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Effect of *Saccharomyces cerevisiae* or *Aspergillus oryzae* cultures and NDF level on parameters of ruminal fermentation

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Abstract

A metabolism trial was conducted to study the effect of two direct-fed microbial cultures (*Saccharomyces cerevisiae*, SC; *Aspergillus oryzae*, AO) and neutral detergent fibre (NDF) level on ruminal fermentation. Six ruminally fistulated Holstein heifers (300 kg body weight) were randomly assigned to a 6 × 6 Latin square where treatments were control groups (CG) at two NDF levels (27 and 37%), and combinations with *S. cerevisiae* (0 or 10 g) or *A. oryzae* (0 or 3 g) cultures. Animals were restricted fed 10.5 kg dry matter day^{-1} (08:00 and 20:00 h). Diets were based on alfalfa hay (40 or 60%), barley grain (42.1 or 20.3%) and corn stover (1.4 or 9.4%). NDF levels of 27% and 37% gave rise to significantly different ruminal pH values (6.21 vs. 6.49, $P < 0.01$) and volatile fatty acid concentrations (73.0 vs. 66.4 mmol l^{-1}, $P < 0.05$). There were interactions ($P < 0.05$) between microbial cultures and NDF level at 6, 9 and 12 h for propionate molar percentage and entodiniomorphid population. In situ alfalfa NDF disappearance at 48 h incubation was increased ($P < 0.05$) by microbial cultures (CG, 53.8^{b}; SC, 56.7^{a,b}; AO, 59.2%^{a}), but was reduced ($P < 0.01$) by the lower NDF level (54.4% vs. 58.7% for 27% and 37% NDF, respectively). According to these results, *Saccharomyces cerevisiae* and *Aspergillus oryzae* cultures increased in situ alfalfa NDF digestion at 48 h and propionate concentration at both levels of NDF.

Keywords: Ruminant; Rumen fermentation; Yeast; *Saccharomyces cerevisiae*; *Aspergillus oryzae*

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1. Introduction

Direct-fed microbial products have improved daily gain and milk production in ruminants (Gomez-Alarcon et al., 1991; Erasmus et al., 1992); however, results have been inconsistent (Williams et al., 1991; Mir and Mir, 1994). In some studies, addition of *Saccharomyces cerevisiae* or *Aspergillus oryzae* have increased fat corrected milk yield (Kellems et al., 1988; Wohlt et al., 1991), whereas no effect was obtained in other experiments (Arambel and Kent, 1988; Sievert and Shaver, 1993). Diet composition and NDF level may have influenced these results.

Therefore, this metabolism trial was conducted to study the effect of two direct-fed microbial cultures *Saccharomyces cerevisiae* and *Aspergillus oryzae*, and dietary neutral detergent fibre (NDF) level on ruminal fermentation in Holstein heifers.

2. Material and methods

Two direct-fed microbial products were evaluated, a yeast culture containing *Saccharomyces cerevisiae* plus growth medium (10 g day^{-1}, Yea-Sacc^{1026}, Alltech Inc., Nicholasville, KY) and a *Aspergillus oryzae* fermentation extract (3 g day^{-1}, Amaferm, BioZyme, St. Joseph, MO), dosed once per day intraruminally, in heifers fed two alfalfa hay levels in the diet (40 and 60% dry matter (DM) basis; Table 1). Six ruminally fistulated Holstein heifers (300 $\pm$ 17 kg body weight) were used in a 6 $\times$ 6 Latin square with factorial arrangement of treatments (3 $\times$ 2), where treatments were control groups without cultures at two NDF levels (27 and 37%), and combinations with *S. cerevisiae* or *A. oryzae* cultures. Each period consisted of 12 days of adaptation and 3 days of sample collection. Animals were restricted fed 10.5 kg DM day^{-1} (08:00 and 20:00 h).

In situ disappearance of NDF was measured incubating 5 g of alfalfa ground through a 2 mm screen, in polyester bags (10 cm $\times$ 20 cm; 60 μm pore size). Duplicate bags were incubated at 3, 6, 12, 24 and 48 h. NDF was determined by procedures outlined by

Table 1
Composition of diets (% DM)

Ingredient	NDF level	
	27%	37%
Alfalfa hay	40.0	60.0
Barley	42.15	20.26
Cottonmeal	9.41	7.67
Molasses	5.0	.68
Corn stover	1.44	9.39
Mineral premix [a]	2.00	2.00
NDF [b] (%)	27.6	36.7
Crude protein [b] (%)	13.2	13.1

[a] Premix composition: Ca, 13.0%, P, 0.5%; Na, 10.9%; Cl, 20.0%; Fe, 430 ppm; Mg, 330 ppm; Mn, 200 ppm; Cu, 80 ppm; Co, 6 ppm; I, 4 ppm; Zn, 80 ppm.
[b] Values determined in the laboratory.

