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Direct-Fed Microbial Supplementation and Health and Performance of Pre- and Postpartum Dairy Cattle: A Field Trial

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ABSTRACT

A double-blind field trial was conducted on a commercial dairy to study the effects of feeding a direct-fed microbial (DFM) product consisting of 2 strains of *Enterococcus faecium* plus *Saccharomyces cerevisiae* yeast on prepartum and postpartum performance of Holstein cows. Treatments consisted of the normal pre- and postfresh TMR supplemented with the DFM (2 g/cow per d) or a placebo. Treatments started approximately 10 d prepartum and continued until about 23 d in milk (DIM). A total of 366 Holstein cows were enrolled in 1 of 2 placebo groups or 2 DFM-supplemented groups. Groups were enrolled consecutively, starting with the placebo treatment. Sample size was limited to 4 groups because the cooperating dairy prematurely terminated the study due to increased health problems in one of the groups. Blood samples were taken during the prefresh period between 2 and 10 d prior to calving and at weekly intervals from 3 to 23 DIM. Blood concentrations of nonesterified fatty acids before calving and β-hydroxybutyrate after calving were not affected by treatment. Supplementation with the DFM product increased milk fat percentage for the first lactation cows and increased milk protein percentage for the second and greater lactation cows during the first 85 DIM. Second-lactation cows fed the DFM product received fewer antibiotic treatments before 85 DIM than cows receiving the placebo. This validated the dairy producer's concern that cows consuming one of the diets (revealed to be the placebo diet after the study was completed) were experiencing more health problems. Most measures of milk yield were numerically increased by supplementation with the DFM product. However, differences in milk yield were not significant. Key covariates for main study outcomes included milk yield in the previous (first) lactation, body condition score prior to calving, days spent in the maternity pen, and stocking density of the prefresh pen.

Key words: dairy cow, direct-fed microbial, milk yield, milk component

INTRODUCTION

The transition period of a dairy cow is defined as 3 wk prior to calving to 3 wk after calving (Grummer, 1995). Dramatic changes that occur during this time impose a metabolic challenge on the dairy cow. Supplementation of transition diets with a variety of direct-fed microbials (**DFM**) has been shown to have numerous benefits in transition diets, including increased ruminal digestion, DMI, and milk production and reduced body temperature (Piva et al., 1993; Higginbotham et al., 1994; McGilliard and Stallings, 1998). The definition of DFM is very broad and may include specific and nonspecific yeast, fungi, bacteria, cell fragments, and filtrates (Beharka and Nagaraja, 1998; Sullivan and Martin, 1999).

Enterococcus faecium produces moderate amounts of lactic acid in the rumen. This could stimulate growth of lactic acid utilizers and stabilize ruminal pH. Nocek et al. (2002) fed a combination of *Enterococcus faecium* and *Lactobacillus plantarum* with *Saccharomyces cerevisiae* to lactating cows receiving a diet high in NFC. Cows fed this DFM combination to 1×10^5 cfu/mL of ruminal fluid had higher ruminal pH nadirs than cows fed to 1×10^6 or 1×10^7 cfu/mL.

A DFM combination providing a daily dose of 5×10^9 cfu of 2 strains of *Enterococcus faecium* and 5×10^9 cfu yeast has been evaluated in 2 controlled studies in transition cows. In both studies DFM were fed from about 21 d prepartum through 70 d postpartum. In the first study (Nocek et al., 2003), the DFM combination significantly reduced early lactation drop in ruminal pH and increased DMI, milk yield, and milk protein percentage in early lactation. Blood glucose and insulin concentrations were significantly higher, and blood NEFA concentrations were numerically lower for cows that received the DFM. In the second study (Nocek

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and Kautz, 2006), the DFM combination significantly enhanced ruminal digestibility of forage, increased milk yield, increased blood glucose, and decreased blood BHBA.

The objective of this study was to determine the effects of a supplemental DFM combination (2 strains of *Enterococcus faecium* and yeast) on the performance of transition dairy cows in a field trial on a commercial dairy.

MATERIALS AND METHODS

Cows, Treatments, and Diets

A total of 366 Holstein dairy cows on a 1,700-cow commercial dairy in southern Wisconsin were entered into a feeding trial to determine the effects of a DFM product on pre- and postpartum performance. The DFM product (Probios TC, Chr. Hansen, Milwaukee, WI) provided 5×10^9 cfu of 2 lactic acid-producing strains of *Enterococcus faecium* and 5×10^9 cfu of *Saccharomyces cerevisiae* per day. Treatments consisted of the normal pre- and postfresh TMR with the DFM product (2 g/cow per d) or a placebo (2 g of carrier alone) incorporated into the custom protein mix for each group. On-farm dairy personnel and the investigators were unaware of which treatment included the DFM until the conclusion of the study.

The study was conducted over a 29-wk period from October 2000 to April 2001. Cows were enrolled in 1 of 4 groups (2 placebo and 2 DFM-supplemented groups). Groups were enrolled consecutively. Sequence of treatment assignments was randomly initiated with the B treatment and followed the sequence B-A-B-A. The identity of the A and B treatments was not revealed to the dairy producer or to the investigators until the study was completed. All eligible cows that consumed the specified diets for at least 10 d before calving and at least 23 d after calving were enrolled in the study. Cows were moved into the prefresh group approximately 21 d before expected calving. Data were collected from each cow until 85 d after calving or until the cow was removed from the herd.

Treatments were switched after approximately 40 second-lactation cows were enrolled in a group. Many cows necessarily received both treatments before calving and therefore were not eligible for enrollment in the study. After several weeks, cows that had received only one diet could again be enrolled.

