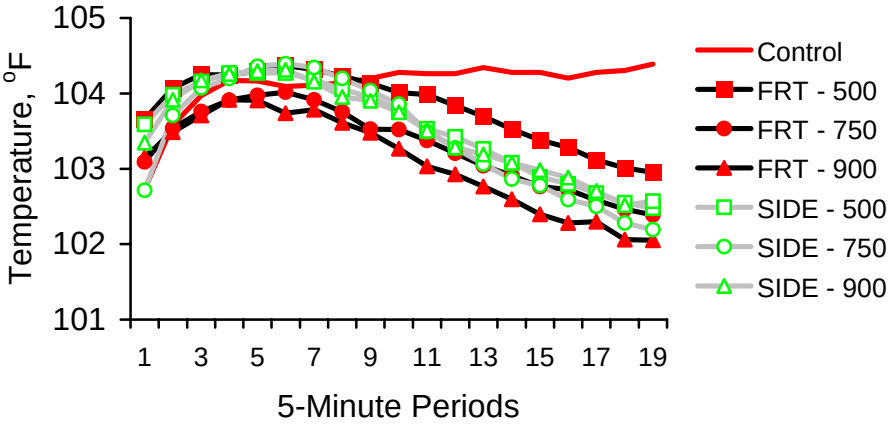
Control = no cooling system, FRT-500 = head to tail airflow at 500 CFM, FRT-750 = head to tail airflow at 750 CFM, FRT-900 = head to tail airflow at 900 CFM, SIDE-500 = right side airflow at 500 CFM, SIDE-750 = right side airflow at 750 CFM, and SIDE-900 = right side airflow at 900 CFM.

Figure 1. Average Respiration Rates of Cows Treated with Different Cooling Systems.



Control = no cooling system, FRT-500 = head to tail airflow at 500 CFM, FRT-750 = head to tail airflow at 750 CFM, FRT-900 = head to tail airflow at 900 CFM, SIDE-500 = right side airflow at 500 CFM, SIDE-750 = right side airflow at 750 CFM, and SIDE-900 = right side airflow at 900 CFM.

Figure 2. Average Rear-udder Skin Surface Temperatures of Cows Treated with Different Cooling Systems.



Control = no cooling system, FRT-500 = head to tail airflow at 500 CFM, FRT-750 = head to tail airflow at 750 CFM, FRT-900 = head to tail airflow at 900 CFM, SIDE-500 = right side airflow at 500 CFM, SIDE-750 = right side airflow at 750 CFM, and SIDE-900 = right side airflow at 900 CFM.

Figure 3. Average Vaginal Temperature of Cows Treated with Different Cooling Systems.

EFFECT OF A NEW TEAT DIP ON SOMATIC CELL COUNT, INCIDENCE OF MASTITIS, AND MILK PRODUCTION IN A COMMERCIAL DAIRY

J. E. Shirley, W. F. Miller, J. Rottinghaus, and E. C. Titgemeyer

Summary

Five hundred and thirty Holstein cows located in a commercial dairy herd near Birdseye, Indiana, were used to evaluate an iodophor (0.50% iodine) teat dip containing a new conditioner. The new teat dip (Dinerin) was evaluated against a common commercial teat dip (WestAgro Iodozyne pre-dip and WestAgro Blockade post dip). The study was conducted February 17 through June 25, 2004. Cows treated with Dinerin teat dip had lower somatic cell counts and produced more milk than those treated with the WestAgro products.

(Key Words: Teat Dip, SCC, Milk Yield.)

Introduction

A new teat dip, Dinerin, developed by KO Manufacturing, Inc. (Springfield, MO) was evaluated in the Kansas State University dairy in 2003 against Westfalia/Surge DERMA-KOTE and TEAT-KOTE 10-3. Although cows treated with Dinerin (0.50% iodine) had fewer cases of mastitis and numerically lower cell counts, a larger study was required to determine statistical relevance. Management and facilities may influence the general effectiveness of a teat dip; thus, the purpose of this study was to evaluate Dinerin teat dip in a commercial dairy located in Indiana. Further, the number of cows used was increased in an effort to evaluate milk response and to provide statistical relevance to the data.

Procedures

Holstein cows (n = 530) located in a commercial dairy herd near Birdseye, Indiana, were used to evaluate a new teat dip. Cows were housed and managed under normal procedures used by the dairy manager. Cows were housed in pens containing 110 to 140 cows per pen. Eight pens were used to accommodate the normal cow-movement procedures used at the dairy. Four pens of cows were treated and 4 pens of cows were controls. Pretreatment milk yield, milk components, and somatic cell count (SCC) were determined and used in covariant analysis. Treatments were balanced for parity, SCC, milk yield, and previous cases of clinical mastitis. Controls cows (n = 232) were treated under the current farm protocol (WestAgro Iodozyne pre-dip and WestAgro Blockade post-dip), and treated cows (n = 299) were pre- and post-dipped with Dinerin. The study was initiated February 17, 2004, and concluded June 25, 2004.

Normal management routine on the farm involved movement of cows among pens, depending on their reproductive status. Treatments were allotted to pens of cows so that cows within a treatment could be moved to another pen within that treatment to accommodate the normal reproductive program. Once cows were assigned to treatment, they remained on that treatment for the duration of the study or until they were dried-off or left the herd. Duration of the study was 90 days.

Data were collected from all cows initially assigned to treatment and from all cows that entered the treatment pens thereafter. Data collected from cows entering the pens after the study was initiated were not used in the analysis unless the cows completed at least 90 days on test. Cows that were dried off or left the herd before 90 days on treatment were not used in the final analysis. Individual cow milk samples were collected every 2 weeks and shipped to the DHI laboratory in Columbia, Missouri, for determination of fat, protein, urea nitrogen, and SCC. Milk weights were recorded each milk sampling date. Milk yields calculated by DHI were used in the analysis. All cases of clinical mastitis were recorded.

Results and Discussion

Data for milk yield, milk composition, and somatic cell count are shown in Table 1. Cows treated with the Dinerin teat dip (0.50% Iodine) produced ($P<0.05$) more milk than did cows treated with the WestAgro product (76.7 vs 74.2 lb daily). Milk fat percentage was similar, but milk protein percentage was greater in milk from cows treated with WestAgro teat dip. No differences were observed in the pounds of milk fat or protein produced daily. The numerical difference in milk fat percentage and the significant difference in milk protein percentage were attributed to a dilution effect because the pounds of milk fat and protein produced daily were similar. Dinerin teat dip reduced ($P<0.05$) somatic cell count, compared with the WestAgro teat dip (365,000 vs. 423,000 counts per mL of milk). A graphical depiction (Figure 1) of somatic cell counts indicates that cows treated with Dinerin teat dip had lower counts during the entire study. The improvement in daily milk yield was attributed to less sub-clinical mastitis, as evidenced by fewer somatic cells in milk from the cows treated with Dinerin teat dip. No difference was observed in the incidence of clinical mastitis.

Conclusion

Dinerin teat dip containing 0.50% Iodine (KO Manufacturing, Inc., Springfield, MO) reduced somatic cells and improved milk yield when compared with another commercial barrier teat dip when applied during late winter and spring in Indiana.

Table 1. Effect of Teat Dips on Production Traits in a Commercial Dairy

	Treatment		
Item	WestAgro	Dinerin	SE
Milk, lb	74.2[a]	76.7	0.74
Fat, %	3.50	3.46	0.02
Protein, %	3.21[a]	3.15	0.009
SCC × 1000	423[a]	365	25.2

[a]Different ($P<0.05$) from Dinerin.

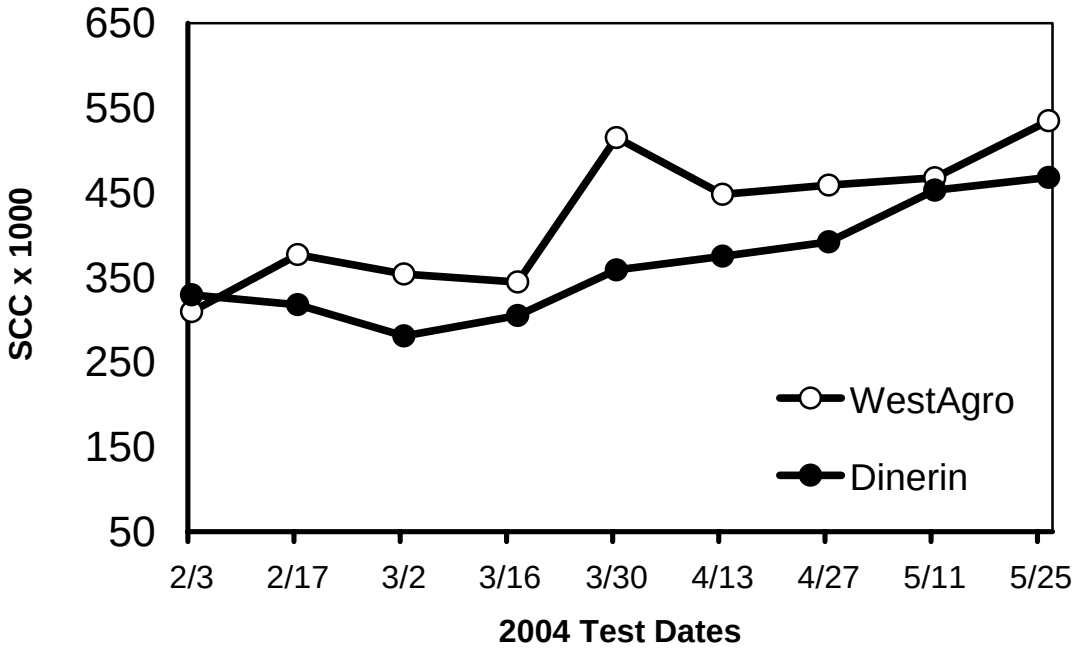


Figure 1. Effect of Teat Dip on Somatic Cell Count (SCC).

JOHNE'S DISEASE: WHERE DO WE GO FROM HERE?

L. C. Hollis

Summary

Johne's disease was characterized as a significant disease in cattle before the start of the 20th century. The disease causes a chronic wasting away and non-responsive diarrhea, coupled with a long incubation period and difficulty in diagnosis until late in the course of disease. As a result, it has become a costly aggravation to dairy producers over the years. Of even greater concern, however, is the more recent incrimination of the causative agent, *Mycobacterium avium* subspecies *pseudotuberculosis* (MAP), as a possible cause of Crohn's disease in humans. Because MAP is present in milk of cows with advanced Johne's disease, and occasionally survives pasteurization, the dairy industry must work proactively to control this disease and reduce the potential for any associated human health risks.

(Key Words: Johne's Disease, Crohn's Disease.)

History

Johne's disease has been a recognized problem in cattle since at least the 19th century. German veterinarians Johne and Frothingham characterized the disease in 1985 and, since that time, our knowledge of its causative agent, pathogenesis, and epidemiology have expanded gradually. According to the 2002 National Animal Health Monitoring Survey for Dairy (http://www.aphis.usda.gov/vs/ceah/cnahs.htm), only 1% of dairy producers had not heard of Johne's disease, 11.4% recognized the name only, 42.3% knew some basics, and 45.3% were fairly knowledgeable about the disease.

Symptoms and Etiology

Johne's disease causes chronic infection of the lower small intestine of ruminants, including dairy and beef cattle; sheep and goats; farm-raised deer, elk and bison; llamas; and wild ruminants (http://www.johnes.org). It is caused by a bacterium, *Mycobacterium avium* subspecies *paratuberculosis* (MAP). The disease spreads through manure-contaminated feed, water, or teats; in milk and colostrum of infected cows; and, occasionally, from mother to calf *in utero*. The organism usually infects young animals, but symptoms normally do not manifest themselves until adulthood. The bacterium grows slowly in the small intestine, causing walls to thicken so nutrients cannot be absorbed. Symptoms include weight loss while maintaining appetite; diarrhea; reduced milk production and fertility; and increased susceptibility to stress, parasites, and concurrent disease.

Based on the results of the 1996 National Animal Health Monitoring Survey for Dairy, it was estimated that 3 to 4% of all dairy cattle are infected with MAP, and that 22% of all herds contain at least 1 MAP-infected animal.

Economic losses from Johne's disease are obviously associated with lost milk production (estimated to range from 2 to 19%), but also result from more insidious problems that are more difficult to measure, such as poor reproductive performance, premature culling, increased susceptibility to concurrent disease, and increased replacement animal costs. National losses are estimated to range from $200 to 250 million per year (http://www.johnes.org).

It is known that MAP passes from infected lactating cows into raw milk. It was previously thought that pasteurization completely destroyed MAP. Recent studies, however, have shown that MAP occasionally survives pasteurization temperatures. The live organism was detected in 2.8% of pasteurized milk cartons purchased from stores in California, Minnesota, and Wisconsin.

Because of the similarity in the nature of lesions and symptoms of the two diseases, there has been speculation that MAP is a cause of a human malady known as Crohn's disease. It, too, is characterized by a long incubation period, with disease rarely appearing before puberty, and by wasting away, chronic diarrhea, granulomatous changes in the lower small intestine, and resistance to treatment. Recent diagnostic efforts using polymerase chain reaction (PCR) technology have detected intact DNA of MAP in a small number of patients having Crohn's disease.

The Next Step

Because of the possibility that MAP may some day be confirmed as the causative agent of Crohn's disease, it would benefit the dairy industry to take a very proactive position and move immediately to initially control, and eventually eradicate, Johne's disease in the nation's cow herds. The Kansas Animal Health Department is currently working with USDA-APHIS in a voluntary program to test Kansas dairy and beef herds in an attempt to identify and eliminate Johne's positive animals from Kansas herds and incorporate best-management practices that are known to reduce the potential for introduction of MAP into a herd or transmission of MAP within a herd.

