Available online at www.sciencedirect.com
ScienceDirect
Animal Feed Science and Technology
145 (2008) 136–150
ANIMAL FEED SCIENCE AND TECHNOLOGY
www.elsevier.com/locate/anifeedsci

Dietary supplementation of ruminant diets with an *Aspergillus oryzae* α-amylase☆

J.M. Tricarico[a,*], J.D. Johnston[b], K.A. Dawson[a]

[a] *Alltech Biotechnology Inc., Nicholasville, KY 40356, USA*
[b] *Ritchie Feed & Seed Inc., Ottawa, Ont. K1B 4V5, Canada*

Accepted 27 April 2007

Abstract

Research in the area of dietary enzyme supplements for ruminant diets has primarily focused on fibrolytic enzymes, while activities involved in the process of starch digestion have been largely ignored. Since starch represents a major component in diets fed to highly productive cattle, the use of enzymatic dietary supplements to manipulate starch digestion in the rumen may allow improved productivity. This review discusses current information on dietary supplementation of calf, dairy and beef cattle diets with an *Aspergillus oryzae* extract containing α-amylase activity. During starch hydrolysis, α-amylase randomly cleaves starch polymers to low molecular weight oligosaccharides and eventually produces maltotriose and maltose from amylose and α-limit dextrins, maltose and glucose from amylopectin. Through its hydrolytic action, supplemental α-amylase hypothetically increases the availability of starch hydrolysis products in the rumen consequently altering the ruminal fermentation process. Data from studies employing lactating dairy cows, steers or rumen-simulating continuous cultures suggest that supplemental α-amylase did not increase ruminal starch digestion but consistently increased butyrate and reduced propionate molar proportions in the rumen. The increase in ruminal butyrate was also reflected in higher blood β-hydroxybutyrate concentrations in both transition and lactating dairy cows. In addition, supplemental α-amylase enhanced ruminal

Abbreviations: ADG, average daily gain; CP, crude protein; DHI, dairy herd improvement; DIM, days in milk; DM, dry matter; DP, degree of polymerization; DU, dextrinizing unit; NDF, neutral detergent fibre; OM, organic matter.

☆ This paper is part of a special issue entitled "Enzymes, Direct Fed Microbials and Plant Extracts in Ruminant Nutrition" guest edited by R. J. Wallace, D. Colombatto and P. H. Robinson.

* Corresponding author at: 3031 Catnip Hill Pike, Nicholasville, KY 40356, USA. Tel.: +1 859 885 9613; fax: +1 859 887 3233.
E-mail address: jtricarico@alltech.com (J.M. Tricarico).

0377-8401/$ – see front matter
doi:10.1016/j.anifeedsci.2007.04.017

epithelium growth in dairy calves, a tissue that preferentially uses butyrate as an energy source. Experiments with pure cultures of ruminal bacteria showed that supplemental α-amylase supported rapid growth of bacteria that cannot grow, or grow slowly, on starch such as *Butyrivibrio fibrisolvens* D1, *Selenomonas ruminantium* GA192 or *Megasphaera elsdenii* T81. In contrast, bacteria that grow rapidly on starch, such as *Streptococcus bovis* S1 or *Butyrivibrio fibrisolvens* 49, did not benefit from α-amylase supplementation. Animal performance studies showed higher weight gain, and longissimus muscle area, in finishing beef cattle fed supplemental α-amylase. Weight gain improvements were primarily mediated through increased dry matter intake, which may be a consequence of reduced ruminal propionate molar proportions reported in other studies. In lactating dairy cattle, supplemental α-amylase increased milk yield, reduced milk fat proportion without reducing milk fat yield and tended to improve milk protein yield when data from 45 commercial herds (approximately 8150 cows) were examined. Currently available data on effects of the *Aspergillus oryzae* α-amylase described here suggest that this enzyme supplement may improve animal productivity by modifying ruminal starch digestion without necessarily increasing starch digestion in the rumen.

Keywords: α-Amylase; *Aspergillus oryzae*; Ruminants

1. Introduction

Digestive processes in livestock and poultry are mediated through the action of enzymes. This has generated interest in using exogenous enzyme preparations as dietary supplements to improve animal productivity by researchers and practicing nutritionists. Successful application of dietary enzyme supplement technology was first achieved with monogastric animals and it is current practice in poultry and pig nutrition (Bedford and Schulze, 1998). Nonetheless, the microbial fermentation process in the rumen has made adoption of dietary enzyme technology more challenging in ruminants.

1.1. Dietary enzymes for ruminants

The vast majority of research in the area of exogenous enzyme supplements for ruminants has focused on fibrolytic enzyme preparations and fiber digestion. Increased ruminal fiber digestion may largely explain improvements in ruminant productivity resulting from dietary supplementation with cell wall-degrading enzymes. Discussion of fibrolytic enzyme supplements is outside the scope of this manuscript and the reader is referred to excellent reviews on this subject by McAllister et al. (2001) and Beauchemin et al. (2003). The list of ruminal effects implicated in the potential mode of action for exogenous enzymes proposed in these reviews include direct hydrolysis, increased ruminal microbial attachment, stimulation of ruminal microbial populations, and synergism with ruminal microbial enzymes. Some of these factors may also have a critical role in the potential mode of action for supplemental α-amylase.

1.2. Exogenous amylases in ruminant diets

Unlike cell wall-degrading enzymes, exogenous amylases have received little attention by ruminant nutritionists. The general perception is that starch digestion by ruminants is

extensive and does not generally limit production in the way that incomplete or slow fiber digestion does. In addition, rapid digestion of excessive amounts of starch in the rumen may lead to ruminal acidosis (Owens et al., 1998) representing a potential concern for inclusion of exogenous amylases in ruminant diets. Consequently, supplementation with exogenous amylases to increase ruminal starch digestion is not warranted. Nonetheless, we hypothesize that exogenous supplemental amylases could be employed to reduce the considerable unexplained variation in ruminal starch degradation within dietary starch sources (Firkins et al., 2001).

