Yeast culture supplementation prevented milk fat depression by a short-term dietary challenge with fermentable starch

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ABSTRACT

Effects of yeast culture on responses to a fermentable starch challenge were evaluated in an experiment with a crossover arrangement of treatments for yeast culture supplementation with 28-d periods and a fermentable starch challenge on the last 2 d of each 28-d period as a split plot within period. Eight ruminally cannulated, midlactation, multiparous Holstein cows (96 ± 14 d in milk) were randomly assigned to treatment sequence. Treatments were yeast culture or control (mix of dry ground corn and soybean meal), top-dressed at 56 g per head per day throughout each period. Diets containing dry ground corn grain were fed from d 1 through 26 of each period. On the last 2 d of each period, the dry ground corn was replaced by finely ground high-moisture corn grain on an equivalent dry matter basis to abruptly increase ruminal fermentability of dietary starch. Response variables were averaged for d 25 and 26 for the dry corn treatment and for d 27 and 28 for the high-moisture corn treatment each period. The fermentable starch challenge decreased dry matter intake by 1.9 kg/d and tended to increase milk yield compared with the dry corn diet. However, effects of the fermentable starch challenge on yield of milk fat varied for the yeast culture and control diets; yield of milk fat decreased from 1.42 to 1.30 kg/d for the control treatment but increased from 1.40 to 1.47 kg/d for the yeast culture treatment. Milk fat concentration tended to decrease from 3.34 to 3.03% during the dietary challenge compared with the base diet for the control treatment but was not affected (mean = 3.32%) by the dietary challenge for the yeast culture treatment. An interaction of treatments was also detected for fat-corrected milk, which increased from 41.0 to 43.0 kg/d for the yeast culture treatment but decreased from 41.6 to 39.8 kg/d for the control diet with the fermentable starch challenge. Frequency of ruminating bouts was decreased by yeast culture compared with control (12.8 vs. 15.7 bouts/d) but not the fermentable starch chal-

lenge. No treatment interactions were observed for any measure of ruminal pH, total or individual volatile fatty acid concentration in ruminal fluid, acetate:propionate ratio, or individual fatty acid isomers in milk fat. Yeast culture supplementation may help prevent depression in milk fat during transition to a diet with highly fermentable starch, but the mechanism responsible remains to be elucidated.

Key words: rumination, high-moisture corn, diet fermentability

INTRODUCTION

Highly fermentable diets can decrease meal size and feed intake (Oba and Allen, 2003), fiber digestibility (Hoover, 1986), and increase flow of *trans* fatty acids (FA) from the rumen resulting in milk fat depression (Bradford and Allen, 2004). Increased ruminal starch fermentation can result in increased partitioning of energy to body condition at the expense of milk yield (Oba and Allen, 2000). Stabilization of ruminal fermentation has been suggested as a benefit of yeast culture (YC) supplementation (Williams et al., 1991). *Saccharomyces cerevisiae* culture stimulated growth of lactate-utilizing and cellulolytic microbes in vitro (Callaway and Martin, 1997), and increasing populations of lactate-utilizing microbes might attenuate transient lactate spikes during diet shifts in vivo (Miller-Webster et al., 2002). Williams et al. (1991) observed an overall decrease in ruminal concentrations of lactic acid in steers fed YC and a reduction in ruminal pH depression 2 h after feeding barley grain. However, effects of YC on ruminal pH have been inconsistent. Although YC decreased peak ruminal lactic acid concentration in lactating cows, no difference was observed for ruminal pH (Erasmus et al., 1992). Others have shown YC to lower ruminal pH (Harrison et al., 1988; Piva et al., 1993) or have no effect on ruminal pH (Wiedmeier et al., 1987; Yoon and Stern, 1996; Robinson and Garrett, 1999). Differences in ruminal pH have also been reported between different YC sources in vitro in continuous culture (Miller-Webster et al., 2002).