REDUCED AGE AT FIRST CALVING: EFFECTS ON LIFETIME PRODUCTION, LONGEVITY, AND PROFITABILITY

M. J. Meyer[1], R. W. Everett[1], and M. E. Van Amburgh[1]

Summary

The primary advantages of reducing age at first calving (AFC) include reducing rearing costs as well as reducing time in which the heifer is only a capital drain on farm resources. The primary disadvantage of reducing AFC is that it is frequently associated with a reduction in first-lactation milk yield. Despite this reduction in first-lactation milk yield, production per year of herd life is typically increased by reduced AFC. Furthermore, although the first lactation yield may be influenced by AFC, future lactations are decidedly not. In addition, stayability and health of cows are not influenced by reduced AFC as long as heifers freshen at an adequate weight. Most analyses indicate that the financial advantage afforded from heifers that freshen at a low AFC seems to at the least offset any milk lost during the first lactation. Furthermore, when the time value of money is considered in this analysis, a reduced AFC (~22 months) seems likely to represent a more fiscally sound management decision. When applying these ideas on the farm, a properly managed feeding and breeding program should permit a first-lactation cow to weigh ~1,210 lb after freshening at 22 months of age. The National Research Council recommends a postpartum weight equal to 82% of her mature body weight. This can be achieved with a maximal prepubertal average daily gain (ADG) of 2 lb/day when a traditional preweaning program is employed or 1.8 lb/day when an intensified preweaning program is employed. Because of the well defined link between inadequate body weight at calving and increased mortality and morbidity in first-lactation cows, achieving this target post-calving body weight is of critical importance.

(Key Words: Heifers, Growth, Age at First Calving.)

Introduction

Between birth and first calving, replacement heifers are not generating income. Instead, this rearing period requires considerable capital expenditures, including feed, housing, and veterinary expenses. These expenses constitute 15 to 20% of the total expenses related to milk production. One approach to reducing this cost is to reduce the amount of time between the birth and first freshening of each heifer. The AFC has a profound influence on the total cost of raising dairy replacements in which older calving heifers are more expensive to raise than younger ones. Furthermore, reducing AFC also can improve the profitability of the enterprise by increasing lifetime milk production and milk production per year of herd life. The AFC can be reduced by a combination of increasing prepubertal ADG and decreasing age at breeding or by reducing age at breeding alone.

Universal recommendations for one particular AFC might be incorrect for all cattle on

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all farms, because the recommendation might not represent the management goals or capabilities of a particular production system or farm. This, however, does not mean that we should ignore AFC. Each dairy has its own set of unique management and environmental conditions that make a universal AFC and body weight after first calving a difficult goal to achieve. The purpose of this paper is to address the impact of reducing AFC on milk production, cow stayability, and farm profitability. Target growth also will be introduced and a basis for its use will be provided.

Milk Yield is Influenced by Age and Body Weight at First Calving and by Prepubertal Daily Gains

The biology involved with the interaction between reduced AFC and first-lactation milk yield is difficult to identify and quantify. This is because reducing AFC is often associated with increased prepubertal daily gain, reduced body weight at calving, or both, which have been shown to influence future milk yield.

Body weight at calving is positively correlated with first-lactation milk yield. A body weight of 1,210 lb is the approximate optimum post-calving weight to maximize first-lactation milk yield in U.S. Holsteins. Indeed, we reported no effects of AFC or prepubertal ADG on first-lactation milk yield when the variation in milk yield associated with post-calving BW was removed by covariate analysis.

The effect of increased prepubertal ADG on first-lactation milk yield in Holsteins is not as well defined. A wealth of data exists describing the negative effect that excessive prepubertal energy intake and increased ADG has on first-lactation milk yield in smaller-framed Danish breeds. The period during which excessive energy intake is thought to negatively influence first-lactation milk yield is between 3 months of age and puberty. It was proposed that average ADG in excess of 0.88, 1.32, and 1.54 lb for Jerseys, Danish Red, and Danish Friesians, respectively, would impair first-lactation milk yield. Using U.S. Holsteins, others observed a significant 5 to 13% reduction in first-lactation milk yield by prepubertal heifers growing at greater than 2.2 lb/day, compared with that of heifers grown at 1.69 to 1.56 lb/day. Contrary to the previous information, a third study reported no significant effect on first-lactation milk yield in U.S. Holsteins fed to gain either 2.18 or 1.72 lb/day. The AFC in that study averaged 24 months, without an effect of prepubertal ADG. These authors proposed that U.S. Holsteins might have a greater mature body weight than the Danish breeds, making them less sensitive to greater rates of gain. It is well documented that fat deposition in early-maturing breeds (i.e., smaller framed) is more responsive to plane of nutrition than larger, late-maturing breeds. Therefore, it is plausible that the smaller Danish breeds experience greater whole-body fat deposition than do United States Holsteins grown at a similar rate. This difference in conditioning at parturition is likely to have a negative influence on health and lactational performance in the smaller breeds.

The data on the effect reduced AFC has on first-lactation milk yield by U.S. Holsteins is variable. Some have observed no effect, whereas others have observed a negative effect. Milk yield in second and subsequent lactations has consistently been unaffected by reduced AFC. One study reported greater lifetime production and greater production per year of herd life in heifers that freshened at 23 vs. 26 months of age. Most of the studies suggest that reducing AFC from an average of 24.7 to 21.9 months resulted in an approximate 4.8% reduction in first-lactation milk yield (Table 1). In those studies, AFC was reduced by either reducing age at breeding alone, or in combination with increasing prepubertal ADG.

Effects of Age at First Calving on Health, Reproduction, and Stayability

In addition to milk yield, stayability of cows in a milking herd can have a profound impact on the profitability of the enterprise; therefore, potential effects of reducing AFC must be considered. Researchers from Brigham Young University addressed this issue by raising 443 U.S. Holsteins on an increased or restricted amount of energy from 6 weeks of age until breeding occurred at 748 lb (supporting 1.96 and 1.74 lb/day for the supplemented and restricted groups, respectively). As a result of the increased nutrient intake and younger age at first breeding, heifers receiving greater energy freshened at 22.4 months, compared with 24.6 months for those receiving a restricted amount of energy. Prebreeding ADG and AFC had no effect on milk yield in the first or subsequent six lactations. Calving difficulty was not different between the two AFC groups. Percentage of heifers in the herd at the start of the first, second, third, fourth, fifth, sixth, and seventh lactations averaged 100, 73, 46, 29, 18, 6, and 2%, respectively, with no difference between the two AFC groups. Likewise, after seven lactations, reasons for cows leaving the herd (including reproductive problems, mastitis, died, low production, reproduction, disease other than mastitis, or crippled) were not affected by prepubertal ADG or AFC.

More recently, researchers from the University of California-Davis evaluated the effects of altering age at breeding to change AFC. Holstein heifers (n = 1,933) on 3 commercial California dairy farms were raised similarly from birth through breeding, then retrospectively were assigned to one of three groups on the basis of AFC. All heifers presumably experienced the same pre- and postpubertal ADG. Differences in AFC were achieved by varying age at first conception. Average AFC for the three resulting groups was 22.3 (n = 514), 23.7 (n = 917), and 25.9 months (n = 474) for low, medium, and high AFC, respectively. Total milk yield in the first 310 days was 22,779; 23,461; and 23,665 lb for low, medium, and high AFC, respectively, with the high- and medium-AFC groups producing significantly more milk than the low-AFC group did.

Conception rate at first AI after freshening and number of inseminations per cow were 27.9% and 3.27, 36.9% and 2.85, and 30.8% and 3.23 for low, medium, and high AFC, respectively. Low- and high-AFC groups had poorer reproductive performance in both categories than did medium AFC. Number of days open for pregnant cows was unaffected by AFC and averaged 121 across the three groups. Furthermore, the fraction of cows pregnant at 310 days in milk averaged 80.6% and did not differ among groups. The percentage of cows having abortion averaged 10% and was not influenced by AFC. Difficulty associated with conception in early-calving heifers could be attributed to a greater energy demand for growth, resulting in less energy available for reproduction, when compared with that of presumably heavier heifers that freshened at an older age.

It has been well documented that calving difficulty has profound negative impacts on the health, reproduction, and stayability of first-lactation cows. Furthermore, heifer size at first calving, but not AFC, is correlated to incidence of dystocia. Supporting this data, the California researchers observed no effect of AFC on calving difficulty scores, indicating that their lowest mean body weight at calving (1,254 lb, low AFC group) was adequate to ensure ease of calf delivery. It should be noted in that study that body weight of pregnant heifers was estimated by using heart girth measurements. These authors also reported that, across all AFC treatments, cows requiring physical assistance with calving had greater mortality (4.9%) than those not having dystocia (2.7%). These facts illustrate the critical role that body weight at first calving

has on the long-term health and stayability of cows.

In the California study, neither the incidence of health problems, culling, or mortality was influenced by AFC. Health problems (and average incidence among the three AFC groups) included retained placental membranes (3.3%), left displaced abomasum (2.9%), lameness (15.0%), and mastitis (19.4%). The AFC did not affect the incidence of these health problems. Likewise, AFC had no affect on the fraction of heifers culled after calving (17.6%) or on mortality (3.9%). There was, however, a tendency for the low-AFC heifers to die earlier in lactation than did the high-AFC cows (20.3 vs. 77.7 d). But the fraction of heifers that died or were sold before 310 DIM averaged 21.7% and was similar across all three AFC groups. In addition, survival analysis showed that the interval from calving to date leaving the herd averaged 255 d and was not affected by AFC.

A group from the University of Florida observed no effect of AFC on survival of first-lactation cows to begin a second lactation for 1,144 Florida Holstein heifers. Taken together, these studies strongly indicate that heifers freshening at ~22 months with adequate post-calving body weights have similar stayability in the herd as those freshening at older ages. The negative effect of freshening heifers under weight is quite profound and cannot be ignored.

Economics of Increased Prepubertal Daily Gains and Reduced Age at First Calving

The economics associated with reducing AFC has been the topic of much debate. Under the assumption that increased prepubertal ADG negatively influences first-lactation milk yield, a researcher from Ohio State University analyzed data from three studies to determine the economically optimal prepubertal ADG for U.S. Holsteins. To assess the financial impact of freshening heifers at a younger age, the net present value or time value of money ("a dollar five years from now is not worth the same as a dollar today") was considered. This assigns economic benefit to heifers generating income sooner by initiating lactation at a younger age. The economic optimum for prepubertal ADG and AFC was estimated to be between 1.98 and 2.42 lb/day and 22.4 and 20.6 months, respectively.

An economic analysis of the California study previously described found average AFC (and 310-d milk yield) for the three resulting groups to be 22.3 months (22,779 lb), 23.7 months (23,461 lb), and 25.9 months (23,665 lb) for low, medium, and high AFC, respectively. Rearing costs for the medium- and high-AFC groups were $40.34 and $107.89, respectively, more than that of the low-AFC group. Income for each AFC group was adjusted for the cost of rearing, estimated feed to increase milk yield, stillbirths, diseases, days open, culling, mortality, labor cost, and the value of milk and calf produced, as well as the value of a cow at the end of the 310-day study. Adjusted income was $119.73 and $9.08 more for the medium- and high-AFC cows, respectively, than for the low-AFC cows. These values were not significantly different, implying that no single AFC had an economic advantage over another. But these researchers did not consider the net present value of money in that analysis. If the net present value had been considered, it would presumably shift the economic advantage to the low-AFC heifers.

A Within-herd Analysis: Effects of AFC on Milk Production and Stayability

We recently evaluated the within-herd effect of AFC on lifetime milk production, stayability, and number of lifetime productive days. Lactation records from 2,519,232 first-lactation cows from 937 herds in California and the Northeast were analyzed by using the test-day model (TDM). The lactations oc-

curred between January 1985 and December 2002. The TDM was employed for this analysis because it describes and accounts for factors that influence lactational performance such as calving year, season, management, and environment. Test-day residuals include the random genetic cow effects and treatment effects (AFC in this instance). These residuals are simultaneously adjusted for herd test day, days in milk, calendar month fresh, pregnancy, and management effects. Test-day analysis is considered to be a more appropriate approach to this type of data analysis because it assumes that global conditions are inappropriate for evaluating management (i.e., AFC) among different herds.

Average AFC for each year was calculated for each of the 973 herds. Within a herd, heifers were then retrospectively assigned to one of five treatments that fit around the herd mean AFC. The AFC treatments consisted of: 1) less than –63 days from the herd-by-year average AFC; 2) –22 to –63 days from the herd-by-year average AFC; 3) –21 to +21 days from the herd-by-year average AFC; 4) +22 to +63 days from the herd-by-year average AFC; and 5) greater than 63 days from the herd-by-year average AFC. Actual AFC across all herds for the five groups was 23.3, 24.3, 25.6, 27.2, and 30.3 months, respectively.

Once assigned to an AFC group, cows were assigned to one or more opportunity groups defined as 3, 4, 5, 6, 7, or 8 years of age. Heifers were assigned to opportunity groups if they had the opportunity to be the age of the group. For example, a cow that has had the opportunity to be 5 years old at the time of test would be assigned to the 3, 4 and 5 year opportunity groups, but not to the 6, 7, or 8 year group. The sum of her 5-year total milk production and the sum of her 5-year total productive days (defined as the number of days lactating) would be averaged with all other cows in the 5-year opportunity group. Likewise, the sum of her 4-year total production and the sum of her 4-year total milk production would be averaged with all other cows in the 4-year opportunity group, and so forth. By the definition of an opportunity group, her data would be included in the 5-year opportunity group even if she had died at 4 years of age. Use of opportunity groups in this analysis permitted the evaluation of total milk produced and total productive days at a given age across treatments (i.e., AFC) while simultaneously discounting the treatments for any treatment-associated differences in early-death loss.

The total number of cows in each of the five AFC treatments and the number assigned to their appropriate opportunity groups is in Table 1. These treatments will be referred to by their average AFC from this point on. The differences in AFC within farm and year are assumed to have arisen from a combination of differences in prepubertal ADG and age at first breeding or from differences in age at first breeding alone.

