Effect of *Aspergillus oryzae* Fermentation Extract (Amaferm®) on In Vitro Fiber Degradation[1]

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ABSTRACT

The influence of *Aspergillus oryzae* fermentation extract (Amaferm®) on in vitro fiber degradation was determined by incubating eight ground fibrous feedstuffs with rumen fluid and buffer inoculum Amaferm® was added at 0, 4, 8, or 12 g/L of fermentation mixture Both NDF and ADF degradabilities were determined after 96 h of incubation Addition of extract had no effect on NDF or ADF degradability of pure cellulose, low endophyte fescue, wheat straw, corn silage, or prairie hay Addition of Amaferm® at .8 or 1 2 g/L increased NDF and ADF degradations of bromegrass hay and alfalfa hay, its addition at 4 or .8 g/L, but not at 1 2 g/L, increased NDF and ADF degradation of high endophyte fescue hay In a second set of in vitro fermentations, selective antimicrobials (penicillin, streptomycin, and cycloheximide) were used to assess the influence of Amaferm® on various microbial groups The enhanced fiber degradation by Amaferm® was attributed to its stimulation of bacterial activity because its addition to whole rumen fluid without or with cycloheximide increased fiber digestion. In contrast, addition of Amaferm® to the whole rumen fluid plus penicillin and streptomycin treatment had no effect on fiber degradation, suggesting that fungal or protozoal activity was not affected by treatment. In conclusion, Amaferm® increased fiber digestibility of certain feedstuffs, and the increase was mediated via stimulation of rumen bacterial, but not fungal or protozoal, activities

(Key words *Aspergillus oryzae* fermentation extract, fiber degradation, rumen fermentation)

Abbreviation key: AFE = *Aspergillus oryzae* fermentation extract, WRF = whole rumen fluid

INTRODUCTION

Considerable efforts have been devoted to manipulating the rumen environment with the goal of improving ruminant production. The result of these efforts is a wide range of feed additives that are capable of influencing some component of rumen metabolism. Ruminants are unique in their ability to utilize fiber and, therefore, should be managed for maximum fiber degradation (22). Research (14, 25) has indicated that some microbial feed additives may increase the nutritive value of feedstuffs by increasing the digestion of dietary fiber

One of several microbial feed additives commercially available is Amaferm® (Bio-Zyme Inc., St Joseph, MO), a fermentation extract of a specific *Aspergillus oryzae* mold (AFE) The addition of AFE increased digestibility of DM, fiber, and CP in vivo (8, 25) and in vitro (6). Additionally, AFE supplementation increased rumen microbial activity in vitro and in vivo, as evidenced by increased VFA concentration and numbers of bacteria, particularly fiber-digesting groups (4, 6). The increased microbial activity and rate of fiber digestion in cows supplemented with AFE sometimes were associated with improved cow performance, such as higher milk production (9, 11, 23). However, production responses were not always found, and they seem to be diet-dependent (9)

Little work has been done on the effect of AFE on the rumen populations of ciliated pro-

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tozoa and fungi (6, 24) The fungal population has high fiber-digesting ability and may contribute to overall fiber digestibility (1) The ciliated protozoal population preys on bacteria, therefore, if AFE were to inhibit the ciliated protozoal population, it may partially account for increased bacterial numbers. Our objectives were to determine the influence of AFE on in vitro fiber degradation of certain feedstuffs and to use selective antimicrobial compounds to assess the effect of AFE on various microbial groups.

MATERIALS AND METHODS

In vitro batch culture fermentations with mixed rumen microorganism were used to determine the influence of AFE on fiber degradations. Rumen fluid was collected from a rumen-cannulated Holstein steer fed an alfalfa hay and concentrate diet (80 20) Rumen contents were squeezed through four layers of cheesecloth into an airtight container and transported to the laboratory The strained rumen fluid was incubated for 30 min at 39°C. Feed particles that rose to the top were removed by vacuum. Strained rumen fluid was used in the preparation of inoculum. McDougall's buffer (15) was diluted using a 1 2 ratio Inoculum (30 ml) was transferred to 50-ml centrifuge tubes containing .5 g of substrate. Substrates tested included alfalfa hay (*Medicago sativa*), bromegrass hay (*Bromus inermis*), high and low endophyte fescue (*Festuca arundinacea*), pure cellulose (Whatman cellulose powder, Whatman Lab Sales Ltd., Hillsboro, OR), wheat straw (*Triticum aestivum*), corn silage (*Zea mays indentata*), and prairie hay [predominantly big bluestem (*Andropogon gerardii*), little bluestem (*Andropogon scoparius*), and Indiangrass (*Sorghastrum nutans*)]. Feedstuffs were ground to pass a 1-mm screen using a Cyclotec mill (Tecator, Inc., Herndon, VA). The AFE was added at 0, .4, 8, or 1 2 g/L of fermentation mixture. Tubes were capped with rubber stoppers equipped with one-way Bunsen valves, incubated at 39°C and vortexed three times daily Samples were removed at 0 and 96 h for NDF and ADF determinations (7, 20) Degradation was calculated as the amount of NDF or ADF that disappeared during the fermentation relative to the initial concentration after correcting for residues in the inoculum Fermentations were set up in triplicate, and the experiment was replicated three times

The substrates (alfalfa hay, bromegrass hay, and high endophyte fescue) that showed a positive response (increased NDF and ADF degradation) with AFE addition were used to assess the influence of AFE on bacterial, fungal, or protozoal contribution to in vitro fiber degradation. The following antimicrobial compounds (Sigma Chemical Co , St. Louis, MO) were added to the fermentation to select for the desired microbial population· 2000 U/ml of penicillin G (1600 U/mg, dissolved in H_2O), 150 U/ml of streptomycin sulfate (650 U/mg, dissolved in H_2O) to inhibit bacteria, and .5 mg/ml of cycloheximide (dissolved in methanol) to inhibit fungi and, possibly, protozoa (26). Tubes receiving no antibiotic or only one antibiotic received methanol to equal the highest amount used. The following treatments were used for each substrate 1) substrate, buffer, and AFE, 2) substrate, buffer, and whole rumen fluid (WRF), 3) substrate, buffer, WRF, and AFE, 4) substrate, buffer, WRF, and antifungal compound; 5) substrate, buffer, WRF, and antibacterial compounds; 6) substrate, buffer, WRF, and antibacterial and antifungal compounds; 7) substrate, buffer, WRF, AFE, and antibacterial compounds; 8) substrate, buffer, WRF, AFE, and antifungal compound; 9) substrate, buffer, WRF, AFE, antibacterial compounds, and antifungal compound (negative control) Tubes were capped with rubber stoppers equipped with one-way Bunsen valves, incubated at 39°C, and vortexed three times daily. Samples were removed at 0 and 96 h for NDF and ADF determinations (7, 20). Degradation was calculated as the amount of NDF or ADF that disappeared during the fermentation relative to the initial concentration after correction for residues in the inoculum, antimicrobial compounds, and AFE supplement. Fermentations were set up in triplicate, and the experiment was replicated three times All data were analyzed using the general linear models procedure of SAS (21) In Experiment 1, effects included in the model were level of AFE (0, .4, .8, or 1 2 g/L), replication and AFE level × replication interaction The effect of AFE was tested with level × replication as the error term. Data from Experiment 2 were analyzed as a split-plot design.

