Influence of Cultures of *Aspergillus oryzae* on Rumen and Total Tract Digestibility of Dietary Components[1]

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ABSTRACT

Three trials were conducted to evaluate the effect of dried cultures of *Aspergillus oryzae* on nutrient utilization by mature Holstein cows fitted with ruminal and duodenal cannulas. In trial 1, four cows (two dry and two lactating) were used to test *Aspergillus oryzae* (3 g/d) and a control treatment at two forage amounts in a 4 × 4 Latin square. Trial 2 compared control, *A. oryzae*, and *Saccharomyces cerevisiae* using six lactating cows in a repeated 3 × 3 Latin square design. For trial 3, four lactating cows were used in a switchback design to compare control to *A. oryzae* treatment. In trials 2 and 3, diets contained 70 and 61% concentrate. A fourth in vitro trial was conducted in conjunction with trial 3 in which rumen fluid was obtained from cows adapted or unadapted to *A. oryzae*. On all trials except high forage in trial 1, *A. oryzae* increased rumen and total tract digestibility of fiber fractions. Rumen VFA and ammonia were not affected by fungal cultures. *Aspergillus oryzae* increased rate of rumen fermentation of alfalfa but not of milo or wheat straw. In vitro disappearance of DM from alfalfa, milo, and wheat straw was increased by *A. oryzae*, and previous adaptation was not required to stimulate in vitro DM digestibility. These results indicate that a primary effect of *A. oryzae* is stimulation of fiber digestion by rumen microbes.

(Key words: *Aspergillus oryzae*, rumen, digestibility)

INTRODUCTION

When added to forages prior to ensiling, cellulases from the fungus *Trichoderma viride* made plant cellulose more digestible (4). In vitro and in vivo fungal additions increased cellulose digestion of hay and rice hulls (7, 16). Van Horn et al. (26) reported a 29% increase in apparent digestion of OM in the rumen with *Aspergillus oryzae* addition to dairy cattle diets. Weidmeier et al. (28) reported increased numbers of cellulolytic bacteria when *A. oryzae*, *Saccharomyces cerevesiae*, or both were fed to rumen-fistulated steers. Increased numbers of anaerobic bacteria were reported by Dawson (8) after supplementation of hay or grain diets with *S. cerevesiae*. The objective of this study was to determine the effect of *A. oryzae* on digestibility of DM and its components in the rumen and total digestive tract of dairy cows.

MATERIALS AND METHODS

Trial 1

Four Holstein cows fitted with ruminal and duodenal cannulas were used in a Latin square design with a 2 × 2 factorial arrangement of treatments. The dietary factors were *A. oryzae* versus control without additive, and low versus high forage in the diet. Forage ratios were 2:1 and 1:2 concentrate to forage. Table 1 describes diet composition. The treatments *A. oryzae* (3 g/d + 87 g of ground sorghum grain) and control (90 g/d of sorghum) were top-dressed in equal portions to a.m. and p.m. meals.

Cows were housed in individual pens with concrete floors and had free access to water, feed, and a mineralized salt block. Feed was provided at 10% in excess of voluntary intake in two meals (0430 and 1640 h), and weighbacks were measured prior to the morning feed-

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TABLE 1 Ingredient and nutrient composition of experimental rations used in the trials

Item	Trial 1		Trial 2	Trial 3
	Low forage	High forage		
	(% DM)			
Ingredients				
Alfalfa, hay	22	63	30	25
Alfalfa, cubes	. .		.	10
Cottonseed, hulls	.		.	4
Cottonseed, whole	11	3	4	15
Cottonseed, meal		. .	6	
Soybean, meal	5	5	.	
Commercial concentrate[1]		.	.	46
Milo, flaked	57	22	50	
Molasses	3	6	9	
Mineral-vitamin mix[2]	1	1	1	
Nutrients				
DM	90	90	93 0	93 9
CP, % of DM	16 0	16 1	14 7	18 0
NDF, % of DM	25 1	32 6	24 3	43 1
ADF, % of DM	15 3	25 5	15 6	29 4
NE_l, Mcal/kg[3]	1 73	1 33	1 69	1 66

[1]Ingredients (%) rolled corn 44, malt pellets, 25, almond hulls 10 alfalfa pellet, 6, wheat mix, 5, cottonseed meal 2 5, molasses, 2 5, dicalcium phosphate, 1 7, urea 1 5, salt, 9, calcium carbonate, .6, trace minerals, 05, vitamin A (IU/kg), 22,000, vitamin D_3 (IU/kg), 8800, vitamin E (mg/kg), 11