Perhaps the most obvious benefit of reducing AFC is its effect on the number of productive days in a cow's lifetime. Total productive days of cows in each AFC treatment are in Table 2. Heifers freshened at a younger age enter the productive phase of their life sooner. Heifers with a younger AFC have an advantage over those freshening at an older age throughout all six opportunity groups. Within a given AFC treatment, number of productive days increases rapidly in the first three opportunity groups, and then its rate of increase slows as cows approach the 8-year opportunity group. This occurs because moving from the 7- to the 8-year opportunity group, for example, likely only adds a few cows that have actually survived this long. Their number of productive days (presumably quite high) is averaged with all other cows in that AFC group, which is likely heavily weighted with cows that died at a younger age and, therefore, had fewer productive days. This causes the increase in number of productive days to slow

in the older opportunity groups. This can be observed happening across all five AFC treatments.

Effect of AFC on number of productive days for the five AFC treatment groups is more clearly illustrated in Figure 1, in which the data are represented as a difference from the 25.6-month-AFC treatment. Again, it is apparent that heifers in the 23.3-month-AFC treatment have the obvious advantage over all other AFC groups. This is despite the average number of productive days decreasing from a high of 59 days more than the 25.6-month AFC group in the 3-year opportunity group to approximately 35 days in the 5- through the 8-year opportunity groups.

The most effective way to evaluate the benefit of reducing AFC and increasing the number of productive days in a cow's lifetime is to consider her lifetime milk production. Lifetime milk production for each of the five AFC treatment groups in the six opportunity groups is given in Table 3. For heifers in the 3-year opportunity group, those that freshened at 23.3 months produced nearly twice the amount of milk as those that freshened at 30.3 months. This trend for increased lifetime milk production continues even to the 8-year opportunity group. As was observed with the total number of productive days, within a given AFC treatment group, the total milk production in each opportunity group increases rapidly from the three- through 5-year opportunity groups, then slows as cows progress through to the 8-year opportunity group. This occurs for the same reason total number of productive days increases at a rapid, then slower, rate.

The differences across the five AFC treatment groups in lifetime milk yield are more visually apparent in Figure 2. Lifetime milk production in this figure is presented as the difference from the 25.6 AFC group, which represents the herds' average AFC. Heifers calving in the 23.3-month-AFC group produced more milk in their lifetime than all other AFC groups through the 5-year opportunity group, with the greatest difference at the 3- and 4-year opportunity groups. In the 3-year opportunity group, few heifers in the 30.3-month-AFC group have freshened, and those that have freshened are likely only in the early stages of their first lactation. Even though the increase in milk yield for the 23.3- and 24.3-month-AFC treatments becomes similar by 6 years, the production and economic advantage lies with the 23.3-AFC group, because they produce more milk sooner. This allows the producer to capitalize on the time value of money discussed previously. In agreement with other data, this increase in lifetime milk production clearly illustrates the advantage of freshening at a younger age.

At first glance, average stayability (percentage survival) for the five AFC treatments (Table 4) seems to weigh heavily against calving at earlier AFC. Other researchers have reported that stayability is not influenced by AFC. Although our data may seem to lead to a different conclusion, it is important to understand that we have calculated stayability by using a different time reference than others have used. They calculated stayability after the conclusion of the first lactations. Therefore, heifers from both high- and low-AFC groups were "exposed" to the same length of lactation, and all had an equal amount of production data upon which culling decisions were made. In our assessment, we compared stayability at a common age; so, for example, in the 3-year opportunity group, heifers in the 23.3-AFC treatments were milking an average of 355 days, whereas those in the 30.3-AFC treatment were milking an average of 166 days (Table 2). Therefore, younger calving heifers have more than twice the amount of production data from which culling decisions could be made and they have a decidedly greater opportunity to be culled. Perhaps most important to note is that the differences in stayability between heifers in the 23.3-, 24.3-,

and 25.6-AFC treatments are quite similar, even at the lowest age opportunity groups. The differences between how we compare stayability across AFC treatments and how others made their estimates is important to understand.

Summary of Within-herd Analysis of AFC

Taken together, these data support management decisions that result in working toward a lower AFC. The increase in lifetime milk production that results from calving at 23.3 months, compared with 25.6, 27.2, or 30.3 months, is substantial and difficult to ignore. Although the lowest average AFC was 23.3 months, it seems safe to conclude that further increases in number of productive days and lifetime milk production would occur when average AFC was further reduced to 22 months. This conclusion is based upon the stayability data and the lifetime-production data from other studies.

A Systematic Approach to Managing Heifers: Target Growth

The target-growth system was first modified for dairy heifers, and further modified and adapted by the National Research Council, to establish a systematic approach to heifer management. The objective of a farm manager should be to determine at what age-weight relationship cattle generate the greatest marginal profit and then manage to that target. Results presented herein indicate that the optimal age and body weight at first calving is around 22 months and 1,210 lbs, but variability in mature body size was not considered in these suggestions. The biologically and economically ideal body weight and age at calving is expected to differ with differing body weight at maturity. Therefore, knowing a herd's mature body weight is required for successful application of this system. At its core, the target-growth program is a straightforward approach to determining, managing, and attaining these age and body-weight targets.

The National Research Council recommended that body weight at first conception and after first calving should be 55% and 82% of mature body weight, respectively. If the mature body weight of the typical high-merit U.S. Holstein is assumed to be 1,474 lb, this would set the target body weight at first conception and after calving at 807 and 1,210 lb, respectively. Naturally, an earlier target AFC requires greater rates of gain to achieve target weights at conception and after calving. With an efficient breeding program, however, AFC can be effectively reduced without requiring excessive prepubertal ADG. An additional approach to control rates of gain between 3 months of age and puberty is to feed for increased preweaning rates when body growth is most efficient.

The third set of targets consists of preweaning ADG and weaning age. Two examples of target-growth solutions are shown in Figures 3 and 4. Both examples illustrate the target weights, ages, and rates of gain for heifers freshening at 22 months and weighing 1,210 lb after calving. All assumptions are similar between the two examples, with the exception of the preweaning program. Figure 3 assumes that a traditional preweaning program is employed that supports an ADG of 1.0 lb/day during the milk-fed phase. Figure 4 assumes an intensified preweaning program that supports a preweaning ADG of 2 lb/day.

Use of an intensified preweaning program yields approximately 56 lb of additional growth after 8 weeks on milk, relative to a traditional calf program. This advantage in body weight at weaning permits less ADG from weaning to conception (2.0 vs. 1.8 lb/day) while still meeting the target age and body weight at conception. This particular intensified preweaning program is, however, more costly than a traditional program simply because more milk (or milk replacer) is required

to achieve the additional weight gain. If a high quality all-milk milk replacer is used, a heifer on this intensified program would require approximately 57 lb more powder to achieve the additional 56 lb of body weight at weaning. Starter also would be consumed starting by approximately 3 weeks of age to support this additional body weight at weaning.

Table 1. Recent Publications Evaluating the Effect of Reduced Age at First Calving (AFC) on First-Lactation Milk Yield

	Prepubertal ADG, lb/day		Weight after calving, lb		AFC, months		First-lactation milk yield, lb		
Study	Late AFC	Early AFC	Late AFC	Early AFC	Late AFC	Early AFC	Late AFC	Early AFC	Milk yield, % change
1	1.63[1]	1.63[1]	1,107	997	26.1	22.9	9,797[2]	9,189	–6.2
2	NR[3]	NR	NR	NR	24.6	22.2	15,367	14,804	–3.7
3	NR	NR	1,276	1,212	23.6	20.6	18,240	16,548	–9.3
3	NR	NR	1,324	1,291	25.6	22.7	17,789	17,310	–2.5
4	1.50	1.84	1,115	1,197	23.0	21.9	20,176[x]	21,173[y]	+4.9
5	1.50	2.07	1,210	1,144	24.5	21.3	21,721[a]	20,651[b]	–4.9
6	1.69	2.46	1,186	1,133	23.6	20.7	18,962[a]	16,507[b]	–12.9
7	1.69	2.49	NR	NR	25.4	22.4	15,745[a]	14,969[b]	–4.9
8	1.65[2]	1.65[2]	1,327	1,256	25.9	22.3	23,665[a]	22,779[b]	–3.7
Mean	-	-	-	-	24.7	21.9	-	-	–4.8

[1]Prepubertal ADG not reported. Rate was calculated on the basis of data included in the paper.
[2]First-lactation milk yields were reported to be similar when AFC was used as a covariate.
[3]Not reported.
[a,b]Means within study having different superscript letters differ ($P<0.05$).
[x,y]Means within study having different superscript letters differ ($P<0.10$).

Table 2. Numbers of Cows Used in the Analysis of Five Age-at-First-Calving (AFC) Treatments and Six Age Opportunity Groups

	AFC treatments, months				
Age	23.3	24.3	25.6	27.2	30.3
– years –	---------- No. of cows ----------				
3	251,399	737,311	824,970	360,487	345,065
4	221,654	638,167	715,156	314,981	305,576
5	193,777	550,140	610,959	272,799	266,159
6	165,115	461,744	515,814	232,651	228,212
7	138,243	383,724	426,985	193,439	191,674
8	110,552	304,365	347,693	159,481	158,338

Table 3. Average Number of Productive Days for Five Age-at-First-Calving Treatments and Six Age Opportunity Groups

	AFC treatments, months				
Age	23.3	24.3	25.6	27.2	30.3
– years –	---------- Productive days ----------				
3	354.8	328.5	296.0	252.4	165.7
4	614.2	593.8	565.7	527.6	450.5
5	796.9	781.0	756.5	723.9	652.4
6	915.9	902.6	880.7	850.6	781.9
7	990.8	975.6	956.1	929.3	861.2
8	1,035.0	1,017.8	998.0	973.1	906.5

Table 4. Average Total Milk Production across Five Age-at-First-Calving (AFC) Treatments and Six Age Opportunity Groups

	AFC treatments, months				
Age	23.3	24.3	25.6	27.2	30.3
– years –	---------- Average total milk production, lb ----------				
3	19,758	18,484	17,345	15,803	10,941
4	34,659	33,609	32,318	30,540	26,224
5	45,445	44,568	43,243	41,503	37,554
6	52,483	51,746	50,490	48,809	45,054
7	56,874	56,005	54,846	53,330	49,632
8	59,424	58,450	57,255	55,858	52,239

Table 5. Average Stayability (Percentage Survival) Across Five Age-at-First-Calving (AFC) Treatments and Six Age Opportunity Groups

	AFC treatment, months				
Age	23.3	24.3	25.6	27.2	30.3
– years –	---------- Survival, % ----------				
3	80.2	81.5	82.6	85.5	90.1
4	56.6	61.0	62.3	64.6	66.8
5	39.9	40.9	41.9	43.4	44.2
6	25.5	25.0	25.7	26.6	27.1
7	13.9	14.1	14.6	15.3	15.4
8	7.4	7.4	7.7	8.2	8.2

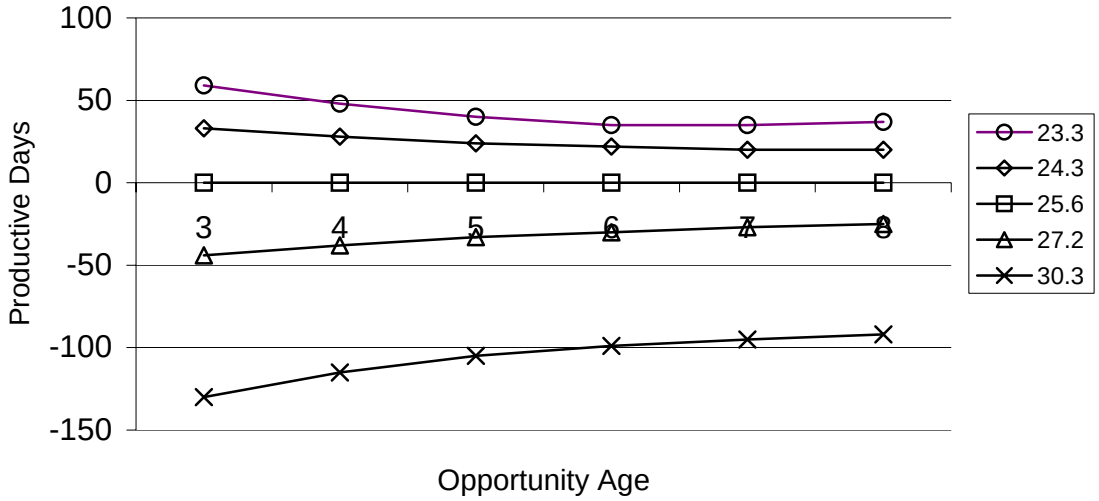

Figure 1. Average Number of Productive Days for Five Age-at-First-Calving (AFC) Treatments and Six Age Opportunity Groups (Difference from mean 25.6-month-AFC treatment).

