

Enzymes in Ruminant Diets

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INTRODUCTION

Exogenous enzymes have been used extensively to remove anti-nutritional factors from feeds, to increase the digestibility of existing nutrients, and to supplement the activity of the endogenous enzymes of poultry (Classen *et al.*, 1991; Bedford, 1993). Researchers in the 1960s examined the use of exogenous enzymes in ruminants (Burroughs *et al.*, 1960; Rovics and Ely, 1962; Rust *et al.*, 1965), but responses were variable and no effort was made to determine the mode of action of these products. Furthermore, production of exogenous enzymes was expensive at the time and it was not economically feasible to apply these preparations at the concentrations necessary to elicit a positive animal response. Recent reductions in fermentation costs, together with more active and better defined enzyme preparations, have prompted researchers to re-examine the role of exogenous enzymes in ruminant production (Chen *et al.*, 1995; Beauchemin *et al.*, 1997; McAllister *et al.*, 1998). Several studies have attempted to define possible modes of action of these additives (Judkins and Stobart, 1988; Feng *et al.*, 1996; Hristov *et al.*, 1998a,b; Yang *et al.*, 1998a). Exogenous enzymes could exert a number of effects, both on the gastrointestinal microflora and on the ruminant animal itself. It is highly probable, therefore, that physiological responses to exogenous enzymes are multi-factorial in origin.

This review will summarize production responses to supplementary exogenous enzymes obtained to date in ruminants. Possible mechanisms by which these products may improve nutrient utilization by ruminants will be discussed and suggestions will be made with regard to

1 strategies that may further enhance the efficacy of these products for ruminants.

2 **Sources of Enzymes**

3 Although enzyme products marketed for livestock number in the hundreds, they are derived
4 primarily from only four bacterial (*Bacillus subtilis*, *Lactobacillus acidophilus*, *L. plantarum*, and
5 *Streptococcus faecium*, spp.) and three fungal (*Aspergillus oryzae*, *Trichoderma reesei*, and
6 *Saccharomyces cerevisiae*) species (Muirhead, 1996). Moreover, it is unlikely that this list of
7 source organisms will expand substantially, given that no petition to the Food and Drug
8 Administration to add a new organism has been successful (Pendleton, 1996).

9 Enzymes are naturally occurring biocatalysts produced by living cells to bring about
10 specific biochemical reactions. In the context of feed additives for ruminants, enzymes are
11 employed to catalyze the degradative reactions by which substrates (i.e., feedstuffs) are
12 digested into their chemical components (e.g., simple sugars, amino acids, fatty acids). These
13 are in turn used for cell growth, either by ruminal microorganisms or by the host animal.

14 Complete digestion of complex feeds such as hay or grain requires literally hundreds of
15 enzymes. Enzyme preparations for ruminants are marketed primarily on the basis of their
16 capacity to degrade plant cell walls and as such, are often referred to as cellulases or
17 xylanases. However, none of these commercial products are preparations of single enzymes;
18 secondary enzyme activities such as amylases, proteases, or pectinases are invariably present.
19 Degradation of cellulose and hemicellulose alone requires a number of enzymes, and
20 differences in the relative proportions and activities of these individual enzymes impacts the
21 efficacy of cell wall degradation by the marketed products. Even within a single microbial
22 species, the types and activity of enzymes produced can vary widely depending on the strain
23 selected and the growth substrate and culture conditions employed (Considine and Coughlan,
24 1989; Gashe, 1992).

1 The diversity of enzyme activities present in commercially available enzyme preparations
2 is advantageous, in that a wide variety of substrates can be targeted by a single product, but it
3 presents problems in terms of quality control and extrapolation of research findings among
4 different preparations. For ruminants, enzyme products are usually standardized by blending
5 crude enzyme extracts to obtain specified levels of one or two defined enzyme activities, such
6 as xylanase and/or cellulase. These products are not currently standardized for secondary
7 activities. In fact, these activities, which may well be affecting the overall effectiveness of a
8 given product, are seldom even measured.

9 **Measurement of enzyme activity**

10 Enzyme activity is assayed by measuring over time either the disappearance of a defined
11 substrate or the generation of a product from the biochemical reaction catalyzed by the enzyme.
12 Activities of enzymes for use in the feed industry are most commonly measured using the latter
13 approach, and are expressed as the amount of product produced per unit time. These
14 measurements must be conducted under conditions closely defined with respect to temperature,
15 pH, ionic strength, substrate concentration, and substrate type, as all of these factors can affect
16 the activity of an enzyme (Headon, 1993). For example, the relative ranking of cellulase activity
17 of three enzyme preparations differs depending on the test substrate (cellulose,
18 carboxymethylcellulose (CMC) or β -glucan) selected for analysis (Table 1). Furthermore,
19 release of reducing sugars from CMC or xylan is not directly proportional to enzyme
20 concentration. Consequently, the ratio of enzyme to substrate will affect enzyme activity
21 estimates (Figure 1, Hristov and McAllister, unpublished data).

22 Enzyme activity can also be assessed using synthetic substrates, which usually consist of
23 chromophores linked to molecules chemically similar to the natural substrate. Enzyme activity
24 is measured as the release of the dye or chromophore (Biely *et al.*, 1985). These synthetic

1 substrates offer uniformity among assays, but are subject to criticism in that they do not
2 represent the substrate found in intact feeds such as cereal grains or forages. Furthermore, the
3 assays used to assess enzyme activity are not representative of the conditions in the digestive
4 tract where ultimately the level and persistence of enzyme activity may be most important. For
5 these reasons, measurement of enzyme activity using traditional assay techniques may have
6 little relevance to the potential efficacy of an enzyme as a feed additive for ruminants.

7 Researchers have attempted to develop biological assays that may be more indicative of
8 the value of a given enzyme preparation for ruminants. These methods usually involve *in vitro*
9 incubation of enzyme and feed with ruminal contents, and measurement of the disappearance
10 of substrates (e.g., cereal grain, straw, hay) representative of those consumed by the animal
11 (Forwood *et al.*, 1990; Varel *et al.*, 1993; Hristov *et al.*, 1996b; 1998a). Alternatively, the amount
12 of gas produced by the mixed culture can be used as an indication of digestion (Iwaasa *et al.*,
13 1998), which enables rapid screening of different enzyme products and application rates.
14 These procedures may provide valuable information on the extent to which exogenous enzymes
15 complement the digestive activity of ruminal microorganisms. However, extrapolation of
16 information from these procedures to whole animal situations is limited (i) by variations in
17 microbial composition among inocula from different donor animals; (ii) by differences in growth
18 of microbial populations in the *in vitro* system versus in the rumen and (iii) by artifactual
19 accumulation of end products that alter enzyme activity. Additionally, these assays do not
20 consider the possible impact of exogenous enzymes on biological parameters such as feed
21 intake, rate of passage or post-ruminal digestion of nutrients.

