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Effect of supplementation of exogenous fibrolytic enzymes on milk yield and composition in early lactating crossbred dairy cows#

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Abstract

A study was conducted to examine the effect of supplementation of exogenous fibrolytic enzymes (EFE) carboxymethylcellulase (CMC) and xylanase (Xy) on milk yield and composition in early lactating crossbred dairy cows fed on total mixed ration (TMR). Fifteen cows within ten days of calving were selected from University Livestock Farm and Fodder Research and Development Scheme (ULF & FRDS), Mannuthy and were randomly divided into three groups. The animals were fed with isonitrogenous TMR (3.5 per cent of body weight) for a period of 90 days and the dietary treatments included; T_1 offered with the control diet (TDN - 60 per cent, CP - 13 per cent), T_2 offered with control diet supplemented with EFE (CMC 3500 units/kg and Xy 16000 units/kg), and T_3 given a diet lower in TDN (TDN - 55 per cent) supplemented with EFE (CMC 3500 units/kg and Xy 16000 units/kg). The results of the study revealed that milk yield remained unaffected (p>0.05) with dietary treatments. Milk composition also remained similar, except for milk urea nitrogen which was lower (p<0.05) in enzyme supplemented groups. Thus, it could be inferred that EFE supplementation in cows during early lactation is more beneficial in diets with low TDN since the milk yield and milk composition of cows fed with TMR having low TDN supplemented with EFE had comparable values with those offered with TMR having higher TDN.

Keywords: *Exogenous fibrolytic enzymes, total mixed ration, early lactating dairy cows*

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In India, agro-industrial by products and crop residues form a major part of feed fed to ruminants. But these feeds are low in protein and energy owing to high fibre and silica. Plant cell wall typically consists of cellulose (35-50 per cent), hemicelluloses (20-40 per cent) and lignin (10–25 per cent) (Sticklen, 2008).

In the ration of dairy cows, dietary fibre has a vital role in preventing sub-acute ruminal acidosis and thereby preventing depression in dry matter intake (DMI), fibre digestion and milk production as well as alterations in milk composition (NRC, 2001).

Rumen microbes are able to synthesize and secrete fibrolytic enzyme complex, favouring the hydrolysis of plant cell wall. Cellulose is degraded by enzymes such as endo-1,4-β-glucanase (carboxymethylcellulase), exo-1,4-β-glucanase and β-glucosidase. Hemicellulose is hydrolysed by xylanase, α-D-galactosidase and β-mannosidase.

But the digestion of fibrous substrates is slow and incomplete in the rumen. Thus, the efficiency of converting forages and crop residues to milk and meat is limited by the poor digestibility of forage cell walls. Hence, suitable treatments are necessary to improve their efficiency. Several recent researches have demonstrated the beneficial effects of supplementing ruminant diets with exogenous enzymes more specifically, exogenous fibrolytic enzymes (EFE) which improves fibre digestibility and consequently, the efficiency of feed utilization by ruminants (Krueger *et al.*, 2008). Hence this study is proposed to evaluate the effects of supplementation of EFE on efficiency of milk production in early lactating crossbred cows.

Materials and methods

Location of study

The study was conducted at the Department of Animal Nutrition, College of Veterinary and Animal Sciences and University Livestock Farm & Fodder Research and Development Scheme (ULF & FRDS), Mannuthy, Kerala, India.

Experimental animals, management and feeding

Fifteen crossbred dairy cows in early lactation (within ten days of calving) were selected for the experiment from ULF & FRDS, Mannuthy. The cows were housed in well ventilated, clean and dry shed with facilities for feeding and watering. They were divided into three groups randomly following completely randomized design. The animals were maintained under uniform feeding and management system throughout the experimental period of 90 days. The cows were fed with isonitrogenous TMR (3.5 per cent of body weight) for a period of 90 days and the dietary treatments were; T_1 offered with the control diet (TDN - 60 per cent, CP - 13 per cent), T_2 offered with control diet supplemented with EFE (CMC 3500 units/kg and Xy 16000 units/kg), and T_3 given a diet lower in TDN (TDN - 55 per cent, CP - 13 per cent) supplemented with EFE (CMC 3500 units/kg and Xy 16000 units/kg). Clean and fresh drinking water was offered to all the animals *ad libitum*. Ingredient compositions of total mixed ration fed to the three experimental groups are given in Table 1.

The fibrolyic enzymes used in the present study were carboxymethyl cellulase and xylanase. These enzymes were procured from Biolaxi Corporation, Thane, Maharashtra, India. The activities of carboxymethyl cellulase and xylanase were 100000 units/g and 100000 units/g, respectively.