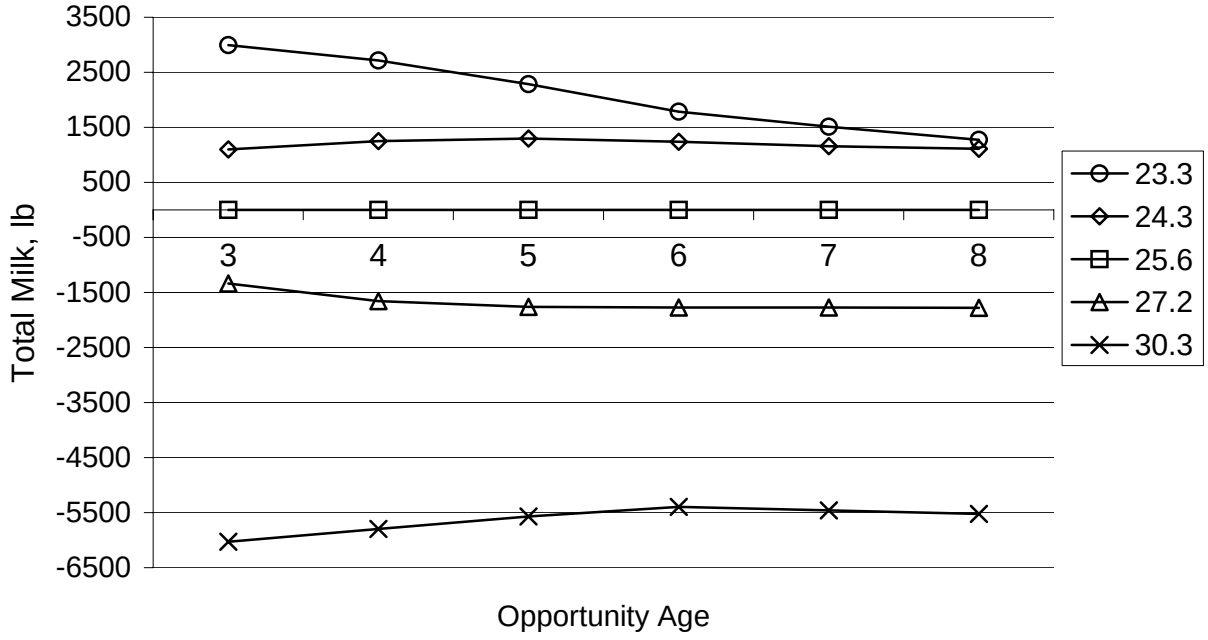

Figure 2. Average Total Milk Production for Five Age-at-First-Calving (AFC) Treatments and Six Age Opportunity Groups (Difference from mean 25.6-month-AFC treatment).

Inputs	
Age first calving, mo	22
Preweaning ADG, lb/day	1.00
Weaning age, weeks	8
Mature BW, lb	1474
BW at conception, % MBW	55%
BW after 1st calf, % MBW	82%

Assumptions	
Birth wt, lb	90
Conceptus wt at birth, lb	132
Conceptus growth in last 1/3 of gest., %	65%
Gestation length, days	279
BW at puberty, lb	620

	Birth	Weaning	Puberty	Conception	Pre-Calving	Post-Calving
BW, lb	90	146	620	811	1341	1209
Age, weeks	0	8	38	56	96	96
Age, days	0	56	268	392	671	671
Age, months	0	0.3	8.8	12.9	22	22
BW, % mature, BW	6%	10%	42%	55%	91%	82%

ADG SOLUTIONS:

Required Rates of Gain	lb/day	
Weaning to conception	1.98	
Conception to pre-calving	1.90	(includes conceptus growth)
Conception to pre-calving	1.43	(does NOT include conceptus growth)

Figure 3. Required Rates of Gain from Birth to Freshening to Meet Target Body Weight and Age at Conception and First Calving When a Standard Preweaning Program is Employed.

Inputs	
Age first calving, mo	22
Preweaning ADG, lb/day	2.00
Weaning age, weeks	8
Mature BW, lb	1474
BW at conception, % MBW	55%
BW after 1st calf, % MBW	82%

Assumptions	
Birth wt, lb	90
Conceptus wt at birth, lb	132
Conceptus growth in last 1/3 of gest., %	65%
Gestation length, days	279
BW at puberty, lb	620

	Birth	Weaning	Puberty	Conception	Pre-Calving	Post-Calving
BW, lb	90	202	620	811	1341	1209
Age, weeks	0	8	42	56	96	96
Age, days	0	56	293	392	671	671
Age, months	0	0.3	9.6	12.9	22	22
BW, % mature BW	6%	14%	42%	55%	91%	82%

ADG SOLUTIONS:

Required Rates of Gain	lb/day	
Weaning to conception	1.81	
Conception to pre-calving	1.90	(includes conceptus growth)
Conception to pre-calving	1.43	(does NOT include conceptus growth)

Figure 4. Required Rates of Gain from Birth to Freshening to Meet Target Body Weight and Age at Conception and First Calving When an Intensified Preweaning Program is Employed.

ACCELERATED GROWTH PROGRAMS FOR DAIRY CALVES

M. J. Brouk

Summary

Accelerated-growth feeding programs are the newest buzz word in calf rearing. Accelerated programs require a milk replacer containing more crude protein and less fat content than traditional milk replacers. These programs are generally phase-feeding programs that increase the amount of milk replacer as the calf advances in age. In addition, changes in the calf starter are necessary to achieve optimal performance. These programs increase weight gain during the liquid-feeding period and may positively impact calf health. Changes in the composition and amount of milk replacer used increase the cost of the accelerated program, compared with that of conventional programs. Gains achieved from an accelerated-growth program during the first few weeks of life are quickly lost if aggressive feeding and management programs are not followed through after weaning. Accelerated-growth calf programs are part of the total heifer rearing program to improve overall lactation efficiency by reaching optimal growth and age-at-first-breeding targets for dairy operations.

(Key Words: Heifers, Calves, Accelerated Growth.)

Introduction

Traditional liquid-feeding programs for dairy calves used milk replacers that contained enough energy and crude protein for maintenance and a minimal amount of growth. Most growth was the result of increased starter intake as the calf advanced in age. These programs generally resulted in 0.5 to 1.0 lb of gain per day from birth through weaning. Typical traditional milk replacers contained 20% crude protein and 20% fat on a solids basis. Whole milk (Holstein) generally contains about 25% crude protein and 29% fat on a dry matter basis. Estimated gain resulting from typical milk-replacer intake is generally about half that of whole milk. It has been recognized for many years that, although milk replacer programs may achieve adequate growth rates, calves allowed to consume more whole milk had greater rates of growth. This improved growth and the goal of achieving faster and more efficient gain led to the development of new, and more aggressive, liquid-feeding programs for young calves. These programs are designed to more closely match the early calf nutrition program with the advanced genetic potential of modern dairy heifers. Using an accelerated calf-growth program, followed by aggressive feeding and management, may result in heifers having greater height, lean tissue, and body weights at younger ages than result from conventional programs, increasing the potential for larger heifers at calving.

Changes in Liquid Feeding

Feeding colostrum for the first 1 to 3 feedings does not change with accelerated programs. Calves must obtain adequate amounts of immunogloblins early in life to develop adequate disease resistance. Traditional milk replacers contain 20% fat and 20% crude protein, and were generally fed at 1 lb of powder per day from birth until weaning. Milk replacers for accelerated programs contain 25 to 28% crude protein and 15 to 16% fat. These are fed in a phase program using about 1.25 lb of powder per day for the first week of life, followed by increasing amounts, reaching 1.8 pounds of powder per day near the end of the

liquid-feeding period. The solids concentration of accelerated programs (14 to 17%) is greater than that of whole milk or traditional milk replacers (13%). Simply increasing the amount of traditional milk replacer powder in a mix will not result in the same concentration of crude protein and fat as that in the accelerated milk replacers.

Changes in Calf Starter

Traditional calf starters may only contain about 18% crude protein (as fed). With accelerated programs, a special starter containing 20 to 22% crude protein (as fed) is required, in combination with the accelerated liquid feeding, to achieve optimal growth. Calves should be consuming 2 lb of a starter diet per day for a minimum of 3 consecutive days before weaning. Reducing liquid feeding by half for the final week before weaning will stimulate intake of the starter diet.

Changes in Post-Weaning Nutrition

If an aggressive post-weaning nutrition program is not adapted, the potential for reduced growth after weaning increases with accelerated growth programs. It is critical that a starter diet with greater nutrient content is fed for 4 to 5 wk after weaning. If growth rate of calves stalls out after weaning, the benefits of the accelerated liquid-feeding programs are soon lost. Good-quality dry forage should be introduced to limit starter diet intake to 5 to 6 lb per calf per day. Maintaining small groups (6 to 10 calves per group) after weaning is another critical factor for the success of these programs. When calves reach 10 to 12 wk of age, switch to a balanced nutrition program to achieve 1.8 to 2.0 lb of gain per day.

Changes in Management

Accelerated calf-feeding programs are not a silver bullet. They require excellent management to realize their maximum full benefit. To maximize genetic potential of calves, increased management is required. Calves need to be provided more milk replacer as age increases. This requires more management and communication with personnel feeding calves. Mistakes in feeding can lead to digestive upsets and increased scouring. Calf stools are generally looser with accelerated programs, and correct identification of scouring and sickness is more difficult. Unnecessary treatment or failure to treat sick calves will significantly reduce the benefits of accelerated calf programs. Because of the increased moisture content of the stool, more bedding is generally required to maintain a comfortable environment for the calf.

Changes in Calf Growth

Increasing lean tissue and bone growth are the most important benefits of accelerated calf programs. According to several studies, heifers should be 2 to 3 inches taller by 3 months of age and contain more lean tissue. In addition, calves should weigh 20 to 30 lb more at weaning than those raised on conventional programs.

Changes in Cost

Accelerated-growth programs generally cost between $35 and $50 more per calf than conventional programs do. Calves grow faster, increase in frame size, and may be healthier than calves raised on conventional programs, but the returns are much more difficult to define. Results from one study indicated that heifers managed for accelerated growth produced more than 900 lb more milk during their first lactation than did conventionally raised heifers. But whole milk fed was obtained by suckling, and the results may not be similar when feeding milk replacers. Further studies to fully explain the benefits of accelerated growth in calves are needed to define the economic benefits of accelerated calf-feeding programs.

PERFORMANCE OF LACTATING DAIRY COWS FED YEAST AND FIBROLYTIC ENZYMES

E. C. Titgemeyer, B. J. Johnson, and J. E. Shirley

Summary

We evaluated the effect of supplementing typical dairy diets with yeast and fibrolytic enzymes on dairy cow performance. Twenty-four Holstein cows were used to evaluate the effects of yeast (Procreatin-7, a live culture of *Saccharomyces cerevisiae*) and various amounts of FP800 (a fibrolytic enzyme mixture) on lactation performance. Treatments were arranged in a 4 × 2 factorial design consisting of 8 treatments: 0, 5, 10, or 15 g of FP800 per day and 0 or 5 g of Procreatin-7 per day. Design and conduct of the experiment allowed at least 10 observations in each of the 8 treatment combinations. Within each 28-day period, the first 2 weeks were used for adaptation to treatment and the next 2 weeks were used for measuring feed intake and milk production. Diets were fed individually to each cow twice daily. The diet contained 22% ground corn, 20% corn silage, 20% wet corn gluten feed, 17% alfalfa, 8% whole cottonseed, and 8% expeller soybean meal. Dietary protein was 19% of dry matter. Treatments were top-dressed to the diets. Cows were milked twice daily. Dry matter intake averaged 64.6 lb/day, milk production averaged 96.8 lb/day, and efficiency of milk production averaged 1.50 lb milk/lb dry matter intake. Dry matter intake, milk production, milk efficiency, and production of all milk components were not changed by addition of either fibrolytic enzymes or yeast. Percentages of fat, protein, and solids-not-fat (SNF) in milk were also not affected by treatment. The results demonstrated no production responses to the addition of fibrolytic enzymes or yeast to the diets of lactating cows under our experimental conditions.

(Key Words: Yeast, Milk Yield, Fibrolytic Enzymes.)

Introduction

A number of experiments have evaluated the effectiveness of live yeast (*Saccharomyces cerevisiae*) cultures on performance of dairy cows. Many of these studies have evaluated performance under conditions of stress to the animal, with the assumption that yeast will reduce some of the digestive disturbances related to variations in feed intake associated with the stressful environment (diet transition or heat stress). Under these conditions, positive responses to yeast cultures have been observed. Applicability of yeast products to a greater segment of the industry, however, has not been as extensively studied.

In a series of experiments at Kansas State University, we have studied in vitro the effects of a number of fibrolytic enzyme mixtures. Among these enzymes, an enzyme mixture containing increased cellulase activity was found to be efficacious in improving in vitro dry matter disappearance of fibrous dairy feeds such as alfalfa, corn silage, corn gluten feed, and soybean hulls. We also observed that responses could be obtained with relatively small amounts of supplementation.

The objective of this study was to evaluate the effects of supplementing typical dairy diets with yeast (live culture of *Saccharomyces*

cerevisiae) and fibrolytic enzymes on milk yield.

Experimental Procedures

Twenty-four Holstein cows were maintained in tie-stalls and used to evaluate the effects of yeast (Procreatin-7, a live culture of *Saccharomyces cerevisiae* produced by Saf Agri, Milwaukee, WI) and various amounts of FP800 (a fibrolytic enzyme mixture produced by Saf Agri) on lactation performance. The cows studied were typical of cows in the dairy industry. They averaged 106 days in milk at the start of the experiment (range: 59 to 239). All cows were treated with bST (Posilac) on the first day of the experiment and biweekly afterward.

The treatments were arranged in a 4 × 2 factorial consisting of 8 treatments: 0, 5, 10, or 15 g of FP800 per day and 0 or 5 g of Procreatin-7 per day. Each of the 8 treatment combinations was designed to be replicated 12 times over four 28-day periods. Due to issues with animal health, however, data were collected from only 21 of the 24 cows.

Within each 28-day period, the first 2 weeks were used for adaptation to treatment and the next 2 weeks were used for measuring feed intake and milk production. Diets were fed individually twice daily to allow for 5 to 10% orts. The diet contained 22% ground corn, 20% corn silage, 20% wet corn gluten feed, 17% alfalfa, 8% whole cottonseed, and 8% expeller soybean meal. Dietary treatments were weighed individually and top-dressed by hand mixing them at each feeding into the top third of the ration within each cow's feed bunk.

Cows were milked twice daily, and milk weights were recorded at each milking. Milk samples were collected weekly (a.m./p.m. composite) and analyzed for lactose, fat, protein, milk urea nitrogen (MUN), and somatic cell count (SCC) by the Heart of America DHI Laboratory, Manhattan, Kansas. Component yields were calculated by multiplying average daily milk weights by the weekly values for milk composition. Cows were weighed and scored for body condition (1 = thin and 5 = fat) at the beginning of the experiment and at the end of each 28-day period.

