

*Review Article***Application and Limitations of Exogenous Fibrolytic Enzymes in Animal Nutrition**

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**Abstract**

Dairy industry and poultry industry in India are facing downfall due to low availability and low digestibility of feeds and forages. Ruminants are fed straw based diet which has a very low digestibility. To increase its digestibility, various exogenous fibrolytic enzymes can be used like cellulase, xylanase, pectinase, amylase etc. These enzymes either can be applied to the feed prior to feeding to hydrolyze the structural carbohydrates or can be directly fed to animals during feeding. The application of these enzymes may improve digestibility of feed, increase feed intake, which may lead to higher feed efficiency, higher growth rate and higher milk production. But care should be taken regarding selection of enzyme, mode and time of application, concentration of enzyme for optimum activity. Therefore, more research is needed to study the specific enzyme for different substrate instead of using a generalized group of enzyme.

**Key words:** Cellulase, Fibrolytic Enzyme, Nonruminants, Ruminants, Xylanase**How to cite:** Satapathy, D., Dutta, T., & Chatterjee, A. (2018). Application and Limitations of Exogenous Fibrolytic Enzymes in Animal Nutrition. International Journal of Livestock Research, 8(8), 21-34. doi: 10.5455/ijlr.20170926065352**Introduction**

In Indian condition, dairy animals are preferably raised up on low quality native grasses, crop residues and agro-industrial by-products because of low availability of fodder and high cost of concentrates. The green roughage, dry roughage and concentrate are deficit up to 62.76, 23.46 and 63.00% of requirement (Kore, 2014). These feeds are low in energy and protein owing to high fiber, lignin and silica. Ruminant animals have the ability to convert low quality feeds into high quality protein due to ruminal microorganisms that synthesize and secrete  $\beta$  1-4 cellulase enzyme complex, thereby allowing hydrolysis of plant cell wall components. High fiber content of fibrous feeds prevents the access of ruminal enzymes to the plant cell wall and reduce nutrient digestibility (Abdel-Aziz *et al.*, 2015; Elghandour *et al.*, 2015; Togtokhbayar *et al.*, 2015). The fodders fed to farm animals have high crude fiber and medium

digestibility. Thus to improve the digestibility, it is important to break down the linkage between cellulose, hemicellulose and lignin. Digestibility of fiber can be improved by different rumen manipulation techniques. Exogenous enzyme application is one of the rumen manipulation techniques that can be used to improve the digestibility of feeds and improved production performance (Rojo *et al.*, 2015; Salem *et al.*, 2015; Valdes *et al.*, 2015). In recent years, use of Exogenous Fibrolytic Enzymes (EFE) has been identified as a promising alternative to improve forage utilization by ruminants (Beauchemin *et al.*, 2003). The potential of enzymes to increase the performance of cattle (Bhasker *et al.*, 2012), buffaloes (Gaafar *et al.*, 2010), lambs (Salem *et al.*, 2012) has been reported previously. Apart from that, broiler industry is also going through a crisis of food supply because of high cost of traditional feeds like maize, soyabean meal etc. Therefore, there should be an approach either to provide alternate feed sources which have fewer digestibilities or to increase the digestibility of those feeds. Digestibility of sunflower meal can be increased by use of exogenous enzyme (Alagawany *et al.*, 2015). Alternate feed sources are known to contain many anti nutritional factors, which are harmful to the animals. Therefore, removal of those antinutritional factors either by different chemicals or by use of antidote enzyme is essential in animal nutrition. Chemicals such as  $\text{NH}_3$ ,  $\text{NaOH}$  can be used to improve the digestibility of feeds and remove deleterious factors, but may also lead to huge loss of DM (Lynch *et al.*, 2014). Therefore, use of exogenous fibrolytic enzyme is on high research interest as digestibility of fiber fraction reaches to a maximum level of 65-70% even under ideal condition (Yang *et al.*, 2011; Chung *et al.*, 2012; Mohamed *et al.*, 2013)