It is not surprising that the potential for amylase supplementation in ruminant diets has been rarely studied and the absence of information on this topic is apparent in the literature. Early studies included amylase in combination with other enzyme activities, none of which were characterized (Burroughs et al., 1960). In more recent studies, amylase activities only represented a minor component of primarily microbial (McGilliard and Stallings, 1998) or predominantly fibrolytic preparations (McAllister et al., 1999; Hristov et al., 2000), while other studies used undefined preparations (Chen et al., 1995) or thermostable α-amylase from *Bacillus licheniformis* (Rojo-Rubio et al., 2001; Mora-Jaimes et al., 2002, Rojo et al., 2005) with the specific objective of increasing starch digestion in sorghum.

The objective of this review is to present and discuss information collected over the last four years on dietary supplementation of calf, dairy and beef cattle diets with an *Aspergillus oryzae* extract primarily containing α-amylase activity.

2. Exogenous α-amylase from *Aspergillus oryzae*

2.1. Exogenous α-amylase characteristics

The dietary amylase supplement (Amaize^{TM}, Alltech Inc., Nicholasville, KY, USA) discussed in this review is based on a powdered *Aspergillus oryzae* extract that contains primarily α-amylase or 1,4-α-D-glucan glucanohydrolase (EC 3.2.1.1) activity. One α-amylase dextrinizing unit (DU) is defined as the quantity of enzyme required to dextrinize soluble starch at the rate of 1 g/h at 30 °C and pH 4.8 according to the procedure described in the Food Chemicals Codex (1996). Final α-amylase concentration in the *Aspergillus oryzae*-based supplement is 600 DU/g. Protease, cellulase and xylanase actvities, determined with hemoglobin, carboxymethyl-cellulase, and brichwood xylan as described by Tricarico et al. (2005), are negligible in this preparation.

2.2. Effects of supplemental Aspergillus oryzae α-amylase on dairy and beef cattle production

Dietary supplementation with the *Aspergillus oryzae* α-amylase preparation improved dairy cattle performance (Table 1). A quadratic increase (P=0.02) in milk production was initially reported by Tricarico et al. (2005) in lactating dairy cows fed a corn grain based diet. These researchers reported a maximum milk yield at 240 DU/kg of dietary dry matter (DM) with an increase of 1.5 kg/d over the non-supplemented control. DeFrain et al. (2005) reported a higher (P=0.03) decrease in DM intake from week 2 to week 1 prepartum, but

Table 1
Effects of supplemental α-amylase from *Aspergillus oryzae* on milk production and composition in lactating dairy cattle (adapted from DeFrain et al., 2005; Tricarico et al., 2005)

	Supplement		
	Control	α-Amylase	Change
DeFrain et al. (2005)[a]			
Number of animals	12	12	
α-Amylase activity (DU/kg DM)	0	662	
Milk yield (kg/d) d 1–70	43.6	44.2	+0.6
Milk yield (kg/d) d 1–21	17.8	17.8	−0.1
Milk fat (g/kg) d 1–21	47.8	41.8	−0.6
Tricarico et al. (2005)[b]			
Number of animals	24	24	
α-Amylase activity (DU/kg DM)	0	240	
Milk yield (kg/d)[c]	29.2	30.7	+1.5
Milk fat (g/kg)	37.1	37.8	+0.7

[a] Supplemental α-amylase was fed from d −21 to 21. DM intake (kg/d) were 12.5 (control) and 11.9 (α-amylase) from d −21 to d 0, and 17.8(control) and 17.7 (α-amylase) from d 1 to d 21.
[b] Supplemental α-amylase was fed at 0, 240, 480 and 720 DU/kg dietary DM. Only data from 0 and 240 DU/kg dietary DM are presented. DMI (kg/d) was 22.7 (control) and 22.9 (α-amylase).
[c] Quadratic effect of α-amylase supplementation (P=0.02).

no differences in early lactation milk production up to 70 days in milk (DIM) in dairy cows fed supplemental α-amylase at 662 DU/kg of dietary DM during the transition period (−21 to 21 DIM).

Dietary supplementation with the *Aspergillus oryzae* α-amylase preparation also improved finishing beef cattle performance in two studies (Tricarico et al., 2007). In both instances, the largest improvements in average daily gain (ADG) occurred during the initial 28 d on feed (Table 2), although overall carcass-adjusted ADG also increased in one experiment. Increases in ADG were apparently a consequence of increased feed intake in both instances and in one experiment the response was predominantly quadratic. Increased DM intake as a result of α-amylase supplementation in finishing beef cattle, but not in lactating dairy cattle, may be a function of different dietary and animal factors in these two production systems. Interestingly, quadratic responses to α-amylase supplementation occurred in lactating dairy and finishing beef cattle and are in general agreement with the frequent occurrence of non-linear responses to exogenous enzyme supplementation that have been reported (*e.g.*, Beauchemin et al., 2003). Other interesting observations are that supplemental α-amylase increased intake and gain with both high moisture and cracked corn in one experiment, but only with cottonseed hulls and not with alfalfa hay in another. The lack of interaction between α-amylase supplementation and corn grain processing suggests no increase in ruminal starch digestion in these studies and that our initial hypothesis that supplemental α-amylase would be more efficacious in diets containing more resistant starch (cracked *versus* high moisture corn) was incorrect. Reasons for the lack of effects of supplemental α-amylase in the alfalfa hay diet are unknown. However, positive responses to α-amylase supplementation reported by Tricarico et al. (2005) in lactating cows and by DeFrain et al. (2005) in prepartum cows occurred with diets con-

Table 2
Effects of supplemental α-amylase from *Aspergillus oryzae* on performance and carcass characteristics in finishing beef cattle (adapted from Tricarico et al., 2007)

	Supplement		
Item[a]	Control	α-Amylase	Change
Exp.1[b]			
Pens per treatment	6	6	
α-Amylase activity (DU/kg DM)	0	950	
DM intake (kg/d)			
d 0–28	7.53	7.84	+0.31
d 0 to end	7.61	7.93	+0.32
ADG (kg/d)			
d 0–28	1.64	1.88	+0.24
Carcass-adjusted to end	1.30	1.42	+0.12
LM area (cm^2)	82.72	88.96	+6.24
Yield grade	2.99	2.89	−0.10
Exp.2[c]			
Pens per treatment	4	4	
α-Amylase activity (DU/kg DM)	0	580	
DM intake (kg/d)			
d 0–28	8.04	9.05	+1.01
d 0 to end	9.13	10.06	+0.93
ADG (kg/d)			
d 0–28	1.98	2.30	+0.32
Carcass-adjusted to end	1.87	2.05	+0.18
LM area (cm^2)	80.04	85.06	+5.02
Yield grade	2.29	2.05	−0.24

[a] Used implants and ionophores (monensin sodium) in both experiments.