Cows within the 4 groups were divided into 2 study populations based on parity. The main experimental population consisted of 163 cows entering their second lactation (about 40 cows per group). Cows in the main study population were intensively monitored for precalving NEFA, BCS, milk production, milk components, estimated DM intake, and postcalving BHBA. A secondary population of 132 first-lactation and 71 second- or greater lactation cows was less intensively monitored for milk production and milk components only. The main and secondary populations of cows were enrolled in the study at the same time and received the same diets. The number of cows in the secondary study populations was not targeted and consisted of any eligible cows that consumed the proper diet during the same time period as the main study population.

Temperature and humidity were recorded every 10 min in the pre- and postfresh pens every day of the study using an automated recording device (Ryan Instruments, HAT Monitor, Redmond, WA). Average temperature and relative humidity values during the study for each cow were calculated based on the days spent in each pen.

On-farm personnel recorded pen moves daily, and a backup of the on-farm record system (Dairy Comp 305, Valley Agricultural Software, Tulare, CA) was created each day. This information was used to determine each cow's daily pen location, dates of pen moves, and daily stocking density for each pen (number of cows in the pen that day divided by the number of free-stalls in the pen). The average stocking density experienced by each cow during the pre- and postfresh periods was calculated from the daily pen stocking densities and each cow's daily pen location. Each pen contained 2 rows of free-stalls (except for the maternity pen, which was loose housing) and a single row of headlocks at the feed bunks. The mean number of headlocks in the pens was 11% greater than the mean number of free-stalls.

The TMR diets offered to the pre- and postfresh groups were sampled weekly at 10 locations along each feed bunk. Feed was sampled immediately after delivery, and samples were collected only from undisturbed portions of the bunk. The composite bunk sample was reduced after mixing into a smaller subsample. Analysis for standard nutrients was made on the subsample using wet chemistry procedures (Cumberland Valley Analytical Services, Maugansville, MD).

Dry matter intake was estimated for each pen by subtracting the estimated amount of feed refused from the amount of feed offered each day. Weights of feed offered and refused each day were obtained from the scales on the feed mixing wagon. Accuracy of the TMR mixer scales was verified monthly on a platform scale with the mixer empty, half-empty, and nearly full. Dry matter intake was adjusted for the number of primiparous cows in each pen using an intake adjustment factor of 0.908 for the prefresh period and 0.808 for the postfresh period. Adjustment factors were derived from data contained in a dairy ration software model (National Research Council, 2001). Estimated DMI was calcu-

lated for each cow based on the days she was present in each pen.

Data Collection and Sampling

Daily milk production for each postpartum cow was recorded using computerized weigh meters in the parlor. Milk weights were collected starting on the third day after calving until 85 DIM. Composite milk samples were collected weekly from cows in the main study population up to 23 DIM during the 0400 h milking using an automated sampler (Milk Meter Sampling Device, WestfaliaSurge Inc., Naperville, IL). The WestphaliaSurge dealer calibrated the milk meters at the beginning of the study according to company calibration guidelines.

Additional milk samples from the main study population were collected monthly as part of the herd's routine DHI testing program. Cows in the secondary study populations were sampled monthly for milk components through d 85. Milk samples from all study populations were refrigerated and analyzed for fat and protein content using infrared techniques by the Ag-Source Milk Analysis Laboratory in Menomonie, WI (MilkOScan 4000 Infrared Analyzer; Foss Technology, Hillerød, Denmark).

Blood samples were collected once weekly via the coccygeal vein from cows in the main study population from the time they entered the prefresh group until 23 DIM. Blood samples from prefresh cows were collected into heparinized tubes and immediately placed on ice. Tubes were later centrifuged at $2,500 \times g$ for 10 min. Plasma was separated, immediately frozen, and later submitted for NEFA analysis. Blood from postfresh cows was collected into tubes without additive and allowed to clot at room temperature. Tubes were later centrifuged at $2,500 \times g$ for 10 min and the serum separated and refrigerated. Serum was later analyzed for BHBA concentration. Blood analyses for BHBA and NEFA were conducted at a commercial laboratory (Marshfield Clinic Veterinary Diagnostic Services, Marshfield, WI) using a Roche Hitachi 911 chemistry analyzer (Roche Diagnostics Corp., Indianapolis, IN), following the procedures of Williamson et al. (1962) for BHBA analysis and the Wako NEFA-C test kit (Wako Chemicals USA, Richmond, VA) for NEFA analysis.

Cows in the main study population were evaluated for BCS using a 5-point scale (Edmonson et al., 1989). Prefresh cows were sampled and scored weekly. However, only the last sample prior to calving was recorded and used in the analysis. If a cow calved less than 48 h after the last prefresh period sample was collected, then her next to last score was recorded instead. Each cow's body condition loss from her prepartum score to her last score (third week after calving) was calculated and recorded as her body condition score drop.

The proportion of cows above threshold concentrations for NEFA and BHBA were also recorded and evaluated. Thresholds concentrations were greater than 400 μM for NEFA and greater than 1,400 μM for BHBA (Oetzel, 2004).

Health outcomes (displaced abomasum, antibiotic treatments excluding treatment of mastitis, and removal from the herd) were recorded until 85 DIM for cows in the main study population. Health outcomes were determined and recorded by the producer. Antibiotic treatments due to clinical mastitis were not included in the analysis. The 85 DIM limit was chosen because it encompassed the time of peak milk yield for most cows. Supplementation of the DFM product stopped at about 23 DIM; therefore, any effects observed from 23 to 85 DIM would indicate a residual treatment effect.

Statistical Analysis and Experimental Design

Data were analyzed by ANOVA using a completely randomized design with subsampling. The experimental unit (i.e., the smallest unit to which the treatment was applied randomly) was the group of cows (n = 4 for each study population). The error term used for testing the treatment effect was treatment by group. Analyses were conducted using SAS Release 8.02 for Windows (SAS Institute, 1999).

