



Effect of exogenous fibrolytic enzymes in total mixed ration on milk yield, composition, feed efficiency in Holstein Friesian crossbred cows

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ABSTRACT

An experiment was conducted to evaluate the effect of exogenous fibrolytic enzymes (EFE; 800 IU/g endo 1,4- β glucanase, 700 IU/g 1 (3),4- β glucanase and 2700 IU/g endo 1,4- β xylanase) @ 240 mg/kg total mixed ration (TMR) feeding on milk and components yield as well as feed utilization efficiency in Holstein Friesian [HF] crossbred cows. Twenty early lactating HF crossbred cows having uniform 4% fat corrected milk (FCM) yield and body weight were used to access the effect on nutrients intake, milk yield, milk composition, feed conversion efficiency and cost of feeding. The crossbred cows fed EFE supplemented TMR produced more milk, 4% FCM and milk fat. The supplementation of EFE resulted in higher milk solid not fat (SNF), protein and lactose as well as higher milk fat and total solids (TS). The consumption of nutrients/kg 4% FCM were less in EFE group cow, whereas only numerical improvement in nutrients intake/kg milk was noticed. The gross protein and energy efficiencies were significantly improved on EFE supplementation. The return over feed cost was 15.87% higher for cows fed EFE supplemented TMR compared to control TMR. In conclusion, feeding of EFE produced significantly higher 4% FCM and milk fat with improved feed conversion efficiency and better economic returns in HF crossbred cows.

Key words: Exogenous fibrolytic enzymes, Feed conversion efficiency, Holstein-Friesian crossbred cows, Milk yield

In India, the majority of livestock subsist on low quality native grasses, crop residues, and agro-industrial by-products. Plant cell walls typically consist of about 35–50% cellulose, 20–35% hemicellulose and 10–25% lignin in the dry mass (Gemedi *et al.* 2014). Chemically, cellulose and hemicellulose is a linear polymer of sugar subunits linked by β -1,4 glycosidic bonds; whereas hemicellulose is sometimes also linked by β -1,3-glycosidic bonds. Microbial enzymes break these bonds but only 10–35% of energy intake is available as net energy owing to incomplete digestion (Krause *et al.* 2003). The exogenous fibrolytic enzymes, viz. cellulases and xylanases, are amongst the two major enzyme groups that are specified to break β -1,4 linkages joining sugar molecules of cellulose and xylans found in plant cell wall components (Beauchemin *et al.* 2003). The meta-analysis revealed variable response of EFE

on milk yield and milk composition which needs reconsideration (Ortiz-Rodea *et al.* 2013). Strategic approach like *in vitro* testing for dose optimization before *in vivo* application and use of cows in early lactation [21 $\pm$ 5 days in milk-DIM] are needed for accurate response on milk yield and milk composition (Adesogan *et al.* 2014). We had hypothesized that supplementation of EFE improves milk and component yield as well as feed utilization efficiency. Considering the above facts, the study aimed to ascertain the effect of EFE supplementation on milk yield, gross milk composition and feed conversion efficiency in early lactating HF crossbred cows.

MATERIALS AND METHODS

An experiment was conducted at Commercial Dairy Farm, Anand, Gujarat, India, as per guidelines of Institutional Animal Ethics Committee. The commercial exogenous fibrolytic enzymes—Roxozyme GT ® (contained 800 IU/g endo 1,4- β glucanase, 700 IU/g 1 (3),4- β glucanase and 2700 IU/g endo 1,4- β xylanase) procured from M/s DSM Nutritional Product Pvt. Ltd., Pune, India; was used @ 240 mg/kg TMR as revealed optimum during *in vitro* study (Lunagariya *et al.* 2017a).

Twenty HF crossbred cows in their 2nd and 3rd lactation [DIM 23.5 $\pm$ 0.92] were selected and allotted to two dietary

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treatments, viz. T₁ (control) and T₂ (exogenous fibrolytic enzymes); ten cows in each group, following individual completely randomized design on the basis of milk yield [13.27±0.52 kg], milk fat content [3.71±0.04%], 4% FCM yield [12.66±0.45 kg] and body weight [457.38±9.51 kg]. Experiment was conducted for 140 days [20 weeks]. Cows were individually fed TMR to meet the CP and TDN requirements (NRC 2001). The ingredient proportions of TMR are given in Table 1. The bi-weekly samples of TMR offered were collected and stored for chemical analysis (AOAC 1995, Van Soest *et al.* 1991). All the cows were housed in well ventilated and hygienic stall and let loose for 2 h each in the morning and evening for exercise. The cows had free access to fresh, clean and wholesome drinking water at tying place and during the time when they were let loose. De-worming of all the lactating cows was carried out using broad spectrum anthelmintic before initiation of the experiment.

