Influence of Addition of Yeast Culture Supplement to Diets of Lactating Cows on Ruminal Fermentation and Microbial Populations

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ABSTRACT

Six ruminally fistulated Holstein cows were utilized in a randomized block design to examine effects of yeast culture supplement on ruminal metabolism and apparent digestibility. Cows were fed a diet of 40% corn silage and 60% concentrate (DM basis). Treatments were control (supplement without yeast cells) and yeast culture supplement. Treatment periods were 6 wk. Ruminal pH, ammonia, molar proportions of acetate and isovalerate, and acetate:propionate ratio were lower and molar proportions of propionate and valerate higher in cows receiving yeast. The concentration of anaerobic bacteria tended to be higher and cellulolytic bacteria concentrations were greater in cows fed yeast than in cows receiving control diet. Supplemental yeast did not affect molar proportions of isobutyrate or butyrate, total VFA, or viable yeast concentrations in ruminal fluid. Ruminal liquid dilution rate and total tract apparent digestibilities were not different between treatments. Rate of disappearance of cellulose in vitro was lower in cows receiving yeast. Less variation in ammonia concentrations and mircobial numbers suggest that ruminal fermentation was more stable in cows receiving yeast culture supplement.

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INTRODUCTION

The effects of yeast culture (YC) on ruminal fermentation and microbial populations have been examined in a limited number of studies. Some reports have demonstrated no effect of YC supplementation on ruminal pH, ammonia, and VFA pattern (1, 26). In contrast, others have reported that YC increased ruminal pH and acetate:propionate ratio in vivo (22) and in vitro (9).

Recently, supplementation of YC has been reported to alter ruminal microbial populations. Addition of YC to diets of nonlactating cows tended to increase the number of total anaerobic bacteria and increased number of cellulolytic bacteria (26). Supplementation of YC to rumen-simulating fermenters increased total anaerobic bacteria concentration and tended to increase cellulolytic bacteria numbers (9).

The response of ruminal microorganisms to YC addition have not been examined in lactating dairy cows consuming diets containing higher amounts of soluble carbohydrates and lower fiber. The objectives of this trial were 1) to examine the effects of YC addition on patterns of ruminal fermentation in lactating cows; 2) to determine the response to YC supplementation on concentrations of rumen bacteria; 3) to evaluate the effects of YC addition on ruminal liquid dilution rate and total tract apparent digestibility; and 4) to compare inocula from animals fed control or YC diets utilizing an in vitro fermentation system.

MATERIALS AND METHODS

Design and Feeding Regimen

Six ruminally fistulated Holstein cows were utilized in a randomized block design. Treat-

ments were control (placebo or YC media without yeast cells, 114 g/cow per d) and YC (114 g/cow per d) with three cows per treatment. (Yeast culture supplement and placebo were provided by Diamond V. Mills, Inc., Cedar Rapids, IA.) At approximately 7 wk postpartum, cows were placed in tie stalls and switched from a diet of corn silage, alfalfa haylage, and concentrate (50% forage and 50% concentrate on dry basis) to experimental diets over a 3-d period. Cows were allowed an additional 4 d to adjust to experimental diets prior to treatments. Treatment periods were 6 wk. Cows were paired into three groups, based on date of calving, in order to compare animals at similar stages of lactation.

Cows were fed twice daily for ad libitum consumption a total mixed ration of corn silage and concentrate consisting of 40% forage and 60% concentrate on a DM basis. Chemical composition (calculated) of complete mixed rations (% DM basis) was DM (as fed), 54.3; CP, 15.0; NDF, 34.2; ADF, 17.5; Ca, .54; P, .62; Mg, .27; K, .99; S, .22. Forage:concentrate ratio was adjusted weekly to ensure proper DM ratio. Yeast culture (57 g/cow) and control supplement (57 g/cow) were top-dressed on rations immediately prior to each feeding.