Samples of feed ingredients were combined by period for analysis. Wet feed stuffs were monitored at least weekly to determine moisture content, and the diet was altered to account for changes in water content. Orts were weighed once daily for calculation of feed intakes. Feed ingredients were analyzed for dry matter, ash, crude protein, neutral detergent fiber (NDF), and acid detergent fiber (ADF).

Results and Discussion

The nutrient composition of the diet (Table 1) was determined by laboratory analysis of individual ingredients. The diet is typical of dairy diets fed in the Midwest. Composition of ingredients was typical of expectations for these feedstuffs. Dietary protein was 19% of dry matter, so protein supplied in the diet should not have limited production by the cows.

Dry matter intake averaged 64.6 lb/day, which is typical for cows maintained under our experimental conditions. Average milk production (96.8 lb/day) and efficiency of milk production (1.50 lb milk/lb dry matter intake) in this experiment were also typical of production levels observed in our herd.

Dry matter intake, milk production, milk efficiency, and production of all milk components were not impacted by the addition of either fibrolytic enzymes or yeast (Table 2). Percentages of fat, protein, and solids-not-fat (SNF) in milk were also not affected by treatment. We were surprised to find that an interaction between yeast and quadratic effect of enzyme amount altered percentages of milk

lactose and SCC. The effect on milk lactose is unusual because lactose secretion drives milk secretion such that its concentration is typically very constant. The effect on SCC can be attributed to a few large SCC values that skewed the results in several treatments.

Table 1. Diet Composition

Ingredient	% of Dry matter
Corn, ground	21.8
Corn silage	20.2
Wet corn gluten feed (Sweet Bran 60)	20.2
Alfalfa	16.8
Whole cottonseed	8.4
Expeller soybean meal (Soybest)	7.6
Limestone	1.34
Fishmeal	1.25
Molasses	0.94
Sodium bicarbonate	0.79
Trace mineral salt[1]	0.33
Magnesium oxide	0.20
Vitamin ADE premix[2]	0.10
Trace mineral premix (Zinpro 4-plex)[3]	0.05
Vitamin E premix[4]	0.02
Sodium selenite premix[5]	0.01
Nutrient	
Organic matter	92.2
Crude protein	19.0
Neutral detergent fiber	29.3
Acid detergent fiber	16.1
Ether extract	4.9

[1]Provided in diet dry matter: 0.32% NaCl, 7.9 ppm Mn, 7.9 ppm Fe, 1.6 ppm Mg, 1.1 ppm Cu, 1.1 ppm Zn, 0.23 ppm I, and 0.13 ppm Co.
[2]Provided per lb diet dry matter: 2,000 IU vitamin A, 1,000 IU vitamin D, and 6 IU vitamin E.
[3]Provided in diet dry matter: 13 ppm Zn from zinc methionine, 7 ppm Mn from manganese methionine, 4.4 ppm Cu from copper lysine, and 0.9 ppm Co from cobalt glucoheptonate.
[4]Provided 3 IU vitamin E per lb diet dry matter.
[5]Provided 0.06 ppm Se in diet dry matter.

Amounts of FP800 fed produced a quadratic change in body weight during each 28-day period. Weight gain was less for cows receiving the intermediate amounts of enzyme (5 and 10 g/day) than for those fed either no enzyme or the largest amount of enzyme (Table 3). Although the effect on efficiency of milk production was not significant, changes in body weight mirrored changes in milk efficiency, suggesting that the slight differences in body weight might be due to repartitioning of energy between milk and body reserves for cows receiving different amounts of enzymes. This relationship demonstrates that the body-weight response to treatment was probably not due to depressions in energy release from the diet for the intermediate enzyme amounts. Changes in body condition did not differ among treatments.

Taken as a whole, the results indicate that no production responses occurred in response to addition of fibrolytic enzymes or yeast to the diets of lactating cows under our experimental conditions. The experiment was conducted from December 3, 2003, through March 24, 2004, so cows were not exposed to any environmental temperature stress during the experiment. Cows were maintained in tie-stalls, and conditions were likely not stressful for the cows. In addition, all cows were at least 59 days in milk, suggesting that stress related to the transition into lactation was limited. Yeast supplementation has been demonstrated to be beneficial to cows maintained under stressful conditions, and our lack of response to yeast supplementation should not be extrapolated to cows maintained under stressful environments.

Responses to enzyme supplementation should be most clearly demonstrated under the minimally stressful conditions of our experiment in which cows could easily respond to changes in energy availability from the diet. It is possible that smaller amounts of the enzyme mixture may have been beneficial. In some previous experiments, responses to enzymes

have been reported at smaller enzyme amounts, with greater amounts of enzymes being unable to yield the same benefits. In our in vitro studies with FP800, we observed that in vitro digestibility was dependent upon the enzyme amounts. In one in vitro study, amounts of FP800 calculated to mimic 1 or 5 g/day fed to a lactating dairy cow yielded a response that was greater than could be achieved with amounts of 15 or 30 g/day. In another in vitro study, responses to FP800 were positive and essentially the same for amounts ranging from 0.3 to 5 g/day. It is unknown if smaller amounts of FP800 would lead to production responses, but this possibility cannot be excluded from our data because amounts of enzyme less than 5 g/day were not tested.

Table 2. Performance of Lactating Dairy Cows Fed Combinations of Yeast and Fibrolytic Enzymes

	No yeast				5 g/day yeast				
	Enzyme, g/day								
Item	0	5	10	15	0	5	10	15	SEM
Cows, no.	10	10	10	12	10	10	10	12	
	lb/day								
DMI	65.7	64.5	63.6	65.2	64.9	63.4	63.9	64.8	0.80
Milk	97.0	97.6	95.6	97.1	97.4	96.9	96.0	96.2	1.6
Milk fat	3.41	3.52	3.32	3.32	3.40	3.40	3.41	3.42	0.093
Milk protein	3.04	3.05	2.95	3.04	3.01	3.03	2.98	3.03	0.059
Milk lactose	4.64	4.81	4.68	4.74	4.78	4.67	4.67	4.67	0.084
Milk SNF	8.57	8.79	8.53	8.69	8.71	8.60	8.55	8.60	0.15
Milk/DMI	1.48	1.52	1.50	1.49	1.50	1.53	1.50	1.49	0.018
	%								
Milk fat	3.54	3.62	3.48	3.43	3.51	3.52	3.57	3.57	0.094
Milk protein	3.14	3.14	3.10	3.14	3.10	3.14	3.13	3.16	0.032
Milk lactose*	4.79	4.92	4.89	4.88	4.91	4.81	4.86	4.85	0.034
Milk SNF	8.85	9.02	8.94	8.96	8.94	8.88	8.92	8.95	0.054
MUN, mg/dL	16.4	16.7	16.7	16.6	17.0	16.7	16.9	16.9	0.37
SCC*, × 1000	687	48	269	292	78	127	719	293	246

*Interaction (P<0.05) between yeast and quadratic effect of enzyme amount.

Table 3. Body Weights (BW) and Body Condition Scores (BCS) and Their Changes in Lactating Dairy Cows Fed Combinations of Yeast and Fibrolytic Enzymes

	No yeast				5 g/day yeast				
	Enzyme, g/day								
Item	0	5	10	15	0	5	10	15	SEM
	lb								
Initial BW	1342	1364	1371	1355	1349	1354	1367	1342	9.9
BW change*	25	3	-7	10	15	7	2	23	9.5
	units								
Initial BCS	2.37	2.62	2.62	2.61	2.50	2.64	2.73	2.59	0.097
BCS change	0.23	-0.04	0.26	0.21	0.05	-0.12	0.05	0.17	0.14

*Quadratic (P < 0.05) effect of enzyme amount.

IMPACT OF DRIED SEAWEED MEAL ON HEAT-STRESSED LACTATING DAIRY CATTLE

B. Cvetkovic, M. J. Brouk, and J. E. Shirley

Summary

Twenty-four lactating Holstein cows were used to determine the production response to the inclusion of brown seaweed in the basal diet during summer heat stress. Cows were blocked by lactation number, days in milk, and energy-corrected milk and then allotted to either a control or control + brown seaweed diet. Cattle on the brown seaweed diet were fed 4 ounces per cow per day for 7 days , and then 2 ounces per cow per day for 14 days, before the start of the experiment. All cattle were housed in a tie-stall barn, fed individually, and milked twice daily. Cows fed brown seaweed produced more ($P<0.01$) milk (77.6 vs 73.8 lb) and milk protein than controls did. But the addition of brown seaweed did not reduce respiration rates, rectal temperature, or rear-udder skin temperature. This indicated a similar heat-stress response for treated and control cows. Other studies have shown a reduction in respiration rates and body temperature when stressed cattle were fed brown seaweed. Further investigation is necessary to determine the factors that resulted in the observed milk and milk-protein responses in this study.

(Key Words: Heat Stress, Nutrition, Milk Production.)

Introduction

Intense summer climates depress animal performance across the United States. High ambient temperature on a dairy farm results in decreased milk production, reproductive performance, and overall animal well being. Previous studies indicated that brown seaweed extract (Tasco-14 seaweed meal) added to the ration of high-producing dairy cows potentially decreases body temperature during heat stress.

The objectives of the study were to quantify the impact of feeding Tasco-14 seaweed meal on feed intake, milk production, milk component concentration, rectal temperature, rear-udder surface temperature, and respiration rate.

Experimental Procedures

Twenty-four Holstein cows were blocked by lactation number, days in milk, and energy-corrected milk and then allotted to either a control diet or control diet containing Tasco-14 seaweed meal. A total mixed ration containing corn silage, whole cottonseed, chopped alfalfa, sweet bran, and grain mix was fed ad libitum twice daily (a.m. and p.m.). Tasco-14 seaweed meal was given as a top-dressing once daily with morning feeding. A total of 12 cows received 4 ounces per cow per day during the first week, and then 2 ounces per cow per day until the end of study. Water was available for ad libitum consumption.

Cows were housed in a tie-stall barn at KSU Dairy Teaching and Research Center for 6 weeks. The first week was an adjustment period, and data were collected during the second through sixth week. Data from weeks 2 and 3 were used to achieve full adjustment to the seaweed, and were not included in the analysis. Rectal temperature, body surface temperature, and respiration rates were measured three times daily for 3 consecutive days per week. Respiration rate was determined by

manual counting of breaths for 15 seconds, rectal temperature was measured with M500 series high-performance digital thermometer (GLA Agricultural Electronics, San Luis Obispo, CA), and body surface temperature was measured at the rear quarter of the udder by using an infrared thermometer (Model 4KM98, Raytek Corporation, Santa Cruz, CA). Milk samples were collected twice weekly and were analyzed for fat, protein, SCC, and milk urea nitrogen by DHIA (Heart of America Lab, Manhattan, KS). Samples of feed components were collected weekly for standard nutritional analysis. During the entire study, all cows were milked twice daily. Milk production and feed intake were recorded daily, as well as the ambient temperature and relative humidity.

Results and Discussion

Similar dry matter intake (Table 1) was observed for cows assigned to the control and seaweed diets. Milk production was greater ($P<0.01$) from cows fed the brown seaweed diet (77.6 lb) than from controls (73.8 lb). Milk fat and protein percentages, as well as the amount of milk fat production, were unaffected by treatment. Amount of milk-protein production was greater ($P<0.01$) from cows fed the brown seaweed than from controls. Treatment did not impact the amounts of energy or fat-corrected milk produced.

Although average respiration rates, rear-udder skin temperatures, and rectal temperatures (Table 2) were less during the morning hours than during the afternoon, no differences were detected between diets.

Milk and milk-protein production were increased when 2 ounces of brown seaweed (per cow per day) was included with a normal diet for lactating dairy cattle. The increase in milk-protein production resulted from greater milk production and a trend for a higher milk-protein percentage when cows were fed brown seaweed. Similar dry matter intakes were observed for control and treatment cattle. Increased milk production in the face of similar intakes resulted in an increase in lactation efficiency, compared with that of the control diet. Improvements in milk and milk protein are not explained by the respiration rate, rear-udder skin temperatures, or rectal temperatures. In this study, the addition of brown seaweed did not reduce the normal heat-stress responses typically observed in dairy cattle. Other studies have shown reduced respiration rates and body temperatures when brown seaweed was fed. Further investigation is required to determine those factors that resulted in the milk and milk-protein production response observed in this study.

Table 1. Production Responses of Heat-stressed Holstein Cows Fed Brown Seaweed Meal

Factor	Diet		SEM	*P* =
	Control	Brown seaweed		
Intake, lb per cow per day	49.9	49.6	1.18	0.800
Milk, lb	73.8	77.6	1.33	0.007
Milk fat, %	3.85	3.61	0.13	0.080
Milk protein, %	3.10	3.21	0.04	0.110
Milk fat, lb	2.79	2.77	0.10	0.840
Milk protein, lb	2.26	2.45	0.05	0.001
ECM[1], lb	76.5	78.9	1.83	0.200
FCM[2], lb	71.3	72.6	1.86	0.520

[1]Energy-corrected milk = 0.327 × lb of milk + 12.95 × lb of milk fat + 7.2 × lb of milk protein.
[2]Fat corrected milk = 0.4 × lb of milk + 15 × lb of milk fat.

