EFFECT OF EXOGENOUS FIBROLYTIC ENZYMES ON RUMINANT PERFORMANCE

A. Hussain, M. Nisa, M. Sarwar, M Sharif and A. Javaid
Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad

The objective of this paper is to examine the role of exogenous fibrolytic enzymes on ruminant productivity. These fibrolytic enzymes have been reported to be effective to increase feed efficiency and ruminant performance. The response of these enzymes is dependent on experimental conditions and is variable. This variation was due to experimental conditions such as the activities and characteristics of the enzymes supplied, variability in enzyme activity, method of providing the enzyme product to the animal and enzyme level in the feed. A limited number of ruminant enzyme products is now commercially available, and this list of products is expected to grow. Random addition of enzymes to diets without consideration for specific situations and substrate targets may disturb or delay the efficiency of enzymes and adoption of enzyme technology on farms.
Keywords: Digestibility, fibrolytic enzymes, fiber, ruminants

INTRODUCTION

Over the years, significant improvements have been achieved in the digestibility of the forages through different chemicals such as sodium hydroxide, hydrogen peroxide, anhydrous ammonia and urea. These chemicals pose different problems when used bt farmers and that is why their use by farming community is limited. As an alternate to these chemicals, fibrolytic enzyme can be used to improve the energy availability for ruminants. The use of exogenous fibrolytic enzymes in the ruminant diet has been reported to improve the fiber digestibility and their use may become common because of absence of their any negative effect on animal health and performance. Vicini *et al.* (2003) studied the impact of fibrolytic enzymes on the digestibility of fibrous diet and concluded that the use of fibrolytic enzymes in ruminant diet comprising mainly fibrous portion could improve digestion and utilization.

Feng *et al.* (1996) demonstrated the effect of exogenous fibrolytic enzymes on dry matter (DM) and neutral detergent fiber (NDF) through *in vitro* technique and reported increased DM and NDF digestibility. Beauchemin *et al.* (2000) used *in vivo* method and reported increased DM and NDF digestibility. In an other study, *In vivo* data indicate improved intake, digestibility, particulate passage, and ruminal degradability when fibrolytic enzymes were added to dry grass immediately before feeding (Feng *et al.*, 1996). A number of studies have shown that the addition of such enzymes to grass or alfalfa before ensiling reduced the concentration of plant structural carbohydrates compared with untreated silages (Jacobs and McAllan, 1991; Stokes, 1992). Improved digestibility of organic matter by sheep (Huhtanen *et al.*, 1985) and milk production (Stokes, 1992; Chen *et al.*, 1994) by cattle have been observed with enzyme-treated silages. Recent research shows that the use of exogenous fibrolytic enzymes holds promise as a means of increasing forage utilization and the productive efficiency of ruminants.

The main objective of this paper is to examine the influence of fibrolytic enzymes on enzyme level, legumes versus grasses, silage, feed intake, digestion kinetics, digestibility, growth performance and milk yield.

Enzyme Level

Some of the variability associated with the use of exogenous enzyme products in ruminant diets is due to supplementation with insufficient or excessive enzyme activity. Kung *et al.* (2000) offered forage (60% corn silage and 40% lucerne hay; on DM basis) treated with increasing levels (0, 1, 2.5 mL/kg of TMR) of an enzyme product to cows. Cows fed the low level of enzyme tended to produce more milk (39.5 kg/d) than those fed the control diet (37.0 kg/d) or those fed the high level of enzyme (36.2 kg/d). Applying fibrolytic exogenous enzymes in a liquid form on to feeds prior to consumption can have a positive effect on animal performance (Rode *et al.*, 1999; Kung *et al.*, 2000). In contrast, infusion of enzymes into the rumen has not been effective (Sutton *et al.*, 2001). There is apparently little or no requirement for a reaction phase or incubation time between treatment and feeding of forages. Lewis *et al.* (1996) observed an increase in total-tract NDF digestibility when an enzyme solution was applied to dry hay prior to feeding, but there was no difference between applying the enzyme immediately before feeding and a 24-h incubation period. *In vitro* studies have reported similar results (Colombatto *et al.*, 2000).

Krause *et al.* (1998) used concentrated enzyme mixture containing mainly cellulase and xylanase, dissolved in water (100 g/L) and then added to barley (15 L/t) during tempering. The barley was tempered by adding 15% water and leaving it overnight. The grain was then rolled and dried. This study resulted to improve NDF and ADF digestibility. Feng *et al.* (1996) applied an enzyme solution directly to grass and observed no effect when added to fresh or wilted forage; however, when it was applied to dried grass, enzymes increased DM and fiber digestibility. Similarly, Yang *et al.* (2000) reported increased milk production and digestibility of the diet when enzymes were added to the concentrate portion of a dairy cow diet, but not when they were added directly to TMR.

Muwalla *et al.*, (2007) used a fungal cellulase derived from Trichoderma spp. The enzyme contained 200-unit/mg cellulase activity at pH 7.0 and he applied this fibrolytic enzyme at a rate of 150 g/ton of forage consumed. Enzyme requirements for 1 ton of the rations were mixed in 500 g of ground corn, mixed with the micronutrient part of the ration and added to the concentrate part at mixing. The diet was mixed biweekly. Miller *et al.* (2008) supplemented the grain portion of a concentrate diet with a fibrolytic enzyme product RG2 in liquid form. The levels of enzyme in treatments included a control (no enzyme added, water only), low RG2 level (1.22 ml RG2/kg DM total ration), medium RG2 level (4x low RG2 rate) and a high RG2 level (8x low RG2 rate). The enzyme product was tested for the activity of enzymes 1, 4-glucanase, endo-1, 4-glucanase, endo-1, 4-xylanase, alpha-amylase and d-glucanase. The RG2 liquid was first diluted with water to 20 ml/kg.