Treatments

According to the manufacturer, the YC supplement used in this trial was compsed of live *Saccharomyces cerevisiae* grown on a media consisting of ground corn, hominy feed, corn gluten feed, wheat middlings, rye middlings, diastatic malt, corn syrup, and cane molasses. In order to eliminate any possible effects of the media, a placebo (or YC media without yeast cells) was chosen as a control. However, when yeast concentrations were determined in both the placebo and YC (Table 1), the placebo was found to contain yeast. The YC contained 2.40×10^6 cfu/g compared with 1.29×10^4 cfu/g for the placebo.

TABLE 1. Yeast concentrations in yeast culture and placebo and estimated intake of yeast (cells/cow per d).

	Viable yeast count[1] (cfu/g)	Estimated intake of yeast (cells/d)
Yeast culture	2.40×10^6	2.74×10^8
Placebo	1.29×10^4	1.47×10^6

[1] Aerobic counts of viable yeast cells on Rose Bengal Agar (with .01% chloramphenicol).

Sampling Procedures

In Vivo. Samples of ruminal ingesta were taken weekly 4 h after morning feeding via fistula and strained through four layers of cheesecloth. A 100-ml aliquot was placed in a 150-ml vial for analysis of pH and fermentation end products (VFA and ammonia). A second 250-ml aliquot was collected by filling a plastic bottle with ruminal fluid to overflowing (to minimize exposure to oxygen), sealing with screw cap, and placing bottle in water (37°C) for transport to lab for determination of the concentrations of microorganisms.

Liquid dilution rate, the proportion of liquid fraction of the rumen replaced per hour, was measured during wk 1, 3, and 6 of trial using a complex of Cr and EDTA as a liquid phase marker (3). Cows were dosed with 950 ml of Cr-EDTA immediately prior to morning feeding. The Cr-EDTA was injected via syringe attached to rubber tubing into widely dispersed areas of rumen, contents of the rumen hand mixed, and 0-h sample taken immediately. Samples were removed from the rumen at 0, 1, 2, 3, 4, 6, 8, and 10 h postfeeding. Samples were taken from six locations in rumen, composited, and a 100-ml aliquot collected. Samples were centrifuged at 10,000 × *g* for 10 min at room temperature and stored at −20°C for later analysis.

Apparent total tract digestibilities of DM, NDF, ADF, hemicellulose, and starch were determined during wk 1, 3, and 6 of trial. Fecal grab samples were collected twice daily for 5 consecutive d. Corn silage and concentrate samples were collected once per week. Daily fecal samples and feed samples were dried in a forced-air oven (50°C) to constant weight and ground in a Wiley mill (1 mm-screen size; Wiley, Philadelphia, PA). Daily fecal samples were composited by week, stored in sealed plastic containers and saved for later analysis.

In Vitro. Samples of ruminal ingesta for in vitro experiments were taken 4 h postfeeding on wk 2 and 5 of experiment. Approximately 250 ml of ruminal fluid (strained through four

layers of cheesecloth) were placed in a plastic bottle, sealed with screw cap, and placed in water (37°C) for transport.

Microbial Counts and Chemical Analysis

In Vivo. Ruminal fluid samples sealed in plastic vials were placed in an anaerobic chamber and diluted in anaerobic dilution solution (4). Viable yeast concentrations were determined by counting colonies on Rose Bengal Chloramphenicol Agar (.1 g chloramphenicol/L of media; Difco Laboratories, Detroit, MI) after 72 h aerobic incubation at 26°C. Due to an inability to identify and enumerate species of yeasts growing on these plates, total yeast counts were defined as total number of colonies growing on Rose Bengal agar. Total anaerobic bacteria count was determined on roll tubes using complete carbohydrate agar prepared with energy-depleted ruminal fluid (2) and counted after 5 d of incubation at 37°C. Cellulolytic bacteria were enumerated using the most probable numbers procedure in cellulose broth after 14 d of incubation at 37°C (6, 17).