Table 2. Impact of Feeding Brown Seaweed Meal on Respiration Rates, Rear-udder Skin Temperature and Rectal Temperature of Heat-stressed Holstein Cows

Item	Diet		SEM	*P* =
	Control	Brown seaweed		
Respiration rate, breaths/min				
Morning	58.1	56.6	2.65	0.90
Early afternoon	69.1	65.0	2.65	0.85
Late afternoon	67.0	66.5	2.65	0.91
Daily average	64.7	62.7	2.09	0.46
Rear-udder skin temperature, °F				
Morning	91.7	91.9	0.39	0.99
Early afternoon	95.1	94.9	0.39	0.99
Late afternoon	96.0	96.1	0.39	0.99
Daily average	94.3	94.3	0.33	0.93
Rectal temperature, °F				
Morning	101.6	101.8	0.13	0.83
Early afternoon	102.0	102.0	0.13	0.99
Late afternoon	102.6	102.6	0.13	0.99
Daily average	102.1	102.1	0.11	0.65

INSEMINATIONS AT ESTRUS INDUCED BY THE PRESYNCH PROTOCOL BEFORE TIMED ARTIFICATION INSEMINATION

J. S. Stevenson and A. P. Phatak

Summary

A controlled field study examined conception rates after two timed-AI (TAI) breeding protocols conducted on two commercial dairy farms. Estrous cycles in postpartum lactating cows were presynchronized with two injections of $PGF_{2\alpha}$ given 14 days apart (Presynch) and then, after 12 days, the standard Ovsynch protocol (injection of GnRH 7 days before and 48 h after an injection of $PGF_{2\alpha}$, with one TAI at 12 to 16 hours after the second GnRH injection) or Heatsynch protocol (injection of GnRH 7 days before an injection of $PGF_{2\alpha}$, followed 24 h later by 1 mg of estradiol cypionate (ECP) and one TAI 48 hours after ECP) was applied. Experimental design allowed for AI to occur any time after the second Presynch injection and during the designed breeding week when estrus was detected. Of the 1,846 first services performed, only 1,503 (rate of compliance = 81.4%) were performed according to protocol. Numbers of cows inseminated, logistic-regression-adjusted conception rates, and days in milk (DIM) were for inseminations made: 1) during 14 days after first Presynch injection (n = 145; 22.6%; 54 ± 0.4 DIM); 2) during 12 days after second Presynch injection (n = 727; 33%; 59 ± 0.2 DIM); 3) during 7 days after the first GnRH injection of Ovsynch or Heatsynch (n = 96; 32.1%; 74 ± 0.5 DIM); 4) after estrus as part of Heatsynch (n = 212; 44.6%; 76 ± 0.3 DIM); 5) after TAI as part of Heatsynch (n = 154; 21.1%; 76 ± 0.4 DIM); 6) after estrus as part of Ovsynch (n = 43; 48.7%; 77 ± 0.7 DIM); and 7) after TAI as part of Ovsynch (n = 271; 24.4%; 77 ± 0.3 DIM). Conception rates when AI occurred after one Presynch injection were less than when AI occurred after two Presynch injections. Conception rates for those inseminated after either Presynch injection did not differ from those inseminated after combined Heatsynch + Ovsynch. Cows in the Ovsynch and Heatsynch protocols inseminated after estrus during the breeding week had greater conceptions rates than those receiving the TAI, but overall conception rates did not differ between protocols. Among cows inseminated after detected estrus, conception was greater for cows in the Heatsynch + Ovsynch protocol (77 ± 0.4 DIM) than for those inseminated after either Presynch injection (54 ± 0.4 or 59 ± 0.2 DIM). We concluded that conception rates after Heatsynch and Ovsynch were similar under these experimental conditions, and that delaying first AI improved fertility for cows inseminated after detected estrus.

(Key Words: Calving Difficulty, Compliance, Conception, Synchronized Estrus.)

Introduction

Various programmed-breeding protocols have been developed to synchronize estrus and ovulation in lactating dairy cows to initiate first services. These include the standard Ovsynch protocol and variations that may include estrogen administration to induce estrus during the breeding week (Heatsynch). The only estrogen product in the United States (estradiol cypionate; ECP) was recently removed from the market. In general, conception or pregnancy rates after either of these two protocols are similar. When estrous cycles are presynchronized, or staged according to days of

the estrous cycle, so that a majority of cows are in mid diestrus (d 5 to 12) at the onset of the Ovsynch protocol, resulting pregnancy rates are enhanced, compared with those of cows beginning the Ovsynch protocol at random stages of the estrous cycle.

A question often asked is whether it is advisable to inseminate cows earlier during the administration of these protocols when estrus is detected after either of the Presynch injections of $PGF_{2\alpha}$ and the cow is at or near the end of the voluntary waiting period (VWP). Conception rates for cows inseminated after estrus tend to be greater or similar to those of cows receiving timed AI (TAI) to which are applied various Ovsynch-like protocols.

A related question is what is the ideal VWP to be applied on individual dairy farms or in the industry today, in which most cows are inseminated after some controlled breeding program. Earlier studies (before application of controlled-breeding programs) in which cows were submitted for AI at predetermined DIM generally reported a significant or numerical trend for increased conception after a longer VWP. A recent study was conducted in which low- and high-producing dairy cows were inseminated at different DIM as part of the Ovsynch protocol. In both milk-production groups, conception rates were improved by delaying first services 3 weeks, from 53 to 59 (14.4%) to 73 to 81 DIM (34.5%) for low-producing cows and from 73 to 81 (28.2%) to 94 to 102 DIM (41.4%) for high-producing cows.

The objective of our study was to determine pregnancy outcomes of two standard breeding protocols (Ovsynch and Heatsynch) in which estrous cycles were presynchronized previously with two injections of $PGF_{2\alpha}$ (Presynch), and inseminations occurred whenever estrus was detected any time after the second Presynch $PGF_{2\alpha}$ injection. Ancillary objectives were to determine compliance to the designed protocols and whether DIM was a significant factor in accounting for different conception rates.

Experimental Procedures

A controlled study was conducted at two (Dairy #3 and Dairy #5) of five Foster Dairy Farms, Hickman, California. Lactating Holstein cows (n = 1,846) that calved between February 2, 2001, and February 1, 2002, were included in the study, which consisted of first postpartum inseminations conducted between March 22, 2001 and April 28, 2002. All five Foster Dairy Farm herds were managed similarly by one central management team, having different herdsmen and inseminators at each dairy. All cows at each dairy were fed a total mixed ration (TMR) to meet or exceed requirements recommended for lactating dairy cows. Diets were mixed from common ingredients located at Dairy #2 to feed cows at Dairy #2 and #3 or at Dairy #5 to feed cows at Dairy #4, #5, and #6. Diets consisted of alfalfa, soybean meal, bypass soya, corn silage, barley, flaked corn, brewer's grains, beet pulp, and added minerals. Cows were milked and fed three times dailym so fresh feed was available when cows returned to pens from the milking parlor after each milking (0400, 1200, and 2000 h). No recombinant bST was used in these herds.

The study was designed to compare conception rates of lactating dairy cows inseminated at first service in response to one of two estrus- and ovulation-synchronization protocols (Ovsynch [injections of GnRH 7 days before and 48 hours after $PGF_{2\alpha}$] and Heatsynch [injection of GnRH 7 days before and an injection of ECP 24 hours after $PGF_{2\alpha}$]). Before applying each protocol, estrous cycles of cows were presynchronized by using two injections of $PGF_{2\alpha}$ (13 to 15 days apart; Presynch), with the second Presynch injection occurring 11 to 12 days before initiating either of the two protocols. Cows were assigned to begin the Presynch injection sequence on the basis of calving dates,

grouped into breeding clusters every 10 days, beginning no sooner than 40 DIM. Doses and sources of hormones were: $PGF_{2\alpha}$ (25 mg; Lutalyse, Pharmacia Animal Health, Kalamazoo, MI); estradiol cypionate (1 mg; ECP; Pharmacia Animal Health, Kalamazoo, MI), and GnRH (100 μg; Cystorelin, Merial Limited, Iselin, NJ). All hormones were administered i.m. in the gluteal muscles.

Experimental protocols were designed for insemination of cows after detected estrus that occurred: 1) during 12 days after the second of two Presynch injections; 2) during 7 days after the first GnRH injection of Ovsynch or Heatsynch (some received $PGF_{2\alpha}$ 7 days later and were inseminated that day); or 3) during the breeding week when the ECP injection of Heatsynch or the second GnRH injection of Ovsynch was administered. Therefore, any cow that was detected in estrus 24 or more hours after the $PGF_{2\alpha}$ injection of the Ovsynch or Heatsynch protocol was eligible for insemination by design. In the absence of previous AI, cows in the Ovsynch protocol were inseminated at 12 to 16 hours after the second GnRH injection and those in the Heatsynch protocol were inseminated at 48 hours after ECP injection.

All hormonal injections and their dates of administration, calving difficulty scores (CDS), calving and breeding dates, AI at estrus or TAI, pregnancy outcomes, etc., were recorded in DHI records (DHI-Provo). As a result, actual dates of hormonal injections and inseminations could be verified to determine protocol compliance. Of the 1,846 inseminations recorded during the experimental period, some cows were inseminated contrary to study design.

Four individuals conducted all inseminations at Dairy #3 and four different individuals conducted inseminations at Dairy #5. The same AI sires were used at both dairies. Therefore, effects of inseminator were confounded with herd. Inseminations associated with estrus were conducted between 8 and 16 hours after detected estrus (a.m. - p.m. rule). Detected estrus was defined to include: 1) visually detected standing to be mounted, 2) tail-chalk rubs, and 3) other secondary signs (mucus or ruffled tail-head hair). Where chalk rubs or secondary signs were detected, cows were often palpated to detect uterine tone for validation of potential accuracy of the suspected estrus. These determinations of defined estrus were judgments made by the inseminators.

Cows were locked up at the feed bunk each morning between 8 and 10 a.m. to conduct hormonal injections, read and apply new tail chalk, check for pregnancy, and perform other health treatments. When cows were detected in standing estrus after morning chalk reads, they were inseminated that evening.

Conception rates (no. of pregnancies ÷ no. of cows inseminated) were calculated from pregnancy checks that were conducted weekly by palpation per rectum of the uterus and its contents at a minimum of 35 to 41 days after last AI.

Results and Discussion

Of the 1,846 first services performed, only 1,503 (81.4%) were performed according to the designed protocol. The breaches in protocol included missed or mistimed injections, hormone-injection sequence errors, and, in some instances, cows receiving both ECP and GnRH during the breeding week. Our protocol dictated that cows were eligible to be inseminated any time once estrus was detected after the second Presynch injection until the scheduled TAI associated with the Heatsynch or Ovsynch protocols. Noncompliant inseminations included those conducted during 14 days after the first Presynch injection (n = 145; 7.9%) and those (n = 198; 10.7%) that did not comply with the synchronization protocol. Of the two deviations from insemination protocol, the latter was

most serious because pregnancy outcomes were most compromised, as measured by actual conceptions rates (22.2%), which included inseminations after detected estrus (conception rate = 26%; n = 130) and TAI (conception rate = 15%; n = 68).

Inseminations made after the first Presynch injection were included in statistical analyses so conception rates after fewer DIM could be examined. Of 1,648 inseminations performed after the five breeding scenarios, 968 (58.7%) occurred after either one (1×PGF) or two (2×PGF) injections of $PGF_{2\alpha}$, or during 7 days after the beginning GnRH injection of Heatsynch or Ovsynch. More ($P = 0.01$) cows in the Heatsynch protocol were inseminated during the designed breeding week, after or in association with expressed estrus (57.9%), than in the Ovsynch protocol (13.6%). These differences between Ovsynch and Heatsynch were consistent across lactation numbers, but more first- and second-lactation cows than older cows were detected in estrus after Heatsynch, compared with those after Ovsynch (treatment × lactation number interaction; $P < 0.01$; first lactation: 63.6 vs. 17.8%; second lactation: 67.8 vs. 9.8%; and third or greater lactation: 47.8 vs. 13.6%, respectively).

Days in milk at each insemination, on the basis of the seven statistically compared protocols, are summarized in Table 1. Cows in 1×PGF were inseminated 5 ± 0.2 d earlier ($P<0.01$) than were cows in 2×PGF, whereas both groups were inseminated 15 to 20 d earlier ($P<0.01$) than cows inseminated during 7 days after the beginning GnRH injection of Heatsynch or Ovsynch, and 17 to 23 days earlier ($P<0.01$) than those that completed the designed Heatsynch and Ovsynch protocols.

Logistic-regression-adjusted mean conception rates are summarized in Table 1 for the five breeding scenarios, as are orthogonal contrasts of adjusted means. Although significant differences were detected for three of six contrasts, some contrasts included confounding treatment protocols with DIM.

The first contrast indicated that the adjusted conception rates increased ($P < 0.05$) from 22.6 to 33% because of the additional injection of $PGF_{2\alpha}$, but DIM at AI also increased ($P < 0.01$) from 54 to 59 (Table 1). Therefore, it is not clear whether the difference in conception occurred because of the additional $PGF_{2\alpha}$ injection or because DIM was longer at AI.

The second contrast compared cows receiving both Presynch injections with those inseminated during 7 days after receiving the beginning GnRH injection of the Heatsynch or Ovsynch protocol. The latter scenario also included some cows that received the $PGF_{2\alpha}$ injection for those two protocols, but were inseminated on that day before receiving subsequent injections associated with Heatsynch (i.e., ECP) or Ovsynch (i.e., GnRH). These cows were likely coming into estrus spontaneously despite the injection of $PGF_{2\alpha}$. Although DIM was longer ($P < 0.01$) for cows in the latter protocol, conception rates (33 vs. 32.1%) did not differ from the 2×PGF cows (Table 1). Again, this contrast confounds treatment (two $PGF_{2\alpha}$ injections vs. two or three $PGF_{2\alpha}$ injections + GnRH) with DIM.

The third contrast also confounds DIM with treatment protocol (detected estrus vs. detected estrus + TAI). Although all cows were inseminated after detected estrus in 1×PGF, 2×PGF, and 2×PGF + GnRH ± PGF groups, only 37.5% of the cows in Heatsynch + Ovsynch were inseminated after, or in association with, expressed estrus. Conception rates (28.3 vs. 31.5%) did not differ between groups (Table 1).

The fourth contrast (TAI: Heatsynch + Ovsynch vs. estrus: Heatsynch + Ovsynch) compared conception rates of cows inseminated after TAI vs. after detected estrus. When treated

with either the Heatsynch or Ovsynch protocol, cows inseminated after, expressed estrus had greater (P<0.001) conception rates (45.3 vs. 23.2%) than those receiving the TAI at similar DIM (Table 1),

The fifth contrast was a comparison of the overall outcomes from the Heatsynch vs. Ovsynch protocols (Table 1). Pregnancy outcomes did not differ between the two protocols. Conception rates of cows inseminated after Heatsynch and Ovsynch, when estrus occurred, were nearly identical, as were conception rates for cows receiving the TAI regardless of protocol (Table 1). Overall numerical differences between Heatsynch (34.7%) and Ovsynch (27.7%) are explained by proportionally more cows having greater conception rates after inseminations associated with expressed estrus (Heatsynch vs. Ovsynch: 57.9 vs. 13.6%), compared with fewer cows having lower conception rates after receiving the TAI.

