Broadening *Horizons*

Role of exogenous enzyme supplementation to improve nutrition and health of ruminants

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Introduction

Feed constitutes the largest cost in livestock production systems. As the cost of formulated rations increases, improving feed efficiency is an important step for farmers to have an economically sustainable business. Improved feed efficiency can also reduce concentrations of certain nutrients in the diet with concomitant reduction in nutrient excretion into the environment, limiting eutrophication and acidification potentials of manure. Exogenous enzymes have been used to improve nutrient utilization in several species of livestock, particularly swine and poultry. In comparison, the use of exogenous enzymes in ruminant diets has been limited, mainly because of the perception that the hydrolytic capacity of the rumen could not be enhanced by enzyme supplementation, and concerns that such enzymes would be ineffective due to ruminal hydrolysis (Adesogan, 2005). Based on the work mainly in non-ruminant animals, efficacy of exogenous enzyme supplementation has

been reported to work in three ways: 1) an increase in feed conversion efficiency, 2) a release of nutrients trapped within insoluble portions of diets, and 3) the release of oligosaccharides which support beneficial microflora while suppressing pathogenic bacteria (Paloheimo et al., 2010). Exogenous enzymes are specific and typically fed to livestock in order to target particular components of the diet such as starch, proteins, or fibre.
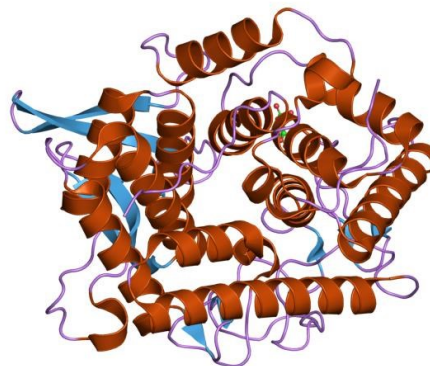
Endo-1,4-beta-xylanase. Credit: Jawahar Swaminathan, European Bioinformatics Institute, via Wikimedia Commons

Classes of exogenous enzymes

Exogenous enzymes supplemented to animal diets include amylolytic enzymes, proteolytic enzymes, β-glucanase, xylanase, and β-mannanase. These enzymes are expected to increase the digestion and thus utilization of dietary starch, protein, β-glucans, arabinoxylans, and mannan, respectively. Here we describe briefly amylolytic, proteolytic and fibrolytic enzymes supplemented to diets for ruminants.

Amylolytic enzymes

Amylolytic, or starch degrading enzymes are often fed with cereal grains such as corn and barley. These amylolytic enzymes can produce as much as 600 metabolizable glucose units by targeting glycosidic bonds (Pérez et al., 2009), thus making them a high energy source. Rojo et al. (2007) reported that the amylolytic enzyme α-amylase was involved in increased starch digestion when tested in ruminally cannulated beef steers. Alternatively, Tricarico et al. (2008) hypothesized that amylolytic enzymes, such as α-amylase, work by cross-feeding mechanisms of ruminal bacteria through oligosaccharides produced by the enzyme, creating modified products of ruminal fermentation. The effect of supplementing exogenous amylolytic enzymes to ruminants has been shown to work in some studies but not in others. For example, Hristov et al. (1998) found no differences in ruminal or total tract digestibility in lactating dairy cattle fed high grain diets and supplemented with polysaccharide degrading enzymes, whereas DeFrain et al. (2005) and Tricarico et al. (2005) found increased proportions of acetate and propionate in steers and lactating dairy cattle supplemented with exogenous α-amylase. Differences in efficacy can be related to several factors including enzyme dose, type of feed, host and management factors (Mendoza et al., 2014).