22 Because viscosity of intestinal digesta is closely correlated with growth and feed efficiency
23 in poultry, viscosity measurements have been used as a standard for assessing the biological
24 value of exogenous carbohydrases for poultry (Sabatier and Fish, 1996). For ruminants,
25 however, the value of enzymes can presently be assessed only through expensive and time

1 consuming production experiments with beef or dairy cattle, which makes screening large
2 numbers of products impractical. This lack of an adequate bioassay for assessing the value of
3 exogenous enzymes is perhaps the greatest impediment to the development of more efficacious
4 enzyme products for ruminants.

5 **Production responses to exogenous enzymes**

6 *Beef Cattle*

7 Evidence that exogenous enzymes could improve average daily gain and feed efficiency in beef
8 cattle was first recorded in a series of ten feeding trials reported almost 40 years ago
9 (Burroughs *et al.*, 1960). When given diets of ground ear corn, oat silage, corn silage or alfalfa
10 hay treated with an enzyme cocktail containing amylolytic, proteolytic and cellulolytic activities
11 (Agrozyme®, Merck Sharp and Dohme Research Laboratories), cattle gained 6.8 to 24.0%
12 more and exhibited feed efficiencies improved by 6.0 to 21.2%, relative to cattle fed untreated
13 control diets. In the same year, four different enzyme preparations (Agrozyme®, Zymo-Pabst®,
14 Rhozyme®, and Takamine®; Merck and Company, Rahway, NJ), given in combination with
15 diethylstilbesterol, were shown to increase gain by cattle fed a corn-alfalfa hay diet by an
16 average of 14.0% (Nelson and Damon, 1960).

17 Further studies confirmed that enzyme supplements could improve average daily gain
18 (ADG) and feed efficiency in cattle fed high silage diets (Rovics and Ely, 1962), but not all
19 responses by feedlot cattle to enzyme supplementation were positive. Leatherwood *et al.*
20 (1960) added a fungal enzyme (Enzyme 19AP®, Rohm and Hass Co.) to a grain supplement for
21 calves fed an alfalfa hay-based diet and found no improvement in the ADG or feed efficiency of
22 the calves. Two enzyme preparations containing primarily amylase and protease activities also
23 failed to increase ADG by cattle given a diet comprising 80% concentrate and 20% chopped
24 alfalfa hay (Clark *et al.*, 1961). In a separate study, Agrozyme® even reduced the ADG of

1 cattle by 20.4% when it was fed with a corn carrier to beef cattle given a corn silage diet (Perry
2 *et al.*, 1960). Similarly, Kercher (1960) found that ADG was reduced when Zymo-Pabst® was
3 fed with a corn carrier to cattle given a diet of steam-rolled barley, alfalfa hay and corn silage.
4 Perry *et al.* (1966) attributed an 18.2% decline in ADG observed in cattle fed Agrozyme® with
5 corn cob diets to a 6.8% reduction in feed intake, because the enzyme had been shown to
6 enhance fiber digestibility in metabolism experiments.

7 Although these early studies provided valuable information on the potential benefits of
8 enzymes for beef cattle, they did little to address the impact on animal responses of factors
9 such as the composition of the diet, types and levels of enzyme activities present, or the method
10 of enzyme application. More recent studies have been designed specifically to address these
11 issues. Different feed types (Beauchemin *et al.*, 1995; Beauchemin *et al.*, 1997), application
12 levels (Beauchemin and Rode, 1996; McAllister *et al.*, 1998; Michal *et al.*, 1996), enzyme
13 products (Pritchard *et al.*, 1996) and enzyme application methods (Beauchemin *et al.*, 1998b;
14 Yang *et al.*, 1998a; Hristov *et al.*, 1998b) have been compared under controlled conditions.
15 Application of different levels (0.25 to 4.0 L tonne⁻¹) of a mixture of xylanase and cellulase
16 products (Xylanase B, Biovance Technologies Inc., Omaha, NE) and cellulase (Spezyme CP®,
17 Genencor, Rochester, NY) increased ADG of steers fed alfalfa hay or timothy hay cubes by 30
18 and 36%, respectively, but had no effect on ADG when applied to barley silage (Beauchemin *et*
19 *al.*, 1995). When this same mixture was applied to a 95% grain diet, feed efficiency of cattle fed
20 barley was improved by 11% but performance of cattle fed corn was unaffected (Beauchemin
21 and Rode, 1996). Application of a different mix of fungal enzyme preparations (Cellulase A,
22 Xylanase B, Finnfed International Ltd. Marlborough, UK) at rates up to 5.0 L tonne⁻¹, however,
23 increased the final weight and ADG of feedlot cattle given diets based on alfalfa silage (Michal
24 *et al.*, 1996; Pritchard *et al.*, 1996) or barley silage (McAllister *et al.*, 1998). Treatment of 82.5%
25 corn diets with “multiple stabilized enzymes” increased ADG and feed conversion by feedlot

1 cattle by 10 and 7.5%, respectively (Weichenthal *et al.*, 1996), and similar improvements in feed
2 efficiency have been reported for cattle fed sorghum-based diets treated with amylase (Krause
3 *et al.*, 1989; Boyles *et al.*, 1992).

4 *Dairy Cattle*

5 The effect of exogenous enzymes on milk production in dairy cows was first examined in the
6 mid 1990s (Chen *et al.*, 1995; Lewis *et al.*, 1995; Stokes and Zheng, 1995) and recently there
7 has been a flurry of research activity in this area (Luchini *et al.*, 1997; Nussio *et al.*, 1997; Kung
8 *et al.*, 1998; Yang *et al.*, 1998a,b; Beauchemin *et al.*, 1998a). As in studies using beef cattle,
9 production responses by dairy cattle to exogenous enzymes have also been variable.

10 Application of enzyme mixtures onto sorghum (Digest M®, Loveland Industries Inc., Greeley,
11 CO) did not improve milk production by Holstein cows (Chen *et al.*, 1995). Similarly, milk
12 responses to exogenous enzymes (Pro-Mote®, Biovance Technologies Inc.) depended on the
13 method of application to barley-based diets (See below, Beauchemin *et al.*, 1998a). Applying a
14 cellulase/xylanase mixture (Finnfeeds International Ltd.) to diets containing 45% to 50%
15 concentrate and alfalfa silage, alfalfa hay or a mixture of alfalfa silage, corn silage and alfalfa
16 hay also failed to increase milk yield (Nussio *et al.*, 1997; Luchini *et al.*, 1997). In contrast,
17 spraying two similar enzyme preparations onto corn silage in a 50% concentrate diet increased
18 milk production by 2.5 kg d⁻¹ without altering milk composition (Kung, 1996). Although it was
19 demonstrated that these enzyme preparations could increase milk production in cows fed alfalfa
20 hay/silage-based total mixed rations (Stokes and Zheng, 1995; Lewis *et al.*, 1995; Sanchez *et*
21 *al.*, 1996), positive responses in milk production were highly dependent on the level of enzyme
22 applied (Sanchez *et al.*, 1996). For example, research at the Lethbridge Research Centre has
23 shown that increasing the amount of enzyme (Pro-Mote®, Biovance Technologies Inc.) applied
24 to alfalfa cubes from 1 g kg⁻¹ to 2 g kg⁻¹ increased milk production from 23.7 kg d⁻¹ (control) to

1 24.6 kg d⁻¹ and 25.6 kg d⁻¹, respectively (Table 2; Yang *et al.*, 1998a). Responses to this
2 enzyme preparation were even more dramatic in early lactation, when treatment of a diet
3 comprising 24% corn silage, 15% alfalfa hay and 61% barley-based concentrate increased milk
4 production by 4 kg d⁻¹. The efficacy of this enzyme was apparently dependent upon the method
5 of its application, as spraying the enzyme onto the total mixed ration did not affect milk
6 production, whereas applying it onto the concentrate achieved the increase (4 kg d⁻¹) in milk
7 production (Beauchemin *et al.*, 1998b).