[b] Exp. 1 used 120 crossbred steers (5 steers/pen) fed alfalfa hay or cottonseed hulls finishing diets for an average 152 d from d 0 to end (slaughter). Only data from cottonseed hulls diets are presented. Main effects of α-amylase on: DMI d 0–28 (P=0.10) and d 0 to end (P=0.27); ADG d 0–28 (P=0.06) and carcass-adjusted to end (P=0.44); LM area (P=0.02). Roughage × α-amylase interaction on: DMI d 0–28 (P=0.85) and d 0 to end (P=0.20); ADG d 0–28 (P=0.02) and carcass-adjusted to end (P=0.11); LM area (P=0.31).

[c] Exp. 2 used 96 crossbred heifer (4 heifers/pen) fed cracked or high moisture corn finishing diets for an average 81 d from d 0 to end (slaughter). Combined data from cracked and high moisture corn diets are presented. Main effects of α-amylase on: DMI d 0–28 (P=0.05) and d 0 to end (P=0.12); ADG d 0–28 (P=0.03) and carcass-adjusted to end (P=0.04); LM area (P=0.12); yield grade (P=0.02). Quadratic effects of α-amylase on: DMI d 0–28 (P=0.06) and d 0 to end (P=0.07); ADG d 0–28 (P=0.05) and carcass-adjusted to end (P=0.04); LM area (P=0.04); yield grade (P=0.02).

taining alfalfa hay or haylage at 300 and 210 g/kg of total forage fed. In contrast, no effects were reported in postpartum cows by DeFrain et al. (2005) or on ruminal fermentation by Hristov et al. (2008) when α-amylase was included in diets containing alfalfa hay or haylage at 450 and 620 g/kg of total forage fed. These observations suggest that a negative interaction may exist between alfalfa and supplemental α-amylase and that additional research is needed to examine potential interactions between exogenous α-amylase and dietary ingredients.

2.3. Effects of supplemental Aspergillus oryzae α-amylase on digestion

Dietary supplementation with the *Aspergillus oryzae* α-amylase preparation apparently does not increase ruminal starch digestion. Ruminal *in situ* starch digestion did not increase in steers or lactating dairy cows fed corn grain based diets containing ensiled forage (Tricarico et al., 2005). Similarly, Hristov et al. (2008) reported no effects on ruminal and total tract starch digestion in lactating dairy cows fed corn grain, barley grain, and alfalfa and grass hay in a more recent study using the same exogenous enzyme preparation. Finally, the possibility that supplemental α-amylase increases intestinal starch digestion is not likely, since the enzyme is inactivated by gastric digestion (M.D. Abney and M.L. Galyean, Texas Tech University, Lubbock, TX, personal communication).

Chen et al. (1995) used a commercial amylase and protease mixture, with the objective of increasing starch digestion in dairy cattle fed sorghum grain, and reported no effects of enzyme treatment on milk production or starch digestion, but observed higher DM, organic matter (OM), crude protein (CP) and neutral detergent fibre (NDF) total tract digestibility in enzyme-treated steam flaked sorghum grain. Unfortunately, a description of the specific amylase present in the mixture, or its concentration, was not provided. Neither Tricarico et al. (2005) nor Hristov et al. (2008) reported changes in ruminal NDF digestion with supplemental α-amylase from *Aspergillus oryzae*. In contrast, supplementation with a thermostable α-amylase from *Bacillus licheniformis* increased ruminal starch digestion both *in vitro* (Rojo-Rubio et al., 2001) and in lambs (Mora-Jaimes et al., 2002; Rojo et al., 2005). Comparison of our results with those reported by these researchers is complicated by the use of different source microorganisms (*Aspergillus oryzae versus Bacillus licheniformis*), enzyme assay conditions, unit definitions, diet composition (primarily corn grain *versus* sorghum grain) and animal species used (*i.e.*, bovines *versus* lambs).

2.4. Effects of supplemental Aspergillus oryzae α-amylase on ruminal fermentation and metabolite concentrations

Dietary supplementation with the *Aspergillus oryzae* α-amylase preparation increased acetate and butyrate and reduced propionate molar proportions (Table 3) in steers, lactating dairy cows, and ruminal-simulating continuous cultures (Tricarico et al., 2005). Supplemental α-amylase also numerically increased ruminal butyrate molar proportions in prepartum dairy cows (DeFrain et al., 2005). Consequently, α-amylase supplementation increased the acetate to propionate ratio; an increase that is not indicative of increased ruminal starch digestion. Benefits of increasing ruminal butyrate molar proportions relative to ruminant productivity are not apparent. However, a review summarizing data from 20 studies reported that ruminal concentrations of butyrate, followed by propionate, had the strongest positive correlation with milk production (Seymour et al., 2005).

The increases in ruminal butyrate molar proportions were also accompanied by increases in serum concentrations of β-hydroxybutyrate and non-esterified fatty acids (Table 4) in prepartum (DeFrain et al., 2005) and lactating dairy cows (Tricarico et al., 2005). Concomitant increases in blood β-hydroxybutyrate, and decreases in blood glucose, have been reported (Huhtanen et al., 1993; Miettinen and Huhtanen, 1996). Nonetheless, α-amylase supplementation did not reduce serum glucose concentrations in lactating cows

Table 3
Effects of supplemental α-amylase from *Aspergillus oryzae* on ruminal butyrate molar proportions and the acetate to propionate (A:P) ratio in lactating dairy cows, steers, continuous cultures (adapted from Tricarico et al., 2005) and transition dairy cows (adapted from DeFrain et al., 2005)

	Supplement		
	Control	α-Amylase	Change
Tricarico et al. (2005)[a]			
Butyrate (mmol/mol)[b]			
Lactating cows	129	141	+12
Steers	128	146	+18
Continuous cultures	178	193	+15
A:P[c]			
Lactating cows	2.85	3.15	+0.30
Steers	2.90	4.08	+1.18
Continuous cultures	1.68	1.75	+0.07
DeFrain et al. (2005)[d]			
Butyrate (mmol/mol)[e]			
Prepartum	91	100	+9
Postpartum	103	110	+7
A:P[f]			
Prepartum	3.53	3.53	0
Postpartum	2.99	2.75	−0.24