TABLE 1 Effect of Amaferm® supplementation on in vitro NDF and ADF degradabilities[1]

	Alfalfa hay	Bromegrass hay	Cellulose	Corn silage	High endophyte fescue	Low endophyte fescue	Prairie hay	Wheat straw
NDF Content of feedstuff, %	53	69	98	59	71	68	70	79
% of NDF Digested by								
Control	36 5[b]	52 7[b]	58 2	62 5	59 1[b]	52 4	54 0	38 3
Amaferm®								
4 g/L	40 2[ab]	56 5[ab]	56 4	60 8	65 0[a]	52 6	51 8	34 9
8 g/L	42 0[a]	57 6[a]	55 8	64 0	65 2[a]	54 0	51 5	36 3
1 2 g/L	42 9[a]	58 3[a]	57 1	61 9	61 3[ab]	52 8	52 8	35 2
SE	8	5	6	9	7	7	6	4
ADF Content of feedstuff, %	38	43	94	29	41	40	49	54
% of ADF Digested by								
Control	25 0[b]	28 8[b]	40 4	23 3	30 5[b]	30 4	26 1	25 9
Amaferm®								
4 g/L	27 7[ab]	31 1[ab]	39 5	25 3	34 2[a]	27 9	26 6	23 7
8 g/L	27 9[ab]	32 5[a]	42 0	25 0	34 4[a]	29 2	27 1	23 1
1 2 g/L	28 0[a]	32 7[a]	41 2	24 8	31 2[ab]	30 3	28 3	25 2
SE	6	6	6	7	.7	6	8	7

[a,b]Column means within each feedstuff with different superscripts differ ($P < .1$)

[1]n = 9

Whole plot tested for differences of level of AFE and replication and interactions between AFE and replication; AFE × replication was the whole-plot error term. The subplot model tested for differences between treatments (antimicrobial compounds) and interactions between AFE and treatments The residual error served as the error term for the subplot. Least squares means were separated using the protected least significant differences test when significant AFE or AFE × treatment effects were detected Significance was declared at $P < .1$.

RESULTS AND DISCUSSION

Addition of AFE had no effect on NDF or ADF degradation of pure cellulose, corn silage, low endophyte fescue, prairie hay, and wheat straw. The initial rate of degradation may have increased, but the overall degradation remained the same between control and AFE-treated fermentation (5, 6). However, AFE addition stimulated NDF and ADF degradations of alfalfa, bromegrass, and high endophyte fescue hay (Table 1). For alfalfa hay, NDF degradation was higher at .8 and 1.2 g/L, and ADF degradation was higher at 1 2 g/L of AFE than the control For bromegrass hay, NDF and ADF degradations were higher at .8 and 1 2 g/L of AFE than the control For high endophyte fescue, NDF and ADF degradations were higher at .4 and .8 g/L, but not at 1.2 g/L, of AFE (Table 1). Therefore, the influence of AFE on in vitro fiber degradability appears to depend on forage type; the reason for this variation is not known Gomez-Alarcon et al (8) reported that AFE increased the rate of in situ rumen fermentation of alfalfa hay, but not of sorghum grain or wheat straw. Why AFE stimulated degradability of high, but not low, endophyte fescue is not clear. However, the in vitro fermentation was not designed for direct comparison of high and low endophyte fescue hays.

In the second set of in vitro fermentations, only alfalfa, bromegrass, and high endophyte fescue hay substrates were tested with selective antimicrobial compounds Overall, the extents of NDF and ADF degradation (Tables 2 and 3) were similar for the WRF (entire microbial population) or WRF plus cycloheximide (bacterial population) treatments Fermentations treated with penicillin plus streptomycin (fungal population) had lower NDF and ADF degradations than treatments with WRF or WRF plus cycloheximide, which agrees with Windham and Akin (26), who reported that bacterial activity was responsible for a signifi-

cant portion of fiber degradation However, fungal activity alone was responsible for 25 to 33% of the NDF and 13 to 18% of the ADF degradabilities. This response may not reflect actual fungal contribution to fiber degradation because colonization of forages by fungi (as determined by sporangial counts on leaf blades) was substantially greater when bacterial activity was inhibited by antibacterial compounds, suggesting a possible biological interaction between fungi and bacteria (2) Antibacterial and antifungal compounds (penicillin plus streptomycin plus cycloheximide) did not completely inhibit microbial activity (negative control); NDF and ADF degradations ranged from 0 to 4% (Tables 2 and 3). The protozoal population may have been responsible for this small digestion, because some spe-

TABLE 2 Effect of Amaferm® supplementation on in vitro NDF degradability

	Substrate		
	Alfalfa hay	Bromegrass hay	High endophyte fescue hay
NDF Content in feedstuff, %	53 2	69 3	71 0
% of NDF Digested by			
Bacteria, fungi, and protozoa			
Whole rumen fluid (WRF)			
Amaferm®			
0 g/L	37 8[a]	55 4[a]	60 0[a]
.4 g/L	42 2[b]	56 8[ab]	64 3[b]
.8 g/L	42 3[b]	60 3[bc]	65 5[b]
1 2 g/L	43 0[b]	61 5[c]	59 2[a]
SE	5	6	6
Bacteria (WRF plus cycloheximide)			
Amaferm®			
0 g/L	32 1[a]	50 8[a]	57 0[b]
.4 g/L	37 8[b]	55 9[b]	62 4[b]
.8 g/L	37 6[b]	56 1[b]	64 9[b]
1 2 g/L	39 2[b]	56 3[b]	55 2[a]
SE	4	5	6
Fungi and protozoa (WRF, penicillin, and streptomycin)			
Amaferm®			
0 g/L	25 4	30 0	31 8
4 g/L	28 1	28 9	31 4
8 g/L	25 7	25 0	32 2
1 2 g/L	28 8	27 5	32 4
SE	8	9	1 1
Negative control (WRF, penicillin, streptomycin, and cycloheximide)			
Amaferm®			
0 g/L	3 2	0	0
4 g/L	4 0	0	3 7
8 g/L	3 5	3 6	0
1 2 g/L	2 8	3 0	2 3
SE	.3	2	2
Amaferm® alone			
Amaferm®			
0 g/L	0	0	0
4 g/L	<1	<1	<1
8 g/L	<1	<1	<1
1 2 g/L	<1	<1	<1