Jafari *et al.* (2005) used enzyme in liquid form with activity 1437, 788, and 7479 μmol per min. per ml for exoglucanase, endoglucanase, and xylanase, respectively. Concentrate enzymes was diluted and sprayed on the wheat straw 1 hour prefeeding. Then wheat straw was mixed in the alfalfa hay with different levels; 0%, 10%, 30% and 30% untreated wheat straw replaced in alfalfa hay.

Legumes Versus Grasses

Fibrolytic enzymes are equally effective in hydrolyzing both legume and grass forages (Chen *et al.* 1994; Selmer-Olsen *et al.* 1993; Chamberlain and Robertson 1992). In other studies (Kung *et al.* 1991 and Weinberg *et al.* 1993) increasing fibrolytic enzyme doses did not produce significant cellulase or pectinase effects on either NDF or ADF in alfalfa hay and mature pea, ryegrass and wheat silage crops respectively. Poutanen *et al.* (1987) observed that bacterial xylanases had different pH, substrate specificity and temperature requirements from fungal enzymes and that 0-xylosidase had a greater activity, compared to bacterial 3-xylosidase. Similarly, Biely *et al.* (1986) and Puls *et al.* (1987) observed that by linking the appropriate xylanolytic enzyme with a complementary forage one could maximize the catalytic power in the xylanase and the addition of extra enzymes such as a-glucuronidase, acetyl xylanesterase and a-arabinosidase in the mixture enhanced the activity of xylanolytic enzymes in hydrolyzing the backbone polymer of xylan. Fibrolytic enzymes with a higher ratio of xylanase activity tend to be more effective in facilitating the *in vitro* digestibility of DM or fiber fractions of legume and grass forages (Spoelstra *et al.*, 1992; Weinberg *et al.*, 1995; Gwayumba and Christensen *et al.*, 1996). Han *et al.*, (1986) observed a 35 percentage units decrease in cellulose content in treated sorghum forage which had been treated with cellulase preparation (10 g enzyme kg" fresh weight) and ensiled for 30 or 60 d. Jaster and Moore (1988) did not observe a change in NDF ADF cellulose hemicellulose and starch from cellulase treated alfalfa hay.

Silage

Several fibrolytic enzymes evaluated as feed additives in ruminant diets were originally developed as silage additives (Feng *et al.*, 1996), such as cellulases and xylanases but now, they are being used in diets. Lewis *et al.* (1999) treated forage with a cellulase / xylanase mixture supplying enzyme as 1 ml/kg of total mixed ration (TMR), on DM basis and observed that cows in early lactation produced 6.3 kg/d (16%) more milk. In the ruminants, their value of enzyme increases more as they appear to survive in the small intestine for a time sufficient to have an effect on target substrates (Morgavi *et al.*, 2001). However, post-ruminal effects of exogenous enzymes on digestion are likely to a significant factor when enzymes are infused into the rumen (Hristov *et al.*, 2000) or added to feed in a manner that allows for easy solubilization in feed. Gwayumba *et al.* (1997) reported that enzymes used for forages especially silage production have two main functions. First, enzymes release free sugars from ensiled plant material to be used by fermentative microbial organisms in producing lactate and acetate, thus lowering the pH and preserving the forage. A second benefit is to increase the cell wall degradation thus lowering the total fiber content of the forage, improving the digestibility of organic matter and may also support higher intakes. It has been observed that the reaction between cellulases and the substrate is complicated by the later being insoluble and therefore hydrolysis is achieved only when the various components of cellulase act synergistically.

The effect of enzyme additives in combination with an inoculant such as lactobacilli on silage quality and animal performance (Fredeen and McQueen 1993) was determined in first and second cut legume-grass mixtures of varying ratios. Two enzyme sources were used: an alfalfa enzyme mix containing cellulase, amylase, glucose oxidase and a grass enzyme mix which was similar to the former additive except it lacked glucose oxidase. Forages were ensiled at 41-42 % moisture content. The resulting silage was not different in DM, ADF, NDF, volatile fatty acids and *in vitro* true digestibility and *in vitro* cell wall digestibility not affected by the treatments. The enzyme response was lacking at all stages of maturity of the grass-legume mixture. Similarly there was no milk yield or composition response in any of the treatments examined, except for a depression of milk fat in alfalfa enzyme mix treated cut forages. There was no effect on cell wall composition and silage quality. However, Jacobs and McAllan *et al.* (1991) found that enzyme treated grass silage reduced apparent digestibility of OM, and later in another study (Jacobs and McAllan. 1992) an increase in apparent OM digestibility in enzyme treated grass silage was observed. Gwayumba *et al.* (1997) discussed that the question of when and in what circumstances enzymes become effective agents in forage improvement is yet to be answered. The observed inconsistencies call for studies to determine the circumstances about pH, temperature, moisture content, incubation time , the forage types, the kinds and forage characteristics or type of processing to facilitate access of enzymes to target cell wall tissues. Determination of the specific enzyme and inoculant types that can give a consistent positive effect as evaluated by animal performance indices such as DM intake, apparent digestibility of organic matter, average daily gain, milk yield and composition.

Feed Intake

Most of the previous studies showed that exogenous fibrolytic enzymes have no effect on nutrient intake but some studies show variable effects. Rodriguez *et al.* (2002) reported that enzymes increased the DM and organic matter (OM) intake, nitrogen intake showing tendency to increase NDF intake. Feng *et al.* (1996) reported that intake of DM was increased by fibrolytic enzyme with dry forages, but not fresh forages. However, fibrolytic enzyme directly fed to the animal, or added to the feed did not change DM intake (Lewis *et al.*, 1996 and Rode *et al.*, 1999). Krause *et al.* (1998) reported that when fibrolytic enzyme sprinkled on forages, intakes of NDF and ADF were lower than expected because of the relatively high proportion of forage. Even though, NDF intakes were some what variable among diets. However, ADF intake was affected by forage source. Eun *et al.* (2005) reported the negative impact of enzymes on the dry matter intake.