Variation in diet fermentability is a regular occurrence on commercial dairy farms because of changes in

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moisture concentration and composition of ingredients, mixing errors, sorting of feeds by animals, and other factors (Stone, 2004). This variation can impair animal health and result in decreased milk yield and efficiency of milk production. Supplementation of YC can potentially improve milk yield and efficiency of conversion of feed to milk by altering populations of microbes in the rumen.

The objective of this experiment was to assess the effects of YC supplementation on ruminal fermentation and production responses to a fermentable starch challenge in lactating dairy cows. We hypothesized that YC treatment would attenuate the decrease in ruminal pH and milk fat yield from a fermentable starch challenge.

MATERIALS AND METHODS

Design and Treatments

Eight ruminally cannulated, midlactation multiparous Holstein cows (96 ± 14 DIM; mean $\pm$ SD) from the Michigan State University Dairy Cattle Teaching and Research Center were assigned randomly to treatment sequence in a crossover design experiment with 28-d periods. Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University before the start of this study. Treatments were YC (Diamond V XP, Diamond V Mills, Cedar Rapids, IA) or control (**CONT**) containing a mix of dry, finely ground shelled corn and soybean meal (48% CP) formulated to be isonitrogenous to YC and top-dressed at 56 g/head daily throughout each period. A fermentable starch challenge was administered as a split plot within each period. Diets containing the dry corn treatment (**DC**) were fed from d 1 through 26 of each period followed by the fermentable starch challenge, in which dry ground corn was replaced with the high-moisture corn treatment (**HMC**) on an equivalent DM basis for the final 2 d of each period (d 27 to 28). At the beginning of the experiment, BW of cows was 620 ± 45 kg and milk yield was 45 ± 5.5 kg/d (mean $\pm$ SD). Samples and data were collected the last 4 d of each period corresponding to the last 2 d of the DC treatment and the 2 d of the dietary challenge (HMC). Diets contained corn silage (67% of forage DM), alfalfa silage (33% of forage DM), corn grain (DC or HMC), expeller soybean meal (SoyPlus, West Central Soy, Ralston, IA), soybean meal (48% CP), distillers grains, a premix of minerals and vitamins, and YC or CONT treatments (Table 1). The diets containing DC treatment were intended to be highly fermentable to ensure detection of treatment effects during the fermentable starch challenge. Therefore, diets were formulated for the minimum concentration of NDF suggested by NRC

Table 1. Ingredients and nutrient composition of experimental diets¹

Item	DC	HMC
Diet ingredient, % of DM		
Distillers grains	6.0	6.0
Soybean meal (48% CP)	8.2	8.2
Dry ground shelled corn	36.2	—
High-moisture corn	—	36.2
Alfalfa silage	11.8	11.8
Corn silage	23.5	23.5
SoyPlus ²	4.6	4.6
Mineral and vitamin mix ³	9.8	9.8
Nutrient composition, ⁴ %		
DM	53.6	52.3
Starch	33.2	35.1
NDF	24.6	23.9
CP	16.5	16.3

¹DC = dry ground corn grain treatment (d 1 to 26 of each period); HMC = high-moisture corn grain treatment (d 27 to 28 of each period).

²SoyPlus (West Central Soy, Ralston, IA).

³Mineral and vitamin mix contained 67.8% dry ground shelled corn, 13.1% limestone, 8.5% magnesium sulfate, 6.4% white salt, 2.9% dicalcium phosphate, 1.3% trace minerals, and vitamin A, D, and E premix.

⁴Values other than DM are expressed as a percentage of dietary DM.

(2001). Diets were formulated to 25% NDF and 17% CP, and mineral and vitamins were formulated according to NRC (2001) recommendations. Dry matter concentration was determined twice weekly for forages, and diets were adjusted when necessary.