[a,b,c]Column means within each treatment with different superscripts differ ($P < .1$)

cies, particularly holotrichs, are not totally inhibited by cycloheximide (26) However, holotrichs are more likely involved in the degradation of the more soluble carbohydrate components of the feed

Addition of AFE to WRF fermentation increased NDF and ADF degradation of bromegrass and alfalfa hays (Tables 2 and 3) The increase ranged from 7 to 12% and from 12 to 15% for NDF and ADF degradation, respectively (Figure 1). Additionally, AFE at 4 or 8 g/L, but not at 1 2 g/L, increased NDF and ADF degradation of high endophyte fescue (Tables 2 and 3) Martin and Nisbet (13) reported that 4 g/L of AFE increased NDF and ADF digestion of bermudagrass, but 1.0 g/L AFE was detrimental Gomez-Alarcon et al. (9) reported increased digestibility of fiber in the rumen and total digestive tract of cows receiving AFE The AFE dose used in our in

TABLE 3 Effect of Amaferm® supplementation on in vitro ADF degradability

	Substrate		
	Alfalfa hay	Bromegrass hay	High endophyte fescue hay
ADF Content in feedstuff, %	37 5	42 6	41 1
% of ADF Digested by			
Bacteria, fungi, and protozoa Whole rumen fluid (WRF)			
Amaferm®			
0 g/L	25 7[b]	29.4[b]	31 0[b]
.4 g/L	28 7[a]	33.0[ab]	34 3[a]
8 g/L	28 8[a]	33 8[a]	34.5[a]
1 2 g/L	28 9[a]	34 3[a]	30.2[b]
SE	4	6	.5
Bacteria (WRF plus cycloheximide)			
Amaferm®			
0 g/L	24 1[b]	26 8[b]	29 0[b]
.4 g/L	28 5[a]	30 6[a]	32 4[a]
8 g/L	27 2[ab]	33 1[a]	33 9[a]
1 2 g/L	27 6[a]	33 3[a]	29 2[b]
SE	3	.2	.3
Fungi and protozoa (WRF, penicillin, and streptomycin)			
Amaferm®			
0 g/L	13 5	15 1	16 8
4 g/L	13 9	16 8	18 0
8 g/L	14 0	15 7	17 2
1 2 g/L	14 3	14 3	15 9
SE	5	.6	.7
Negative control (WRF, penicillin, streptomycin, and cycloheximide)			
Amaferm®			
0 g/L	1 2	0	0
4 g/L	1 0	0	1 5
8 g/L	5	.6	5
1 2 g/L	0	0	1 3
SE	1	1	2
Amaferm® alone			
Amaferm®			
0 g/L	0	0	0
4 g/L	<1	<1	<1
8 g/L	<1	<1	<1
1 2 g/L	<1	<1	<1

[a,b]Column means within each treatment with different superscripts differ ($P < 1$)

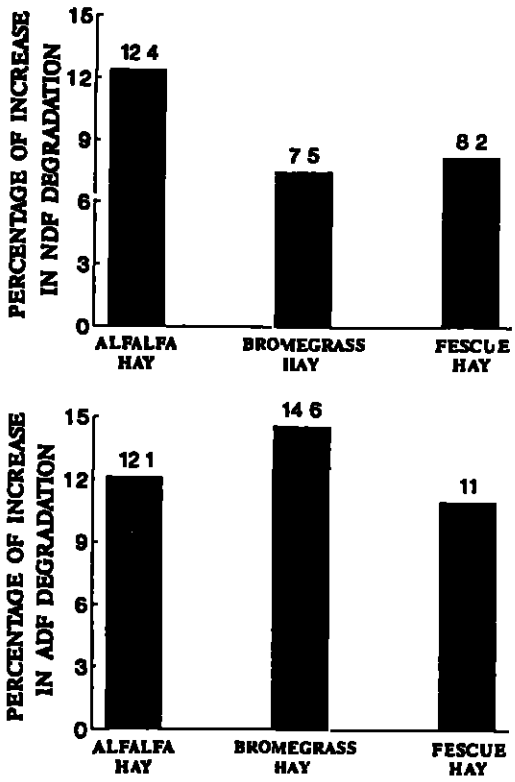

Figure 1. The mean percentage of increase in NDF and ADF degradation with supplementation of 4, 8, or 1 2 g/L of Amaferm® fermentation mixture for alfalfa and bromegrass hay substrates and 4 or 8 g/L of Amaferm® for high endophyte fescue hay substrate

vitro study was similar to that used by Martin and Nisbet (13) However, these doses are higher (four- to eightfold) than the current recommended dose of 2 to 6 g/d per animal in production ruminant diets. The rumen inoculum for our in vitro fermentations was from a steer that was not fed AFE These data agree with results of Gomez-Alarcon et al (8), but not with those of Arambel et al. (3), who reported that, in order to demonstrate an AFE effect, the rumen inoculum had to be from animals adapted to AFE.

The addition of .4, 8, or 1 2 g/L AFE to the WRF plus cycloheximide (bacteria) treatment increased NDF and ADF degradation of alfalfa and bromegrass hays, and addition of 4 or 8 g/L of AFE increased NDF and ADF degradation of high endophyte fescue (Tables 2 and 3)

Addition of AFE to the WRF plus penicillin plus streptomycin treatment had no effect on NDF or ADF degradation, which suggests that AFE had no effect on fungal or protozoal activity Frumholtz et al. (6) reported that the protozoal population decreased with the addition of AFE to the rumen simulator fermenter (Rusitec®). However, the protozoal population in Rusitec® is often lower and less stable than that in vivo (10). Additionally, AFE had no effect on the growth of pure cultures of rumen fungi *Neocallimastix frontalis, Neocallimastix patriciarum,* and *Piromonas communis* (17) Therefore, the enhanced fiber degradation by AFE was attributable to its stimulation of bacterial activity. However, interactions between fungi and bacteria may enhance the fibrolytic activity of fungi (16) and may be the reason for the trend ($P = .14$) for higher NDF digestion with WRF than with WRF plus cycloheximide for the alfalfa hay and bromehay substrates (Table 2).