The sixth contrast compared conception rates of cows after detected estrus at different DIM (Estrus: 1×PGF + 2×PGF vs. estrus: Heatsynch + Ovsynch). This contrast verified that conception rates were increased (P<0.01) when inseminations, based solely on estrus, occurred after more DIM (31.3 vs. 45.3%; Table 1).

One might argue that the additional hormonal treatments (GnRH, $PGF_{2\alpha}$, and ECP [Heatsynch cows only]) applied to cows in the Heatsynch and Ovsynch protocols could account for observed differences. This was not true, however, in a recent study in which low- and high-producing dairy cows were inseminated after an Ovsynch protocol applied at different DIM. In both milk-production groups, conception rates were improved by delaying first services 3 weeks, from 53 to 59 DIM (14.4%) to 73 to 81 DIM (34.5%) for low milk-producing cows and from 73 to 81 DIM (28.2%) to 94 to 102 (41.4%) for high-producing cows.

A large percentage of the cows calved unassisted (CDS = 1; 1,282 of 1,846; 69.4%). Numbers of cows with scores of 2 (14%), 3 (14.8%), 4 (2%), and 5 (0.3%) made up the remaining proportions. Greater CDS was associated negatively with subsequent conception rate. For every 1-unit (range of 1 to 5) increase in calving difficulty, conception rate was reduced (P<0.01) by 4.8 ± 1.4%. Those having a CDS of 1 or 2 had greater (P<0.05) conception rates than those having a CDS of 3 or more (33.8 vs. 24.6%). Poorer fertility of cows having calving difficulty, because of its associated uterine pathology, is consistent with other reports.

Herd had no effect on pregnancy outcomes. All of the probability values for herd were > 0.4. This is attributed to both herds being fed the same TMR from a common feed supply, being managed under similar policies, and using common AI sires, even though different people detected estrus and performed inseminations at each dairy. Overall conception rates between herds differed by less than 0.5 percentage points.

Averaged across all treatment protocols, conception rates among lactation numbers tended to differ. No treatment × lactation number interaction was detected. First-lactation cows tended (P = 0.10) to have greater conception rates than did second-lactation cows (34.5 vs. 27.7%), and cows in their third or greater lactation tended (P = 0.08) to differ from conception rates of second-lactation cows (34.7 vs. 27.7%). Our results tend to confirm those in a recent study of 1,584 lactating cows, in which first-lactation cows had greater conception rates than older cows when all inseminations were performed after an Ovsynch protocol. Further, less-fertile, older cows also produce more milk, which may account for some reduction in conception rates, although increased milk yields are confounded with age. That second-lactation cows had poorer conception rates in our study

indicates that greater attention must be addressed to factors known to influence subsequent fertility, such as body condition and feed intake, and to other health issues during first lactations, first dry and subsequent transition periods, and early postpartum after second calvings.

We have demonstrated that one of the challenges in implementing various reproductive-management schemes is compliance to protocols. Nearly 19% of the cows were inseminated off-protocol. This is not an issue of concern for those cows in which inseminations occurred after detected estrus, but when TAI was administered to cows that received an improper injection sequence, pregnancy outcomes were compromised. Most serious infractions included those in which injections were given out of sequence, were given on the wrong dates, or were not given as designed. Compliance is difficult to monitor because documentation may be inaccurate or missing.

Our results clearly show that cows inseminated after detected estrus at similar DIM are more fertile than those receiving TAI. Nonetheless, given the poor rates of detected estrus (including missed observations and lack of estrus expression), TAI has proved to be an important tool for achieving pregnancies. In many studies, conception rates are nearly equal to, or greater than, for cows inseminated after estrus, but pregnancy rates (no. of pregnancies ÷ no. of cows attempted to AI) are often similar or greater because proportionally more cows of similar fertility are inseminated.

For cows inseminated at estrus at various DIM, those inseminated after 75 DIM had greater conception rates than those inseminated before 60 DIM. Establishing the appropriate VWP for individual herds is essential. Herd history for pregnancy outcomes after first services can be examined to determine if there is justification for delaying inseminations to achieve improved conception rates. In earlier studies in which no ovulation control was employed, various VWP were tested. Most studies demonstrated nearly similar conception rates for cows inseminated earlier versus later postpartum, but cows inseminated earlier generally required more services per pregnancy, partly because that measure (services per pregnancy) does not account for services made for cows that fail to conceive and are eventually culled.

Evaluation of synchrony protocols should include reproductive performance traits (e.g., herd estrus-detection rates) in addition to costs of administering protocols. A recent study found that Ovsynch used to initiate first services improved reproductive performance in two herds (reduced days to first services and days open; reduced culling for infertility in one herd), but AI based on detected estrus was economically superior in another herd, whereas Ovsynch was superior in the second herd because of poorer estrus-detection rates. Days open and culling were the major cost factors of those evaluated in their economic analysis. Inseminations associated with Ovsynch (TAI), compared with those made after detected estrus in response to $PGF_{2\alpha}$, have greater impact on net returns in summer months than during cooler months when estrus-detection rates tend to be greater.

Declining conception rates have been reported for lactating dairy cows since the 1950s, in the face of milk yields per cow that have increased 3.3 times. Our results indicate that the VWP should be extended in some herds to allow for improved fertility that may occur by delaying inseminations. Because of ovulation control and the benefits of increased persistency of lactation for cows treated with bST, a shorter VWP seems less critical, particularly when a longer VWP may result in improved pregnancy outcomes. Using ovulation control prevents prolonged and excessively variable intervals to first services. Further, and more important,

because fewer than half of cows conceive at first AI, use of various tested resynchronization protocols for cows diagnosed not pregnant guarantees that cows are re-inseminated within 2 to10 days of their not-pregnant diagnosis.

Table 1. Conception Rates at First Services in Lactating Dairy Cows After Various Protocols

	Protocol[1]						
			2×PGF +	Heatsynch (HS)		Ovsynch (OVS)	
Item	1×PGF	2×PGF	GnRH ± PGF	Estrus	TAI	Estrus	TAI
No. of cows	145	727	96	212	154	43	271
Average DIM at AI	54 ± 0.4 [a] (40-89)	59 ± 0.2 [b] (51-102)	74 ± 0.5 [c] (65-95)	76 ± 0.3 [d] (71-118)	76 ± 0.4 [d] (73-106)	77 ± 0.7 [d] (73-84)	77 ± 0.3 [d] (73-110)
Pregnancy rate[2], %	22.6	33.0	32.1	44.6	21.1	48.7	24.4
Orthogonal contrasts:							
1×PGF vs. 2×PGF	22.6* (145)	33.0 (727)					
2×PGF vs. 2×PGF + GnRH ± PGF		33.0 (727)	32.1 (96)				
1×PGF + 2×PGF + 2×PGF + GnRH ± PGF vs. HS + OVS		28.3 (968)				31.5 (680)	
TAI (HS + OVS) vs. estrus (HS + OVS)				23.2*** (425)		45.3 (255)	
Heatsynch vs. Ovsynch				34.7 (366)		27.7 (314)	
Estrus only: 1×PGF + 2×PGF vs. HS + OVS		31.3** (872)				45.3 (255)	

[a,b,c,d] Means having different superscript letters differ ($P<0.01$).

*$P<0.05$.

**$P<0.01$.

***$P<0.001$.

[1]Cows were inseminated after a single injection of $PGF_{2\alpha}$ (1×PGF); after the second of two injections of $PGF_{2\alpha}$ given 14 days apart (2×PGF); after the first GnRH injection of Ovsynch or Heatsynch (some received PGF 7 days later and were inseminated that day; 2×PGF + GnRH ± PGF); after estrus induced by estradiol cypionate (ECP) injection of Heatsynch (GnRH injection 7 d before $PGF_{2\alpha}$ and ECP given 24 hours after $PGF_{2\alpha}$); timed AI at 48 hours after the $PGF_{2\alpha}$ injection of Heatsynch; after estrus following the $PGF_{2\alpha}$ injection of Ovsynch (injection of GnRH 7 days before and 48 hours after $PGF_{2\alpha}$); and timed AI at 12 to 18 hours after the second GnRH injection of the Ovsynch protocol.

[2]Means are based on analyses by logistic regression.

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RESYNCHRONIZING ESTRUS AND OVULATION IN OPEN COWS AND HEIFERS

J. S. Stevenson and S. M. Tiffany

Summary

We compared outcomes of two protocols used to resynchronize estrus and ovulation in dairy females after found open at pregnancy checks. Replacement heifers and lactating cows in which AI occurred 41 ± 1 day earlier were presented every 2 to 3 weeks for a pregnancy check by ultrasonography. Ovaries were scanned, follicles were mapped and sized, presence of corpus luteum was noted, and GnRH was injected (day 0) . Females received $PGF_{2\alpha}$ 7 days later (day 7) and then were assigned randomly to either receive estradiol cypionate (ECP) 24 hours after $PGF_{2\alpha}$ (day 8; Heatsynch; n = 230) or a second GnRH injection after $PGF_{2\alpha}$ (day 9; Ovsynch; n = 224). Those detected in estrus were inseminated, whereas the rest received a timed AI (TAI) between 65 and 74 hours after $PGF_{2\alpha}$. Few females (5.1%) were inseminated between open diagnosis and day 8. On day 10, more ECP- than GnRH-treated females were inseminated after detected estrus (24 vs. 6%). Overall, more Ovsynch than Heatsynch females received a TAI (82 vs. 62%). Conception rates tended to be greater for females inseminated after estrus (37%) than after TAI (29%), and the tendency was more pronounced for those treated with Heatsynch (41 vs. 27%) than for those treated with Ovsynch (33 vs. 31%). Conception rates for females having elevated progesterone 7 days after the not-pregnant diagnosis were greater than conception rates of those having low progesterone in Heatsynch (42%; n = 133 vs. 25%; n = 55) and Ovsynch protocols (33%; n = 142 vs. 15%; n = 45). Conception rates were greater in heifers than in lactating cows (43 vs. 28%), regardless of protocol employed. Although overall pregnancy outcomes were similar in response to either the Ovsynch or Heatsynch protocol, inseminations performed after detected estrus before the scheduled TAI reduced days to eventual conception and tended to increase conception rates, particularly after Heatsynch.

(Key Words: Synchronized Estrus, Ovulation, Cysts.)

Introduction

Earlier identification of nonpregnant females is one way to reduce prolonged inter-insemination intervals that occur because of poor efficiency in detecting post-insemination estrus. Treating nonpregnant cows with $PGF_{2\alpha}$ between 27 and 29 days after a previous AI induced regression of the corpus luteum (CL) before subsequent insemination after detected estrus or timed AI (TAI), and reduced days to re-insemination and to conception. Other options may include applying various TAI protocols to females that are diagnosed open. Applying the Ovsynch protocol or substituting estradiol cypionate (ECP) for GnRH in an Ovsynch-like protocol (known as Heatsynch) are viable options. Administering ECP to females in proestrus has been found to induce estrus, preovulatory LH surge, ovulation, and normal corpus luteum (CL) development in dairy heifers and dairy cows. Two previous studies have found that conception rates of Heatsynch-treated heifers are not different from those in heifers inseminated after detected estrus, and those in lactating cows after Heatsynch are similar to those after Ovsynch.

The objective of the present study was to determine fertility after applying the Ovsynch or Heatsynch protocols to dairy females that were diagnosed open by transrectal ultrasonography. An ancillary objective was to determine whether ovarian status at the initiation of the two protocols influenced subsequent pregnancy outcomes for either protocol.

Experimental Procedures

Lactating Holstein cows (n = 414) were housed in either tie stalls or free stalls at the Kansas State University Dairy Teaching and Research Center. Replacement Holstein heifers (n = 40) were housed in free stalls with an adjacent dirt lot and concrete feed apron behind the feed bunk. Females were presented for a pregnancy check every 2 to 3 weeks between August 2000 and November 2002, consisting of 46 separate groups. Days since AI at pregnancy diagnosis averaged 41 ± 1 day (range: 27 to 200). Slightly more than 91% of females were between days 27 and 53 since last AI. Those few females having prolonged intervals since AI had been pregnant, but aborted and had returned to estrus.

Pregnancy status was determined (presence of uterine fluid plus a CL, detection of embryo, or both) by using transrectal ultrasonography (real time, B-Mode, linear array, diagnostic, ultrasound scanner equipped with a 5-MHz transducer, Aloka 500V, Wallingford, CT). In open females, ovaries were scanned, follicles were mapped and sized by using electronic calipers (average of vertical and horizontal measures), and the presence of CL was noted. Ovarian characteristics quantified were number of CL, number of follicles $\geq$10 mm on each ovary and their total per female, diameter of the largest and second-largest follicle (excludes the largest cystic structure[s] in those females bearing cysts), and diameter of all cystic structures (defined later).

After the not-pregnant diagnosis and ovarian examination, all females received i.m. 100 µg of GnRH (d 0; Cystorelin, Merial, Iselin, NJ). Females were then blocked by lactation number (1 vs. 2+), and replacement heifers were balanced by body weight and age before random assignment to one of two treatments: 1) 25 mg of $PGF_{2\alpha}$ (day 7; Lutalyse, Pharmacia Animal Health, Kalamazoo, MI) 7 days after not-pregnant diagnosis, plus 1 mg of ECP (day 8; ECP, Pharmacia Animal Health, Kalamazoo, MI) 24 hours after $PGF_{2\alpha}$ (Heatsynch; n = 230); or 2) 25 mg of $PGF_{2\alpha}$ 7 days after not-pregnant diagnosis, plus a second GnRH injection 48 hours after $PGF_{2\alpha}$ (day 9; Ovsynch; n = 224).