Data and Sample Collection

Throughout the experiment, cows were housed in individual tie stalls. Access to feed was blocked between 1000 and 1130 h, and orts and feed offered were weighed and recorded for each cow during each collection period. Cows were fed to 110% of expected intake. Water was available ad libitum in each stall. Stalls were bedded with sawdust and cleaned twice daily. Dry matter intake was recorded for each cow daily. Samples of diet ingredients (0.5 kg) and orts (12.5%) were collected on d 25 to 28 of each period. Diet ingredients and ort samples for individual cows were composited (d 25 and 26 for the DC treatment diet and d 27 and 28 for the HMC treatment diet). Cows were milked twice daily at 0500 and 1700 h, and milk was sampled at each milking from d 25 to 28 of each period. Cows were milked in a milking parlor on d 1 to 24 of each period and were milked in their tie stalls on d 25 to 28 of each period. Two samples were taken from each cow at each milking; one sample was immediately frozen for later determination of FA profiles and the other was collected in a sealable tube containing a preservative and refrigerated for later determination of milk composition. Body weights were measured immediately before the experiment and

on d 28 of each period. Body condition was scored by 3 trained investigators on a 5-point scale where 1 = thin and 5 = fat, as described by Wildman et al. (1982), immediately before the experiment and on d 28 of each period for each animal. Feeding and chewing behavior and ruminal pH were continuously monitored (every 5 s) on d 25 to 28 of each period using a computerized data acquisition system (Dado and Allen, 1993). From d 25 to 28 of each period, pH probes were removed at 1000 h to check calibration and were recalibrated if necessary. Daily mean, minimum, maximum, variation range, and hours below pH 6.0 unweighted or weighted by the deviation in pH units were calculated. Response variables were averaged for d 25 and 26 for the DC treatment and for d 27 and 28 for HMC treatment each period. Rumen fluid was sampled every 3 h with an indwelling sampling device weighted in the ventral rumen on d 26 and 27 of each period and analyzed for short-chain FA and ammonia.

Sample and Statistical Analysis

Diet ingredients and orts were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. All samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for NDF, CP, and starch. Concentrations of NDF were determined according to Van Soest et al. (1991, method A), and CP was analyzed according to Hach et al. (1987). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide; glucose concentration was measured with a glucose oxidase method (Sigma Chemical Co., St. Louis, MO).

Milk samples were analyzed for fat, true protein, and lactose by midinfrared spectroscopy (AOAC, 1990) by Universal Labs (East Lansing, MI). Milk samples were composited across d 25 and 26 and across d 27 and 28 of each period for determination of FA profile so that equal amounts of milk fat were contributed from each sample composited. Composite samples were centrifuged at $17,800 \times g$ for 30 min at 8°C, and approximately 350 mg of fat cake was extracted according to Hara and Radin (1978). Methyl esters were formed according to Christie (1982) as modified by Chouinard et al. (1999). Fatty acids were quantified by gas chromatography (Clarus 500, Perkin-Elmer Corp., Norwalk, CT) according to Kramer et al. (1997) using a SP-2560 capillary column (100 m $\times$ 0.20 mm i.d. with 0.02- μ m film thickness; Supelco, Bellefonte, PA). Oven temperature was 70°C for 4 min then increased 13°C/min to 175°C and was held for 27 min before being increased again at 4°C/min to 215°C and held for 31 min. Helium was used as the carrier gas, and the total run time was 80 min.

Each sample was run at 2 split modes (1:20 and 1:100) to quantify low- and high-abundance FA, respectively. Rumen fluid was analyzed for concentration of major VFA and lactate by HPLC (Waters Corp., Milford, MA) according to Oba and Allen (2003). Ammonia concentration was determined for centrifuged rumen fluid samples according to Broderick and Kang (1980).

Data were statistically analyzed using the MIXED procedure of SAS (version 9.1.3) according to the following model:

$$Y_{ijkl} = \mu + C_i + P_j + T_k + CPT_{ijk} + D_l + TD_{kl} + e_{ijkl}$$

where μ = overall mean; C_i = random effect of cow ($i = 1$ to 8); P_j = fixed effect of period ($j = 1$ to 2); T_k = fixed effect of YC ($k = 1$ to 2); CPT_{ijk} = interaction of cow, period, and YC; D_l = fixed effect of fermentable starch challenge ($l = 1$ to 2); TD_{kl} = interaction of YC and fermentable starch challenge; and e_{ijkl} = residual.