Table 1. Composition of TMR offered to HF crossbred cows

Ingredient	Kg (DM)
Cotton seed cake	9.62
Maize grounded	9.62
Compounded concentrate mixture	11.50
Maize cake	5.77
Soybean DOC	1.92
Mineral mixture	0.38
Bypass fat	0.38
Sodium bicarbonate	0.38
Salt	0.38
Green fodder (maize/sorghum)	22.80
Wheat straw	22.80
Mix straw (40% soybean, 40% green gram and 20% gram)	14.40
Total	100.00

TMR, Total mixed ration; HF, Holstein Friesian; DM, dry matter; DOC, de-oiled cake

Animals were machine milked twice daily (5:30 AM and 5:30 PM) and the milk samples were drawn at bi-weekly interval in proportion to morning and evening milk production from individual animals. The milk samples were analyzed for milk fat, solids-not-fat (SNF), milk protein and lactose content as per BIS (1981) using Milkoscan. The 4% FCM yield was calculated using Gains formula (ICAR 2013). The feed conversion efficiency was calculated as the amount of DM [kg], CP [g] and TDN [kg] required to produce one kg of whole milk or 4% FCM. The gross and net protein efficiency as well as gross and net energy efficiency was calculated as per formula described by Purushothaman *et al.* (2008) except milk energy was calculated as FCM × 750 and for protein efficiency, nitrogen content was multiplied by 6.25.

The cost of feeding and income from milk under the two groups was worked out from the data of daily feed intake, actual purchase price of feeds and fodder, and sale

of milk on the basis of fat and SNF content. Economic efficiency was expressed as the daily feed cost, price of milk, and the ratio of feed cost to price of milk. The experimental data was subjected to one way analysis of variance (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

The chemical composition of total mixed ration (TMR) is given in Table 2. The nutrients content of TMR was sufficient to satisfy the nutrient requirement of cows.

Nutrients intake and body weight of cows: Average body weight changes and nutrient intake of HF crossbred cows under control TMR [T₁] and exogenous fibrolytic enzymes (EFE) supplemented TMR [T₂] differed non-significantly (P>0.05) (Table 3).

Table 2. Proximate composition (% on DM basis) of TMR

Parameter	TMR On-Farm study
CP	11.01±0.08
EE	4.04±0.08
CF	24.82±0.32
NFE	52.56±0.41
Total Ash	7.57±0.08
Silica	2.19±0.04
Calcium	1.06±0.02
Phosphorus	0.45±0.01
NDF	56.80±0.53
ADF	28.66±0.23
Cellulose	18.70±0.04
Hemicellulose	28.14±0.44

DM, Dry matter; TMR, total mixed ration; CP, crude protein; EE, ether extract; CF, crude fibre; NFE, nitrogen free extract; NDF, neutral detergent fibre; ADF, acid detergent fibre.

Table 3. Body weight and nutrient intake (kg) of lactating crossbred cows

Parameter	Treatment	
	T ₁	T ₂
Initial body weight (kg)	455.21±14.84	459.55±12.58
Final body weight (kg)	450.28±14.68	454.84±12.56
Change in body weight (kg)	-4.93±0.40	-4.71±0.42
DM intake (kg/day)	14.16±0.54	15.15±0.45
CP intake (kg/day)	1.559±0.06	1.669±0.05
DCP intake (kg/day)	0.854±0.03	0.914±0.03
TDN intake (kg/day)	8.190±0.31	8.764±0.26
DM (kg/100 kg body weight)	3.16±0.14	3.34±0.10
CP (kg/100 kg body weight)	0.348±0.02	0.367±0.01
DCP (kg/100 kg body weight)	0.191±0.01	0.201±0.01
TDN (kg/100 kg body weight)	1.829±0.08	1.930±0.06
DM (g/kg W ^{0.75})	145.34±5.96	153.96±4.16
CP (g/kg W ^{0.75})	16.00±0.66	16.96±0.46
DCP (g/kg W ^{0.75})	8.76±0.36	9.28±0.25
TDN (g/kg W ^{0.75})	84.05±3.45	89.04±2.40

Mean without superscript within parameter differ non-significantly (P>0.05). HF, Holstein Friesian; DM, dry matter; CP, crude protein; DCP, digestible crude protein; TDN, total digestible nutrients.