To maximize pregnancy outcomes in both treatments, any female detected in estrus after the initial GnRH injection, but at least 24 hours before the scheduled TAI, was inseminated 8 to 16 hours after first detected estrus (a.m. - p.m. rule). Females were observed for estrus at least twice daily (morning and late afternoon), in addition to other casual observations during the work day (7:30 a.m. to 5:00 p.m) and while various groups of cows were moved to the milking parlor (5:30 to 10:00 a.m and 5:30 to10:00 p.m.). In the absence of previous insemination, all remaining females received a TAI at 65 to 74 hours after $PGF_{2\alpha}$ (16 to 20 hours after GnRH or 46 to 50 hours after ECP). After treatment inseminations, pregnancy outcome was determined as described previously by using transrectal ultrasonography between 33 and 40 days after AI.

Blood samples were collected from females in 39 of 46 groups (83.2% of females) before the GnRH injection (day 0), before $PGF_{2\alpha}$ (day 7), and 24 hours after $PGF_{2\alpha}$ (day 8) for later radioimmunoassay analysis of blood concentrations of progesterone (P4) in serum.

Ovarian scans and blood collected before injections for P4 analysis were used to classify females into one of four ovarian status groups:

anestrus, follicular cysts, luteal cysts, and cycling. Females were classified to be anestrus (n = 20) when serum concentration of P4 was < 1 ng/mL on days 0, 7, and 8 and few follicles > 10 mm were detected on day 0. Females having follicular cysts (n = 12) had multiple follicles, including at least one follicle > 20 mm in diameter, and had concentrations of P4 < 1 ng/mL on day 0. Females having luteal cysts (n = 12) had multiple follicles, including at least one follicle > 20 mm in diameter, and had serum concentration of P4 ≥ 1 ng/mL on day 0. Cycling females (n = 344) had normal ovarian structures and various concentrations of P4 in blood serum, but none had serum concentrations of P4 < 1 ng/mL at all three sampling times.

Results and Discussion

Cumulative percentages of all inseminations conducted after detected estrus during 10 days after not-pregnant diagnosis are illustrated in Figure 1. By day 8 (day of ECP injection), 5.2% of females pre-assigned to Heatsynch and 4.9% pre-assigned to Ovsynch had been inseminated. On day 9 (day of GnRH injection), another 9.6% were inseminated in the Heatsynch treatment and another 6.7% were inseminated in the Ovsynch treatment. Of those inseminated on day 10, more ($P < 0.05$) females were inseminated after detected estrus in the Heatsynch (23.5%; n = 54) than in the Ovsynch (6.3%; n = 14) protocol. Not shown in Figure 1 are all remaining females that received a TAI on day 10. More ($P<0.01$) Ovsynch (82.1%; n = 184) than Heatsynch (61.7%; n = 142) females received the TAI.

No differences in conception rates were detected between treatments, when considering all inseminations (Table 1). Replacement heifers had greater ($P<0.05$) conception rates than lactating cows (Table 1). Conception rates also tended ($P<0.10$) to be greater for females inseminated after detected estrus than for those receiving one TAI (37.1%; n = 128 vs. 28.9%; n = 326) in both protocols (Table 1). Conception rates for the 76 females inseminated during 48 hours after ECP were 38.2%, compared with 31% of the 29 Ovsynch females inseminated during the same period, including those that received GnRH midway during the same 48-hour period. For females inseminated after detected estrus, average days from treatment AI until when conception finally occurred were fewer ($P<0.01$) than for those receiving the TAI (45 ± 10 [n = 81] vs. 76 ± 7 [n = 174]).

For females having concentrations of P4 ≥ 1 ng/mL seven days after treatment initiation, regardless of subsequent treatment (Table 1), conception rates were greater ($P<0.001$) than those having < 1 ng/mL P4 (37.5%; n = 275 vs. 20%; n = 100). Concentrations of P4 in females of different ovarian and cycling status are illustrated in Figure 2. Cycling females and those having luteal cysts had greater ($P<0.05$) concentrations of P4 on day 0 (day of GnRH) than did anestrous females and those bearing follicular cysts. These average differences were maintained on day 7, except in those having follicular cysts, in which serum P4 increased to concentrations similar to those having luteal tissue. In all 12 females having follicular cysts on day 0 (serum P4 was < 1 ng/mL), only 2 failed to have an increase in serum P4 ≥ 1 ng/mL by 7 days after GnRH injection. By day 8, 24 hours after $PGF_{2\alpha}$, only anestrous females had less ($P<0.05$) serum P4 than all others.

Conception rates for these females of different ovarian and cycling status are summarized in Table 1. Because of limited numbers of observations in all but the cycling group, potential differences in conception rates were not statistically detectable, but data are presented for informational purposes.

Eight of the 24 cystic females (only 1 heifer, which had a follicular cyst; otherwise, 4 follicular and 4 luteal cysts) expressed estrus after not-pregnant diagnosis on the day of TAI,

except for 1 female having a luteal cyst that expressed estrus 1 day before TAI. On the basis of our initial classifications of the cystic structure before serum P4 concentrations were assessed, we identified 3 cows with a luteal cyst and 21 females with follicular cysts. In retrospect, on the basis of serum P4, we had classified incorrectly 9 of the 21 females having follicular cysts, because each of the 9 had elevated serum P4 on day 0 and, therefore, were luteal, rather than follicular, cysts. Concentrations of P4 on the day of not-pregnant diagnosis for females having follicular cysts were less ($P<0.001$) than those in females bearing luteal cysts (0.5 ± 0.5 vs. 3.3 ± 0.5 ng/mL).

We have demonstrated that early-pregnancy diagnosis of dairy females via transrectal ultrasonography is a practical means of identifying nonpregnant females for prompt reinsemination. That process was facilitated by applying either the Ovsynch or Heatsynch protocol after transrectal ultrasonographic diagnosis of no pregnancy. Although overall pregnancy outcomes were similar between protocols, females that expressed estrus and were re-inseminated before the scheduled TAI tended to have greater conception rates and did eventually conceive sooner than those receiving the TAI.

It is unfortunate that the estrogen (ECP) used in the Heatsynch protocol is no longer available in the U.S. market. The concept, however, of applying an early open diagnosis, whether it be by transrectal ultrasonography or palpation, and promptly re-inseminating, is valid by any means, including using the Ovsynch protocol. When early diagnosis of pregnancy by ultrasonography is not possible, more frequent detection by palpation (e.g., weekly vs. biweekly) on a herd basis reduces the interinsemination interval and increases AI submission rate. When AI submission rate is increased, increases in total pregnancy rate should result because submission rate is one of two factors that determines overall pregnancy rate (i.e., product of AI submission rate × actual conception rate).

Table 1. Conception Rates in Dairy Females after Applying the Ovsynch or Heatsynch Protocol

	Protocol[1]	
Item	Heatsynch	Ovsynch
	--- (% [no.])---	
Overall[2]	34.1 (230)	31.8 (224)
Lactation number[3]		
0	46.2 (20)	40.1 (20)
1	29.5 (108)	22.4 (92)
2+	26.8 (102)	32.8 (112)
AI after detected estrus[4]	41.2 (88)	33.0 (40)
Timed AI	27.1 (142)	30.6 (184)
Serum P4 7 days after not pregnant diagnosis[5]		
High (≥ 1 ng/mL)	42.2 (133)	32.9 (142)
Low (< 1 ng/mL)	25.3 (55)	14.6 (45)
Cycling	34.8 (172)	33.8 (174)
Anestrus	15.6 (13)	16.2 (7)
Follicular cyst	25.8 (6)	38.4 (6)
Luteal cyst	53.1 (6)	28.2 (6)
Unknown	37.4 (33)	24.7 (31)

[1]Heatsynch = injection of GnRH 7 days before an injection of $PGF_{2\alpha}$, followed in 24 hour by 1 mg of ECP and one fixed-time AI (TAI) 42 to 50 hours after ECP. Ovsynch = injection of GnRH 7 days before and 48 hours after an injection of $PGF_{2\alpha}$, with one fixed TAI at 16 to 22 hours after the second GnRH injection. Adjusted least-squares percentages are illustrated.
[2]Includes all inseminations since treatment initiation on day 0.
[3]Replacement heifers differed ($P<0.05$) from lactating cows.
[4]Pregnancy rate for AI after detected estrus (37.1%; n = 128) tended ($P<0.10$) to be greater than that after TAI (28.9%; n = 326).
[5]Females with concentration of P4 ≥ 1 ng/mL 7 days after treatment initiation had greater ($P<0.001$) pregnancy rates than those with P4 < 1 ng/mL(37.5%; n = 275 vs. 20%; n = 100).

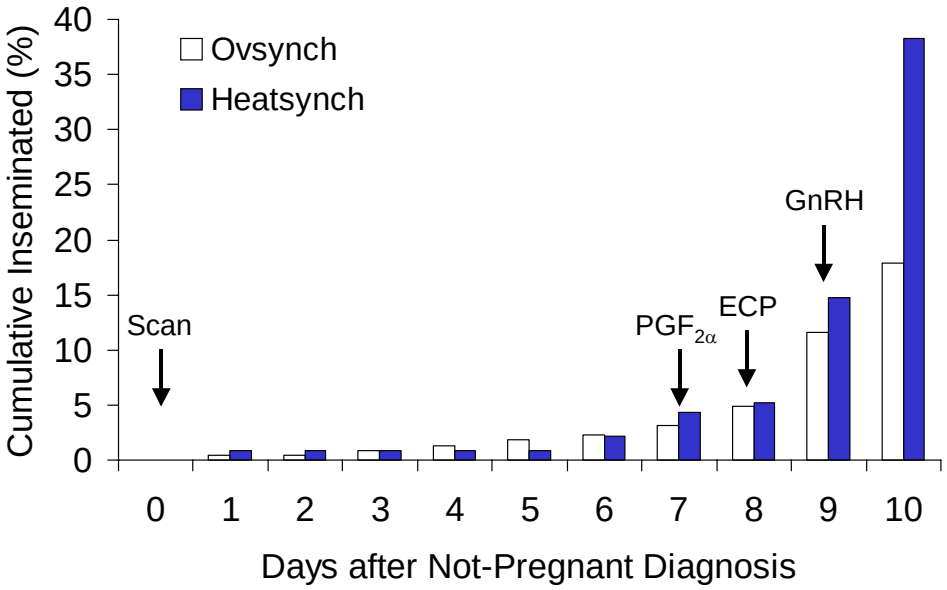

Figure 1. Cumulative Percentages of Females Inseminated after Detected Estrus During 10 Days after the Not-pregnant Diagnosis and Initiation of Treatments, as a Proportion of All Females Inseminated. More (P<0.05) females were inseminated after detected estrus on day 10 in the Heatsynch (23.5%; n = 54) than Ovsynch (6.3%; n = 14) protocols. Not shown on day 10 are all remaining females that received a TAI in the Ovsynch (82.1%; n = 184) and Heatsynch (61.7%; n =142) protocols.

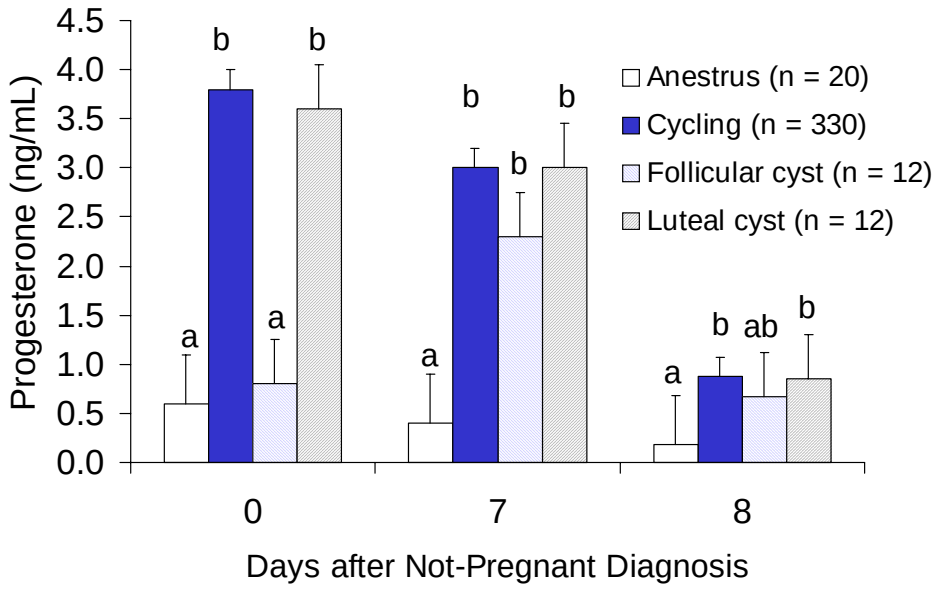

Figure 2. Serum Concentrations of Progesterone in Dairy Females Classified According to Ovarian Structures and Progesterone Status After the Not-pregnant Diagnosis and Initiation of Treatments. Injections of GnRH were administered on day 0, $PGF_{2\alpha}$ on day 7, and ECP on day 8 (those in Heatsynch protocol) or GnRH on day 9 (those in the Ovsynch protocol). Bars within day having different superscript letters differ ($P<0.05$).

INDEX OF KEY WORDS

Indexer's note: The numbers indicate the first page of each article that uses the listed key word.

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BIOLOGICAL VARIABILITY AND CHANCES OF ERROR

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

In some of the articles herein, you will see the notation "P<.05". That means the probability of the differences resulting from chance is less than 5%. If two averages are said to be "significantly different," the probability is less than 5% that the difference is from chance, or the probability exceeds 95% that the difference resulted from the treatment applied.

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

In other papers, you may see an average given as 2.5 ± .1. The 2.5 is the average; .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 all the research reported herein, statistical analyses are included to increase the confidence you can place in the results.

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