Main effects of YC treatment were tested with the mean square error for the interaction of cow, period, and YC, and main effect of the fermentable starch challenge and its interaction with YC were tested using residual mean square error. Treatment effects were declared significant at $P \leq 0.05$ and tendencies for treatment effects at $P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$ and tendencies for interactions at $P \leq 0.15$. When interactions among main effects were significant, treatment means were separated using Tukey's Honestly Significant Difference test (SAS version 9.1.3, SAS Institute Inc., Cary, NC).

RESULTS

Significant interactions of treatments were detected for yields of milk fat and 3.5% FCM ($P < 0.01$; Table 2). Increased starch fermentability (HMC compared with DC) increased milk fat yield (1.40 to 1.47 kg/d) for YC but decreased it (1.42 to 1.30 kg/d) for CONT. Milk yield tended to increase ($P = 0.07$) for HMC compared with DC. Milk fat concentration was not affected by the fermentable starch challenge for YC but tended to decrease for CONT (3.34 to 3.03%; interaction $P = 0.11$). Increased starch fermentability increased yield of 3.5% FCM from 41.0 to 43.0 kg/d for YC but decreased it from 41.6 to 39.8 kg/d for CONT (interaction $P < 0.01$). The HMC treatment decreased concentrations of milk protein (2.99 vs. 2.93%, $P < 0.01$) and SNF (8.83 vs. 8.72, $P < 0.01$) compared with DC, but yields of milk protein, lactose, and SNF were not affected by treatment.

Table 2. Milk production response to yeast culture (YC) and starch fermentability treatments¹

Item	Control		YC		SEM	P-value		
	DC	HMC	DC	HMC		FS	YC	FS × YC
Yield, kg/d								
Milk	43.0	43.6	42.6	44.8	1.7	0.07	0.72	0.26
FCM (3.5%)	41.6	39.8	41.0	43.0	1.3	0.80	0.32	0.004
Milk fat	1.42	1.30	1.40	1.47	0.06	0.47	0.20	0.006
Milk protein	1.28	1.27	1.27	1.31	0.04	0.51	0.51	0.19
Milk lactose	2.22	2.23	2.18	2.28	0.08	0.14	0.89	0.29
SNF	3.79	3.79	3.75	3.90	0.12	0.25	0.78	0.24
Milk composition, %								
Fat	3.34	3.03	3.32	3.31	0.21	0.09	0.17	0.11
Protein	2.99	2.92	2.99	2.94	0.06	0.005	0.79	0.46
Lactose	5.16	5.13	5.13	5.10	0.06	0.22	0.25	0.88
SNF	8.84	8.73	8.81	8.71	0.14	0.006	0.40	0.77
DMI, kg/d	27.5	25.4	27.7	26.0	1.11	0.006	0.71	0.76
3.5% FCM/DMI	1.55	1.60	1.50	1.67	0.06	0.02	0.91	0.15
BW change, kg/28 d			14.0	10.3	6.1	— ²	0.69	
BCS change, /28 d			0.08	0.03	0.06	— ²	0.50	

¹FS = main effect of fermentable starch challenge (DC = dry ground corn grain treatment, d 1 to 26; HMC = high-moisture corn grain treatment, d 27 to 28); YC = main effect of yeast culture; FS × YC = interaction of dietary challenge and yeast culture.

²Main effect of conservation method was not measured.

Dry matter intake decreased 1.9 kg/d by HMC compared with DC ($P = 0.02$, Table 2) because of decreased meal frequency (9.9 vs. 8.4 meals/d, $P = 0.03$, Table 3). The reduction in DMI for HMC compared with DC with no effect on 3.5% FCM resulted in increased efficiency of milk production as determined by 3.5% FCM/DMI (1.53 vs. 1.64, kg/kg; $P = 0.02$, Table 2). Number of ruminating bouts per day decreased by YC

compared with CONT (15.7 vs. 12.8, $P = 0.04$, Table 3). This resulted in numerical but not significant ($P = 0.13$) decreases in total ruminating time and number of ruminating chews per day. Water intake and drinking behavior were not affected by treatment.