### Sources of Enzymes

Enzyme products are derived primarily from four bacterial (*Bacillus subtilis*, *Lactobacillus acidophilus*, *L. plantarum* and *Streptococcus faecium*), three fungal (*Aspergillus oryzae*, *Trichoderma reesei* and *Saccharomyces cerevisiae*) species and some yeasts (Subramaniam and Vimala, 2012). Enzymes are produced from bacteria and fungi by fermentation process. Fermentation has been classified into Solid State Fermentation (SSF) and Submerged Fermentation (SmF) mainly based on the type of substrate used during fermentation. In solid state fermentation, solid substrates like bran, bagasses are used. In this case, the fermentation takes place in a slow and steady manner but for a very long period which leads to controlled release of nutrients. But in case of submerged fermentation or liquid fermentation process, liquid substrates like broth, molasses are used. These types of substrates are used in a very rate leading to rapid production of enzymes. So the substrates should be replaces or supplemented with nutrient at a faster rate (Subramaniam and Vimala, 2012).

SmF is usually implemented in case of bacterial enzyme production, due to the requirement of higher water potential (Chahal, 1983). SSF is preferred when enzymes have to be extracted from fungi, which require lesser water potential. More than 75% of the industrial enzymes are produced using SmF. One of the major reason being that SmF supports the utilization of genetically modified organisms to a greater extent than SSF. Fungi produce more cellulase from bacteria still isolation of cellulase from bacteria are now becoming more popular. Because of the following reasons-

1. Higher growth rate of bacteria
2. Bacterial cellulase are more complex and provide better function
3. Bacteria can sustain in highly variable environment (Sujani and Seresinhe, 2015).

Cellulase are produced using both fungi and bacteria with more emphasis on the use of fungi because of their capability to produce ample amounts of enzymes (Subramaniam and Vimala, 2012) and often less complex than bacterial cellulase and easy for extraction and purification. Bacteria, yeasts and filamentous fungi have been identified as suitable candidates to produce xylanases (Kamble and Jadhav, 2012). According to another study, new xylanase producing Gram positive bacteria has been isolated from termite gut (Matteotti *et al.*, 2012).

### Types of Enzymes

The types and activity of enzymes produced can be diverse depending on the strain selected, the substrate they are grown on and the culture conditions used (Gashe, 1992; Lee *et al.*, 1998). A huge number of enzymes are needed for degradation of structural carbohydrate of plant cellwall (Morgavi *et al.*, 2012). Common commercially available enzymes are cellulases and xylanases, but a lot of secondary enzymes are also available in the market like amylases, proteases, esterases, or pectinases. Further carbohydrate breaking enzymes or group of enzymes can be classified as endoglucanases and exoglucanases based up on their site of action (Zhang and Lynd, 2004). For degradation of protein fraction, amyolytic (Noziere *et al.*, 2014; Gencoglu *et al.*, 2010) and proteolytic (Eun and Beauchemin, 2005; Vera *et al.*, 2012) enzymes can also be applied. Fibrolytic enzymes are mainly of two types i.e. for cellulose hydrolysis and for hemicellulose hydrolysis. Enzymes for cellulose hydrolysis are endoglucanase, cellobiohydrolase,  $\beta$ -glucosidase, while enzymes for hemicellulose hydrolysis are endoxylanases,  $\beta$ -1,4-xylosidases. Some other enzymes are also included under fibrolytic enzymes like acetyl xylan esterase, ferulic acid esterase,  $\alpha$ -D-glucuronidase,  $\alpha$ -L-arabinofuranosidase.

