Influence of *Aspergillus oryzae* Fermentation Extract on Forage Intake, Site of Digestion, In Situ Degradability, and Duodenal Amino Acid Flow in Steers Grazing Cool-Season Pasture[1,2]

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ABSTRACT: Ten ruminally and duodenally cannulated (326 ± 28 kg) and four esophageally fistulated (394 ± 23 kg) steers grazing cool-season pasture throughout the growing season were used to evaluate the influence of *Aspergillus oryzae* fermentation extract (AO) supplementation on intake, forage nutrient utilization, and duodenal amino acid flow. Steers grazed a predominately smooth brome (*Bromus inermis* L.) pasture, and measurements were taken in three periods (June, July, and August). Steers were dosed daily at 0700 via the ruminal cannula with AO (2 g of AO per steer daily; DM basis) or not supplemented with AO Each period consisted of 18 d for adaption to AO and 7 d for collection Forage N was greater, and ADF was lower ($P < 10$), in June than in July and August Ruminal pH, ammonia, total VFA concentration, and VFA proportions were not affected ($P > .10$) by AO supplementation. In vitro DM digestibility (percentage) and forage OM intake (grams/kilogram of BW) were greater ($P < .10$) for steers supplemented with AO. Ruminal and total tract NDF and ADF digestibilities were lower in June and greater during July ($P < .10$) in steers supplemented with AO Total tract percentage of N disappearance was lesser during June and greater ($P < .10$) during July for steers receiving AO Total, essential, and nonessential amino acid flows were increased ($P < .10$) by both AO supplementation and advancing season. In situ rate of ruminal CP degradation was not affected ($P < .10$) by treatment during any period. The slowly degraded forage N fraction, as determined by an in situ technique, was increased ($P < .10$) during July and August in steers that received AO relative to unsupplemented steers Our data indicate that supplementation with AO of steers grazing cool-season pasture altered intake and digestion measurements, but that responses seemed to vary with season

Key Words· Steers, Grazing, Intake, Digestion, *Aspergillus oryzae*

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Introduction

Aspergillus oryzae fermentation extract (Amaferm, AO, Biozyme, St. Joseph, MO) has been the subject of considerable research with dairy cows (Harris et al., 1983, Kellems et al, 1990). Gomez-Alarcon et al. (1990) reported increased intake and digestibility of NDF and ADF in response to AO supplementation with Holstein cows fed a medium-concentrate diet These results are supported by digestibility data from lambs fed a 10% corn diet (Judkins and Stobart, 1988). In contrast, others have shown no change in fiber digestion in response to AO (Firkins et al , 1990, Oellermann et al., 1990) Using sheep, Fondevila et al. (1990) suggested that rate, but not extent, of fiber degradation may be increased by AO supplementation Although data with sheep and dairy cattle indicate that AO may have a positive effect on fiber digestion, little research has been done with beef cattle. Moreover, information concerning duodenal amino acid flow in beef cattle grazing pasture throughout the growing season is lacking and the effect of fungal extracts on amino acids reaching the small intestine has not been evaluated Therefore, our objectives were to evaluate the influence of AO on intake, fermentation, rate of passage, in situ NDF and CP degradation, site of digestion, and duodenal amino acid flow in steers grazing cool-season pasture throughout the growing season.

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Materials and Methods

Experimental Periods

Ten ruminally and duodenally cannulated (326 ± 28 kg) and four esophageally fistulated Hereford steers (394 ± 23 kg) freely grazed a 10 5-ha, cool-season pasture (*Bromus inermis*) from mid-May to the end of August 1989. Cannulation techniques provided for humane treatment of animals, adhered to locally approved procedures, and were similar to those described by Streeter et al. (1990) Duodenal cannulas were placed cranial to the biliary duct, and postoperative care consisted of both topical and intramuscular application of antibiotics as outlined by Caton et al. (1987) Steers were assigned randomly to control or supplementation groups. Supplementation consisted of 2 g of AO steer^{-1} d^{-1} delivered through the ruminal cannula via a gelatin capsule

Three 24-d experimental periods were conducted throughout the growing season (June, July, and August). The first 18 d of each period served as an adaptation phase to AO (AO was not dosed continually between periods) and the last 6 d were used for sample collection On d 13 of each period, esophageal masticate samples were collected. Esophageal forage was collected three to five times on d 13 (from sunrise until early afternoon). At each collection time, steers were fitted with screen-bottomed collection bags, esophageal cannulas were removed, and steers were allowed to graze freely for approximately 30 min. Esophageal masticate was composited within steer across sampling times and subsampled for chemical composition. The remaining masticate was then composited within treatment across animals and divided into portions for estimation of in situ degradability and particulate passage rate.