The animals were milked twice a day, at 5 A.M. in the morning and 2 P.M. in the afternoon. The data on milk yield, recorded twice a day, was used to calculate the daily milk yield for each animal throughout the experimental period. Morning and evening milk samples were collected from individual animals every fortnight and pooled samples were analysed for milk composition.

Milk analysis

The collected milk samples were analysed for total solids, protein (AOAC, 2016) and fat (IS: 1224, 1977). From the above data, the solids not fat (SNF) was calculated. The milk urea nitrogen (Bector *et al.,* 1998) was also analysed.

Statistical analysis

Data obtained on the various parameters were analysed statistically as per Snedecor and Cochran (2014) by analysis of variance (ANOVA) technique, using the software Statistical Product and Service Solutions (SPSS) version 24.0.

Results and discussion

The per cent chemical composition of ration fed to experimental animals is shown in Table 2.

Milk production

Consolidated data on fortnightly average milk production of experimental lactating cows maintained on three treatments $T_{1,}$ T_2 and T_3 are given in Table 3. The average milk production of experimental animals maintained on three experimental rations T_1, T_2 and T_3 were 11.48±0.33, 12.4±1.52 and 11.49±0.6 kg, respectively. There was no significant difference (p>0.05) in daily milk production of experimental lactating cows maintained on the three dietary treatments. The values of average milk production obtained in the present study was comparable with those reported by Chacko *et al.* (2016) in lactating crossbred cows fed with TMR having 65 per cent TDN and the values ranged from 11.63 to 12.67 kg. In agreement with the results obtained in the present study, Peters *et al*. (2015) and Romero *et al.* (2016) reported that milk yield in lactating Holstein cows did not get improved by supplementation of EFE (having xylanase and endoglucanase activities) at a rate of 114400 units of xylanase and 35200 units of endoglucanase per kg DM of TMR and 26926 units of xylanase and 2714 units of endoglucanase per kg of TMR, respectively. Zilio *et al*. (2019) also observed that application of fibrolytic enzymes (having xylanase) at the

Table 1. Ingredient composition of TMR offered to cows maintained on three dietary treatments

Ingredients	Percentage composition of TMR		
	T_1	T_2	T_3
Maize	21	21	10
Rice polish	8.5	8.5	4.5
Deoiled rice bran	8	8	10
Corn gluten fibre	15.5	15.5	10.5
Coconut cake	10	10	15.5
Alfalfa	4	4	5.5
Straw	30	30	41
Salt	0.5	0.5	0.5
Calcite	1.5	1.5	1.5
Mineral mixture	1	1	1
Total	100	100	100
Xylanase (per kg of TMR)	-	16000 units	16000 units
Carboxymethyl cellulase (per kg of TMR)		3500 units	3500 units
Vit AB_2D_3K, g/100kg*	20	20	20

*Composition per gram: Vitamin A – 82,500 I.U, Vitamin B_2 – 50 mg, Vitamin D_3 – 12,000 I.U and Vitamin K – 10 mg.

Table 2. Chemical composition[1] of the rations fed to experimental lactating cows, in Percentage

Parameter	Dietary treatments		
	T1	T2	T3
Dry matter	90.42±0.02	90.42±0.02	90.50±0.01
Crude protein	13.46±0.08	13.46±0.08	13.37±0.06
Ether extract	4.00±0.02	4.00±0.02	3.83±0.04
Crude fibre	14.89±0.24	14.89±0.24	16.75±0.23
Total ash	10.80±0.01	10.80±0.01	12.50±0.01
Nitrogen free extract	56.85±0.25	56.85±0.25	53.55±0.25
Acid insoluble ash	4.51±0.05	4.51±0.05	5.61±0.04
Calcium	0.90±0.01	0.90±0.01	0.94±0.03
Phosphorus	0.56±0.02	0.56±0.02	0.55±0.02
Neutral detergent fibre	56.00±0.20	56.00±0.20	59.48±0.21
Acid detergent fibre	26.53±0.12	26.53±0.12	28.58±0.23

[1]Values expressed on DM basis, average of six values with S.E.

