Proceedings

Western Section
American Society of Animal Science

Volume 42

***Robert A. Bellows**, President*
***Duane L. Garner**, President-Elect*
***Ray A. Field**, Secretary-Treasurer*
***Ian D. Smith**, Director at Large*

University of Wyoming

Department of Amimal Science

Laramie, Wyoming

August 6-9, 1991

Proceedings, Western Section, American Society of Animal Science

Vol. 42, 1991

INFLUENCE OF ASPERGILLUS ORYZAE FERMENTATION EXTRACT (AMAFERM) AND BARLEY SUPPLEMENTATION ON IN SITU NUTRIENT DEGRADABILITY AND RUMINAL PH IN STEERS FED LOW-QUALITY HAY[1,2,3]

R.M. Westvig, J.S. Caton and D.O. Erickson
North Dakota State University
Fargo, ND 58105

ABSTRACT

Four steers fitted with indwelling ruminal cannulas were arranged in a 4 x 4 Latin square to evaluate the effects of Aspergillus oryzae (AO) fermentation extract and barley supplementation on in situ forage nutrient utilization and ruminal pH. Steers were fed a basal hay (8.1% CP) ad libitum. Treatments were arranged in a 2 x 2 factorial with Amaferm (AO; 2 g/head daily) and barley (2.73 kg/head daily) supplementation. Dacron bags containing basal forage were ruminally incubated at 0, 4, 8, 12, 16, 24, 36, 48 and 72 h. Ruminal fluid samples were taken at -2, 0, 1, 3, 6, 9, 12, 15 and 24 h after feeding. Main effect means for in situ dry matter (DM) and neutral detergent fiber (NDF) degradabilities were lower (P<.05) in barley compared with non-barley supplemented steers at 16, 36, 48 and 72 h. Steers fed AO had higher (P<.06) in situ DM and NDF degradabilities at 16 h compared with non-AO supplemented steers. In addition, AO supplementation tended (P<.16) to enhance in situ NDF degradability above controls at 8 and 36 h of incubation. Moreover, AO supplementation resulted in higher (P<.04) crude protein (CP) in situ degradability at 16 h compared with steers not fed AO. Barley supplementation had no influence (P>.10) on in situ forage CP degradability. Ruminal pH was depressed in steers fed barley (P<.10) at 3, 6 and 9 h post supplementation compared with steers not fed barley In addition, AO supplementation increased (P<.10) ruminal pH above non-AO supplemented steers at 1 and 24 h post AO feeding. Supplementation of AO tended (P<.16) to evaluate ruminal pH at 9 h after supplementation. These data indicate that barley supplementation reduces (P<.10) DM and NDF in situ degradation and ruminal pH at various times measured. Feeding AO resulted in higher DM, NDF and CP degradabilities at 16 h of incubation and occasionally altered ruminal pH.

Key Words: In Situ, Degradability, Barley, Aspergillus Oryzae

Introduction

Use of low quality forages in pen fed cattle rations have often been limited to mid-gestation, where nutrient requirements are low. In addition, cattle grazing native range in the northern great plains may be consuming low quality forage from fall dormancy until spring. Limiting nutrient characteristics of these forages prevents optimal production and often dictates the use of supplements regardless of stage of production. Research is needed in the area of supplementation to find ways to improve the utilization of low to medium quality roughages while at the same time increasing the energy content of the diet (Lusby and Wagner, 1987). However, supplementing large amounts of readily fermentable energy sources such as barley, corn or molasses can reduce both forage intake and rate and extent of fiber digestion (Chase and Hibberd, 1985; Mould et al., 1983). These results may partially be explained by decreased ruminal pH, which in turn adversely effect cellulolytic bacteria (El Shazly et al., 1961; Mould and Orskov, 1983).

Forage intake, digestibility and cellulolytic bacteria have been shown to increase with the addition of AO (Caton et al, 1990; Wiedmeier et al., 1987). Conversely, fungal additives have not altered rates of passage (Arambel and Wiedmeier, 1986; Wiedmeier et al., 1987). Recent in situ work (Fondevila et al., 1990; Gomez-Alarcon et al., 1990) has shown that AO can increase the rate of digestion of highly fibrous feedstuffs. Several experiments concerning the influence of AO on nutrient digestion and utilization by dairy cattle at both high and low planes of nutrition have been reported, while relatively few exist for beef cattle. Therefore, the objectives of the current experiment were to determine the effects of barley and AO supplementation on in situ nutrient degradability and ruminal pH in steers fed a low-quality hay.