[a] Supplemental α-amylase was provided at: 0, 240, 480 and 720 DU/kg dietary DM to lactating cows (data from 0 and 240 DU/kg dietary DM are presented); 0, 360 and 720 DU/kg dietary DM to steers (averages for 1–11 h after feeding from 0 and 360 DU/kg dietary DM are presented); 0 and 1200 DU/kg dietary DM to continuous cultures (averages for 72–120 h are presented).
[b] Significance for butyrate molar proportions: main effects of α-amylase supplementation in lactating cows ($P<0.05$) and continuous cultures ($P=0.03$).
[c] Significance for A:P: main effect of α-amylase supplementation in lactating cows ($P<0.05$) and α-amylase by time interaction in steers ($P=0.07$).
[d] Supplemental α-amylase was provided at 0 and 662 DU/kg dietary DM from d −21 to 21 (prepartum and postpartum averages are presented).
[e] Significance for butyrate molar proportions: main effects of α-amylase supplementation prepartum ($P=0.14$).
[f] Significance for A:P: α-amylase by day interaction prepartum ($P<0.04$; 3.60 *vs.* 3.33 d −21 and 3.46 *vs.* 3.74 d −7 for control and α-amylase, respectively).

at 240 DU/kg DM (Tricarico et al., 2005) and only tended to increase postpartum plasma glucose concentrations at 662 DU/kg dietary DM (DeFrain et al., 2005). It is conceivable that increased productivity in cattle may arise from effects of α-amylase supplementation on ruminal fermentation and the concomitant changes in metabolite concentrations that imply differences in nutrient metabolism in supplemented cattle.

2.5. *Effects of supplemental Aspergillus oryzae α-amylase on ruminal development in calves*

Ruminal development is stimulated by microbial VFA production and especially by butyrate and propionate (McLeod and Baldwin, 2000). Approximately 0.90 of ruminal butyrate may be absorbed by rumen tissue providing energy for rumen wall thickening,

Table 4
Effects of supplemental α-amylase from *Aspergillus oryzae* on circulating β-hydroxybutyrate (BHBA), non-esterified fatty acids (NEFA) and glucose in lactating (adapted from Tricarico et al., 2005) and transition dairy cows (adapted from DeFrain et al., 2005)

	Supplement		
	Control	α-Amylase	Change
Tricarico et al. (2005)[a]			
BHBA (μmol/l)	434	492	+58
NEFA (μEq/l)	160	192	+32
Glucose (mg/l)	468	474	+6
DeFrain et al. (2005)[b]			
BHBA (μmol/l)			
Prepartum	376	603	+227
Postpartum	895	990	+95
NEFA (μEq/l)			
Prepartum	115	373	+258
Postpartum	535	471	-64
Glucose (mg/l)			
Prepartum	689	715	+26
Postpartum	640	693	+53

[a] Supplemental α-amylase was provided at 0, 240, 480 and 720 DU/kg dietary DM to lactating cows. Metabolite concentrations in serum are presented for 0 and 240 DU/kg dietary DM. Main effects of α-amylase supplementation for BHBA and NEFA ($P<0.05$).

[b] Supplemental α-amylase was provided at 0 and 662 DU/kg dietary DM from d −21 to 21. Metabolite concentrations in plasma are presented as averages for prepartum and postpartum. Effects of α-amylase supplementation for BHBA and NEFA prepartum ($P<0.01$) and glucose postpartum ($P=0.08$).

and papillae and capillary development (Weigand et al., 1975). Therefore, supplementation with a butyrate enhancing additive may be beneficial to ruminal epithelium development. This hypothesis was examined with neonatal dairy calves by providing supplemental α-amylase from *Aspergillus oryzae* in the first 200 g of calf starter consumed daily at 0, 6,762 or 13,524 DU/d in an initial study and 0, 4,710 or 9,420 DU/d in a second study. Dietary supplementation with the α-amylase preparation enhanced ruminal epithelium development in both studies, as evidenced by increased papillae length and width (A.J. Heinrichs, Penn State University, State College, PA, USA, unpublished), suggesting that a dose of supplemental α-amylase that is adequate to improve ruminal epithelium development in calves is between about 7000 and 9000 DU/d.

3. *Aspergillus oryzae* α-amylase mode of action

3.1. Ruminal starch hydrolysis

Starch is composed of an insoluble linear polymer of glucose bound by α-1,4 linkages (amylose) and a highly branched polymer with α-1,6 bonds at each branch point (amylopectin). The process of starch digestion involves α-amylase, which cleaves internal α-1,4 linkages of the polymer backbone randomly and releases low molecular weight oligosaccha-

rides (maltodextrins), and isoamylase, which cleaves the α-1,6 linkages of the amylopectin branch points. Glucoamylase and β-amylase cleave glucose and maltose from amylase non-reducing ends. Although ruminal bacteria, protozoa and fungi are all involved in ruminal starch digestion, the contributions of protozoa and fungi are not clearly defined.

Examination of the amylolytic activity of the predominant starch-digesting ruminal bacteria, and their hydrolysis products, can give some insight into potential modes of action for supplemental α-amylase in the rumen. The ruminal bacteria with the highest capacity for starch digestion are *Ruminobacter amylophilus* and *Streptococcus bovis*, followed by *Prevotella ruminicola* and some *Butyrivibrio fibrisolvens* strains like 49 and A38 (Cotta, 1988). All of these ruminal bacteria produced mixed oligosaccharides as a result of amylose digestion in the laboratory (Cotta, 1988). *Butyrivibrio fibrisolvens* 49, *Ruminobacter amylophilus* H18, *Streptococcus bovis* JB1 and *Prevotella ruminicola* 23 produced primarily maltose through maltotetraose. *Butyrivibrio fibrisolvens* A38 also produced maltopentaose and maltohexaose, and *Prevotella ruminicola* B_14 also produced maltoheptaose. In addition, prolonged exposure to the enzyme decreased the larger, and concurrently increased the smaller, oligosaccharide products from amylose hydrolysis. This pattern of hydrolysis is consistent with production of endo-acting enzymes by all studied bacteria whose activity is similar to that of α-amylase. Genes encoding α-amylase activity have since been cloned from *Streptococcus bovis* (Clark et al., 1992; Cotta and Whitehead, 1993) and *Butyrivibrio fibrisolvens* (Rumbak et al., 1991) providing further support for this hypothesis. Therefore, it is likely that starch in the rumen is hydrolyzed to a variety of products ranging from glucose to maltoheptaose that could be used as growth substrates by a variety of ruminal microorganisms.