Continuous Outcomes with Single Measures. Effect of DFM on continuous outcomes with single measures (or outcomes calculated as single means) was determined using mixed models. Covariates available for inclusion in the final models were recorded on an individual cow basis and included previous lactation 305-d mature equivalent milk production, diet composition, temperature, humidity, stocking density, days spent in the prefresh group, and days spent in the maternity pen. Because there were up to 44 possible covariates per outcome, covariates were first screened for inclusion in the final models by evaluating their correlation to each outcome using the CORR procedure of SAS. Covariates with significant Pearson product-moment correlations ($P < 0.05$) were included in a backwards elimination model in the REG procedure of SAS. Covariates included ($P < 0.10$) in the regression model were made available for the final mixed model. Group was entered into the model as a random effect. The final mixed model, using the MIXED procedure of SAS, was determined by manual backward elimination of eligible covariates until all remaining covariates were significant in the model ($P < 0.05$).

Residual plots were visually evaluated as a test for the assumption of normal distribution of the data. Data for BHBA and NEFA were transformed (natural log) to reduce heteroscedasticity of the residual plots. The least squares means and standard errors for these outcomes were reported from model results using untransformed data.

Continuous Outcomes with Repeated Measures. Effect of DFM on continuous outcomes with repeated measures (daily milk yield, milk fat test, milk fat yield, milk protein test, and milk protein yield) was determined using repeated measures analysis in mixed models as described by Littell et al. (1998). The model included auto-correlation using the spatial powers covariance structure in the MIXED procedure of SAS. Data available for this analysis included milk components that were measured on wk 1, 2, and 3 plus additional DHI tests before 85 DIM. All available daily milk weights from 3 to 85 DIM were also included in the analysis. Covariates based on DIM considered for inclusion in the mixed model for repeated measures in the main study population were the linear, squared, cubic, and quadratic effects of DIM, the inverse of the same, and all interactions of DIM variables with treatment. These were screened using a backwards elimination model in the REG procedure of SAS. Covariates eligible for inclusion in the mixed models were those included in the regression model ($P < 0.10$) plus previous lactation 305-d mature equivalent milk production, stocking density of the prefresh group, and days spent in the maternity pen. The model was reduced by manual backward elimination of eligible covariates until all were significant in the final model ($P < 0.01$ for outcomes with denominator degrees of freedom > 500, and $P < 0.05$ for all other outcomes). Some cubic transformations of DIM could not be included in the final models because they rendered the least-squares means nonestimable. Excluding these terms from the models resulted in negligible changes in *P*-values for treatment effects.

Repeated measures data from cows in the secondary populations were analyzed using similar methods, except that inverse measures of DIM were not considered, and all eligible covariates were entered into the mixed models without prior screening in a regression analysis. Because previous ME milk production was a key covariate for milk yield in the second-lactation cows and because first-lactation animals had no record of previous milk yield, their data were analyzed separately from the data for the second and greater lactation cows. Lactation and treatment by lactation were eligible covariates for models involving the second and greater lactation cows.

Residual plots from the repeated measures analyses were visually evaluated as a test for the assumption of normal distribution of the data. There was no evidence of heteroscedastity in the residual plots, and no data transformations were necessary.

Proportional Outcomes with Single Measures. Effect of DFM on proportional outcomes with single measures (NEFA concentration >400 μM, BHBA concentration >1,400 μM, removal from the herd, antibiotic treatment, and displaced abomasum) was determined using logistic regression in a mixed model (Glimmix macro of SAS). Covariates with Pearson product-moment correlations $P < 0.05$ were eligible for inclusion in the final logistic regression model. Group was considered a random effect in the model. Covariates were eliminated by manual backwards elimination until all were significant ($P < 0.05$) in the final model.

Criteria for Significance. Only 2 degrees of freedom were available for testing treatment effects in all of the models. Significance was declared at $P < 0.10$ for treatment effects, unless otherwise noted, because of the few degrees of freedom. Values presented are least-squares means with the pooled standard error of the difference.

RESULTS AND DISCUSSION

Descriptive statistics of the experimental groups are presented in Table 1. Values for all variables did not differ ($P > 0.10$) by treatment. No maximum temperature exceeded the upper critical temperature of about 21°C for lactating dairy cows.

Nutrient composition of diets offered is presented in Table 2. Composition of prepartum diets for the placebo and DFM treatments generally did not differ ($P > 0.10$). The placebo treatment had significantly lower crude fat concentration, although both treatments were below the maximum recommendation of 4.5% dietary fat, DM basis (National Research Council, 2001). Samples of prepartum TMR containing the DFM product had significantly lower DCAD. All prepartum diets had relatively low DCAD due to the addition of anionic salts.

Postfresh diets were also very similar for TMR samples containing the DFM product and the placebo. Chlorine was significantly higher in the postfresh placebo samples, but DCAD was not different between the diets. Postpartum diets were relatively high in fiber (about 38% NDF, DM basis) and low in NFC (about 32%, DM basis).

The cause of significant differences between the composition of diets containing the placebo or the DFM product was not investigated. Variation in feed ingredient composition is expected in the time period between sampling and analyzing a feed ingredient, so some differences between diets over time were expected.

