MERIT RESEARCH JOURNALS
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Merit Research Journal of Agricultural Science and Soil Sciences (ISSN: 2350-2274) Vol. 7(12) pp. 170-176, December, 2019
Available online http://meritresearchjournals.org/asss/index.htm

DOI: 10.5281/zenodo.3595985

Review

A Review on Effects of Exogenous Fibrolytic Enzymes in Ruminant Nutrition

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Abstract

Ruminant can convert plant biomass that human can't use directly to high quality protein-meat and milk, which is important to agriculture and human society. However, the conversion efficiency is dependent on digestibility of plant cell walls. The conversion efficiency is low as well as the digestibility of plant material at present. The enzyme application method can vary in a wide range from applying to forage at harvesting, at ensiling, at feeding and the portion of feed which is mixed with enzyme may also vary from to forage to concentrate to a part of totally mixed rations. The positive effects of exogenous enzymes enzymes on growth production of dairy cattle have been demonstrated definitively, but the information required to improve the consistency and increase the magnitude of these responses is unfortunately still lacking. Comparisons between experiments are exceedingly difficult, because many enzyme products are poorly defined. Further, several studies have shown that over-application of enzyme is possible, such that increased application costs are not recovered by corresponding improvements in animal performance. In general, exogenous enzymes that used to enhance the feed digestibility will be one of most important ruminant additives in future, though they have variability at present.

Keywords: Fibrolytic enzyme, Ruminal microorganisms, Plant cell wall, Nutrition, Dairy cattle

INTRODUCTION

Feed cost represents 40 to 60% of the total cost of production in dairy farms (Bozic *et. al.*, 2012), and because of that nutritionists are constantly in search of alternatives to increase animals' feed utilization which can be accomplished by enhancing diet digestibility of feedstuffs. The fiber is a major component of the forage dry matter. Moreover, the rumen environment affects fiber digestion (McDonald *et al.*, 2011). Increasing fiber digestibility is a common practice as an attempt to reduce feed costs and ensure greater financial returns. Forages continue to be the most important components of the diets fed to ruminant animals even under intensive concentrate feeding systems (Beauchemin *et al.*, 2013). However, the energy available from forages is significantly limited by large quantities of fiber materials forming plant cell walls and as a consequence, it limits feed intake and animal performance (Jung and Allen, 1995). To increase efficiency and precision of dairy cattle nutrition, novel feeding and feed ingredient technologies need to be continually implemented on the farm in order to reduce operation costs and meet nutrient demands for greater milk production.

Dairy producers aim to procure alternatives that provide the lowest cost source of nutrients that most closely matches the nutrient requirements of the dairy cow. Fiber digestion limitations have motivated producers to feed higher starch diets. Dietary starch plays an important role in the diet of high producing dairy cows, providing an energy-dense substrate, especially critical during early lactation when glucose requirements are high (Van Vuuren *et al.*, 2010). However, dietary starch concentration can be challenging and lead to consequences that impair the rumen ecosystem (Enermark, 2008) leaving cows at greater risk of developing sub-acute ruminal acidosis (SARA), a common digestive disorder frequently caused by feeding a diet containing highly fermentable carbohydrates, with inadequate physically effective fiber required for adequate rumen buffering (Plaizier *et al.*, 2008).

Methods that increase fiber digestion are likely to play a role in improving energy availability of ruminant diets and reducing feed costs (Vinici *et al.* 2003). Not just health concerns, but also increases in feed prices (Gencoglu *et al.*, 2010) have prompted producer's interest to feed direct fed microbial (DFMs) and exogenous fibrolytic enzymes (EFE) which can be an alternative to enhance feed utilization by improving fiber digestibility, and increasing energy utilization per unit of feed, culminating in a reduction of feed costs (Beauchemin *et al.*, 2008).