3.2. Effects of α-amylase supplementation on growth of ruminal bacteria

Data from our studies suggest that supplemental α-amylase from *Aspergillus oryzae* does not increase ruminal starch digestion, but shifts ruminal fermentation to a higher molar proportion of butyrate and acetate at the expense of propionate, presumably by modifying microbial metabolism or microbial populations in the rumen. Our hypothesis is that supplemental *Aspergillus oryzae* α-amylase produces maltodextrins that provide substrate, and a competitive advantage, to non-amylolytic organisms that produce butyrate and acetate from starch hydrolysis products.

A series of experiments were conducted to examine effects of α-amylase supplementation on growth of representative strains of ruminal bacteria on starch. Pure cultures of *Butyrivibrio fibrisolvens* strains D1, 49 and A38, *Streptococcus bovis* S1, *Megasphaera elsdenii* T81 and *Selenomonas ruminantium* GA192 were grown anaerobically at 37 °C on medium 10 containing soluble potato starch (1.0 g/l) as the sole carbohydrate source. Enzyme treatment was applied immediately prior to bacterial inoculation by adding 0.1 ml of a solution to provide a final concentration of 0.06 DU/ml. Control cultures received 0.1 ml of a solution prepared with fermentation solubles (enzyme carrier). Microbial growth was estimated in each culture by measuring optical density at 600 nm over time. Each experiment consisted of either two or three replicates per treatment. As expected, *Streptococcus bovis* S1 and *Butyrivibrio fibrisolvens* 49 grew rapidly on starch-containing medium and α-amylase supplementation had no effects on their growth (Fig. 1). *Butyrivibrio fibrisol-*

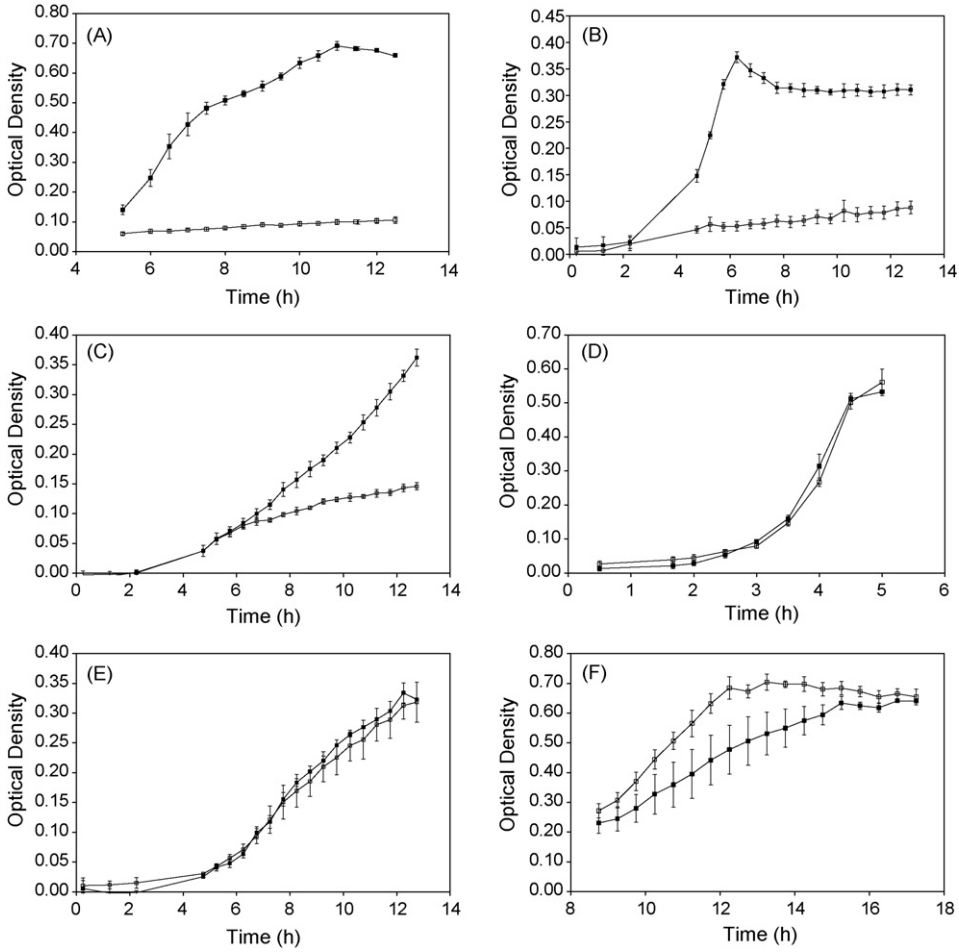

Fig. 1. Growth (optical density at 660 nm) on soluble potato starch (1 g/l) in the absence (empty square) or presence (solid square) of supplemental α-amylase (0.06 DU/ml) by (A) *Butyrivibrio fibrisolvens* D1; (B) *Selenomonas ruminantium* GA192; (C) *Megasphaera elsdenii* T81; (D) *Streptococcus bovis* S1; (E) *Butyrivibrio fibrisolvens* 49; and (F) *Butyrivibrio fibrisolvens* A38.

vens A38 grows equally well on maltose and starch (Cotta, 1988) and grew more slowly in the presence of supplemental α-amylase in this experiment. Conversely, *Butyrivibrio fibrisolvens* D1, *Selenomonas ruminantium* GA192 and *Megasphaera elsdenii* T81 only grew poorly or not at all on starch. However, these non-amylolytic species grew rapidly when supplemental α-amylase was included in the starch-containing medium (Fig. 1).