Nadeau *et al.* (2000) reported that Cellulase combined with inoculant or formic acid increased total DM intake by 8 and 13%, respectively in lambs, compared with control silage, when averaged across different plant species. These treatments had similar effects on the digestible DM intake. As NDF concentration of forage is a good predictor of the voluntary DM intake by sheep. So, the higher DM intake of the silage treated with cellulase and inoculant or formic acid may have been associated with their lower NDF concentrations compared with the control silages. Additionally, the scientist demonstrated that silage fermentation characterisics have been associated with voluntary intake of silage and thus, the improved fermentation of silages treated with cellulase and inoculant or formic acid compared with the control silages may be another reason for the increased DM intake of these silages. Some other studies indicated significant positive effects on DM intake with enzyme additives (Chen and Stokes, 1992 and Fredeen and McQueen, 1993). The effects of the inoculants on feed DM intake are more likely to be non significant than significant.

Muwalla *et al.* (2007) conducted a study to evaluate the effect exogenous fibrolytic enzymes on high concentrate diet in awassi lambs, a local breed of Jordan. He demonstrated the results that the supplement of these fibrolytic enzyme in the high concentrate diet have no effect on the intake of DM, OM, crude protein, and NDF because all the results were similar for lambs fed diet with or without the fibrolytic enzyme. Metabolizable energy intake was also not affected by the enzyme supplementation. Such results may be due to the lower fiber content of the high concentrate diet and fibrous portion which was the actual target substrate of the fibrolytic enzymes. Miller *et al.* (2008) conducted a study to determine effects of a xylanase/endoglucanase exogenous enzyme product in growing lambs and fed barley or sorghum grain-based diets and reported that exogenous enzyme did not change voluntary feed intakes in the lambs.

A similar inconsistency was observed (Selmer-Olsen *et al.*, 1994) that an inoculant ensiled with low DM perennial rye grass had no effect on DM intake. Similarly, Mir and Mir *et al.* (1994) and Mir *et al.* (1995) observed no improvement in DM intake and nutrient intake in animals fed inoculant. Direct-fed fibrolytic enzymes mixed with alfalfa hay also did not affect the DM intake in lactating cows (Lewis *et al.*, 1995).

Digestion Kinetics

Rodriguez *et al.* (2007) reported that in lambs, exogenous fibrolytic enzymes increased the soluble fraction of DM and ruminal *in situ* disappearance rate of DM and neutral detergent fiber (NDF). Exogenous fibrolytic enzymes improved ruminal disappearance rates of DM and NDF, but had no impact on lamb performance or ruminal fermentation. In contrast, increasing polysaccharidase concentration lowered the resulting silage pH and NDF content but did not affect *in situ* DM disappearance (Naakashima *et al.*, 1988). However, the degradation rate constant was increased from 4.6 % h in control diet to 8.1 % h for 10 g enzyme/kg DM in the rice straw. Similarly, Selmer-Olsen *et al.* (1993) observed significant decreases in NDF and ADF fractions of forages treated with increasing concentration of cellulase/hemicellulase enzymes. Chamberlain and Robertson (1992) found that digestible OM disappearance 60.9, 62.4 and 63.5 % in ryegrass silage responded positively to xylanase levels 0, 218, 517 ml respectively. Hydrolytic removal of arabinose units from the xylan polymer may increase rate of cellulose degradation. The response to xylanolytic based enzyme preparation supports the suggestion that xylose: arabinose ratio may be an important factor affecting cell wall degradability.

Nutrient Digestibility

Rodriguez *et al.* (2002) reported that alfalfa hay showed higher *in vivo* digestibility for DM, OM, and CP, and higher N retention, whereas NDF and hemicellulose digestibility were higher for ryegrass hay. For DM and hemicellulose during the first 12 h, whereas for NDF (3, 48, and 72 h) and ADF (3, 24, 48, and 72 h) disappearance was larger for ryegrass hay. Increases in NDF digestibility by fibrolytic enzymes have been reported for silages, barley straw (Krause *et al.*, 1998), and forages (Lewis *et al.*, 1996). Rode *et al.* (1999) found that fibrolytic enzymes significantly increased DM, OM, NDF, ADF, and CP digestibility in dairy cows. Fibrolytic enzymes sprinkled on forages have increased digestibility of DM (Feng *et al.*, 1996) and NDF (Feng *et al.*, 1996; Lewis *et al.*, 1996). Krause *et al.* (1998) also reported that applying a fibrolytic enzyme mixture to tempered, rolled barley increased total tract ADF digestion by 14% in diets containing barley and straw and by 55% in diets containing barley and silage. Even though digestion of DM and NDF was not improved by enzyme treatment of grain but ADF digestion substantially increased. In diets that contain silage, enzymes increased ADF digestion by 55%, and, in diets containing straw, the increase was 14%. There was a positive effect of enzyme treatment on fiber digestion.

Hristov *et al.* (2000) reported that exogenous polysaccharide degrading enzymes could potentially improve nutrient digestion of ruminants, postruminally not only by modifying the feed but also enzyme resistance to proteolysis. Kung *et al.* (2000) reported that treatment of feeds with enzymes just prior to feeding can improve digestibility via a number of different mechanisms including direct hydrolysis, enhanced microbial attachment, changes in gut viscosity, complementary actions with ruminal enzymes, changes in the site of nutrient digestion and improvements in palatability and changes in patterns of feed consumption could also occur.

