

IMPROVING THE NUTRITIVE VALUE OF FORAGE BY APPLYING EXOGENOUS ENZYME

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Abstract
The use of exogenous enzymes in animal nutrition dates back to the mid-1920s, however, nowadays the development of interdisciplinary sciences exploiting molecular methods create new opportunities and deliver new tools to assess effectiveness of their utilization. Effectiveness of enzymes in animal nutrition depends on (i) type, (ii) source, (iii) level of supplemented enzymes, (iv) the type of diet fed, (v) animal health and (vi) animal productivity. In most tropical countries, the ruminant feed is based on fibrous resources with a cell wall content between 40 and 70% of dry matter, of which less than 50% is quickly digested, which generates high excretion of nutrients to the environment and low productivity in their production systems. Recently, forage cell wall digestibility has undergone significant improvements through exogenous enzyme technology. In terms of enzyme technology, the two most popular enzyme complexes are those of the cellulase and hemicellulase families, generally known to be multi-component enzymes that when added to forage could possibly assist in the preservation of forages, especially silage. Enzymes can be applied to straw in their pure form or through inoculation with appropriate cell wall degrading microbes. It is acknowledged that enzyme preparations with specific activities can be used to drive specific metabolic and digestive processes in the gastrointestinal tract and may increase natural digestive processes to improve the availability of nutrients and feed intake thereafter The use of enzymes has a positive relation with palatability, feed intake, rumen microbial N synthesis, digestibility, and improves animal performance as milk production, live weight gain, feed efficiency and immunity. Enzyme preparations for ruminants are produced through microbial fermentation, beginning with seed culture and growth media. In general, Bacillus subtilis, Lactobacillus acidophilus, L. plantarum, and Streptococcus faecium, spp. are the source of bacterial enzymes. Fungal enzymes generally come from Aspergillus oryzae, Trichoderma reesei, and Saccharomyces cerevisiae species.

Key words: Exogenous, Enzyme, Forage, nutritive value;

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1. INTRODUCTION

Forage is the main nutritional components for ruminants throughout the world (Wilkins, 2000). It is most important components of the diets fed to ruminant animals even under intensive concentrate feeding systems (Beauchemin *et al*., 2013). Ruminants are able to convert low quality feeds into food of high biological value for human beings. The ruminant's digestive process is not 100% efficient. Because, under most feeding conditions, neutral detergent fiber (NDF) digestibility in the ruminant digestive tract is less than 65%, and ruminal NDF digestibility is often less than 50% (Beauchemin and Holtshausen, 2010). Increasing fiber digestibility is a common practice as an attempt to reduce feed costs and ensure greater financial returns. Roughages, especially crop residues constitute the major ingredients of ruminants' diets in developing countries, while the complex network formed by structural carbohydrates and lignin in crop residues limits the digestibility and efficient utilization of forages by ruminants. Many attempts have been made to increase the digestibility of forages. Among this, use of exogenous enzymes has been received considerable attention for long time (Chung *et al.,* 2012).

The improvement of forage digestibility, evaluation, utilization and increased productive efficiency of animals has been the focused area of forage research for many decades. Biological treatments of some by-products are very essential in order to degrade lignocellulosics into lignin, cellulose, hemicellulose, and improve CP content. Animal uses enzymes in the digestion of feed, those produced either by the animal itself or by microbes present in the digestive tract. Hence, the supplementation of animal feeds with exogenous enzymes to increase the efficiency of digestion can be seen as an extension of the animal's own digestive process (Elghandour *et al*. 2015; Salem *et al*. 2015).

The use of enzymes has a positive relation with palatability, feed intake, rumen microbial N synthesis, digestibility, and improves animal performance as milk production, live weight gain, feed efficiency and immunity (Arriola *et al.* 2011; Gado *et al.* 2011). Mendoza *et al* (2014) stated that, exogenous xylanases and cellulases are the most commonly used fibrolytic enzymes for ruminants.

