



Improved dairy production through enzyme supplementation

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ABSTRACT

The rumen ecosystem has the ability to transform low grade nutrients to high quality products owing to the numerous micro-flora colonies it harbours which produce different types of degrading enzymes. It has been assumed that normal rumen flora is able to digest only a small portion of the cellulosic biomass enteric rumen. This provides numerous opportunities for improving digestion via enhancing digestibility through degradation pathways in rumen. The modern animal nutrition science has utilized this knowledge to commercially harness enzymes for improving nutrient availability for production enhancement. Broadly categorized as fibrolytic, proteolytic and amylolytic, these enzymes act synergistically with the naturally available enzymes in rumen. Enzyme supplementations improve the digestibility of fibre and increase nutrient absorption and energy availability for production activities across physiological status of the animal. This review summaries response of large lactating ruminants to the external enzyme (*in vivo*) supplementation in terms of actual milk production, milk composition, body weights, dry matter intake and digestibility of nutrients, as well as to assess the economic benefit in terms of additional expenses incurred and benefit derived with increase in milk production.

Key words: Amylolytic, Cellulolytic, Enzyme, Solid state fermentation, Xylanolytic

The increased demand of animal products in developing countries requires advanced and scientific approach to increase animal production with available feed resources. Major problem is to fill the gap between the nutrient requirements of an animal and availability of feed, because majority of livestock depend on agriculture by-products which are generally low in protein content, high in crude fibre and having low digestibility coefficients (Kholif *et al.* 2005). The actual conversion of feed/fodder especially forages with high fibre content is not efficient, thereby limiting the overall digestive process in the rumen. Because of incomplete digestion, only 10 to 35% of energy intake is available as net energy (Krause *et al.* 2003). This significantly influences animal performance and cost of production.

Intrinsically, the action of enzymes produced from the buccal cavity and the gastro-intestinal tract help to digest the poor quality fodder, besides the action of ruminal micro-flora. The most inefficient is the digestion of the fibre content of fodder, which can be made more efficient with the application of external enzymes, which help to destroy the linkages between cellulose, hemicellulose and lignin in order to increase the availability of nutrients to the animal. 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 (Tricarico *et al.* 2005, Wang *et al.* 2001, Colombatto *et al.* 2003). Most commercial enzyme products contain more than one active enzymes and are designed to target digestion. These products are blends with varying concentrations of xylanases, glucanases and cellulases, as well as amylases, proteases and lipases.

Source of enzymes

Exogenous enzymes are primarily derived from bacterial and fungal species. List of suitable sources for enzyme production is given in Table 1.

Production techniques

Enzyme products are produced by two fermentation processes:

1. Solid State Fermentation (SSF)
2. Submerged Fermentation (SmF) (Murad and Azzaz 2010)

SSF is best suited for those micro-organisms which require less moisture like fungi, whereas SmF is primarily meant for cultivation of bacteria that require high moisture content. Commercial enzyme production mainly uses SmF method as the method allows better control of the conditions during fermentation (Subramaniyam and Vimala 2012)

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Table 1. Source of enzyme

Type of enzyme	Source	Enzyme	Reference
Cellulolytic	Bacterial	<i>Lactobacillus acidophilus</i>	Sujani and Seresinhe (2015)
		<i>L. plantarum</i>	
Xylanolytic	Bacterial	<i>Bacillus subtilis</i>	Qinnghe <i>et al.</i> (2004)
		<i>Streptococcus faecium</i>	
	Fungal	<i>Ruminococcus Fibrobacteres</i>	Sujani and Seresinhe (2015)
		<i>Aspergillus</i>	
		<i>Trichoderma</i>	
		<i>Trichoderma longibrachiatum</i>	
Amylolytic	Fungal	<i>Trichoderma reesei</i>	Tricarico <i>et al.</i> (2008)
		<i>Aspergillus niger</i>	
		<i>Aspergillus oryzae</i>	

Role in cell wall digestion

The enzymes used in ruminant nutrition are mainly classified into three categories as fibrolytic, amylolytic and proteolytic enzymes.

Fibrolytic enzymes: Exogenous fibrolytic enzymes can be further categorized based on their specific activity, i.e. cellulases and xylanases. Cellulases cause hydrolytic digestion of cellulose, and convert it into primary products such as glucose, cellobiose and cello-oligosaccharides. This conversion is the result of activity of three enzymes namely endo- β -1,4-glucanases, exo- β -1,4-cellobiohydrolases and β -glucosidases. The endo- β -1,4-glucanases, and exo- β -1,4-cellobiohydrolases act together to hydrolyze cellulose into small cello-oligosaccharides. These oligosaccharides (mostly cellobiose) are further hydrolyzed by a core β -glucosidase into glucose (Sukumaran *et al.* 2005). Whereas xylanolytic enzymes group includes endoxylanase, β -xylosidase, α -glucuronidase, α -arabinofuranosidase and acetyl xylan esterase. Xylanases are a group of glycoside hydrolase enzymes which degrade the linear polysaccharide xylan into xylose by catalyzing the hydrolysis of glycosidic linkage (β -1,4) of xylosides.

Amylolytic enzymes: Amylases catalyze hydrolysis of internal α -1,4-glycosidic linkages in starch and convert this complex structure into low molecular weight products, such as glucose, maltose and maltotriose units. Klingerman *et al.* (2009) found increase of 3.9 kg of milk and 3.6 kg of 3.5% FCM/d in lactating HF cattle following supplementation with enzyme having amylolytic activity in TMR. Noziere *et al.* (2014) found that the addition of an exogenous amylase preparation in Corn silage-based diets with 20 or 30% starch improved the ruminal digestibility of starch from 75% to 81%, but total tract digestibility of starch was not altered. Amylase supplementation tends to enhance the total volatile fatty acids concentration in the rumen without affecting production, intake of dry matter

and digestibility of fibre (neutral detergent fibre and acid detergent fibre) or ruminal digestion. Tricarico *et al.* (2005) found increased proportions of acetate and propionate in ruminally fistulated HF steers and lactating dairy cattle supplemented with exogenous α -amylase @ 0, 240, 480, 720 dextrinizing units (DU)/kg of TMR. Enzyme supplementation response is greatly influenced by dose, type of feed, host and management factors (Mendoza *et al.* 2014).

Proteolytic enzymes: These enzymes convert protein into simple amino acids by degradation. These enzymes have ability to target nitrogen cross-linkages in the cell wall of forages and therefore particularly effective in diets having low digestibility. Nitrogen in cell walls accounts for approximately 7 to 11% of total cell wall content (Aufrère 1994). Degradation of cell wall-bound nitrogen provides additional nitrogen to digestible nitrogen pool, resulting in higher production of fermentable organic matter in rumen (Colombatto and Beauchemin 2009). It has also been suggested that the exogenous protease enzyme works synergistically with the endogenous enzymes for enhancing overall feed digestibility (Colombatto *et al.* 2003).

Factors influencing enzyme activity

Level of enzyme incorporation: Positive responses in production are highly dependent on the supplementation level of enzyme (Lewis *et al.* 1999). Yang *et al.* (1998) observed increase in milk production by 0.9 to 2 kg/animal/day with the increase in dose rate of enzyme supplementation @ 1 to 2 g/kg applied on alfalfa cube, respectively. Contrary to this, Kung *et al.* (2000) suggested the maximum response in milk production (39.5 kg/d) with the low level of enzyme application as compared to control (37 kg/d) and those fed the higher level of enzyme (36.2 kg/d), with similar results reported by Beauchemin *et al.* (2000) and Shekar *et al.* (2010). Supplementing enzyme (cellulase and xylanases) at three levels in dairy cattle namely low (1.25 ml/kg of forage DM), medium (2.5 ml/kg of forage DM) and high (5.0 ml/kg of forage DM), Lewis *et al.* (1999) concluded that maximum response is with medium level supplementation as compared to low or high level of supplementation. Several other studies have also shown that over-application of enzymes does not recover the enzyme supplementation cost over and above the improvement in animal performance (Beauchemin *et al.* 1995, McAllister *et al.* 1999).