Plant cell wall and dietary fiber

The metabolism of carbohydrates by ruminal microorganisms results in the production of volatile fatty acids, which in turn, supply around 60 – 80% of the host animals' total caloric requirements. The conversion of complex plant polysaccharides, resistant to mammalian enzymatic hydrolysis, to energy is beneficial to both the host animal and the microbial symbionts (Knapp *et al.*, 2014). In this context, forages continue to be the most import components of the diets fed to ruminant animals, even under intensive concentrate feeding systems. Animal performance, however, is greatly correlated with voluntary feed intake which is negatively affected by plant cell wall concentration when animals consume high-forage diets (Jung and Allen, 1995). Cell walls affect intake by contributing to ruminal fill which is critically determined by its concentration and rate of passage parameters (Jung and Allen, 1995). Plant cell walls are composed of four major polymeric building components, three polysaccharides - cellulose, hemicelluloses and pectin, and the polyphone lignin (Glass *et al.*, 2013). However, plant cell wall structure configuration and composition vary depending on plant tissue, age and cell type, and also within each cell wall layer (Ding and Himmel, 2006).

Plant cell wall or fiber digestibility by animals depends on the association of its major components, cellulose and hemicelluloses with the primary factor lignin controlling nutrient availability (Van Soest and Wine, 1967). Lignin is a highly branched polyphenolic macromolecule strongly resistant to chemical and biological degradation and can account for about 10 - 25% of the plant dry matter. Lignin is linked in a network to cellulose and xylose with ester, phenyl and covalent bonds with an important role in protecting the plants against invasion by pathogens and insects (Sticklen, 2008). Lignin is the most important single fiber component limiting nutrient animal accessibility to nutrients; however, digestibility of plant cell wall is more regulated by how the components of the cell wall are arranged than by its proportions (Jung and Deetz 1993). Both cellulose and hemicelluloses are slowly digested by rumen microbes, but can be completely digested in the absence of lignin (Weimer, 1998). Fiber concentration the feed material is a critical component of animal nutrition. The concentration and digestibility of NDF in feeds is closely related to the variation in dry matter digestibility (DMD) which determines how much nutrient is available to the animal. Neutral detergent fiber has a negative relationship with DMD which is related to the energy in the feed available to the animal and so is linked to animal performance (Mertens, 2002). Moreover, NDF fraction plays an important role in rumen health. It stimulates the appropriate motility of the rumen to promote rumination, secretion of saliva to regulate ruminal pH, and development of the ruminal mat that optimizes the fermentation processes (Zebeli and Metzler. 2012). The effectiveness of fiber in meeting ruminant minimum requirements is measured not only by the aNDF measurement itself but within combination with fiber's physical properties, such as particle size. This effectiveness has been traditionally referred as the ability of fiber to maintain milk fat production and animal health. Effective fiber is divided into two different concepts, physically effective NDF (peNDF) and effective NDF (eNDF). The peNDF is related to physical characteristics of fiber (primarily particle size) that influence chewing activity and the biphasic nature of ruminal contents (buoyant mat of large particles on a pool of liquid and small particles). Also, peNDF supply additional buffering to the rumen and help to modify rumen pH. The eNDF is related to the sum total ability of a feed to replace forage or roughage in a ration.

Rumen microbial population's ability to degrade fiber can be affected by type of forage, harvest, and fermentation or processing methods (Galloway *et al.*, 1991). Factors such as hybrid selection, maturity at harvest, starch content and length of fermentation prior to feeding can impact corn silage digestibility and subsequent milk yield. For instance, Johnson *et al.*, (1999) reported greater DMI and increased milk production ranging from 0.2 to 2.0 kg/d when cows were fed mechanically processed corn silage vs. non-processed corn silage. Moreover, Oba and Allen, (2000) reported significant increases in milk production (41.7 vs. 38.9 kg/d) of dairy cows when diets containing either brown midrib corn silage (49% NDFD) or normal corn silage (39.4% NDFD) were fed.

Dairy nutritionists and dairy producers are constantly seeking strategies to both increase the digestibility of forage and also reduce its variability in digestibility as these factors are likely to play a role in animal performance. Oba and Allen, (2000) suggested that each unit-increase in neutral detergent fiber digestibility (NDFD) was associated with 0.17 kg increase in DMI and a 0.25 kg increase in 4% fat-corrected milk. Furthermore, these authors also suggested that digestibility of NDF should be measured more routinely to assess forage quality effects on animal performance. Moreover, DMD and NDFD of forages are not constant and feed additives that increase DMD, NDFD, and therefore DMI are needed to increase cow's performance and increase feed efficiency.