[2]Dicalcium phosphate, .3%, salt, .3% trace minerals, .05%, vitamin A, 70,000 IU

[3]Esumated from NRC (19)

ing Milking was at 0500 and 1700 h Cows were adapted to treatments for at least 7 d after which feed intake and milk production were recorded

Treatment periods were for 21 d During the last 10 d, 24 g/d Cr_2O_3 were administered twice daily in two 12-g gelatin capsules Fecal grab samples were collected twice daily the last 5 d, and duodenal samples were collected every 4 h the last 3 d. Fecal and duodenal samples were frozen immediately and stored at –5°C until further analysis

On the last day of each period, rumen samples were collected every 2 h from 0420 to 1630 h After measuring the pH, rumen samples were acidified, frozen, and stored at –5°C. Samples of feed, orts, feces, and digesta were dried at 55°C in a forced air oven, ground in a Wiley mill to pass a 2-mm screen, and analyzed for DM, OM, and CP (3), NDF, and ADF (9), chromium by atomic absorption spectrophotometry using a multielement hollow cathode lamp at 357.9 nm with an air-acetylene flame after digestion with sulfuric acid and rediges-tion with periodic acid. Purines in digesta and in rumen bacteria were determined by the method of Zinn and Owens (29) Thawed rumen samples were centrifuged at 800 × *g* for 5 min in a refrigerated centrifuge (4°C) Supernatant was analyzed for ammonia (6) and VFA by GLC with a 80/120 Carbopack B-DA/4 Carbowax 20-m column. Part of the supernatant was further centrifuged at 16,300 × *g* for 20 min in a refrigerated centrifuge (4°C) to separate bacterial cells The ratio of purines to protein in bacterial cells and digesta was used to estimate bacterial mass in digesta

Trial 2

Six Holstein cows in mid to late lactation fitted with duodenal cannulas were used in a replicated 3 × 3 Latin square design Treatments consisted of control, *A oryzae*, and *S cerevesiae* (3 + 87 g sorghum grain daily) *Saccharomyces cerevesiae* was provided by Diamond V Mills, Inc, Cedar Rapids, IA The diet contained 70% concentrate and 30% forage

(Table 1). Treatment administration and cow management were as in trial 1, except experimental periods were 28 d (7 d of adjustment and 21 d of treatment). Because all cows were not equipped with rumen fistulas, ruminal samples were collected only from two cows per treatment and used to measure content of CP and purines in rumen bacterial cells as described for trial 1

Trial 3

Four lactating Holstein cows fitted with ruminal cannulas were used in a crossover design to study the effect of *A. oryzae* on certain rumen parameters Cows in trial 3 were housed in 4 × 12-m dirt pens equipped with lock-in stanchions for sampling Two cows also were equipped with duodenal cannulae so that rumen digestibility estimates could be made Diets contained 61% concentrate and 39% forage plus the control or *A oryzae* supplements as described (Table 1). Feeding, milk production evaluation, and chromium oxide administration were as in previous trials, and periods lasted for 28 d

To determine feed passage rates, 300 g of ytterbium-treated corn and 150 g of dysprosium-treated alfalfa were prepared by soaking according to the procedures of Goetsch and Galyean (10) and were introduced into the rumen of each cow at 0800 h on d 23 of treatment. Previous studies showed less than 3% migration of markers added in this manner (17). Fecal samples were collected at 0, 8, 12, 16, 20, 24, 32, 40, 48, 60, and 72 h after administration of marked feeds and frozen at −5°C until analyzed After thawing, samples were dried and ground as in trial 1 and analyzed for markers using the acid-digested filtrate of the ashed sample by atomic absorption spectrophotometry as indicated by Poore (21). Calculations of passage rates were according to the model of Grovum and Williams (11) for the descending part of the fecal decay curve. Composite fecal samples were analyzed for OM, CP, NDF, and ADF as described in trial 1.