### Mechanism of Action of Enzymes

Primary target of exogenous enzymes are cellulose and hemicellulose. Cellulose is a branched complex structure. Therefore, degradation is not simple and needs a group of enzymes. Cellulases are a group of

enzymes that hydrolyse cellulose or  $\beta$ -(1,4)-glucan. Enzymes belonging to this class are cellobiohydrolases, endoglucanases and  $\beta$ -glucosidases or cellobiases. Cellobiohydrolases act on crystalline parts of cellulose, whereas endoglucanases are believed to cleave at the amorphous regions of the polymer. These two enzymes produce cellobiose from cellulose. Finally, cellobiose is acted by cellobiase or  $\beta$ -glucosidases to produce glucose that is simple sugar which can be digested or utilized by the animals (Rabinovich *et al.*, 2002).

Xylan is the major component of hemicellulose and is, after cellulose, the second most abundant polysaccharide in nature. Xylans account for 30–35% of the cell wall material of annual plants (grasses and cereals), 15–30% of hardwoods and 7–10% of softwoods. They are also branched compounds, which need a group of enzymes known as xylanases for degradation. Partial hydrolysis of hemicellulose effective for viscosity reduction. However, for complete hydrolysis synergistic action of several xylanases is needed. The side-chain cleaving ‘accessory’ enzymes remove the substituent groups and the 1,4- $\beta$ -D-xylosidase cleaves xylobiose and xylooligosaccharides into xylose monomers (Shallom and Shoham, 2003). The accessory enzymes for total hydrolysis of arabinoxylan include  $\alpha$ -L-arabinofuranosidase, acetyl xylan esterase and feruloyl esterase, P- coumaric acid esterase and  $\alpha$ -D-glucuronidase. Hydrolysis by xylanases of cereal xylans releases oligosaccharides consisting of xylose or xylose and arabinose residues (Paloheimo *et al.*, 2010).

### Application of Enzymes

Application of enzymes can be done through different methods such as pre-treatment on the feeds for a period of time or at the time of feeding or providing directly into the rumen. Which is the best method, is yet to be finalized. But it can be stated that method of application of enzyme should be selected basing on the type of feed and feed components. Adding enzymes to the feed prior to feeding provides enough time for the enzymatic action on fibrous carbohydrates leading to hydrolysis of the fibers and availability of monomer units to animals. Yang *et al.* (2000) reported that fibrolytic enzyme addition to concentrates one month before feeding increased diet digestion and milk production by dairy cows. Again, Shadmanesh (2014) observed increase in milk yield and SNF by feeding TMR mixed with fibrolytic enzyme prior to feeding. However, Lynch *et al.* (2014) reported no effect of exogenous fibrolytic enzyme application during ensiling of alfalfa on nutritive value, rather more loss of DM when applied along with ferulic acid esterase inoculum.

### Effect of Enzymes on Dairy Cattle

Mohamed *et al.* (2013) reported that supplementing fibrolytic enzyme (with TMR at the time of feeding) to early lactating dairy cows increased milk production significantly without affecting DMI.

Supplementing cellulase and xylanase mixture (1:1) @1.5g/kg DM to Sahiwal cows increased the CF and NDF digestibility leading to increased milk production (Miachio and Thakur, 2007). Gado *et al.* (2009), Holtshausen *et al.* (2011) and Arriola *et al.* (2011) also reported similar results. Klingerman *et al.* (2009) observed significant increase in milk production without affecting milk fat and milk protein due to supplementation of exogenous fibrolytic enzyme. Tewoldebrhan *et al.* (2017) also reported improved feed conversion efficiency and lowered somatic cell count without affecting the milk production and milk composition in Holstein cows when supplemented with  $\beta$ -mannanase @ 0.1% of DM. However, a nonsignificant change in milk production was observed by Diler *et al.* (2014) due to supplementation of direct fed microbials and enzyme mixture. Similar result was also observed by Elwakeel *et al.* (2007). However, they suggested that slight differences of milk production might be due to repartitioning of energy between milk and body reserves for cows receiving enzymes.