Method of enzyme application: Enzyme activity is greatly influenced by the type and nature of substrate. The application methods vary from feed treated before feeding (e.g. silage making, forage harvesting) to application of enzyme at the time of feeding with total mixed rations (TMR), application to the hay, concentrate and even direct application into the rumen. The enzyme-feed specificity should be given a special consideration while selecting method of application (Hvelplund *et al.* 2009). Beauchemin *et al.* (1999) reported the method of enzyme application plays important role along with the type of feed. Spraying of exogenous enzyme onto TMR did not affect milk

Table 2. Effect of exogenous enzyme on milk production and composition review

Name of enzyme used (Reference)	Spp.	Rate of inclusion (Method of inclusion)	% Change in milk production	% Changes in milk constituents				Others	Interpretation
				Fat	Protein	Lactose	SNF		
Cellulase and xylanase Gaafar <i>et al.</i> (2010)	Buffalo	8000 U Cellulase and 20000 U Xylanase (Mix with roughage leaving overnight)	15.6	5.4	5.0	6.3	5.3	% change in total solids and ash were 5.39 and 14.28	Milk production and fat % significantly increase in treatment group
Cellulase and xylanase Azam <i>et al.</i> (2017)	Nili Ravi Buffalo	10 g/d/head (with basal diet sorghum forage and conc.)	12.2	0.3	1.5	2.1	2.2	NR	Milk yield was increase with increase enzyme supplement then dropped. Milk composition varies within treatment. But overall remain unaffected
		15 g/d/head (with basal diet sorghum forage and conc.)	17.6	3.5	1.8	1.5	2.5		
		20 g/d/head (with basal diet sorghum forage and conc.)	10.1	-4.7	2.3	2.1	3.4		
Cellulase and xylanase Shekhar <i>et al.</i> (2010)	Murrah Buffalo	T1= 1.5 g/kg (Mix with TMR) T2= 3 g/kg (Mix with TMR)	12.9	1.3	0	0	0.96	Total solids % change were 0.54 and 1.64 in T1 and T2 resp.	Milk production increase significantly with the low dose of enzyme whereas milk composition NS
Cellulase, xylanase, α -amylase and protease EI-Aziz <i>et al.</i> (2012)	Buffalo	(LFE) = 0.89 unit cellulose, 0.058 unit xylanase, 3.39 units α -amylase and 1.56 units proteases (with crushed flax seed diet) (HFE)= 7.05 unit cellulose, 2.32 unit xylanase, 61.5 units α -amylase and 29.2 units proteases (with crushed flax seed diet)	-1.3	9.9	-0.7	2.0	-3.6	% change in total solids were 1.7 and -8.1 in LFE and HFE respectively	NS
Pectinase, cellulase, acidic protease, xylanase, α -amylase, phytase, glucoamylase and cellulose Azzaz <i>et al.</i> (2013)	Buffalo	2 g/head/day (30 units pectinase + 4 units cellulase) (Mix with Conc. feed mixture)	3.7	-2.9	-8.3	-4.3	-11.6		NS
		2 g/head/day (1000 units acidic protease + 30 units pectinase + 25 units xylanase + 20 units α -amylase + 10units phytase + 5units glucoamylase + 4 units cellulase) (Mix with Conc. feed mixture)	5.2	-2.3	-2.1	-3.8	-3.9	% change in total solids were 3.3 and 2.1 in group 1 and 2 respectively	
			5	-4.8	-2.4	-3.6	-0.6		

(Table 2 Contd...)

Name of enzyme used (Reference)	Spp.	Rate of inclusion (Method of inclusion)	% Change in milk production	Changes in milk constituents				Others	Interpretation
				Fat	Protein	Lactose	SNF		
Pectinase, cellulase, acidic protease, xylanase, α -amylase, phytase, glucoamylase and cellulose Morsy <i>et al.</i> (2015)	Egyptian Buffalo	40 g/head/dayVeta-zyme (VET) (<i>Lactobacillus acidophilus</i> , 2000 protease, pectinase, xylanase, 550 α -amylase, phytase, glucoamylase, 400 cellulase units/g	8.9	3.5	4.4	NR	NR	% change in milk casein and milk urea nitrogen was 9.4 <i>vs.</i> 14.9 and -25.9 <i>vs.</i> -25.3 in VET and TOM groups, respectively	Significant increase in actual milk production as well as fat, protein and milk casein content while significantly decrease in milk urea nitrogen
		40 g/head/day Tomoko (TOM) (<i>Aspergillus awamori</i> , 1000 protease, 30 pectinase, 25 xylanase, 20 α -amylase, 10 phytase, 5 glucoamylase, 4 cellulase units/g	4.5	1.6	9.0				
Xylanase Mohamed <i>et al.</i> (2013)	HF Cattle	15g/d/head (Mix with TMR)	3.8	-1.4	1.5	0.6	1.2	NR	Milk production is significantly improve while no change in milk composition was observed
Xylanase Bassiouni <i>et al.</i> (2010)	HF Cattle	1 g/ kg	8.2	3.5	2.2	0.7	1.0	% change in total solids and ash were 1.8 and -1.4 respectively	
Cellulase Bernard <i>et al.</i> (2010)	HF Cattle	4800 units/head/day (corn silage + alfalfa hay)	2.2	-1.6	0	-0.6	NR	NR	NS
		4800 units/head/day (T85 haylage diet)	-1.2	1.4	2.2	0.6			
Cellulase/hemicellulase Titi (2003)	HF Cattle	150 g/tonnes (Forage)	27.2	3.5	10	NR	0.5	% change in total solids were 1.3	Milk production increases significantly, milk composition remain unchanged except protein shows significant increase
Cellulase and xylanase Dehghani <i>et al.</i> (2011)	HF Cattle	2.5 g/kg	3.2	-6.5	2.1	NR	NR	NR	NS
	HF (PHF red)	5.0 g/kg	-3.2	-0.9	1.7				
Cellulase and xylanase Bilik <i>et al.</i> (2009)		15 g/d/head (Mix with Conc. Part of TMR)	7.2	-0.5	4.8	0.4		% decrease in milk solids were -4.4	

(Table 2 Contd...)

Name of enzyme used (Reference)	Spp.	Rate of inclusion (Method of inclusion)	% Change in milk production	% Changes in milk constituents			Others	Interpretation
				Fat	Protein	Lactose		
Cellulase and xylanase Beauchemin <i>et al.</i> (1999)	HF Cattle	2.5 g/kg (Mix with TMR)	3.3	3.2	1.5	0.2	NR	Actual milk production was not affected by supplement whereas 4% FCM milk was significant
Cellulase and xylanase Schingoethe <i>et al.</i> (1999)	HF Cattle	2000 + 7500 units/kg	3.5	2.7	0	NR	NR	Actual milk production not affected while 3.5% FCM was significantly higher because fat% was significantly higher
		2850 + 10700 units/kg	5.9	4.5	0.4			
		4300 + 16050 units/kg	3.8	2.4	0.2			
Cellulase and xylanase Yang <i>et al.</i> (2000)	HF Cattle	50 mg/kg TMR (Sprayed on forage)	-0.3	-5.9	-1.5	-1.9	NR	Overall treatment significantly not affect milk composition
		50 mg/kg conc (Sprayed on conc.)	5.9	-4.4	-1.5	0		
Cellulase and xylanase Reddish <i>et al.</i> (2007)	Dairy Cattle	10 g/d/Cow (Dried enzyme on TMR)	-0.2	4.4	-0.7	0.4	NR	NS
Cellulase and xylanase Dunda (2015)	HF Cattle	300 g/tonne (Mix with conc.)	0.4	1.6	0.3	NR	0.24	Fat and total solids changed significantly
Cellulase and xylanase Lewis <i>et al.</i> (1999)	HF Cattle	1.65 ml/kg (Sprayed on forage)	5.0	4.18	1.53	NR	NR	Improve milk production as well as fat %
Cellulase and xylanase Dean <i>et al.</i> (2013)	HF Cattle	EC=4 g/d/head (Mix with conc.)	-6.6	2.9	5.5	NR	NR	Cow fed ETMR produces more fat as compare to other treatment group but all over composition and milk production remain unaffected
		ETMR= 4 g/d/head (Mix with TMR)	-2.4	8.7	5.5			
		EF= 4g/d/head with forage	-5.7	2.7	4.1			
		ES= 1.3 g/kg DM (Mix with silage)	-2.4	1.3	-0.3			
Cellulase and xylanase Bowman <i>et al.</i> (2002)	HF Cattle	1 g/cow/d of TMR (Conc.)	2.0	2.5	0.5	-0.4	NR	All over milk composition not affected, cow received enzyme with concentrate produced more milk but not significantly change
		1 g/cow/d of TMR (Pelleted Supplement)	-2.0	3.8	0.8	-0.6		Milk protein and fat decrease with increase in enzyme supplement
		1 g/cow/d of TMR (Premix)	-5.1	6.9	2.5	-1.3		
Cellulase and xylanase Kung <i>et al.</i> (2000)	HF Cattle	2 L/tonne (Sprayed on forage portion of TMR)	6.7	3.78	1.6	NR	NR	
		5 L/tonne (Sprayed on forage portion of TMR)	-2.2	-10	-5.7			

(Table 2 Contd...)