Non-fiber carbohydrates and dietary starch

Carbohydrates are broadly classified as either nonstructural or structural. Structural carbohydrates comprise those found in plant cell walls and nonstructural carbohydrates (NSC) are found inside the cells of plants. Nonstructural carbohydrates are the major source of energy for high producing dairy cows and include sugar, starches, organic acids and fructans (NRC, 2001). However, from a ruminant feeding perspective, non-fibrous carbohydrates (NFC) is mostly considered by nutritionists when formulating rations for dairy cows. Non-fibrous carbohydrates include pectin as one of its components as this structural polymer is rapidly and completely fermented by microorganisms in the rumen. Plant NFC can be calculated as, NFC = 100 - (%NDF + %CP + %Fat + %Ash) (Grant, 2005; NRC, 2001). Due to rumen health concerns, such as acidosis, the maximum concentration of NFC should be limited to 32-42 percent of the total ration dry matter (DM) (Nocek, 1997).

Starch and sugar are the major components of on NFC plant fraction. Sugars, the second major component of NFC after starch, are highly water-soluble carbohydrates supplying a rapid source of energy to rumen microbes which may alter the rumen microbial ecology to increase fiber digestion (Chamberlain *et al.*, 1993). However, dietary situations influence the optimum feeding rate of between 2.5 and 5% of supplemental sugar (Firkins *et al.* 2008). Compared to starch and structural carbohydrates, microbes spend less energy to reduce sugars to smaller units (Golder *et al.*, 2012) which means that rumen microbes can utilize sugars at faster rates and use this energy to grow more rapidly and increase their capability to degrade more fiber. Rate of carbohydrate fermentation can be beneficial in the extent as it might result in the effective capture of rumen degradable protein (RDP) and increase the supply of metabolizable protein (MP) (Broderick and Radloff, 2004).

Feeding diets differing in starch and NFC concentrations as a prepartum feeding strategy for optimal postpartum intakes and better health performance has been more controversial than clearly defined. The transition period defined 3 weeks before calving and 3 weeks after calving is the most stressful time of a cows' life. Although energy demand increases in late gestation and early lactation, feed intake typically decreases (Grummer, 1995). Rapid growth of the fetus can account for the decrease in prepartum intakes due to extra abdominal compression and reduction in the rumen capacity, and after calving feed intake continually increases and peak around 9 – 13 weeks of lactation (Kertz *et al.*, 1991). The period from parturition until peak milk production is the most critical phase for a dairy cow (Schingoethe *et al.*, 1993).

During early lactation, dietary starch plays an important role in the diet of high producing dairy cows, providing an energy-dense substrate, especially critical during periods when glucose requirements are high (Van Vuuren *et al.*, 2010) but cows struggle to meet these demands due to low DMI and high milk yields, often leading to a postpartum energy balance deficit (Spurlock *et al.*, 2012). However, glucose availability for direct absorption is low in ruminants due to high carbohydrate fermentation rates in the rumen (Baird *et al.*, 1980). In fact, starch is efficiently fermented by microorganisms in the rumen to short-chain fatty acids, which from these propionate, valerate, and isobutyrate can be utilized as glucogenic precursor for net synthesis of glucose to fulfill high priority of lactose in the mammary gland. Nevertheless, propionate is known to be the most abundant of the three glucogenic acids (15 - 40%) and by far the predominant substrate for gluconeogenesis in ruminants for lactose demand. High producing dairy cows produce over 2 kg of lactose daily and have specific requirements for glucose especially during early lactation. Failure in supplying glucose may not only lead to decreases in milk yields but also can cause disorders in fat metabolism occasioning direct and indirect health problems (van Knegsel *et al.*, 2005). The mammary gland utilizes 60 to 85% of the total glucose used in lactating ruminants, lactose synthesis by itself accounts for 50 to 85% of mammary glucose utilization (Annison *et al.*,1974) Glucose requirements and status are critically dependent on state of lactation and level of milk production in dairy cattle, which is closely related to endogenous glucose production supported by endocrine changes during peak lactation (Hammon *et al.*, 2010). Decreases in tissue responsiveness to insulin and a reduction in glucose uptake into insulin-sensitive organs (muscle and adipose tissue) are part of homeorhetic changes that takes place after parturition and thus favors glucose uptake into the mammary gland (Komatsu *et al.*, 2005). Insulin resistance ensures that body reserves can occur to support mammary gland requirements (Bauman, 2000).