Effects of α-amylase supplementation on growth of *Butyrivibrio fibrisolvens* D1 were further examined using commercial maltodextrin products (Maltrin®, Grain Processing Corporation, Muscatine, IA, USA) with various degrees of polymerization (DP) as carbohydrate source (Table 5). Microbial growth conditions and monitoring were as described

Table 5
Saccharide composition (g/kg) of commercial maltodextrins (Maltrin®, Grain Processing Corporation, Muscatine, IA, USA) used as a carbohydrate source for *in vitro* growth of *Butyrivibrio fibrisolvens* D1 in the absence and presence (0.06 DU/ml) of α-amylase from *Aspergillus oryzae*

	Maltrin® product code				
	M440	M500	M550	M580	M600
Dextrose equivalence[a]	5	10	15	18	20
Average theoretical MW[b]	3600	1800	1200	1000	900
Average degree of polymerization[c]	221	111	74	62	58
Composition (g/kg DM basis)					
Glucose (DP1)	3	8	13	16	23
Maltose (DP2)	9	29	48	58	74
Maltotriose (DP3)	14	44	67	78	91
Maltotetraose (DP4)	14	38	55	61	68
Maltopentaose (DP5)	13	34	47	54	63
Maltohexaose (DP6)	18	57	84	102	119
Maltoheptaose (DP7)	24	68	91	102	100
>Maltoheptaose (>DP7)	905	722	595	529	462

[a] Dextrose equivalence (DE) is a quantitative measure of the degree of starch polymer hydrolysis (DE of starch = 0 and DE of dextrose or glucose = 100).
[b] MW: molecular weight.
[c] Degree of polymerization (DP) refers to the number of glucose units joined in the molecule and is presented.

above. Growth of *Butyrivibrio fibrisolvens* D1 was similar in the presence or absence of supplemental α-amylase with maltodextrins providing an average DP of 11.1 or less (Fig. 2). However, α-amylase supplementation increased *Butyrivibrio fibrisolvens* D1 growth with a maltodextrin providing an average DP of 22.1. These results confirm that *Butyrivibrio fibrisolvens* D1 can grow efficiently on low DP maltodextrins and that supplemental α-

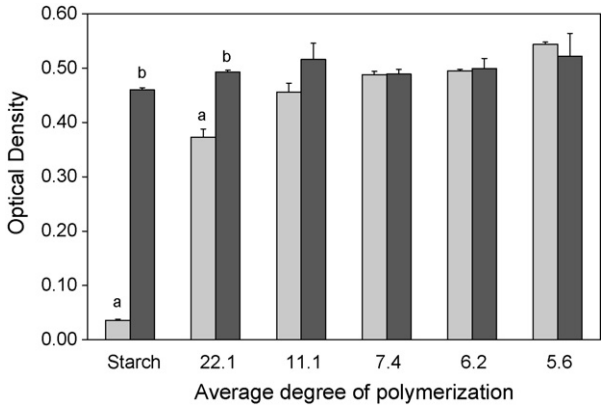

Fig. 2. Growth (optical density at 660 nm) of *Butyrivibrio fibrisolvens* D1 after incubation for 15 h on soluble potato starch (1 g/l) or oligosaccharides (maltodextrins) of varying average degree of polymerization in the absence (light grey) or presence (dark gray) of supplemental α-amylase (0.06 DU/ml) from *Aspergillus oryzae*. Means within a substrate with different superscripts differ (P<0.05).

amylase activity can provide these hydrolysis products from starch or maltodextrins with a higher DP.

3.3. A comprehensive hypothesis

Maltodextrins produced from hydrolysis of native starch can be used by a wide variety of ruminal bacteria including amylolytic and non-amylolytic species. Our studies, and those by Cotta (1992), showed that although *Selenomonas ruminantium* and *Megasphaera elsdenii* grow poorly on starch, both are able to grow rapidly on maltodextrins. Similarly, cellodextrins produced by cellulolytic bacteria can be used by non-cellulolytic species (Russell, 1985) and xylooligosaccharides from xylan hydrolysis can be used by non-xylanolytic species (Cotta, 1993). These observations suggest that cross-feeding mechanisms are a general feature of the ruminal microbial ecosystem and those microorganisms that utilize hydrolysis products from other species will contribute to ruminal fermentation (Van Soest, 1982).

The hypothetical mode of action proposed for the specific *Aspergillus oryzae* α-amylase preparation may be applicable for fibrolytic exogenous enzymes as well. The comprehensive hypothesis would be that exogenous enzymes hydrolyze complex carbohydrates (*i.e.*, starch, cellulose and xylans) into oligosaccharides (*i.e.*, malto-, cello- and xylo-oligosaccharides) that support cross-feeding in the rumen. The oligosaccharide cross-feeding hypothesis is compatible with reports of improved total tract digestibility (Rode et al., 1999), pre-ingestive (Hristov et al., 1998) and ruminal effects (Yang et al., 1999), feed-enzyme specificity (Colombatto et al., 2003a), structural changes rendering the polymers more amenable to degradation (Nsereko et al., 2000), increased bacterial attachment (Wang et al., 2001), stimulation of ruminal microbial populations (Nsereko et al., 2002), and synergism between exogenous and endogenous ruminal enzymes (Morgavi et al., 2000).

The oligosaccharide cross-feeding hypothesis is also attractive because it is consistent with most of the controversial features described for exogenous enzymes in the literature. First, increased reducing sugar concentrations resulting from exogenous enzyme supplementation in the absence of ruminal microbes have been described in some instances (Hristov et al., 1998), although it is not an absolute requirement and cannot fully explain responses to enzyme supplementation (Beauchemin et al., 2004). Production of oligosaccharides is a function of enzyme activity that does not necessarily result in increased reducing sugar concentrations. Second, ruminal digestion is generally considered a first order kinetic process with substrate availability, rather than enzyme concentration, as the limiting factor (Weimer, 1998). Production of oligosaccharides from polymers by exogenous enzyme action would effectively increase substrate availability by exposing new sites for hydrolytic attack in the polymer by polymer-degrading bacteria, and by exposing the oligosaccharides for cross-feeding by microbes that would not normally have access to it. This increase in substrate availability would also explain the increase in the initial rate of digestion reported for fibrolytic enzyme supplements (Wallace et al., 2001; Colombatto et al., 2003b). Third, low enzyme doses may be beneficial while high enzyme doses that increase overall ruminal enzymatic activity are not required to obtain improvements in digestion and performance (Beauchemin et al., 2004). Fourth, the cross-feeding mechanisms resulting from oligosaccharide production by exogenous enzyme action may explain the increased production of

propionate from fiber digestion or acetate and butyrate from starch digestion. This concept agrees with the classic example of two-species microbial interaction for propionate production from cellulose; whereas *Fibrobacter succinogenes* produces cellulose fragments and succinate from cellulose that are in turn converted to acetate, propionate and carbon dioxide by *Selenomonas ruminantium* (Van Soest, 1982). Finally, and most importantly, the oligosaccharide cross-feeding hypothesis is consistent with the frequent occurrence of quadratic responses to enzyme supplementation. Low exogenous enzyme concentrations would not produce enough oligosaccharides for effective cross-feeding to occur while high enzyme doses, or prolonged exposure to enzymes, would extensively hydrolyze polymers to di- and mono-saccharides thereby failing to support an effective cross-feeding mechanism.

