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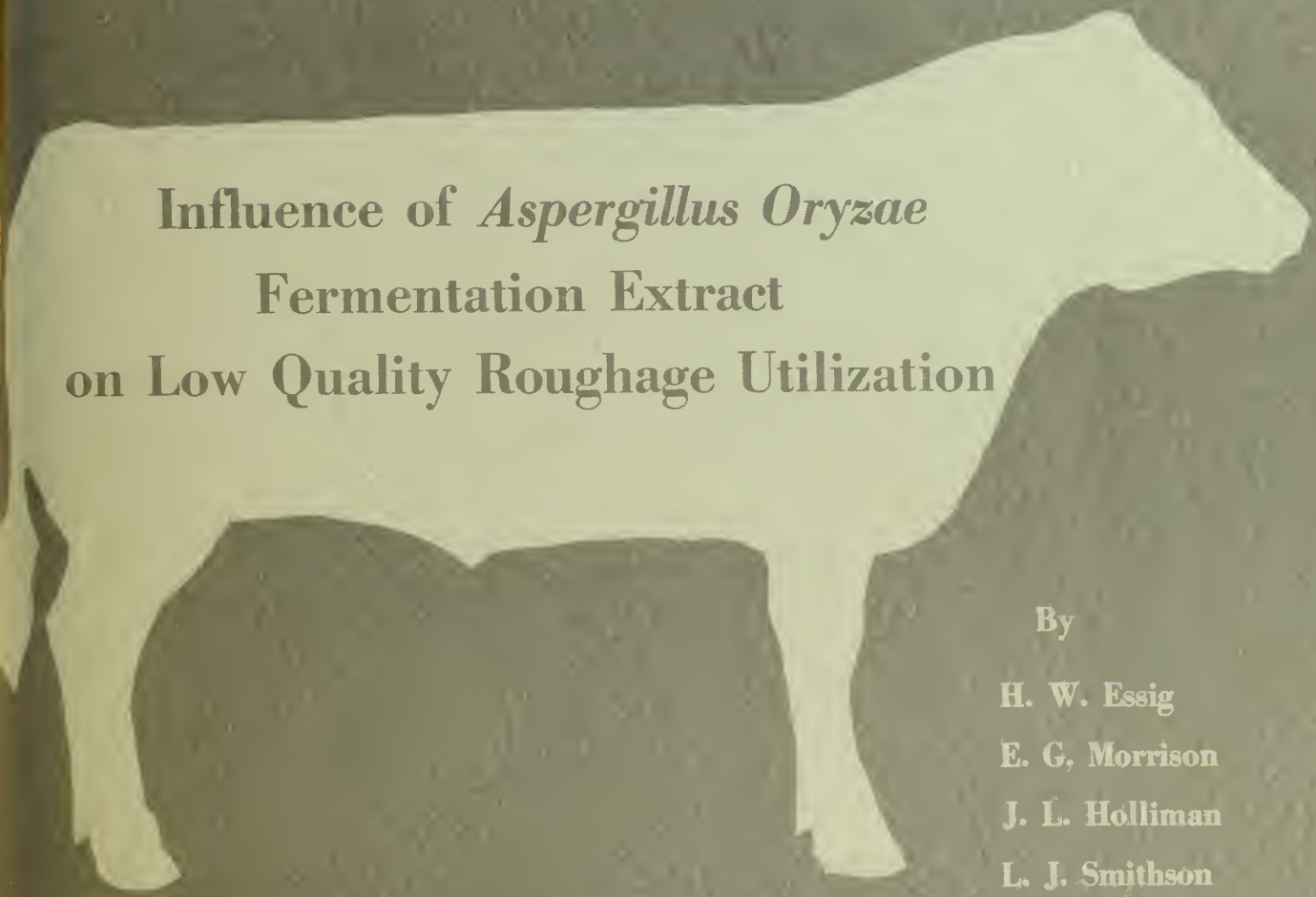

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Influence of *Aspergillus Oryzae* Fermentation Extract on Low Quality Roughage Utilization

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Wintering beef cows on roughages supplemented with a protein source is a common practice in the southeastern United States. Winter feeding of low-quality roughages has increased importantly in recent years, and this has created a need for more precise determination of the protein supplementation required for efficient winter feeding of beef cows.