Exogenous fibrolytic enzyme feeding resulted in increased sugar release from fibers leading to increased TVFA concentration, decreased rumen pH. Rumen liquor protein and nitrogen concentration was at optimum concentration by supplementing exogenous fibrolytic enzyme (240mg/kg TMR) which indicated better utilization of carbohydrate and protein in nonpregnant Gir and crossbred dairy cows (Lunagariya *et al.*, 2017). Supplementing exogenous fibrolytic enzymes with corn or sugarcane silage had no significant effect on eating behavior, nutrient intake, rumen fermentation pattern, and microbial protein synthesis. But this supplementation increased NDF digestibility and N absorption of low quality forage like sugarcane silage in dairy cows (Gandra *et al.*, 2017). Similar to this other studies also revealed no effect of fibrolytic enzyme supplementation on feed intake (Peters *et al.*, 2015; Silva *et al.*, 2016). But Romero *et al.* (2016) reported increased DMI, OMI and CPI due to supplementation of exogenous enzyme (cellulase and xylanase) @ 1ml or 3.4ml per kg Bermuda grass based TMR without affecting the milk composition. Beauchemin and Holtshausen (2010) suggested that the stage of lactation plays a vital role for effectiveness of exogenous fibrolytic enzyme application in case of dairy cows. Proteolytic enzyme supplementation resulted in increased digestibility of DM, OM however decreased the DMI and Milk yield. Although, dairy efficiency that is milk/DMI was increased (Eun and Beauchemin, 2005).

### Effect of Enzymes on Beef Cattle

In case of beef steers, DMI and DMD were found to be increased after due to application of fibrolytic enzyme after harvesting of Bermudagrass (Krueger *et al.*, 2008). In another study by Varges *et al.* (2013), they reported no effect of fibrolytic enzyme on body weight gain, feed conversion efficiency but improved carcass characteristics and tenderness. Atrian and Shahryar (2012) found that application of fibrolytic enzyme @ 14ml/10kg alfalfa hay had a positive effect on daily gain and DMI. Again they stated that there should be a limitation of application i.e. application @19ml/10kg hay had a negative effect on

growth performance. Contradicting to these studies Salem *et al.* (2011) reported no significant growth in goat due to fibrolytic enzyme supplementation while apparent digestibility of NDF and ADF was increased. A study conducted by Balci *et al.* (2007) exhibited better daily weight gains, total weight gains and feed conversion rates. Positive effect of fibrolytic enzyme on Barley and wheat based dried distillers grain has been observed by He *et al.* (2014 and 2015). In an experiment on beef cattle, Romero *et al.* (2013), reported that digestibility of hay depend on its maturity i.e. 5 week mature hay had higher digestibility while that of 13 week hay had low digestibility even after fibrolytic enzyme application. Russell *et al.* (2016) reported decreased NDF digestibility and non-differed ADF digestibility of whole shell corn due to addition of enzyme in beef steers.

### Effect of Enzymes on Buffaloes

Buffaloes are known to consume more amount of straw and fodder than that of cattle. But, poor utilization capacity of forages by the animal is main constraint in buffalo husbandry. Therefore, there is high need for improving the digestibility of the forages in case of buffaloes. Several experiments have been carried out regarding effect of exogenous fibrolytic enzymes on buffalo performance. Through in vitro experiment, Malik and Bandla (2010) specified a dose of exogenous enzyme and when that dose was supplemented to male buffalo calves along with probiotics, digestibility of OM, ADF and NDF was improved significantly leading to higher ADG and feed efficiency. Nawaz *et al.* (2016) also reported similar results. However, contradictory result has been observed by Reddy *et al.* (2016) who reported no effect of exogenous fibrolytic enzyme and live yeast culture on performance of buffalo bulls. Similarly, addition of enzyme to urea treated straw had no effect on digestibility of wheat straw in case of buffalo because of rapid production of ammonia and alkaline pH (Rehman *et al.*, 2014). Thakur *et al.* (2010) defined the dose of the enzyme (Cellulase and xylanase) in an experiment and suggested that supplementation of enzyme @ 1.5gm/kg DM of TMR had more pronounced effect on growth performance in buffalo as compared to 3gm/ kg DM and non supplemented group. Shekhar *et al.* (2010) and Mosy *et al.* (2016) have reported improved nutrient digestibility and higher milk production due to addition of exogenous fibrolytic enzyme in case of Murrah buffalo and Egyptian buffalo, respectively. Supplementation of enzyme decreased the rumen pH, increased the ruminal TVFA, ruminal ammonia and nitrogen fraction which is an indication of better digestibility of feeds (Rajamma *et al.*, 2014).