According to Alvarez *et al*. (2009), due to the increased dry matter (DM) and crude protein (CP) soluble fractions of diets upon fibrolytic enzyme addition, the reducing sugars produced would provide energy that would lead to rapid microbial growth. Increased ruminal bacteria numbers could lead to increased microbial colonization of the feed particles. Meale *et al* (2014) also reported that, a synergistic relationship between exogenous enzymes and rumen microbiota, and an increase in bacterial attachment are other likely modes of action of exogenous enzymes in the rumen. Exogenous enzymes increased microbial growth and production of MP (Gado *et al*., 2009). As different researcher investigated, enzyme treatments decreased the retention time of digesta in the rumen, positive responses would be expected because of the enzymes acting on structures of the plant cell walls and increasing access of ruminal microbes to the potentially fermentable fiber (Sutton *et al.* 2003).

The conversion rate of the enzymes is reduced when insufficient substrate is available to saturate the enzyme. The enzyme can be inhibited or completely inactivated at temperatures of 70 to 80 ºC or in presence of chemicals (heavy metals) and natural inhibitors (Lehninger *et al.*, 2005).
Therefore, the objective of this current topic paper is to review on improving the nutritive value of forage by applying exogenous enzyme.

2. LITRETURE REVIEW

Worldwide demands for animal based products are increasing in a booming rate thus emphasizing the essentiality of applying strategies to improve animal productivity. Ruminal energy utilization can be improved by enzyme feed additives with fibrolytic properties. The enzyme feed additive increase the quantity of enzymes that are available to digest structural plant parts in the rumen to increase utilization of fibrous feedstuff (Chung *et al*., 2012). Generally, enzyme supplementation makes more flexibility when formulating a diet since the ingredient quality or animal digestibility capacity can be manipulated (Paloheimo *et al*., 2011). Research on effects of forage cell wall degrading or fibrolytic enzymes started as early as the 1960's as reviewed by Holtshausen and Beauchemin (2010).

Fibrolytic enzyme preparation ready to be used, and delivered through a feed ingredient such a molasses-based liquid feed (MLF), for instance, might reduce on-farm labor and increase the acceptance of this new technology by producers while decreasing the errors of enzyme preparation. Moreover, MLF is designed to provide sugar into diets and also improve particle adhesion. This ingredient feature can help to enhance binding of the enzyme with the feed substrate, which may increase the resistance of enzyme to proteolysis in the rumen. The use of EFE to improve forage quality can also replace other expensive strategies that increase fiber digestibility such as treatments with physical agents such as heat, steam, and pressure, or with chemicals such as acids, alkalis, NH3, and ozone. Those alternatives are likely to require a bigger investment of capital and have essentially a high energy intensive utilization for physical methods such as steam or pressure explosion, the potential of pelleting, chopping, or grinding which might result in limiting salivary buffering of ruminal acids. Moreover, the corrosive and/or hazardous nature of chemicals such as NH3 and NaOH might add potential for excessive DM losses following hydrolysis (Lynch *et al*., 2013; Adenogan *et al*., 2014).

2.1. Biological Treatments of Forage

The efficiency by which ruminants obtain energy from structural plant polysaccharides and, in turn, produce high quality meat and milk protein is increasingly important if the demands of an expanding human population are to be fulfilled (Meale *et al*., 2014). Different methods have been attempted to improve forage digestiblity for ruminant animals. This includes treatment with physical agents such as heat, steam, and pressure; with chemicals such as acids, alkalis, and NH3; with biological agents such as white rot fungi; via natural selection, breeding, or molecular engineering and enzyme technology.

Dai (2007) mentioned that, enzymes are at the core of biological treatments used to reduce lignin or liberate carbohydrates in straw. Enzymes of have been used alone () or in combination with physical and/or chemical treatments (Pedersen *et al*., 2010). Enzymes used in fiber digestion are series of endo and exo-enzymes, which were usually originated from either fungi (Trichoderma, Aspergillus, etc.) or bacteria (Bacillus sp.) (Ghorai *et al*., 2009; Nagaraja ,2012).

The EFE, like other feed enzymes, are of natural origin and non-toxic. Graminha *et al* (2008) reported that, EFE are mostly commercial products of microbial fermentation of *Trichoderma* and *Aspergillus* on safe, simple and inexpensive solid agricultural and agro industrial residues . These organisms are generally recognized as safe and are therefore non toxic, non pathogenic and do not produce antibiotics. These enzymes are often used at low concentrations and are easy to apply to feed.