Animal welfare and production performance concerns in addition to recent increases in feed costs, especially grains, and consequent reductions in income over feed cost have encouraged dairy producers to use moderate starch diets (Ferraretto *et al.*, 2011). Replacing a portion of the diet’s concentrate for ingredients with greater fiber content can lower the price of final diets and also decrease disturbances in rumen metabolism (Zebeli and Metzler-Zebeli, 2012).

Direct fed microbials

Feeding antibiotics to animals was prohibited in the European Union (EU) in 2006 due to concerns over increasing bacterial antibiotic resistance in humans, as sub-therapeutic doses of antibiotics had been used in feed to promote growth and maintain health in farm animals (Prieto *et al*., 2014). The growing concern regarding the use of antibiotics in animal production has increased the interest of exploring alternatives to antimicrobial feed additives (Martin *et al*., 1999). Direct fed microbial, traditionally referred to as “probiotic” are live or viable naturally occurring organisms commonly used as supplements in animal production with the goal to confer a health benefit to the host by including improved establishment of beneficial gut microflora (NRC, 2001). Different probiotic strains have been shown to successfully improve growth performance and to reduce enteric disease in pigs, poultry, ruminants and cultured fish (Gaggìa *et al*., 2010).

Direct fed microbial have three primary ways to influence ruminants, 1) as an additive for silage or haylage or as a preservative for hay; 2) to replace or decrease the use of antibiotics in stressed cattle; 3) to enhance feed efficiency and increase milk production in dairy cows and body weight gain in beef cattle (Yoon and Stern 1995). The term DFMs has included specific and nonspecific yeast, fungi, bacteria, cell fragments, and filtrates (Knowlton 2002). The most common DFMs interventions of ruminal fermentation to promote desirable intestinal micro flora, improve nutrient utilization and stabilize pH to promote rumen health, include the supplementation of fungal cultures (*Aspergillus oryzae, Saccharomyces cerevisiae*) and lactate producing (*Enterococcus*) and lactate-utilizing (*Proprionibacterium*) bacterial species as well as *Bifidobacterium spp*., and *Bacillus spp* (FAO, 2013).

Fungal species are also commonly fed to animals as a blend with bacterial species such as Enterococcus strains which have been selected for raising nadir pH in the rumen, and increasing daily mean rumen pH (Oetzel *et. al*., 2007). Enterococcus strains in a combination with yeast product have an enhanced lactation cow performance by increasing milk protein percentage and improving health status by decreasing the number of antibiotic treatments which might be a promising alternative to enhance performance of transition dairy cows (Oetzel and McGuirk ,2007). The combination of bacterial and fungal strains has also been supplemented to feedlot cattle fed a high-grain diet as an attempt to decrease the risk of acidosis and improve feed digestion. Beauchemin *et al*., (2003) showed that although *Enterococcus faecium* caused only small shifts in microbial ecosystem when supplemented to feedlot cattle fed high grain diets, it appeared to be metabolically active.

It is relevant to consider that dietary changes influence populations of ruminal microbes, which can be highly specific to the ruminant host, and if properly manipulated can improve the nutritional management of ruminants (Mullins *et al*., 2013). Evidence supports that rumen microbial populations adapt to dietary changes (Ramirez *et al*., 2012). Modern animal production should effectively take advantage of feed additives as rumen manipulators to increase animal productivity. Despite the range of promising benefits of Bacillus pumilus including enhanced fiber digestibility and increased feed efficiency, there is still a lack of information on the effects of its supplementation to ruminant animals. Research should consider investigating the benefits of relevant DFMs such as Bacillus pumilus that can improve animal performance and economic returns.

Effect of Exogenous fibrolytic enzymes in Ruminant

Increase in feed prices, especially grains, and declines in enzyme costs have prompted interest in using enzyme additives in dairy cattle diets to increase nutrient utilization, and assure profitability and sustainability of the animal production activity (Beauchemin *et al*., 2008). Methods that increase fiber digestion are likely to play a role in improving energy availability of ruminant diets and reducing feed costs (Vinici *et al*., 2003), as forage digestibility continues to limit the intake of available energy by ruminants, and correspondingly, contributes to excessive nutrient excretion by livestock (Chung *et al*., 2013). Forages contain about 30 to 70 percent NDF, and NDF digestibility in the ruminant digestive tract is typically less than 65 percent of North American diets (with about 50 percent NDF degradability in the rumen) but can be considerably higher for some grasses and grass silage-based diets (Huhtanen *et al*., 2009).