The AFE contains few live cells (17) and likely could not have grown in the in vitro conditions provided. However, *A oryzae* possesses a wide range of enzymatic activities (19), including carboxymethycellulase activity (12), which could have facilitated fiber digestion. Addition of AFE alone (buffer without WRF) did not degrade any of the substrates provided. Therefore, the effect of AFE on fiber degradation was mediated through bacterial activity in the rumen The AFE increased total anaerobic and cellulolytic bacterial numbers in vitro and in vivo (6, 25) Also, Beharka et al (4) reported that calves supplemented with AFE had higher counts of fiber-digesting rumen bacteria than unsupplemented calves The reasons for bacterial stimulation by AFE include rumen pH stabilization (6), enhanced nutrient uptake (18), and provision of some unknown growth factors (13).

CONCLUSIONS

The AFE appeared to stimulate NDF and ADF degradation of certain feedstuffs, including both legumes and grasses This increase in degradability appeared to be a consequence of stimulation of bacterial activity and not of fungal or protozoal activities.

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#54 EFFECTS OF Aspergillus oryzae EXTRACT (AMAFERM) ON RUMINAL FIBROLYTIC BACTERIA AND IN VITRO FIBER DEGRADATION. A.A. Beharka* and T.G. Nagaraja, Dept. of Animal Sci., Kansas State University, Manhattan, Kansas 66506 (613-532-5654)

The effect of Amaferm on growth of pure cultures of ruminal cellulolytic, hemicellulolytic and pectinolytic bacteria (*Fibrobacter succinogenes*, *Butyrivibrio fibrisolvens*, *Eubacterium cellulosolvens*, *Ruminococcus flavefaciens*, *R. albus*, *Prevotella* (*Bacteroides*) *ruminicola*, and *Lachnospira multiparus*) was determined. Bacteria were grown in anaerobic, complete carbohydrate rumen fluid medium with filter-sterilized Amaferm at 0, 2 or 5% of the medium. The medium was inoculated with late-log-phase culture and growth was monitored by measuring absorbance. The addition of Amaferm to the medium increased (P<.1) the specific growth rate of *Ruminococcus albus* (.71 vs .61) and *Fibrobacter succinogenes* (.35 vs .26). Amaferm had no effect on growth of other fibrolytic bacteria. Selective antimicrobial compounds (penicillin, streptomycin, and cycloheximide) were used to assess the influence or Amaferm on bacterial and fungal contributions to in vitro fiber degradation. A variety of ground, fibrous substrates (0.5g) were incubated with ruminal fluid inoculum (1:2 ruminal fluid to buffer). Amaferm was added at 0, .4, .8 or 1.2 g/l. NDF and ADF digestibilities were determined after 96 h incubation. Addition of Amaferm increased (P<.l) NDF and ADF digestion of brome, and alfalfa hay. Amaferm addition at .4 or .8 g/l, and not 1.2 g/l, increased NDF and ADF digestion of high endophyte fescue. The enhanced fiber degradation by Amaferm was attributed to its stimulation of bacterial activity Amaferm did not appear to stimulate fungal activity. Addition of Amaferm had no effect on NDF or ADF digestion of pure cellulose, low endophyte fescue, wheat straw, corn silage and prairie hay. In conclusion, Amaferm appears to stimulate NDF and ADF digestibility of certain feedstuffs and this increase in digestibility maybe a consequence of growth stimulation of some fibrolytic bacteria.

INTRODUCTION

1. Amaferm supplementation has been reported to:
   - increase fiber digestibility.
   - increase total and fibrolytic bacterial numbers.
   - increase VFA concentration.
2. It has been proposed that Amaferm supplementation may increase the nutritive value of feedstuffs by increasing the digestion of dietary fiber.
3. Little work has been done to determine which fibrolytic bacteria are being stimulated.
4. The effect of Amaferm on the ruminal protozoa and fungi populations is unknown.
   - The fungal population has been shown to have high fiber digesting ability.
   - Inhibition of the protozoa population which can prey on bacteria may account for increased bacterial numbers.

OBJECTIVE

To determine the effect of Amaferm on the growth rate of selected pure cultures of ruminal bacteria, with and without antimicrobial compounds and on the extent of degradation of forage components by the different microbial populations.

PROCEDURES

A. THE EFFECT OF AMAFERM ON BACTERIAL GROWTH

1. Pure cultures of ruminal bacteria were grown in anaerobic, complete carbohydrate rumen fluid medium with filter sterilized Amaferm at 0, 2 or 5% of the medium.
2. The medium was inoculated with a late-log-phase culture.
3. Growth was monitored by measuring absorbance.

B. THE INFLUENCE OF AMAFERM ON BACTERIAL AND FUNGAL CONTRIBUTION TO IN VITRO FIBER DEGRADATION

1. A variety of ground fibrous substrates (alfalfa hay, brome hay, high and low endophyte fescue hay, pure cellulose, wheat straw, corn silage and prairie hay (0.5g) were incubated with ruminal fluid inoculum (1:2 rumen fluid to buffer).
2. Amaferm was added at 0, 4, 8 or 1.2 g/l.
3. Selective antimicrobial compounds were added: penicillin G (P) streptomycin sulfate (S) and cycloheximide (C).
4. Treatements were as follows (in triplicate):
   a. substrate + buffer (B)
   b. substrate + rumen fluid (RF) + B
   c. substrate + RF + B + Amaferm
   d. substrate + B + Amaferm
   e. substrate + RF + B + P + S
   f. substrate + RF + B + P + S + Amaferm
   g. substrate + RF + B + C
   h. substrate + RF + B + C + Amaferm
   i. substrate + RF + B + P + S + C
   j. substrate + RF + B + P + S + C + Amaferm
5. NDF and ADF digestibilities were determined after 96 H.