Proteolytic enzymes

Proteolytic enzymes are responsible for the degradation of protein to release amino acids throughout the digestive tract; mainly within the rumen and small intestine. These enzymes can be effective in diets with low digestibility due to their ability to target nitrogen cross-linkages in the cell wall of forages. Nitrogen in cell walls may account for only 7 to 11% of total cell wall content (Aufrère, 1994). However it could be argued that degradation of cell wall-bound nitrogen may provide additional nitrogen to digestible nitrogen pool, and increase the total amount of fermentable organic matter in the rumen (Colombatto and Beauchemin, 2009). In ruminants, supplementations of exogenous proteolytic enzymes are not as common as in non-ruminants because of an abundance of endogenous proteolytic enzymes
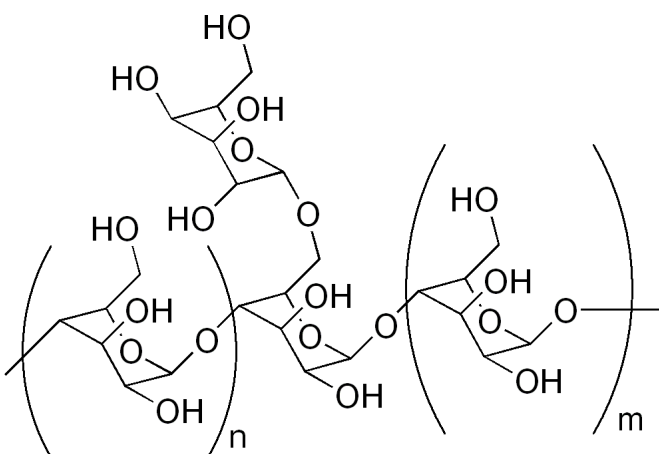
produced by the microorganisms in the rumen. However, Eun and Beauchemin (2005) studied the effects of exogenous proteolytic enzymes in lactating Holstein cows fed high or low forage diets and found increased acid detergent fibre, neutral detergent fibre, and hemicellulose digestibilities in enzyme-supplemented low forage diets, showing an efficacy of proteolytic enzymes in ruminant diets. The authors also observed decreased dry matter intake associated with decreased milk yields in enzyme-fed cows masking the benefits of improved nutrient digestibility. It is difficult to explain why dry matter intake decreased when enzymes were fed. However, ammonia nitrogen concentration in the rumen increased for the exogenous proteolytic enzyme in both groups of cows fed high (17.2 vs. 14.5 mg/dL) or low (13.1 vs. 10.4 mg/dL) forage diets. Lazzarini et al. (2009) demonstrated that dry matter intake decreased as rumen ammonia nitrogen concentration exceeds 15.3 mg/dL in cattle; therefore, the increased rumen ammonia concentration may have negatively affected intake in the experiment of Eun and Beauchemin (2005). There has been some efforts to improve efficacy of proteolytic enzyme supplementation in ruminants through identification of different types of proteases. A number of studies have shown exogenous alkaline and serine-type proteases to have a potential to increase feed digestibility in ruminants (Beauchemin et al., 2004; Eun and Beauchemin, 2007; Colombatto and Beauchemin, 2009). This may be due to their abilities to bypass rumen degradation and thus contribute to protein digestion in the small intestine. It has also been suggested that exogenous proteases may work closely with endogenous enzymes by potentially providing endogenous enzymes greater access to nutrients particularly in cell wall matrices (Colombatto et al., 2003), thus creating a symbiotic relationship between endogenous and exogenous enzymes and enhancing overall feed digestibility. Such a relationship could be quite beneficial in improving nutrient availability and feed conversion efficiency of ruminants.

Fibrolytic enzymes

Ruminants typically consume diets containing relatively high amounts of forage, which contains cell