The objectives of this study were: 1) to compare cottonseed meal and *Aspergillus oryzae* fermentation extract (Vita-Ferm®)[1] in wintering rations for beef cows; 2) to determine nutrient digestibility of low quality roughages supplemented with cottonseed meal or *A. oryzae;* and 3) to measure the presence and utilization of metabolic intermediates and end products.

EXPERIMENTAL PROCEDURE

This study consisted of two separate trials. One, in which cows were fed a wintering ration and then fed a protein supplement during the summer months after the cows had calved, was conducted at the Brown Loam Branch Experiment Station, Raymond, Mississippi during winter of 1973-74 and spring-summer of 1974. The second was a digestibility experiment to measure the influence of protein materials on the digestibility of low-quality cotton by-products (roughages) and was conducted by the Animal Science Department, Mississippi State University during the spring of 1974.

Study 1---This study with wintering rations was started by weighing and dividing 39 cows into two groups on December 18, 1973. All cows were allowed to graze dormant bermudagrass pasture and were fed Coastal bermudagrass hay free choice. One group was fed a *76 percent cottonseed meal-24 percent salt* supplement, the other *Vita-Ferm 6-2-2*. Supplements were fed free choice from a covered bunker.

All cows were weighed at the end of the wintering period and moved the following day (March 8) to bermudagrass pasture. The group that previously had received *Vita-Ferm 6-2-2* was changed at that time to *Vita-Ferm cow-calf supplement* fed free choice.

All cows were weighed again on April 5 (after all had calved) to establish a base for determining gain during the summer grazing period. Supplementation with cottonseed meal-salt was discontinued at this time and the cows that previously had access to this mixture were retained on bermudagrass pasture (*without supplement*) until they were weighed at the end of the trial (October 16). Cows with access to *Vita-Ferm* cow-calf supplement were moved to another pasture (two-thirds bermudagrass and one-third fescue-white clover) about six weeks before the trial was terminated.

Study 2: Digestion Trial 1---Eighteen steers of similar age and weight were identified and stratified according to weight and breed across three groups of six animals. Each group of six animals was composed of three Angus steers and three Hereford steers. The three dietary treatments consisted of (1) gin trash, (2) gin trash plus *A. oryzae* (283.5 g of Vita-Ferm 6-2-2 per head daily) and (3) pelleted gin trash.[2] The conventional method of total collection for seven days was used to calculate digestibility values. Urine and feces were collected for use in chemical analyses and calculation of digestible energy and nitrogen retention values.

Study 2: Digestion Trial 2---The

[1]Vita-Ferm® 6-2-2 and Vita-Ferm cow-calf products of Biozyme Enterprises, Division of St. Joseph Feed and Supply Co., Inc., St. Joseph, MO 61504, were the sources of **Aspergillus oryzae** *fermentation extract. Biozyme Enterprises provided the Vita-Ferm products and a grant-in-aid for this study.*

[2]Gin trash was fed at 1.25 times maintenance requirement, computed by the equation:

$$\text{Quantity of feed per steer} = \frac{74.5\ (\text{wt. in lb. to } .75 \text{ power})}{\text{Estimated DE of feed}} \times 1.25$$

Table 1. Diet Ingredient Composition and Calculated Analysis of Dietary Treatments in Digestion Trial 2 at Mississippi State University, 1974.

Ingredients and analyses	Diet		
	Basal	Basal plus Vital-Ferm®6-2-2	Basal plus CSM
CSH, percent	76.8	76.8	76.8
CSM, percent	23.2	23.2	23.2
A. oryzae, g/head daily	—	283.5	—
CSM, g/head	—	—	181.44
Calculated analyses:			
CP, percent	12.0	—	—
DE, kcal/kg	2129.0	—	—

18 steers used in Trial 1 were randomized and used in a second digestion study to determine the influence of additives on digestible energy and nitrogen retention of cottonseed hull rations (Table 1). The three dietary treatments were: (1) basal CSH-CSM; (2) basal plus *A. oryzae* (283.5 g of Vita-Ferm 6-2-2 per head daily); and (3) basal plus 181 g of CSM (equivalent to crude protein in Vita-Ferm). The basal diet was fed at 1.25 times the maintenance requirement as calculated in Digestion Trial 1.