Name of enzyme used (Reference)	Spp.	Rate of inclusion (Method of inclusion)	% Change in milk production			% Changes in milk constituents			Others	Interpretation
			Fat	Protein	Lactose	Fat	Protein	Lactose		
Cellulase and xylanase Yang <i>et al.</i> (1999)	HF Cattle	1 g/kg LH (Alfalfa hay)	3.8	4.4	1.1	NR	NR	NR	NR	No change in fat%, whereas lactose and protein increases significantly. Milk production significantly influence by enzyme addition
		2 g/kg HH (Alfalfa hay)	8.0	3.6	0.8					
		1 g/kg HT (Alfalfa hay+conc)	6.8	3.9	1.3					
Cellulase and xylanase Rode <i>et al.</i> (1999)	HF Cattle	1.3 g/kg (Mix with TMR)	10.0	-6.5	-2.3	NR	NR	NR	NR	Enzyme supplementation have potential to improve milk production because enhances nutrient digestibility but need to adjust to avoid fat depression NS
Cellulase and xylanase Lopuszanka and Bilik (2011)	HF (PHF red)	15 g/d/head (Mix with TMR)	7.6	4.9	-0.4	NR			% change in milk casein and total solids were 5.6 and 2.4, respectively	NS
Cellulase and xylanase Bordeny <i>et al.</i> (2015)	HF Cattle	15 g/d/head (Mix with TMR)	11.3	2.6	1.2	1.05			% change in total solids were 5.13	Actual milk production and Fat % change significantly rest are remain unaffected NS
Cellulase and xylanase Silva <i>et al.</i> (2015)	HF Cattle	8 g/head/day (mix with TMR) 16 g/head/day (mix with TMR) 24 g/head/day (mix with TMR)	-0.6 0.12 -1.3	0.32 0 0.3	0.4 0 0.2	NR	NR	NR	NR	Enzyme supplement did not significantly improve the milk production but toward higher side in low conc. diet Milk yield is significantly improved. However, increased milk yield not accompanied by increase milk component Overall composition and milk yields remain unaffected NS
Cellulase, xylanase and esterase Arriola <i>et al.</i> (2011)	HF Cattle	3.4 mg/g of TMR (Low Conc diet) 3.4 mg/g of TMR (High Conc. diet)	-0.6 -3.9	2.2 -0.7	NR	NR			NR	
Cellulase, xylanase, protease, amylase Gado <i>et al.</i> (2009)	Brown Swiss Cattle	40 g/d/head (TMR)	22.6	2.9	0	NR			NR	
Cellulase, xylanase, and β -Glucanase Elwakeel <i>et al.</i> (2007) Xylanase and Endoglucanase Holtshausen <i>et al.</i> (2011) Xylanase and endoglucanase Miller <i>et al.</i> (2008)	HF Cattle	5 g/d/head (TMR)	0.7	2.3	0	2.7	1.9	NR	NR	Overall enzyme did not affect the milk production and composition
		10 g/d/head (TMR)	-1.4	-1.6	-1.2	2.1	1.0			
	HF Cattle	15 g/d/head (TMR)	0.2	-3.1	0	1.9	1.2			
		0.5 mL/kg (TMR)	0.5	-3.0	2.0	0	NR	NR		
		1 mL/kg (TMR)	-0.5	-0.9	2.7	0.43				
Xylanase and endoglucanase Chung <i>et al.</i> (2012)	HF Cattle	Low = 2.15 ml/kg (with barley grain conc.) High = 4.30 ml/kg (with barley grain conc.) Low = 2.15 ml/kg (with sorghum grain conc.) High = 4.30 ml/kg (with sorghum grain conc.) 0.5 ml/kg (mix with TMR) 1 ml/kg (mix with TMR)	-1.0 -4.2 -1.4 2.8 1.3 0.8	1.5 -1.7 0 -5.0 NR	0.6 0 0.6 0 NR	-0.4 -0.8 0.4 0.60 NR	NR	NR	NR	NR

Table 3. Effect of enzyme supplementation on body weight

Name of fibrolytic enzyme used	Spp.	Rate of inclusion (method of inclusion)	% Change in Body weight	Interpretation
Cellulase and xylanase Shekhar <i>et al.</i> (2010)	Murrah buffalo	T1=1.5 g/kg (Mix with TMR)	0.5	NS
Cellulase and xylanase Dean <i>et al.</i> (2013)	HF Cattle	T2=3 g/kg (Mix with TMR)	-0.5	
		EC=4 g/d/ head (Mix with conc.)	0.31	NS
		ETMR=4 g/d/ head (Mix with TMR)	-1.4	
		EF=4 g/d/ head (Mix with forage)	-2.3	
		ES=1.3 g/kg DM (Mix with silage)	-1.7	
Cellulase and xylanase Lopuszanka and Bilik (2011)	HF (PHF red)	15 g/d/head (Mix with TMR)	-0.3	NS
Cellulase and xylanase Bilik <i>et al.</i> (2009)	HF (PHF red)	15 g/d/head (Mix with Conc. Part of TMR)	0.18	NS
Cellulase and xylanase Yang <i>et al.</i> (1999)	HF Cattle	1 g/kg LH (Alfalfa hay)	0.3	NS
		2 g/kg HH (Alfalfa hay)	0.8	
		1 g/kg HT (Alfalfa Hay + Conc)	-0.3	
Cellulase and xylanase Kung <i>et al.</i> (2000)	HF Cattle	2 L/tonne (Sprayed on forage portion of TMR)	1.5	NS
		5 L/tonne (Sprayed on forage portion of TMR)	-2	
Cellulase and xylanase Rode <i>et al.</i> (1999)	HF Cattle	1.3 g/kg (Mix with TMR)	-4.8	NS
Cellulase, xylanase and esterase Arriola <i>et al.</i> (2011)	HF Cattle of TMR (Low Conc. diet)	3.4 mg/g	-0.5	NS
		3.4 mg/g of TMR (High Conc. diet)	-3.9	
Endoglucanase and xylanase Holtshausen <i>et al.</i> (2011)	HF Cattle	0.5 mL/kg (TMR)	1.5	Significantly decrease
		1 mL/kg (TMR)	-0.3	
Phytase and cellulose Knowlton <i>et al.</i> (2007)	HF Cattle	297 g/tonne (Alfalfa silage)	0.9	NS
β -glucanase, xylanase, and endocellulase Beauchemin <i>et al.</i> (2000)	Dairy Cattle	1.22 L/tonne (TMR)	0.5	NS
		3.67 L/tonne (TMR)	0.3	

+, Increase; -, Decrease; NR, Not reported; NS, Non-significant

production while application on to concentrate achieved increase in milk production by 4 kg/d. This matches the results of an earlier study by Kung (1996) who found spraying two similar enzyme preparations onto corn silage in a 50% concentrate diet increased milk production by 2.5 kg/d without altering milk composition. Similarly, Yang *et al.* (2000) reported increase in milk production with the concentrate feeding (37.4 kg/d) as compared to TMR (35.2 kg/d), when similar dose enzyme (50 mg/kg DM) was applied to concentrate and TMR ration. Sutton *et al.* (2003) reported favorable responses are towards higher side when enzyme is sprayed @ 2 kg/1000 kg TMR, probable explanation to this is greater opportunity for enzyme-induced fibre hydrolysis when enzyme is applied directly in TMR when applied to the concentrate or infused directly into the rumen.

Direct infusion of enzyme into the rumen was reported to be ineffective (McAllister *et al.* 1999), while contrary reports of increased fibrolytic activity in ruminal fluid are also available (Giraldo *et al.* 2008).

Although the enzyme did not affect the total

concentration of Volatile Fatty Acids (VFAs) in ruminal fluid significantly, but the molar proportions of propionate did increase and the ratio of acetate : propionate decreased. Beauchemin *et al.* (2000) also found that total VFA concentration was not affected by fibrolytic enzyme supplementation. The proportion of acetate was higher in cows fed on low levels of fibrolytic enzyme compared with control, while the acetate proportion was intermediate for cows fed on high concentration of fibrolytic enzyme. This suggests change in the ruminal population of bacteria due to enzyme supplementation. Interestingly for same supplementation methods, Lewis *et al.* (1999) and Hristov *et al.* (2008) found opposite results.

Application of enzyme preparations on fresh forage have also been found to be less effective (Feng *et al.* 1996) due to increased passage rate (Beauchemin *et al.* 2003) and inhibitory compounds (Nsereko *et al.* 2000). Yang *et al.* (1999) reported that the response of enzyme feeding is best when enzyme is applied on dry feeds including hay and grain. Further, Yang *et al.* (2000) reported that addition of enzyme to concentrate mixture one month before feeding

increases milk production and feed digestibility in dairy cattle. Similarly, researchers reported pre-treatment of feed with enzyme results in improved performance of large dairy animal (Wang *et al.* 2001, Giraldo *et al.* 2004). Enzyme application at the time of harvesting of Bermuda grass hay improves digestibility as well as intake of crude protein (CP) and dry matter (DM) (Krueger *et al.* 2008). Above studies suggest that addition of enzyme prior to feeding improves the fibre digestibility by altering the structure and making it more susceptible for degradation.

Researchers also reported that addition of exogenous enzymes to the high moisture feed is more advantageous as the high water content favours enzymatic hydrolysis of cell wall (Beauchemin *et al.* 1999). Similar reports suggest that the application of enzyme during ensiling improves fermentative and nutritive values of corn and Bermuda grass silage (Colombatto *et al.* 2003, Dean *et al.* 2005). However, Hvelplund *et al.* (2009) reported increase in NDF content in enzyme treated silage results in decrease in digestibility by 14 and 19% units, as compared to un-treated silage. These variability's in results warrant further examination of application methods in relation to efficacy of production.

