INFLUENCE OF DONOR ANIMAL ADAPTATION TO ADDED YEAST CULTURE AND/OR ASPERGILLUS ORYZAE FERMENTATION EXTRACT[1] ON IN VITRO RUMEN FERMENTATION[2]

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ABSTRACT

Influence of donor animal adaptation to added yeast and/or fungal culture was evaluated on an in vitro rumen fermentation system. In vitro dry matter disappearance tended to increase in the donor animals that were better adaptated to fungal treatments An adaptation to the microbial additives also increased in vitro ammonia nitrogen and branch chain volatile fatty acids These data indicate that in vitro fermentation results may be improved by prior adaptation of the donor animal to certain microbial additives.

INTRODUCTION

In recent years researchers have used fungal cellulase preparations to increase dry matter (1, 2), fiber (2, 3), and protein (2) digestion in ruminants. However, Leatherwood et al. (4) indicated that fungal cellulase addition had no effect on feed utilization. Tegbe and Zimmerman (5) demonstrated that incorporation of yeast single cell protein into diets of growing pigs improved nitrogen digestibility and retention. However, Johnson and Remillard (6) indicated that the addition of brewers yeast decreased overall diet dry matter, energy and carbohydrate digestibility. Results from in vitro experiments have also varied. Daniels and Hashim (1) demonstrated that in vitro dry matter digestibility (IVDMD) was highest with addition of 375 mg of a cellulase preparation per kilogram of substrate. IVDMD decreased when more than 375 mg/kg was added, perhaps due to metabolite accumulation. Michalowski (7) also attributed the failure to increase IVDMD to metabolite accumulation.

This study determined whether rumen fluid inoculum from ruminants adapted to yeast and/or fungal cultures affected in vitro fermentation.

MATERIALS AND METHODS

In a series of in vitro fermentation trials, a rumen-fistulated heifer (weighing approximately 590 kg) was fed each of four treatments as follows: 1) basal ration (ad libitum alfalfa hay); 2) basal ration plus 90 gm yeast culture(YC)[3]/day; 3) basal ration plus 2.63 gm

[1] Amaferm (Patent No. 3043748). BioZyme Enterprises, Inc., St. Joseph MO.

[2] Supported in part by grants from Diamond V Mills, Inc. and BioZyme Enterprises, Inc.

[3] Diamond V Miles, Inc., Cedar Rapids, IA

Aspergillus oryzae fermentation extract (AO)[4]/day; 4) basal ration plus 90 gm AO PREMIX[5]/day. The heifer was adapted to each treatment 21 days prior to collection of rumen fluid inoculum. Rumen fluid was obtained 3.5 h after the morning feeding from just below the hay mat in the ventral sac and was strained through four layers of cheesecloth.

Alfalfa hay ground through a 1 mm mesh screen was the substrate in the in vitro fermentation experiment. Each treatment was tested on two alternate days. To 2.0 gm of substrate previously weighed into 250 ml centrifuge bottles was added 40 ml rumen fluid inoculum and 160 ml mineral buffer (8). Centrifuge bottles were flushed with CO_2, capped with Bunsen valves and incubated for 12 and 24 h at 39°C. The direct addition of YC, AO and AO PREMIX was at levels of 1.8, 0.053 and 1.8 mg/ml of the in vitro fermentation solution, respectively. At the end of 12 and 24 h, in vitro dry matter disappearance (IVDMD) was determined by centrifugation at 1,200 x g for 10 minutes followed by decantation of the supernatant and drying of residue at 60°C for 72 h. An aliquot of the supernatant from the 24 h fermentation sample was acidified by placing 9 parts supernatant with 1 part 6N HCE. The mixture was then clarified by centrifugation at 25,000 x g for 20 minutes and analyzed for volatile fatty acids (VFA) by gas chromatography using 5% PNFS and 1% H_3PO_4 on Anakrour A90/100 column[6] at 110°C and ammonia nitrogen (NH_3-N) using Nessler's reagent (9).

All data were analyzed by two-way analysis of variance with treatment means being tested by least significant difference if F tests were significant.

RESULTS AND DISCUSSION

The in vitro dry matter disappearance (IVDMD) of alfalfa hay is shown in Table 1. Overall, IVDMD's were unaffected by YC and/or AO/AO PREMIX addition. It is doubtful that lack of difference was a result of metabolite accumulation, since there was no treatment by time interaction. However, for AO treatments, donor animal adaptation to slightly increase IVDMD.