On d 18 at 0700 of each collection period (June 19, July 24, and August 21) 200 mL of Co-EDTA (746, 813, and 835 mg of Co for Periods 1, 2, and 3, respectively; Uden et al , 1980) were administered intraruminally as a fluid phase marker. Ruminal samples were collected at 0, 3, 6, 9, 12, 15, and 24 h relative to dosing of Co-EDTA (0-h ruminal sample taken before Co was dosed). Ruminal samples were analyzed immediately for pH with a portable pH meter (Model SA230, Orion, Cambridge, MA) fitted with combination electrode. Ruminal samples were strained through four layers of cheesecloth, and the fluid portion was acidified with 7.2 *N* H_2SO_4 at the rate of 1 mL of acid/100 mL of strained ruminal fluid. Samples were transported to the laboratory and stored frozen (−10°C).

On d 19 at 0700, Yb-labeled hay (Teeter et al., 1984) was dosed into the rumen and fecal samples were taken at 0, 4, 8, 12, 16, 20, 26, 30, 34, 42, 48, 52, 60, 68, 78, 86, 94, and 106 h after dosing. The dose of Yb varied with period and ranged from 62 to 4.12 g Fecal samples were stored frozen (−10°C) Also on d 19, sampling for site of digestion measurements was initiated. Chromic oxide was used as a digesta flow marker and dosed twice daily (8 g at 0800 and 1900 in gelatin capsules; 16 g of total chromic oxide daily) for 5 d before and throughout the collection period. Duodenal (approximately 200 mL) and fecal samples were taken at 8-h intervals in a system that allowed for every other hour during a 24-h period to be sampled. As a result, samples were collected at 0900 and 1700 on d 19, 0100, 1100, and 1900 on d 20, 0300, 1300, and 2100 on d 21; and 0500 on d 22 Duodenal and fecal samples were frozen immediately (−10°C) and stored. Cannula loss and flow problems resulted in data from seven steers (four AO, three control) during Period 1 and six steers (three per treatment) during Periods 2 and 3 being used for statistical analyses

In situ degradability measurements began on d 20 Dacron bags (Ankom Products, Fairport, NY; 10 cm × 20 cm; 53 ± 10 μm pore size) containing approximately 5 g of dried (50°C, forced-air oven) esophageal masticate were introduced into the rumen at 1900. Three bags containing forage and one blank were sealed with a #8 rubber stopper and two #19 rubber bands. Dacron bags from various incubation times were held in a large mesh bag (38 cm × 46 cm) fitted with a nylon zipper. Incubation times were 0, 4, 8, 12, 16, 24, 36, 48, and 72 h. The 72-h bag was introduced into the rumen at 1900 on d 20 as described above. All bags were removed from the rumen at 1900 on d 23 of each period. Bags were washed until rinse water was clear and then dried at 50°C, desiccated, and weighed Residue samples were obtained from the bags and stored until they were analyzed. On d 22 at 0700, ruminal fluid was collected from each steer and used as inoculum for IVDMD (Tilley and Terry, 1963). Three tubes containing dried and ground (2-mm screen) esophageal masticate from individual treatment groups were inoculated with fluid from each respective steer. On d 23, just after removing the Dacron bags, 1,500 mL of ruminal fluid (strained through four layers of cheesecloth) were collected from each steer, preserved with .15 *M* NaCl in 37% (vol/vol) formaldehyde, and stored until bacteria were separated.

Laboratory Analyses

Esophageal masticate and intestinal and fecal samples were analyzed for DM, ash, ADF, and N by standard procedures (AOAC, 1990). Analysis for NDF was conducted by the method of Robertson and Van Soest (1981). Dacron bag residues were analyzed for DM, N, and NDF as outlined above. Esophageal masticate samples were analyzed for soluble N by the .15 *M* NaCl procedure of Waldo and Goering (1979). Acid detergent insoluble N was determined on masticate samples by conducting N analysis on ADF residues. Duodenal, fecal, and dietary samples were analyzed for indigestible ADF (Krysl et al., 1988)

Bacterial cells were isolated from 1,500 mL of ruminal fluid by differential centrifugation as outlined by Merchen and Satter (1983). Freeze-dried, isolated bacterial cells (IBC), duodenal samples, and Dacron bag residues were analyzed for purine concentration by the procedure of Zinn and Owens (1986). Dry matter, ash, and N were conducted on IBC by procedures noted above

Fecal samples were prepared for Cr analysis as outlined by Williams et al (1962) Chromium concentration was determined by atomic absorption spectroscopy (air-plus-acetylene flame)

Ruminal samples were thawed at room temperature and centrifuged at 10,000 × *g* for 10 min, and the fluid portion was analyzed for Co by atomic absorption spectroscopy (air-plus-acetylene flame). Supernatant fractions also were analyzed for ammonia concentrations by the colorimetric procedure of Broderick and Kang (1980). Ruminal fluid portions were analyzed by gas chromatography (Packed Column, 140°C, N gas carrier, Shimadzu Scientific Instruments, Columbia, MD) for VFA (Goetsch and Galyean, 1983) using 2-ethylbutyric acid as an internal standard.