For rates of digestion, triplicate nylon bags (8 × 25 cm; 50-μ pore size) containing 4 g of milo, alfalfa hay, or wheat straw were tied to a weighted chain with nylon twine and incubated in the rumen starting d 19 of the experimental period for 3, 6, 12, 18, 24, 36, 48, 72, or 96 h Incubations were started so that all bags would be removed from rumens at the same time After removal, bags were gently washed several times with cold tap water until the wash water became clear Nonincubated bags containing the feed samples were washed similarly to determine amount of soluble DM. Dry matter residue after 96 h of incubation was considered indigestible The difference between the insoluble and indigestible DM fractions was considered the potentially digestible DM (PDDM) for which the rates of digestion were calculated by regressing the natural log of percentage of remaining PDDM against time (23)

For the in vitro trial, the Tilley and Terry (25) technique was used with urea added to the buffer Triplicate sample tubes containing .5 g of either milo, alfalfa hay, or wheat straw were incubated with rumen contents from control cows with or without added *A oryzae* (25 mg/L) or with rumen contents from cows fed *A oryzae* Microbial incubation was 48 h and the acid-pepsin incubation was for 24 h, both were incubated at 40°C. Samples were filtered in preweighed Gooch crucibles with glass wool

Results from the three trials were analyzed using analysis of variance and, when appropriate, orthogonal contrast procedures described by Steel and Torrie (24) Results of trial 1 were analyzed as a 2 × 2 factorial arranged in a 4 × 4 Latin square Trial 2 data were analyzed as a replicated 3 × 3 Latin square and trial 3 (in vivo) was a simple crossover design with 2 treatments. Animal passage rates, PDDM, and digestion rates of PDDM for the various feeds as affected by *A oryzae* treatment were tested by analysis of variance using a crossover design (24). In vitro digestibilities of DM (25) of the different feeds were tested by orthogonal comparisons for effects of *A oryzae* added to the diet or directly to the incubation media

RESULTS AND DISCUSSION

Cows were fed for ad libitum intake in all trials and differed greatly in DM intakes (Table 2) both within and between trials. Two of four cows in trial 1 were dry and consumed about 1 0% of their body weight as DM; the lactating cows consumed 2 5%. The lactating cows in trial 2 consumed about 2.5% of body weight as DM, and those in trial 3 consumed 3.5% Trials were not designed to evaluate effect of addi-

TABLE 2 Effect of *Aspergillus oryzae* (AO) on feed intake and total tract digestibility of nutrients Summary of three trials [1]

Dietary treatment	DM Intake	DM	OM	CP	NDF	ADF
	(kg/d)	Digestion coefficient (%)				
Trial 1[2]						
L, C	10 9	72 6	74 6	67 9	50 2	34 1
L, AO	12 6	72 4	74 2	69 9	54 8	38 5
H, C	11 5	67 8	74 7	74 4	66 8	52 1
H, AO	9 4	71 2	72 9	73 5	67 3	51 9
SEM	3 3	4 3	1 5	5 6	1 3	1 1
Trial 2[3]						
C	16 1	66 1	67 1	69 5	47 5	18 0
SC	16 7	66 8	69 5	72 3	51 3	32 2
AO	17 0	66 7	69 2	71 8	50 2	32 8
SEM	7	1 3	9	.8	1 6	5 6
Trial 3						
C	23 6[a]	63 8[a]	66 0	74 1	39 6[a]	21 3
AO	26 2[b]	67 9[b]	69 3	76 1	47 0[b]	30 0[b]

[a,b]Means in the same column within a trial with different superscripts differ ($P<.05$)

[1]SC = *Saccharomyces cerevisiae*, C = control, L = low forage H = high forage

[2]Concentrate effect significant ($P<.01$) for ADF and NDF, also, interaction effect significant ($P<.05$) for ADF and NDF

[3]Orthogonal contrasts C vs SC and AO significant ($P<.05$) for CP, NDF, and ADF digestion coefficients, SC vs AO did not differ ($P>.25$)

tives on milk production because of stressful management during sampling and the short length of experimental periods However, in trial 2, milk production was 17.8, 16.8, and 15 8 kg/d for the control, *S cerevesiae*, and *A oryzae* treatments, respecuvely, while in trial 3, control cows produced 28 4 kg/d and *A oryzae* cows 29 3

Total tract digestibilines for the three trials are summarized in Table 2 Differences within trials in DM digestibility were small and higher ($P<.05$) for *A oryzae* only for trial 3 Trends were similar for digestibility of OM and CP Greatest differences were observed for digestibilities of NDF and ADF in all trials In trial 1, ADF and NDF digestion was higher ($P<.01$) for the high than low forage diet, and *A. oryzae* had no effect ($P>.10$) on high forage Lower fiber digestion might be expected in low forage diets, because a major proportion of that fiber was from cottonseed, which is lower in digestibility than alfalfa fiber

On the low forage diet in trial 1, *A oryzae* partially restored fiber digestibility with increases of 9% ($P<.05$) for NDF and 13% ($P<.05$) for ADF. These results support those of the trial of Huber and Higginbotham (15) where an *A oryzae* supplement increased milk production of cows fed normal forage but not high forage diets.

