Effect of Yeast Culture and *Aspergillus oryzae* Fermentation Extract on Ruminal Characteristics and Nutrient Digestibility[1]

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ABSTRACT

Four nonpregnant and nonlactating Holstein cows fitted with ruminal fistulas were assigned to each of four diets in a 4 x 4 Latin square design Dietary treatments were 1) basal diet containing 50% concentrate, 2) basal diet plus 90 g/d yeast culture, 3) basal diet plus 2 63 g/d *Aspergillus oryzae* fermentation extract, 4) basal diet plus 90 g/d of *A oryzae* fermentation extract and yeast culture Cows were fed diets at a rate of 86 g DM/kg BW$^{.75}$ for 14 d adaptation followed by an 8-d collection period Digestibility of dry matter was increased by *A oryzae* and *A oryzae* and yeast culture combination treatments Digestibility of CP was increased regardless of fungal culture addition Hemicellulose digestibility, percent ruminal cellulolytic organisms, and acetate to propionate ratio were increased by the addition of fungal supplements

INTRODUCTION

Feeding cereal grains to increase the energy density of cattle diets has increased performance but resulted in a negative associative effect in digestion of structural carbohydrates The negative effect is presumably due to alteration in cellulolytic bacteria habitat (12) One solution has been to feed buffers to maintain optimal ruminal pH (7) of 6 7 to 7 0 (15) Another method to maintain structural carbohydrate digestion in high cereal grain diets is daily ruminal inoculation with organisms capable of maintaining cellulolytic activity in the ruminal habitat produced by high cereal grain diets Jahn (11) adapted rumen bacteria in batch cultures to specific habitats and inoculated lactating dairy cattle intraruminally with the cultures Inoculated cattle produced 17% more fat-corrected milk Addition of a fungal culture to the diet of lactating dairy cows improved milk fat content (13) Huber (personal communication) reported increased fat-corrected milk production when the diet of dairy cows was supplemented with the same *Aspergillus oryzae* fermentation extract used in this study. The purpose of this study was to measure the effect of supplemental viable fungal cultures on ruminal fermentation characteristics and nutrient digestibility in cattle fed 50% concentrate diets

MATERIALS AND METHODS

Four nonpregnant and nonlactating Holstein cows (averaging 659 kg) fitted with ruminal fistulas were assigned each of four dietary treatments (Tables 1 and 2) Cows were fed individually at 0400 and 1600 h daily and had free access to clean water Diets were fed for a 14-d adaptation followed by an 8-d collection period Supplements were topdressed on the basal rations

On d 1 to 3 of the collection period, fecal grab samples were taken during the 0400 and 1600 h feeding Diet samples were taken daily starting 2 d prior to the fecal collection period Fecal samples were initially dried for 72 h in a forced air oven at 60°C Laboratory DM was determined in a forced air oven at 100°C for 24 h Feed and dried fecal samples were Wiley mill ground (1-mm screen) and analyzed for CP (2), ADF, NDF (17), and acid insoluble ash (AIA)

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TABLE 1 Composition of diets

Item	IFN[1]	Diets (DM basis) Basal	Basal + YC[2]	Basal + AO/YC[3]	Basal + AO[4]
			(%)		
Chopped alfalfa hay	1-00-063	33 38	33 38	33 38	33 38
Rolled barley	4 07 939	25 87	24 88	24 88	24 88
Beet pulp with molasses	4-00-672	17 00	17 00	17 00	17 00
Chopped barley straw	1-00 498	15 00	15 00	15 00	15 00
Wheat bran	4-05-190	8 50	8 50	8 50	9 46
Trace-mineralized salt		25	25	25	25
YC	7-05 520		99		
FC/YC				99	
AO	5 06-152				03

[1] International Feed Number

[2] Yeast culture Diamond V Mills, Inc Cedar Rapids IA

[3] Vitaferm (Amaferm plus yeast culture vitamins and minerals) BioZyme Enterprises Inc St Joseph MO

[4] Amaferm (*Aspergillus oryzae* fermentation extract) BioZyme Enterprises Inc St Joseph, MO

(16) Acid insoluble ash was used as an internal marker to determine apparent nutrient digestibility

On d 4, ruminal liquid volume (LV) was determined at 0 3, 6, and 9 h postfeeding (0400 h) using the method of Alexander et al (1) except 500 ml of Cr-EDTA acid solution (4) were used as the water soluble marker instead of polyethylene glycol A 300 ml sample of mixed rumen digesta was taken at each time period A 50-ml aliquot was used to determine DM by drying for 72 h in a forced air oven at

TABLE 2 Estimated nutrient content of diets[1]