2.2. Role of Exogenous Fibrolytic Enzymes

The challenge in successful farming with ruminants, be it dairy cattle, beef cattle or sheep and goats lies in the effective utilization of feed resources, as feeding costs present the largest component of production costs. Of the feeds typically utilized, forage composes the largest part and hence presents a logical area of research for the improvement there of. Exogenous fibrolytic enzymes present one way of improving fibre digestibility. Enzymes are proteins that increase the rates of chemical reactions (Cammack, 2006). In enzymatic reactions, the molecules at the beginning of the process, called substrates, are converted into different molecules, called products.

Beauchemin *et al* (2013) stated that methods that increase fiber digestion are likely to play a role in improving energy availability of ruminant diets and reducing feed costs, as forage digestibility continues to limit the intake of available energy by ruminants, and correspondingly, contributes to excessive nutrient excretion by livestock. Increase in feed prices, especially grains, and declines in enzyme costs have prompted interest in using enzyme additives in ruminant diets to increase nutrient utilization and assure the sustainability of animal production activity (Beauchemin *et al*., 2008).

Enzymes can enhance feed utilization use by increasing the rate and extent of pre-ingestive, ruminal, and postruminal fiber hydrolysis, digestion, and degradation, by increasing the ruminal passage rate, by increasing ruminal microbial numbers and/or attachment, by stimulating ruminal microbes, and by decreasing digestive fluid viscosity (Adesogan et al., 2014). Plant cell wall digestion is complex as its three major polysaccharides building components cellulose, hemicellulose and pectin are cross-linked with lignin, a polyphenolic macromolecule strongly resistant to chemical and biological degradation (Glass *et al*. 2013). Plant cell walls are also linked with enzymes, structural proteins, and proteoglycans, forming an intricately linked network that provides strength and durability to its structure (Popper *et al*., 2011). Therefore, numerous enzymes are required in the process of plant cell wall digestion. The use of exogenous fibrolytic enzymes (EFE) is an alternative to increase the ruminal hydrolysis of cell wall contents due to increase in the microbial binding capacity to digest, stimulation of microbial populations, and the synergy between the enzymes synthesized in the rumen and polysaccharides contained in such products.

2.3. Types, Sources and Extraction of Enzymes

Exogenous enzymes which use in ruminant nutrition can be characterized into three main categories as fibrolytic, amylolytic and proteolytic enzymes. In additional to major categories of enzymes, phytase which is extensively used in monogastric feeding is also becoming popular in ruminant feeding. As described by Zhang and Lynd, (2004), exogenous fibrolytic enzymes can be classified further based on their specific activity as cellulase, which hydrolyze the fiber of plant cell wall to glucose, cellobiose or cellooligosaccharides with combined activity of three enzymes namely endoglucanase, exoglucanases and $-glucosidase. Xylanase, that catalyzes the hydrolysis of 1,4-beta-D-xylosidic linkages in xylans that are constituents of hemicellulose, a structural component of plant cell walls.

Enzyme products are derived primarily from four bacterial (*Bacillus subtilis*, *Lactobacillus acidophilus*, *L. plantarum* and *Streptococcus faecium*, spp.), three fungal (*Aspergillus oryzae*,*Trichoderma reesei* and

Saccharomyces cerevisiae) species and some yeasts. 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 (Subramaniyam and Vimala, 2012) and often less complex than bacterial cellulase and easy for extraction and purification. *Trichoderma reesei* is the main microbe used commercially to produce large quantities of cellulases and hemicellulases (Paloheimo *et al*., 2010); however, it exhibits maximum cellulose degradation efficiency at pH 5 (Adav *et al*., 2011; Glass *et al*., 2013). Therefore, alternative microorganisms that secrete copious quantities of cellulase with high degradation efficiency under ruminal conditions are needed (Adesogan *et al*., 2014).