4. Conclusions

Dietary supplemental α-amylase from *Aspergillus oryzae* may improve ruminant productivity by modifying ruminal starch digestion without necessarily increasing starch digestion in the rumen. The proposed hypothetical mode of action for α-amylase involves production of oligosaccharides from amylose and amylopectin that can be used by amylolytic and non-amylolytic bacteria in cross-feeding mechanisms that modify the resulting products of fermentation in the rumen. The hypothesis of oligosaccharide cross-feeding is also consistent with various observations associated with exogenous fibrolytic enzymes that have been reported.

Acknowledgements

The authors acknowledge the contributions to the collaborative research efforts of the following individuals: A.M. Gehman, A.J. Heinrichs, C.M. Jones, S.I. Kehoe, J.M. DeFrain, A.R. Hippen, K.F. Kalscheur, K.C. Hanson, D.L. Harmon, K.R. McLeod, S.M. Speight, G.A. Harrison, A.E. Kozenski, L. Johnston, M.D. Abney, J.D. Rivera, J.J. Cranston, N.A. Elam, J.F. Gleghorn, J.T. Richeson, and M.L. Galyean.

References

Beauchemin, K.A., Colombatto, D., Morgavi, D.P., Yang, W.Z., 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci. 81, E37–E47.

Beauchemin, K.A., Colombatto, D., Morgavi, D.P., Yang, W.Z., Rode, L.M., 2004. Mode of action of exogenous cell wall degrading enzymes for ruminants. Can. J. Anim. Sci. 84, 13–22.

Bedford, M.R., Schulze, H., 1998. Exogenous enzymes for pigs and poultry. Nutr. Res. Rev. 11, 91–114.

Burroughs, W., Woods, W., Ewing, S.A., Greig, J., Theurer, B., 1960. Enzyme additions to fattening cattle rations. J. Anim. Sci. 19, 458–464.

Chen, K.H., Huber, J.T., Simas, J., Theurer, C.B., Yu, P., Chan, S.C., Santos, F., Wu, Z., Swingle, R.S., DePeters, E.J., 1995. Effect of enzyme treatment or steam-flaking of sorghum grain on lactation and digestion in dairy cows. J. Dairy Sci. 78, 1721–1727.

Clark, R.G., Hu, Y.-J., Hynes, M.F., Salmon, R.K., Cheng, K.-J., 1992. Cloning and expression of an amylase gene from *Streptococcus bovis* in *Escherichia coli*. Arch. Microbiol. 157, 201–204.

Colombatto, D., Morgavi, D.P., Furtado, A.F., Beauchemin, K.A., 2003a. Screening of exogenous enzymes for ruminant diets: relationship between biochemical characteristics and in vitro ruminal degradation. J. Anim. Sci. 81, 2628–2638.

Colombatto, D., Mould, F.L., Bhat, M.K., Morgavi, D.P., Beauchemin, K.A., Owen, E., 2003b. Influence of fibrolytic enzymes on the hydrolysis and fermentation of pure cellulose and xylan by mixed ruminal microorganisms in vitro. J. Anim. Sci. 81, 1040–1050.

Cotta, M.A., 1988. Amylolytic activity of selected species of ruminal bacteria. Appl. Environ. Microbiol. 54, 772–776.

Cotta, M.A., 1992. Interaction of ruminal bacteria in the production and utilization of maltooligosaccharides from starch. Appl. Environ. Microbiol. 58, 48–54.

Cotta, M.A., 1993. Utilization of xylooligosaccharides by selected ruminal bacteria. Appl. Environ. Microbiol. 59, 3557–3563.

Cotta, M.A., Whitehead, T.R., 1993. Regulation and cloning of the gene encoding amylase activity of the ruminal bacterium *Streptococcus bovis*. Appl. Environ. Microbiol. 59, 189–196.

DeFrain, J.M., Hippen, A.R., Kalscheur, K.F., Tricarico, J.M., 2005. Effects of dietary alpha-amylase on metabolism and performance of transition dairy cows. J. Dairy Sci. 88, 4405–4413.

Firkins, J.L., Eastridge, M.L., St-Pierre, N.R., Noftsger, S.M., 2001. Effects of grain variability and processing on starch utilization by lactating dairy cattle. J. Anim. Sci. 79 (E. Suppl.), E218–E238.

Food Chemicals, Codex, 1996. Food Chemicals Codex, fourth ed. National Academy Press, Washington, DC, USA.

Hristov, A.N., McAllister, T.A., Cheng, K.J., 1998. Effect of dietary or abomasal supplementation of exogenous polysaccharide-degrading enzymes on rumen fermentation and nutrient digestibility. J. Anim. Sci. 76, 3146–3156.

Hristov, A.N., McAllister, T.A., Cheng, K.J., 2000. Intraruminal supplementation with increasing levels of exogenous polysaccharide-degrading enzymes: effects on nutrient digestion in cattle fed a barley grain diet. J. Anim Sci. 78, 477–487.

Hristov, A.N., Basel, C.E., Melgar, A., Foley, A.E., Ropp, J.K., Hunt, C.W., Tricarico, J.M., 2008. Effect of exogenous polysaccharide-degrading enzyme preparations on ruminal fermentation and digestibility of nutrients in dairy cows. Anim. Feed Sci. Technol. 145, 182–193.