Goering and Van Soest (1970). Barley samples (5 g) were also incubated to determine in situ starch disappearance. Starch was determined from glucose released from residual sample using the method of Herrera-Saldana and Huber (1989) modified by Wester (1989). After incubation, bags were removed and rinsed with cool water several times in a bucket, until the water ran clear.

NDF was determined by procedures outlined by Goering and Van Soest (1970) modified by the addition of 150 μl of Takaterm L-340 30 min after digestion was initiated, in order to eliminate residual starch (Plata et al., 1994).

Ruminal fluid samples from the ventral sac were taken on day 13 during the collection period at 0, 3, 6, 9 and 12 h after feeding. Ruminal fluid pH was measured immediately after sampling and then 50 ml of ruminal fluid were stored in a freezer (−20°C) for further analysis.

Ruminal fluid samples were also used to count protozoa, and to determine volatile fatty acid (VFA) and ammonia-N concentrations. VFA concentrations were determined by gas chromatography in samples prepared with metaphosphoric acid (Erwin et al., 1961). Ammonia-N was measured by the indophenol method (McCullough, 1967).

Table 2
Main effects of microbial culture and NDF level on ruminal pH, and in situ disappearance of NDF and starch

Time	Treatment [a]						SE [b]	Significant effects [c]
(h)	C-27	A-27	S-27	C-37	A-37	S-37		
In situ NDF disappearance (%)								
3 [d]	7.45	9.29	9.26	10.3	7.36	11.8	1.59	
6	9.92	9.35	9.49	13.2	12.7	12.2	1.52	NDF
9	14.2	15.2	16.0	20.3	20.78	20.0	2.50	NDF
12	17.0	14.9	17.6	26.3	25.5	27.8	2.47	NDF
24	34.3	38.3	35.9	37.0	42.4	40.9	2.68	
48	50.5	58.9	53.7	56.9	59.3	59.8	2.11	NDF, MC
Mean	22.2	58.9	53.7	56.9	59.3	59.7	2.79	
In situ starch disappearance (%)								
3 [d]	44.9	45.9	38.7	46.9	45.9	38.8	4.08	
6	51.8	51.8	47.5	50.1	54.8	62.6	3.64	
9	59.2	62.5	54.0	61.7	59.9	62.9	3.83	
12	54.3	64.7	56.5	62.3	63.5	60.8	3.69	
Mean	52.5	64.7	56.5	62.3	63.5	60.8	4.35	
Ruminal pH								
0 [e]	6.10	6.36	6.27	6.72	6.65	6.62	0.07	NDF
3	6.06	6.18	6.17	6.37	6.36	6.47	0.05	NDF
6	5.97	6.20	6.12	6.39	6.38	6.34	0.08	NDF
9	6.15	6.24	6.28	6.47	6.41	6.44	0.08	NDF
12	6.26	6.44	6.35	6.69	6.54	6.55	0.07	NDF
Mean	6.11	6.28	6.24	6.53	6.47	6.48	0.08	

[a] C, control; A, *Aspergillus oryzae*; S, *Saccharomyces cerevisiae*; 27, 27% NDF; 37, 37% NDF.
[b] Standard error.
[c] Main effects ($P < 0.05$): NDF, neutral detergent fibre level; MC, microbial culture.
[d] Hours of incubation.
[e] Hours after feeding.

Ruminal protozoa were counted with a Sedgewick-Rafter cell as described by Mendoza et al. (1993) and the results expressed as numbers per millilitre of ruminal contents (Dehority, 1984).

Data were analysed as a 6 × 6 Latin square design with a 3 × 2 factorial arrangement of treatments with time effects as a subplot (Steel and Torrie, 1980) using the GLM procedure of the Statistical Analysis Systems Institute Inc. (1985).

3. Results and discussion

In situ alfalfa NDF disappearance was increased ($P < 0.05$) only at 48 h incubation by microbial cultures (CG, 53.8[b]; SC, 56.7[a,b]; AO, 59.2%[a]), but was reduced ($P < 0.01$) by the lower fibre level during most times of incubation (Table 2). Improvement in NDF digestibility using *Saccharomyces cerevisiae* in low quality forages has been reported previously (Weidmeier et al., 1987; Ayala et al., 1992); however, other studies show no effect on in situ NDF digestion of alfalfa (Roa-Vega et al., 1993). This effect could be the result of increments in total and cellulolytic bacterial populations observed with the addition of yeast culture (Harrison et al., 1988; Dawson et al., 1990) or fungal culture (Weidmeier et al., 1987; Fondevilla et al., 1990).