Chemical Analyses---Chemical analyses of the feed and feces for digestion Trials 1 and 2 included dry matter (DM), gross energy (GE) and nitrogen; to determine digestible dry matter (DDM), digestible energy(DE), energy digestion coefficient (EDC) and nitrogen retention (NR). Rumen fluid was obtained to be used in the IVDM determinations (Goering and Van Soest, 1970) and volatile fatty acid analyses (Rogillio and Essig, 196 for both digestion trials. Rumen fluid was obtained by a stomach tube before feeding and at two and four hours after feeding.

RESULTS AND DISCUSSION

Study 1---There was no difference in average weight change of cows fed either cottonseed meal or Vita-Ferm during the winter feeding period (Table 2). Essig and Valentine (1964) indicated that enzymes from *A. oryzae* had no effect on gains of steers fed a finishing diet. There was a difference ($P < .01$) in final condition score in favor of those cows fed Vita-Ferm (Table 2).

Cows that were given access to Vita-Ferm and bermudagrass-fescue-clover six weeks before weaning gained more ($P < .01$) than did those given no supplement and allowed access to bermudagrass (Table 2). It is our opinion that the difference in the summer treatment was due to the influence of white clover during the final six weeks of the study. When data from the winter period and summer period trials were combined, there was a difference ($P < .01$) in daily gains---again this was probably due to the clover being available for six weeks to the Vita-Ferm treatment group and not to the control group.

Feed cost for cows given hay and cottonseed meal-salt in the winter feeding period was $29.61 per cow, compared with $40.12 for cows fed Vita-Ferm and grass hay during the winter feeding period (Table 2). The cost of feeding Vita-Ferm during the summer grazing period was $19.93 per cow. Therefore, total feed cost per cow for the control group was $29.61, compared with $60.05 for those given access to Vita-Ferm from December 18 through October 16 (Table 2). The additional 87 lbs of gain by cows given Vita-Ferm would not have covered the cost of the supplement at cow prices below 35 cent per pound.

Calf performance as measured by weaning weight, average daily gain, type score, and condition score, was not influenced by feeding Vita-Ferm (Table 3). Generally the calf is the only product sold and there was no advantage for the inclusion of Vita-Ferm during the winter feeding and summer grazing periods.

Study 2: Digestion Trial 1---There were no differences ($P < .0$ in DDM, DE or EDC values for steers fed gin trash or gin trash plus *A. oryzae* diets (Table 4). These results are in agreement with Theurer *et al.* (1959), Burroughs *et al.* (1960) and Tillman (1960) who reported that the addition of enzymes to a diet had no effect on the

Table 2. Performance, Feed Consumption and Feed Costs of 39 Bred Beef Cows on Winter Feeding Supplemented with Cottonseed Meal-Salt or Vita-Ferm®6-2-2 Followed By Summer Grazing with no Supplement or with Vita-Ferm Cow-Calf Supplement, MAFES Brown Loam Branch, Winter 1973-74 and Spring-Summer 1974.

Item	Period and supplement					
	Winter 12/18/73 to 4/5/74		Spring-summer 4/5 to 10/16/74		Winter and spring-summer 12/18/73 to 10/16/74	
	CSM-salt[1]	Vita-Ferm[1]	None	Vita-Ferm[1]	CSM-salt	Vita-Ferm
Cows per treatment, no.	19	20	19	20	19	20
Days, no.	108	108	194	194	302	302
Avg initial wt, lb.	930	933	874	868	930	933
Avg final wt, lb.	874	868	997	1087	997	1086
Avg wt change, lb.	-56a	-65a	123a	219b	67a	154b
Avg initial condition score[2]	9.4	9.4	—	—	—	—
Avg final condition score[2]	8.6a	9.3b	—	—	—	—
Avg daily ration, lb.						
Bermudagrass hay	17.91	17.83	—	—	17.91	17.83
CSM-salt (76-24)	1.68	—	—	—	1.68	—
Vita-Ferm 6-2-2	—	1.31	—	—	—	1.31
Vita-Ferm cow-calf supplement	—	0.467	—	0.467	—	0.467
Total feed cost/cow, $[3]	29.61	40.12	—	19.93	29.61	60.05

[1]CSM-salt (76% CSM to 24% salt) fed only in wintering period, Vita-Ferm 6-2-2 fed 12/18/73 to 3/07/74, Vita-Ferm cow-calf supplement fed from 3/08/74 to 10/16/74.