Huhtanen, P., Miettinen, H., Ylinen, M., 1993. Effect of increasing ruminal butyrate on milk yield and blood constituents in dairy cows fed a grass silage-based diet. J. Dairy Sci. 76, 1114–1124.

McAllister, T.A., Oosting, S.J., Popp, J.D., Mir, Z., Yanke, L.J., Hristov, A.N., Treacher, R.J., Cheng, K.J., 1999. Effect of exogenous enzymes on digestibility of barley silage and growth performance of feedlot cattle. Can. J. Anim. Sci. 79, 353–360.

McAllister, T.A., Hristov, A.N., Beauchemin, K.A., Rode, L.M., Cheng, K.-J., 2001. Enzymes in ruminant diets. In: Bedford, M., Partridge, G. (Eds.), Enzymes in Farm Animal Nutrition. CABI Publishing, Oxon, UK, pp. 273–298.

McGilliard, M.L., Stallings, C.C., 1998. Increase in milk yield of commercial dairy herds fed a microbial and enzyme supplement. J. Dairy Sci. 81, 1353–1357.

McLeod, K.R., Baldwin, R.L., 2000. Effects of diet forage: concentrate ratio and metabolizable energy intake on visceral organ growth and in vitro oxidative capacity of gut tissues in sheep. J. Anim Sci. 78, 760–770.

Miettinen, H., Huhtanen, P., 1996. Effects of the ratio of ruminal propionate to butyrate on milk yield and blood metabolites in dairy cows. J. Dairy Sci. 79, 851–861.

Mora-Jaimes, G., Barcena-Gama, R., Mendoza-Martinez, G.D., Gonzalez, S.S., Herrera-Haro, J.G., 2002. Performance and ruminal fermentation in lambs fed sorghum grain treated with amylases. Agrociencia 63, 31–39.

Morgavi, D.P., Beauchemin, K.A., Nsereko, V.L., Rode, L.M., Iwaasa, A.D., Yang, W.Z., McAllister, T.A., Wang, Y., 2000. Synergy between ruminal fibrolytic enzymes and enzymes from *Trichoderma longibrachiatum*. J. Dairy Sci. 83, 1310–1321.

Nsereko, V.L., Morgavi, D.P., Rode, L.L.M., Beauchemin, K.A., McAllister, T.A., 2000. Effects of fungal enzyme preparations on hydrolysis and subsequent degradation of alfalfa hay fiber by mixed rumen microorganisms in vitro. Anim. Feed Sci. Technol. 88, 153–170.

Nsereko, V.L., Beauchemin, K.A., Morgavi, D.P., Rode, L.M., Furtado, A.F., McAllister, T.A., Iwaasa, A.D., Yang, W.Z., Wang, Y., 2002. Effect of a fibrolytic enzyme preparation from *Trichoderma longibrachiatum* on the rumen microbial population of dairy cows. Can. J. Microbiol. 48, 14–20.

Owens, F.N., Secrist, D.S., Hill, W.J., Gill, D.R., 1998. Acidosis in cattle: a review. J. Anim Sci. 76, 275–286.

Rode, L.M., Yang, W.Z., Beauchemin, K.A., 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. J. Dairy Sci. 82, 2121–2126.

Rojo, R., Mendoza, G.D., Gonzalez, S.S., Landois, L., Barcena, R., Crosby, M.M., 2005. Effects of exogenous amylases from *Bacillus licheniformis* and *Aspergillus niger* on ruminal starch digestion and lamb performance. Anim. Feed Sci. Technol. 124, 655–665.

Rojo-Rubio, R., Mendoza-Martinez, G.D., Crosby-Galvan, M.M., 2001. Use of thermostable amylase from *Bacillus licheniformis* on in vitro starch digestion of sorghum and corn. Agrociencia 35, 423–427.

Rumbak, E., Rawlings, D.E., Lindsey, G.G., Woods, D.R., 1991. Cloning, nucleotide sequence, and enzymatic characterization of α-amylase from the ruminal bacterium *Butyrivibrio fibrisolvens* H17c. J. Bacteriol. 173, 4203–4211.

Russell, J.B., 1985. Fermentation of cellodextrins by cellulolytic and noncellulolytic rumen bacteria. Appl. Environ. Microbiol. 49, 572–576.

Seymour, W.M., Campbell, D.R., Johnson, Z.B., 2005. Relationships between rumen volatile fatty acid concentrations and milk production in dairy cows: a literature study. Anim. Feed Sci. Technol. 119, 155–169.

Tricarico, J.M., Johnston, J.D., Dawson, K.A., Hanson, K.C., McLeod, K.R., Harmon, D.L., 2005. The effects of an *Aspergillus oryzae* extract containing alpha-amylase activity on ruminal fermentation and milk production in lactating Holstein cows. Anim. Sci. 81, 365–374.

Tricarico, J.M., Abney, M.D., Galyean, M.L., Rivera, J.D., Hanson, K.C., McLeod, K.R., Harmon, D.L., 2007. Effects of a dietary *Aspergillus oryzae* extract containing alpha-amylase activity on performance and carcass characteristics of finishing beef cattle. J. Anim. Sci. 85, 802–811.

Van Soest, P.J., 1982. Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, NY, USA.

Wallace, R.J., Wallace, S.J., McKain, N., Nsereko, V.L., Hartnell, G.F., 2001. Influence of supplementary fibrolytic enzymes on the fermentation of corn and grass silages by mixed ruminal microorganisms in vitro. J. Anim. Sci. 79, 1905–1916.

Wang, Y., McAllister, T.A., Rode, L.M., Beauchemin, K.A., Morgavi, D.P., Nsereko, V.L., Iwaasa, A.D., Yang, W., 2001. Effects of an exogenous enzyme preparation on microbial protein synthesis, enzyme activity and attachment to feed in the Rumen Simulation Technique (Rusitec). Brit. J. Nutr. 85, 325–332.

Weigand, E., Young, J.W., McGilliard, A.D., 1975. Volatile fatty acid metabolism by rumen mucosa from cattle fed hay or grain. J. Dairy Sci. 58, 1294.

Weimer, P.J., 1998. Manipulating ruminal fermentation: a microbial ecological perspective. J. Anim. Sci. 76, 3114–3122.

Yang, W.Z., Beauchemin, K.A., Rode, L.M., 1999. Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. J. Dairy Sci. 82, 391–403.