Table 1. Descriptive statistics of the experimental groups from a population of 163 second-lactation cows in a field trial to evaluate a DFM[1] fed before and after calving

Variable	DFM			Placebo			Pooled SE	P^2 <
	Group 2	Group 4	Mean	Group 1	Group 3	Mean		
Cows	40	40	—	40	43	—	—	—
Start date (first calving)	11/22/00	3/7/01	—	10/15/00	1/13/01	—	—	—
End date (last calving)	12/4/00	4/8/01	—	10/31/00	2/13/01	—	—	—
ME,[3] kg/305 d	10,545	11,943	11,224	10,022	11,311	10,669	672	0.61
Initial BCS[4]	3.43	3.48	3.45	3.09	3.45	3.27	0.13	0.43
Prefresh period								
Prefresh,[5] d	22.0	25.1	23.5	19.7	17.1	18.4	1.4	0.12
Maternity,[6] d	5.8	5.1	5.5	6.1	6.6	6.4	0.4	0.28
NEFA int.,[7] d	4.8	5.4	5.1	5.9	4.7	5.3	0.5	0.83
Stocking,[8] %	87.0	83.0	85.0	91.3	127.4	109.4	12.9	0.31
Temperature,[9] °C	1.2	1.4	1.3	13.1	–3.1	5.0	5.7	0.69
Humidity,[10] %	73.3	69.9	71.6	66.5	79.6	73.1	4.8	0.85
Postfresh period (calving to 23 DIM)								
Stocking, %	86.6	71.1	78.8	80.7	77.7	79.2	5.6	0.97
Temperature, °C	–4.3	5.9	0.8	10.1	–3.1	3.5	5.9	0.78
Humidity, %	75.6	69.2	72.4	71.1	77.8	74.4	3.3	0.70

[1]Direct-fed microbial (Probios TC, Chr. Hansen, Milwaukee, WI) containing 5×10^9 cfu of bacteria (2 specific *Enterococcus faecium* strains) and 5×10^9 cfu of yeast.

[2]*P*-value for treatment effect.

[3]Previous lactation mature equivalent milk production.

[4]Body condition score prior to calving.

[5]Days in the prefresh period.

[6]Days in the maternity pen.

[7]Interval from NEFA sample collection to calving.

[8]Stocking density, calculated as cows in the pen divided by free-stalls in the pen.

[9]Mean daily temperature recorded in the pens.

[10]Mean daily humidity recorded in the pens.

Measurement of nutrient intakes was important because placebo and DFM-supplemented groups were not concurrent in this study design. Intake of all measured nutrients was estimated on an individual cow basis and included as potential covariates in the final models for treatment effects. Most nutrients were included as significant covariates in one or more of the final models (data not shown), indicating that for this field trial it was important to intensively collect information about diet and include it in the statistical analysis. There was no apparent pattern to the inclusion of nutrient intakes in the final models; this indicated that feed delivery and nutrient intakes were well controlled on the farm and did not overly influence study outcomes.

Prepartum Performance

Estimated DM intake and average blood NEFA concentration were not affected ($P > 0.10$) by DFM supplementation (Table 3). Groups receiving the DFM supplementation had numerically higher DMI and numerically lower but statistically nonsignificant NEFA concentrations. Nocek et al. (2003) also reported no effect of DFM supplementation on precalving NEFA.

Stokol and Nydam (2005) reported that blood NEFA concentrations were more stable at 24°C if collected into tubes containing EDTA or tubes without anticoagulant compared with collection into tubes containing heparin. Samples stored at 4°C were stable regardless of the type of tube used for collection. Because samples in this trial were placed in ice immediately after collection, the use of heparin as the anticoagulant should not have affected the stability of blood NEFA.

All cows lost BCS (about 0.41 units) from the last score taken before calving to the third week of lactation (Table 3). Treatment did not affect ($P > 0.10$) BCS loss.

Postpartum Performance

Average and peak milk yield, DIM at peak, milk components, estimated DM intake, and blood BHBA results from the main population of cows for the first 3 wk of lactation are presented in Table 3. No outcomes were affected ($P > 0.10$) by treatment. Postpartum outcomes were also evaluated separately for wk 1, 2, and 3 (data not shown). No outcomes were affected by treatment on wk 1, 2, or 3.

Repeated measures analyses indicated that milk fat percentage was not affected by DFM supplementation

Table 2. Composition of diets offered to 163 second-lactation cows in a field trial to evaluate a DFM[1] fed before and after calving

	Prefresh period				Postfresh period			
Variable	DFM	Placebo	Pooled SE	P^2 <	DFM	Placebo	Pooled SE	P^2 <
DM, %	56.0	56.3	0.4	0.60	60.5	61.8	1.6	0.62
NE_L,[3] Mcal/kg DM	1.54	1.43	0.03	0.13	1.55	1.56	0.02	0.74
Crude fat, % of DM	4.0	3.0	0.1	0.01	3.9	4.0	0.2	0.59
CP, % of DM	16.4	17.9	0.6	0.23	16.8	18.3	0.9	0.36
SIP,[4] % of DM	6.6	6.6	0.5	0.99	6.4	5.8	0.4	0.42
ADF, % of DM	28.7	30.9	1.5	0.40	26.3	24.8	0.6	0.23
NDF, % of DM	41.2	42.9	2.0	0.61	39.7	36.5	1.1	0.18
NFC,[5] % of DM	30.7	27.8	2.5	0.50	32.0	32.8	2.3	0.84
Ash, % of DM	7.68	8.41	0.43	0.35	7.64	8.39	0.43	0.34
Ca, % of DM	1.06	1.03	0.06	0.77	1.01	1.16	0.08	0.31
Cl, % of DM	1.06	1.15	0.06	0.38	0.54	0.67	0.02	0.04
Mg, % of DM	0.44	0.42	0.01	0.41	0.30	0.32	0.02	0.42
P, % of DM	0.32	0.34	0.03	0.66	0.41	0.45	0.01	0.20
K, % of DM	1.64	2.10	0.20	0.25	1.70	1.98	0.10	0.19
Na, % of DM	0.02	0.04	0.00	0.18	0.18	0.14	0.04	0.52
S, % of DM	0.29	0.30	0.02	0.87	0.27	0.26	0.02	0.77
DCAD,[6] mEq/kg of DM	−48	43	21	0.09	191	216	29	0.59

[1]Direct-fed microbial (Probios TC, Chr. Hansen, Milwaukee, WI) containing 5×10^9 cfu of bacteria (2 specific *Enterococcus faecium* strains) and 5×10^9 cfu of yeast.