8 *Lambs*

9 It was shown in the 1960s that feeding a mixture of amylolytic, cellulolytic and proteolytic
10 enzymes (Agrozyme®; 1.5, 3 and 6 g d⁻¹), as well as a potent proteolytic enzyme (Ficin®, Merck
11 and Company; 5, 10 and 20 mg d⁻¹) did not alter feed conversion or the ADG of fattening lambs
12 fed ground corn or alfalfa hay (Theurer *et al.*, 1963). Recently, we have also found that fibrolytic
13 enzymes (Finnfeeds International Inc.) did not increase feed intake or ADG by lambs fed alfalfa
14 hay- or barley-based diets (McAllister *et al.*, 1998).

15 *Learning from animal experiments*

16 The positive effects of exogenous enzymes on growth production both by beef and by dairy
17 cattle have been demonstrated definitively, but the information required to improve the
18 consistency and increase the magnitude of these responses is unfortunately still lacking.
19 Comparisons among experiments are exceedingly difficult, because many enzyme products are
20 poorly defined. Further, several studies have shown that over-application of enzyme is
21 possible, such that increased application costs are not recovered by corresponding
22 improvements in animal performance (Beauchemin *et al.*, 1996; McAllister *et al.*, 1998). Thus,
23 application of one enzyme preparation at a given concentration provides little information with

1 regard to the potential effect on animal performance of a different application level, let alone a
2 different product. Method of application also influences production responses; they have been
3 shown to differ between dry forage, fresh forage and silage (Feng *et al.*, 1996; Beauchemin *et*
4 *al.*, 1995), and if the enzyme is infused directly into the rumen, applied to the complete diet or to
5 the concentrate component only (Lewis *et al.*, 1996; McAllister *et al.*, 1998; Beauchemin *et al.*,
6 1998b). It is obvious that many factors may influence enzyme efficacy in ruminants. Therefore,
7 an understanding of the modes of action by which enzymes improve nutrient utilization in
8 ruminants is key to obtaining consistent positive responses to exogenous enzymes over a broad
9 range of diets and animal types.

10 **Modes of Action**

11 Upon initial consideration, exogenous enzymes might be expected to alter feed utilization in
12 ruminants either through their effects on the feed prior to consumption, or through their
13 enhancement of digestion in the rumen and/or in the post-ruminal digestive tract (Figure 2). In
14 actuality however, all of these possible modes of action are intertwined and enzyme-mediated
15 alteration of the feed prior to consumption likely has ramifications on ruminal and post-ruminal
16 digestion of nutrients. Preconsumptive effects of exogenous enzymes may be as simple as the
17 release of soluble carbohydrate or as complex as the removal of structural barriers (Figure 2A)
18 that limit the microbial digestion of feed in the rumen. Within the rumen, exogenous enzymes
19 could act directly on the feed or could indirectly stimulate digestive activity through synergistic
20 effects on ruminal microorganisms (Figure 2B). Exogenous enzymes may remain active in the
21 lower digestive tract, contributing to the post-ruminal digestion of fiber or could indirectly
22 improve nutrient absorption in the lower tract by reducing viscosity of intestinal digesta (Figure
23 2C). They may also supplement enzyme activity in the feces, thereby contributing by
24 accelerating decomposition of waste (Figure 2D). Ultimately, the goal of enzyme

1 supplementation is to improve the efficiency of feed utilization in ruminants and reduce waste
2 production. Undoubtedly, the mode of action of exogenous enzymes in ruminants is
3 exceedingly complex and continues to be a major focus of the research presently being
4 conducted with these additives.

5 *Preconsumption effects*

6 There is ample evidence that exogenous enzymes can release reducing sugars from feedstuffs
7 prior to consumption (Beauchemin and Rode, 1996; Hristov *et al.*, 1996a,b). However, the
8 degree of sugar release depends on both the type of feed and the type of enzyme. For
9 example, only two of eleven enzyme preparations tested released significant amounts of
10 reducing sugars from barley silage (Hristov *et al.*, 1996a). Moreover, the preparations most
11 effective at releasing reducing sugar from alfalfa hay were not those that released the most
12 reducing sugars from barley silage. There is evidence that exogenous enzymes may be more
13 effective when applied to dry forage as opposed to wet forage (Feng *et al.*, 1996; Beauchemin
14 *et al.*, 1998b). At first this seems improbable, given that the role of water in the hydrolysis of
15 soluble sugars from complex polymers is a fundamental biochemical principle (Lehninger,
16 1982). However, feed offered to ruminants is seldom absolutely dry; even feeds that
17 nutritionists would describe as “dry” (e.g., grain, hay) contain 6 to 10% moisture. Release of
18 soluble sugars from these “dry” feeds suggests that their water content is sufficient to enable
19 hydrolysis.

20 Release of sugars from feeds arises at least partially from the solubilization of NDF and
21 ADF (Hristov *et al.*, 1996a; Gwayumba and Christensen, 1997). This is consistent with
22 observed increases in the soluble fraction and rate of *in situ* digestion (Feng *et al.*, 1996; Hristov
23 *et al.*, 1996a; Dong, 1998; Hristov *et al.*, 1998a; Yang *et al.*, 1998a). However, most studies
24 have not found exogenous enzymes to improve the extent of *in situ* or *in vitro* DM digestion

1 (Feng *et al.*, 1996; Hristov *et al.*, 1996a). These results suggest that enzyme additives only
2 degrade substrates that would be naturally digested by the endogenous enzymes of the rumen
3 microflora. Using scanning electron microscopy, we have observed that high concentrations of
4 fibrolytic enzymes can cause digestive pits in the cell walls of barley straw (Figure 3). However,
5 similar degradation was not observed when enzymes were applied at the concentration
6 recommended by manufacturer (McAllister *et al.*, unpublished data).