Table 3. Fortnightly average milk production[1] of lactating cows maintained on three experimental rations, kg

Fortnight	Daily milk production (kg)			p-value
	T_1	T_2	T_3	
1	12.18±0.61	13.91±1.66	13.41±0.58	0.520[ns]
2	12.30±0.53	13.57±1.82	12.78±0.74	0.750[ns]
3	12.19±0.23	12.77±1.54	11.61±0.71	0.717[ns]
4	11.37±0.45	12.01±1.53	10.82±0.71	0.711[ns]
5	10.75±0.38	11.62±1.25	10.27±0.74	0.551[ns]
6	10.12±0.38	10.55±1.46	10.05±0.55	0.919[ns]
Mean	11.48±0.33	12.40±1.52	11.49±0.60	0.745[ns]

[1]Mean values are based on five replicates with S.E.; ns- non significant

Table 4. Fortnightly average milk composition[1] of lactating cows maintained on three experimental rations, %

Parameter	Treatment	Fortnights						Mean±S.E
		1	2	3	4	5	6	
Fat (%)	T1	3.86±0.29	3.64±0.32	3.68±0.28	3.56±0.32	3.64±0.34	3.56±0.35	3.66±0.30
	T2	3.70±0.27	3.64±0.36	3.70±0.27	3.76±0.27	3.68±0.23	3.76±0.25	3.71±0.26
	T3	3.90±0.29	3.70±0.31	3.46±0.22	3.54±0.29	3.68±0.36	3.72±0.27	3.67±0.27
SNF (%)	T1	8.30±0.25	8.56±0.24	8.60±0.21	8.73±0.26	8.78±0.19	8.83±0.23	8.63±0.20
	T2	8.62±0.13	8.74±0.19	8.79±0.08	8.84±0.08	8.94±0.09	8.98±0.05	8.81±0.08
	T3	8.58±0.21	8.60±0.21	8.85±0.13	8.81±0.18	8.68±0.21	8.75±0.19	8.71±0.16
Total solids (%)	T1	12.16±0.07	12.20±0.20	12.28±0.14	12.29±0.21	12.42±0.27	12.39±0.27	12.29±0.19
	T2	12.32±0.15	12.38±0.17	12.49±0.21	12.60±0.20	12.62±0.25	12.74±0.21	12.52±0.19
	T3	12.48±0.27	12.30±0.21	12.31±0.13	12.35±0.19	12.36±0.20	12.47±0.19	12.38±0.18
Protein (%)	T1	2.69±0.07	2.74±0.07	2.75±0.07	2.73±0.06	2.73±0.10	2.77±0.09	2.74±0.07
	T2	2.85±0.07	2.76±0.06	2.78±0.06	2.80±0.07	2.82±0.08	2.84±0.09	2.81±0.07
	T3	2.70±0.06	2.73±0.07	2.77±0.04	2.76±0.02	2.78±0.04	2.78±0.07	2.76±0.04
MUN (mg/dL)	T1	13.06±0.12[a]	13.36±0.19[a]	13.46±0.14[a]	13.60±0.11[a]	13.74±0.13[a]	13.82±0.11[a]	13.51±0.12[a]
	T2	11.10±0.15[b]	11.28±0.15[b]	11.36±0.11[b]	11.46±0.12[b]	11.52±0.14[b]	11.60±0.08[b]	11.39±0.12[b]
	T3	11.34±0.15[b]	11.48±0.08[b]	11.66±0.09[b]	11.72±0.17[b]	11.86±0.18[b]	11.90±0.16[b]	11.66±0.12[b]

[1]Mean values are based on five replicates with S.E.

[a,b]Means in the columns bearing different superscripts differ significantly at p<0.01

rate of 51 IU of xylanase per kg DM of diet, had no effect in improving the milk yield of lactating Holstein cows. However, Shadmanesh (2014) and Arif *et al*. (2019) observed increased milk production in early lactating dairy cows and buffaloes, respectively, when they fed rations supplemented with 620-1500 units of xylanase. This discrepancy between studies could be due to differences in activity, dose and mode/time of application of enzymes and physiological status of animals (Beauchemin *et al*., 2003).

Milk composition

The composition of milk, viz. fat, total solids, solids not fat, protein and milk urea nitrogen collected from experimental animals at fortnightly intervals are given Table 4.

The average fat content in milk of experimental animals fed on the three experimental rations T_1, T_2 and T_3were 3.66±0.30, 3.71±0.26 and 3.67±0.27 per cent, respectively and were similar (p>0.05) in all the three treatment groups. In accordance with present study, Zilio *et al*. (2019) also could not observe any improvement on milk fat percent in lactating Holstein cows supplemented with commercial enzyme product having xylanase (51 IU/kg DM of diet) @ 12 g/day. Similarly, Miachieo and Thakur (2007) did not observe any effect on milk fat content in lactating

Sahiwal cows fed with TMR supplemented with EFE containing equal proportion of xylanase and cellulase at a rate of 11700 IU of xylanase and 6705 IU of cellulase per kg DM.