### Effect of Enzymes on Sheep and Goat

In case of goats, supplementation of fibrolytic enzyme with TMR improved NDF and ADF digestibility by 10 and 9.1%, respectively. It also resulted in higher concentration of TVFA and acetate with higher bacterial count. So feed efficiency was found to be higher because of enzyme supplementation

(Yuangklang *et al.*, 2017). Bhasker *et al.* (2013) observed similar rumen fermentation pattern in sheep due to cellulose-xylanase mixture. But they also observed no significant effect on rumen pH, DMI and rumen nitrogen content. Higher growth rate in Mangolian lambs were also observed due to cellulase supplementation by Togtokhbayar *et al.* (2017). High doses of Exogenous Fibrolytic Enzymes (EFE) (0, 5, 10g/ Kg DM of oat straw) were evaluated for their effects on lamb performance (Bueno *et al.*, 2013). Resulting from enzyme treatment, intake decreased linearly ( $p < 0.04$ ) with increasing enzyme doses without changing the weight gain, feed conversion, digestibility and ruminal fermentation parameters. Similar results of unchanged weight gain and dry matter intake were also evident in the study by Torres *et al.* (2013). *Salix babylonica* leaves extract along with enzyme had a pronounced effect on meat quality as compared to enzyme supplementation alone (Cayetano *et al.*, 2013; Valdes *et al.*, 2015). Wang and Xue (2016) also reported no significant effect of enzyme supplementation on nutrient digestibility, nitrogen retention, energy metabolism and methane emission in Boer goats. Contradicting to these Salem *et al.* (2015) reported improved feed intake, nutrient digestibility, nitrogen balance and rumen fermentation pattern in case of sheep, due to supplementation of *Atriplex halimu* and enzyme cocktail.

Exogenous enzyme supplementation may lead to increased feed intake and digestibility of forages leading to increased weight gain. This has been supported by Mijinyawa *et al.* (2016), who observed increased feed intake, weight gain and average daily gain in bucks fed sugarcane bagasse with enzyme supplementation were significantly ( $p < 0.05$ ). Nutrients digestibility followed similar pattern except for Dry matter (DM), CP and CF which were not significant. They concluded that urea treatment with enzyme supplementation has positive effect on performance of fattening Red Sokoto bucks. Another study was conducted to investigate the effects of adding cellulolytic enzyme “Asperozym” or Tomoko® to the diets on the performance of goats. The diets supplemented with either enzymes showed significantly ( $p < 0.05$ ) increased digestibility of all nutrients with significant effect on rumen liquor parameters (Kholif and Aziz, 2014).

### **Effect of Enzymes on Non-Ruminant Animals**

In an experiment on growing pigs, Schertz *et al.* (2016) reported 7% faster growth rate ( $p = 0.03$ ) due to supplementation of exogenous enzyme with protease and carbohydrase activity. Lu *et al.* (2016) also reported higher body weight gain, ADG and apparent total tract digestibility because of supplementation of xylanase,  $\beta$ -glucanase and phytase. Contradicting to this, O’Shea *et al.* (2014) reported no effect of xylanase and protease on pigs, which are fed diets based on rapeseed meal, and dried distillers grain despite of higher ileal digestibility. They suggested that that this might be due to lower average daily feed intake. Yang *et al.* (2017) reported that supplementing xylanase improved the crude protein digestibility while phytase supplementation improved digestibility of calcium and phosphorus. However,

supplementing both enzymes impaired the effectiveness of xylanase leading to reduced NDF digestibility. This might be due to alteration in hindgut flora composition because of phytate that led to deactivate xylanase.