7 Although exogenous enzymes do effect release of soluble carbohydrates, the amount
8 liberated represents only a minute portion of the total carbohydrate present in the diet. It is
9 difficult to attribute observed enzyme-associated production responses solely to the generation
10 of soluble carbohydrates prior to consumption, given that comparable increases in yield were
11 not seen when up to 9% of total dietary DM was supplied as molasses (Wing *et al.*, 1988).
12 Additionally, there is ample evidence that through associative effects soluble carbohydrates can
13 actually depress fiber digestion in ruminants (Huhtanen, 1991). Some exogenous enzyme
14 preparations contain soluble carbohydrates, which can affect *in vitro* gas production (Varel *et*
15 *al.*, 1993), but it is unlikely that they contribute significant levels of soluble carbohydrate to the
16 feed at practical application levels. Thus, it is most likely that at least a portion of the positive
17 production responses observed to accompany enzyme supplements is due to alterations in
18 ruminal or post-ruminal digestion.

19 *Ruminal effects*

20 Direct hydrolysis: Until recently it was assumed that upon introduction into the rumen,
21 exogenous enzymes would be rapidly degraded by the array of proteases produced by ruminal
22 microorganisms (Kung, 1996). Indeed, fungal cellulases incubated with ruminal fluid were
23 rapidly degraded to that extent that after 6 h of incubation, less than 25% of their original activity
24 remained (Kopency *et al.*, 1987; Vandevoorde and Verstraete, 1987). However,

1 experimentation with other enzyme products showed that CMCase activity and xylanase activity
2 remained constant after 6 h of incubation with ruminal fluid (Hristov *et al.*, 1998b). Furthermore,
3 exogenous enzymes increased xylanase and cellulase activity in the rumen (Hristov *et al.*,
4 1998a,b,1999) and there is evidence that declining exogenous enzyme activity in ruminal fluid is
5 associated both with inactivation of the enzyme and with their outflow with the fluid phase of
6 ruminal contents (Figure 4; Hristov *et al.*, 1996b).

7 The fact that exogenous enzymes remain active in the rumen raises the possibility that
8 they may improve digestion through the direct hydrolysis of ingested feed. Several researchers
9 have shown that exogenous enzymes can enhance fiber degradation by ruminal
10 microorganisms *in vitro* (Forwood *et al.*, 1990; Varel *et al.*, 1993; Hristov *et al.*, 1996a; Feng *et*
11 *al.*, 1996; Dong *et al.*, 1999) and *in situ* (Lewis *et al.*, 1996). This effect has been confirmed in
12 some (Beauchemin *et al.*, 1998a; Yang *et al.*, 1998a) but not in all (Firkins *et al.*, 1990; Varel
13 and Kreikemeier, 1994) studies conducted using cattle with ruminal and duodenal cannulae.
14 Although adding exogenous enzymes may increase the activity of xylanases and cellulases in
15 ruminal fluid, enzyme activity in the fluid usually represents less than 30% of the total enzyme
16 activity in the rumen, the remainder being associated with the feed particles (Minato *et al.*, 1966;
17 Brock *et al.*, 1982). For example, applying fibrolytic enzymes to a grass hay diet for sheep prior
18 to consumption increased endoglucanase activity and xylanase activity in ruminal fluid, but this
19 activity accounted for only 0.5% of the total endoglucanase activity in the rumen (Table 3; Dong,
20 1998). Given that exogenous enzymes represent only a small fraction of the ruminal enzyme
21 activity, and that the ruminal microbiota is inherently capable of readily digesting fibre (McAllister
22 *et al.*, 1994), it is difficult to envision how exogenous enzymes would enhance ruminal fibre
23 digestion through direct hydrolysis.

24
25 Synergism with ruminal microorganisms: Enhancement of fibre digestion in the rumen would

1 seem more feasible if these products are working synergistically with ruminal microbes.
2 Logically, this concept implies that exogenous enzyme preparations contain enzymatic activities
3 that would normally be limiting to digestion of plant cell walls by ruminal microorganisms.
4 Limitations to plant cell wall digestion in the rumen could result from insufficient quantities or
5 types of enzyme production by the ruminal microbes, from an inability of degradative enzyme(s)
6 to interact with the target substrates, or from conditions in the rumen not being optimal for
7 enzyme activity (e.g., low ruminal pH). At least 21 different enzymatic activities have been
8 identified as being involved in the hydrolysis of the structural polysaccharides of the plant cell
9 wall, all of which are produced by a normally functioning ruminal microflora (White *et al.*, 1993).
10 Researchers have shown that extracts from *Aspergillus oryzae* can increase the number of
11 ruminal bacteria (Newbold *et al.*, 1992a,b) and can work synergistically with extracts from
12 ruminal microorganisms to enhance release of soluble sugars from hay (Newbold, 1995). The
13 extent of cross-linking by *p*-coumaryl and feruloyl groups to arabinoxylans has been identified
14 as one factor that limits the digestion of plant cell walls (Hatfield, 1993). *Aspergillus oryzae* has
15 been shown to produce an esterase capable of breaking the ester bridges formed between ferulic
16 and *p*-coumaric acids and arabinoxylan (Tenkanen *et al.*, 1991); these are the activities that
17 have been proposed to function synergistically with ruminal microorganisms (Varel *et al.*, 1993).
18 However, many of the ruminal fungi (e.g., *Neocallimastix* spp., Borneman *et al.*, 1990) and
19 some species of ruminal bacteria (e.g., *Fibrobacter succinogenes*, McDermid *et al.*, 1990;
20 *Butyrivibrio fibrisolvens*, Dalrymple *et al.*, 1996) produce esterases capable of hydrolyzing
21 linkages with phenolic acids. The fact that exogenous enzymes usually only increase the rate
22 and not the extent of digestion (Varel *et al.*, 1993; Feng *et al.*, 1996; Hristov *et al.*, 1996a)
23 suggests that the activities contributed by these additives are not novel to the ruminal
24 environment. Recent work in which oligonucleotide 16S rRNA probes are used to study rumen
25 ecology suggests that there may be several species of fibrolytic ruminal bacteria (e.g., clostridia,

1 ruminococci) that have yet to be cultured in the laboratory (Forster and Whitford, 1998). If this is
2 the case, these microorganisms may also be contributing to the diverse array of enzyme
3 activities required for efficient digestion of plant cell walls.

4 Hydrolysis of cellulose and hemicellulose is accomplished either by free enzymes or by
5 cellulosomal structures comprising multiple enzymes bound non-covalently to form an organized
6 complex (Teeri, 1997). Aerobic fungi, the principal commercial source of exogenous enzymes,
7 hydrolyze plant cell walls by means of free enzymes, whereas hydrolysis of plant cell walls by
8 *Clostridium* spp. and the anaerobic ruminal fungi involves cellulosomal structures (Beguin *et al.*,
9 1998; Ljungdahl *et al.*, 1998). There is also evidence that the ruminococci may rely on a
10 cellulosome-like multi-enzyme complex for fibre degradation (Flint *et al.*, 1998; Ohara *et al.*,
11 1998). Destruction of these multi-enzyme complexes during the extraction process may explain
12 why enzymes from mixed ruminal microorganisms failed to release as much soluble sugar from
13 hay and straw as extracts from *A. oryzae* (Newbold, 1995).