Possible mode of action

The important mechanism of action in application of exogenous enzymes is the release of reducing sugars from feed ingredients (Beauchemin *et al.* 1995). Forsberg *et al.* (2000) reported that release of carbohydrates provides sufficient additional available carbohydrates to encourage rapid microbial growth and shortening the lag time required for microbial colonization on the substrate.

The enzyme supplementation in cattle feed may exert its effect in the following ways:

- Increase the number of bacterial colonies as a result, hydrolytic capacity of the rumen increases (Yang *et al.* 1999, Morgavi *et al.* 2000a, Wang *et al.* 2001) which is directly proportional to *in situ* dry matter disappearance rate, when alfalfa cubes treated with exogenous enzyme prior to consumption (Yang *et al.* 1998)
- Exogenous enzymes work synergistically with rumen micro-flora (Morgavi *et al.* 2000b).
- Enzyme-mediated reductions in viscosity could improve nutrient absorption in the small intestine of dairy animals. Hristov *et al.* (1998) found that the reduction in enzyme mediated viscosity was associated with 1.2 and 1.5% increase in total tract digestibility of DM, when enzyme was applied to feed or directly infused into abomasum's, respectively.
- Enzymes may also help in decomposition of feces by accelerating the process.

Effect of enzyme addition on animal performance parameters

Effect on milk production and milk constituents: Fortified and supplement diets have major impact on the microflora population in rumen and therefore it is widely accepted that

there will be changes in milk production performance of the animal. Studies have demonstrated dietary addition of enzymes, either to forage or concentrate portion, increased milk production in enzyme treated group as compared to control group. However overall milk composition is not likely to change with enzyme application though changes in some constituents have been reported. Table 2 enlists various studies and the salient findings in this regard.

Above review indicates that milk fat and protein contents are more likely to be affected by the exogenous supplementation of fibrolytic enzymes, because of alteration in ruminal acetate:propionate ratio, which results in increase in fat content of milk (Dean *et al.* 2012). Diler *et al.* (2014) reported that fat % is also affected by milk production; if there is marked increase in milk production the milk fat content decreases (negatively correlated traits) or it may remain unaffected. However, Bordeny *et al.* (2015) found contradictory results, i.e. significant increase in actual milk production with significant increase in milk fat content as well.

An imbalance between RDP (rumen degradable protein) and RUP (rumen undegradable protein) may cause a alter milk protein percentage. If ruminal degradability of feed nitrogen is increased, without a compensatory increase in microbial protein synthesis, it will result in reduced levels of metabolizable protein and absorbed amino acids for the synthesis of milk protein (Eun and Beauchemin 2005). Increase in milk protein content may be ascribed to increased microbial protein synthesis in the rumen by the use of enzyme additive (Zheng *et al.* 2000).

Lactose is the least variable among the milk constituents, though some studies have also reported increase in milk lactose in response to enzyme supplementation, which may be attributed to improved digestibility of nutrients. Specifically, the increase in ruminally fermented OM, which resulted in a numerical downward shift in the ratio of acetate to propionate, would have increased delivery of glucogenic precursors to the mammary gland (Yang *et al.* 1999).

Effect on body weights: Addition of enzyme supplement to the ration of dairy animals did not exert any significant effect on body weights of large ruminants, though some studies have reported contrary findings. Table 3 enlists various studies and the salient findings in this regard.

Supplementation of enzyme to the dairy animals did not affect the body weights significantly, which may be due to the fact that increase in nutrient availability got utilized in milk production rather than body reserve (Lewis *et al.* 1999). Contrary to this, improvement in body weights by enzyme addition may be because of change in ruminal volatile fatty acid proportion which results in increase in adipose tissue lipogenesis (Rode *et al.* 1999).

Effect of enzyme supplement on dry matter intake: Studies show that fortifying feed with exogenous enzyme(s) enhances the nutrient digestibility but not dry matter intake and may/ may not lead to increase in the efficiency of production. Table 4 enlists various studies and the salient findings in this regard.

Table 4. Effect of enzyme on dry matter intake

Name of fibrolytic enzyme used and Reference	Spp.	Rate of inclusion (Method of inclusion)	% Change in DMI	Interpretation
Cellulase and xylanase <i>Azam et al. (2017)</i>	Nili Ravi Buffalo	10/g/d/head (With basal diet and conc.) sorghum forage	3.3	DMI increases with the increase in enzyme supplementation
		15/g/d/head (-do-)	5.5	
		20 g/d/head (-do-)	8.2	
Cellulase and xylanase <i>Shekhar et al. (2010)</i>	Murrah buffalo	T1=1.5 g/kg (Mix with TMR)	6.6	NS
		T2=3 g/kg (Mix with TMR)	-1.2	
Cellulase / hemicellulase <i>Titi (2003)</i>	HF Cattle	150 g/tonne (Forage)	0.1	NS
Protease E <i>Sucu et al. (2014)</i>	HF Cattle	4/g/d/head (Mix with TMR)	-3.7	Significantly decrease
Protease Eun and <i>Beauchemin (2005)</i>	HF Cattle	1.25 mL/kg conc. Portion of TMR (High forage)	-4.6	Significantly decrease
		1.25 mL/kg conc. Portion of TMR (Low forage)	-9.6	
Xylanase <i>Mohamed et al. (2013)</i>	HF Cattle	15/g/d/head (Mix with TMR)	0.2	NS
Xylanase <i>Bassiouni et al. (2010)</i>	HF Cattle	1 g/ kg	1.8	NS
Cellulase <i>Bernard et al. (2010)</i>	HF Cattle	4800 units/head/day (corn silage + alfalfa hay + T85 haylage diet)	-0.4	NS
Cellulase and xylanase <i>Dehghani et al. (2011)</i>	HF Cattle	2.5 g/kg	13.9	DMI is significantly increase in lower dose of enzyme as compared to high dose and control
		5.0 g/kg	7.2	
Cellulase and xylanase <i>Shadmanesh (2014)</i>	HF Cattle	15 g/head/day (Mix with TMR)	0.5	NS
Cellulase and xylanase <i>Dunda (2015)</i>	HF Cattle	300 g/tonne (Mix with conc.)	0.2	NS
Cellulase and xylanase <i>Bordeny et al. (2015)</i>	HF Cattle	15 g/d/head (Mix with TMR)	0.3	NS
Cellulase and xylanase <i>Dean et al. (2013)</i>	HF Cattle	EC=4 g/d/head (Mix with conc.)	3.3	Overall DMI remain unchanged while body weights increases
		ETMR=4 g/d/head (Mix with TMR)	7.1	
		EF=4 g/d/head (Mix with forage)	-4.8	
		ES=1.3 g/kg DM (Mix with silage)	4.3	
Cellulase and xylanase <i>Lopuszanka and Bilik (2011)</i>	HF (PHF red)	15 g/d/head (Mix with TMR)	3.6	NS
Cellulase and xylanase <i>Gaafar et al. (2010)</i>	Buffalo	8000 U Cellulase and 20000 U Xylanase (Mix with roughage leaving overnight)	4.02	Significantly increase
Cellulase and xylanase <i>Reddish et al. (2007)</i>	Dairy Cattle	10 g/d/cow (Dried enzyme on TMR)	-2.5	NS
Cellulase and xylanase <i>Kung et al. (2000)</i>	HF Cattle	2 L/tonnes (Sprayed on forage portion of TMR)	2.3	NS
		5 L/tonne (-do-)	-0.9	
Cellulase and xylanase <i>Sutton et al. (2003)</i>	HF Cattle	2 kg/tonnes (Sprayed on TMR)	2.2	NS
		2 kg/tonnes (Sprayed on Conc.)	0.9	
		2 kg/tonnes (Infuse in rumen)	-1.2	
Cellulase and xylanase <i>Bowman et al. (2002)</i>	HF Cattle	1 g/cow/d of TMR (Conc.)	0.4	NS
		1 g/cow/d of TMR (Pelleted Supplement)	1.7	
		1 g/cow/d of TMR (Premix)	0.4	DMI is not affected wether enzyme added to TMR or conc.
Cellulase and xylanase <i>Yang et al. (2000)</i>	HF Cattle	50 mg/kg TMR (Sprayed on forage)	5.2	
		50 mg/kg conc. (Sprayed on conc.)	2.1	
Cellulase and xylanase <i>Yang et al. (1999)</i>	HF Cattle	1 g/kg LH (Alfalfa hay)	1.47	NS
		2 g/kg HH (Alfalfa hay)	1.47	
		1 g/kg HT (Alfalfa Hay + Conc.)	1.9	

(Table 4 Contd...)