In case of poultry birds, exogenous enzyme supplementation is needed to increase the feeding value of raw materials, to reduce the variation in nutrient quality of ingredients and to reduce the incidence of wet litter (Bedford, 2000). Broiler farmers are taking a step back from feeding soyabean meal to birds because of its high cost. Therefore, unconventional feed for protein sources are being used. One of the unconventional feed is sunflower meal (SFM). Alagawany *et al.* (2017) reported that SFM could replace soyabean meal up to 50% with addition of exogenous enzyme (xylanase, protease and amylase) and could provide improved growth performance, activity of digestive enzymes and carcass traits. Nikam *et al.* (2016 and 2017) also suggested that using of enzymes like xylanase,  $\beta$ -D-glucanase, cellulase, mannanase and pectinase to diets based on guar meal, rapeseed meal, cottonseed meal improves body weight gain and feed intake. Naik *et al.* (2017) and Santhi *et al.* (2014) have observed similar results of exogenous enzyme supplementation on broilers and turkey, respectively.

### **Limitations or Challenges for Use of Exogenous Enzyme**

Though use of exogenous enzymes have a lot of advantages, its use is limited because of its price and again some scientists reported no effect of this type of supplementation in animals fed with concentrate based diet. Before use of enzymes, some basic knowledge regarding enzyme and substrate should be acquired for optimum result.

### **Specificity of the Exogenous fibrolytic Enzyme to the Substrate**

A particular enzyme can break a specific linkage. Therefore, enzymes are feed specific according to their chemical structure. Example- Enzymes effective for improving dry matter digestibility of corn silage were different from enzymes required for alfalfa hay digestion (Colombatto *et al.*, 2003).

### **Time of Application**

As discussed earlier time of application of enzyme for optimum action is essential. Whether the enzyme should be fed to the animal during feeding or application of enzyme should be done on the feed just before feeding should be analyzed as per the type of feed.

### **Rate and Level of Enzyme Application**

Low dose of enzyme application may not exploit its optimum hydrolytic potential. Similarly overdose also may lead to molecular crowding leading to decreased dry matter intake and digestibility.

(Bommarious *et al.*, 2008). Optimum level of enzyme should be applied on the feed and when used in combination, optimum ratio of enzymes should be identified for optimum result (Thakur *et al.*, 2008)

### **Influence of pH and Temperature**

Enzyme activity is pH specific and enzyme activity reaches its optimum potential at a particular range of temperature. By studying the effect of 18 commercially available endoglucanase and xylanase at different temperatures and pH, Adesogan *et al.* (2014) reported that 78 and 83% of these enzymes exhibited endoglucanase and xylanase activity at 50°C, however, 77 and 61% had optimal activity at pH 4 to 5, respectively.

### **Inappropriate Choice**

Choice of enzyme for hydrolysis of structural carbohydrate should be substrate specific. Inappropriate choice of enzyme may hydrolyse other available sugars leading to less effectiveness of feed. Some exogenous fibrolytic enzymes were developed for other applications such as textiles, food, nonruminant diets or paper. Therefore, such EFE often lack sufficient potency and specificity for improving the use of fibrous ruminant feeds (Adesogan *et al.*, 2014).

### **Conclusion**

Exogenous enzymes can be used in ruminant and non-ruminant nutrition in an appropriate way and at proper concentration to improve the feed utilization capacity of animal, to improve the digestibility of feed, to improve the growth rate and weight gain and also to improve the milk production. Appropriate choice of the enzyme should be done basing on the target feed chemical composition of target feed. Future studies can be carried out giving emphasis on enzyme specificity and enzyme mode of action.

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