EFFECT OF AMAFERM ON BACTERIAL SPECIFIC GROWTH RATE

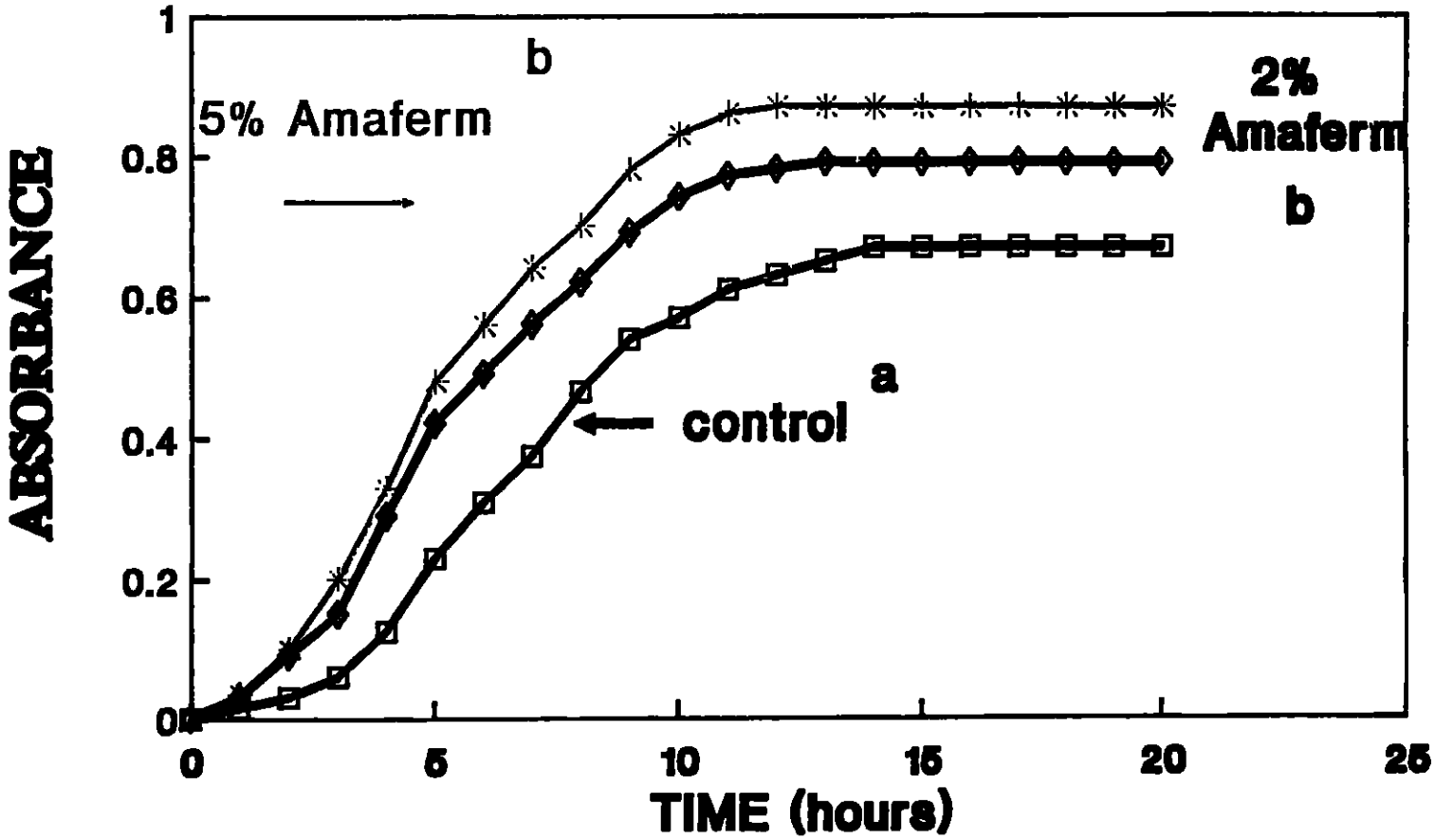
BACTERIAL CULTURE	Effect of Amaferm
Anaerovibrio lipolytica	0
Bacteroides amylophilus	0
Bacteroides (Prevotella) ruminicola	0
Bifidobacterium globosum	0
Butyrivibrio fibrisolvens	0
Eubacterium cellulosolvens	0
Fibrobacter succinogenes	+
Fusobacterium biotype A	0
Fusobacterium biotype B	0
Lachnospira multiparus	0
Lactobacillus vitulinus	0
Lactobacillus ruminis	0
Megasphaera elsdenii	+
Ruminococcus albus	+
Ruminococcus flavefacians	0
Streptococcus bovis	0
Selenomonas ruminantium	+
Veillonella alcalescens	0

Effect Of Amaferm Supplementation On In Vitro NDF Digestion With Antimicrobial Compounds.