Name of fibrolytic enzyme used and Reference	Spp.	Rate of inclusion (Method of inclusion)	% Change in DMI	Interpretation
Cellulase and xylanase Silva <i>et al.</i> (2015)	HF Cattle	8 g/head/day 16 g/head/day 24 g/head/day	2.6 1.1 3.4	DMI linearly affected by enzyme supplementation
Cellulase and xylanase Lewis <i>et al.</i> (1999)	HF Cattle	1.65 ml/kg (Sprayed on forage)	9.26	DMI numerically increase but not statistically significant
Cellulase and xylanase Rode <i>et al.</i> (1999)	HF Cattle	1.3 g/kg (Mix with TMR)	1.6	NS
Cellulase and xylanase Beauchemin <i>et al.</i> (1999)	HF Cattle	2.5 g/kg (Mix with TMR)	0.9	NS
Cellulase and xylanase Granzin (2005)	HF Cattle	4 g/cow/day (grain-based concentrate) –Primiparous –Multiparous	14.3 6.8	NS, no interaction between feed intake and parity
Cellulase and xylanase Schingoethe <i>et al.</i> (1999)	HF Cattle	2000 + 7500 units/kg 2850 + 10700 units/kg 4300 + 16050 units/kg	3.9 –1.4 8.6	NS
Cellulase, xylanase and esterase Arriola <i>et al.</i> (2011)	HF Cattle	3.4 mg/g of TMR (Low Conc diet) 3.4 mg/g of TMR (High Conc. diet)	–5.9 –7.4	NS
Cellulase, xylanase, and β -Glucanase Elwakeel <i>et al.</i> (2007)	HF Cattle	5 g/d/head (TMR) 10 g/d/head (TMR) 15 g/d/head (TMR)	–0.5 –0.9 –0.2	NS
Cellulase, xylanase and ferulic acid esterase (FAE) Dhiman <i>et al.</i> (2002)	HF Cattle	Cellulase, xylanase @ 1.3 L/t = C (Applied on forage) Cellulase, xylanase @ 0.002 L/t = C–LF (Applied on forage) FAE @ 0.002 L/t = LF (Applied on forage) FAE @ 0.03L/t = HF (Applied on forage)	1.5 –5.1 –2.9 6.6	NS
Cellulase, xylanase, protease, amylase Gado <i>et al.</i> (2009)	Brown Swiss Cattle	40 g/d/head (TMR)	13.0	Significantly increase
Cellulase, xylanase, endoglucanase, β -Glucosidase, β -xylosidase, α -L-arabin of ruranosidase, α -L-arabonopyranosidase, and α -D-glucuronidase Vicini <i>et al.</i> (2003)	HF Cattle	1.25 L/tonnes (Sprayed on forage) 2 L/tonnes (Sprayed on TMR)	2.8 0.9	NS
Xylanase and Amylase Histrov <i>et al.</i> (2008)	HF	Amylase @ 10 g/cow/day (Intraruminal) Xylanase @ 10 g/cow/day (Intraruminal) Amylase + xylanase @ 10 g/cow/day (Intraruminal)	6.6 6.2 0	NS
Xylanase and Endoglucanase Holtshausen <i>et al.</i> (2011)	HF Cattle	0.5 mL/kg (TMR) 1 mL/kg (TMR)	–6.5 –9.4	Significantly decrease
Xylanase and Endoglucanase Miller <i>et al.</i> (2008)	HF Cattle	Low = 2.15 ml/kg (With barley grain conc.) High = 4.30 ml/kg (With barley grain conc.) Low = 2.15 ml/kg (With sorghum grain conc.) High = 4.30 ml/kg (With sorghum grain conc.)	–5.5 0 2.8 0.9	NS
Xylanase, α -glucanase, and endocellulase Beauchemin <i>et al.</i> (2000)	Dairy Cattle	1.22 L/tonnes (TMR) 3.67 L/tonnes (TMR)	7.5 5.7	Low level of enzyme application significantly improve DMI

(Table 4 Contd...)

Name of fibrolytic enzyme used and Reference	Spp.	Rate of inclusion (Method of inclusion)	% Change in DMI	Interpretation
Endo 1-4 β , glucanase, Endo 1-3 (4) β -glucanase, 1-4 β -xylanase Peter <i>et al.</i> (2010)	HF Cattle	6.2 mL/kg (TMR)	-0.6	NS
Endo 1-4 β , glucanase, Endo 1-3 (4) β -glucanase, 1-4 β -xylanase Peter <i>et al.</i> (2015)	HF Cattle	3.9 ml/kg (Mixed with TMR) 3.8 ml/kg (-do-)	4.8 3.2	NS
Enzyme A and B Naik (2004)	Crossbred Cattle (Kankrej $\times$ Jersey)	Enzyme A = live yeast, xylanase, phytase, cellulase, β -glucanases, pectinase, amylase, protease, α -galactosidase and lipase (Mix at the time of conc. Preparation) Enzyme B = phytase, cellulase, amylase, protease, invertase and lipase (Mix at the time of conc. preparation)	9.8 1.2	NS
Phytase and cellulose Knowlton <i>et al.</i> (2007)	HF Cattle	297 g/tonnes (Alfalfa silage)	-3.1	NS
Fibrolytic enzyme Khanh <i>et al.</i> (2012)	HF Cattle	50 mg/kg TMR 50 mg/kg fermented TMR	0.09 3.4	NS

+, Increase; -, Decrease; NR, Not reported; NS, Non-significant

In the above tabulated review indicates that the dry matter intake (DMI) remains fairly unaffected by the exogenous enzyme supplementation while some researchers also show influence of enzyme supplements on DMI. Lewis *et al.* (1999) stated that the possible reason for numerically high DMI is increase in the particulate passage rate if the digestibility of the DM and NDF remain unaffected. The rate of passage of digesta also depends on the type of substrate used. Eun and Beauchemin (2005) reported that enzyme application with concentrate feeding results in increase in DMI as the concentrate causes less rumen fill, has lower lignified polysaccharide concentration and requires less rumination, a combination of these peculiarities results in increased passage rate of digesta.

Beauchemin *et al.* (2000) suggested that enzyme supplementation in higher concentrations leads to contrasting results by competing with the bacteria for binding sites on feed particles, which reduces overall digestibility of feed, besides reducing the chewing of feed/fodder, thereby resulting in lower ruminal pH and digestive capacity. Similarly, Lewis *et al.* (1996) states that over application of enzymes may lead to excessive binding of enzymes to substrates which might hamper attachment to fibre by rumen micro-organisms. They further suggested that because of excessive binding, release of antinutritional factors is another consequence of concern. While addition of enzyme results in increased nutrient digestibility, which increases solubilisation of cellular contents, but it need not affect the feed intake of animal (Vansoest *et al.* 1991).

Effect of enzyme supplement on digestibility of nutrient: Several studies show that the addition of exogenous enzymes to the diet of animal enhances digestion by breaking down the cell wall component, which are

unavailable to the animal due to complex plant cell wall structure. Table 5 enlists various studies and their salient findings in this regard.

Treating feed/fodder with enzyme enhances the digestibility of nutrients specially change in crude fibre digestibility, which may be associated with synergism between external enzyme and ruminal enzyme (Morgavi *et al.* 2000). Researchers also reported that by the external application of enzyme, microbial colonization increases (Rode *et al.* 1999, Beauchemin *et al.* 2000), which has positive correlation with dry matter disappearance rate (Yang *et al.* 1999). Beauchemin *et al.* (2000) revealed that exogenous fibrolytic enzymes may aid in exposing additional cell wall cites for bacterial attachment and thereby permitting more complete digestion of the diet ultimately improving the digestive process.

Effect on economic efficiency: Supplementing enzymes to the large ruminants has economic impact in terms of reducing average daily feed intake cost, increased milk production, increased FCM produced and more ECM produced. Table 6 shows different studies and their salient findings in this regard.

The increase in economic efficiency is associated with increased milk production because of the enzyme treatment and at the same time, no differences in average daily feed cost because feed intake remained mostly unchanged (Titi 2003). Contrary to this, Lunagariya (2016), found that economic efficiency is not affected by enzyme addition because increase in milk production requires increased nutrient supply to dairy cattle resulting in increase in average daily feed cost.