[2]*P*-value for the effect of treatment within the specified period.

[3]NE_L estimate based on the Ohio equation (National Research Council, 2001).

[4]Soluble intake protein.

[5]NFC calculated as (100 − crude fat − CP − NDF − ash).

[6]Calculated as milliequivalents of (Na + K) − (Cl + S).

Table 3. Single-measure outcomes from 163 second-lactation cows in a field trial to evaluate a DFM[1] fed before and after calving

Variable	n	DFM	Placebo	Pooled SE	*P* <
Prefresh period					
Estimated DMI,[2] kg/d	163	14.2	13.1	1.4	0.65
Blood NEFA, μ*M*	160	187	200	27	0.50
Postfresh period					
Milk yield 3-23 DIM,[3] kg/d	163	39.8	37.9	1.6	0.44
Peak milk,[4] kg	149	50.9	51.5	1.2	0.70
DIM at peak milk, d	149	46.3	44.1	2.2	0.48
Milk fat,[5] %	126	4.22	4.50	0.16	0.39
Milk fat yield, kg/d	126	1.67	1.69	0.06	0.84
Milk protein,[5] %	126	3.35	3.34	0.12	0.95
Milk protein yield, kg/d	126	1.59	1.10	0.33	0.41
DMI, kg/d	163	20.2	21.8	1.6	0.56
BCS drop[6]	132	0.41	0.41	0.33	0.99
Blood BHBA,[7] μ*M*	129	848	559	81	0.21

[1]Direct-fed microbial (Probios TC, Chr. Hansen, Milwaukee, WI) containing 5×10^9 cfu of bacteria (2 specific *Enterococcus faecium* strains) and 5×10^9 cfu of yeast.

[2]Estimated DMI calculated from daily pen feed intake and adjustment for the proportion of first lactation cows in the pen on each day.

[3]Average daily milk yield from daily milk weights obtained between 3 and 23 DIM.

[4]Highest daily milk yield based on a 5-d rolling average of all daily milk weights from 3 to 85 DIM.

[5]Average of the 3 weekly milk composition measurements taken in each of the first 3 wk of lactation.

[6]Body condition score decrease from prefresh to the third week of lactation.

[7]Average of the 3 blood BHBA determinations taken in the first 3 wk of lactation.

Table 4. Repeated measures of milk yield and milk components for 163 second-lactation cows from 3 to 85 DIM in a field trial to evaluate a DFM[1] fed before and after calving

Variable	OBS[2]	DFM	Placebo	Pooled SE	$P <$
Milk yield,[3] kg	77.1	43.4	42.5	2.5	0.71
Milk fat, %	4.5	4.06	3.88	0.11	0.30
Milk fat yield, kg/d	4.5	1.57	1.58	0.08	0.99
Milk protein, %	4.5	3.19	3.13	0.04	0.07
Milk protein yield, kg/d	4.5	1.29	1.27	0.07	0.85

[1]Direct-fed microbial (Probios TC, Chr. Hansen, Milwaukee, WI) containing 5×10^9 cfu of bacteria (2 specific *Enterococcus faecium* strains) and 5×10^9 cfu of yeast.

[2]Mean number of observations recorded per cow from 3 to 85 DIM.

[3]All recorded daily milk weights from 3 to 85 DIM.

for the main population of cows from 3 to 85 DIM (Table 4) and for all the second- and greater lactation cows (Table 5). Milk fat percentage was higher ($P < 0.10$) for first-lactation cows that received the DFM product (Table 5) and particularly in very early lactation (Figure 1). The interaction terms treatment × DIM and treatment × DIM^2 were included ($P < 0.05$) in the final model for milk fat percentage in first-lactation cows.

Increased milk fat percentage in very early lactation is often associated with adverse events such as excessive negative energy balance, rapid mobilization of body fats, and subclinical ketosis (Duffield and Bagg, 2002). However, neither mean blood BHBA concentration nor the proportion of cows with elevated BHBA concentrations was increased in cows receiving the DFM product in the first week of lactation.

Nocek et al. (2003) reported numerically but not significantly increased milk fat percentage when the DFM product was supplemented. The magnitude of the increase was greatest in the first week of lactation, which is consistent with the results of the current field study. In contrast, DFM supplementation significantly decreased milk fat percentage in another trial (Nocek and Kautz, 2006). These results indicate an inconsistent effect of the DFM product on milk fat percentage. Other factors or interactions may be more important determinants of milk fat percentage in early lactation.

Supplementation with the DFM product increased ($P < 0.10$) milk protein percentage from 3 to 85 DIM for the main study population (Table 4) and for the second and greater lactation cows (Table 5). Increases in milk protein percentage were greatest in the first weeks of lactation (Figure 2). The interaction terms treatment × DIM and treatment × DIM^2 were included ($P < 0.01$) in the final models for milk protein percentage.

Total milk protein yield was not increased by DFM supplementation, and the magnitude of the milk protein percentage response was biologically small, al-

Table 5. Repeated measures of milk yield and milk components for 132 first-lactation and 234 second- or greater lactation cows from 3 to 85 DIM in a field trial to evaluate a DFM[1] fed before and after calving

Variable	OBS[2]	DFM	Placebo	Pooled SE	$P <$
Lactation = 1 (n = 132)					
Milk yield,[3] kg/d	76.8	29.2	30.8	0.8	0.14
Milk fat, %	2.3	3.63	3.33	0.19	0.08
Milk fat yield, kg/d	2.3	1.12	1.01	0.04	0.19
Milk protein, %	2.3	3.07	3.00	0.03	0.12
Milk protein yield, kg/d	2.3	0.96	0.92	0.11	0.45
Lactation > 1[4] (n = 234)					
Milk yield, kg/d	76.8	44.2	42.3	1.5	0.47
Milk fat, %	3.9	3.95	3.77	0.08	0.47
Milk fat yield, kg/d	3.9	1.57	1.54	0.03	0.39
Milk protein, %	3.9	3.13	3.10	0.04	0.03
Milk protein yield, kg/d	3.9	1.26	1.30	0.02	0.30

[1]Direct-fed microbial (Probios TC, Chr. Hansen, Milwaukee, WI) containing 5×10^9 cfu of bacteria (2 specific *Enterococcus faecium* strains) and 5×10^9 cfu of yeast.