[2]Condition score: extremely fat, 16; above average, 13; average, 10; below average, 7; extremely thin, 4.

[3]Feed prices: grass hay, $30 ton; cottonseed meal, $155/ton; salt, $34/ton; Vita-Ferm 6-2-2, $310/ton; Vita-Ferm cow-calf supplement, $440/ton.

a,b,Means in a row for each period followed by a different letter differ (P <.01).

gestibility of dry matter, organic atter and cellulose. DDM, DE and DC values for steers fed gin trash and gin trash plus Vita-Ferm were higher (P < .05) than values for steers fed pelleted gin trash. These results are in agreement with researchers who have reported lower digestibility values for

Table 3. Performance of Calves from 39 Cows on Winter Feeding Supplemented with Cottonseed Meal-Salt or Vita-Ferm®6-2-2, Followed By Summer Grazing with No Supplement or with Vita-Ferm Cow-Calf Supplement, MAFES Brown Loam Branch, Winter 1973-74 and Spring-Summer 1974.

Item	Supplement[1]	
	Winter: CSM-salt Summer: None	Winter: Vita-Ferm 6-2-2 Summer: Vita-Ferm cow-calf supplement
Calves, no.	19	20
Birth wt, lb.	68.9	72.2
Weaning wt, lb.	379	387
ADG, birth to weaning, lb.	1.28	1.29
Wt per day age, lb.	1.56	1.59
Avg age at weaning, days	251	245
Type score at weaning[a]	11.8	12.0
Condition score at weaning[b]	9.68	10.1

[1]See table 2 for supplement regimes.

aType score: avg good, 10; high good, 11; low choice, 12.

bCondition score: below avg, 7; avg, 10; above avg, 13.

pelleted hay than for non-pelleted hay (Reynolds and Lindahl, 1960; Johnson *et al.,* 1964).

There were no significant differences (P < .05) in nitrogen retention per gram of feed consumed or nitrogen retention percent for steers fed gin trash or gin trash plus *A. oryzae* diets (Table 4). These results are in agreement with Theurer *et al.* (1959), Tillman (1960) and Theurer (1960) who indicated that supplementation with enzymes had no effect on nitrogen retention in cattle. Nitrogen retention values for steers fed gin trash plus *A. oryzae,* although not significant, were larger than the other two treatments. Nitrogen retention values were lower (P < .05) for steers fed pelleted gin trash than for those fed the other two experimental diets. These results agree with the work of Esplin *et al.* (1957) and Jentseh *et al* (1971) who reported that pelleted rations lowered nitrogen retention.

IVDMD values were not different (P < .05) for the experimental diets (Table 4). These results are in general agreement with the work of Bowden and Church (1959) who, in a series of *in vitro* experiments, reported that enzyme additions failed to increase cellulose digestion. Johnson *et al.* (1964) reported no difference in IVDMD values for pelleted and unpelleted hay rations. They further stated that, when rate of passage of pelleted feedstuffs was controlled as in the *in vitro* procedure, dry matter disappearance was similar to that of non-pelleted feedstuffs.

There were differences (P < .05) in change of rumen fluid acetic acid concentration at two hr post-feeding due to experimental diets (Table 5). Rumen fluid acetic acid concentration changed negativ for steers fed gin trash plus *oryzae.* Steers fed pelleted gin tra had the largest increase in rum fluid acetic acid concentrati change at two hr postfeedi Values of concentration chan for steers fed gin trash plus *oryzae* decreased (P < .05). Johns and Dyer (1964) indicated that use of enzymes in cattle ratio tended to lower rumen fluid V concentrations. Slightly hig positive changes in concentrat of rumen fluid acetic acid at t

Table 4. Digestible Dry Matter (DDM), Digestible Energy (DE), Energy Digestion Coefficient (EDC), Nitrogen Retention (NR), and IVDMD Values of Gin Trash, Pelleted Gin Trash and Gin Trash Plus Vita-Ferm®6-2-2 Fed to 18 Steers, Mississippi State University, 1974.