Fortifying diet of large dairy ruminants with enzymes has positive effect on production traits and digestibility,

Table 5. Effect of enzyme supplement on nutrient digestibility

Name of fibrolytic enzyme used and Reference	Spp.	Rate of inclusion	Method of inclusion	% change in digestibility coefficient							Interpretation
				DM	OM	CP	CF	EE	NDF	ADF	
Cellulase and xylanase Gaafar <i>et al.</i> (2010)	Buffalo	8000 U Cellulase and 20000 U Xylanase	Mix with roughage leaving overnight	5.1	5.8	3.5	7.0	2.96	NR	NR	Digestibility coefficient were significantly higher than control group
Cellulase and xylanase Azam <i>et al.</i> (2017)	Nili Ravi Buffalo	10/g/d/head 15/g/d/head 20 g/d/head	With basal diet sorghum forage and conc.	0.2	NR	0.2	NR	NR	0.2	NR	Increase level of enzyme application linearly improve the digestibility
Cellulase and xylanase Shekhar <i>et al.</i> (2010)	Murrah Buffalo	T1=1.5 g/kg T2=3 g/kg	Mix with TMR	3.9	4.46	4.3	10.8	7.3	7.6	8.5	Overall digestibility remain unaffected however, the fibre digestibility increase with the lower enzyme application
Pectinase, cellulase, acidic protease, xylanase, α -amylase, phytase, glucoamylase and cellulose Azzaz <i>et al.</i> (2013)	Buffalo	2 g/head/day (30 units pectinase + 4 units cellulase) 2 g/head/day (1000 units acidic protease + 30 units pectinase + 25 units xylanase + 20 units α -amylase + 10 units phytase + 5 units glucoamylase + 4 units cellulase)	Mix with Conc. feed mixture	10.6	9.1	4.5	14.6	-4.6	17.9	10.2	Digestibility of EE and CP remain unaffected, while digestibility of DM, OM, CF, ADF, NDF and NFE were highly significant.
Pectinase, cellulase, acidic protease, xylanase, α -amylase, phytase, glucoamylase and cellulose Morsy <i>et al.</i> (2015)	Buffalo	40 g/head/day Veta- zyme (<i>Lactobacillus</i> <i>acidophilus</i> , 2000 protease, pectinase, xylanase, 550 α - amylase, phytase, glucoamylase, 400 cellulase units/g) 40 g/head/day Tomoko (<i>Asper</i> <i>gillus awamori</i> , 1000 protease, 30 pectinase, 25 xylanase, 20 α -amylase, 10 phytase, 5 glucoamylase, 4 cellulase units/g	Mix with TMR	11.4	12.4	8.3	NR	3.1	12.3	9.2	Digestibility is significantly affected by enzyme supplementation except for EE
				6.0	8.4	7.5		2.3	8.3	8.7	

(Table 5 Contd....)

Name of fibrolytic enzyme used and Reference	Spp.	Rate of inclusion	Method of inclusion	% change in digestibility coefficient							Interpretation	
				DM	OM	CP	CF	EE	NDF	ADF		NFE
Cellulase and xylanase Dunda (2015)	HF Cattle	300 g/tonnes	Mix with conc.	0.5	1.0	0.2	0.2	0.1	NR	NR	0.9	Digestibility of nutrient increases
Cellulase and xylanase Dean <i>et al.</i> (2013)	HF Cattle	EC=4 g/d/ head	Mix with conc.	-3.3	NR	0.2	NR	NR	0.6	NR	NR	Digestibility not influenced by enzyme addition however
		ETMR=4 g/d/head	Mix with TMR	-0.2		1.9			0.6			OM digestibility is increase
		EF=4 g/d/ head	Mix with forage	-3.2		0.2			1.8			in barley silage– based TMR
		ES=1.3 g/kg DM	Mix with silage	-2.9		2.8			-3.9			but not significant
Cellulase and xylanase Barbadikar <i>et al.</i> (2012)	Cross bred calves	0.025/kg	With TMR	0.3	0.29	NR	0.24	5.24	NR	NR	-0.3	Digestibility of ether increases in treatment group but statistically digestibility of all component remain unaffected
Cellulase and xylanase Kung <i>et al.</i> (2000)	HF Cattle	2 L/ton	Sprayed on forage	NR	NR	NR	NR	NR	-2.2	-3.5	NR	NS
		5 L/ton	portion of TMR						7.2	-7.1		
Cellulase and xylanase Lewis <i>et al.</i> (1999)	HF Cattle	1.65 ml/kg	Sprayed on forage	-1.8	NR	NR	NR	NR	-1.4	NR	NR	NS
Cellulase and xylanase Rode <i>et al.</i> (1999)	HF Cattle	1.3 g/kg	Mix with TMR	11.9	NR	NR	NR	NR	20	32.2	NR	Digestibility of DM and NDF improve significantly
Cellulase and xylanase Granzin (2005)	HF Cattle	4 g/cow/day (grain –based concentrate)	Primiparous	6.2	-3.3	0.9	0.4	NR	NR	NR	NR	NS
			Multiparous	2.5	-0.9	-0.6	0.4					
Cellulase and xylanase Beauchemin <i>et al.</i> (1999)	HF Cattle	2.5 g/kg	(Mix with TMR)	NR	NR	NR	NR	NR	8.0	11.2	NR	Digestibility coefficients were significantly higher in treatment group
Cellulase and xylanase Bowman <i>et al.</i> (2002)	HF Cattle of TMR	1 g/cow/d	Conc.	11.5	10.8	NR	NR	NR	25.5	25.5	NR	Cow received Conc. had a significantly higher DM
			Pelleted Supplement	7.7	7.2				14.2	15.1		and OM total tract digestion
Cellulase and xylanase Yang <i>et al.</i> (1999)	HF Cattle	1 g/kg LH	Premix	1.2	0.9				5.8	8.7		Digestibility of NDF and OM was significantly higher in HH group while digestibility of ADF was not affected by treatment
		2 g/kg HH	(Alfalfa hay)	NR	2.3	NR	NR	NR	6.2	1.9	NR	NS
		1 g/kg HT	(Alfalfa hay)		4.0				12.4	8.9		
			(Alfalfa hay + conc)		3.3				9.5	2.7		
Cellulase and xylanase Yang <i>et al.</i> (2000)	HF Cattle	50 mg/kg TMR	Sprayed on forage	2.8	4.5	7.8	NR	NR	7.74	11.6	NR	NS
Cellulase and xylanase Sutton <i>et al.</i> (2003)	HF Cattle	50 mg/kg conc.	Sprayed on conc.	4.2	5.7	6.8			3.9	5.97		Overall digestibility is not affected
		2 kg/t	Sprayed on TMR	1.4	1.4	NR	NR	NR	0.6	-5.4	NR	
Cellulase, xylanase and esterase Arriola <i>et al.</i> (2011)	HF Cattle	3.4 mg/g of TMR	Sprayed on conc.	0.9	0.8				1.4	-4.6		Digestibility is improve with the enzyme application
			Infuse in rumen	0	0				-0.6	2.2		
			Low Conc diet	3.9	NR	4.5	NR	NR	5.9	8.8	NR	
		High Conc diet	4.1		7.3				7.9	8.6		

(Table 5 Contd...)

Name of fibrolytic enzyme used and Reference	Spp.	Rate of inclusion	Method of inclusion	% change in digestibility coefficient							Interpretation		
				DM	OM	CP	CF	EE	NDF	ADF		NFE	
Xylanase and Amylase Histrov <i>et al.</i> (2008)	HF	Amylase @ 10 g/cow/day	Intraruminal	2.2	2.4	NR	NR	NR	5.0	NR	Digestibility of DM and OM is significantly decreased in xylanase + amylase group whereas, NDF remain unaffected		
		3.1		2.3				5.8					
		5.7		-5.4				1.5					
Cellulase, xylanase, protease, amylase Gado <i>et al.</i> (2009) ProteaseEun and Beauchemin (2005)	Brown Swiss Cattle HF Cattle	Amylase + xylanase @ 10 g/cow/day 40 g/d/head	TMR	12.0	11.0	NR	NR	NR	39.7	32.6	NR	Total tract digestibility of DM, OM, NDF and ADF is higher in treatment group Digestibility improve significantly by addition of enzyme irrespective of forage content in diet Apparent digestibility of DM, NDF, and ADF tended to increase with the enzyme addition	
		High forage Low forage	2.2	1.9	NR	NR	NR	3.7	12.8	NR	25.8		35.9
			10.4	9.4									
Phytase and cellulose Knowlton <i>et al.</i> (2007)	HF Cattle	297 g/tonnes	Alfalfa silage	7.7	NR	NR	NR	NR	19.1	25.3	NR	Digestibility of DM and OM changes significantly	
α -glucanase, xylanase, and endocellulase Beauchemin <i>et al.</i> (2000) Enzyme A and B Naik (2004)	Dairy Cattle Crossbred Cattle (Kankrej $\times$ Jersey)	1.22 L/tonnes 3.67 L/tonnes	TMR	4.0	3.3	NR	NR	NR	2.5	13.0	NR	Significant effect on digestibility of CP and EE	
			0	0.2				7.2	-7.42				
		Mix at the time of conc. preparation	3.6	3.9	21	-1.6	16	5.9	-2.6	NR			
Fibrolytic enzyme Khanh <i>et al.</i> (2012)	HF Cattle	Enzyme A=live yeast, xylanase, phytase, cellulase, β – glucanases, pectinase, amylase, protease, α -galactosidase and lipase Enzyme B=phytase, cellulase, amylase, protease, invertase and lipase	TMR Fermented TMR	2.6	2.5	14	-0.6	9.8	3.5	-3.9		Digestibility of DM, OM, ADF and NDF significantly affected. While CP digestibility remain unaffected	
		50 mg/kg 50 mg/kg	5.6	4.9	1.2	NR	NR	12.2	24.2	NR	-3.2		-1.5
			0.1	-1.5	0.2								

+, Increase; –, Decrease; NR, Not reported; NS, Non-significant.