Nowak *et al.* (2003) reported that cellulase and xylanase improved DM, ADF and NDF ruminal disappearance after a short time of incubation, 4 and 6 hours. Disappearance of DM, ADF and NDF of wheat straw in the rumen after 6 h increased by 3.9, 3.5, 3.9%, respectively. Disappearance of the DM, ADF and NDF of TMR incubated for 6 h in the rumen was also improved significantly by 7.2, 3.1 and 2.9%, respectively. Eun *et al.* (2005) reported the improved fiber digestion and nitrogen utilization by using the proteolytic enzymes in the diet for lactating ruminants. Cruywagen *et al.* (2007) also reported that addition of enzyme increased neutral detergent fiber, acid detergent fiber, and hemicellulose degradation more at pH 6.50 and 6.72 as compared to the lower pH. Colombatto *et al.* (2003) treated alfalfa stems with mixtures of xylanases and cellulases which resulted to increase the rate of fermentation and *in vitro* organic matter (OM) degradability. Similarly, *in vitro* evaluation showed that enzymes increased the rate of silage degradation about 11% by 24-h post-inoculation and improved the nutritive quality (Colombatto *et al.*, 2004). A similar demonstration was given by Chamberlain and Robertson *et al.* (1992) in which one enzyme resulted in increased and the other decreased the *in vitro* OM disappearance. It was observed (Selmer-Olsen, 1994) that both *in vitro* DM disappearance and crude fiber composition of timothy and red clover were not affected by inoculant or enzyme or combined enzyme and inoculant treatments. An inoculant ensiled with low DM perennial rye grass had no effect on cell wall fractions digestibility, however a significant improvement in DM digestibility was observed (Keady and Steen, 1994).

Muwalla *et al.* (2007) reported that the supplement of fibrolytic enzyme in the high concentrate diet have no effect on the digestibility of DM, OM, crude protein, and NDF for lambs fed diet with or without the fibrolytic enzyme. Miller *et al.* (2008) reported that xylanase/endoglucanase exogenous enzyme product did not effected the total tract digestibility of NDF and starch in growing lambs fed barley or sorghum grain-based diets compared to the lambs fed exogenous enzymes untreated diets. But the lambs fed the sorghum diet exhibited a linear increase in total tract acid detergent fiber digestibility with increasing rate of exogenous enzymes treatment. He reported that N balance also increased linearly, potentially due to improved ruminal protein availability.

Hainze *et al.* (2003) studied the digestion effects of a commercial product that contained microbial xylanase and cellulase in horses. It was reported that digestibility of DM was about 7% lower for the alfalfa- based diet than for other diets, and a diet vercesenzyme interaction was observed such that digestibility of DM in the alfalfa-based diet was decreased while in the other diet called as sweet feed-based diet was increased when enzyme was administered. Digestibility of NDF tended to be about 6 % lower for the alfalfa based diet than for other diets. Similar results were observed for another diet vercese nzyme interaction by the same scientist and it was suggested that exogenous fibrolytic enzymes have the potential to improve digestibility of plant cell wall constituents in typical feeds fed to horses. But the scientist was unable to discuss that why exogenous fibrolytic enzymes were ineffective when administered with the alfalfa-based diet and actually resulted in decreased digestibility of DM and plant cell wall constituents.

However, Titi and Tabbaa (2004) demonstrated the increase in the digestibility of DM, OM, NDF, and acid detergent fiber (ADF) in Awassi lambs fed a concentrate based diet supplemented with fibrolytic enzymes. The true mechanism to increase the digestibility is not known, however, it may be through increased viable rumen bacteria (Nsereko *et al.*, 2002), increased rate of ruminal bacterial growth and flow, and hence a greater absorption of amino acids in small intestine, which would have also significant impact on the supply of metabolizable protein to the animal.

The extent of digestibility of forage and the daily feed intake has direct consequences on production in ruminant animals. Any factor that will increase rate of degradability of low quality fibers could increase digestibility, ultimately improve productivity. The enzyme systems that have been tested in forage trials were mixtures. The relative activities of the various enzymes included in these mixtures were not provided in the reports. In addition different reports use different ways of reporting enzyme concentration and activity making it difficult to compare results.

Eun *et al.* (2006) conducted an in vitro experiment using six enzyme products and showed that ammonia pretreatment or supplemental exogenous enzyme preparations enhanced *in vitro* degradation of rice straw, but the effectiveness of exogenous enzymes was enhanced when they were used with ammoniated rice straw rather than with untreated rice straw. The mechanism whereby ammonia pretreatment improved the efficacy of exogenous enzymes for enhancing feed digestion remains uncertain. The possible reason discussed by the scientist is the synergy between ammonia pretreatment and exogenous enzyme addition for *in vitro* degradation of rice straw that ammoniation may disrupt the lignin–carbohydrate complex thereby increasing the accessibility of fiber to exogenous and endogenous enzymes. But the ability of exogenous enzymes to cleave the esterified bonds within lignin–carbohydrate complexes may be limited, and therefore, their effect on the extent of *in vivo* digestibility may also be limited. Furthermore, the scientist added that enzymes may increase the availability of soluble carbohydrates to the microorganisms.

Direct-fed fibrolytic enzymes mixed with hay did not affect the NDF digestibility in lactating cows (Lewis *et al.*, 1995). In two *in vitro* experiments using fibrolytic enzymes on alfalfa and tall fescue hay (Hunt *et al.*, 1995) it was observed that even though the isolated fibrolytic enzymes had potential for improving forage digestibility, they conceded that the relationship between commercial enzymes and rumen bacteria was still too inconsistent for any benefits to be derived from enzymatic treatment of forages.

