Influence of Yeast Culture on Ruminal Microbial Metabolism in Continuous Culture

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ABSTRACT

A continuous culture study was conducted to evaluate the effect of two different yeast cultures on ruminal microbial metabolism. The treatments were a) control lactation ration, b) yeast culture 1 (YC1, Diamond-V XP) and c) yeast culture 2 (YC2, A-Max), both fed at an equivalent of 57 g/head per day. The results showed that both yeast culture products increased dry matter (DM) digestion, propionic acid production, and protein digestion compared with the control. Yeast culture 1 demonstrated an increase in molar percentage of propionic acid, a reduction in acetic acid, and a lower mean nadir (daily low) pH compared with YC2. Ruminal cultures treated with YC digested more protein and contributed less bypass N than control. Supplementing YC2 resulted in a tendency for higher microbial N/kg DM digestion than YC1. Yeast culture 1 resulted in production of rumen microbes containing less protein and more ash than YC2. These results support previous research findings that yeast culture does influence microbial metabolism, and specific yeast cultures may have different modes of action.

(Key words: yeast culture, ruminal metabolism, microbial protein)

Abbreviation key: YC = yeast culture.

INTRODUCTION

Dietary inclusion of yeast culture has been shown to improve DMI and milk production in early-lactation dairy cattle (Wohlt et al., 1991; Shaver and Garrett 1995; Dann et al., 2000). Although these production responses are cited, the specific mode of action still remains elusive. There are several proposed modes of action associated with yeast culture as summarized by

Wallace (1994). In his scheme, removal of oxygen from the rumen environment by *Saccharomyces cerevisiae* plays a prominent role in increasing bacterial viability. Hession et al. (1992) suggest yeast have a limited ability to grow in the ruminal environment; therefore, the fact that yeast may grow in the rumen and directly stimulate a response is unlikely. However, popular theories suggest yeast culture provides various growth factors, pro-vitamins, and/or micronutrients that help stimulate the growth of the ruminal bacteria in the rumen (Wiedmeier et al., 1987; Beharka and Nagaraja, 1991; Newbold et al., 1995). One theory indicates the key influence of yeast culture would be to stimulate lactic acid utilizing bacteria (Nisbet and Martin, 1991a; Williams et al., 1991; Callaway and Martin, 1997). This action would result in a reduction of lactic acid; thus, an increase in the daily low (nadir) pH, resulting in a more stabilized ruminal environment. A higher pH would create an environment more conducive to the growth of rumen cellulolytic bacteria (Harrison et al., 1988; Beharka and Nagaraja, 1991; Yoon and Stern, 1996), ultimately increasing fiber digestion, feed intake, and therefore, production response.

Another popular theory is associated with yeast having a positive influence on ammonia uptake (Dawson 1987; Williams and Newbold, 1990). This could improve microbial protein production and efficiency or both, thus providing an increased supply of amino acids post-ruminally to the cow to serve in stimulating a production response (Erasmus et al., 1992). Others (Wiedmeier et al., 1987; Carro et al., 1992; Hession et al., 1992) have not observed a reduction in ammonia concentration. There are several yeast products on the market with nuances in their manufacturing process that may have an influence on performance; however, very few studies have been conducted to compare yeast culture in the same experimental environment. The objective of this study was to determine nutrient digestion and metabolism of ruminal microorganisms in continuous culture when fed either a control ration or control ration with different yeast culture products.

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Table 1. Diet composition and chemical analyses.

Item	% of DM		
Ingredient			
Corn silage			20.38
Haylage			32.96
Ground corn			29.20
Soybean meal, 44% CP			16.31
Urea			0.28
MgO			0.13
TM Salt			0.19
Limestone			0.56
Analyses	Corn silage	Haylage	TMR
CP, %	6.29	19.94	17.06
Soluble protein, % CP	49.95	61.30	35.00
NDF, %	47.43	41.56	33.20
ADF, %	29.88	30.13	21.67
NSC, ¹ %	31.04	9.42	33.82
Starch, %	29.50	5.93	29.51
Sugar, %	1.54	3.49	4.31
Ether Extract, %	2.63	3.06	2.79
Ash, %	4.55	9.91	5.93
Calculated NFC, ² %	39.10	25.53	41.02

NSC¹ = Enzymatic determination of starch and sugar.

NFC² = 100 - (%NDF + %CP + %EE + %Ash).

MATERIALS AND METHODS

A lactating dairy ration was formulated to support 40 kg/d of milk production. Dietary ingredient and nutrient composition are shown in Table 1. This study comprised three treatments: a) control, the lactation ration alone; b) yeast culture 1 (YC1), the lactation ration plus Diamond-V XP yeast culture, Cedar Rapids, Iowa; and c) yeast culture 2 (YC2), the lactation ration plus A-Max yeast culture concentrate, Vi-Cor, Mason City, Iowa. A lactating dairy ration was formulated to support 40 kg/d of milk production with a predicted DMI of 24.5 kg/d. The yeast concentration in the diets was based on both manufacturers' recommended daily yeast intake of 57g/d in that quantity of feed. Based on these recommended doses, yeast cultures were fed at a rate of 2.3mg/g of DM. The final concentration in the continuous cultures was 0.2 g/L.