wall fractions that have complex links and limit their degradation. Insoluble cell wall content in forages could be about 40-70% of total forage DM (Van Soest et al., 1994), which has the potential to be digested through enzymatic activity. In addition, energy intake is limited by forage digestibility and contributes to excessive manure excretion in ruminants (Beauchemin et al., 2003). Fibrolytic enzymes hydrolyze non-starch polysaccharides in plant cell walls by targeting the main constituents including cellulose and hemicellulose. Hemicelluloses also have a substantial role in maintaining cell wall structure in plants, and account for about a quarter of total plant biomass (Scheller and Ulvskov, 2010). Hemicellulose is a heterogeneous group of poly-saccharides with β-1-4 linkages which includes xyloglucans, glucuronoxylans, glucuronoarabino-xylans, glucomannans and galactomannans (Scheller and Ulvskov, 2010). The most common types of fibrolytic enzymes that break down hemicelluloses in the rumen include xylanase, β-glucanase, and β-mannanase. Survival of exogenous fibrolytic enzymes from the rumen to the small intestine have been widely debated because of high proteolytic activity in the rumen. Fontes et al. (1995) showed that certain types of fibrolytic enzymes, such as cellulase and xylanase, resist degradation in the rumen and can have beneficial impacts in the small intestine. In addition, the authors indicated that mixing enzymes with their substrate would increase their chances of bypassing the rumen as some enzymes have shown greater resistance to ruminal degradation when

Segment of galactomannan. Credit: Edgar 181, Wikimedia Commons

presented with their substrates. Nonetheless, several studies investigating the efficacy of various types of exogenous fibrolytic enzymes in ruminants have shown mixed results. Salem et al. (2013) studied the effects of the exogenous fibrolytic enzyme mixture namely ZADO on feed intake, digestibility, and rumen fermentation in beef steers. The authors concluded that the supplementation of ZADO was associated with an increase of nutrient digestibility of approximately 12% and improved feed conversion efficiency. However, not all enzyme mixtures have proven to be effective and, in some cases, have proven to have negative effects on digestion. For example, Wallace et al. (2001) tested various combinations of fibrolytic enzymes such as xylanase, endoglucanase, cellobiohydrolase and β-glucosidase to determine the efficacy of enzyme supplementation on fibre digestion in ruminally cannulated sheep. Although enzyme activities tended to be stronger in the rumen of sheep fed exogenous enzymes than sheep in the control groups, the authors did not observe any significant effects on ruminal fermentation at the provided dietary inclusion rates. Negative impacts of exogenous enzymes have been recorded in some studies using fibrolytic enzymes in high concentrate diets. The reason for feeding fibrolytic enzymes with high grain diets was that the breakdown of insoluble fibre may provide more optimal pH levels in the rumen as well as decrease risks for nutritional diseases such as acidosis (Beauchemin and Holtshausen, 2010). Miller et al. (2008) applied the enzyme Roxazyme G2 (endo-1,4-beta-xylanase, endo-1,4-beta-glucanase and endo-1,(3)4-beta-glucanase) to high barley diets and high sorghum diets fed to beef steers, and found adverse results. The authors reported that Roxazyme G2 was associated with decreased starch digestibility in sorghum diets and increased urinary N excretion, which may be associated with decreased nutrient utilization. The large discrepancies between the outcomes of studies using amylolytic, proteolytic, and fibrolytic enzymes could be related to variability in enzyme concentrations, feed types, or energy content of the diets. Several reviews have already been published on enzymes in general and specifically fibrolytic enzymes (e.g., Kung, 2001; McAllister et al., 2001; Beauchemin et al., 2003, 2004). However, one class of enzymes, β-mannanase, has not received as

much attention. Therefore, the following sections will be focused on β-mannanase and its substrate β-mannan.

Fibrolytic enzymes targeting β-mannan

β-Mannan

The polysaccharide β-mannan is an important component of the plant cell walls and can be classified into 4 groups: pure mannan, glucomannan, galactomannan, and galacto-glucomannan (Moreira and Filho, 2008). β-mannans are linear polysaccharides with repeating β-1-4-mannose, β-1-6 galactose, β-1-6 glucose structures attached to the β-mannan backbone. Galactomannan is commonly found in coconut, locust bean, guar, and legumes, whereas glucomannan is commonly found in the konjac plant (Scheller and Ulvskov, 2010). Higher concentrations of galactomannans are most abundant in legumes, but can be prevalent in other seeds as well (Scheller and Ulvskov, 2010).

The livestock industry continues to integrate plant by-products as feed to reduce feed costs. By-products commonly used in livestock diets that contain relatively greater amounts of β-mannans include palm kernel meal (32%), soybean hulls (8%), and soybean meal (3%).