The HMC treatment increased ruminal VFA concentrations compared with DC (117.4 vs. 111.5 mM, $P = 0.03$) mainly from increased butyrate concentration

Table 3. Feeding behavior response to yeast culture (YC) and starch fermentability treatments¹

Item	Control		YC		SEM	P-value		
	DC	HMC	DC	HMC		FS	YC	FS × YC
Eating								
Meals, no./d	10.7	8.6	9.1	8.2	0.9	0.03	0.20	0.35
Meal length, min	32.9	36.0	34.9	38.3	3.7	0.25	0.49	0.95
Meal size, kg	2.61	2.89	2.83	3.18	0.26	0.18	0.30	0.89
Intermeal interval, min	97.9	118.2	125.8	123.9	17.3	0.56	0.32	0.48
Eating chews, no./meal	2,467	2,715	2,679	2,985	319	0.23	0.32	0.90
Eating chews, no./d	22,963	22,014	23,639	21,748	1,413	0.05	0.89	0.48
Eating time, ² min/d	257	252	255	245	14.2	0.15	0.77	0.58
Eating rate, kg/min	0.085	0.083	0.085	0.100	0.006	0.27	0.20	0.12
Ruminating								
Ruminating bouts, no./d	15.5	15.8	12.6	12.9	1.2	0.50	0.04	1.00
Ruminating length, min	28.8	29.7	31.1	31.1	1.5	0.76	0.24	0.74
Ruminating interval, min	56.8	58.3	93.2	65.0	14.5	0.35	0.18	0.30
Ruminating chews, no./bout	1,653	1,744	1,809	1,794	119	0.68	0.30	0.57
Ruminating chews, no./d	25,552	27,240	23,320	22,518	2,453	0.67	0.13	0.24
Ruminating time, ² min/d	428	447	390	383	35	0.72	0.13	0.43
Ruminating rate, no./min	60.7	61.2	60.3	60.8	2.2	0.47	0.57	0.95
Total chews, no./d	48,515	49,255	46,959	44,266	3,038	0.45	0.33	0.20
Total chew time, min/d	685	700	645	628	42	0.93	0.24	0.41
Water intake, L/d	95.0	93.8	96.3	93.8	5.2	0.53	0.81	0.83
Drinking bouts, no./d	10.4	11.4	9.5	10.7	1.4	0.16	0.29	0.90

¹FS = main effect of fermentable starch challenge (DC = dry ground corn grain treatment, d 1 to 26; HMC = high-moisture corn grain treatment, d 27 to 28); YC = main effect of yeast culture; FS × YC = interaction of dietary challenge and yeast culture.

²Actual time spent chewing (not a product of number of bouts and bout length).

Table 4. Ruminal VFA, lactate, and ammonia concentration response to yeast culture (YC) and starch fermentability treatments¹

Item	Control		YC		SEM	P-value		
	DC	HMC	DC	HMC		FS	YC	FS × YC
Total VFA, mM	110.7	115.5	112.2	119.3	3.2	0.03	0.51	0.64
Acetate	65.4	66.3	66.1	67.6	1.85	0.32	0.63	0.80
Propionate	26.1	28.5	25.5	27.6	1.95	0.06	0.66	0.86
Butyrate	13.9	15.1	15.1	18.6	1.49	0.02	0.28	0.18
Isobutyrate	1.10	1.13	1.19	1.29	0.08	0.30	0.18	0.54
Valerate	2.06	2.20	2.04	2.13	0.16	0.12	0.73	0.75
Isovalerate	1.98	2.00	2.06	2.05	0.17	0.98	0.69	0.87
Branch chain	3.09	3.13	3.24	3.34	0.24	0.55	0.42	0.81
Acetate:propionate	2.59	2.54	2.66	2.61	0.20	0.60	0.67	1.00
Lactate, mM	0.35	0.48	0.36	0.25	0.11	0.87	0.30	0.20
Ammonia, mg/dL	7.11	6.42	8.07	7.42	0.49	0.01	0.13	0.93

¹FS = main effect of fermentable starch challenge (DC = dry ground corn grain treatment, d 1 to 26; HMC = high-moisture corn grain treatment, d 27 to 28); YC = main effect of yeast culture; FS × YC = interaction of dietary challenge and yeast culture.