Eun *et al.* (2007) used enzyme products, in the *in vitro* experiment, including 13 endoglucanases and 10 xylanases, and discussed that all enzyme treatments, alone or in combination, increased DM and fiber degradability significantly. However, the study showed that there is no difference in the digestibility of alfalfa either enzyme was used in combinations or alone the component. The total volatile fatty acid production was not affected by enzyme treatments although some products changed the acetate to propionate ratio. Nadeau *et al.* (2000) discussed that addition of inoculant or formic acid to cellulase-treated silage tended to increase the NDF digestibility. The scientist demonstrates that it may be probably related to degradation of the easily digestible portion of NDF by cellulase during ensiling, leaving the less-digestible portion of NDF for microbial degradation in the rumen and in the lower tract.

Fiber Digesting Activities

The focus of most enzyme-related research for ruminants has been on plant cell wall degrading enzymes. Cellulose and hemicellulose, the major structural polysaccharides in plants, are converted to soluble sugars by enzymes collectively referred to as cellulases and hemicellulases. The types of cellulases and hemicellulases can differ substantially among commercial enzyme products, and differences in the relative proportions and activities of these individual enzymes may have an impact on the efficacy of cell wall degradation by these products. Cellulose is hydrolyzed through a complex process involving cellulases, and numerous specific enzymes contribute to cellulase activity. However, the main enzymes involved in degrading the xylan core polymer to soluble sugars are xylanases and β-1, 4 xylosidase. Xylanases are specific for the internal β-1, 4 linkages within the xylan backbone. Typically, the enzyme products evaluated for ruminants are blends of various cellulases and xylanases that were originally produced and marketed for other uses. Thus, ruminant enzyme products have been reformulated as new information about production and strain selection became available. Kung *et al.* (2000) compared two different enzyme products with similar cellulase and xylanase activities in the diets of lactating dairy cows. Only one of the two enzyme products resulted in an increase in milk yield.

There is increasing evidence that plant cell wall degrading enzymes stimulate fiber digestion in the rumen (Feng *et al.*, 1996; Yang *et al.*, 1999). The response has been shown to be due to enzymatic activities, but the key enzymes involved have not been identified. Identification of the important enzyme activities would provide a rationale for designing more effective enzyme products for ruminants. Wallace *et al.* (2001) used six enzyme products to examine the relationship between enzyme activities gas production using grass and corn silage. A significant positive correlation was reported between cellulase activity and gas production from grass silage. Xylanase and endoglucanase, regardless of ensiling temperature, can be helpful in improving the nutritive quality of maize silage when applied at ensiling stage (Colombatto *et al.*, 2004). Jafari *et al.* (2005) used enzyme in liquid form with activity 1437, 788, and 7479 µmol per min. per ml for exoglucanase, endoglucanase, and xylanase, respectively and found the controversial effects on the wool characteristics of the lambs.

Growth Performance

Improvement in the animal performance through efficient utilization of existing feed resources is always a need and interest of farmers and research workers. Exogenous fibrolytic enzymes proved to be a choice to attain this purpose. A limited number of these enzymes have been introduced commercially in the market. Their use in the diet for ruminant animals increased the DM, NDF, acid detergent fiber (ADF) digestibility, milk production and growth performance, especially of diets with higher fibrous material.

Studies reported that adding fibrolytic enzymes to forage diets can improve fiber digestibility (Feng *et al.*, 1996). The results of adding fibrolytic enzymes to high grain diets have been surprisingly more consistent than those for high forage diets. Krause *et al.* (1998) reported a 28% increase in ADF digestibility using a similar enzyme product added to a high-concentrate diet. Cruywagen *et al.* (2007) also reported that adding enzyme in the forage diet improved the lamb growth rate and feed conversion ratio. He suggested that enzymes can be widely introduced in the animal feed industry. Muwalla *et al.* (2007) reported that the supplement of fibrolytic enzyme in the high concentrate diet have no effect on the final body weight, average daily gain, and feed to gain ratio for awassi lambs. Miller *et al.* (2008) reported that xylanase/endoglucanase exogenous enzyme product supplementation did not improve live weight gain, feed conversion efficiency or wool growth performance in growing lambs fed barley or sorghum grain-based diets.

An inoculant ensiled with low DM perennial rye grass had no effect on average daily gain weight (Keady and Steen *et al.*, 1994). Jafari *et al.* (2005) used ewe lambs to determine the substitution effects of treated wheat straw with exogenous fiber-degrading enzymes for alfalfa hay on wool characteristics. It was reported that final fleece weight, wool production per day, feed conversion to wool, final staple length, wool and hair crimp per centimeter of length were not affected by replacing treated wheat straw for alfalfa hay, but replacing 20%, and 30% treated, and 30% untreated wheat straw for alfalfa hay numerically increased mean final diameter of fiber. The scientist concluded that treated wheat straw with exogenous fiber-degrading enzymes can be replaced for alfalfa hay in naieni breed replacement ewe lamb diets. Similarly improvements in the wool characteristics of lambs have been shown (Jafari *et al.*, 2004). Exogenous firolytic enzymes reported to be very effective to improve the average daily gain of steers (Beauchemin et al., 2003; Beauchemin and Rode, 1996) while the similar results have been shown in lambs (Jafari *et al.*, 2004). Titi and Lubbadeh (2004) studied the effect of cellulose enzyme supplement on weaning weights of lambs and kids and resulted the increased weaning weight during the last 2 months of pregnancy and the first 2 months of lactation.

Milk Production Performance

In a number of studies in dairy cattle, the responses to exogenous fibrolytic enzymes have been discussed.