Materials and Methods

Four Hereford steers (293 kg) fitted with ruminal cannulae were arranged in a 4 x 4 Latin square. Each period of the Latin square consisted of a 14 d adaptation period and an 8 d collection period. Treatments were arranged as a 2 x 2 factorial within the Latin square with Aspergillus oryzae (AO; $2 g \cdot head \cdot d^{-1}$) fermentation extract (Amaferm) and barley ($2.73\ kg \cdot head \cdot d^{-1}$) supplements as the main effects. Both supplements were offered once daily at 0900. Steers were housed individually and fed prairie grass hay (8.1% CP) ad libitum as the basal forage. Isonitrogenous supplements were formulated to meet NRC (1984) requirements for .9 kg of daily gain. Protein levels were adjusted using soybean meal (SMB; Table 1). Limestone was added daily (25 g and 45 g to control and barley fed steers, respectively) to balance calcium and phosphorus, and trace mineral blocks were offered free choice.

Dacron bags (10 x 20 cm; pore size approximately 50 microns), purchased from Ankom[4], were filled with approximately 5 g ground (2 mm screen) prairie grass hay, sealed with a rubber stopper

[1] Scientific Paper No. 1916 of the North Dakota Agric. Exp. Sta., Fargo.
[2] Research partially supported by Bio-zyme Inc., St. Joseph, MO. 64502.
[3] Dept. of Anim. and Range Sci.

[4] Ankom Inc., Spencerport, NY 14559.

and incubated in the rumen for 0, 4, 8, 12, 16, 24, 36, 48 and 72 h. After incubation, all bags were removed from the rumen and washed in tap water until rinse water was clear. Bags were then dried (60°C), dessicated and reweighed. Grab samples of dietary forage and grain were taken from each period and analyzed separately. Samples of refused hay were taken periodically throughout each collection period and analyzed. Forage and diet samples were analyzed for DM and CP content (AOAC, 1984). In situ forage NDF and dietary NDF and ADF were measured by the non-sequential method of Goering and Van Soest (1970).

Rumen samples were taken at -2, 0, 1, 3, 6, 9, 12, 15 and 24 h post supplementation. Samples were immediately analyzed for pH using a combination electrode.

Data were analyzed by analysis of variance (Cochran and Cox, 1957). Rumen samples measured over time were handled as a split-plot within a Latin square (Gill and Hafs, 1971) All data were analyzed using a general linear model of statistical analysis systems (SAS, 1982).

Results and Discussion

No barley x AO interactions were detected; therefore, main effect means are reported except in Table 5. Barley supplementation decreased ($P < .10$) in situ DM at 4 h and DM and NDF ($P < .05$) forage degradability at 16, 36, 48 and 72 h post-supplementation compared with non-barley supplemented steers (Tables 2 and 3). There was also a tendency ($P < .13$) for barley supplementation to decrease both DM and NDF degradability at 24 h. Depressions in DM degradability agree with results found by Ulmer et al. (1990) when pen fed steers were fed similar amounts of barley. Mould et al. (1983) also showed decreases in DM degradation (in situ) but not until barley supplementation reached 60% of the diet. Decreases in DM and NDF degradability of forage could possibly be attributed to changes in the bacterial population due to grain supplementation (Stewart, 1977).

Barley supplementation had no effect ($P > .10$) on in situ CP degradability. Correction for microbial attachment has the potential to alter these values, especially with forage residues (Varvikko and Lindberg, 1985). Microbial N correction was shown to have a major influence on estimates of feed N disappearance for reed canary grass but had only a minor effect on lucerne hay estimates (Kennedy et al., 1984). True N disappearance of reed canary grass was greater than the apparent feed N disappearance from washed nylon bags. Decreases in cellulose digestion in situ are often accompanied by a decrease in in situ N digestion (Lindberg, 1981; Lindberg and Varvikko, 1982)

In contrast to barley treatments, AO supplementation increased ($P < .10$) in situ DM, NDF and CP degradability of forage at 16 h compared with the non-AO supplemented steers (Tables 2, 3 and 4). In addition, AO supplementation tended ($P < .16$) to enhance in situ DM and NDF degradabilities at 8 and 36 h, and CP degradability at 8 h.