Donor adaptation to both yeast and AO increased ($P<.05$) NH_3-N levels (Table 2). The yeast culture probably provided factors that stimulated proteolytic bacteria. The fungal culture treatment provided *Aspergillus oryzae*, which has been shown to be actively proteolytic (10). Further verification of increased rumen protein degradation is provided by significant increases ($P<.05$) in branch chain volatile fatty acids. Allison and Bryant (11) observed that increased rumen branched chain volatile fatty acid (VFA) concentration was a result of deamination and decarboxylation of branched chain amino acids. The acetate:propionate (Ac/Pr) ratio tended to increase in rumen fluid

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

[5] Vitaferm (Amaferm + YC + vitamins/minerals), BioZyme Enterprises, Inc., St. Joseph, MO

[6] Analabs GCM-109. Unit of Foxboro Analytical, 80 Republic Dr., North Haven, CT

TABLE 1. EFFECT OF DONOR ANIMAL ADAPTATION TO SUPPLEMENTAL YEAST AND ASPERGILLUS ORYZAE FERMENTATION EXTRACT ON IN VITRO DRY MATTER DISAPPEARANCE

Treatment	IVDMD
	--%--
Control	46.9
YC[a]	
Unadapted	48.9
Adapted	47.0
AO[b]	
Unadapted	46.6
Adapted	51.9
AO PREMIX[c]	
Unadapted	48.9
Adapted	51.3
SE[d]	2.1

[a] Yeast Culture. Diamond V. Mills, Inc., Cedar Rapids, IA.
[b] Amaferm (Aspergillus oryzae fermentation extract). BioZyme Enterprises, Inc., St. Joseph, MO.
[c] Vitaferm (Amaferm + YC + vitamins/minerals). BioZyme Enterprises, Inc., St. Joseph, MO.
[d] Standard error.

TABLE 2. EFFECT OF DONOR ADAPTATION OF SUPPLEMENTAL FUNGAL CULTURES ON IN VITRO AMMONIA NITROGEN AND VOLATILE FATTY ACID CONCENTRATION.

Item	Treatment							
	Control	YC[a]		AO[b]		AO PREMIX[c]		SE[d]
		Unadapted	Adapted	Unadapted	Adapted	Unadapted	Adapted	
NH3-N (mg/dl)	18.7^{gh}	14.2^{h}	44.6^{e}	15.7^{h}	21.6^{fg}	14.7^{h}	24.8^{f}	4.2
VFA (µmole/ml)	43.9^{g}	44.10^{g}	47.3^{fg}	40.1^{g}	59.6^{e}	41.3^{g}	56.3^{ef}	17.6
Acetate (Molar%)	68.4	66.8	67.9	65.4	70.3	66.6	66.9	2.2
Propionate	18.8^{ef}	19.4^{ef}	17.1^{fg}	19.6^{ef}	16.0^{g}	20.1^{e}	17.9^{efg}	.9
Isobutyrate	.8	.9	1.0	1.0	1.0	.8	.9	.01
Butyrate	8.7	9.0	9.5	9.9	8.8	8.9	10.0	.3
Isovalerate	1.2^{f}	1.4^{ef}	1.5^{e}	1.5^{ef}	1.3^{ef}	1.2^{f}	1.4^{ef}	.01
Valerate	2.2^{f}	2.6^{ef}	3.0^{e}	2.6^{ef}	2.6^{ef}	2.4^{ef}	3.0^{e}	.1
Acetate/ Propionate	3.6^{f}	3.4^{f}	4.0^{ef}	3.3^{f}	4.4^{e}	3.3^{f}	3.7^{f}	.1

[a] Yeast Culture. Diamond V. Mills, Inc., Cedar Rapids, IA.
[b] Amaferm (Aspergillus oryzae fermentation extract). BioZyme Enterprises, Inc., St. Joseph, MO.
[c] Vitaferm (Amaferm + YC + vitamins/minerals). BioZyme Enterprises, Inc., St. Joseph, MO.
[d] Standard error.
[e,f,g,h] Means in the same row with different superscripts differ (P<.05).

collected from the donor animal adapted to the fungal culture. Similar trends were observed in vivo (2).

Results indicate that at least a 21-day adaptation to yeast and AO fermentation extract for ruminants is necessary to properly evaluate rumen fermentation characteristics in vitro. More research is needed to confirm the validity of this hypothesis.

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