Such as, Kung *et al.* (2002) treated the forage portion of the total mixed ration with cellulose and xylanase. He found that pH is an important factor for the enzyme activity and suggested that these enzymes can be used to improve the milk production. Lewis *et al.* (1999) treated forage with a cellulase/xylanase mixture supplying enzyme as1 mL/kg of total mixed ration (TMR), on DM basis) and observed that cows in early lactation produced 6.3 kg/d (16%) more milk. However, higher and lower levels of the same enzyme product were less effective. Rode *et al.* (1999) applied an enzyme product to the concentrate portion of a diet (supplying 1.3 g/kg of TMR on a DM basis) and observed a 3.6 kg/d or 10% increase in milk production for cows in early lactation. Yang *et al.* (2000) added an enzyme mixture to the concentrate, and cows in early lactation produced 2 kg/d or 5.9% more milk. However, there was no response when the same enzyme was added to the TMR. Thus from these studies, it is clear that exogenous enzymes can be effective for ruminants, but it is important to determine the conditions that are most likely to result in positive responses. Some other studies also reported the improvement in the production of milk yield of dairy cows using fibrolytic enzymes in ruminant nutrition such as (Beauchemin *et al.*, 2000).

In general, results with beef cattle and dairy cows indicate a positive response to enzymes, but the results are variable. Although this variability may be viewed as an indication that feed enzyme additives are not a suitable technology for ruminants, we believe that much of the variability can be attributed to factors such as enzyme type, level of supplementation, method of enzyme application, and the energy balance of the test animals. Enzyme treatments produced inconsistent effects on milk yield, Chen and Stokes (1992) treated alfalfa hay and observed a significant increase in milk yield whereas, Fredeen and McQueen (1993) observed a decrease in milk yield from enzyme treated grass hay. However, Lewis *et al.* (1995) reported that cows consuming treated hay produced more milk even though they lost more body weight. While in contrast, in studies of enzyme treated forages, Chen *et al.* (1994) and Fredeen and McQueen (1993) point out that the response by dairy cows fed enzyme treated cereal silages was poor.

The effects of enzyme treatments such as alfazyme and cornzyme on the nutritive value of hay crop silage and corn silage were evaluated with Jersey and Holstein cows (Chen and Stokes, 1992) and resulted that cornzyme did not affect DM intake in these cows but significantly reduced milk yield by 5.1 % whereas alfazyme improved both DM intake (3.2 %) and milk yield by 6.2 %. In a related trial a mixed grass-legume forage was treated with an enzyme product or a commercial inoculant or both inoculant and enzyme combined and ensiled in four bunker silos (Stokes *et al.*, 1992). Combining the inoculant and the enzyme products gave no advantages in silage fermentation or animal performance; instead the combined products were actually antagonistic. The results exemplify the inconsistent effects of silage enzymes on animal performance. Prior to using enzymes in such combinations enzyme/inoculants/animal synergistic association should be established.

REFERENCES

Beauchamin, K.A. and Rode, L.M. 1996. Use of feed enzymes in ruminant nutrition. In animal science research and development: Meating future challenges. (Ed.) Rode, L.M. Proceedings of 1996 Candian Society of Animal Science Annual Meeting Lethbridge, AB. pp. 103-130.

Beauchemin, K.A., D. Colombatto, D.P. Morgavi and W.Z. Yang. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci. 81, E37–E43.

Beauchemin, K.A., L.M. Rode, M. Maekawa, D.P. Morgavi and R. Kampen. 2000. Evaluation of a non-starch polysaccharidase feed enzyme in dairy cows diets. J. Dairy Sci. 83: 543–553.

Biely, P., C.R. Mackenzie, J. Puls and H. Schneider. 1986. Cooperativity of esterases and xylanases in the enzymatic degradation of acetyl xylan. J. Biotech. 4: 731-733.

Chamberlain, D.G. and S. Roberston. 1992. The effects of addition of various enzyme mixtures on the fermentation of perennial ryegrass silage and on its nutritional value for milk production in dairy cows. Anim. Feed Sci. Technol. 37: 257-264.

Chen, J. and M.R Stokes. 1992. Effects of enzyme treatments on the preservation and nutritive value of hay crop silage and corn silage. J. Dairy Sci. 75: 272.

Chen, J., M.R Stokes and C.R. Wallace. 1994. Effects of enzyme-inoculant systems on preservation and nutritive value of haycrop and corn silages. J. Dairy Sci. 77: 501-512.

Colombatto, D., F.L. Mould, M.K. Bhat, R.H. Phipps and E. Owena. 2004. *In vitro* evaluation of fibrolytic enzymes as additives for maize (*Zea mays* L.) silage II. Effects on rate of acidification, fibre degradation during ensiling and rumen fermentation. Anim. Feed Sci. Technol. 111: 129-143.

Colombatto, D., F.L. Mould, M.K. Bhat, R.H. Phipps and E. Owena. 2004. *In vitro* evaluation of fibrolytic enzymes as additives for maize (*Zea mays* L.) silage. I. Effects of ensiling temperature, enzyme source and addition level. Anim. Feed Sci. Technol. 111: 111–128.

Colombatto, D., F.L. Mould, M.K. Bhat and E. Owen. 2007. Influence of exogenous fibrolytic enzyme level and incubation pH on the in vitro ruminal fermentation of alfalfa stems. Anim. Feed Sci. Technol. 137: 150–162

Colombatto, D., F.L. Mould, M.K. Bhat and E. Owen. 2003. Use of fibrolytic enzymes to improves the nutritive value of ruminant diets. A biochemical and in vitro rumen degradation assessment. Anim. Feed Sci. Technol. 107: 201–209.