14 Evidence has also been presented that cellulosomes may play a role in adhesion of
15 microbial cells to their substrates (Pell and Schofield, 1993; Beguin *et al.*, 1998). The process
16 of adhesion is absolutely essential for efficient digestion either of forages or cereal grains in the
17 rumen (McAllister *et al.*, 1994; 1996). Cellulose binding domains may be involved in the
18 attachment of ruminal bacteria to cellulose (Pell and Schofield, 1993). Presently it is not known
19 if exogenous enzymes block microbial adhesion sites on the surface of feeds, or expose
20 additional ones. Administering an aqueous solution of mixed fibrolytic enzymes (3.3% vol vol⁻¹)
21 directly into the rumen of cannulated sheep actually lowered DM digestion (McAllister *et al.*,
22 1998), and similar results were reported when enzymes were infused into beef steers (Lewis *et al.*,
23 1996). These studies suggest that enzymes are not as efficacious when ruminally infused
24 as when applied directly to the feed. Alternatively, this response may be enzyme-dependent,
25 given that administration of other enzymes directly into the rumen did not affect DM digestion

1 (Hristov *et al.*, 1999). Treating alfalfa cubes with exogenous enzymes prior to consumption
2 increased bacterial colonization and *in situ* DM disappearance of forage between 3 h and 24 h
3 of ruminal incubation (Yang *et al.*, 1998a). These responses were supported by a concurrent
4 numerical increase in digestibility of fibre in the rumen, and by significantly increased
5 digestibility of fibre in the total gastrointestinal tract (Yang *et al.*, 1998a).

6 Considering the low fiber content of high concentrate diets, it is surprising that fibrolytic
7 enzymes have improved feed digestion (Krause *et al.*, 1998) and performance of cattle fed high
8 cereal grain diets (Beauchemin *et al.*, 1997; Iwassa *et al.*, 1997). An explanation of this
9 phenomenon may come from comparing the pH optima of the fibrolytic enzymes produced by
10 ruminal microorganisms with the pH optima of exogenous fibrolytic enzymes produced by
11 aerobic fungi. It is well documented that growth of fibrolytic bacteria is inhibited (Russell and
12 Dombrowski, 1980), and that fibre digestion is severely compromised when pH falls below 6.2
13 (Hoover *et al.*, 1984). Most of the fibrolytic enzymes produced by ruminal microorganisms
14 function optimally at pH above 6.2 (Greve *et al.*, 1984; Matte and Forsberg, 1992). In contrast,
15 the pH optima of fibrolytic enzymes produced by aerobic fungi typically range from 4.0 to 6.0
16 (Gashe, 1992; Muzakhar *et al.*, 1998). This point is illustrated by an experiment in which the
17 extent to which *Trichoderma longibrachiatum* enzymes enhanced gas production was shown to
18 increase as the pH declined from 6.5 to 5.5 (Table 4; Morgavi *et al.*, unpublished data). Further,
19 although a decline in pH from 6.5 to 5.5 reduced DM disappearance from corn silage in mixed
20 ruminal cultures supplemented with *T. longibrachiatum* enzymes, the negative effect of low pH
21 on DM disappearance was more pronounced in the absence of added enzyme (Table 4).
22 Ruminal pH can be below 6.0 for a significant portion of the day in dairy cattle (Nocek, 1998;
23 Yang *et al.*, 1998a) and in feedlot cattle (Krause *et al.*, 1998). Under these conditions,
24 exogenous enzymes could make a significant contribution to ruminal fiber digestion. The
25 higher fibre content of barley, as compared to corn, may explain why exogenous enzymes

1 improved feed conversion in finishing cattle fed barley grain but did not affect feed conversion
2 by finishing cattle fed corn (Beauchemin *et al.*, 1997).

3 Some evidence also suggests that non-enzymatic factors in crude enzyme extracts may
4 work synergistically with ruminal microorganisms. In early experiments with fungal cellulases,
5 boiling the extract for 20 minutes failed to reduce its effect on cellulose digestion *in vitro*
6 (Bowden and Church, 1959). In that study, adding valine or proline to the incubation medium
7 enhanced cellulose digestion to the same extent as adding fungal cellulases. A more recent
8 study showed that autoclaved *A. oryzae* extract (8% vol vol⁻¹) enhanced cell wall degradation *in*
9 *vitro* (Varel *et al.*, 1993). The researchers attributed the effect to the presence of soluble
10 substrate in the extract, but concluded that it would not be relevant at the concentration of
11 extract (i.e., 0.067 mg ml⁻¹) expected at recommended *in vivo* dosages. The effects of non-
12 enzymatic factors are likely expressed more *in vitro* than *in vivo*, because microbial populations
13 have limited adaptive capabilities *in vitro*, and they are exposed to higher concentrations of the
14 additive due to the absence of flow or dilution. Enzyme extracts often contain preservatives to
15 prolong their shelf life, as well as emulsifying agents (e.g., surfactants) that maintain the
16 enzymes in suspension and facilitate application of the product to the feed. Work in our
17 laboratory has shown that surfactants can alter microbial activity and feed degradation in the
18 rumen (McAllister *et al.*, 1999). Unfortunately, few enzyme manufacturers list the non-
19 enzymatic components of their products and the consequent difficulty in distinguishing between
20 non-enzymatic and enzymatic effects of enzyme products continues to hamper progress toward
21 defining the mode of action of these products.

22 Digesta flow: Enzymes have been shown to enhance (Feng *et al.*, 1996; Dong, 1998) and to
23 exert no effect (Beauchemin *et al.*, 1998a; Yang *et al.*, 1998a) on the rate of passage of
24 particulate matter from the rumen. Increases in passage rate can be associated with a faster

1 rate of particle size reduction in the rumen and a corresponding increase in feed intake
2 (Mertens *et al.*, 1984). Although exogenous enzymes have improved both intake and digestion
3 in some experiments (Stokes and Zheng, 1995; Sanchez *et al.*, 1996), in others enhanced
4 digestion is not associated with an increase in feed consumption or particulate passage rate
5 (Judkins and Stobart, 1988; Beauchemin *et al.*, 1998a; Krause *et al.*, 1998; Kung *et al.*, 1998).
6 These studies suggest that some portion of the effects of exogenous enzymes may be post-
7 ruminal.

8 *Post-ruminal effects*

9 Experiments in our laboratory have shown that exogenous enzymes not only heighten fibrolytic
10 activity in the rumen, but also increase fibrolytic activity in the small intestine (Hristov *et al.*,
11 1998a,b,1999). This phenomenon was particularly evident with xylanase activity.
12 Supplementary enzymes increased duodenal xylanase activity by 30% (Hristov *et al.*, 1998a).
13 In the same study, enzyme supplementation increased cellulase activity at the small intestine by
14 only 2 to 5%, because the enzymes were largely inactivated by the low pH and pepsin in the
15 abomasum. Other researchers have reported that xylanases from both mesophilic and
16 thermophilic microorganisms resist proteolysis (Fontes *et al.*, 1995), possibly due to their high
17 degree of glycosylation (Gorbacheva and Rodionova, 1977), but our work has shown that
18 acidity, not pepsin, is the primary factor responsible for the inactivation of xylanases in the
19 abomasum (Hristov *et al.*, 1998a). At pH 2.0 to 2.6 (Sturkie, 1970) gastric fluids in the gizzard
20 of poultry are typically less acidic than digesta in the stomach of swine or in the abomasum of
21 ruminants, and they have shorter residence times. This difference in gastrointestinal physiology
22 may explain why positive responses to enzyme supplementation are more consistent in poultry
23 than in swine or ruminants (Campbell and Bedford, 1992; Baas and Thacker, 1996).