In steers grazing cool season pasture, AO supplementation resulted in lower in situ DM degradabilities in June and August, but had higher in situ DM disappearance in July at 48 h of incubation (Caton et al., 1990). Conversely, in vitro DM digestibility was enhanced ($P < .10$) by AO in all periods (Caton et al., 1990). Other studies have indicated enhanced in vitro digestibilities of various diets (Gomez-Alarcon et al., 1990) as well as increased rate of in situ degradability (Fondevila et al., 1990; Gomez-Alarcon et al., 1990) resulting from AO supplementation.

Simple effect means at 16 h (Table 5) indicate that AO increased ($P < .07$) DM and NDF degradability compared with other treatments. Crude protein degradability at 16 h was increased ($P < .10$) by AO supplementation compared with hay and barley treatments. Reasons for AO affects at only 16 h are unknown. Error in dacron bag residual nitrogen resulting from bacterial contamination can be high, especially in forages (Nocek, 1988). Correction for microbial contamination and calculation of rate of digestion may give more insight into differences observed in this study.

Split-plot analysis revealed treatment by time interactions for pH, therefore ruminal pH values are presented by time (Table 6) Steers fed barley exhibited a characteristic depression ($P < .10$) in ruminal pH at 3, 6 and 9 h post-supplementation compared with steers not fed barley Lowered ruminal pH is the result of the production of volatile fatty acids (VFA) as well as other acids (such as lactic) and weaker buffering power of concentrates compared with that of forages (Slyter, 1976). Less production and recycling of saliva, an important physiological buffer, is required with the consumption of concentrates. Concentrates generally increase the production of propionate which has a lower pKa than that of acetate, thus further lowering the buffering capacity of rumen fluid.

In contrast to barley supplementation, addition of AO increased ($P < .10$) ruminal pH above non-AO supplemented steers at 1 and 24 h post AO feeding, and had a tendency ($P < .14$) to elevate ruminal pH at 9 h Although not significant, ruminal pH values were numerically higher for AO compared with control steers at all times measured and would appear to indicate a quicker recovery from postfeeding ruminal pH depression

In vitro results reported by Frumholtz et al. (1989) suggested that AO eliminated the fall in pH associated with the addition of grain. Conversely, at most times measured, the results of this experiment tend to agree with those of Arambel and Wiedmeier (1986), Gomez-Alarcon et al. (1990) and Wiedmeier et al. (1987) who suggested that AO supplementation had no effect on ruminal pH.

Implications

Data from the present study appears to confirm reductions in in situ digestion of DM and NDF associated with supplementation of a highly fermentable starch as well as reductions in ruminal pH. These data also indicate that AO supplementation in beef steers fed a low-quality hay may only have marginal affects on in situ forage degradability and ruminal pH.

Literature Cited

AOAC. 1990. Official Methods of Analysis (15th Ed). Association of Official Analytical Chemists, Washington, D.C.

Arambel, M.D. and R.D Wiedmeier 1986. Effect of supplemental *Saccharomyces cerevisiae* and(or) *Aspergillus oryzae* on rumen fermentation. J. Dairy Sci. 69(Suppl 1):188.

Caton, J.S, D.O. Erickson, D A Carey and D.W. Ulmer. 1990. Influence of fungal extract supplementation on forage intake, digestibility and ruminal fermentation in steers grazing cool season pasture throughout the grazing season. Proc. Western Sect Am. Soc. Anim. Sci. 41:311.

Chase, C.C. and C.A. Hibberd. 1987 Utilization of low-quality native grass hay by beef cows fed increasing quantities of corn grain. J. Anim. Sci. 65.557.

Cochran, W.G. and G.M. Cox. 1957. Experimental Design (2nd Ed.). John Wiley and Sons, New York

El-Shazly, K., B.A. Dehority and R.R. Johnson. 1961. Effect of starch on the digestion of cellulose in vitro and in vivo by rumen microorganisms J. Anim. Sci. 20:268

Fondevila, M, C.J. Newbold, P.M. Hatten and E.R. Orskov. 1990. A note on the effect of *Aspergillus oryzae* fermentation extract on the rumen fermentation of sheep given straw. Anim Prod. 51:422

Frumholtz, P.P., C.J. Newbold and R.J. Wallace. 1989. Influence of *Aspergillus oryzae* fermentation extract on the fermentation of a basal ration in the rumen simulation technique (Rusitec). J. Ag. Sci., Cambridge 113.169.