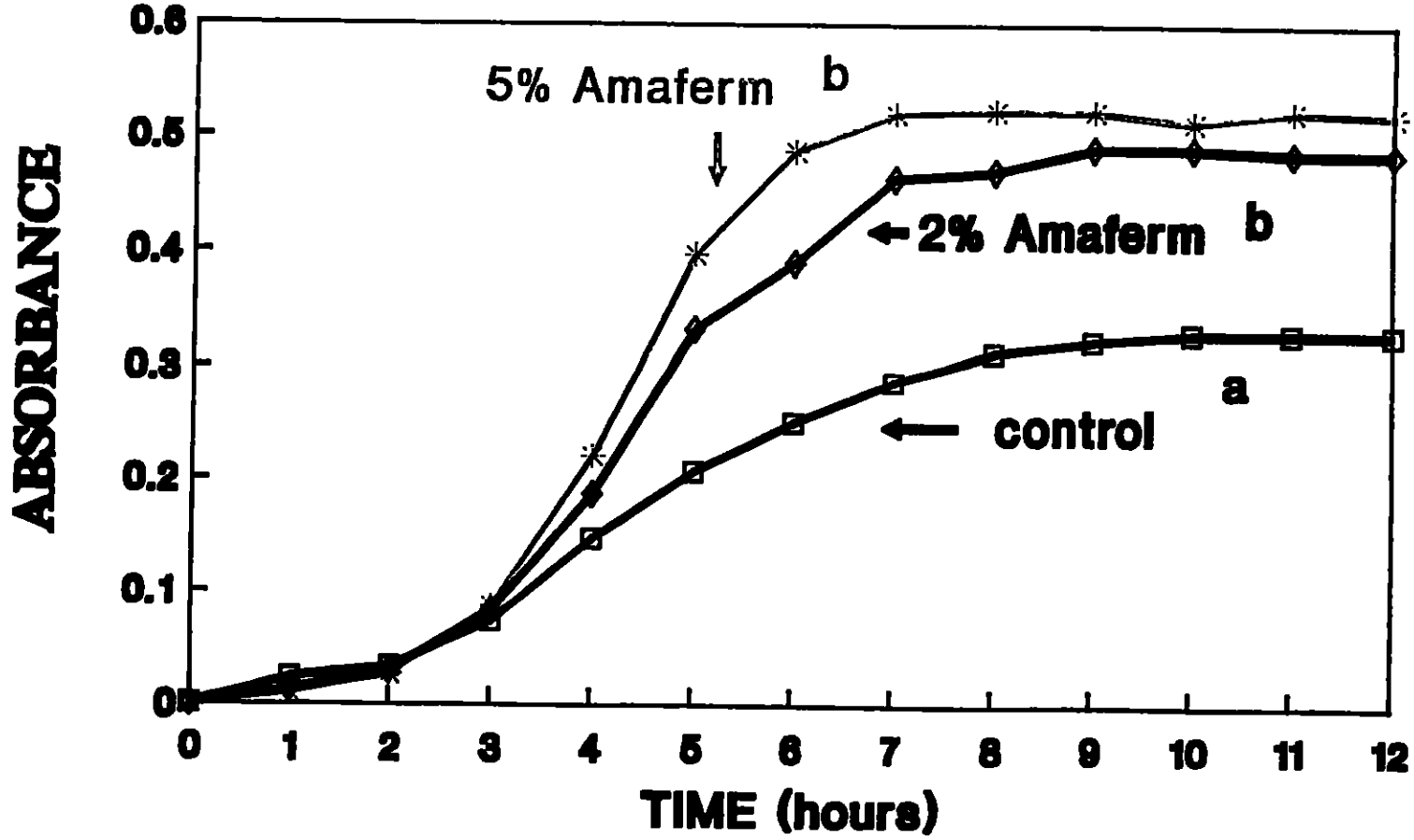
	FEEDSTUFF		
	alfalfa	brome	fescue
% NDF in feedstuff	53.2	69.3	71.0
% NDF digested by:			
1. bacteria + fungi + protozoa (whole rumen fluid, WRF)			
no Amaferm	37.8[a]	55.4[a]	60.0
AO = 1.2 g/l	43.0[b]	61.5[b]	59.0
2. bacteria (WRF + C)			
no Amaferm	32.1[a]	50.8[a]	57.0
AO = 1.2 g/l	39.2[b]	56.3[b]	55.2
3. fungi & protozoa (WRF + penicillin & streptomycin)			
no Amaferm	25.4	30.0	31.8
AO = 1.2 g/l	28.8	27.5	32.4
4. negative control (WRF + P + S + C)			
no Amaferm	3.2	0	0
AO = 1.2 g/l	2.8	3	2.3
5. Amaferm alone (no RF)			
no Amaferm	0	0	0
AO = 1.2 g/l	<1	<1	<1

[ab] Means down a column with different superscripts differ (P<.1).

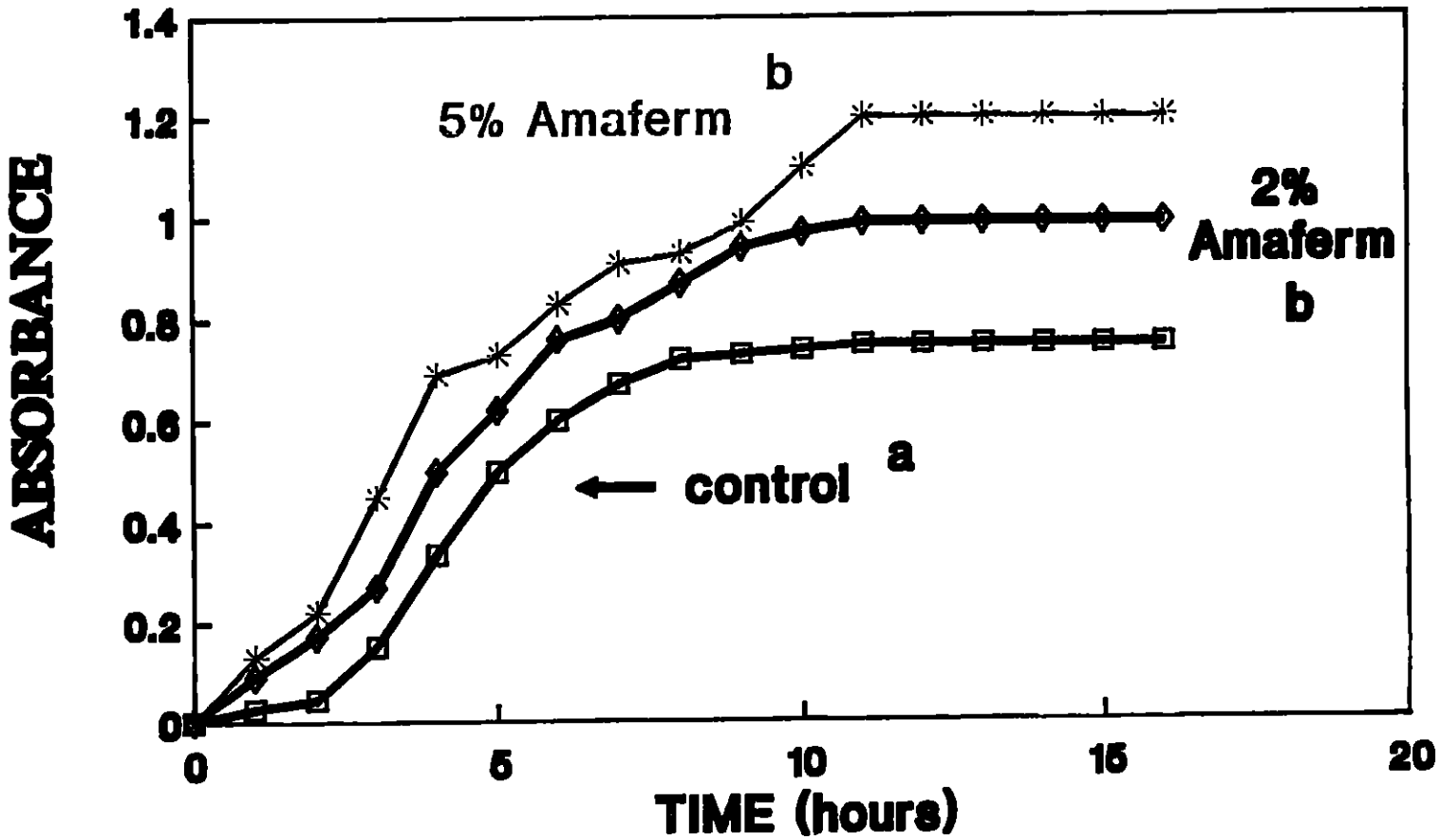
Effect of Amaferm on the specific growth rate of Megasphaera elsdenii. Lines with uncommon superscripts differ (P<.1)