In trial 2, orthogonal contrasts with control treatments showed that *S cerevesiae* and *A oryzae* fungal treatments increased ($P<.05$) CP, NDF, and ADF digestibilities despite relatively small differences Weidmeier et al (28) observed increases above controls in digestibility of DM, CP, and hemicellulose with *S cerevesiae* and *A oryzae*. A later report from Utah (5) showed no response to *S cerevesiae* Harrison et al (13) indicated a depression in NDF and ADF digestibilities with a commercial yeast extract Other fungal additives have increased fiber digestibility in steers (7) and cows (26)

Table 3 summarizes rumen digestibilities of the three trials as calculated from the composition and flow of digesta to the duodenum Previous work (27) showed that chromic oxide was a suitable marker using the sampling schedule employed in this study, but variation was large In trial 1, no differences in DM, NDF, or ADF digestibility in the rumen were detected ($P>.10$), but *A oryzae* numerically increased ADF digestibilities by 20%

In trial 2, *A. oryzae* increased ($P<.05$) NDF digestibility and numerically increased that of ADF. Trial 3 showed a 36% advantage in NDF digestibility and 41% in ADF for *A oryzae*

TABLE 3 Effect of *Aspergillus oryzae* (AO) on rumen digestibility of DM, NDF, and ADF, truly fermentable organic matter (TFOM), and efficiency of microbial yields (Yom, g microbial protein/g TFOM) Summary of three trials [1]

Dietary treatment	DM	NDF	ADF	TFOM	Yom
	(%)				
Trial 1[2]					
L, C	48.5	43 1	30 4	64 3	183
L, AO	45 1	44 6	36 2	67.8	206
H, C	63 9	66 2	47.5	79 6	.134
H, AO	62 5	67 3	45 7	83 4	195
SEM	8 1	9 3	9 7	7.8	010
Trial 2[3]					
C	26 1	40 9	16 4	58 0	192
SC	37 7	53 1	29 3	54.7	197
AO	42 8	56 6	30 8	58 9	188
SEM	5 7	4 2	11 3	8 9	020
Trial 3					
C	25 2	27 7[a]	19 5[a]	53 0	.171
AO	26 9	37.6[b]	27 4[b]	53 7	181
SEM	2 1	3 4	1 6	3 5	010

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

[1]C = control, L = low forage, H = high forage, SC = *S cerevisiae*

[2]For Yom, C < AO ($P<05$)

[3]Orthogonal contrasts C vs SC and AO significant ($P<05$), SC vs AO did not differ ($P>25$)

treatment and strongly supports *A oryzae* effects observed on high concentrate diets in trials 1 and 2. Fermentation of OM after correction for microbial mass was not affected by treatments in any of the trials In trial 1, an increase in efficiency of microbial yield was observed ($P<05$) for the *A oryzae* treatment at both forage intakes Weidmeier et al (28) showed an increase in microbial numbers with fungal cultures In trials 2 and 3, however, no significant effect in microbial efficiency was observed

Analyses of the rumen contents from trial 1 are in Table 4. Neither pH, total VFA, nor individual VFA were affected by *A oryzae* There was a trend toward lower pH, higher total VFA, and a lower acetate to propionate ratio (2 64 vs 3.01) on high concentrate diets, but numbers may have been insufficient to detect real differences. These results are in agreement with others for *A oryzae* (33) but different from those observed with yeast supplementation (17)

Rumen ammonia was relatively high for all treatments, but variation was too high to detect significant effects Van Horn et al (26) and Harris et al (12) reported reductions in rumen ammonia with fungal additives. However,

TABLE 4 Effect of *Aspergillus oryzae* treatment on rumen fermentation (trial 1) [1]

	Low forage		High forage		
Item	C	AO	C	AO	SEM
Rumen pH	6 1	6 3	6 7	6.6	2
Total VFA, *mM*	84 2	90.8	72 7	78 1	7 6
Acetate, %	62.0	60 9	66 8	64 2	4 3
Propionate, %	23 6	22 8	21 4	22 1	1 6
Butyrate, %	9 6	12 0	10 1	11 3	9
Rumen ammonia, mg/dl	20 6	35 1	22 6	25 4	6.8

[1]C = Control, AO = *A oryzae* None of the differences was significant (two cows/treatment)

TABLE 5 Influence of *Aspergillus oryzae* (AO) addition on ruminal passage rate of corn and alfalfa hay (trial 3)[1]