Fecal samples were extracted for Yb analysis with .05 *M* EDTA and analyzed for Yb by atomic absorption spectroscopy (nitrous oxide-plus-acetylene flame) Ytterbium concentrations were then fitted to a one-compartment, nonlinear model (Krysl et al, 1988).

Duodenal samples were hydrolyzed with 6 *N* HCl before amino acid analysis (Blackburn, 1978). Hydrolyzates were subjected to precolumn derivatization with *o*-phthaldialdehyde on a microbore column via HPLC (Hewlett Packard 1090 LC, Palo Alto, CA).

Calculations

Fecal output was calculated by dividing Cr dose (grams) by Cr concentration in the feces (grams/gram of feces) Dry matter forage intake (grams) was determined by dividing fecal output by forage in vitro DM indigestibility Organic matter intake was calculated by multiplying DMI by dietary OM factor Intake (grams) was divided by average individual animal BW (kilograms, steers were weighed unshrunk at the beginning and end of each period) for each period, and forage intake was expressed as grams/kilogram of BW

Ruminal fluid passage rate data were calculated by regressing the natural logarithm of ruminal Co concentration on time, the absolute value of the slope was used as rate of passage (percentage/hour) Fluid volume was estimated by extrapolating to time 0 and dividing by Co dose. Fluid flow was determined by multiplying dilution rate by volume, and turnover was calculated as the inverse of the slope. Particulate digesta passage rate (percentage/hour) was estimated by multiplying k_1 by .59635 (Krysl et al, 1988). Total mean retention time (TMRT) was determined by summing tau and the quantity of $2/k_1$

Ruminal and postruminal digestibilities were estimated using the marker ratio technique (Merchen, 1988). Duodenal OM flow was calculated by multiplying apparent ruminal indigestibility of OM by intake, microbial N flows were determined by using purines as a microbial marker (Zinn and Owens, 1986) Microbial protein synthesis was calculated by multiplying duodenal microbial N flow by 6 25. Microbial efficiency was estimated by dividing duodenal microbial N flow (grams) by the quantity (kilograms) of OM truly fermented.

In situ rate of NDF digestion and lag time were estimated by the nonlinear model of Mertens and Loften (1980). Crude protein fractions and rate of in situ disappearance were determined with the model of Ørskov and McDonald (1979). This model calculates an instantaneously degraded CP fraction (Fraction A) and a slowly degraded CP fraction (Fraction B). Rates derived from this model are calculated on the slowly degraded fraction (NRC, 1985). Both the NDF and CP rates were calculated using nonlinear regression procedures (Marquardt method) of SAS (1985). In situ CP residues were corrected for microbial contamination by using purines as a microbial marker (Messman et al., 1992).

Statistical Analyses

Data were analyzed as a split-plot design by analyses of variance (Cochran and Cox, 1957) The statistical model included effects for treatment, steer within treatment, period, and period × treatment. Steer within treatment was used as the error term to test treatment effects. When period × treatment interactions were noted ($P < .10$), treatment effects were analyzed within period. Ruminal VFA, ammonia, and pH data were analyzed as a split-split-plot (Gill and Hafs, 1971). The model included effects for treatment, steer within treatment, sampling time, sampling period, and all interactions. Steer within treatment was used as the error term for treatment. When treatment × sampling period × sampling time interactions were not evident ($P > .10$), data were averaged across sampling period and time The GLM procedure of SAS (1985) was used for all statistical computations.

Results and Discussion

Dietary chemical composition (Table 1) was largely unaffected ($P > .10$) by treatment. In general, these data agree with those of Olson et al. (1992) and suggest that supplementation of grazing steers with fermentation products has little influence on chemical composition of forage consumed Esophageal masticate forage collected during August contained less ($P < .10$) N, soluble N, insoluble N, and ADIN and more NDF and ADF than masticate collected during June.