Gill, D L. and H.D. Hafs. 1971. Analysis of repeated measurements of animals. J. Anim. Sci. 33:331.

Goering, H.K. and P.J. Van Soest. 1970 Forage fiber analysis (apparatus, reagents, procedures and some applications). USDA-ARS Handbook No. 379. ARS, USDA, Washington, D.C.

Gomez-Alarcon, R A., C. Dudas and J.T. Huber. 1990. Influence of cultures of *Aspergillus oryzae* on rumen and total tract digestibility of dietary components. J. Dairy Sci. 73:703.

Kennedy, P.M, G.P. Hazelwood and L P. Milligan. 1984 A comparison of methods for the estimation of the proportion of microbial nitrogen in duodenal digesta, and of correction for microbial contamination in nylon bags incubated in the rumen of sheep. Br. J. Nutr. 52:403.

Lindberg, J.E. 1981. The effect of basal diet on the ruminal degradation of dry matter, nitrogenous compounds and cell walls in nylon bags. Swedish J. Agric. Res. 11:159.

Lindberg, J E. and T. Varvikko. 1982. The effect of bag pore size on the ruminal degradation of dry matter, nitrogenous compounds and cell walls in nylon bags. Swedish J Agric. Res. 12:165.

Lusby, K.S. and D G. Wagner. 1987. Effects of supplements on feed intake. In: F.N. Owens (Ed) Proceedings. Feed Intake by Beef Cattle, November 20-22, 1986. pp. 173-181. Anim. Sci. Dept, Ag Expt. Station, Oklahoma State Univ., Stillwater, Oklahoma.

Mould, F.L. and E.R. Orskov. 1983. Manipulation of rumen fluid pH and its influence on cellulolysis in sacco, dry matter degradation and the rumen microflora of sheep offered either hay or concentrate. Anim. Feed Sci. Technol. 10:1.

Mould, F.L., E.R. Orskov and S.O. Mann. 1983. Associative effects of mixed feeds. 1. Effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolysis in vivo and dry matter digestion of various roughages. Anim. Feed Sci. Technol. 10.15.

Nocek, J.E. 1988. In situ and other methods to estimate ruminal protein and energy digestibility: A review. J. Dairy Sci. 71:2051.

NRC. 1984. Nutritional requirements of beef cattle (6th Ed) National Academy Press, Washington, D C.

SAS. 1982. SAS User's Guide: Statistics. SAS Inst Inc., Cary, NC.

Slyter, L.L 1976. Influence of acidosis on rumen function J. Anim. Sci. 43.910.

Stewart, C.S. 1977 Factors affecting the cellulolytic activity of rumen contents. Appl. Environ. Microbiol. 33:497.

Ulmer, D N., J S. Caton, S.L. Boyles and D.O. Erickson. 1990 Influence of increasing level of barley supplementation on forage intake, digestibility and ruminal fermentation in beef steers fed medium quality hay. J. Anim. Sci. 68(Suppl. 1):571.

Varvikko, T. and J.E. Lindberg. 1985. Microbial nitrogen in nylon bag residues quantified by feed ^{15}N dilution. Br. J Nutr. 54:473.

Wiedmeier, R D., M.J Arambel and J.L. Walters. 1987. Effect of yeast culture and *Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient digestibility. J. Dairy Sci. 70.2063.

TABLE 1. DAILY SUPPLEMENTAL INTAKE AND CHEMICAL COMPOSITION OF DIETARY COMPONENTS FED STEERS (DRY MATTER BASIS).

Item	Hay	Barley	SBM
Composition (%)			
DM	94.8	91.1	93.5
ASH	8.9	2.5	6.6
NDF	74.3	18 0	14.0
ADF	44.1	5.5	8.9
CP	8.1	16.3	48.8
OM	91.1	97.5	93.4
Amount fed (g)			
Supplemented	ad lib	2727	157
Control	ad lib	0	677

TABLE 2. INFLUENCE OF BARLEY AND AMAFERM (AO) SUPPLEMENTATION ON IN SITU DRY MATTER DEGRADABILITY OF PRAIRIE GRASS HAY INCUBATED IN THE RUMEN OF STEERS (MAIN EFFECT MEANS).