However, with the refining knowledge on microbiology the isolation and characterization of novel cellulase from bacteria are now becoming more popular. Behind this high acceptance there are several reasons like (1) Bacteria often have a higher growth rate than fungi allowing for higher recombinant production of enzymes, (2) Bacterial cellulases are often more complex and are in multi-enzyme complexes providing increased function and synergy and (3) Bacteria inhabit a wide variety of environmental and industrial niches like thermophilic or psychrophilic, alkaliphilic or acidophilic and halophilic strains (Immanuel *et al*., 2006). Large scale production of exogenous enzymes combines the disciplines of microbiology, genetics, biochemistry and engineering with the basic principle, fermentation (Sadhu and Maiti, 2013). . Enzyme preparations for ruminants are produced through microbial fermentation, beginning with seed culture and growth media. Once the fermentation is complete, the enzyme protein is separated from the fermentation residues and source organism. Although the microorganisms from which the enzymes are derived only constitute a very limited group, 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 (Meale *et al*., 2014).

Fermentation methods are divided into two categories as Solid State Fermentation (SSF) and Submerged Fermentation (SmF) (Murad and Azzaz, 2010).

The SSF is the cultivation of microorganisms on moist solid substrates, like bran, bagasse, paddy straw and other agricultural waste and paper pulp and SMF utilizes free flowing liquid substrates, such as molasses and broth (Subramaniyam and Vimala, 2012). The SSF is best suited for fermentation techniques involving fungi and microorganisms that require less moisture content while SmF is commonly practiced with microorganisms such as bacteria that require high moisture content. Approximately 90% of the commercial enzymes are produced by SmF method as the method allows better control of the conditions during fermentation. The SSF method develops a tight contact with the insoluble substrate therefore achieving higher substrate concentration for fermentation. Since SSF involves relatively little liquid when compared with SmF, downstream processing from SSF is theoretically simpler and less expensive

Table 1: Cellulase and xylanase producing microorganisms and optimum conditions for the production (Source :- Sadhu and Maiti, 2013) .

Enzyme and microorganism	Optimum PH	Optimum temperature (C^0
Cellulase	6.1	55
Bacillus licheniformis		
Bacillus sp(alkalophilic)	9.0	-
Bacillus subtilis	5.5	60
Cellulomonas uda	5.5-6.5	45-50
Cell vibrio gilvus	7.6	<40
Thermomonosphoras fusca	6.0	74
Microbispora fusca	5.5-7.2	-
Pseudomonas fluorescens	7.0	35
Bacteriodes cellulosolvens	6.4	39
Zylanase	7.0	30
Penicillium canescens		
Streptomyces spp.	7.2	28
Thermomyces lanuginus	6.0	40
Acremonium furcatum	-	30
Aspergillus niger	5.0	28
Cochliobolus sativus	4.5	30

2.4. Method of Application of Enzymes

Wallace *et al*., (2001), stated that, the beneficial impact of addition of EFE depends on several factors such as diet composition, type of enzyme preparation, enzyme stability, specific enzyme activities, amount of enzyme added and application method. There are several enzyme application methods widely used but the most effective method is yet to be recognized. The application methods vary from a pretreatment of the feed for a period of time before feeding (e.g., silage making, forage harvesting) to application at the time of feeding (application to the hay, in Totally Mixed Rations (TMR), concentrate), even the direct application to the rumen. As enzyme activity strictly depends on the type of feed the enzyme-feed specificity should be given a special consideration when selecting an appropriate method (Hvelplund *et al*.2009). Different domestic ruminants at various stages of production have been used. Various types of forages have been fed, and the enzyme products in those studies were given to the animals in diverse ways at the time of feeding; sprayed onto forage, added to concentrate, sprayed onto the total mixed ration (TMR), added as dry powder to feed, or ruminally infused (Beauchemin and Holtshausen, 2010).The DM degradation was increased when enzymes were pre-incubated with the forage. These findings highlighted the importance of adsorption and binding of the enzyme to substrate before feeding to allow proper attachment and protection against degradation by rumen proteases and creating a stable enzyme–feed complex (Elwakeel et al., 2007). Because enzymes did not impact DMI, they are not responsible for ruminal pH differences reported by Krause and Oetzel (2006).