Ruminal VFA, pH, and ammonia concentrations were not affected ($P > .10$) by AO supplementation These data agree with other research (Fondevila et

Table 1 Dietary chemical composition as influenced by *Aspergillus oryzae* fermentation extract (AO) in steers grazing cool-season pasture throughout the growing season

Item	Treatment			Period			
	Control	AO	SE	June	July	August	SE
No of observations	6	6	—	4	4	4	—
OM, % of DM	86 1	86 8	01	87 8	86 5	85 0	01
	%, OM basis						
NDF	72 8	69 8	1 17	67 7^{a}	70 4^{ab}	75 9^{b}	1 57
ADF	43 7	42 6	54	37 4^{a}	43 1^{b}	49 1^{c}	1 13
N	2 18	2 24	16	2 77^{a}	1 82^{b}	2 05^{b}	16
Soluble N	69	75	04	97^{a}	55^{b}	63^{b}	07
Insoluble N	1 49	1 50	15	1 80^{a}	1 27^{b}	1.41^{b}	11
ADIN	23	23	02	15^{a}	23^{b}	31^{c}	02

[a,b,c]Row means within treatment and period main effects that do not have common superscripts differ (P < 10)

al , 1990; Gomez-Alarcon et al , 1990, Oellermann et al , 1990; Newbold et al., 1991) and demonstrate that AO has little influence on these ruminal measurements. A transient increase in acetate in response to AO supplementation was noted by Firkins et al. (1990), but the reason for this response to AO was unclear. Oellermann et al. (1990) reported an inconsistent decrease in ruminal pH when AO was fed to nonpregnant, nonlactating Holstein cows consuming a 25% concentrate diet Elimination by AO of the decrease in pH associated with grain supplementation has been noted in vitro (Frumholtz et al , 1989). In addition, feeding AO in conjunction with grain supplements lessened the decrease in ruminal pH at 1 and 24 h after supplementation (Westvig et al., 1991). However, most reports in the literature agree with our data and suggest that ruminal pH is not affected greatly by supplemental AO (Firkins et al , 1990; Fondevila et al., 1990, Gomez-Alarcon et al., 1990, Newbold et al., 1991).

Forage intake (grams/kilograms of BW) was increased (P < .10) by AO supplementation (Table 2). Other research (Adams et al , 1981) with steers fed medium-concentrate diets also demonstrated intake responses from feeding yeast products. Responses in our study were predominantly a result of the consistent increase (P < .10) in IVDMD in steers supplemented with AO. However, although not different (P > .10), fecal output was 7% greater in steers supplemented with AO than in control steers In addition, using OM digestibilities calculated from IADF data (Table 3), averaged across period within treatment, (66.1 vs 67 4% for control and AO-supplemented steers, respectively, Table 3) and fecal

Table 2 Influence of *Aspergillus oryzae* fermentation extract (AO) on ruminal fermentation, in vitro digestibility, and forage intake in steers grazing cool-season pasture throughout the growing season[a]

Item	Treatment			Period			
	Control	AO	SE	June	July	August	SE
Fermentation							
pH	6 47	6 48	03	6 41	6 53	6 48	05
Total VFA, m*M*	94 3	92.3	2 50	86 1^{b}	88 9^{b}	104 8^{c}	2 95
Acetate, mol/100 mol	68 0	67 4	41	66 0^{b}	70 1^{c}	67 0^{d}	39
Propionate, mol/100 mol	18 0	18 5	35	19 4^{b}	17 9^{c}	17 5^{d}	.28
Butyrate, mol/100 mol	9 8	9 8	24	10 1^{b}	8 9^{c}	10 4^{b}	35
Ammonia, mg/dL	19 7	18 6	93	13 6^{b}	8 8^{c}	35 1^{d}	1 19
Intake/digestion							
No of observations	13	15	—	10	9	9	—
IVDMD, %	59 3^{b}	65 2^{c}	1 09	67 5^{b}	60 1^{c}	59 2^{c}	1 41
Fecal output, g of OM	2,934	3,140	129 9	2,402^{a}	3,355^{c}	3,354^{c}	132 9
Intake, g of OM	7,742^{b}	9,772^{c}	464 1	7,960	9,095	9,216	537 4
Intake, g of OM/kg BW	25 4^{a}	31 5^{c}	.8	28 9	28 2	28 2	18

[a]Number of observations for ruminal pH, VFA, acetate, propionate, and butyrate were 84, 105, 63, 63, and 63 for control, AO, June, July, and August, respectively

[b,c,d]Row means within treatment and period main effects that do not have common superscripts differ (P < 10)

Table 3 Influence of *Aspergillus oryzae* fermentation extract (AO) on site and extent of organic mat r (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) digestion in s eers grazing cool-season pasture throughout the growing season[a]