24 Administering large quantities of enzymes (400 g d⁻¹) directly into the rumen can

1 significantly increase cellulase activity in the small intestine (Hristov *et al.*, 1999). Exogenous
2 enzymes supplemented in this manner flow mainly with the fluid phase of ruminal contents
3 (Hristov *et al.*, 1999) and at these elevated feeding levels, a portion of the cellulases even
4 escapes inactivation by the low pH and pepsin in the abomasum. We have observed that
5 increased xylanase activity in the small intestine is associated with a decline in intestinal
6 viscosity (Table 5; Hristov *et al.*, 1998b, 1999). Because viscosity of duodenal digesta
7 increases with increasing levels of grain in the diet (Mir *et al.*, 1998), enzyme-mediated
8 reductions in viscosity could improve nutrient absorption in the small intestine of cattle fed grain
9 diets. Reduced intestinal viscosity was associated with 1.2% and 1.5% increases in total tract
10 digestibility of DM when enzymes were applied to the feed or infused into the abomasum,
11 respectively (Hristov *et al.*, 1998a). However, intestinal viscosity in cattle is only between 1 and
12 2 cP (Mir *et al.*, 1998) whereas intestinal viscosity in poultry may exceed 400 cP (Bedford,
13 1993). Improved growth performance in poultry supplemented with enzymes is often associated
14 with 10-fold reductions in intestinal viscosity (Bedford, 1993; Graham, 1996). Consequently, it is
15 difficult to comprehend how the relatively modest declines in intestinal viscosity observed in
16 ruminants supplemented with high levels of enzymes results in a substantial improvement in
17 nutrient absorption in the small intestine.

18 In studies with dairy cows fed barley grain diets, improvements in total tract digestion
19 were attributed primarily to an improvement in the digestibility of fiber and starch in the lower
20 tract (Beauchemin *et al.*, 1998a). Hydrolysis of complex carbohydrates by exogenous enzymes
21 in the small intestine and subsequent absorption of released sugars would offer energetic and
22 nitrogen balance benefits to the animal that would not be accessible if these substrates
23 remained undigested or were fermented by microbial populations residing in the large intestine.
24 It is possible that exogenous enzymes work synergistically with the microbes even in the large
25 intestine, given that we have determined xylanase activity in the feces to increase linearly with

1 increasing levels of enzyme supplementation (Table 5; Hristov *et al.*, 1999). This heightened
2 fibrolytic activity may have important ramifications with regard to the rate at which fecal material
3 decomposes in the environment.

4 **Steps toward improving exogenous enzymes for ruminants**

5 *Match the enzyme to the feed*

6 Not all exogenous enzymes are equally effective at digesting complex substrates such as alfalfa
7 and barley grain (Table 6). Feedstuffs are exceedingly complex structurally, and our lack of
8 knowledge of the factors that limit the rate and extent of feed digestion impedes our engineering
9 of enzyme preparations designed to overcome constraints to feed digestion. With some feeds,
10 specific targets can be identified. In corn, for example, the protein matrix surrounding the starch
11 granules, as opposed to the properties of the starch itself, dictates the extent and rate of starch
12 digestion in that grain (McAllister *et al.*, 1993). Thus, exogenous enzymes designed to improve
13 the utilization of corn should contain proteases capable of digesting the protein matrix and
14 exposing starch granules to digestion by endogenous ruminal or host enzymes. An exogenous
15 preparation containing amylase but not protease activity would not be expected to substantially
16 improve utilization of corn by ruminants. In straw, the major barriers to microbial digestion are
17 apparently silica, wax and cutin (Bae *et al.*, 1997) whereas in unprocessed grain, the pericarp
18 dictates the extent of grain digestion (Wang *et al.*, 1998). Enzyme preparations that digest the
19 structural components limiting the extent of feed digestion in the rumen would be expected to be
20 more efficacious than preparations that just increase the rate of feed degradation in the rumen.
21 Many enzyme preparations are currently in use, with no attempt being made to define the types
22 or activities of the enzymes they contain. Such random employment of enzymes on feeds,
23 without consideration for specific substrate targets, will only discourage or delay adoption of
24 exogenous enzymes for more standard use in the animal production industry. Ultimately,

1 enzyme cocktails should be designed specifically to overcome the constraints limiting digestion
2 of a particular type of feed. Component enzymes in such cocktails might vary even for a given
3 forage, targeting particular maturity levels and structural barriers. Recent developments in
4 biotechnology make it feasible to engineer such enzyme cocktails containing xylanase and β -
5 glucanase activities, but we presently lack the technology for specific production of many other
6 potentially important enzymes (e.g., cutinase, ferulic acid esterase, acetylxylin esterase,
7 arabinofuranosidase).

8 *Lower enzyme cost*

9 Once the mechanisms of action and specific targets for degradative exogenous enzymes have
10 been identified, steps can be taken to optimize the application of these preparations.
11 Application concentrations can be minimized by ensuring that the preparations contain the
12 enzyme activities most likely to elicit an improvement in feed utilization. In some instances, the
13 activity of crude enzyme preparations may be increased by including specific enzymes most
14 likely to overcome the constraints to feed digestion. Recently, application of recombinant DNA
15 technology has enabled manufacturers to increase the volume and efficiency of enzyme
16 production, and to create new products (Ward and Conneely, 1993; Hodgson, 1994). Genes
17 encoding superior enzymes can be transferred from organisms such as anaerobic bacteria and
18 fungi, typically impractical for commercial production, into well characterized industrial microbial
19 production hosts (e.g., *Aspergillus* and *Bacillus* spp.). Expression of genes encoding novel
20 enzymes in plants such as canola and potato may be a particularly effective means of lowering
21 the cost of enzyme production (Pen *et al.*, 1993; van Rooijen and Moloney, 1994). At the
22 Lethbridge Research Centre, a β -glucanase gene from the ruminal bacterium *Fibrobacter*
23 *succinogenes* has been expressed in a number of lines of potato, and a 10-fold difference in
24 level of expression has been observed among lines (Table 7). The line exhibiting the highest

1 expression levels is now being evaluated in diets for poultry. It is possible that similar
2 technologies (e.g., grasses expressing cutinase, esterase) may have applications in ruminant
3 production.

4 **Conclusion**

5 The use of feed enzymes in the monogastric animal production industry has increased
6 dramatically in recent years and numerous commercial products are presently being marketed.