Table 6. Effect of enzyme supplementation on economic efficiency

Name of fibrolytic enzyme used and Reference	Spp.	Rate of inclusion	% increase in economic efficiency	Interpretation
Cellulase and xylanase Gaafar <i>et al.</i> (2010)	Buffalo	8000 U Cellulase and 20000 U Xylanase (Mix with roughage leaving overnight)	15.3	Economic efficiency increased significantly with increasing dietary fibre content
Xylanase Bassiouni <i>et al.</i> (2010)	HF Cattle	1 g/kg (Berseem hay, corn silage and rice straw)	8.2	Best results obtain in corn silage + enzyme
Cellulase/ hemicellulase Titi (2003)	HF Cattle	150 g/tonnes (Forage)	37.9	*
Cellulase and xylanase P.M. Lunagariya (2016)	HF Cattle	240 mg/kg (mix with TMR)	4.7	NS

*,Significant; NS, Non-significant.

especially in case of crude fibre. This enhances the scope of utilization of low quality high fibrous feed in economic and efficient way. However, the results are inconsistent showing further need of species-specific enzyme combination products with dose standardisation.

REFERENCES

- Arriola K G, Kim S C, Staples C R and Adesogan A T. 2011. Effect of fibrolytic enzyme application to low- and high-concentrate diets on the performance of lactating dairy cattle. *Journal of Dairy Science* **94**(2): 832–41.
- Aufrère J, Boulberhane D, Graviou D and Demarquilly C. 1994. Comparison of *in situ* degradation of cell-wall constituents, nitrogen and nitrogen linked to cell wall for fresh lucerne and 2 lucerne silages. *Annales de zootechnie* **43**(2): 125–34.
- Azam B, Tahir M N, Shahzad F, Ghaffar A, Abbas G and Gohar M. 2017. Exogenous fibrolytic enzymes addition in concentrate ration of Lactating Nili Ravi buffaloes: Effects on milk production and diet digestibility. *Pakistan Journal of Zoology* **49**(4).
- Azzaz H H, Murad H A, Kholif A M, Morsy T A, Mansour A M and El-Sayed H M. 2013. Increasing nutrients bioavailability by using fibrolytic enzymes in dairy buffaloes feeding. *Journal of Biological Sciences* **13**(4): 234.
- Bassiouni M I, Gaafar H M A, Mohi A M A, Metwally A M and Elshora M A H. 2010. Evaluation of rations supplemented with fibrolytic enzyme on dairy cows performance 3. Productive performance of lactating Friesian cows. *Livestock Research Rural Development* **22**: 117.
- Beauchemin K A. 1999. Use of feed enzymes in ruminant nutrition. *Canadian Journal of Animal Science* **79**: 243–46.
- Beauchemin K A, Colombatto D, Morgavi D P and Yang W Z. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants 12. *Journal of Animal Science* **81**(14_suppl_2): E37–E47.
- Beauchemin K A, Rode L M and Sewalt V J H. 1995. Fibrolytic enzymes increase fibre digestibility and growth rate of steers fed dry forages. *Canadian Journal of Animal Science* **75**(4): 641–44.
- Beauchemin K A, Rode L M, Maekawa M, Morgavi D P and Kampen R. 2000. Evaluation of a non-starch polysaccharidase feed enzyme in dairy cow diets. *Journal of Dairy Science* **83**(3): 543–53.
- Bernard J K, Castro J J, Mullis N A, Adesogan A T, West J W and Morantes G. 2010. Effect of feeding alfalfa hay or Tifton 85 bermudagrass haylage with or without a cellulase enzyme on performance of Holstein cows. *Journal of Dairy Science* **93**(11): 5280–85.
- Bilik K, Niwińska B and Łopuszańska-Rusek M. 2009. Effect of adding fibrolytic enzymes to periparturient and early lactation dairy cow diets on production parameters. *Annals of Animal Science* **9**(4): 401–13.
- Bowman G R, Beauchemin K A and Shelford J A. 2002. The proportion of the diet to which fibrolytic enzymes are added affects nutrient digestion by lactating dairy cows. *Journal of Dairy Science* **85**(12): 3420–29.
- Chung Y H, Zhou M, Holtshausen L, Alexander T W, McAllister T A, Guan L L and Beauchemin K A. 2012. A fibrolytic enzyme additive for lactating Holstein cow diets: Ruminal fermentation, rumen microbial populations, and enteric methane emissions. *Journal of Dairy Science* **95**(3): 1419–27.
- Colombatto D and Beauchemin K A. 2009. A protease additive increases fermentation of alfalfa diets by mixed ruminal microorganisms *in vitro*. *Journal of Animal Science* **87**(3): 1097–1105.
- Colombatto D, Mould F L, Bhat M K and Owen E. 2003. Use of fibrolytic enzymes to improve the nutritive value of ruminant diets: a biochemical and *in vitro* rumen degradation assessment. *Animal Feed Science and Technology* **107**(1–4): 201–09.
- da Silva T H, Takiya C S, Vendramin T H A and Renno F P. 2015. Effect of fibrolytic enzymes on performance of dairy cows. *Zootecnia: Otimizando Recurso e Potencialidades, Belo Horizonte-MG*. 19 a 23 de Julho, pp:1–3.
- Dean D B, Adesogan A T, Krueger N and Littell R C. 2005. Effect of fibrolytic enzymes on the fermentation characteristics, aerobic stability, and digestibility of bermudagrass silage. *Journal of Dairy Science* **88**(3): 994–1003.
- Dean D B, Staples C R, Littell R C, Kim S and Adesogan A T. 2013. Effect of method of adding a fibrolytic enzyme to dairy cow diets on feed intake digestibility, milk production, ruminal fermentation, and blood metabolites. *Animal Nutrition and Feed Technology* **13**(3): 337–57.
- Dhiman T R, Zaman M S, Gimenez R R, Walters J L and Treacher R. 2002. Performance of dairy cows fed forage treated with fibrolytic enzymes prior to feeding. *Animal Feed Science and Technology* **101**(1–4): 115–25.
- Diler A, Kocuyigit R, Yanar M and Aydin R. 2014. Effect of feeding