Item	June			July			August		
	Control	AO	SE	Control	AO	SE	Control	AO	SE
No of observations	4	3	—	3	3	—	3	3	—
OM digestib lity %									
Total[b]	83 6[c]	78 7[d]	33	59 1[c]	67 3[d]	58	57 1	56 1	1 08
Ruminal[b]	72 3[c]	65 2[d]	1 31	39 1[c]	50 1[d]	72	31 8	29 2	1 92
Postruminal	11 3	13 4	96	19 9[c]	17 0[d]	87	25 3	26 3	1 49
NDF digestibility, %									
Total[b]	82 3[c]	76 4[d]	49	57 6[c]	61 4[d]	74	59 5[c]	55 9[d]	58
Ruminal[b]	81 6[c]	76 2[d]	94	54 7[c]	62 4[d]	1 06	52 5	51 6	1 90
Postruminal	7	-3	68	2 6[c]	-1 2[d]	66	7 1	4 3	1 92
ADF digestibility %									
Total[b]	77 2[c]	71 4[d]	42	47 7[c]	55 4[d]	36	51 3	50 8	63
Ruminal[b]	76 7[c]	69 7[d]	.80	43 5[c]	52 4[d]	2 13	50 9	47 1	1 37
Postruminal	4	1 6	1 00	4 4	3 1	1 71	4	3 6	80
Postruminal digestibility, % of entering									
OM[b]	40 5	38 3	1 60	32 7	34 1	1 39	37 1	37 0	1 53
NDF	3 2	-1 4	3 50	5 6	-3 2	1 51	14 7	8 6	3 66
ADF	1 4	5 0	3 49	7 4	6 4	2 7	8	6 8	1 49

[a]Calculated using indigestible ADF
[b]Treatment × sampling period interactions were present ($P < 10$)
[c,d]Means within a period and row that do not have common superscripts differ ($P < 10$)

output from Table 2 to calculate intake, results in a 9 7% increase in OM intake (8,784 vs 9,632 g) in steers fed AO. Gomez-Alarcon et al. (1990) reported increased in vitro digestibility of alfalfa hay when inoculum that originated from dairy cows fed AO was compared with inoculum from control cows Other reports concerning effects of AO on in vitro digestion have not shown changes in DM disappearance (Frumholtz et al., 1989; Newbold et al., 1991) These differences may be explained by different in vitro techniques and whether inoculum sources were adapted to AO.

Analyses of site of digestion and microbial efficiency data resulted in treatment × sampling period interac-

Table 4 Influence of *Aspergillus oryzae* fermentation extract (AO) on site and extent of N digestion, duodenal N flow, and microbial protein synthesis in steers grazing cool-season pasture throughout the growing season[a]

Item	June			July			August		
	Control	AO	SE	Control	AO	SE	Control	AO	SE
No of observations	4	3	—	3	3	—	3	3	—
Intake, g/d	201 5[c]	240 6[d]	12 37	148 7[c]	188 2[d]	13 30	165 8	219 1	21 60
Duodenal flow, g/d	108 2[c]	157 6[d]	9 60	188 6	207 0	23 47	288 1	348 2	38 94
Microbial, g/d	30 3[c]	52 8[d]	3 37	60 7	72 3	10 71	81 6	116 0	16 30
Nonmicrobial, g/d	77 9[c]	104 8[d]	6 69	127 8	134 7	13 50	206 5	232 2	22 73
Digestibility, %									
Total[b]	84 6[c]	81 0[d]	40	52 0[c]	64 5[d]	64	38 6	40 4	2 29
Ruminal[b]	45 2[c]	33 3[d]	2 69	-33 9[c]	-9 4[d]	2 21	-88 0	-83 7	7 65
Postruminal	39 4[c]	47 7[d]	2 55	86 2[c]	74 2[d]	2 59	128 3	122 6	8 80
Postruminal, % of entering	71 8	71 3	1 24	64 3[c]	67 7[d]	1 09	68 1	66 6	2 00
Microbial protein synthesis, g/d	189 3[c]	329 9[d]	21 07	379 6	451 7	66 95	509 9	725 0	101 86
Microbial efficiency, g of microbial N/kg of OM truly fermented[b]	5 9[c]	9 5[d]	.34	19 1[c]	13.8[d]	1 10	32 7	40 4	3 66

[a]Digestibilities calculated using indigestible ADF
[b]Treatment × sampling period interactions were present ($P < .10$)
[c,d]Means within a period and row that do not have common superscripts differ ($P < 10$)

Table 5. Influence of *Aspergillus oryzae* fermentation extract (AO) on ruminal digesta kinetics in steers grazing cool-season pasture throughout the growing season

	Treatment			Period			
Item	Control	AO	SE	June	July	August	SE
No. of observations	13	15	—	10	9	9	—
Fluid dilution rate, %/h	13.7	13.1	.87	16.5[a]	11.0[b]	12.6[b]	.72
Fluid volume, L	43.2	54.3	7.24	37.8[a]	54.5[b]	54.2[b]	4.44
Fluid flow rate, L/h	5.3	6.8	.92	6.0	5.6	6.5	4.44
Fluid turnover time, h	8.2	8.1	.55	6.2	9.9	8.3	5.18
Particulate flow, %/h	3.9	3.5	.79	4.6[a]	3.2[b]	3.3[b]	.21
Total mean retention time, h	46.4	48.7	2.59	37.5[a]	53.4[b]	51.7[b]	2.00
First appearance of marker, h	13.7	13.7	.93	11.2[a]	14.5[b]	15.4[b]	1.20