[2]Mean number of observations recorded per cow from 3 to 85 DIM.

[3]All recorded daily milk weights from 3 to 85 DIM.

[4]Includes the main study population (163 second-lactation cows) plus an additional 71 second- and greater lactation cows from the secondary study population.

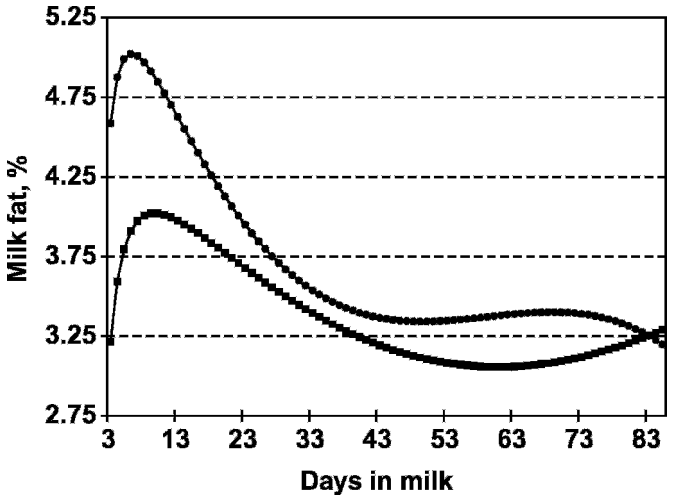

Figure 1. Predicted values from the final model for milk fat percentage of 132 first-lactation cows receiving a direct-fed microbial product (—●—) or a placebo (—■—). The direct-fed microbial product was Probios TC (Chr. Hansen, Milwaukee, WI), which contained 5×10^9 cfu of bacteria (2 specific *Enterococcus faecium* strains) and 5×10^9 cfu of yeast. Pooled SE = 0.04%.

though statistically significant. Changes in milk protein percentage of about 0.03 percentage units are unlikely to be detected on the farm.

Nocek at al. (2003) also found increased milk protein percentage when the DFM product was supplemented, and the increase was of a greater magnitude (about 0.15 percentage units). The improvement in milk protein

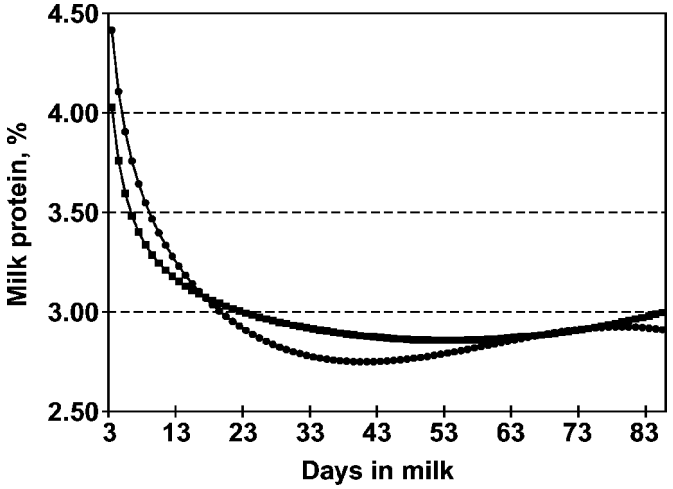

Figure 2. Predicted values from the final model for milk protein percentage of 234 second- and greater lactation cows receiving a direct-fed microbial product (—●—) or receiving the placebo (—■—). The direct-fed microbial product was Probios TC (Chr. Hansen, Milwaukee, WI), which contained 5×10^9 cfu of bacteria (2 specific *Enterococcus faecium* strains) and 5×10^9 cfu of yeast. Pooled SE = 0.19%.

percentage was not present immediately after calving but increased with DIM. Nocek and Kautz (2006) reported numerically increased milk protein (0.01 percentage units) in cows receiving the DFM product. These cows had considerably increased milk yield, which could explain why the increase in milk protein percentage was not greater. Improved rumen function and increased microbial protein yield could account for the consistent findings of improved milk protein percentage or milk protein yield in cows receiving the DFM product.

Cows supplemented with DFM had fewer antibiotic treatments ($P < 0.10$) than cows receiving the placebo (Table 6). Reasons for antibiotic treatment of the DFM-supplemented cows were metritis (13 cows), lameness (5 cows), displaced abomasum (1 cow), and unknown infection (1 cow). Reasons for antibiotic treatments in the cows receiving the placebo were metritis (21 cows), lameness (3 cows), displaced abomasum (1 cow), and unknown infection (10 cows). These results should be interpreted with caution. All diagnoses and treatment decisions were made by the producer, and detailed reasons for antibiotic treatments were not recorded. Other proportional outcomes were not affected by DFM. More detailed health data and larger sample sizes would be needed to clarify the effect of DFM on the general health of dairy cows during early lactation.

Daily milk yield, peak milk yield, milk fat yield, and milk protein yield were not affected by DFM supplementation (Tables 3 through 5). Previous studies with the same DFM product documented significant increases in milk yield of 4.6% (Nocek et al., 2003) and 6.0% (Nocek and Kautz, 2006). Sample size in the current study was very small and lacked statistical power to declare significance for similar numerical increases.