7 In many instances, the mechanisms and modes of action of these preparations have been
8 defined. In contrast, few commercial preparations of exogenous enzymes exist for ruminants
9 and many of these just now entering the marketplace. Although positive responses in animal
10 performance have been observed, results have been inconsistent. Characteristics of the
11 ruminant digestive tract (e.g., complex microbial populations producing numerous endogenous
12 enzymes) complicates elucidation of the mechanisms of exogenous enzyme action in
13 ruminants. There is evidence that exogenous enzymes initiate digestion of feeds prior to
14 consumption and that they can improve feed digestion in the rumen and lower digestive tract.

15 The challenge for researchers is to determine the modes of action, singly or in combination, that
16 enable exogenous enzymes to improve feed efficiency and increase growth and milk
17 production.

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34 production of dairy cows. *Journal of Animal Science* 76, Suppl. 1, 320.Suppl. 1, 320.

1 **TABLE 1. Relative activities of three commercial enzyme preparations on substrates that**
 2 **reflect cellulase activity**

Product	% Protein	Substrate ^z		
		Cellulose ^y	CMC ^x	β-Glucan
1	11.5	102.1	737.9	1016.4
2	21.3	126.1	727.1	1042.1
3	15.6	225.6	699.7	1638.3

9 ^zFor all substrates, activity is expressed as nmol reducing sugar released mg⁻¹ product min⁻¹,
 10 after a 3-h incubation of the substrate with the enzyme product in 0.2 M sodium phosphate (pH
 11 6.0) buffer at 39°C.

12 ^yMicrocrystalline cellulose

13 ^xCarboxymethylcellulose

14 (Adapted from Hristov *et al.*, 1996b)

TABLE 2. Dry matter intake, milk yield and composition of lactating cows fed enzyme-treated diets

Item	Diet ^z				SE ^y
	Control	LH	HH	HT	
DMI					
kg d ⁻¹	20.4	20.7	20.7	20.8	0.7
% of BW	3.29	3.39	3.32	3.42	0.14
BW (kg)	621	623	626	619	3
Milk yield ^x (kg d ⁻¹)					
Actual	23.7 ^b	24.6 ^{ab}	25.6 ^a	25.3 ^{ab}	0.6
4% FCM	22.4 ^b	22.9 ^{ab}	24.6 ^a	24.2 ^a	0.7
SCM	22.2 ^b	23.2 ^{ab}	24.4 ^a	24.2 ^a	0.7
Milk composition (%)					
Fat	3.79	3.70	3.78	3.76	0.11
Protein	3.36	3.41	3.48	3.49	0
Lactose	4.56 ^b	4.61 ^{ab}	4.60 ^{ab}	4.62 ^a	0
Milk DMI ⁻¹ (kg kg ⁻¹)	1.2	1.22	1.29	1.25	0.1

^{a,b}Within a row, means bearing unlike superscripts differ ($P < 0.05$).

^zLH = low level of enzyme added to cubed alfalfa hay; HH = high level of enzyme added to cubed alfalfa hay; HT = enzyme added to both cubed alfalfa hay and concentrate.

^ySE = Standard error.

^xFCM = fat-corrected milk; SCM = solids-corrected milk.

1 **TABLE 3. Effect of exogenous enzymes on endoglucanase and xylanase activities^z in the**
 3 **rumen of sheep fed grass hay**

		% of Total Ruminal Activity			
		CON	ENZ	CON	ENZ
8	Activity in liquid phase (units ml ⁻¹ h ⁻¹)				
9	Endoglucanase	0.026	0.029 ^b	–	–
10	Xylanase	0.315 ^a	0.344 ^b	–	–
11	Activity in particulate phase (units g ⁻¹ DM h ⁻¹)				
12	Endoglucanase	3.14	3.12	–	–
13	Xylanase	2.37	28.27	–	–
14	Total activity in liquid phase (units × 10 ³)				
15	Endoglucanase	0.14	0.165	3.6	4.6
16	Xylanase	1.76	1.95	5.4	6.4
17	Total activity in particulate phase (units × 10 ³)				
18	Endoglucanase	3.79	3.45	96.4	95.4
19	Xylanase	30.5	28.54	94.6	93.6

20 ^{a,b}Within a row, means bearing unlike superscripts differ ($P < 0.05$).

21 ^zEndoglucanase activity was standardized against a commercial enzyme preparation from
 22 *Penicillium funiculosum* (EC 3.2.1.4, Sigma Chemical Co., St. Louis, MO) and xylanase activity
 23 was standardized against a commercial xylanase activity from *Aspergillus niger* (EC3.2.18,
 24 Sigma Chemical). Incubations were conducted in sodium phosphate buffer (pH 6.5) at 39°C for
 25 30 min. Adapted from Dong, 1998.

1 **TABLE 4. Effect of pH and *Trichoderma longibrachiatum* enzyme preparations on *in vitro***
 2 **gas production and dry matter disappearance from corn silage during 48 h of incubation**
 3 **with mixed ruminal cultures^z**

4	Item	pH	No enzyme	Enzyme	
5				Autoclaved	Unautoclaved
6					
7	Gas production (ml)	6.5	9.4	10.4	11.9
8		6.0	7.3 ^a	8.0 ^a	9.8 ^b
9		5.5	6.4 ^a	7.1 ^a	9.0 ^b
10	DM disappearance (%)	6.5	32.1 ^a	31.5 ^a	36.2 ^b
11		6.0	23.6 ^a	23.5 ^a	31.8 ^b
12		5.5	23.2 ^a	22.8 ^a	32.7 ^b

13 ^{a,b}Within a row, means bearing unlike superscripts differ ($P < 0.05$).

14 ^zConsecutive batch culture techniques were used to adapt mixed ruminal microorganisms to each
 15 respective pH prior to incubation. Morgavi *et al.*, unpublished data.

TABLE 5. Enzyme activity^z in duodenal digesta and feces of cattle with and without enzyme supplementation

Item	Experiment 1 ^y			Experiment 2 ^x				<i>P</i> ^w
	Control	EF	EA	Control	EFL	EFM	EFH	
Ruminal contents								
CMCase	42.7	51.9	41.1	43.3	48.0	48.6	52.9	***
Xylanase	215.2	298.1	208.1	179.1	237.3	264.0	299.1	***
Amylase	190.1 ^a	160.5 ^{ab}	95.9 ^b	144.1	138.1	137.9	144.0	NS
Viscosity (cP)	3.16 ^{AB}	2.99 ^B	3.34 ^A	3.33	3.06	2.90	2.74	***
Duodenal contents								
CMCase	ND	0.75	2.20	0.18	0.79	1.89	6.32	***
Xylanase	3.6 ^a	45.1 ^b	109.1 ^b	ND	6.72	13.7	37.4	***
Amylase	0.98	0.82	0.77	0.92	0.87	0.98	2.88	***
Viscosity (cP)	1.73	1.56	1.54	1.75	1.53	1.73	1.49	***
Feces								
CMCase	NM	NM	NM	30.7	19.6	26.5	46.0	NS
Xylanase	NM	NM	NM	658.5	686.0	832.1	900.0	***
Amylase	NM	NM	NM	447.3	479.2	512.1	450.2	NS
Total tract								
digestibility (%)	80.2	81.4	81.9	74.9	76.2	75.3	74.5	NS

^zExpressed as nmol reducing sugars released ml⁻¹ min⁻¹.