[a,b]Row means within treatment and period that do not have common superscripts differ ($P < .10$)

tions ($P < .10$). Specific interactions are noted in Tables 3 and 4. Total tract digestion (as calculated with IADF, Table 3) of OM, NDF, and ADF were decreased in June ($P < .10$) and increased ($P < .10$) in July in steers supplemented with AO. Moreover, ruminal digestion (percentage of intake) followed the same pattern as total tract digestion. Post ruminal OM and NDF digestion were decreased ($P < .10$) by feeding AO during July. No differences ($P > .10$) were noted in postruminal digestion when expressed as a percentage of amounts entering the small intestine. Gomez-Alarcon et al. (1990) reported increased digestibility of DM and NDF by dairy cows in response to AO. Judkins and Stobart (1988) reported improved cell wall digestion in response to AO in sheep fed a forage corn diet. Conversely, Firkins et al. (1990) reported that AO did not affect digestibility by Holstein heifers fed high-forage diets.

Nitrogen intake was greater (Table 4; $P < .10$) during June and July by steers supplemented with AO than by control steers. Microbial and non-microbial N flowing to the duodenum were greater ($P < .10$) in June and unaffected in July and August by AO. Moreover, ruminal N digestion was decreased ($P < .10$) and postruminal N digestion increased ($P < .10$) during June in response to AO. In July, N digestion was greater ($P < .10$) ruminally and lower ($P < .10$) postruminally in steers supplemented with AO than in control steers. Microbial protein synthesis was greater ($P < .10$) in steers fed AO than in control steers during June, no other differences in microbial protein synthesis were noted. Microbial efficiency (Table 4) was increased ($P < .10$) in June and decreased ($P < .10$) in July in AO relative to control steers. Firkins et al. (1990) reported no differences in N digestion and microbial efficiency in heifers consuming high-forage diets with or without AO supplementation. Reasons for varying responses by period in microbial efficiency in our study are unclear.

Fluid and particulate digesta kinetics were unaffected by treatment (Table 5). Fluid dilution

Table 6. Influence of *Aspergillus oryzae* fermentation extract (AO) on in situ neutral detergent fiber digestion in steers grazing cool-season pasture throughout the growing season

	June			July			August		
Item	Control	AO	SE	Control	AO	SE	Control	AO	SE
No. of observations	5	5	—	4	5	—	4	5	—
Incubation time, h									
4[a]	−1.6	−2.7	1.23	9.2	9.0	1.14	7.3[b]	6[c]	.83
8	14.4	13.4	2.78	19.8	19.8	2.25	17.9[b]	11.2[c]	2.11
12[a]	35.7[b]	27.3[c]	2.91	31.2	34.5	3.40	21.5	20.2	1.53
16[a]	49.0[b]	39.7[c]	2.21	40.1	38.8	2.49	29.7	30.9	3.47
24[a]	62.4	58.2	2.45	55.2	50.2	2.28	38.5	44.6	4.10
36[a]	—	—	—	62.9	62.5	.94	52.4	57.4	1.50
48[a]	76.1[b]	71.8[c]	1.45	66.5	66.5	.35	56.8	60.5	1.51
72[a]	78.7[b]	76.5[c]	.78	72.9[b]	71.6[c]	.47	63.7	65.9	1.23
Rate, %/h[a]	9.13	7.60	.78	6.58	5.86	.77	4.03[b]	5.62[c]	.61
Lag, h	4.34	3.88	.57	4.09[b]	2.03[c]	.69	2.87	3.93	1.02

[a]Treatment × sampling period interactions were present ($P < .10$)
[b,c]Means within a period and row that do not have common superscripts differ ($P < .10$)

Table 7. Influence of *Aspergillus oryzae* fermentation extract (AO) on in situ crude protein disappearance in steers grazing cool-season pasture throughout the growing season[a]

	June			July			August		
Item	Control	AO	SE	Control	AO	SE	Control	AO	SE
No. of observations	5	5	—	4	5	—	4	5	—
Incubation time, h									
0	35.5	34.3	1.32	45.6	43.9	1.44	40.7	35.2	2.48
4[b]	36.2	38.1	2.62	47.2	42.8	1.98	46.2	37.4	1.30
8[b]	42.8[c]	49.1[d]	1.83	52.8	52.3	2.73	54.3[c]	47.3[d]	1.91
12	58.1	59.8	1.97	62.5	62.5	3.39	57.3[c]	53.3[d]	1.77
16	68.7	67.1	3.19	68.5	64.9	2.23	62.6	58.5	2.84
24[b]	78.8	80.5	1.71	77.1[c]	72.2[d]	1.40	64.1	64.3	2.20
36	—	—	—	80.7	79.1	1.24	72.1	72.2	.95
48	87.8	86.6	1.05	82.1	84.5	1.36	72.2	71.4	1.29
72	91.2	88.7	1.72	86.0	85.8	1.04	76.8	74.9	1.17
Fraction A, %[b]	28.6	30.0	1.60	41.9	40.0	1.59	40.4[c]	32.3[d]	1.19
Fraction B, %[b]	68.8	63.1	2.23	46.3[c]	50.1[d]	1.30	37.3[c]	44.5[d]	2.01
Rate, %/h	4.63	5.56	.53	5.00	4.32	.58	5.40	5.43	1.13