Item	Diets (DM basis) Basal	Basal + YC[2]	Basal + AO/YC[3]	Basal + AO[4]
Metabolizable energy, Mcal/kg	2 41	2 41	2 41	2 41
Crude protein, %	13 32	13 30	13 32	13 32
Neutral detergent fiber, %	42 75	42 66	42 68	42 75
Acid detergent fiber, %	25 03	25 01	25 04	25 03
Calcium, %	64	64	69	64
Magnesium, %	26	26	29	26
Phosphorus, %	32	32	35	32
Potassium, %	1 75	1 75	1 80	1 75
Sulfur, %	26	26	27	26
Cobalt, mg/kg	33	33	55	33
Copper, mg/kg	12 41	12 38	14 41	12 41
Manganese mg/kg	36 77	36 65	40 91	36 80
Zinc mg/kg	33 61	33 60	49 82	33 64

[1] Nutrient analysis based on International Feed Number and manufacturer's guaranteed analysis

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[3] Vitaferm (Amaferm plus yeast culture vitamins and minerals) BioZyme Enterprises Inc St Joseph MO

[4] Amaferm (Patent No 3043748) BioZyme Enterprises Inc St Joseph MO

60°C. A 200-ml aliquot was strained through four layers of cheesecloth and pH measured on the filtrate using a combination electrode. A portion of the filtrate was acidified by placing nine parts filtrate with one part 6 N HCl. The mixture was then clarified by centrifugation at 25,000 × g for 20 min and analyzed for VFA using gas chromatography with a 5% neopentyl glycosuccinate and 1% H_3PO_4 on Anakrom A90/100 column[3] at 115°C and NH_3N using specific ion electrode. Another 30-ml portion of the filtrate was centrifuged at 25,000 × g for 20 min and analyzed for Cr using a modification of the method of Fenton and Fenton (8). Ten milliliter aliquots of clarified ruminal fluid were placed in a 25 ml volumetric flask and evaporated in a forced air oven at 100°C. Chromium standards were prepared similarly and contained 10, 5, 2.5, 1.25, .625, and 3125 mg Cr/dl. Chromium content of the residue was determined by digestion with 5 ml of the solution described by Fenton and Fenton (8), and percent transmittance was measured at a wavelength of 430 nm using a spectrophotometer[4]. The difference in Cr content of the ruminal fluid before and after the addition of 500 ml of Cr EDTA into the rumen was used to determine LV.

On d 5, a pulse dose of 1000 ml of Cr-EDTA solution (4) was hand mixed with rumen digesta immediately prior to the 0400 h feeding. Samples of rumen digesta were collected at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 h postdosing and analyzed for Cr as described. Slope of the natural log of Cr content versus time was defined as the ruminal liquid dilution rate (LDR).

On d 6 to 7, 200 g of Cr mordanted dietary fiber (9) was mixed with the 0400-h ration. Rumen digesta samples were collected at 0, 2, 4, 6, 8, 10, 12, 16, 20, 24, 32, 40, and 48 h postfeeding and dried in a forced air oven for 72 h at 60°C. Samples were ground in a Wiley mill (1-mm screen) and analyzed for Cr (8). Ruminal particulate rate of passage was defined as the slope of the natural log of the chromium content versus time.

On d 8, rumen digesta was hand mixed and samples collected at 3 h after the 0400 h feeding and analyzed for total viable bacteria and cellulolytic bacteria using habitat stimulating medium in anaerobic roll tubes described by Hungate (10). Four replicate samples at two serial dilutions were used for rolltube evaluations. Cellulolytic bacteria were counted based on visual identification of clear zones produced by bacteria in the cellulose media.

Data were analyzed using a model for the Latin square design. The model included dietary treatments, cows, and time periods. When main effect means were significant, treatment means were separated using Duncan's new multiple range test (14).

RESULTS AND DISCUSSION

Ruminal digesta flow kinetics are presented in Table 3. Ruminal LV, LDR, PROP, and liquid outflow rate were not affected by treatment. Ruminal fermentation characteristics are presented in Table 4. Ruminal pH, VFA, and NH_3N were unaffected by treatment.

Effect of the fungal supplements on rumen bacteria are in Table 5. Fungal supplements tended to increase viable bacteria numbers. Cellulolytic bacteria numbers were increased by nearly 40% by fungal supplements ($P < .05$). The use of yeast culture (YC) supplement tended to increase the percent of bacteria that were cellulolytic, and supplements that contained *A. oryzae* fermentation extract (AO) increased percent cellulolytic bacteria by 27% ($P < .05$). Because YC is not cellulolytic, the yeast may have provided stimulatory factors for cellulolytic bacteria, such as B vitamins or branched-chain VFA (6). Only 2.63 g/d of AO supplement were fed, therefore, stimulatory factors probably would not explain the increased number and proportion of cellulolytic bacteria. Cellulolysis by *A. oryzae* is a more probable explanation. The AO/YC supplement may have provided both stimulating factors and cellulolytic organisms. The AO/YC was also fortified with vitamins and minerals (Table 2) that may have stimulated cellulolytic bacteria (6).