	Diet		
Item	Gin trash	Pelleted gin trash	Gin trash plus Vita-Ferm 6-2-2
DDM, %	55.81a	48.88b	53.14a
DE, kcal/kg	2373a	1943b	2227a
EDC, %	54.95a	47.14b	52.17a
NR, %	42.26a	31.81b	44.68a
Mg N/g feed	6.77a	5.00b	7.64a
IVDMD, %	55.04a	54.94a	56.23a

a,bMeans in the same row not followed by a common letter differ significantly (P<.05).

Table 5. Change in Volatile Fatty Acid (VFA) Content of Rumen Fluid Taken at Two and Four Hours Post-Feeding From 18 Steers Fed Gin Trash, Pelleted Gin Trash and Gin Trash Plus Vita-Ferm®6-2-2, Mississippi State University, 1974.

		Change in VFA concentration					
Diet	Sampling time	Acetic	Propionic	Butyric	Isovaleric	Valeric	TVFA
				mM/liter			
Gin trash	2 hr	6.72a	.12	-.09	-.03	.01	7.48
	4 hr	10.06a	.49	.98	.8	.06	11.77
Pelleted gin trash	2 hr	9.60a	.96	-.64	-.06	-.05	11.52
	4 hr	12.63a	.28	-.03	-.03	-.03	12.82
Gin trash plus Vita-Ferm 6-2-2	2 hr	-9.45b	-2.63	-.05	.03	.02	-7.09
	4 hr	-12.85b	-3.25	-.99	.03	.01	-17.07

a,bMeans in the same column not followed by a common letter differ significantly (P<.05)

nd four hr postfeeding for steers
ed pelleted gin trash are in agree-
ient with Kromann and Meyer
.972) who reported greater concen-
rations of VFA in the ingesta of
heep fed finely ground or pelleted
oughage diets. Also, there was a
reatment difference (P < .05) in
umen fluid acetic acid concentra-
on change at four hr postfeeding.
Changes in the four-hr concentra-
on of rumen fluid acetic acid from
teers fed gin trash and pelleted gin
rash did not differ significantly
P < .05); however, changes in
alues of rumen fluid from steers
ed pelleted gin trash appeared to
e larger at two and four hr, which
ight be indicative of the larger
DDM, DE and EDC values
reviously discussed.

There were no differences
P < .05) in the two or four hr rumen
luid concentration changes of
ropionic, butyric, isovaleric,
aleric and TVFA. The addition of
A. *oryzae* in general appeared to
lecrease propionic and butyric acid
oncentration change in rumen
luid at the two- and four-hr
ostfeeding sampling. This is in
greement with Johnson and Dyer
1964) who indicated that the use of
nzymes in cattle diets tended to

Table 6. Digestible Dry Matter (DDM), Digestible Energy (DE), Energy Digestion Coefficient (EDC), Nitrogen Retention (NR), and IVDMD Values of Cottonseed Hulls-Cottonseed Meal, Cottonseed Hulls-Cottonseed Meal Plus Cottonseed Meal and Cottonseed Hulls-Cottonseed Meal Plus Vita-Ferm® 6-2-2 Fed to 18 Steers, Mississippi State University, 1974.

	Diet		
Item	Basal	Basal plus CSM	Basal plus Vita-Ferm 6-2-2
DDM, %	47.82	45.58	43.05
DE, kcal/kg	2097	2008	1840
EDC, %	46.79	44.54	41.60
NR, %	41.28	44.86	42.15
Mg N/g feed	7.68	9.54	8.26
IVDMD, %	47.96	53.53	48.99

Values were not significantly different (P<.05)

lower VFA concentration.

Study 2: Digestion Trial 2--- There were no differences (P < .05) in DDM, DE and EDC values among steers fed three dietary treatments (Table 6). These results are in agreement with Theurer *et al.* (1959), Burroughs *et al.* (1960) and Tillman (1960), who reported that supplementing diets with enzymes had no effect on the digestibility of dry matter, organic matter and cellulose. Steers fed the CSH-CSM basal diet appeared to have higher DDM, DE and EDC values than those fed the other dietary treatments.