In the present study, supplementation of fibrolytic enzymes @ 240 mg/kg TMR resulted in improvement in DM and nutrients (CP, DCP and TDN) intake on daily total, per cent body weight and metabolic body weight basis. The nutrients intake was higher ($P>0.05$) on feeding TMR supplemented with EFE [8,000 units endo-1,4- β glucanase, 18,000 units endo-1,3 (4)- β glucanase and 26,000 units 1,4- β xylanase/ml] @ 3.8 and 3.9 ml/kg DM to early and mid-lactating Holstein cows, respectively as reported by Peters *et al.* (2015). Same trend on nutrients intake was reported by Morsy *et al.* (2015) in multiparous Egyptian buffaloes.

Milk and components yield: The crossbred cows fed EFE supplemented TMR produced 12.34% more milk ($P>0.05$) and 15.70% more 4% FCM ($P<0.05$) in comparison to crossbred cows fed control TMR (Table 4). The FCM yield curve (Fig. 1) of EFE group cows remained higher throughout the experiment. The statistical analysis revealed that milk fat ($P<0.01$), SNF ($P<0.05$), and TS ($P<0.01$) contents and daily milk fat yield ($P<0.05$) of HF crossbred cows fed EFE supplemented TMR were higher in comparison to cows fed control TMR, whereas protein and lactose yield difference between the two groups were non-significant ($P>0.05$).

Table 4. Daily milk and components yield (kg) of lactating crossbred cows

Parameter	Treatment	
	T ₁	T ₂
Milk yield (kg/day)	13.37 $\pm$ 0.39	15.02 $\pm$ 0.47
FCM yield (kg/day)	12.80 $\pm$ 0.68	14.81 $\pm$ 0.67*
Milk fat %	3.75 $\pm$ 0.03	3.94 $\pm$ 0.03**
Milk SNF %	8.99 $\pm$ 0.01	9.20 $\pm$ 0.02*
Milk TS %	12.74 $\pm$ 0.08	13.14 $\pm$ 0.05**
Milk protein %	3.28 $\pm$ 0.01	3.31 $\pm$ 0.01
Milk lactose %	5.04 $\pm$ 0.01	5.06 $\pm$ 0.02
Milk fat (kg/day)	0.50 $\pm$ 0.02	0.59 $\pm$ 0.03*
Milk SNF (kg/day)	1.20 $\pm$ 0.07	1.38 $\pm$ 0.06
Milk TS (kg/day)	1.70 $\pm$ 0.09	1.97 $\pm$ 0.09
Milk protein (kg/day)	0.44 $\pm$ 0.01	0.50 $\pm$ 0.01
Milk lactose (kg/day)	0.67 $\pm$ 0.02	0.76 $\pm$ 0.02

^{a,b}Mean with different superscript in a row differ significantly ($P<0.05$, $P<0.01$). FCM, fat corrected milk; SNF, solid not-fat; TS, total solid.

The milk fat, SNF and TS contents and yield of FCM and milk fat were significantly improved owing to better nutrient availability due to EFE supplementation as body weight changes of cows were similar under both treatments. The significant improvement in milk fat and numerical improvement in milk SNF, TS, protein and lactose content was reported on feeding fibrolytic enzymes @ 1 g/kg DM (Bassiouni *et al.* 2010) and @ 15 g/animal/day (El-Bordeny *et al.* 2015) to lactating Holstein Friesian cows. Same trend was also reported in buffaloes by Gaafar *et al.* (2010).

Feed conversion efficiency and cost of feeding: The DM, CP, DCP and TDN intake to produce each kg whole milk

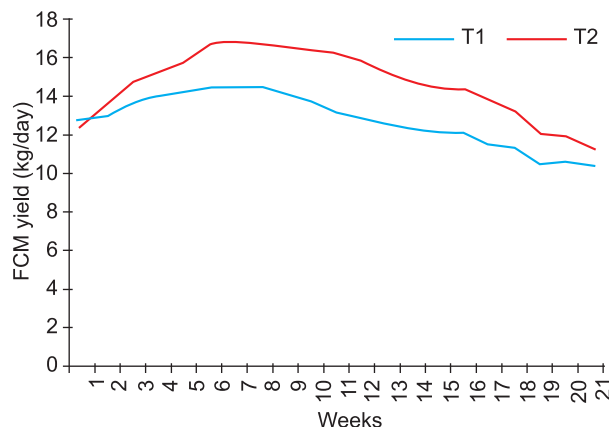


Fig. 1. 4% Fat corrected milk (FCM) of crossbred cow.