Continuous Culture System

A 12-unit continuous culture system similar to that described by Hoover et al. (1976) was used. Each fermenter had a working volume of 1164 ml, and all diets were fermented in triplicate under the following conditions: liquid dilution rate: 12%/h, solids retention time: 22 h, temperature: 39°C, feed intake/24 h: 100 g of DM (2×), pH: monitored hourly. Inocula for the fermenters were obtained from two ruminally cannulated, lactating Holstein cows. Rumen fluid was pooled before inoculating fermenters. Diets were fed automatically in two equal feedings at 12-h intervals.

The artificial saliva of Weller and Pilgrim (1974) was continuously infused at a rate to provide the 12%/h liquid flow for fermentation periods of 10 d. The first 7 d were for equilibration. During the last 3 d the effluents were collected in an ice bath and a 1-L sample was composited and saved for analysis.

After the effluent was collected on d 10, the contents of the fermenters were allowed to settle and the upper fluid layer was used for collection of microbes. Two 250-ml samples were taken from each fermenter and centrifuged at 4°C for 20 min at 200× g. The supernatants were centrifuged for 15 min at 30,000 × g, the pellets were combined, resuspended in saline, and again centrifuged at 4°C for 15 min at 30,000 × g. The supernatants were discarded and the pellets were resuspended in 20 ml of a 50:50 mixture of distilled water and methanol and centrifuged for 15 min at 30,000 × g. The supernatants were poured off, and the pellets were resuspended in distilled water and lyophilized.

Chemical Analyses

The feed DM was determined by an oven drying at 100°C for 24 h. Effluent DM was determined by centrifuging a 34- to 40-g sample of effluent at 30,000 × g for 45 min. The supernatant was discarded and the particulate matter was dried at 100°C for 24 h and reweighed. For digestibility determinations, DMD and OMD were corrected for microbial DM and OM. Determination of the NDF and ADF content in the feed was by the methods of Goering and Van Soest (1978) with modifications by Van Soest (1990). The adaptations for NDF and ADF analysis of continuous culture effluents were described by Crawford et al. (1983). Total N in feed, effluents, bacterial and ammonia was determined according to AOAC (1990) using an automated Tecator digestion system (Tecator, Inc., Herndon, VA). Ether extraction of the feed was performed according to AOAC (1990). Analysis of VFA was performed in accordance with the gas chromatographic separation procedure (Anonymous, 1975). The GC was a Varian model 3300 with an FID detector (Varian, Inc., Palo Alto, CA). The column was a 2-m × 2-mm glass column packed with 10% SP-1200/1% H₃HPO₄ on 80/100 chromosorb WAW (Supelco, Inc. Bellefonte, PA). Effluent and bacterial concentrations of purines were determined by the procedures of Zinn and Owens (1986). The sugars and starches of the feeds and effluents were determined by the procedure of Smith (1969), except that ferricyanide was used to detect reducing sugars.

Statistics

Data were analyzed using ANOVA, the general linear model of SAS (1982). Treatment sum of squares were

Table 2. Digestion coefficients for dry and organic matter, fiber and nonstructural carbohydrates.

Item	Treatments			SE	Contrasts, <i>P</i> =	
	Control	YC1 ¹	YC2 ²		Control vs. YC	YC1 vs. YC2
Digestion, %						
DM	66.6	71.6	69.0	1.5	0.010	NS
OM	49.7	51.6	49.3	1.9	NS	NS
NDF	44.5	44.8	46.6	2.2	NS	NS
ADF	39.5	43.3	48.3	3.8	NS	NS
NSC ³	79.3	82.1	80.1	2.2	NS	NS
Total carbohydrate ⁴ , g/d	41.2	43.2	41.4	1.4	NS	NS

¹YC1 = Yeast culture 1, Diamond-V XP, Cedar Rapids, IA.²YC2 = Yeast culture 2, A-Max Concentrate, Mason City, IA.³Includes sugar and starch.⁴g NDF + nonstructural carbohydrate digested per day.

partitioned into orthogonal comparisons: Control versus YC and YC1 versus YC2. The following model was used in the statistical analysis: $Y_{ij} = \mu + \alpha_i + e_{ij}$, μ = overall mean, α_i = *i*th treatment effect, e_{ij} = random error.