^yEF, enzyme applied to feed, EA, enzyme infused into abomasum. Adapted from Hristov *et al.*, 1998.

^xEnzyme introduced directly into the rumen at rates of 0 (Control) or 100, 200 and 400 g d⁻¹ (EFL, EFM and EFH, respectively). Adapted from Hristov *et al.*, 1999.

^wLinear effect of enzyme supplementation of enzyme activity ($P < 0.001$).

^{a,b}Within a row and experiment, means bearing unlike superscripts differ ($P < 0.05$).

^{A,B}Within a row and experiment, means bearing unlike superscripts differ ($P < 0.10$).

1 **TABLE 6. Release of reducing sugars² from alfalfa hay and hulless barley by a number of**
 2 **commercial fibrolytic enzyme products**

3 4 5 6 Product	Substrate	
	Alfalfa hay	Hulless barley
7 A	379.0	4980.32
8 B	102.8	1384.4
9 C	122.3	1785.5
10 D	106.4	1201.8
11 E	30.2	387.0
12 F	31.0	489.5
13 G	134.7	1808.7
14 H	156.1	2780.3
15 I	170.7	2424.9
16 J	62.8	2558.3

17 ²Activity is expressed as ppm of glucose released by the product (present at 0.250 mg ml⁻¹)
 18 under standardized test conditions.

1 **TABLE 7. Accumulation of glucanase in the leaf and tuber tissue of seven transgenic**
 2 **lines² of potato (*Solanum tuberosum*)**

Line	Glucanase (as % of total protein)	
	Leaf Tissue	Tuber tissue
1	0.011	0.004
2	0.016	0.005
3	0.020	0.011
4	0.031	0.023
5	0.067	-
6	0.105	0.050
7	0.047	0.021

13 ²Beta-glucanase from *Fibrobacter succinogenes* was expressed in potato using the cauliflower
 14 mosaic virus (CAMV) 35S promoter (Armstrong *et al.*, 1999).

FIGURE CAPTIONS

1

2

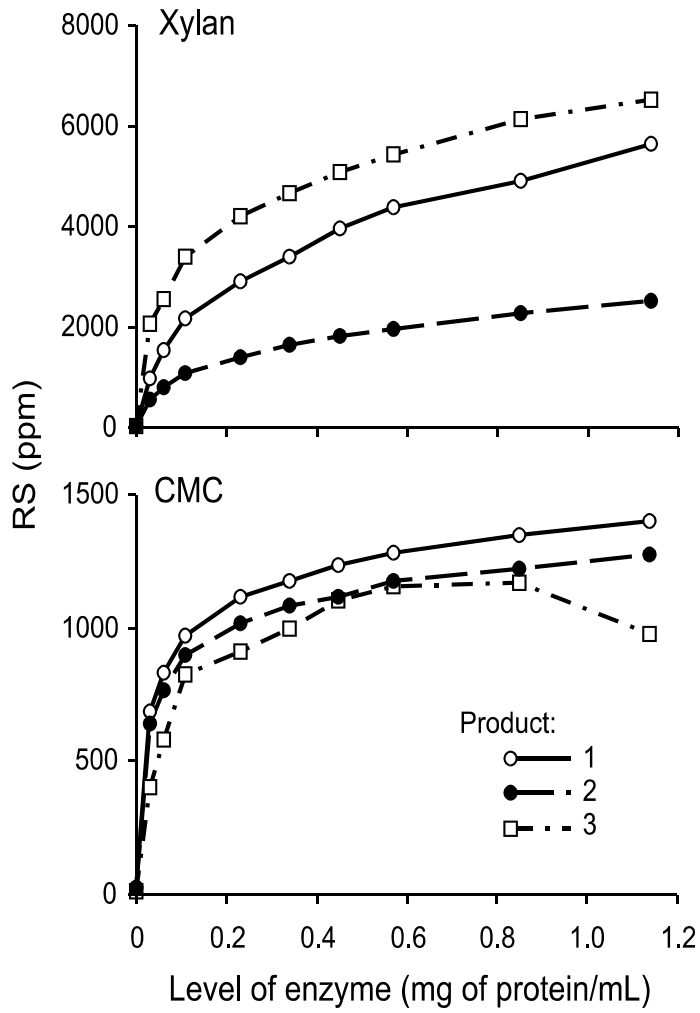
3 **Figure 1.** Release of reducing sugars from xylan and carboxymethylcellulose (CMC) during a 5-
4 h incubation with three commercially available enzyme preparations. Note that the relationship
5 between reducing sugar release and enzyme concentration is not linear, indicating that
6 concentration of enzyme selected can affect estimates of activity. (Hristov and McAllister,
7 unpublished).

8 **Figure 2.** Possible modes of action of exogenous enzymes in ruminants. (A) Prior to
9 consumption, exogenous enzymes may partially digest feed or weaken structural barriers that
10 impede microbial digestion in the rumen. (B) In the rumen, exogenous enzymes may hydrolyze
11 feed directly or work synergistically with ruminal microorganisms to enhance feed digestion. (C)
12 In the small intestine, exogenous enzymes may improve nutrient absorption by reducing
13 intestinal viscosity, or by hydrolyzing substrates that escape ruminal digestion. (D) In the feces,
14 exogenous enzymes may increase the rate of decomposition.

15 **Figure 3.** Scanning electron micrographs of barley straw (A) untreated or (B) incubated in a
16 1:10 dilution of concentrated enzyme product containing cellulases and xylanases (Finnfeeds
17 International Ltd.). Note that this exceedingly high concentration of the enzyme caused visible
18 degradation of the barley straw. In other studies, enzymes were applied at levels recommended
19 by the manufacturer (e.g., 2.0 L tonne⁻¹) and no visible damage of the surface of the straw was
20 observed (data not shown). Bars = 250 Fm. (McAllister, Bae and Cheng, unpublished).

21 **Figure 4.** Decline in endocellulase and β -glucanase activities in the rumen during a 15-h period
22 following administration of two enzyme products directly into the rumen. Note that decline of

1 both enzyme activities differs between the two products, and that the declines are related to the
2 passage of fluid from the rumen, as measured by the decline in Cobalt-EDTA. RS: Reducing
3 sugars. (Adapted from Hristov *et al.*, 1996b).



Enzyme mode of action

Rumen

- ↑ • Microbial numbers
- ↑ • Microbial attachment
- ↓ • Particle size
- ↑ • Rate of passage
- ↑ • Digestion

Small intestine

- ↓ • Viscosity
- ↑ • Digestion

Large intestine

- ↑ • Digestion



Preconsumption



Decomposition