The pH of each sample was determined within 1 h of collection via a Corning 150 pH/Ion Analyzer (Scientific Instruments, Medfield, MA). Sample was then stored at −20°C prior to analysis for ruminal VFA and ruminal ammonia. Volatile fatty acid concentrations in thawed ruminal fluid were determined by gas chromatography (11) on a Varian Aerograph (Varian, Palo Alto, CA) using 10% SP-100/1% H_3PO_4 on Chromosorb WAW packing (Supleco, Inc., Bellefonte, PA) in a 4 mm by 182.9-cm column. Chromatographs were adjusted for internal standard (cyclohexanone) and were integrated for VFA concentrations by a Perkin-Elmer M-1 Computing Integrator (Perkin-Elmer Corp., Norwich, CT). Ruminal ammonia concentrations were determined by the method of Imler et al. (13) using a Technicon Autoanalyzer II (Technicon Instruments Corp., Tarrytown, NY).

Ruminal samples for liquid dilution rate determination were thawed, wet ashed, and analysis of chromium concentration determined by atomic absorption (Model 560, Perkin-Elmer Corp., Norwich, CT) as described by Binnerts et al. (3). Liquid dilution rate was calculated as the slope of $\log_{10}$ Cr concentration versus time. Zero time marker concentrations were estimated by back extrapolation of the dilution regression equation (21).

Corn silage, concentrate and fecal samples were analyzed for acid-insoluble ash (AIA) by the 2 N HCl method of Van Keulen and Young (24). Feed and fecal samples were also analyzed for NDF (25), ADF (12), and starch (16). Hemicellulose content was calculated as the difference between NDF and ADF content of samples. Apparent digestibilities were calculated using AIA as an internal indicator.

In Vitro. In vitro cellulose (Sulka-Floc, Brown Paper Co.) disappearance was determined using a modification of the method of Tilley and Terry (23). Ruminal fluid samples that had been sealed in plastic vials were placed in an anaerobic chamber and blended 1:1 with McDougall buffer (19), which had been previously bubbled with CO_2 in a 37°C water bath. The sample was then removed from the chamber and 50 ml of ruminal fluid-buffer mixture under a CO_2 gas phase) was placed in 125-ml serium bottles (American Scientific Products, McGaw Park, IL) containing .500 ± .001 g of Sulka-Floc (under a CO_2 gas phase). Four ml of urea solution (6.5 g urea/L distilled water) was added to each bottle and the bottles were sealed with rubber stoppers and aluminum seals (Wheaton Scientific, Millville, NJ). Three bottles with Sulka-Floc were prepared for each sample. To account for potential substrates contained in inoculum, one serum bottle per sample was prepared with inoculum and no added Sulka-Floc as a blank.

Serum bottles were incubated at 37°C in a shaking water bath. After 24 h, gas production in serum bottles was measured by water displacement in an inverted graduated cylinder and 10 ml aliquots of incubation fluid were removed via syringe (18.5 G needle) for VFA and ammonia determination and stored at −20°C. After incubation, four drops of isoamyl alcohol, 4 ml of 2.2 N HCl, and approximately .2 g of pepsin were added to each bottle. Bottle contents were then gently mixed and bottles stoppered and incubated for an additional 24 h at 37°C to digest protein.

Following pepsin digestion, contents of serum bottles were filtered (with suction) through preweighed filter paper (Whatman #4), dried at 100°C for 24 h and dry residue determined. In vitro cellulose disappearance was

determined by the formula:

$$\% \text{ Cellulose disappearance} = \frac{[S_{DM} - (R_{DM} - B_{DM})]}{S_{DM}} \times 100$$

Where S_{DM} = substrate dry weight, R_{DM} = residue dry weight, and B_{DM} = blank dry weight.

Thawed ruminal fluid samples from cultures were analyzed for VFA concentrations and ammonia as previously described. Net VFA production and gas production from cellulose were determined by subtracting VFA and gas produced in blanks.

Statistical Analysis

Analysis of variance was performed as a randomized block with repeated measures (7) using the general linear model of SAS (20). Least squares means (20) were computed using wk 1 to 6 data (18 observations per treatment). Data were tested for homogeneity of variance using an F statistic computed to test for equality of variances between treatments (20). Differences in treatment means of variables demonstrating nonhomogenous variance were estimated using a *t* test procedure (20).