The close association of enzymes with feed may enable some form of pre- ingestive attack of the enzymes upon the plant fiber and/or enhance binding of the enzymes to the feed, thereby increasing the resistance of the enzymes to proteolysis in the rumen. . Exogenous enzymes may be expected to be more effective when applied to high-moisture feeds (such as silages) compared to dry feeds because of the higher moisture content. Exogenous fibrolytic enzymes have been evaluated to improve fiber digestibility in diets and feedstuffs for ruminants (Elwakeel et al., 2007 , Pinos-Rodríguez et al., 2008 and Reddish and Kung, 2007), using different application procedures to enhance the action mechanism of enzymes in these feeds (and Adesogan, 2005). Enzymes on dry form have been solubilized in water and sprayed directly onto feeds before feeding (Colombatto et al., 2003), applied in a liquid form directly onto the feed (Beauchemin et al., 2004) or as a dry additive directly onto the feed (Pinos-Rodríguez et al., 2005 and Reddish and Kung, 2007).

2.5. Enzyme Application at Ensiling

Enzyme application at ensiling is practically attractive because uniform distribution throughout the forage is ensured when enzymes are applied using properly calibrated sprayers on forage harvesters. If effective, such enzymes should hydrolyze plant cell walls into simple sugars that can be used as fermentable substrates by homolactic bacteria. Several silage additives contain a mixture of inoculant bacteria and fibrolytic enzymes in order to ensure that sufficient homofermentative bacteria are available to utilize the sugars released by enzyme action and dominate the fermentation. Several studies have also demonstrated that enzyme application especially in the presence of microbial inoculants improves the fermentation of tropical grasses (Adesogan et al., 2004), and wheat silage.Although some studies have also shown that enzyme application improves the fermentation of corn silage (Colombatto et al., 2004). Fibrolytic enzymes are often used in silages to enhance the degradation of plant cell walls carbohydrates to fermentable sugars, which could be used by LAB during ensiling (Zhang *et al*. 2010; Yu *et al*. 2011). Fibrolytic enzymes are often used in silages to enhance the degradation of plant cell walls carbohydrates to fermentable sugars, which could be used by LAB during ensiling (Zhang *et al*. 2010; Yu *et al*. 2011).

Clearly enzyme application at ensiling to forage containing low sugar contents is logical because of potential sugar release from enzymeinduced fibrolysis, but the response depends on the enzyme activities and treatment conditions (Adesogan, 2005). It was reported that enzyme application could increase milk production in cows fed total mixed rations based on alfalfa and corn silage (Beauchemin *et al.*, 2007), positive responses in milk production (68 +/- 31 d in milk, 46.9 +/- 9.1 kg of milk/d) were highly dependent on the level of enzyme applied (Kung *et al.*, 2009; Bilik and Lopuszanska-Rusek, 2010).

2.6. Enzyme Application at Feeding.

Enzyme application to diets at feeding is attractive because the fermentable substrates released by enzyme action can be directly fermented by ruminal bacteria, thereby releasing energy for the host animal. However care is needed to ensure an even distribution of the small quantity of enzyme that is typically added. Nevertheless, several studies have demonstrated that enzyme application at feeding improves milk production in dairy cows and improves average daily gain in beef cattle (Adapted from Kung (2001b) and (Colombatto and Adesogan, 2005)).

Table 2: Summary tables on enzyme types, Application methods and main effects.

Researchers	**Enzyme description**	**Pre incubation enzyme substrate interaction**	**Substrate /feed**	**Animal studied**	**Digestion effects reported**	**Production effect and general comments**
Alvared et al.,2009	Zylanases (43.4lu) Cellulase(31.0lu)	24h	Higher fibre diet (>500g/kg DMNDF)	Steers	Insacco Increased DM and CP fraction .a. No effect on DM b,or c.Icreased CP ,c	No effects reported on DMI,ADG,or Feed conversion Pre incubation effects suggested