The average solids not fat (SNF) content in milk from experimental animals fed on the three experimental rations T_1, T_2 and T_3 were 8.63±0.20, 8.81±0.08 and 8.71±0.16 per cent, respectively. There was no significant difference ($p>0.05$) between three treatments in any of the fortnights. Similar to present findings, milk SNF content was not affected by supplementation of commercial enzyme product having xylanase (100 XU/g) in the TMR (15 g/head/day) of lactating Holstein cows (El-Bordeny *et al.,* (2015). Furthermore, Arif *et al*. (2019) reported that supplementation of commercial enzyme product (having xylanase and endoglucanase activity) in early lactating Nilli Ravi buffaloes did not produce any improvement ($p>0.05$) milk SNF per cent.

The average total solids content in milk of experimental animals fed on three experimental rations T_1, T_2 and T_3 were 12.29±0.19, 12.52±0.19 and 12.38±0.18 per cent, respectively. Statistical analysis of the data on total solids per cent revealed that there was no significant difference ($p>0.05$) between three treatments in any of the fortnights. In agreement with the results of the present study, Lunsin *et al*. (2021) did not observe any effect on milk total solids content, when four levels of fibrolytic enzymes (0, 1.2, 2.4, and 3.6 g/kg DM of TMR) having 4900 units cellulase, 18000 units of xylanase, 10000 units of beta-glucanase and 17000 units amylase activities, when fed to lactating Holstein cows. Similarly, Miachieo and Thakur (2007) did not observe any effect on milk total solids content in lactating Sahiwal cows fed with wheat straw based TMR supplemented with a one : one mixture of cellulase and xylanase @ 1.5 g/kg DM and the values ranged from 13.3 to 13.4 per cent.

The average protein content in milk from experimental animals fed on the three experimental rations T_1, T_2 and T_3were 2.74±0.07, 2.81±0.07 and 2.76±0.04 per cent, respectively and were similar ($p>0.05$) in all the three treatment groups. The values obtained in present experiment is comparable with previous study conducted by Jayaraj *et al*. (2021) in lactating cows fed with a ration having higher CP (20.93 per cent) and values were ranged from 2.80 to 2.93 per cent. In agreement with the present study, no effect on milk protein content by supplementation of a one : one mixture of xylanase and cellulase @ 1.5 g/kg DM of wheat straw based TMR was reported in lactating Sahiwal cows (Miachieo and Thakur, 2007) and in early lactating Holstein cows fed with TMR supplemented with EFE (containing 8,000 units/mL of endo-1,4-β glucanase, 18,000 units/mL of endo-1,3(4)-β glucanase and 26,000 units/mL of 1,4-β xylanase) at a rate of 4.4mL/kg DM (Peters *et al*., 2015).

The average milk urea nitrogen (MUN) content in milk of experimental animals fed on the three experimental rations T_1, T_2 and T_3 were 13.51±0.12, 11.39±0.10 and 11.66±0.12 mg/dL, respectively. It was observed that MUN content was higher ($p<0.05$) in the animals fed diet unsupplemented with enzyme (T_1) on comparison to animals offered feed supplemented with EFE (T_2 and T_3). However, the values of MUN observed in T_2 and T_3 were similar ($p>0.05$). These findings were in accordance with those made by Lopuszanska-Rusek and Bilik, (2011) who observed that supplementing an enzyme preparation (having xylanase and cellulase activities) at a dose rate of 15 g/day in early lactating Polish Red and White Holstein-Friesian cows resulted in decrease of milk urea content compared to control group. Similarly, Knowlton *et al.* (2007) observed a tendency to reduce MUN content of milk in lactating Holstein cows supplemented with EFE (containing 5,000 units of phytase activity/g and 15,000 units of cellulase activity/g) at a dose rate of 4,455 IU cellulase and 1,485 IU phytase /kg DM of TMR.

Lower MUN in enzyme supplemented groups in the present study might be due to the improved efficiency of utilization of nitrogen by rumen microbes, since better release of usable energy can improve nitrogen utilization by rumen microbes without elevating the rumen ammonia level. Yang *et al*. (1999) reported that supplementation of fibrolytic enzymes enhanced microbial protein synthesis in lactating cows.

The findings of this study suggest that fibrolytic enzymes in feed have some interaction with the efficiency of nitrogen utilization and more research is warranted to investigate the effects of supplementing fibrolytic enzymes especially on rumen microbial protein synthesis.

Conclusion

On summarizing the results, it could be inferred that dairy cows fed with TMR having less TDN content, when supplemented with EFE had comparable milk production and composition with those cows fed high TDN ration. Thus, it can be concluded that application of EFE such as xylanase and carboxymethyl cellulase is beneficial in feeding practices of crossbred dairy cows on paddy straw-based rations.

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Conflict of interest

The authors declare that they have no conflict of interest.

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