	Barley		Amaferm		
Time, h	Control	Barley	Control	AO	SE[a]
	%				
0	21.00	21.06	20.88	21.17	.22
4	22.41[b]	22.98[c]	22.59	22.80	.29
8	25.79	26.06	25.32	26.53	.70
12	30.31	29.39	29.03	30.67	1.75
16	35.26[b]	31.61[c]	31.76[b]	35.11[c]	1.46
24	43.67	39.70	40.28	42.46	2.55
36	54.46[b]	48.08[c]	49.13	53.42	2.54
48	60.46[b]	54.55[c]	56.43	58.58	2.09
72	67.84[b]	64.34[c]	66.06	66.13	1.42

[a]Standard error of main effect means, n = 8.
[bc]Main effect means within barley or AO having differing superscript differ (P<.05).

TABLE 3. INFLUENCE OF BARLEY AND AMAFERM (AO) SUPPLEMENTATION ON IN SITU NEUTRAL DETERGENT FIBER (NDF) DEGRADABILITY OF PRAIRIEGRASS HAY INCUBATED IN THE RUMEN OF STEERS (MAIN EFFECT MEANS).

	Barley		Amaferm		
Time, h	Control	Barley	Control	AO	SE[a]
	%				
0	7.01	8.14	8.09	7.06	1.23
4	8.16	8.34	8.23	8.28	.52
8	12 30	12.94	11.88	13.35	.91
12	17.99	17.00	16.28	18.71	2.26
16	24.43[b]	19.41[c]	19.42[b]	24.40[c]	2.04
24	35.33	28.65	30.29	33.70	3.67
36	49.37[b]	39 96[c]	41.60	47.73	3.68
48	56.68[b]	48.27[c]	50.89	54.06	3.04
72	67.76[b]	60.95[c]	63.29	63.42	1.78

[a]Standard error of main effect means, n = 8.
[bc]Main effect means within barley or AO having differing superscript differ (P<.05).

TABLE 4. INFLUENCE OF BARLEY AND AMAFERM (AO) SUPPLEMENTATION ON IN SITU CRUDE PROTEIN DEGRADATION OF PRAIRIEGRASS HAY INCUBATED IN THE RUMEN OF STEERS (MAIN EFFECT MEANS).

Time, h	Barley		Amaferm		SE[a]
	Control	Barley	Control	AO	
	%				
0	30.73	31.19	30.41[b]	31.52[c]	.44
4	29.79	30.50	30.67	29.62	.87
8	30.99	32.92	30.87	33.04	1.28
12	35.16	36.98	35.34	36.79	1.37
16	40.22	37.98	37.00[b]	41.20[c]	1.63
24	47.26	44.75	45.19	46.82	2.09
36	56.38	54.54	53.92	57.00	2.26
48	62.77	59.64	59.99	62.42	2.25
72	69.66	68.32	69.42	68.56	1.32

[a]Standard error of main effect means, n = 8.
[bc]Main effect means within barley or AO having differing superscript differ (P<.05).

TABLE 5. INFLUENCE OF BARLEY AND AMAFERM (AO) SUPPLEMENTATION ON 16 H IN SITU DM, NDF AND CP DEGRADABILITIES (SIMPLE EFFECT MEANS).

Item	Hay	Barley	AO	Barley + AO	SE[a]
	%				
DM	33.08[b]	30.45[b]	37.45[c]	32.79[b]	1.46
NDF	21.23[b]	17.62[b]	27.63[c]	21.20[b]	2.04
CP	37.32[b]	36.67[b]	43.13[c]	39.29[bc]	1.63

[a]Standard error of simple effect means, n = 4.
[bc]Simple effect means within a row having different superscript differ (P<.10).

TABLE 6 INFLUENCE OF BARLEY AND AMAFERM (AO) SUPPLEMENTATION ON RUMINAL PH OF STEERS (MAIN EFFECT MEANS).

Time, h	Barley		Amaferm		SE[a]
	Control	Barley	Control	AO	
-2	6.54	6.58	6.53	6.58	.06
0	6.45	6.49	6.44	6.50	.05
1	6.40	6.42	6.36[b]	6.47[c]	.03
3	6.16[b]	5.87[c]	5.97	6.06	.09
6	6.10[b]	5.63[c]	5.84	5.88	.13
9	6.02[b]	5.69[c]	5.73	5.97	.14
12	6.11	6.00	5.94	6.17	.18
15	6.16	6.04	6.08	6.12	.13
24	6.34	6.40	6.28[b]	6.46[c]	.07

[a]Standard error of main effect means, n = 8.
[bc]Main effect means within barley or AO having differing superscript differ (P<.10).