Diets in this trial averaged about 29.3% NFC in the prepartum groups and about 32.4% NFC (DM basis) in the postfresh groups. These diets may not have been high enough in NFC to cause a ruminal acidosis problem, which the DFM product was designed to avert. General guidelines for providing adequate fiber in the diet and maintaining optimal DMI include total NDF between 25 to 35%, maintaining a minimum of 18% forage NDF, and feeding 33 to 40% NFC (Valadares Filho et al., 2000).

Field Study Design

Nonregulatory feeding trials in commercial settings can be useful in evaluating new nutritional supplements. Field studies can estimate the frequency of success under a wide range of conditions and suggest management and environmental factors that impact such success. Results can be used to determine potential

Table 6. Proportional outcomes from 163 second-lactation cows in a field trial to evaluate a DFM[1] fed before and after calving

	DFM			Placebo			
Variable	Group 2	Group 4	Mean	Group 1	Group 3	Mean	$P <$
Prefresh period							
Blood NEFA >400 μM, %	2.5 (1/40)	8.1 (3/37)	5.2 (4/77)	7.5 (3/40)	9.3 (4/43)	8.4 (7/83)	0.78
Postfresh period							
Blood BHBA[2] >1,400 μM, %	12.5 (3/24)	11.8 (4/34)	12.1 (7/58)	8.3 (3/36)	22.9 (8/35)	15.5 (11/71)	0.30
Displaced abomasum, %	5.0 (2/40)	5.0 (2/40)	5.0 (4/80)	2.5 (1/40)	7.0 (3/43)	4.8 (4/83)	0.96
Antibiotic treatment, %	20.0 (8/40)	30.0 (12/40)	25.0 (20/80)	32.5 (13/40)	51.1 (22/43)	42.2 (35/83)	0.09
Removal by 85 DIM,[3] %	5.0 (2/40)	2.5 (1/40)	3.8 (3/80)	5.0 (2/40)	18.6 (8/43)	12.0 (10/83)	0.67

[1]Direct-fed microbial (Probios TC, Chr. Hansen, Milwaukee, WI) containing 5×10^9 cfu of bacteria (2 specific *Enterococcus faecium* strains) and 5×10^9 cfu of yeast.

[2]Proportion of cows with blood BHBA >1,00 μM in any of the 3 samples collected in the first 3 wk of lactation.

[3]Cows removed from the herd by 85 DIM because of death or culling.

economics impacts of a new practice and serve as a basis to make changes in protocols on real farms. Field studies are generally not appropriate for elucidating mechanisms or modes of action (St-Pierre and Jones, 1999).

Field studies with an on-off design are appealing because they can be conducted in commercial dairies without splitting transition cows into concurrent treatment and control groups. However, it can be practically challenging to acquire sufficient experimental units in an on-off field study. This limitation may be somewhat offset by reduced variance among experimental units, because each data point is a group mean (St-Pierre and Jones, 1999).

Another inherent difficulty in field studies is lack of control over management decisions. This study was constrained by management factors that were outside of the control of the investigators. The initial study design for this study called for the enrollment of 8 treatment groups of approximately 40 cows each. Power estimates using bootstrap simulation techniques showed that a difference of 1.7 kg of daily milk could be declared significant ($P < 0.05$ and a power estimate of 0.80) with this sample size. However, our final sample size was only 4 groups of cows.

Several factors contributed to the smaller than expected number of treatment groups enrolled in the study. The study was designed to take advantage of the cooler seasons so that heat stress would not confound results. However, weather conditions still caused difficulties in this study. In December 2000, extremely cold temperatures (Figure 3) caused freezing of the manure flush system. Passageways were covered with ice, making it difficult for the cows to walk. We observed cows struggling to reach their stalls and staying in them for long periods of time once they reached them. To avoid bias caused by these unusual weather conditions, the group of cows enrolled in the study during this time period was removed from the analysis.

Other difficulties prolonged the time needed to complete the field study and reduced the number of groups of cows that could enter it. Many cows did not spend the minimum 10 d required in the prefresh group and were not included in the study. This slowed the enrollment of groups. Finally, after the fourth group was enrolled in the study, the farm managers perceived that cows experienced more health problems on treatment B compared with treatment A, and requested that we not use treatment B in any future groups. This decision prematurely ended the study once the fourth and final group (treatment A) was finished.

The identity of the treatments was revealed after the study ended. The farm managers had correctly assumed that treatment A contained the DFM product. Statistical analysis of herd data then confirmed that the number of antibiotic treatments was significantly higher when the placebo was fed (Table 6). This illustrates a common difficulty in field studies—if the product tested is obviously better than the placebo, it is difficult (and in some circumstances unethical) to continue the study.

The initial power estimates also did not account for the unexpectedly large variation in individual cow milk yields. The placebo group had a standard deviation of 8.2 kg for milk yield from 3 to 23 DIM. Our statistical power estimates (using data from previous studies) used 6.8 kg as the standard deviation for daily milk yield. The larger standard deviation meant that 20 groups (instead of 8) would have been needed to detect a milk yield response of 1.7 kg.