As expected, ruminal pH was increased ($P < 0.05$) in rations with 37% NDF during all sampling times (Table 2). The lower pH is associated with the amount of starch and fermentable carbohydrates (Weiss et al., 1989). The addition of *Saccharomyces cere-*

Table 3
Effects of microbial culture and NDF level on ammonia-N and VFA concentration

Time	Treatment [a]						SE [b]	Significant effects [c]
(h)	C-27	A-27	S-27	C-37	A-37	S-37		
Ammonia-N (mg dl^{-1})								
0 [d]	12.0	18.3	16.4	14.6	13.3	12.7	2.27	
3	12.4	18.9	17.7	17.8	17.9	20.6	1.66	MC
6	9.96	10.7	13.3	12.7	10.5	11.6	1.55	
9	8.4	10.5	9.0	11.4	7.71	9.18	1.43	
12	9.36	11.2	8.57	10.3	8.54	9.50	0.82	
Mean	10.4	13.9	12.9	13.4	11.6	12.7	3.96	
Total VFA (mM)								
0 [d]	80.2	81.7	75.1	77.6	69.0	69.2	4.95	
3	76.8	84.5	86.8	78.4	80.0	86.8	6.17	
6	71.7	83.6	92.7	88.7	73.3	87.9	5.07	MC × NDF
9	76.4	77.2	76.0	78.9	72.2	76.0	4.33	
12	73.18	73.3	74.8	67.5	68.4	63.3	3.91	NDF
Mean	75.6	80.0	81.0	78.2	72.6	76.6	12.12	

[a] C, control; A, *Aspergillus oryzae*; S, *Saccharomyces cerevisiae*; 27, 27% NDF; 37, 37% NDF.
[b] Standard error.
[c] Main effects and/or interaction ($P < 0.05$): NDF, neutral detergent fibre level; MC, microbial culture; MC × NDF, MC × NDF interaction.
[d] Hours after feeding.

visiae or *Aspergillus oryzae* did not affect ruminal pH (Table 2). Results in the literature are conflicting and effects are confounded with intake and NDF level (Harrison et al., 1988; Dawson et al., 1990; Beharka et al., 1991; Williams et al., 1991; Caton et al., 1993); however, in this study intake was restricted.

There was no effect of either microbial culture or NDF level on in situ starch digestion (Table 2). This could explain the inconsistent response to microbial fed cultures in feedlot diets (Mir and Mir, 1994). Regarding the effect of NDF level on starch digestion, some studies (e.g. Cole et al., 1976) have shown that ruminal starch digestion was depressed by increments of forage in the diet and this was associated with the rate of passage. Results from this study suggest that the rate of starch digestion is not affected by the ruminal environment associated with forage.

At 3 h post feeding, the $N\text{-}NH_3$ concentration was lower ($P < 0.05$: Table 3) for control groups (15.0^a mg dl^{-1}) than for *Saccharomyces cerevisiae* (19.15^b mg dl^{-1}) and *Aspergillus oryzae* (18.44^b mg dl^{-1}; Table 3) groups. In most of the studies, microbial cultures have no effect on ammonia nitrogen or in situ degradability of protein (Wohlt et al., 1991; Erasmus et al., 1992; Sievert and Shaver, 1993).

Table 4
Effects of microbial culture and NDF level on molar proportion of VFA

Time	Treatment [a]						SE [b]	Significant effects [c]
(h)	C-27	A-27	S-27	C-37	A-37	S-37		
Acetate (%)								
0 [d]	71.3	72.0	71.7	72.9	72.5	74.4	0.75	NDF
3	70.9	71.7	70.8	71.5	72.5	70.0	0.57	
6	71.1	72.4	71.0	72.4	72.3	73.1	0.63	NDF
9	71.5	71.9	71.9	72.1	72.7	73.4	0.65	
12	72.4	72.7	72.8	73.3	73.4	73.4	0.69	
Mean	71.4	72.1	71.7	72.4	72.7	72.8	1.62	
Propionate (%)								
0 [d]	15.7	14.6	15.7	14.8	15.9	15.2	0.41	MC×NDF
3	17.2	15.9	17.0	16.4	17.4	17.8	0.47	
6	16.8	15.1	16.2	15.8	16.6	16.7	0.28	MC×NDF
9	16.2	15.6	15.7	15.2	16.0	16.1	0.33	MC×NDF
12	15.3	14.1	15.1	14.7	15.9	15.8	0.38	MC×NDF
Mean	16.2	15.0	15.9	15.4	16.6	16.3	0.94	
Butyrate (%)								
0 [d]	11.8	12.3	12.1	11.0	10.0	10.2	0.72	NDF
3	11.8	12.3	12.1	11.0	10.0	10.2	0.77	NDF
6	12.0	12.9	12.7	11.7	10.2	10.1	0.64	NDF
9	12.2	13.2	12.3	12.7	11.1	10.4	0.65	NDF
12	12.1	13.0	12.0	11.8	10.6	10.7	0.65	NDF
Mean	12.0	12.7	12.2	11.6	10.4	10.3	0.80	