RESULTS AND DISCUSSION

There was a tendency for increased ($P = 0.10$) DM digestion for YC compared with control (Table 2). There were no effects ($P > 0.05$) of either YC on other nutrient digestibility parameters. There was a numerical trend for increased OM and NS carbohydrate digestibility for YC1, whereas digestion of NDF and ADF was numerically higher for YC2. Others have shown an effect of YC addition in supporting an increase of fiber and protein digestibility (Wohlt et al., 1991). The difference in DM and OM digestion is due to buffer salt contamination of the effluent.

Both YC increased ($P = 0.004$) while total VFA compared with control YC1 resulted in more ($P = 0.04$) total VFA produced than YC2 (Table 3). Both YC resulted in less ($P = 0.02$) acetic and more ($P = 0.03$) propionic and valeric acid than control. Yeast culture 1 had reduced acetic ($P = 0.05$) and increased ($P = 0.04$) propionic acid percentages compared to YC2. There was no effect ($P > 0.05$) of either yeast culture on other acids. The relative proportions of acetic and propionic acid resulted in an AP ratio narrower ($P = 0.03$) for both YC treatments compared with control, with YC1 demonstrating a greater decrease ($P = 0.05$) than YC2. A further clarification of AP ratio is gained by examining the actual mmoles of acetic and propionic acid per day. The change in AP ratio for YC1 was caused by a marked increase ($P = 0.01$) in propionic acid production.

Mean daily fermentation pH was not affected by either YC compared with control; however, YC1 was lower ($P = 0.04$) than YC2. When the daily pH data was

Table 3. VFA production, molar ratios, and average daily fermenter pH.

Item	Treatments			SE	Contrasts, <i>P</i> =	
	Control	YC1 ¹	YC2 ²		Control vs. YC	YC1 vs. YC2
Total VFA, mmoles/d	370	426	398	7.6	0.004	0.04
Molar acid percentages:						
Acetic	57.3	47.1	53.2	1.7	0.02	0.05
Propionic	20.4	32.0	23.6	2.2	0.03	0.04
Isobutyric	1.02	0.72	0.83	0.17	NS	NS
Butyric	18.1	16.0	18.7	0.9	NS	NS
Isovaleric	0.62	0.53	0.70	0.06	NS	0.08
Valeric	2.60	3.70	3.0	0.2	0.03	0.08
A-P ratio	2.82	1.48	2.36	0.26	0.03	0.05
mmoles/day:						
Acetic	212	200	212	9	NS	NS
Propionic	75	137	93	9	0.01	0.01
Average pH	6.30	6.17	6.37	0.05	NS	0.04

¹YC1 = Yeast culture 1, Diamond-V XP, Cedar Rapids, IA.²YC2 = Yeast culture 2, A-Max Concentrate, Mason City, IA.

Table 4. Fermentation pH by hours after feeding.

pH, hours after feeding	Diets			SE	Contrasts, <i>P</i> =	
	Control	YC1 ¹	YC2 ²		Control vs. YC	YC1 vs. YC2
0	6.55	6.43	6.60	0.07	NS	NS
2	6.29	6.01	6.37	0.08	NS	0.02
4	6.09	6.00	6.21	0.07	NS	0.07
6	6.11	6.03	6.21	0.05	NS	0.05
8	6.22	6.16	6.32	0.05	NS	0.09
12	6.51	6.41	6.54	0.04	NS	0.07

¹YC1 = Yeast culture 1, Diamond-V XP, Cedar Rapids, IA.²YC2 = Yeast culture 2, A-Max Concentrate, Mason City, IA.

contrasted statistically in 2-h intervals (Table 4), the fermentation pH for YC1 was lower than YC2 at 2 ($P = 0.02$), 4 ($P = 0.07$), 6 ($P = 0.05$), 8 ($P = 0.09$), and 12 h ($P = 0.07$). The numerical increase in NDF (3.9%) and ADF (10.4%) digestion for YC2 compared to YC1 (Table 2) and the increase in acetic relative to propionic (Table 3) could have contributed to the modified pH profile. Others (Dawson et al., 1990) showed a similar occurrence in vitro, where total VFA and molar proportion of propionic acid increased and acetic acid decreased with a concomitant reduction in pH. While pH did not drop below 6.0, pH reductions of rumen fluid in the 5.8 to 6.2 range that are short in duration and of a cyclic nature will cause a moderate depression in fiber digestion (Hoover, 1986).