β-mannans are typically known for their structures' resistance to solubility that creates high viscosity in feed and therefore promotes anti-nutritive properties in animal feeds (Chauhan et al., 2012), especially in non-ruminant species such as poultry

Soybean hulls. Photo credit: Denis Bastianelli, CIRAD, via Feedipedia

and swine (Jackson et al., 1999; Rainbird et al., 1984; Saenphoon et al., 2013). The viscosity of β-mannan has been proposed to be the reason for depressed digestion and absorption in the gut as well as decreased pancreatic enzyme activity within the small intestine of non-ruminants (Ikegami et al., 1990; Isaksson et al., 1982). Additionally, β-mannans can greatly affect gastrointestinal health by initiating innate immune signalling receptors (Ausubel, 2005) and by over-stimulation of immune functions of the mucosal walls in the intestine (Chauhan et al., 2012; Peng et al., 1991; Duncan et al., 2002; Zhang and Tizzard, 1996). Due to immune signalling and stimulation, β-mannan is associated with decreased nutrient utilization, thus requiring surplus nutrient intake to reach the production potential (Peng et al., 1991; Zhang and Tizzard, 1996). Jackson et al. (2004) also reported β-mannan from soybean meal to decrease animal performance by increasing proliferation in macrophages, monocytes, and cytokines, major components of innate immune system. Additionally, several studies link β-mannan to reduced gastric emptying and inhibited insulin-glucose dependent peptide (GLP) and insulin-like growth factor-1 (IGF-1) (Nunes and Malmlöf, 1992; Morgan et al., 1985: Leeds et al., 1980; Sambrook and Rainbird, 1985). Although extensive research has been conducted on the metabolic and immunologic effects of β-mannan in non-ruminants, very little is known about the mode of action in ruminant diets. With the expanding interests of exogenous enzyme supplementation for ruminants, there is a substantial need for further investigation.

β-mannanase

Several studies have investigated the effectiveness of β-mannanase as an exogenous enzyme to be supplemented in ruminant and non-ruminant diets because of its tolerance to a wide range of pH levels, temperature, and gastric juices such as pepsin and trypsin (Cai et al., 2011; Tang et al., 2016; Yang et al., 2015). Jackson et al. (2004) suggested that β-mannanase acts in three main ways: 1) reduction of feed viscosity in the small intestine, 2) improvement of energy metabolism, and 3) decreased unwarranted immune system stimulation. It has been suggested that β-mannanase is able to increase digestibility of minerals, crude protein, and dry matter through the reduction of digesta viscosity and increased enzymatic activity in the intestine of growing pigs (Lv et al., 2013). β-mannanase hydrolyzes the glycosidic

bonds between mannan structures and thus improves nutritive values of feeds (Ahirwar et al., 2016; Cai et al., 2011; Liu et al., 2015). Pettey et al. (2002) implied that β-mannanase supplementation could provide an energy advantage of as much as 100 kcal/kg when supplied in a low energy diet. Hydrolysis of β-mannan by β-mannanase releases mannan-oligosaccharides of various lengths shown to improve gastrointestinal health or overall health and performances of ruminants (Tewoldebrhan et al., 2017).

Efficacy of β-mannanase

Non-ruminants

β-mannanase supplementation has proven to be highly effective in increasing feed conversion ratio, average daily gain, and carcass weight of poultry (e.g. Cho and Kim, 2013; Jackson et al., 1999, 2003, 2004; Wu et al., 2005; Li et al., 2010) and swine (e.g., Pettey et al., 2002; Yoon et al., 2014; Kim et al., 2013; Lv et al., 2013). Additionally, increases in egg weight and overall egg production has been linked to β-mannanase supplementation in poultry (Jackson et al., 1999). Lee et al. (2003) found that supplementation of β-mannanase not only removed growth depression and increased feed conversion efficiency, but also mitigated ileal viscosity in broilers fed a ration containing at least 2.5% guar hulls. Furthermore, increased ileal digestibility of amino acids such as leucine and phenylalanine (Mok et al., 2013) and dry matter (Yoon et al., 2014) have been reported in pigs fed β-mannanase. β-mannanase appears to reduce viscosity in the ileum and thus create an environment that promotes nutrient digestibility and gastrointestinal health.