[a] C, control; A, *Aspergillus oryzae*; S, *Saccharomyces cerevisiae*; 27, 27% NDF; 37, 37% NDF.
[b] Standard error.
[c] Main effects and/or interaction ($P < 0.05$): NDF, neutral detergent fibre level; MC, microbial culture; MC×NDF, MC×NDF interaction.
[d] Hours after feeding.

Table 5
Effects of microbial culture and NDF level on protozoa population

Time	Treatment [a]						SE [b]	Significant effects [c]
(h)	C-27	A-27	S-27	C-37	A-37	S-37		
Entodinomorphids ($\times 10^3$)								
0 [d]	912	2630	2137	3019	1288	2818	29	MC×NDF
3	1445	2187	1513	1778	831	912	35	
6	977	2570	2570	2691	1230	1318	30	MC×NDF
9	1230	1862	1479	1905	891	1380	25	MC×NDF
12	2137	3311	3235	3162	2454	1071	32	MC×NDF
Mean	1340	2512	2187	2511	1339	1500	25	
Holotrichs ($\times 10^3$)								
0 [d]	165	141	85	79	54	57	6	NDF
3	60	66	53	74	56	81	6	MC
6	66	109	85	104	46	30	6	NDF, MC×NDF
9	123	141	85	79	54	57	8	NDF
12	81	144	74	112	138	64	7	MC
Mean	99	120	76	90	70	58	8	

[a] C, control; A, *Aspergillus oryzae*; S, *Saccharomyces cerevisiae*; 27, 27% NDF; 37, 37% NDF.
[b] Standard error.
[c] Main effects and/or interaction ($P < 0.05$): NDF, neutral detergent fibre level; MC, microbial culture; MC×NDF, MC×NDF interaction.
[d] Hours after feeding.

There was an interaction ($P < 0.05$) between microbial cultures and NDF at 6 h for VFA concentration, and a NDF level effect at 12 h (Table 3). For 37% NDF, propionate molar percentage was reduced ($P < 0.05$) in treatments without microbial culture, but showed an increment with microbial cultures. Similar results were reported for *Saccharomyces cerevisiae* (Malcom and Kiesling, 1990; Williams et al., 1991; Plata et al., 1994), whereas no changes in acetate or propionate have been observed with *Aspergillus oryzae* (Sievert and Shaver, 1993; Caton et al., 1993). However, in vitro studies suggest that *Aspergillus oryzae* stimulates lactate-utilising bacteria, which increases propionate production (Nisbet and Martin, 1991).

The molar proportion of acetate was increased by the 37% NDF level at 0 and 6 h, and butyrate during all sampling times (Table 4). The differences observed in VFA patterns are associated with substrate availability and microbial populations (Song and Kennelly, 1990); there were elevated numbers of noncellulolytic bacteria in the 27% NDF treatment compared with high numbers of cellulolytic bacteria with 37% NDF.

There were interactions ($P < 0.05$) between microbial cultures and NDF at 0, 6, 9 and 12 h for entodiniomorphid populations (Table 5). Entodiniomorphid counts were increased ($P < 0.05$) in groups with microbial cultures control when NDF was 37%; however, for microbial cultures, there was a reduction in population when the NDF level changed from 27 to 37%. Increments of ciliates with *Saccharomyces cerevisiae* (Ayala et al., 1992; Plata et al., 1994) and *Aspergillus oryzae* (Fondevilla et al., 1990) have been observed previously.

Protozoa holotrichs did not show consistent effects (Table 5) that could be associated with the migration or sequestration of holotrichs in the rumen wall or the digesta (Matanobu and Iriki, 1989).

According to the results of this trial, the addition of *Saccharomyces cerevisiae* or *Aspergillus oryzae* cultures increased in situ alfalfa NDF digestion at 48 h and propionate concentration at both levels of NDF. Direct-fed microbial cultures did not have direct effects on ruminal pH or in situ starch digestion.

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