RESULTS AND DISCUSSION

Ruminal pH and End Products

Ruminal pH and ammonia are presented in Table 2. Ruminal pH was lower in cows receiving YC than in cows receiving control supplement (P = .002). Cows consuming YC also tended to have lower ruminal ammonia concentrations (P = .15).

Ruminal VFA patterns were altered by addition of YC (Table 2). Molar proportion of acetate was lower (P = .007) and molar proportion of propionate was greater (P = .006) in cows receiving YC. The shift in molar proportions of acetate and propionate resulted in a lower acetate:propionate ratio (P = .01) in ruminal fluid of animals receiving YC. Supplemental YC also resulted in an increase in the molar proportion of total isoacids (isobutyrate plus isovalerate plus valerate) (P = .03). Higher molar proportions of valerate in cows fed YC (P = .03) accounted for this increase. Total VFA concentration did not differ between treatment groups.

TABLE 2. In vivo ruminal pH, ammonia, and volatile fatty acids (mol/100 mol).

Item	Treatment				Probability of no difference		
	Control		Yeast		Trt[a]	Week[b]	Trt X week[c]
	$\bar{X}$	SE	$\bar{X}$	SE			
pH	5.64	.04	5.40	.03	.002	.08	.14
Ammonia, mg/L	125.8	14.5	87.5	6.7	.15	.73	.83
VFA, mol/100 mol							
Acetate	53.1	.72	48.2	.71	.007	.07	.40
Propionate	27.4	1.02	30.0	.95	.006	.08	.02
Isobutyrate	1.4	.17	1.6	.09	.58	.06	.06
Butyrate	14.7	.47	14.5	.73	.79	.28	.33
Isovalerate	1.6	.09	1.1	.07	.06	.03	.03
Valerate	2.5	.10	4.6	.30	.02	.08	.31
Isoacids[1]	5.0	.15	7.3	.30	.03	.22	.94
Total VFA, m*M*	172.2	7.2	184.5	5.6	.48	.13	.29
Acetate:propionate	2.00	.09	1.65	.08	.01	.05	.04

[a]Effect of treatment (Trt) on variable.

[b]Effect of week on variable.

[c]Interaction of treatment and week.

[1]Isobutyrate plus isovalerate plus valerate.

Comparisons between this study and others are difficult due to differences in diets and intake. Lower ruminal pH and acetate:propionate ratio in cows fed YC is in contrast to previous studies (9, 22). Although the reason for a decrease in ruminal pH with addition of YC is unclear, the pH shift does account for the shift in molar percentage of acetate and propionate. When ruminal pH drops below 6.0, molar percentage of propionate increases with minor decreases in pH (8). The decrease in molar proportion of isovalerate and increase in molar proportion of valerate when YC was fed may also be related to lower ruminal pH (10). Lower ruminal ammonia concentrations in animals fed YC is in agreement with Dawson and Newman (9).

Ruminal Microbial Populations

Viable yeast, total anaerobic bacteria, and cellulolytic bacteria concentrations are in Table 3. Addition of YC to diets resulted in nearly a twofold increase in the concentrations of yeast cells in ruminal fluid. However, there was not a significant effect of yeast supplementation on yeast concentrations in ruminal fluid. Cows receiving cupplemental YC also tended to have greater total anaerobic bacteria concentrations in ruminal fluid than animals receiving control diets ($P = .22$). The concentration of cellulolytic bacteria was greater in animals receiving YC ($P = .03$).

Increased numbers of total anaerobic bacteria and cellulolytic bacteria with addition of YC culture are in agreement with other studies (9, 26). The mechanism by which YC causes an increase in bacterial amounts is not known at this time. Greater concentrations of total anaerobic bacteria and cellulolytic bacteria may explain why ruminal ammonia concentrations are lower in cows fed YC. Ammonia is the preferred source of N for a large proportion of the ruminal microbial population (5) and incorporation of ammonia into ruminal bacteria has been demonstrated (18). Lower concentrations of ammonia in the rumen of cows fed YC may reflect increased transportation of ammonia into microbial protein.