In addition to production related improvement, health benefits of supplementing β-mannanases have been reported more often in non-ruminant species. For example, Cho and Kim (2013) found reduced plasma low-density lipoprotein cholesterol in broilers fed β-mannanase, indicating potential increases in dietary fat digestibility and availability of polyunsaturated fatty acids.

Increased insulin secretion through increased glucose absorption has been observed in laying hens (Jackson et al., 1999), which is also in agreement with

Kim et al. (2013), who observed an increased glucose absorption in swine. In addition, Kim et al. (2003) concluded that increases in insulin, and potential increases in circulating insulin like growth factor-1 (IGF-1) could be the basis for observed growth rate increases in swine fed β-mannanase.

Increased insulin and IGF-1 have been linked to enhanced embryonic development, fertility, and increased first service conception rates (Patton et al., 2007; Matsui et al., 1995). In addition, Jackson et al. (2003) studied the effects of β-mannanase on disease challenged broiler chickens and found that supplementation of the enzyme drastically reduces intestinal lesions cause by *Eimeria* spp. and *Clostridium perfringens*. Li et al. (2010) determined supplementation of β-mannanase fed to broilers improved health status as indicated by reduced concentrations of serum immunoglobulins. The authors concluded that β-mannanase potentially removes inhibitory effects on pancreatic enzymes and thereby improves nutrient digestibility in broilers (Li et al., 2010).

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Ruminants

There has been little research conducted on the efficacy of β-mannanase in ruminant diets, however, the observations in those studies closely agree with the nutritional and health benefits found in non-ruminants. Lee et al. (2014) reported an increase in overall growth, feed conversion efficiency, and nitrogen utilization in goats fed exogenous β-mannanase. Similarly, Seo et al. (2016) reported increased average daily gain in growing beef heifers. Most recently, β-mannanase was supplemented in a

corn silage and alfalfa hay-based diet fed to mid-lactating Holstein dairy cows (Tewoldebrhan et al., 2017). The authors reported that cows receiving β-mannanase increased milk yield per unit of dry matter intake and milk protein yield per kilogram of crude protein intake. Additionally, the somatic cell count in milk decreased in enzyme supplemented cows. Azevedo et al. (2016) demonstrated that β-mannanase supplementation reduced plasma concentration of acute phase protein, Haptoglobin, in early-lactating Holstein cows fed β-mannanase indicating favorable effects on overall health status and stress. Nabte-Solis (2009) reported that β-mannanase supplementation could improve nutritive value of soy protein in dairy calves and could thereby help relieve some cost of raising dairy calves usually fed more expensive whey protein supplements.

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In summary, exogenous enzymes including β-mannanase have been proven to be effective in non-ruminant diets for decades and continue to be used. The effectiveness of supplementing ruminant diets with exogenous enzymes has been highly variable. This is likely due to variability in type of enzyme used, species, physiological status, rumen pH, gastrointestinal conditions, enzyme dosage, and nutrient composition of diets (Beauchemin et al., 2003; Mendoza et al., 2014). The few studies that have been conducted so far on effectiveness of β-mannanase in ruminants have shown beneficial nutritional effects in goats, beef and lactating cows.

In addition, there is some indication of health benefits from β-mannanase in ruminants as well. More research is needed to assess the mechanism of actions, long-term effect of β-mannanase enzyme in ruminants, particularly lactating dairy cows, in relation to improving feed conversion efficiency, health status, and perhaps reproductive efficiency, and cost effectiveness. As far as other exogenous enzymes are concerned, the conditions under which they elicit positive effects need to be better understood. Studies on survival of the exogenous enzymes in the rumen and use of cost-effective dose of enzymes for ruminants that is effective in such a large rumen volume are also required.

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