- direct fed–microbials plus exogenous feed enzyme on milk yield and milk composition of Holstein Friesian cows. *Veterinarija Ir Zootechnika* **65**(87).
- Dunda B A. 2015. Effect of supplementation of probiotic and enzyme on performance. Doctoral dissertation. Maharashtra Animal and Fishery Sciences University, Nagpur.
- El-Aziz M A, Kholif S M and Morsy T A. 2012. Buffalo's milk composition and its fat properties as affected by feeding diet supplemented with flaxseed or fibrolytic enzymes in early lactation. *Journal of Life Sciences* **4**(1): 19–25.
- El-Bordeny N E, Abedo A A, El-Sayed H M, Daoud E N, Soliman H S and Mahmoud A E M. 2015. Effect of exogenous fibrolytic enzyme application on productive response of dairy cows at different lactation stages. *Asian Journal of Animal and Veterinary Advances* **10**: 226–36.
- Elwakeel E A, Titgemeyer E C, Johnson B J, Armendariz C K and Shirley J E. 2007. Fibrolytic enzymes to increase the nutritive value of dairy feedstuffs. *Journal of Dairy Science* **90**(11): 5226–36.
- Eun J S and Beauchemin K A. 2005. Effects of a proteolytic feed enzyme on intake, digestion, ruminal fermentation, and milk production. *Journal of Dairy Science* **88**(6): 2140–53.
- Gaafar H M A, Abdel-Raouf E M and El-Reidy K F A. 2010. Effect of fibrolytic enzyme supplementation and fibre content of total mixed ration on productive performance of lactating buffaloes. *Slovak Journal of Animal Science* **43**: 147–53.
- Gado H M, Salem A Z M, Robinson P H and Hassan M. 2009. Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. *Animal Feed Science and Technology* **154**(1–2): 36–46.
- Giraldo L A, Ranilla M J, Tejido M L and Carro M D. Effects of enzyme application method on *in vitro* rumen fermentation of tropical forages. *Journal of Animal and Feed Sciences* **13**(2004): 63–66.
- Granzin B C. 2005. Effects of a fibrolytic enzyme supplement on the performance of Holstein Friesian cows grazing kikuyu. *Tropical Grasslands* **39**(2): 112–16.
- Holtshausen L, Chung Y H, Gerardo-Cuervo H, Oba M and Beauchemin K A. 2011. Improved milk production efficiency in early lactation dairy cattle with dietary addition of a developmental fibrolytic enzyme additive. *Journal of Dairy Science* **94**(2): 899–907.
- Hristov A N, Basel C E, Melgar A, Foley A E, Ropp J K, Hunt C W and Tricarico J M. 2008. Effect of exogenous polysaccharide-degrading enzyme preparations on ruminal fermentation and digestibility of nutrients in dairy cows. *Animal Feed Science and Technology* **145**(1–4): 182–93.
- Khanh T T M, Vasupen K, Bureenok S, Wachirapakorn C and Yuangklang C. 2012. Effect of fibrolytic enzymes supplementation on rumen fermentation and digestibility in dairy cow fed straw-based diet. *Khon Kaen Agricultural Journal* **40**(2): 141–44.
- Kholif A M, El-Ashry M A, El-Alamy H A, El-Sayed H M, Fadel M and Kholif S M. 2005. Biological treatments banana wastes for feeding lactating goats. *Egyptian Journal of Nutrition and Feeds* **8**(2): 149–62.
- Klingerman C M, Hu W, McDonell E E, DerBedrosian M C and Kung L. 2009. An evaluation of exogenous enzymes with amylolytic activity for dairy cows. *Journal of Dairy Science* **92**(3): 1050–59.
- Knowlton K F, Taylor M S, Hill S R, Cobb C and Wilson K F. 2007. Manure nutrient excretion by lactating cows fed exogenous phytase and cellulase. *Journal of Dairy Science* **90**(9): 4356–60.
- Krause D O, Denman S E, Mackie R I, Morrison M, Rae A L, Attwood G T and McSweeney C S. 2003. Opportunities to improve fibre degradation in the rumen: microbiology, ecology, and genomics. *FEMS Microbiology Reviews* **27**(5): 663–93.
- Kung Jr L, Treacher R J, Nauman G A, Smagala A M, Endres K M and Cohen M A. 2000. The effect of treating forages with fibrolytic enzymes on its nutritive value and lactation performance of dairy cows. *Journal of Dairy Science* **83**(1): 115–22.
- Lewis G E, Hunt C W, Sanchez W K, Treacher R, Pritchard G T and Feng P. 1996. Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage-based diet fed to beef steers. *Journal of Animal Science* **74**(12): 3020–28.
- Lewis G E, Sanchez W K, Hunt C W, Guy M A, Pritchard G T, Swanson B I and Treacher R J. 1999. Effect of direct-fed fibrolytic enzymes on the lactational performance of dairy cows. *Journal of Dairy Science* **82**(3): 611–17.
- Łopuszańska-Rusek M and Bilik K. 2011. Influence of pre- and post-partum supplementation of fibrolytic enzymes and yeast culture, or both, on performance and metabolic status of dairy cows. *Annals of Animal Science* **11**(4): 531–45.
- Lunagariya P M. 2016. *Effect of Supplementing Fibrolytic Enzymes on Rumen Fermentation and Milk Yield in Dairy Cows*. Doctoral Dissertation, AAU, Anand.
- Mendoza G D, Loera-Corral O, Plata-Pérez F X, Hernández-García P A and Ramírez-Mella M. 2014. Considerations on the use of exogenous fibrolytic enzymes to improve forage utilization. *Scientific World Journal*.
- Miller D R, Granzin B C, Elliott R and Norton B W. 2008. Effects of an exogenous enzyme, Roxazyme® G2 Liquid, on milk production in pasture fed dairy cows. *Animal Feed Science and Technology* **145**(1–4): 194–208.
- Mohamed D E D A, Borhami B E, El-Shazly K A and Sallam S M A. 2013. Effect of dietary supplementation with fibrolytic enzymes on the productive performance of early lactating dairy cows. *Journal of Agricultural Science* **5**(6): 146.
- Morsy T A, Kholif A E, Kholif S M, Kholif A M, Sun X and Salem A Z. 2016. Effects of two enzyme feed additives on digestion and milk production in lactating Egyptian buffaloes. *Annals of Animal Science* **16**(1): 209–22.
- Murad H A and Azzaz H H. 2010. Cellulase and dairy animal feeding. *Biotechnology* **9**(3): 238–56.
- Naik P A. 2004. 'Manipulation of rumen fermentation by enzymes'. Doctoral Dissertation. AAU, Anand.
- Nozière P, Steinberg W, Silberberg M and Morgavi D P. 2014. Amylase addition increases starch ruminal digestion in first-lactation cows fed high and low starch diets. *Journal of Dairy Science* **97**(4): 2319–28.
- Nsereko V L, Beauchemin K A, Morgavi D P, Rode L M, Furtado A F, McAllister T A and Wang Y. 2002. Effect of a fibrolytic enzyme preparation from *Trichoderma longibrachiatum* on the rumen microbial population of dairy cows. *Canadian Journal of Microbiology* **48**(1): 14–20.
- Peters A, Lebzien P, Meyer U, Borchert U, Bulang M and Flachowsky G. 2010. Effect of exogenous fibrolytic enzymes on ruminal fermentation and nutrient digestion in dairy cows. *Archives of Animal Nutrition* **64**(3): 221–37.
- Peters A, Meyer U and Dänicke S. 2015. Effect of exogenous fibrolytic enzymes on performance and blood profile in early

- and mid-lactation Holstein cows. *Animal Nutrition* **1**(3): 229–38.
- Prabhakar B M. 2012. 'Effects of feeding fibrolytic enzymes on growth performance of cross-bred calves.' Doctoral Dissertation, Anand Agricultural University, Anand.
- Qinnghe C, Xiaoyu, Y, Tiangui N, Cheng J and Qiugang M. 2004. The screening of culture condition and properties of xylanase by white-rot fungus *Pleurotus ostreatus*. *Process Biochemistry* **39**(11): 1561–66.
- Reddish M A and Kung L. 2007. The effect of feeding a dry enzyme mixture with fibrolytic activity on the performance of lactating cows and digestibility of a diet for sheep. *Journal of Dairy Science* **90**(10): 4724–29.
- Rode L M, Yang W Z and Beauchemin K A. 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. *Journal of Dairy Science* **82**(10): 2121–26.
- Schingoethe D J, Stegeman G A and Treacher R J. 1999. Response of lactating dairy cows to a cellulase and xylanase enzyme mixture applied to forages at the time of feeding. *Journal of Dairy Science* **82**(5): 996–1003.
- Shekhar C, Thakur S S and Shelke S K. 2010. Effect of exogenous fibrolytic enzymes supplementation on milk production and nutrient utilization in Murrah buffaloes. *Tropical Animal Health and Production* **42**(7): 1465–70.
- Subramaniyam R and Vimala R. 2012. Solid state and submerged fermentation for the production of bioactive substances: a comparative study. *International Journal of Natural Sciences* **3**(3): 480–86.
- Sucu E, Nayeri A, Sanz-Fernandez M V, Upah N C and Baumgard L H. 2014. The effects of supplemental protease enzymes on production variables in lactating Holstein cows. *Italian Journal of Animal Science* **13**(2): 3186.
- Sujani S and Seresinhe R T. 2015. Exogenous enzymes in ruminant nutrition: A review. *Asian Journal of Animal Sciences* **9**(3): 85–99.
- Sukumaran R K, Singhania R R and Pandey A. 2005. Microbial cellulases-production, applications and challenges. *Journal of Scientific and Industrial Research* **64**: 832.
- Sutton J D, Phipps R H, Beever D E, Humphries D J, Hartnell G F, Vicini J L and Hard D L. 2003. Effect of method of application of a fibrolytic enzyme product on digestive processes and milk production in Holstein-Friesian cows. *Journal of Dairy Science* **86**(2): 546–56.
- Titi H H. 2003. Evaluation of feeding a fibrolytic enzyme to lactating dairy cows on their lactational performance during early lactation. *Asian Australasian Journal of Animal Science* **16**(5): 677–84.
- Tricarico J M, Johnston J D and Dawson K A. 2008. Dietary supplementation of ruminant diets with an *Aspergillus oryzae* α -amylase. *Animal Feed Science and Technology* **145**(1–4): 136–50.
- Tricarico J M, Johnston J D, Dawson K A, Hanson K C, McLeod K R and Harmon D L. 2005. The effects of an *Aspergillus oryzae* extract containing alpha-amylase activity on ruminal fermentation and milk production in lactating Holstein cows. *Animal Science* **81**(3): 365–74.
- Van Soest P V, Robertson J B and Lewis B A. 1991. Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**(10): 3583–97.
- Vicini J L, Bateman H G, Bhat M K, Clark J H, Erdman R A, Phipps R H and Hard D L. 2003. Effect of feeding supplemental fibrolytic enzymes or soluble sugars with malic acid on milk production. *Journal of Dairy Science* **86**(2): 576–85.
- Wang Y, McAllister T A, Rode L M, Beauchemin K A, Morgavi D P, Nsereko V L and Yang W. 2001. Effects of an exogenous enzyme preparation on microbial protein synthesis, enzyme activity and attachment to feed in the rumen simulation technique (Rusitec). *British Journal of Nutrition* **85**(3): 325–32.
- Yang W Z, Beauchemin K A and Rode L M. 1999. Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. *Journal of Dairy Science* **82**(2): 391–403.
- Yang W Z, Beauchemin K A and Rode L M. 2000. A comparison of methods of adding fibrolytic enzymes to lactating cow diets. *Journal of Dairy Science* **83**(11): 2512–20.