Ruminal Liquid Dilution Rate

Ruminal liquid dilution rate (Table 4), expressed as percent per hour, was not affected by inclusion of YC in diets. Liquid dilution rate was 10% higher in cows fed YC than cows fed placebo, although difference did not approach significance. Adams et al. (1) and Wiedmeier et al. (26) both reported that inclusion of YC supplement in the diet resulted in a nonsignificant increase in liquid dilution rate. An increase in liquid dilution rate may account for an increase in the number of bacteria in the rumen. Increasing the dilution rate in a rumen-simulating chemostat tended to increase the concentration of total anaerobic bacteria (14).

TABLE 3. Viable yeast, total anaerobic bacteria, and cellulolytic bacteria concentrations in ruminal fluid (per ml).

Item	Treatment				Probability of no difference		
	Control		Yeast		Trt[a]	Week[b]	Trt × week[c]
	$\bar{X}$	SE	$\bar{X}$	SE			
Yeast (log_{10})	5.39	.26	5.67	.22	.43	.92	.99
Total anaerobic bacteria (log_{10})	9.89	.08	10.09	.05	.22	.31	.69
Cellulolytic bacteria (log_{10})	7.82	.09	8.08	.06	.03	.47	.98

[a]Effect of treatment (Trt) on variable.

[b]Effect of week on variable.

[c]Interaction of treatment and week.

TABLE 4. Ruminal liquid dilution rate and apparent digestibility.

Item	Treatment				Probability of no difference		
	Control		Yeast		Trt[a]	Week[b]	Trt X week[c]
	$\bar{X}$	SE	$\bar{X}$	SE			
Liquid dilution rate, %/h	11.7	.8	12.9	1.6	.52	.41	.91
Apparent digestibility, %							
DM	65.1	3.1	60.0	2.6	.37	.001	.49
NDF	47.7	4.8	39.3	3.6	.40	.004	.66
ADF	45.6	6.2	33.4	4.6	.28	.002	.39
Hemicellulose	49.3	13.2	43.9	9.8	.55	.15	.95
Starch	96.2	.5	96.3	.4	.89	.31	.79

[a]Effect of treatment (Trt) on variable.

[b]Effect of week on variable.

[c]Interaction of treatment and week.

Apparent Digestibility

Apparent digestibilities, as estimated by AIA internal indicator, are in Table 4. Addition of YC to diets did not affect apparent digestibilities of DM, NDF, ADF, hemicellulose, or starch. Yeast culture addition to diets of ruminants has been reported to increase apparent digestibilities of CP and hemicellulose (26). The lack of an increase in apparent digestibility of the fiber fraction of the ration when YC was fed is in contrast to expectations given the increase in number of cellulolytic bacteria in the rumen. One explanation might be that although YC stimulated an increase in the number of cellulolytic bacteria, the activity of these organisms was somehow decreased. The procedure used to enumerate cellulolytic bacteria indicates only that the number of bacteria capable of degrading cellulose was increased. Jung and Varel (15) noted that increases in the number of cellulolytic bacteria did not correspond to increases in digestion of cell wall, cellulose, or hemicellulose.

In Vitro Effects of Yeast Culture

The in vitro procedure utilized in this trial did not test the effects of addition of YC to in vitro incubations. It did, however, provide information that allows comparison of ruminal fluid from animals consuming the control diet and the YC-supplemented diet. Mean in vitro cellulose disappearance (Table 5) in ruminal fluid of cows receiving YC was lower than that for controls (P = .08). Total VFA produced from cellulose also tended to be lower for yeast culture than controls (P = .16). Mean in vitro gas production and ammonia were not affected by treatment. However, in vitro ammonia was higher in the YC group in wk 2 (P = .09) and higher in controls in wk 5 (P = .08).