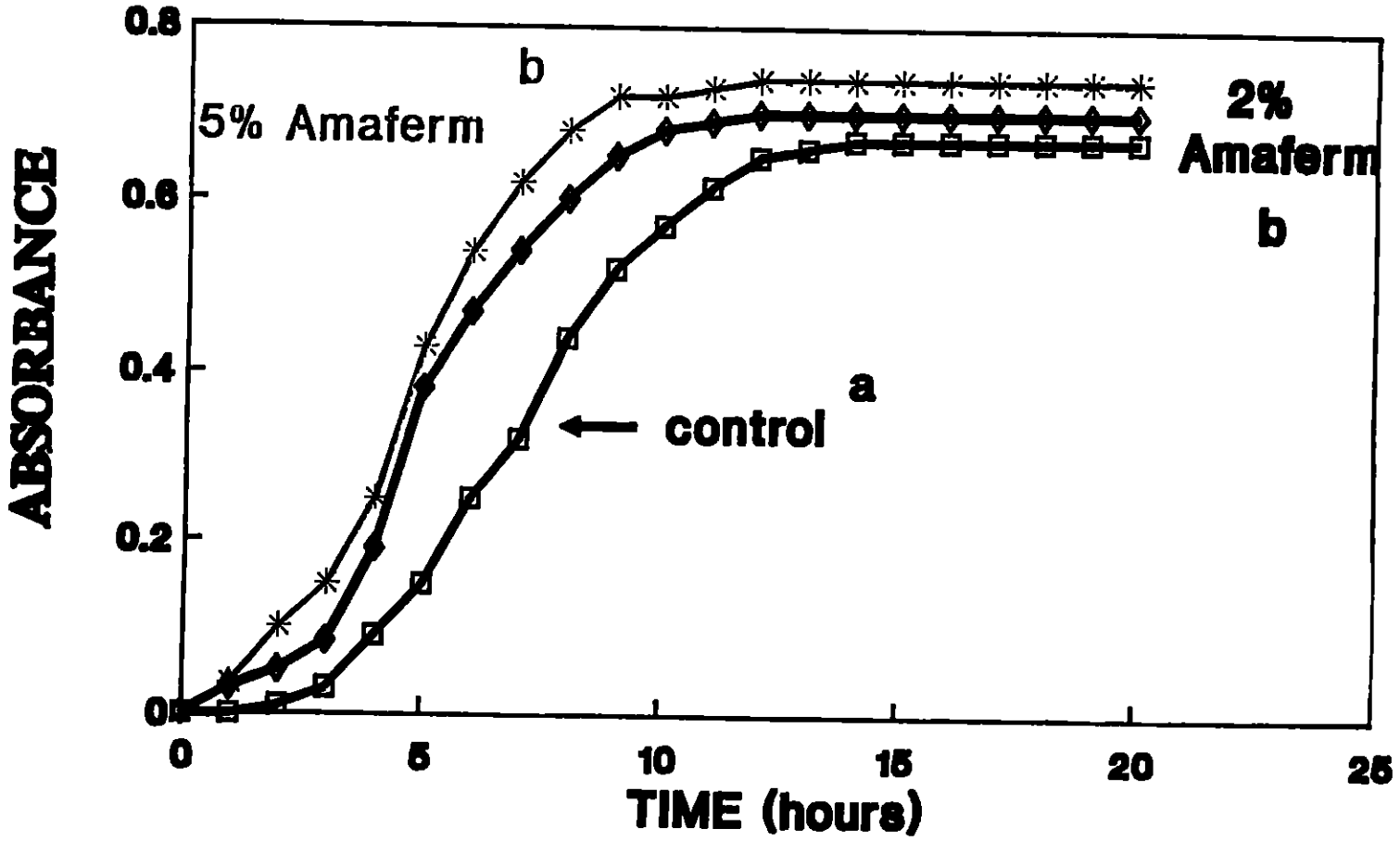
Effect of Amaferm on the specific growth rate of Ruminococcus albus. Lines with uncommon superscripts differ (P<.1).



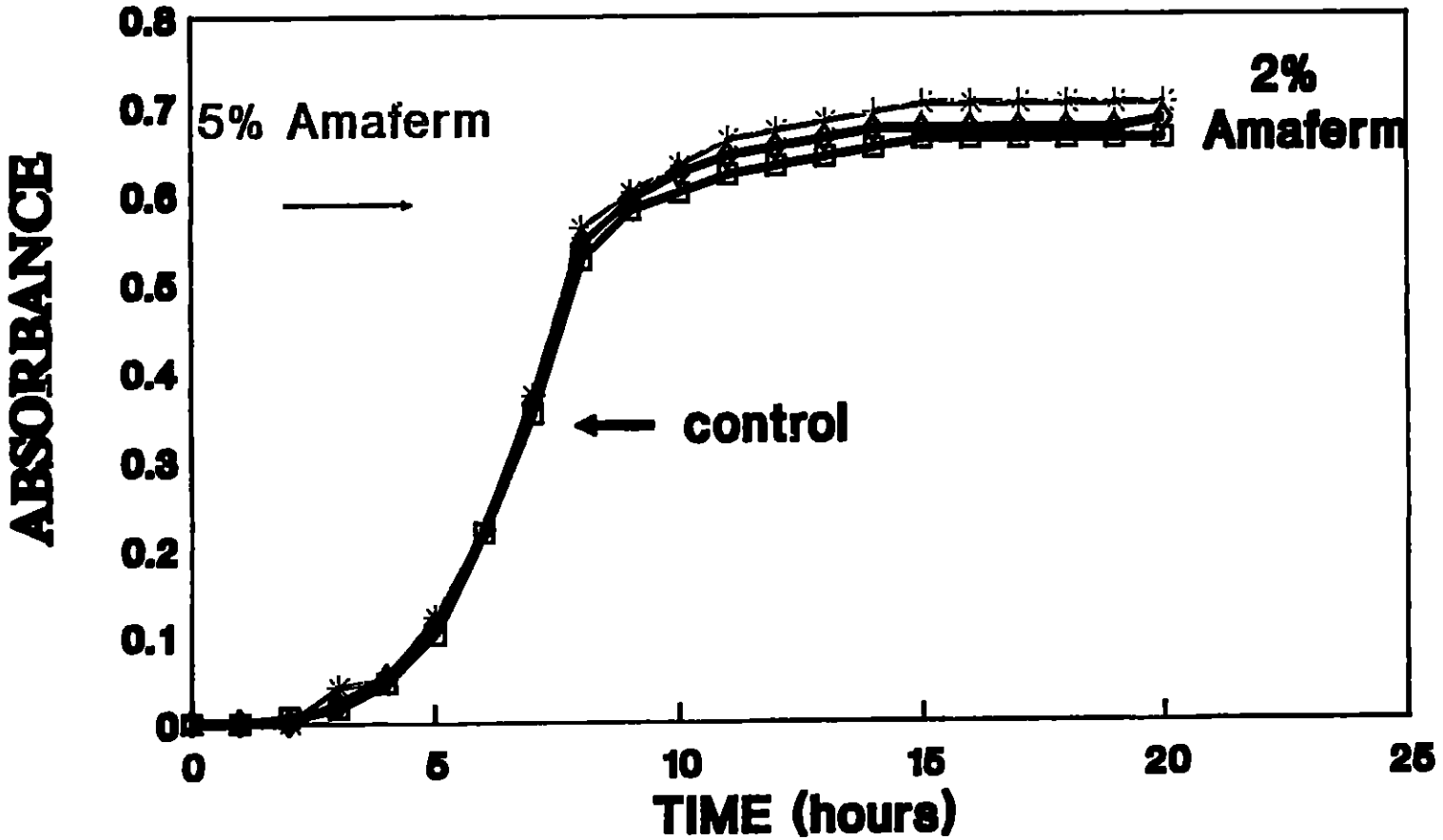
Effect of Amaferm on the specific growth rate of Selenomonas ruminantium. Lines with uncommon superscripts differ (P<.1)