Alvared et al.,2009	Xylanase(43.4lu) Cellulases(31.0lu)	24h	Wheat middling and oat straw	Steers	Increased disappearance of ADF (wheat middling) ,and NDF (oat)	Pre incubatede effect suggest
Bala et al.,2009	Cellulase Xylanase		Enzyme added to concentrate fed at 500g/d	Lactating crossbred Beetle-sannen goats	Increased digestibility of DM,OM,CP,ADF,ncreased microbial protein	Improved milk yield, fat, soluble non fat (SNF) improved BWT
Dean et al.,2008	Commercial enzymes: Protaese, Biocellulase X-20 CA and Biocellulase A-20	Substrate treated with 3 week pirior to feeding and stored in plastic containers	Tropical grass; Coastal Bermuda grass hay and Pensacola bahiagrass hay	Buffered rumen fluid from Lactating cows	x-20 and A-20 resulted in reduced fibre concentrations ,increased initial (a),and letter phases (48 hr) of IVDM. extent of digestion	Higher application rates increased effects Amoniation yielded Superior result to enz. Treated
Eun et al.,2007 a	Virous EFE (Endoglucanase and zylanase)	Pre-incubation allowed	Lucern hay ,corn silage	Buffered rumen fluid of lactating holetein cows	Improved NDF degradability of 20 and 60 % for lucern hay and corn silage .respectively.	Superior results were obtained with the optimum dose rate

Re Searchers	Enzyme description	Pre incubation enzyme substrate interraction	Substrate /feed	Animal studied	Digestion effects reported	Production effect and general comments
Kruwagen andvanzyl ,2008	Cyophilized and fresh fibrolytic enzyme	None	High 920g/kg DM forage and low 600 g/kg DM forage diets	Dehne merino lamb	-	Improved BWT gains, improved feed conversion ratio. Fresh enzyme cocktail were reported to be superior
Giraldo et al,2008	Fibrolytice containing endoglucanose and zylanase from trichodema langibranchiatum	70 grass :30 concentrate	0h	sheep	Increased ruminally insoluble potential degradable fraction .Dm and fractional rate of degradation. Increased propionate and decreased ace:pro	Positive effects obtained without an enzyme substrate interaction
Girado et al .,2008b	Fibrolytic enzymes from	24 hrs	700,500 and 300g	Buffered rumen	Increased true digestibility of	Reported effects were the greatest at

trichoderma viride ,Aspergillus	forage /kg diet forge /kg diet DM.Grass hay	fluid from merino sheep in Rusitec fermentors	substrate DM, Increased total VFA ,acetate and propionate	8hrs incubation

2.7. Production Responses in Ruminants

Apart from fibrolytic enzymes there is evidence that exogenous proteolytic enzyme could increase the total tract digestibilities of DM, Organic Matter (OM), Acid Detergent Fiber (ADF) and NDF with larger increases in digestibility of cows though the feeding of proteolytic enzyme unexpectedly decreased feed intake of cows (Eun and Beauchemin, 2005).

Dairy cattle :- From a study done by Mohamed *et al*. (2013) they found that supplementation of early lactating dairy cow diet with fibrolytic enzymes (Enzyme was added to the TMR at the time of feeding), did not cause any significant changes in dry matter intake. But with the supplementation of exogenous fibrolytic enzymes milk yield was improved significantly ($p<0.003$) (41.0 vs. 39.5 kg/cow/day) compared to untreated dairy cows. In addition, the energy corrected milk (40.6 vs. 39.4 kg) and feed efficiency in early lactating dairy cows were improved significantly 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 cellulase enzyme supplementation, respectively.

A recent study by Holtshausen *et al*. (2011) screened five doses of a fibrolytic enzyme additive (AB Vista, Marlborough, UK), and further assessed its efficacy *in situ* before the enzyme additive was fed to lactating Holstein dairy cows. The enzyme product improved fat corrected milk production efficiency in a dose dependent manner up to 11.3%; however, DMI decreased. Similarly, Arriola et al. (2011a) screened varying amounts of a fibrolytic enzyme product *in situ* before conducting a feeding trial. Milk production efficiency was increased in cows fed this enzyme product with a low-concentrate diet as compared with those fed either an untreated low-concentrate diet or a high-concentrate diet (treated or untreated). Therefore, it is evident that careful attention needs to be paid to the type and dose of enzymes being applied to dairy cattle diets (Meale et al., 2014).