Evidence for a Stabilizing Effect of Yeast

When data were tested for homogeneity of variance, animals consuming YC exhibited less variation in some variables than those animals fed the control supplement (Table 6). Ammonia concentrations, both in vivo and in vitro, were less variable in animals consuming YC. Total bacteria and cellulolytic bacteria concentrations also tended to be less variable in cows fed YC than those fed the control supplement. In vitro gas production and total VFA production also had lower standard errors in animals fed YC than those fed control supplement. Less variation in these measurements suggests a more stable fermentation in the rumens of animals fed YC compared to those fed the control supplement.

Yeast Concentrations in Supplements

By using the placebo as a control, any difference between treatments could be attributed to an effect of yeast or "fermentative meta-

TABLE 5. In vitro[1] cellulose disappearance, gas production from cellulose, VFA production from cellulose, and ammonia.

Item	Treatment				Probability of no difference		
	Control		Yeast		Trt[a]	Week[b]	Trt × week[c]
	$\bar{X}$	SE	$\bar{X}$	SE			
Cellulose disappearance, %							
wk 2	56.4	2.1	42.6	3.4	.003		
wk 5	52.2	.8	42.5	3.0	.01		
Mean	54.3	1.2	42.5	2.2	.08	.26	.28
Gas production from cellulose, ml							
wk 2	60.4	10.0	53.4	3.9	.53		
wk 5	68.0	4.6	60.1	3.3	.17		
Mean	64.2	5.3	56.8	2.6	.41	.22	.94
Total VFA from cellulose, m*M*							
wk 2	47.88	8.48	31.57	5.05	.26		
wk 5	50.37	3.13	35.92	1.65	.0009		
Mean	49.13	4.40	33.75	2.63	.16	.45	.84
Ammonia, mg/L							
wk 2	239.9	21.4	281.1	3.7	.09		
wk 5	316.6	25.5	265.4	5.9	.08		
Mean	273.3	18.6	278.2	3.9	.92	.0001	.0001

[a]Effect of treatment (Trt) on variable.

[b]Effect of week on variable.

[c]Interaction of treatment and week.

[1] Incubation time = 24 h.

TABLE 6. Effect of yeast on variation between treatments.

Item	Treatment				Test of homogenity of variance[a]
	Control		Yeast		
	$\bar{X}$	SE	$\bar{X}$	SE	
In vivo					
Ammonia, mg/L	125.8	14.5	87.5	6.7	.003
Total anaerobic bacteria (log_{10})	9.89	.08	10.09	.05	.04
Cellulooytic bacteria (log_{10})	7.82	.09	8.07	.06	.16
In vitro					
Gas production from cellulose, ml	64.2	5.3	56.8	2.6	.04
Ammonia, mg/L	273.3	18.6	278.2	3.9	.0001
Total VFA, mmol/L from cellulose	49.13	4.40	33.75	2.63	.04

[a]Probability of variance being equal between treatments.

bolites" produced in media by yeast fermentation and not the media itself. If the effect of YC on ruminal fermentation is due to yeast cells, then because that the placebo also contained yeast, less difference between treatments existed than was intended. Corn silage fed in this trial also contained viable yeast cells (1.24×10^5 cfu/g of wet silage). A cow consuming 20 kg/d (as fed basis) of corn silage would also consume 2.48×10^9 yeast cells/d from silage alone. This is approximately nine times the number of yeast cells added by feeding 114 g/d of YC (2.74×10^8 yeast cells). Despite the large background population of yeast provided by corn silage and the presence of yeast in the control supplement, differences in ruminal fermentation and microbial populations existed between animals fed the two supplements.

CONCLUSIONS

Supplementation of YC to diets of lactating cows changed the pattern of the end products of ruminal fermentation, suggesting a shift in metabolic activities of ruminal microflora. Cows consuming YC had higher concentrations of total anaerobic and cellulolytic bacteria in the rumen. However, increases in the number of cellulose degraders in the rumen did not translate into increased fiber digestion. Less variation in ammonia concentrations and microbial numbers suggests that ruminal fermentation was more stable in animals fed YC than in control animals.

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