Intensive collection of covariate data (individual cow data, environmental data, and nutrient intake data) is essential for on-off study designs. Covariates included in the final models for this study were inspected for their extent and pattern of inclusion. Milk yield in the previous lactation was the key covariate for the main study population (data not shown). This covariate was highly significant ($P < 0.001$) in almost every measure of milk yield evaluated. It was also included in most of

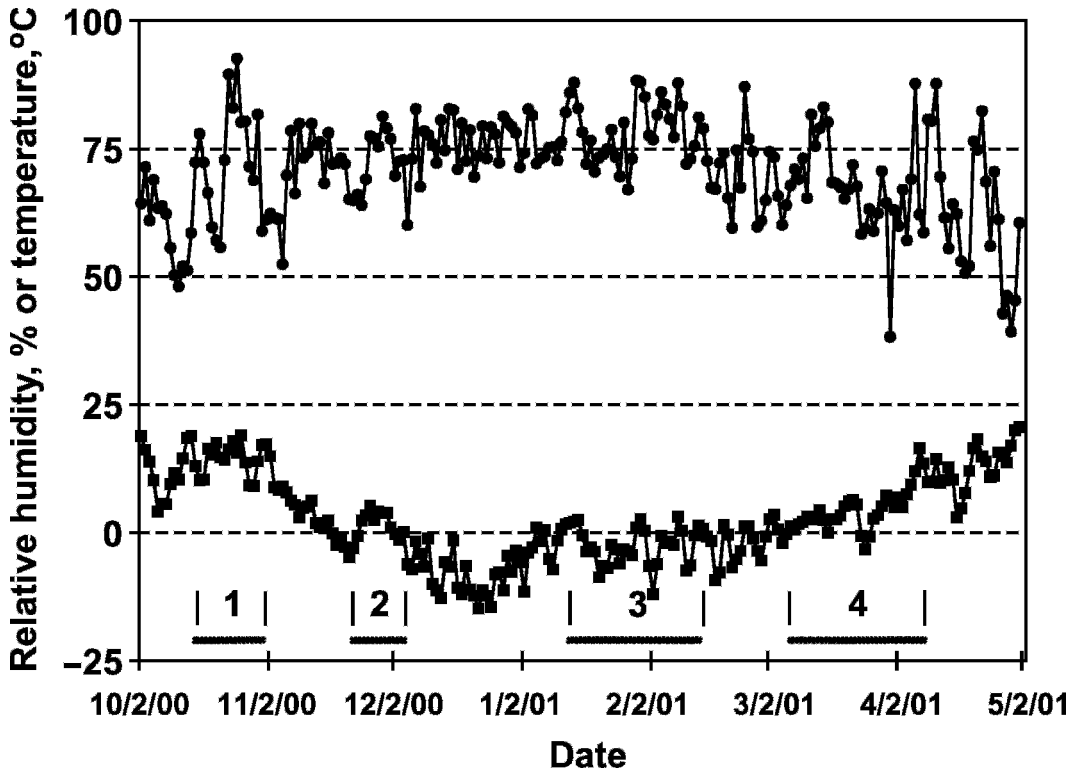

Figure 3. Relative humidity (—●—) and temperature (—■—) measured in the prefresh group during the field trial. Fresh dates for cows in each group are indicated by |___|.

the milk fat and protein yield final models. Using the final repeated measures model for milk yield from 3 to 85 DIM for the second and greater lactation cows, an increase of 500 kg of previous ME milk was associated with an increase in daily milk yield of 0.8 kg (about 1.8%). The direction of this outcome was expected, but its magnitude was unexpectedly large and illustrates the importance of including previous milk yield in the analysis of this field study.

Body condition score prior to calving was also an important covariate in this study (data not shown). Increasing body condition score prior to calving was consistently associated ($P < 0.05$) with increased body condition drop after calving, increased blood BHBA concentration, and increased milk yield. Increasing dry cow body condition score 0.25 units increased milk yield (from 3 to 85 DIM) by 0.8 kg for the main study population and increased blood BHBA concentration by about 100 μM. These results were expected, and the inclusion of BCS as a covariate in this field study was important.

Days spent in the maternity pen, where most cows were moved several days before expected calving, were included in many of the final models and had negative implications (data not shown). Increasing days in the maternity pen increased prefresh blood NEFA concentrations, increased postfresh BHBA concentrations, decreased milk protein content, decreased milk protein yield, and increased risk for removal from the herd by 85 DIM ($P < 0.05$). Based on the final model for average blood BHBA concentration, increasing time in the maternity pen by 2 d increased BHBA by 50 μM. These results indicate that it is important to include pen movement in the data collected from a field study and that prolonged stays in a maternity pen prior to calving may negatively affect dairy cow health and production.

Stocking density of the prefresh pen was also an important covariate in the statistical analysis of this field trial. Increased stocking densities prior to calving decreased ($P < 0.05$) milk yield after calving. The final model for 3 to 85 DIM milk yield for the first lactation cows showed that a 10% increase in prefresh stocking density resulted in a 0.7-kg decrease in daily milk yield. These results indicate that pen stocking density should be recorded or controlled in field studies and that increasing stocking densities may adversely affect subsequent milk production.

Actual days prior to calving for the last NEFA sample and actual DIM for the first milk component test were highly significant in the final models for NEFA and wk-1 milk components, respectively. These results indi-

cate that actual days relative to calving should be recorded and included as covariates in the statistical analyses of field studies with weekly sample collection. Many outcomes change rapidly around calving, and it is not sufficient to assume that they are equal if collected within the same week.

Interpretation of the covariate data from this study should be approached with caution because the observed effects are associative and do not establish causation. The study was designed to prospectively control supplementation with the DFM product; all other effects were evaluated retrospectively.

CONCLUSIONS

Supplementation with the DFM product increased milk fat percentage for the first lactation cows, increased milk protein percentage for the second and greater lactation cows during the first 85 DIM, and decreased the number of antibiotic treatments for the second-lactation cows before 85 DIM compared with cows receiving the placebo. Most measures of milk yield were numerically increased by DFM supplementation, but the differences were not significant. Field studies can be used to evaluate products that may enhance transition dairy cow performance. However, a large number of groups are required to demonstrate significant differences in proportional outcomes and outcomes with high variability. Analysis of key covariates revealed that previous lactation milk production was positively associated with milk yield and that increasing body condition score prior to calving was associated with increased milk yield, BHBA concentrations, and body condition loss after calving. Increasing days spent in the maternity pen before calving was negatively associated with cow health and performance, and overcrowding before calving was associated with decreased postpartum milk yield.

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