Nutrient digestibility data are presented in Table 6. Digestibility of DM was increased by 4.2% with supplements containing AO ($P < .05$). Digestibility of CP increased ($P < .01$) with all fungal supplements, the AO/YC supplement

[3]Analabs GCM 109. Unit of Foxboro Analytical, North Haven, CT.

[4]Spectronics 601, Bausch and Lomb.

TABLE 3 Effect of treatments on ruminal digesta flow kinetics

Item	Diets				SE
	Basal	Basal + YC[1]	Basal + AO/YC[2]	Basal + AO[3]	
Liquid volume, L	50 92	51 31	52 37	52 60	55
Liquid dilution rate, %/h	8 90	9 52	6 67	7 31	67
Liquid outflow rate, L/h	4 53	4 88	3 49	3 85	35
Particulate rate of passage, %/h	4 52	5 11	3 39	3 62	39

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TABLE 4 Effect of treatments on ruminal fermentation characteristics

Item	Diets				SE
	Basal	Basal + YC[1]	Basal + AO/YC[2]	Basal + AO[3]	
pH	6 34	6 34	6 38	6 44	04
Total VFA mmol/L	64 60	66 40	63 40	67 90	3 00
Acetate, molar %	69 30	68 80	69 50	69 30	68
Propionate, molar %	14 00	13 80	13 40	13 10	31
Butyrate, molar %	12 40	13 00	12 70	13 30	33
Acetate propionate[4]	4 96	5 08	5 20	5 30	15
% Branched chain VFA	4 25	4 46	4 43	4 31	27
NH_3N, mg/dl	18 21	18 72	19 32	19 27	35
NH_3N pool, g	9 27	9 61	10 19	10 14	21

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[4] Acetate pool to propionate pool

TABLE 5 Effect of treatments on ruminal bacteria

Item	Diets				SE
	Basal	Basal + YC[1]	Basal + AO/YC[2]	Basal + AO[3]	
Total viable bacteria, $\times 10^8$/ml	196 2	255 0	257 3	223 5	16 5
Cellulolytic bacteria, $\times 10^8$/ml	25 0[a]	39 8[b]	45 6[b]	39 1[b]	3 8
% Cellulolytic bacteria	12 9[a]	15 4[ab]	18 0[b]	17 5[b]	9

[a,b] Means in the same row with different superscripts differ ($P < 05$)

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TABLE 6 Effect of treatments on total tract nutrient digestibility

Item	Diets				
	Basal	Basal + YC[1]	Basal + AO/YC[2]	Basal + AO[3]	SE
	(%)				
Dry matter	77 0[a]	79 1[ab]	81 0[b]	79 8[b]	62
Crude protein	79 5[c]	82 2[de]	84 4[e]	81 6[d]	63
Acid detergent fiber	69 3	70 0	72 6	71 0	89
Hemicellulose	76 3[a]	80 5[b]	83 5[b]	80 8[b]	1 03

[a b] Means in the same row with different superscripts differ ($P < 05$)

[c d e] Means in the same row with different superscripts differ ($P < 01$)

[1] Yeast culture Diamond V Mills Inc Cedar Rapids IA

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resulted in the largest increase The YC supplement probably provided factors stimulatory toward proteolytic bacteria, whereas the AO supplement was actively proteolytic (5) The AO.YC supplement provided both factors Hemicellulose digestibility was increased 6 5% with the fungal supplements ($P < 05$) Increased digestibility of structural carbohydrates was concurrent with increased number and proportion of cellulolytic organism measured (Table 5) and tendency toward reduced particulate and liquid passage rates (Table 3)

The addition of viable fungal supplements containing AO and YC to the diets of cattle fed moderate amounts of concentrate is a means to increase digestibility of the structural carbohydrate portion of the diet The combination of YC which probably provided stimulatory factors for rumen bacteria and AO, which produces cellulase enzymes was most beneficial Although AO does not produce the enzymatic machinery to depolymerize completely structural carbohydrates to simple sugars it does produce enzymes that cause partial depolymerization (5) and it aids rumen cellulolytic bacteria in complete depolymerization of cellulosic material to simple sugars (3) Further research on dose-response at different forage to concentrate ratios and characterization of metabolites of AO are needed to elucidate mode of action

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