Supplementing dairy cow diets with exogenous fibrolytic enzymes (EFE) has the potential to improve plant cell wall digestibility and therefore, the efficiency of feed utilization (Meale et al., 2014). Several studies with early lactation cows (<100 days in milk [DIM]) reported a significant higher milk performance due to EFE supplementation (Gado et al., 2009). Other feeding trials with early lactation cows did not find significant effects of EFE supplementation on milk yield (Arriola et al., 2011, Elwakeel et al., 2007, Holtshausen et al., 2011, Miller et al., 2008c). Inconsistencies of results may be due to differences in energy status of experimental cows, diet composition, type and activity of enzyme used, and method of application (Adesogan et al., 2014 and Beauchemin and Holtshausen, 2010). Only a few studies using mid-lactation cows reported significant but lower effects of EFE supplementation on milk yield.

B. *Beef cattle:* Some recent research have assayed the effect of fibrolytic enzyme products in high forage diets fed to growing cattle and steers (Cranston and Krehbiel, 2007; Krueger *et al.*, 2008).
The ultimate goal of using enzymes in beef cattle feed is to increase ADG and feed conversion efficiency. Although responses to exogenous enzymes are expected to be greater in beef cattle fed roughage-based diets as compared with high-grain diets, many exogenous enzyme formulations have shown promising effects in cattle fed barley-based finishing diets (Beauchemin and Holtshausen, 2010). Vargas *et al*. (2013) conducted a research to determine the impact of different levels of a fibrolytic enzyme in a finishing diet on steer performance and carcass characteristics.
Sheep: Gomaa *et al*. (2012) utilized exogenous anaerobic bacterial enzyme in a different way by growing green barley on enzyme treated rice straw and then by feeding to Ossimi sheep to investigate digestibility parameters. Adding enzyme rice straw grown barley significantly increased ($p<0.05$) TDN%, digestibility coefficients, ruminal ammonia-N, total volatile fatty plasma total protein values. Another study was conducted (Arce-Cervantes *et al*., 2013) by supplementing a Lignocellulolytic Extract (LE), extracted from the thermo-tolerant

basidiomycete to observe the effects on the intake, digestibility, feed efficiency, growth and productive performance of lambs. Average daily gain and digestibility were improved ($p<0.05$) with LE supplementation (60 and/or 120 mL). Improved cellulase activity in the rumen and a reduction of butyric acid were observed.
Titi and Lubbadeh (2004) conducted another research with the main objective of investigating the influence of fibrolytic enzyme treatment on birth weight of lambs and they found that birth weight was not significantly affected but the weaning weights of lambs were increased ($p<0.05$).
Mota *et al*. (2011) conducted a study to evaluate the effect of exogenous enzyme, glucoamylase from *Aspergillus niger* addition in finishing diets for lambs. The average daily gain (kg), intake (kg dayG1) and were not affected by the treatments.Supplementation of exogenous fibrolytic enzyme in sheep diet did not change the *in situ* disappearances of DM, NDF and CP of the roughage mixture but the *in situ* Microbial Protein Synthesis (MPS) was increased significantly (Van de Vyver and Useni, 2012). Enhanced TDN, CP digestibility with normal blood and rumen parameters in sheep were observed by Gomaa *et al*. (2012) after treating rations with exogenous enzymes. As suggested by the study of Avellaneda *et al*. (2009) fibrolytic enzymes reduced duodenal ADF flow and forestomach digestion and there were no enzyme effects on DMI, N balance, ruminal degradation, total tract digestion, ruminal fermentation, as well as ruminal protozoal counts.
Goat: Supplementing dairy goat concentrate with a fibrolytic enzyme mixture enhanced DM and OM *in vivo* total tract digestibility (Gonzalez *et al*., 2008). A study by Hussain *et al*. (2014) witnessed that the enzyme supplementation with the TMR resulted in 31.25% increase in net profit by improving the average daily weight gain significantly (83.49 g in treated group compared with 68.33 g in control) and by a non-significant ($p = 0.2875$) reduction of feed.

Table 3: livestock species wise response for the exogenous enzyme supplementation.