Cruywagen, C.W. and W.H.V. Zyl. 2007. Effects of a fungal enzyme cocktail treatment of high and low forage diets on lamb growth, Anim. Feed Sci. Technol. 934-936.

Eun, J.S. and K.A. Beauchemin. 2007. Assessment of the efficacy of varying experimental exogenous fibrolytic enzymes using in vitro fermentation characteristics. Anim. Feed Sci. Technol. 132: 298–315.

Eun, J.S. and K.A. Beauchemin. 2005. Effects of a proteolytic feed enzyme on intake, digestion, ruminal fermentation, and milk production. J. Dairy Sci. 88: 2140–2153.

Eun, J.S., K.A. Beauchemin, S.H. Hong and M.W. Bauer. 2006. Exogenous enzymes added to untreated or ammoniated rice straw: Effects on in vitro fermentation characteristics and degradability. Anim. Feed Sci. Tech. 131: 86–101.

Feng, P., C.W. Hunt, G.T. Pritchard and W.E. Julien. 1996. Effect of enzyme preparations on *in situ* and *in vitro* degradation and *in vivo* digestive characteristics of mature cool-season grass forage in beef steers. J. Anim. Sci. 74: 1349–1357.

Fredeen, A.H. and R.E. McQueen. 1993. Effect of enzyme additives on quality of alfalfa/grass silage and dairy cow performance. Can. J. Anim. Sci. 73: 581-591.

Gwayumba, W. and D.A. Christensen. 1996. The effect of fibrolytic enzymes on protein and carbohydrate degradation fractions in forages. Canadian Society Animal Science, 46th Annual Conference, Lethbridge, AB. pp. 2.

Hainze, M.T.M., R.B. Muntifering and C.A. McCall. 2003. Fiber digestion in horses fed typical diets with and without exogenous fibrolytic enzymes. J. Equine Vet. Sci. 23, No. 3: 111-115.

Han, F. 1986. Pretreatment of coastal bermudagrass with sodium hydroxide and cellulose before ensiling. J. Dairy Sci. 69: 1837.

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

Huhtanen, P., K. Hissa, S. Saakkola, and E. Poutiainen. 1985. Enzymes as silage additives. Effect on fermentation quality, digestibility in sheep, degradability in sacco and performance in growing cattle. J. Agric. Sci. 57: 284

Hunt, C.W., P. Feng, R. Treacher and G.T. Pritchard. 1995. Effect of fibrolytic enzyme additives on in vitro degradability of alfalfa and tall fescue. Proc. Western Sect. ASAS 46: 349.

Jacobs, J.L. and A.B. McAllan. 1991. Enzymes as silage additives. 1. Silage quality, digestion, digestibility and performance in growing cattle. Grass Forage Sci.46: 63-73.

Jacobs, J.L. and A.B. McAllan. 1992. Protein supplementation of formic acid and enzyme-treated silages. 1. Digestibilities, organic matter and fiber digestion. Grass Forage Sci. 47: 103 113.

Jafari, A., M.A. Edriss and M. Alikhani. 2004b. Type and concentration effects of fibrolytic enzymes on wool characteristic in Bakhtiari replacement ewe lambs. Proceedings of the Science of Changing Climates. University of Alberta, Edmonton, Canada.

Jafari, A., M.A. Edriss, M. Alikhani and G. Emtiazi. 2005. Effects of treated wheat straw with exogenous fibre-degrading enzymes on wool characteristics of ewe lambs. Pak. J. Nutr. 4(5): 321-326.

Jaster, E.H. and K.J. Moore. 1988. Fermentation characteristics and feeding value of enzyme treated alfalfa haylage. J. Dairy Sci. 71: 705-711.

Keady, T.W.J. and R.W.J. Steen. 1994. Effects of treating low dry-matter grass with a bacterial inoculant on the intake and performance of beef cattle and studies on it's mode of action. Grass Forage Sci. 49: 438-446.

Krause, M., K.A. Beauchemin, L.M. Rode, B.I. Farr and P. Norgaard. 1998. Fibrolytic enzyme treatment of barley grain and source of forage in high-grain diets fed to growing cattle. J. Anim. Sci. 76: 2912–2920.

Kung, L., Jr., R.J. Treacher, G.A. Nauman, A.M. Smagala, K.M. Endres and M.A. Cohen. 2000. The effect of treating forages with fibrolytic enzymes on its nutritive value and lactation performance of dairy cows. J. Dairy Sci. 83: 115–122.

Kung, L., Jr., R.S. Tung, K . Maciorowski, K. Buffum, K. Knutsen and W.R. Aimutis. 1991. Effects of plant cell-wall-degrading enzymes and lactic acid bacteria on silage fermentation and composition. J. Dairy Sci. 74: 4284.

Lewis, G.E., C.W. Hunt, W.K. Sanchez, R. Treacher, G.T. Pritchard and P. Feng. 1996. Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage-based diet fed to beef steers. J. Anim. Sci. 74: 3020–3028.

Lewis, G.E., W.K. Sanchez, R. Treacher, C.W. Hunt and G.T. Pritchard. 1995. Effect of direct-fed fibrolytic enzymes on lactational performance of mid-lactation Holstein cows. Proceedings, Western Section, American Society of Animal Science and Western Branch Canadian Society of Animal Science. July 6th-8h, Lethbridge, AB. 46: 310-313.

Lewis, G.E., W.K. Sanchez, C.W. Hunt, M.A. Guy, G.T. Pritchard, B.I. Swanson and R.J. Treacher. 1999. Effect of direct-fed fibrolytic enzymes on the lactational performance of dairy cows. J. Dairy Sci. 82: 611–617.