Table 5. Feed conversion efficiency of lactating crossbred cows

Parameter	Treatment	
	T ₁	T ₂
DM intake (kg/kg milk)	1.071 $\pm$ 0.03	1.015 $\pm$ 0.01
CP intake (g/kg milk)	117.96 $\pm$ 2.76	111.77 $\pm$ 1.63
DCP intake (g/kg milk)	64.60 $\pm$ 1.51	61.21 $\pm$ 0.89
TDN intake (kg/kg milk)	0.620 $\pm$ 0.01	0.587 $\pm$ 0.01
DM intake (kg/kg FCM)	1.115 $\pm$ 0.02	1.028 $\pm$ 0.01**
CP intake (g/kg FCM)	122.76 $\pm$ 2.33	113.22 $\pm$ 1.40**
DCP intake (g/kg FCM)	67.23 $\pm$ 1.28	62.01 $\pm$ 1.40**
TDN intake (kg/kg FCM)	0.645 $\pm$ 0.01	0.595 $\pm$ 0.01**
Gross protein efficiency (%)	27.92 $\pm$ 0.69	29.64 $\pm$ 0.43**
Gross energy efficiency (%)	26.52 $\pm$ 0.47	28.71 $\pm$ 0.39**
Net protein efficiency (%)	50.97 $\pm$ 1.25	54.11 $\pm$ 0.79*
Net energy efficiency (%)	46.37 $\pm$ 0.77	47.70 $\pm$ 0.37

^{a,b}Mean with different superscript differ significantly ($P<0.05$, ** $P<0.01$). DM, Dry matter; CP, crude protein; DCP, digestible crude protein; TDN, total digestible nutrients.

Table 6. Costs of feeding of HF crossbred cows

Parameter	Treatment		Improvement over control (%)
	T ₁	T ₂	
Cost of feeding (₹/cow/day)	172.06 $\pm$ 6.54	190.24 $\pm$ 5.70	-10.56
Return from sale of milk (₹/cow/day)	365.20 $\pm$ 20.08	423.16 $\pm$ 19.13	15.87
Ratio of return over feed cost (₹)	2.11 $\pm$ 0.05	2.22 $\pm$ 0.03	5.21

HF, Holstein Friesian; EFE, exogenous fibrolytic enzymes.

was numerically improved ($P>0.05$) in EFE supplemented TMR group [Table 5], whereas improvement was significant ($P<0.01$) on 4% FCM production. The gross protein and energetic efficiency improved ($P<0.01$) by 29.64 and 26.52% on feeding TMR supplemented with EFE @ 240 mg/kg TMR. The net protein efficiency improved significantly (3.14%) under EFE supplemented group (T₂). The returns over feed cost was ₹ 0.11 higher for cows fed EFE supplemented TMR in comparison to cows fed control TMR.

The milk production efficiency and return over feed cost improved on supplementation of exogenous fibrolytic enzymes in the present study. The milk production efficiency improved ($P < 0.05$) linearly with increasing level of enzyme supplementation (Holtshausen *et al.* 2011). An improved ($P = 0.001$) milk:feed ratio was observed in Holstein dairy cows on feeding TMR supplemented with exogenous enzymes @ 15 g/cow/day during 12 weeks trial period (Mohamed *et al.* 2013). Whereas the milk production efficiency was statistically not affected when cows were fed TMR supplemented with fibrolytic enzymes (Miachio and Thakur 2007). Significantly ($P < 0.01$) higher nitrogen utilization efficiency (18.70 vs. 16.32%) of Holstein cows was reported by El-Bordeny *et al.* (2015) on feeding TMR supplemented with fibrolytic enzyme. Titi (2003) reported significantly ($P = 0.02$) higher income over cost on feeding EFE (150 g/t forage) supplemented diet in lactating Friesian cows. The improved ratio of income over feed cost might be due to improved energy availability and efficient utilization of nutrients. The exogenous fibrolytic enzymes feeding release energy that may be utilized for milk yield during early lactation or storage of energy as body fat during late lactation or dry period in dairy cattle. The feeding of exogenous fibrolytic enzymes to crossbred cows after peak lactation (Lunagariya *et al.* 2017b) was without improvement in milk yield and feed conversion efficiency; but the higher body weight gain of cows (6.17 kg more) at the end of experiment and that resulted in improved calf weight (+2.50 kg) even though having more female calf (female to male ratio 5:3 vs. 3:5).

The supplementation of EFE @ 240 mg/kg TMR resulted in significantly more 4% FCM and fat yield as well as improvement in milk fat, SNF and TS contents. The DM and nutrients intake were numerically higher with similar body weight change. The gross protein and energy utilization were also significantly superior for cows fed EFE supplemented TMR. The supplementation of EFE might have released more energy which reflected in increase 4% FCM yield. The ratio of income over feed cost of cows fed EFE supplemented TMR was improved over control TMR. In conclusion, feeding of EFE @ 240 mg/kg TMR in early lactating HF crossbred cows resulted in higher 4% FCM yield, milk fat yield, feed conversion efficiency and better economic returns.

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