Protein digestibility and ammonia N were increased ($P = 0.05$ and 0.08 , respectively) while NAN and bypass N flow/d were decreased ($P = 0.07$ and 0.04 , respectively) by inclusion of YC compared with control (Table 5). This should have made the N more available for microbial growth and while not statistically significant ($P > 0.10$), both treatments had numerically higher microbial out-

puts than did the control. Efficiency expressed as microbial N produced per kilogram of both DM and carbohydrate digested were not affected by YC. However, YC2 tended ($P = 0.10$) to be higher in microbial N produced per kilogram of DMD. Analysis of microbes harvested from the fermenters showed evidence of differences in microbial composition (Table 6). Microbes harvested from YC2 had a higher ($P = 0.004$) protein content than did those from YC1, resulting in estimated CP values of 52.2 and 59.6% for YC1 and YC2, respectively. The protein content of the control was 56.1%. It appeared that the protein content decrease observed in YC1 was associated with an increase ($P = 0.03$) in ash compared with YC2. Based on analysis of the YC provided by the manufacturers, YC would only account for 0.4 and 0.2% of the microbial N and ash for YC2 and YC1, respectively.

When fungal cultures were supplemented in ruminant diets, there was a stimulatory effect in specific ruminal bacteria. Shifting the microbiota caused an increase in microbial protein synthesis and changes in microbial amino acid profiles (Beharka and Nagaraja,

Table 5. Nitrogen partitioning, microbial growth, and microbial efficiency.

Item	Treatments			SE	Contrasts, <i>P</i> =	
	Control	YC1 ¹	YC2 ²		Control vs. YC	YC1 vs. YC2
CP digested, %	64.2	68.8	70.6	1.8	0.05	NS
NAN, g/d	2.85	2.78	2.76	0.03	0.07	NS
Ammonia N, mg/dl	5.53	7.46	8.27	0.90	0.08	NS
Bypass N, g/d	1.09	0.94	0.89	0.05	0.04	NS
Microbial N, g/d	1.77	1.83	1.87	0.05	NS	NS
Efficiencies:						
Microbial N/kg DMD ³	26.5	25.6	27.1	0.6	NS	0.10
Microbial N/kg CHOD ⁴	42.5	42.9	44.1	1.9	NS	NS
Feed N, % ⁵	90.5	88.0	87.0	1.4	NS	NS

¹YC1 = Yeast culture 1, Diamond-V XP, Cedar Rapids, IA.²YC2 = Yeast culture 2, A-Max Concentrate, Mason City, IA.³Microbial N produced per kilogram of DM digested.⁴Microbial N produced per kilogram of total carbohydrate digested.⁵Digested feed N converted to microbial N, %.

Table 6. Composition of the microorganisms.

Item	Treatments				Contrasts, <i>P</i> =	
	Control	YC1 ¹	YC2 ²	SE	Control vs. YC	YC1 vs. YC2
Nitrogen, %	8.98	8.35	9.54	0.18	NS	0.004
Ash, %	12.24	28.52	11.42	1.80	NS	0.03

¹YC1 = Diamond-V XP, Cedar Rapids, IA.²YC2 = A-Max Concentrate, Mason City, IA.

1991; Dawson and Hopkins, 1991). Erasmus et al. (1992) showed supplementation of YC tended to increase microbial protein synthesis in dairy cows and significantly altered the amino acid profile of the duodenal digesta. McLeod et al. (1990) showed no differences in microbial protein synthesis. The increase in protein concentration for YC2 bacteria would be expected to provide greater nutrient value to the host.

The control ration was well balanced for maximizing microbial growth. In spite of this, yeast culture effects were observed for DM digestion, pH, VFA production, and AP ratios. The small increases in DM digestion (Table 2), which was similar for both YC, was primarily due to increases in protein digestion. Although both cultures were growing on similar amounts of carbohydrate, VFA production (Table 3) was increased by both YC compared to the control. This appears to be associated with more efficient VFA production pathways involving higher propionic acid. VFA produced/g of carbohydrate digested for control, YC1 and YC2 were 8.98, 9.86, and 9.61, respectively. Higher VFA, especially propionic acid, are important in terms of enhanced lactose production in milk volume. A high propionic and somewhat lower acetic acid production, along with a significant decrease in pH for YC1, could be potentially detrimental for DM intake and butterfat production. Although the higher propionic acid production that resulted from feeding YC would be expected to have a positive effect on milk volume, changes in protein metabolism are also of great importance in the early lactating cow. Microbial protein is high quality bypass protein. The increased microbial N produced per day for YC2 would help explain the increased efficiency based on digested DM. Applying the DM digestibility and efficiency values to a lactating cow consuming 54 lb of DM/d, total microbial protein for control and YC2 would be 2613 and 2869 g/d, respectively, a difference of 256 g of microbial protein/d. This is a significant contribution to amino acid delivery postruminal.

CONCLUSIONS

Supplementing diets with YC increased DM digestion, total VFA production, and propionic acid produc-

tion. Yeast culture 1 had a lower ruminal pH than YC2. Supplementing YC2 resulted in a tendency for higher microbial N/kg of DMD than YC1. Yeast culture 1 resulted in production of rumen microbes containing less protein and more ash than YC2. Yeast culture supplementation does influence ruminal microbial metabolism, and some YC may have different modes of action and, therefore, more pronounced performance results than other YC.

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