(16.9 vs. 14.5 mM, $P = 0.02$) and a tendency for increased propionate concentration (29.0 vs. 25.8 mM, $P = 0.06$). Ruminal ammonia concentration was decreased by HMC compared with DC (7.59 vs. 6.92 mg/dL, $P = 0.01$), whereas concentration of lactate was low (<0.5 mM) and not affected by treatment (Table 4). No main effects of YC or interactions of treatments on concentrations of VFA, lactate or ammonia, or ratio of acetate to propionate were detected.

The HMC treatment decreased mean ruminal pH from 6.04 to 5.91 ($P < 0.01$, Table 5) and increased fraction of time less than pH 6.0 from 9.1 to 11.8 h/d ($P = 0.03$) compared with DC. Time under pH 6.0, weighted by the deviation in pH units, increased from 327 to 533 unit hours ($P = 0.01$) for HMC compared with DC. The HMC treatment also tended to decrease minimum (5.41 vs. 5.31, $P = 0.08$) and maximum (6.61 vs. 6.56, $P = 0.09$) daily ruminal pH. No main effects of YC or interactions of treatments were observed for any measure of ruminal pH.

Most milk FA were unaffected by treatment (individual FA data not shown). No effect of treatment was observed for FA less than C16 as a percentage of total milk FA, but C16:0 increased (28.4 vs. 27.7%, P

< 0.01) and total C18 FA tended to decrease (37.8 vs. 38.9%, $P = 0.10$, Table 6) for HMC compared with DC. No effect of treatment was observed for total *cis* C18:1 FA (21.4%), *trans* 18:1 FA (2.22%), or *trans*-10, *cis*-12 conjugated linoleic acid (CLA; 0.01%).

DISCUSSION

Effects of YC on yields of milk and milk fat have been inconsistent. Although no previous experiments that we know of have evaluated effects of YC on milk production response to a fermentable starch challenge, others have detected an interaction between YC supplementation and diet fermentability. A previous experiment with a similar feeding rate (60 g/d) of the same YC product used in our experiment detected a significant interaction between YC and diet forage NDF concentration (Wang et al., 2001). Yeast culture treatment increased yields of milk fat and 3.5% FCM for the 21% forage NDF diet but not for the 17% NDF diet (interactions: $P = 0.12$ of milk fat yield and $P = 0.10$ for 3.5% FCM yield). Although decreased forage NDF concentration is often associated with increased diet fermentability, the reverse is more likely in this instance. The 17%

Table 5. Ruminal pH response to yeast culture (YC) and starch fermentability treatments¹

Item	Control		YC		SEM	P-value		
	DC	HMC	DC	HMC		FS	YC	FS × YC
Mean	6.04	5.90	6.04	5.91	0.07	0.003	0.95	0.83
Variance	0.13	0.14	0.11	0.13	0.02	0.34	0.51	0.48
Range	1.24	1.22	1.14	1.27	0.09	0.35	0.77	0.22
Minimum	5.37	5.31	5.45	5.31	0.08	0.07	0.58	0.48
Maximum	6.62	6.53	6.60	6.58	0.07	0.09	0.84	0.25
Time <6.0, h/d	8.84	12.01	9.37	11.52	1.81	0.03	0.99	0.64
Area <6.0, unit h	339	557	314	509	119	0.01	0.78	0.88

¹FS = main effect of fermentable starch challenge (DC = dry ground corn grain treatment, d 1 to 26; HMC = high-moisture corn grain treatment, d 27 to 28); YC = main effect of yeast culture; FS × YC = interaction of dietary challenge and yeast culture.