[a]Fraction A = rapidly degraded portion and Fraction B = slowly degraded portion of forage CP.
[b]Treatment × sampling period interactions were present ($P < .10$).
[c,d]Means within a period and row that do not have common superscripts differ ($P < .10$).

(percentage/hour) decreased and fluid volume (liters) increased ($P < .10$) with advancing season. Total mean retention time (hours) was greater ($P < .10$) and particulate passage rate (percentage/hour) lower ($P < .10$) in July and August than in June. Others (Judkins and Stobart, 1988; Westvig, 1992) have shown no response in fluid or particulate passage rate when AO was fed to sheep and steers, respectively.

Analyses of in situ NDF and CP degradability data yielded treatment × sampling period interactions ($P < .10$); therefore, data are presented by sampling period. Specific interactions are indicated on Tables 6 and 7. In situ NDF disappearance was lower ($P < .10$) in June at 12, 16, 48, and 72 h in steers supplemented

Table 8. Influence of *Aspergillus oryzae* fermentation extract (AO) on duodenal amino acid (AA) flow in steers grazing cool-season pasture throughout the growing season

	Treatment			Period			
Item	Control	AO	SE	June	July	August	SE
No. of observations	10	9	—	7	6	6	—
	g/d						
Histidine	17.3[a]	23.7[b]	1.8	12.6[a]	18.4[b]	30.6[c]	2.1
Arginine	44.2	55.8	4.1	30.9[a]	44.9[b]	74.1[c]	4.9
Valine	46.3[a]	62.0[b]	4.6	33.0[a]	51.1[b]	78.4[c]	5.6
Phenylalanine	41.1[a]	55.9[b]	4.1	30.2[a]	43.3[b]	72.1[c]	6.1
Isoleucine	40.2[a]	54.3[b]	4.0	29.2[a]	43.9[b]	68.6[c]	4.9
Leucine	63.4[a]	85.8[b]	6.0	46.6[a]	67.6[b]	109.8[c]	8.0
Threonine	46.5[a]	65.0[b]	4.4	35.0[a]	53.2[b]	79.2[c]	5.5
Lysine	54.8[a]	75.0[b]	5.1	41.9[a]	61.5[b]	91.4[c]	6.3
Aspartic Acid	90.1[a]	128.5[b]	8.8	68.1[a]	105.3[b]	154.5[c]	10.7
Glutamic Acid	89.1[a]	134.6[b]	9.4	72.9[a]	105.7[b]	156.9[c]	11.3
Serine	42.4[a]	58.0[b]	3.6	31.4[a]	48.2[b]	71.1[c]	5.0
Glycine	51.7	65.3	5.1	37.1[a]	52.5[b]	85.9[c]	5.0
Alanine	56.5[a]	79.8[b]	5.2	43.5[a]	63.7[b]	97.2[c]	7.0
Tyrosine	34.4[a]	48.9[b]	3.2	26.6[a]	38.0[b]	60.1[c]	4.2
Total flow	718.1[a]	992.7[b]	69.2	539.1[a]	797.3[b]	1229.8[c]	85.8
Essential AA[d]	353.9[a]	477.6[b]	34.0	259.5[a]	383.8[b]	604.1[c]	43.2
Nonessential AA[e]	364.1[a]	515.1[b]	35.2	279.6[a]	413.5[b]	625.8[c]	42.7

[a,b,c]Means within treatment and period main effects that do not have common superscripts differ ($P < .10$).
[d]Essential AA = HIS + ARG + VAL + PHE + ILE + LEU + THR + LYS.
[e]Nonessential AA = ASP + GLU + SER + GLY + ALA + TYR.