Miller, D.R., R. Elliott and B.W. Norton. 2008. Effects of an exogenous enzyme, Roxazyme® G2 Liquid, on digestion and utilisation of barley and sorghum grain-based diets by ewe lambs. Anim. Feed Sci. and Tech. 140: 90–109.

Mir, P.S. and Z. Mir. 1994. Effect of live-yeast culture and lasolacid supplementation on the performance of growing-finishing steers fed alfalfa-silage. com-silage and high grain diets. Can. J. Anim. Sci. 74.

Mir, Z., E.Z. Jan, J.A. Robertson, P.S. Mir and D.H. McCarteney. 1995. Effects of microbial inoculant and moisture content on preservation and quality of round baled alfalfa. Can. J. Anim. Sci. 75.

Morgavi, D.P., K.A. Beauchemin, V.L. Nsereko, L.M. Rode, T.A. McAllister, A.D. Iwaasa, Y. Wang and W.Z. Yang. 2001. Resistance of feed enzymes to proteolytic inactivation by rumen microorganisms and gastrointestinal proteases. J. Anim. Sci. 79: 1621–1630.

Muwalla, M.M., S.G. Haddad and M.A. Hijazeen. 2007. Effect of fibrolytic enzyme inclusion in high concentrate fattening diets on nutrient digestibility and growth performance of Awassi lambs. Livest. Sci. 111: 255–258.

Nakashima, Y., E.R. Orskov, P.M. Hotten, K. Ambo and Y. Takase. 1988. Rumen degradation of straw. 6. Effect of polysaccharidase enzymes on degradation characteristics of ensiled rice straw. Anim. Prod. 47: 421-427.

Nadeau, E.M., J.R. Russell and D.R. Buxton. 2000. Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant, and formic acid fed to lambs. J. Anim. Sci. 78: 2980-2989.

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

Nowak, W., H. Kruczynska and S. Grochowska. 2003. Effect of fibrolytic enzymes on dry matter, ADF and NDF ruminal disappearance and intestinal digestibility. Czech J. Anim. Sci. (5): 191–196.

Poutanen, K., M. Ratto, J. Puls and L. Viikari. 1987. Evaluation of different microbial xylanolytic systems. J. Biotech. 6: 49-60.

Puls, J., O. Schmidt and C. Granzow. 1987. Alpha-glucuronidase in two microbial xylanolytic systems. Enzyme Microb. Technol. 9: 83-88.

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

Rodriguez, J.M. P., Mendoza, S.S. Gonzalezc, P.H. Robinsond, G. Mendozae and G.A. Lvareza. 2007. Effects of exogenous fibrolytic enzymes on ruminal fermentation and digestibility of total mixed rations fed to lambs. Anim. Feed Sci. Technol.

Rodriguez, J.M.P., S.S. Gonzalez, G. D. Mendoza, R. Barcena, M.A. Cobos, A. Hernandez and M.E. Ortega. 2002. Effect of exogenous fibrolytic enzyme on ruminal fermentation and digestibility of alfalfa and rye-grass hay fed to lambs. J. Anim. Sci. 80: 3016–3020.

Selmer-Olsen, I. 1994. Enzymes as silage additives for grass-clover mixtures. Grass Forage Sci. 49: 305-315.

Selmer-Olsen, I., A. R. Henderson, S. Robertson and R. McGinn. 1993. Cell wall digesting enzymes for silage. 1. The fermentation of enzyme-treated ryegrass in laboratory silos. Grass Forage Sci. 48: 45-54.

Spoeltra, S.F., P.G.V. Wikselaar and B. Harder. 1992. The effect of ensiling whole crop maize with a multi-enzyme preparation on the chemical composition of the resulting silages. J. Sci. Food Agric. 60: 223-228.

Stokes, M.R. 1992. Effects of an enzyme mixture, an inoculant, and their interaction on silage fermentation and dairy production. J. Dairy Sci. 75: 764.

Sutton, J.D., R.H. Phipps, D.E. Beever, D.J. Humphries, G.F. Hartnell and J.L. Vicini. 2001. Comparison of different methods of administration on the effect of fibrolytic enzymes on digestive processes in lactating cows. J. Dairy Sci. 84: 37.

Titi, H.H. and M.J. Tabbaa. 2004. Efficacy of exogenous cellulase on digestibility in lambs and growth of dairy calves. Livest. Prod. Sci. 87: 207–214.

Titi, H.H. and W.F. Lubbadeh. 2004. Effect of feeding cellulase enzyme on productive responses of pregnant and lactating ewes and goats. Small Rumin. Res. 52: 137–143.

Vicini, J.L., H.G. Bateman, M.K. Bhat, J.H. Clark, R.A. Erdman, R.H. Phipps, M.E. Amburgh, G.F. Hartnell, R.L. Hintz and D.L. Hard. 2003. Effect of feeding supplemental fibrolytic enzymes or soluble sugars with malic acid on milk production. J. Dairy Sci. 86: 576–585.

Wallace, R.J., S.J.A. Wallace, N. McKain, V.L. Nsereko and G.F. Hartnell. 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.

Weinberg, Z.G., G. Ashbell, A. Azrieli and I. Brukental. 1993. Ensiling peas, ryegrass and wheat with additives of lactic acid bacteria and cell wall degrading enzymes. Grass Forage Sci. 48: 70-78.

Weinberg, Z.G., G. Ashbell, Y. Hen and A. Azrieli. 1995. The effect of cellulase and hemicellulase plus pectinase on aerobic stability and fiber analysis of peas and wheat silages. Anim. Feed Sci. Tech. 55: 287-293.

Yang, W.Z., K.A. Beauchemin,and L.M. Rode. 2000. A comparison of methods of adding fibrolytic enzymes to lactating cow diets. J. Dairy Sci. 83: 2512–2520.

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