Table 6. Milk fatty acid profile (% of total fatty acids) response to yeast culture (YC) and starch fermentability treatments¹

Item	Control		YC		SEM	P-value		
	DC	HMC	DC	HMC		FS	YC	FS × YC
Fatty acid, % of total								
<C16:0	26.75	26.82	27.14	27.77	1.03	0.58	0.46	0.66
C16:0	27.66	28.08	27.80	28.78	0.60	0.004	0.33	0.19
Total C18 ²	39.15	38.55	38.64	37.11	1.15	0.10	0.21	0.46
C18:1 <i>trans</i>	2.14	2.28	2.29	2.18	0.29	0.88	0.80	0.19
C18:1 <i>cis</i>	21.83	21.80	21.46	20.65	0.75	0.31	0.25	0.34
Conjugated linoleic acid (<i>cis</i> -9, <i>trans</i> -11)	0.45	0.48	0.48	0.49	0.03	0.22	0.36	0.51
Conjugated linoleic acid (<i>trans</i> -10, <i>cis</i> -12)	0.016	0.008	0.008	0.008	0.005	0.27	0.41	0.37

¹FS = main effect of fermentable starch challenge (DC = dry ground corn grain treatment, d 1 to 26; HMC = high-moisture corn grain treatment, d 27 to 28); YC = main effect of yeast culture; FS × YC = interaction of dietary challenge and yeast culture.

²Includes C18:0 plus C18:1, C18:2, and C18:3 isomers.

forage NDF diet had lower concentrations of corn grain and corn silage and a much greater concentration of soyhulls, which decreased the NSC concentration of the diet from 37.9 to 32.8%. Therefore, the 21% forage NDF diet was likely more fermentable than the 17% forage NDF diet, and treatment effects are consistent with those in our experiment.

Williams et al. (1991) reported that 10 g/d of live yeast plus growth medium (*Saccharomyces cerevisiae*; YEA-SAC, Alltech Biotechnology Center, Nicholasville, KY) increased FCM yields compared with control for the greater concentrate diet only (interaction: $P = 0.06$) when evaluated at 2 different forage:concentrate ratios (50:50 and 40:60). The experiment was conducted with 2 different forage sources: grass hay and ammoniated wheat straw. Although the yeast treatment increased FCM for the 60% concentrate diet for both forages, the increase was because of opposite effects on yields of milk and milk fat for each forage treatment. Although the authors attributed the increase in FCM yield to a greater DMI for cows supplemented with the yeast, the mechanism by which yeast supplementation increased FCM was dependent upon forage source, which likely affected digestion kinetics in the rumen (not measured).

The reduction in DMI by HMC compared with DC is consistent with reduction of feed intake with increased starch fermentability and ruminal propionate production (Allen, 2000; Allen et al., 2005). Oba and Allen (2003) reported that HMC decreased DMI compared with DC in a 32% starch diet because of a reduction in meal size, despite an increase in meal frequency. The reduction in DMI by HMC compared with DC in the current experiment was because of decreased meal frequency despite a numerical increase in meal size. The reason for the different feeding behavior response causing the reduction in feed intake between the current experiment and that reported by Oba and Allen (2003) is not known. However, cows in the current experiment

were slightly later in lactation (96 vs. 55 DIM at initiation of treatments) and had greater milk yield (43.5 vs. 38.6 kg/d), and differences in the source of corn affecting digestion kinetics and rate of propionate production might have had an effect.

Yields of milk protein, lactose, and SNF were not affected by treatment, so reductions in concentrations of milk protein and SNF for HMC compared with DC were likely because of increased milk yield. We were not able to determine the mechanism for the interaction of treatments on yield of milk fat in this experiment. Specific FA that are intermediates in biohydrogenation of dietary polyunsaturated FA cause milk fat depression by downregulation of expression of genes for key lipogenic enzymes (Harvatine and Bauman, 2006). These specific FA are only produced during altered ruminal fermentation (Bauman and Griinari, 2003). The first FA to be recognized was *trans*-10, *cis*-12 CLA (Baumgard et al., 2000), followed more recently by *trans*-9, *cis*-11 CLA and *cis*-10, *trans*-12 CLA (Bauman et al., 2008). No main effects or interactions of treatments were detected for concentration of *trans*-10, *cis*-12 CLA. *Trans*-9, *cis*-11 CLA and *cis*-10, *trans*-12 CLA were not detected with our methods. Although we cannot explain treatment effects by CLA, we cannot rule out that specific FA produced from altered ruminal fermentation inhibited milk fat production in this experiment, because other inhibitory FA may be discovered in the future.