Table 9. Influence of *Aspergillus oryzae* fermentation extract (AO) on profile of amino acids (AA) entering the duodenum of steers grazing cool-season pasture throughout the growing season

Item	June			July			August		
	Control	AO	SE	Control	AO	SE	Control	AO	SE
No. of observations	4	3	—	3	3	—	3	3	—
	g/100 g of AA								
Histidine[a]	2.4	2.3	.08	2.2	2.4	.11	2.6	2.4	.11
Arginine[a]	6.2[b]	5.5[c]	.06	5.8	5.5	.08	6.4[b]	5.7[c]	.12
Valine	6.1	6.1	.16	6.4	6.4	.07	6.6[b]	6.2[c]	.06
Phenylalanine	5.7	5.5	.13	5.7[b]	5.2[c]	.12	5.7	5.9	.18
Isoleucine	5.4	5.4	.13	5.5	5.5	.08	5.7	5.5	.08
Leucine	8.8	8.6	.09	8.7[b]	8.3[c]	.11	8.9	8.9	.11
Threonine	6.4[b]	6.6[c]	.04	6.6	6.7	.07	6.4	6.5	.09
Lysine[a]	7.8	7.8	.07	7.9[b]	7.5[c]	.06	7.4	7.5	.03
Aspartate	12.0	13.1	.47	12.9	13.3	.15	12.4	12.7	.11
Glutamate	13.0[b]	13.8[c]	.29	12.6[a]	13.9[c]	.29	12.2[b]	13.2[c]	.17
Serine[a]	5.8	5.8	.07	6.2[b]	5.9[c]	.07	5.8	5.8	.07
Glycine	7.6[b]	6.4[c]	.22	6.8	6.5	.21	7.3	6.8	.32
Alanine	7.9[b]	8.2[c]	.04	8.0	8.0	.05	7.8	8.0	.09
Tyrosine	4.9	4.9	.06	4.7[b]	4.8[c]	.04	4.8[b]	5.0[c]	.04

[a]Treatment × sampling period interactions were present ($P < .10$).
[b,c]Means within a period and row that do not have common superscripts differ ($P < .10$).

with AO; however, rate (percentage/hour) and lag time (hours) were unaffected ($P < .10$) in June by feeding AO. Disappearance of NDF was decreased ($P < .10$) at 72 h in July and at 8 h in August in steers fed AO. During July, lag time (hours) was less ($P < .10$) in AO than in control steers (2.03 vs 4.09 h, respectively). Rate of NDF digestion was greater ($P < .10$) in AO-supplemented than in unsupplemented steers during August. In contrast, Firkins et al. (1990) reported no changes in rate of digestion in response to AO supplementation. Gomez-Alarcon et al. (1990) reported increased rates of DM digestion in Holstein cows supplemented with AO consuming alfalfa hay. Likewise, rate of DM disappearance in our study (data not shown) was greater ($P < .10$) in steers supplemented with AO than in control steers during August (5.92 vs 4.33%/h, respectively).

In situ rate of CP digestion (corrected for bacterial attachment) agrees with values reported by Messman et al. (1992) and was not altered by treatment during any sampling period. During June, total in situ forage CP degradation (data not shown) calculated by the model of Ørskov and McDonald (1979) was lower ($P < .10$) in AO than in control steers. This finding is likely a result of increased N intakes in AO compared with control steers. During July and August, AO supplementation resulted in a larger ($P < .10$) slowly degraded forage CP fraction (Fraction B) than this fraction in controls. Reasons for shifts in forage CP fractions in response to treatment (Table 7) remain unclear and may warrant further investigation.

Total duodenal essential and nonessential amino acid (AA) flow (grams/day) was increased ($P < .10$) in steers supplemented with AO compared with control steers; this response resulted from increases in individual amino acid flows ($P < .10$, Table 8). The increase in duodenal AA flow was likely a result of increased N intake (Table 4). Total essential, nonessential, and individual duodenal AA flows were lowest ($P < .10$) in June, intermediate in July, and greatest ($P < .10$) in August. Increased AA flow with advancing season was likely a result of increased microbial protein synthesis from June to August (Table 4).

Analyses of AA profiles reaching the duodenum (grams/100 grams of AA) resulted in treatment × sampling period interactions ($P < .10$). Specific interactions are shown in Table 9. Profiles of some essential AA were less ($P < .10$) in June, July, and August in steers supplemented with AO than in control steers; these AA were arginine and glycine in June, phenylalanine, leucine, and lysine during July, and arginine and valine in August. Glutamic acid (grams/100 grams of AA) was increased by AO supplementation during each experimental period, and tyrosine proportions were increased in steers supplemented with AO during both July and August. Reasons for these shifts in AA are unclear.

Implications

Using a fermentation extract of *Aspergillus oryzae* as a supplement for cattle grazing cool-season (bromegrass) pasture can alter forage intake and digestion. Increased intake and digestion (during July) in response to supplemental *Aspergillus oryzae* fermentation extract indicates that feeding the extract

may improve utilization of summer-dormant, cool-season pasture. Forage nutrient utilization seemed to be decreased by extract feeding when forage nutrients were plentiful (June) Further research evaluating supplemental *Aspergillus oryzae* extract during summer dormancy seems warranted

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