However, the efficacy of fibrolytic enzymes in improving animal’s performance while increasing feed conversion in ruminants has been variable (Beauchemin *et al*., 2008). The variability of responses to exogenous fibrolytic enzymes can be attributed to the durations of feeding period, stage of lactation that cows are fed, and inappropriate choice of enzymes with lack of sufficient potency and specificity for improving digestibility under ruminal conditions (Adesogan *et al*., 2014). It is recommended that feeding trial should be followed only after previous in vitro model evaluation of EFE on ruminal temperature and pH conditions. In order to optimize enzyme activity, it needs to be placed in an ideal media with proper temperature, pH and have comparable subtract. According to a study mentioned in a literature review by Adenogan *et al*., (2014) an evaluation of 18 commercial EFE showed that 78 and 83% of them exhibited optimal endoglucanase and xylanase activities, respectively, at 50°C, and 77 and 61% had optimal activities at pH 4 to 5, respectively, indicating that most would likely act suboptimal in the rumen.

Ariola *et al*., (2011) reported that multiparous or primiparous cows fed low-concentrate diets treated with fibrolytic enzymes had a better performance than cows fed untreated high-concentrations diets, which leads to the conclusion that greater forage proportions can be fed to dairy cows without jeopardizing milk production, lowering the cost of diets and reducing risk of rumen acidosis. Enzyme additives increase the rate of fiber digestion, which can provide more digestible energy to the animal for growth or milk production (Beauchemin *et al*., 2008). Dairy animals usually calve for the first time at about 24 months of age as this maximizes the economic benefit (Hoffman and Funk, 1992). However, animals are not yet physically mature at this stage; they require nutrients for their own continued growth in addition to that of their developing calf (Coffey, 2006).

Mohamed *et al*. (2013) reported that supplementation of early lactating dairy cow diet with fibrolytic enzymes, did not cause any significant changes in dry matter intake. But with the supplementation of exogenous fibrolytic enzymes milk yield was better significantly. In addition, the energy corrected milk and feed efficiency in early lactating dairy cows were improved significantly better compared to the control group. These results consistent with outcome of Lopuszanska-Rusek and Bilik, (2011) where they observed enhanced milk production with xylanase-esterase supplementation and a tendency of improving DMI and milk production with xylanase and cellulose enzyme supplementation, respectively. But they hypothesized that slight differences of milk production might be due to repartitioning of energy between milk and body reserves for cows receiving enzymes. Similar results, non-significant changes of milk production with the supplementation of direct fed microbial and enzyme mixture was observed by Diler *et al*., (2014). A few studies suggested exogenous enzymes, especially phytase can be used to decrease manure nutrient excretion which is environmental friendly. (Knowlton *et al.,* 2007) stated that feeding cows with enzyme formulation decreased fecal DM, NDF and ADF excretion and reduced fecal excretion of nitrogen and phosphorous.

In a study by Chung *et al*., (2013) they disclosed the effect of enzyme additive on Volatile Fatty Acid production, ammonia nitrogen flow, pH, or population densities of total protozoa, bacteria and methanogens in ruminal fluid reported unchanged. Increasing the level of enzyme supplement in the diet also linearly increased enteric methane production, even when adjusted for feed intake or milk production. However, Peters *et al*., (2010) inspected the influence of fibrolytic enzyme supplementation with TMR prior to feeding on ruminal fermentation, microbial protein synthesis, nutrient digestion and milk yield and composition and there were no significant differences in any of the parameters inspected.

CONCLUSIONS

Supplementation of exogenous enzymes with ruminant diets shows beneficial effects on feed utilization, growth and production performance in ruminants though some debatable issues need to be further revised. Therefore, future studies are highly recommended with the special emphasis on feed specific enzyme activity, method of supplementation and optimum dosage of enzymes. For the benefit of all interested parties, there should be standardization in the way in which enzyme products are described in scientific literature.

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