Lack of effect of YC on ruminal pH is consistent with most previous reports with lactating dairy cows (Wiedmeier et al., 1987; Erasmus et al., 1992; Yoon and Stern, 1996). However, diet fermentability was more moderate for previous experiments with dairy cows, especially compared with our fermentable starch challenge. In an experiment with steers, YC limited the reduction in ruminal pH after a challenge with barley (Williams et al., 1991). In that study, ruminal lactate concentration peaked at approximately 8 mM, which

likely contributed to the decline in ruminal pH. We failed to detect effects of YC on any measure of ruminal pH. It is possible that high buffering capacity of the rumen combined with low lactate concentrations limited our ability to detect effect of YC. The large expected increase in fermentation acid production by HMC compared with DC had little effect on ruminal pH (≤ 0.14 pH units) despite the high-starch ($>32\%$) and low-NDF ($<26\%$) diets indicating high buffering capacity of rumen contents. Lactate is more effective at decreasing ruminal pH than the VFA, because it has a lower acid dissociation constant and is not readily absorbed from the rumen. Even if YC stimulated lactate-utilizing bacteria as shown by Callaway and Martin (1997), low lactate concentrations limited effects of YC on ruminal pH. We conclude that YC effect on milk fat yield was not related to its effect on ruminal pH.

Effects of HMC compared with DC on ruminal VFA concentrations were consistent with increased production of fermentation acids. Relative production of propionate and butyrate compared with acetate was likely greater than reflected by the measured concentrations, because rate of absorption of VFA increases with chain length as pH declines (Dijkstra et al., 1993). Lack of main effects or interactions for YC on concentrations of total and individual VFA were consistent with most in vivo studies reported in the literature (Yoon and Stern, 1996; Putnam et al., 1997; Robinson and Garrett, 1999). Although concentrations of VFA in rumen fluid do not necessarily reflect production rates because of differences in absorption rates among VFA, it is unlikely that VFA caused the interaction of YC and starch fermentability on yield of milk fat in our experiment. Studies that have reported effects of YC on VFA concentrations reported increased propionate and decreased acetate concentrations in vivo (Harrison et al., 1988) and increased propionate production in continuous culture (Miller-Webster et al., 2002). This is inconsistent with YC effects on yield of milk fat observed in this experiment, because increased propionate and decreased acetate production should both decrease milk fat yield according to other theories of milk fat depression. Increased propionate production and subsequent increases in gluconeogenesis might decrease circulating plasma FA available for uptake by the mammary gland through actions of insulin (McClymont and Vallance, 1962), and decreased acetate supply might limit substrate for milk fat synthesis (Van Soest, 1963).

The reduction in frequency of ruminating bouts and weak tendency for reduction in ruminating chews and ruminating time per day by YC is a novel finding to our knowledge. Reduction in rumination with no effect on ruminal pH by YC further indicates that buffer capacity of rumen contents was relatively high, because flow

of salivary buffers in saliva was likely decreased (Allen, 1997). We are unable to ascertain the mechanism for the reduction in rumination. It is unlikely that ruminating behavior was altered by osmotic effects, because total ruminal VFA concentrations, and likely osmolality (not measured), were similar for YC and CONT. It is possible that YC decreased rumination by increasing ruminal NDF digestion, thereby decreasing ruminal distension (not measured); YC has been reported to stimulate growth of cellulolytic bacteria in vivo with increased ruminal fiber digestibility (Wiedmeier et al., 1987). Alternatively, YC might contain a bioactive component that affected rumination. Effects of YC on ruminating behavior should be investigated further.

CONCLUSIONS

Yeast culture supplementation in diets of lactating dairy cows may decrease the incidence of milk fat depression commonly observed when ruminal starch fermentability is rapidly increased. Further research to elucidate the mechanisms involved is required to better understand the benefits of feeding YC in the diets of lactating dairy cows.

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