Effect of Amaferm on the specific growth rate of Fibrobacter succinogenes. Lines with uncommon superscripts differ (P< 1)



Effect of Amaferm on the growth rate of Prevotella (Bacteroides) ruminicola. No treatment effect (P>.1).



SUMMARY

1. The addition of Amaferm to the growth medium increased (P<.1) the growth rate of the fibrolytic bacteria *Ruminococcus albus* (.71 vs .61) and *Fibrobacter succinogens* (.35 vs .26).
2. Amaferm had no effect on growth of other fibrolytic bacteria.
3. Additionally, Amaferm supplementation increased the growth rate of *Megasphaera elsdenii* (.44 vs .33) and some strains of *Selenomonas ruminantium* (.77 vs .67).
4. Amaferm increased (P<.1) NDF and ADF digestion of brome and alfalfa hay. Amaferm addition at .4 or .8 g/l and not 1.2 g/l increased NDF and ADF digestion of high endophyte fescue hay.
5. The enhanced fiber degradation by Amaferm was attributed to its stimulation of bacterial activity. Amaferm did not appear to stimulate fungal activity.
6. Addition of Amaferm had no effect on NDF or ADF digestion of pure cellulose, low endophyte fescue hay, wheat straw, corn silage and prairie hay.

In conclusion, Amaferm appears to stimulate NDF and ADF digestibility of only certain feedstuffs, and this increase in digestibility may be a consequence of growth stimulation of some fibrolytic bacteria.

TRIAL SUMMARY

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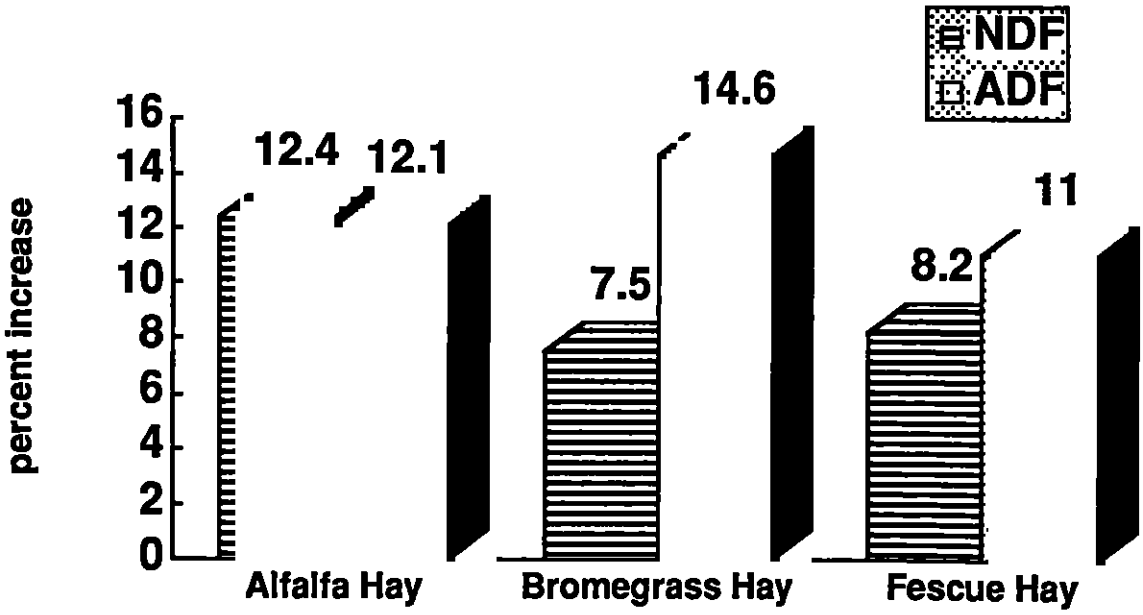
j. Dairy Science. 1993, 76:812-818

EFFECT OF ASPERGILLUS ORYZAE FERMENTATION EXTRACT *(AMAFERM)* ON IN VITRO FIBER DEGREDATION

- AMAFERM was added at levels of 0, 0.4, 0.8 or 1.2 gm/L to the fermentation mixture containing one of 8 ground fiberous feedstuffs.
- NDF and ADF degradabilities were determined after a 96 hour incubation.
- AMAFERM increased NDF and ADF degredations of bromegrass hay, alfalfa hay and high endophyte fescue hay.
- The enhanced fiber degredation by AMAFERM is attributed to its stimulation of bacterial activity.
- AMAFERM alone has no effect on fiber digestion.

Effect of Aspergillus oryzae Extract (AMAFERM) on In Vitro Fiber Degradation



Supplemented with 0.4, 0.8 or 1.2 g/L of AMAFERM.
Alfalfa significantly better at all levels at P< 10
Bromegrass significantly better at 0.8 and 1.2 levels at P<.10
High endophyte fescue significantly better at 0.4 and 0 8 levels at P<.10.