	C	AO	SEM
	——— (%h^{-1}) ———		
Corn	9 1	10 2	1 89
Alfalfa	4 2	3 6	0 22

[1]Markers ytterbium for corn, dysprosium for alfalfa C = control (four cows/treatment)

Arambel et al (2) reported that *A oryzae* increased rumen ammonia and branched-chain VFA concentrations in vitro They suggested that *A oryzae* promoted greater protein degradation and that branched-chain VFA are products of protein degradation Boing (5) observed that *A oryzae* has proteolytic activity

In trial 3, dynamics of the rumen digestion were studied by determining the effect of *A oryzae* treatment on rates of passage and fermentation No differences in rates of passage of corn or alfalfa were observed (Table 5), supporting data of Weidmeier et al. (28) for *A oryzae* and *S cerevesiae*. Rates of corn passage were more than twice as fast as and are consistent with other data with lactating dairy cows (20, 22).

Treatment had no effect on PDDM (Table 6), which appears to be inherent in the specific feedstuff (18) Digestion rates of PDDM were similar for control and *A oryzae* treatments for milo and wheat straw ($P > 10$); but *A oryzae* increased ($P < 05$) the rate of PDDM digestion for alfalfa hay, reflecting a more active metabolism of microbes fermenting alfalfa and was probably associated with the increased rumen digestibilities of NDF and ADF (28). Weidmeier et al (28) showed increased numbers of cellulolytic bacteria with *A oryzae* addition, and Harrison et al (13) and Dawson (8) reported similar findings with yeast. Greater numbers of fiber-digesting organisms may explain the increased rate of alfalfa digestion

Effects of *A oryzae* may not be limited to increased bacterial numbers Microscopic observations in our laboratory (22) revealed numerous colonies of fungi similar in appearance to normal rumen fungi were attached to feed particles collected at the duodenum of cows fed *A oryzae*, but such fungi were essentially absent in duodenal contents of control cows Rumen fungi have a complete array of enzymes to digest fibrous materials (18)

Table 7 presents the results from in vitro studies The control treatment had lower ($P < .01$) extent of digestion for milo, alfalfa hay, and wheat straw DM than treatments where *A oryzae* was added to rumen inocula from control cows or where rumen inocula was taken from cows fed *A oryzae* The magnitude of the response to *A oryzae* was similar in both conditions However, the response was greater with forages than with milo grain Arambel et al (2) reported that in order to reproduce the *A*

TABLE 6 Potentially digestible DM (PDDM) and rates of digestion for milo, alfalfa hay, and wheat straw DM determined in situ[1]

Item	Milo	Alfalfa hay	Wheat straw
Soluble fraction %	57 7	61 3	51 9
PDDM, %			
C	91 6	58 2	44 1
AO	92 1	59 2	46 2
SEM	8	.4	1 8
Digestion rate of PDDM, %h^{-1}			
C	6 62	7 79[a]	5 65
AO	6 66	8 80[b]	4 88
SEM	.11	.27	74

[a,b]Means within the same column with different superscripts are different ($P < .05$)

[1]Four cows per treatment (trial 3). C = control, AO = *Aspergillus oryzae*

TABLE 7 Effect of *Aspergillus oryzae* (AO) treatment on in vitro digestibility of DM of different feedstuffs.[1]

	Treatment[2]		
Feedstuff	C	C + AO	AO
	——— (%) ———		
Milo[3]	84 6	91 1	91 0
Alfalfa hay[3]	50 4	61 8	59 4
Wheat straw[3]	49 6	65 8	66 8

[1]Incubation in buffered medium for 48 h followed by 24 h of pepsin digestion (30)

[2]C = control

[3]Orthogonal comparison of C vs C + AO and AO was significant ($P < .01$), but C + AO vs. AO was not ($P > 50$). SE = 1.51

oryzae effect in vitro, the rumen inoculum had to be obtained from adapted animals However, other authors have observed a positive response by direct addition of fungal cultures to in vitro systems (4, 7, 16). Because *A oryzae* effects can be reproduced in vitro, study of the mechanism of increased digestion becomes more plausible These results and those discussed previously suggest that *A oryzae* affects digestion differently in the different feedstuffs and that immediate response might be obtained by addition of *A. oryzae* to ruminant diets In conclusion, addition of the *A oryzae* or *S cerevesiae* cultures to ruminant diets generally increased in vivo digestibility of fiber in the rumen and the total digestive tract These increases were supported by in situ increases in rate of DM disappearance of alfalfa hay and in vitro increases in DM digestibility of grain and forages These results help explain the higher milk yields resulting from feeding *A oryzae* to lactating cows

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