Type of enzyme	Method of application	Response	Reference
Dairy cattle Fibrolytic (Cellulase and Zylanase) phytase	Added to TMR,Concentrate, At ensiling, at feeding ,added to diet at time feeding	DM digestibility*,Fecal DM, NDF* ADF*,N and P* Apparent digestibility of DM*,ADF* and NDF*	Dean et al .(2007) Knowlton *et al* .(2007)
NM Fibrolytic	NM Added to TMR	Milkyield*,and composition* AFA*,NH3*,protozoa Bacteria*methanogens and CH4	Diler *et al* .(2014) Chung *et al* .(2012)
Fibrolytic Fibrolytic	NM Added to TMR	DMI*,milk yield*, DMI*,milk yield	Holtshausen et al. (2011) Lopuszanska-Ruseka and Bilik (2011)
Fibrolytic proteolytic	Nm Added to Pelleted supplement	DMI*,Milk yield*,and composition *,tota ltract digestibility of DM*,OM*,NDF*, ADF* FI*,Milk protein * ,Milk fat* and lactose	Elwakeel *et al*.(2007) Eun and Beauchemin (2005)
Beef cattle fibrolytic	Sprayed to forage at harvesting ,bailing or before feeding	Digestibility of DM*,NDF*,Final BW*,ADG*	Kruger *et al*.(2008)
Fibrolytic fibrolytic Sheep fibrolytic	Added to TMR Added to TMR Sprayed to forage 1h before feeding, Added to concentrate	Hotcarcass yield*,DMI*,BW*,ADG*,Digestiblity of DM*,CP*ADF*NDF* ,DWG*,AWG* ADG*,digestibility *,butric acid *, and rumen cellulose activity*,live weight*,Chemical composition*hot carcass weight*.	Vargas *et al*.((2013) Vera et al.(2012) Arce –Cervante et al (2013) Cayetano *et al*.(2012)
Goat Fibrolytic, proteolytic Fibrolytic	Added to concentrate Added to concentrate	Nutrient digestibility*,digestibility of DM*,OM*,CP*,NDF and ADF	Selame *et al*.(2012) Bala et al .(2009)

3. CONCLUSION

Ruminant production systems throughout the world are based on available natural pastures and harvest crop residues. These are of poor nutritive value as they consist of highly lignified stems specially in tropical areas and its utilization is limited by low quality (high fiber and low energy contents) and lack of the constant supply of grasses and legumes. Increasing the efficiency with which forage is digested by the ruminal micro-organisms has been the subject of extensive investigations for over a century. Forage digestibility has been improved by several biotechnological products: among this exogenous enzyme is the most important one. The use of enzymes has a positive relation with palatability, feed intake, rumen microbial N synthesis, digestibility, and improves animal performance as milk production, live weight gain, feed efficiency and immunity . In the past two decades, the application of exogenous fibrolytic enzymes (EFE) has demonstrated to have the potential to increase forage utilization by rumen microbes and improve production efficiency Enzyme products are derived primarily from four bacterial (*Bacillus subtilis*, *Lactobacillus acidophilus*, *L. plantarum* and *Streptococcus faecium*, spp.), three fungal (*Aspergillus oryzae*, *Trichoderma reesei* and *Saccharomyces cerevisiae*) species and some yeasts. Exogenous enzymes which use in ruminant nutrition can be characterized into three main categories as fibrolytic, amylolytic and proteolytic enzymes.

Exogenous fibrolytic enzymes can be classified further based on their specific activity as cellulase, and zylanase. The beneficial impact of addition of EFE depends on several factors such as diet composition, type of enzyme preparation, enzyme stability, specific enzyme activities, amount of enzyme added and application method.Enzymes have been applied to TMR, hay, ensiled forages, concentrate, supplement, or premix and Exogenous enzymes may be expected to be more effective when applied to high-moisture feeds (such as silages) compared to dry feeds because of the higher moisture content. Enzyme application to diets at feeding is attractive because the fermentable substrates released by enzyme action can be directly fermented by ruminal bacteria, thereby releasing energy for the host animal. However care is needed to ensure an even distribution of the small quantity of enzyme that is typically added.

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