There were no differences (P < .05) for nitrogen retention per gram of feed consumed or nitrogen retention percent between steers fed the three experimental diets (Table 6). Nitrogen retention values for steers fed the basal diet

Table 7. Change in Volatile Fatty Acid (VFA) Content of Rumen Fluid Taken at Two and Four Hours Post-Feeding From 18 Steers Fed Cottonseed Hulls-Cottonseed Meal, Cottonseed Hulls-Cottonseed Meal Plus Cottonseed Meal and Cottonseed Hulls-Cottonseed Meal Plus Vita-Ferm® 6-2-2, Mississippi State University, 1974.

		Change in VFA concentration					
Diet	Sampling time	Acetic	Propionic	Butyric	Isovaleric	Valeric	TVFA
				mM/liter			
Basal	2 hr	6.460	.501	1.490	-.030	-.003	9.510
	4 hr	2.301	-1.748	.811	-.014	.007	1.390
Basal plus CSM	2 hr	6.400	2.910	3.710	.029	.010	13.840
	4 hr	4.735	2.500	2.013	.010	-.031	9.231
Basal plus Vita-Ferm 6-2-2	2 hr	8.320	2.160	1.540	-.030	-.010	11.700
	4 hr	8.688	1.268	.426	-.054	-.004	10.330

Values were not significantly different (P<.05).

appeared to be lowest, while steers fed the cottonseed meal-supplemented diet appeared to have retained the most nitrogen. These results are in agreement with Theurer *et al.* (1959), Tillman (1960) and Theurer (1960), who indicated that supplementation with enzymes had no effect on nitrogen retention. In general, supplementation of the CSH-CSM basal ration with CSM or *A. oryzae* had no effect on nitrogen retention per gram of feed consumed or nitrogen retention percent.

IVDMD values were not different ($P < .05$) for the three dietary treatments (Table 6). These results are in agreement with the work of Bowden and Church (1959), who, in a series of *in vitro* experiments, observed that the addition of enzymes failed to increase cellulose digestion.

There were no significant differences in change of acetic, propionic, butyric, isovaleric, valeric or TVFA concentration of rumen fluid at two- or four-hr postfeeding due to experimental diets (Table 7). These results ag with the work of Yang *et al.* (19 and Rust *et al.* (1965), who repor no significant differences in V concentration changes due to zyme supplementation. The tre of VFA changes was larg positive. This agrees with Shep *et. al.* (1958) and Stewart *et.* (1958) who indicated that V concentration increased af feeding and decreased four to six later.

SUMMARY

There was no difference in weight change of cows fed bermudagrass hay and cottonseed meal or bermudagrass hay and Vita-Ferm 6-2-2 during the winter feeding period. When Vita-Ferm cow-calf supplement was fed to cows during the summer grazing period and, when these cows were placed on a bermuda-fescue-clover pasture six weeks before weaning of the calves, there was a significant difference in weight change when compared with the treatment group that remained on bermudagrass without supplement. The total feeding cost per cow from December 18 to October 16 was $30.44 more for the cows fed Vita-Ferm than for those cows given cottonseed meal during the winter and no protein during the summer.

There were no differences in weaning weight, birth weight, average weight per day of age, type score and weaning condition for calves from cows given either the control treatment or those given the Vita-Ferm treatment.

No differences ($P < .05$) were detected in energy digestion coefficients (EDC), digestible energy (DE), digestible dry matter (DDM), nitrogen retention (NR) or IVDMD values due to the addition of *A. oryzae* to gin trash. EDC, DE, DDM and NR values were lower ($P < .05$) for steers fed pelleted gin trash than for those fed non-pelleted gin trash. There were no significant differences in EDC, DE, DDM and NR values due to additives (cottonseed meal or *A. oryzae*) to the basal CSH-CSM diets fed to steers during Digestion Trial 2. In Dig tion Trial 1 VFA concentrations rumen fluid except acetate at tw and four-hr samplings were i different due to diets. Acetic a concentration change was lov ($P < .05$) in rumen fluid of steers f gin trash plus *A. oryzae* than i steers fed only gin trash. Additi of *A. oryzae* appeared to lower t total VFA concentration in t rumen fluid. No significa differences were detected in rum fluid VFA concentration due experimental diets in Digesti Trial 2. *In vitro* dry matter d appearance values for experime tal diets did not differ ($P < .05$) either Digestion Trial 1 or Dig tion Trial 2.

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