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Effect of exogenous fibrolytic enzymes supplementation to improve voluntary intake, availability of nutrients and growth performance in weaned crossbred calves

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ABSTRACT

The present study was carried out to evaluate the effect of supplementation of exogenous fibrolytic enzymes (EFE) on voluntary feed intake, availability of different nutrients and growth performance in weaned crossbred calves. Fifteen weaned healthy female Jersey crossbred calves (weight 79.73±3.46 kg; age 234.5±11.33 days) were divided equally into three groups viz., Control (T_0), Treatment-1 (T_1) and Treatment-2 (T_2). Growth trial was conducted on these calves for 90 days. Animals in each treatment group were fed *ad lib.* total mixed ration (TMR) (CP 12%, TDN 65%) individually for 90 days. Calves under control (T_0) group were fed *ad lib.* TMR (without EFE). Calves under T_1 and T_2 were fed *ad lib.* TMR supplemented with EFE cellulase and xylanase @ 8000 and 16000 IU/kg DM of TMR and with EFE cellulase and xylanase @ 12000 and 24000 IU/kg DM of TMR, respectively. Significantly greater DMI per unit body weight (kg/100 kg BW and g/kg $W^{0.75}$) was recorded in enzyme supplemented groups compared to control group; however, difference between T_1 and T_2 was non-significant. Addition of EFE in the TMR diet of calves increased TDN and DCP intake (g/kg $W^{0.75}$) in T_1 and T_2 than T_0. Supplementation of EFE also enhanced TDN (%), DCP (%), DNDF (%), DE (Mcal/kg of feed) and ME (Mcal/kg of feed) value of the diets under T_1 and T_2 than T_0. Average daily weight gain (g/d/calf) was estimated greater in T_1 and T_2 than T_0. The feed conversion efficiency was similar among three treatments. Whereas, plasma total protein and albumin concentration increased significantly due to supplementation of EFE in the diet of calves. Therefore, it may be concluded that supplementation of exogenous fibrolytic enzymes (cellulase and xylanase @ 8000 and 16000 IU/kg TMR DM) may boost growth potential of finisher Jersey crossbred calves with enhanced intake of nutrients.

Keywords: Calves, Exogenous fibrolytic enzymes, Growth, Glucose, Intake, Nutrients

Ruminants in the tropical and subtropical regions of the world largely depend on forage plants as an important source of nutrients. The digestibility and available energy of the fibre is low because the cell wall of the plant is an interwoven matrix of polymers that forms dynamic and complex structures (Hatfield *et al*. 1999). These complex structures are regarded to be hurdles for microbial invasion and also limit their access to digestible plant cell wall networks. The amounts of cellulose, hemicellulose, lignin, pectin, and minerals in forage cell walls vary depending on the species and growing stage of the plant (Carrillo-Díaz *et al*. 2022).

Therefore, efficient use of fibrous feed in the livestock production system is limited (Hatfield *et al*. 1999) in spite of having several processing techniques. The use of exogenous fibrolytic enzymes (EFE) as a biological treatment method has got more attention by researchers (McAllister *et al*. 2003, Sujani and Seresinhe 2015, Iannaccone *et al*. 2022). The exogenous enzymes used in ruminant diet are from fungal (largely *Trichoderma longibrachiatum*, *Aspergillus oryzae* and *Aspergillus niger*); bacterial (*Bacillus* spp., *Penicillium funiculosum*) or rumen bacterial (Gado *et al*. 2009) origin with high cellulase and hemicellulase activity. Cellulases (β-glucosidases, endo-β-glucanases and exo-β-glucanases or cellobiohydrolases) and xylanases (endo-β-xylanases, β-xylosidases, arabinofurosidases, glucuronidases and acetylxylanesterases) are the enzymes which release soluble sugars by breaking links in cellulose and hemicellulose (Tirado-González *et al*. 2021). Several researchers reported increase in fibre digestion due to supplementation of EFE; consequently, improved nutrient utilization pattern in large ruminants (Kady *et al*. 2006, Shekhar *et al*. 2010, Salem *et al*. 2013) as well as in small ruminants (Sheikh *et al*. 2017, El-Bordeny *et al*. 2017, Abid *et al*. 2020). Some studies showed EFE in the ruminant diet increased animal performance in terms of greater body weight gain (Wang *et al*. 2018, Lourenco *et al*. 2020, Mousa *et al*. 2022). Meanwhile, few studies

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(Bueno *et al*. 2013, Tewoldebrhan *et al*. 2017, Hassan and Almaamory 2019) reported either a negative or no effect on nutrient utilization and animal performance due to addition of EFE in the ruminant diet. Therefore, the study was carried out to observe the effect of supplementation of exogenous fibrolytic enzymes (EFE) on voluntary nutrients intake, availability of different nutrients, blood parameters and growth performance in weaned crossbred calves reared under intensive system of feeding.

MATERIALS AND METHODS

Site of the experiment: Animal experiment was conducted in the cattle yard complex of Eastern Regional Station of ICAR-National Dairy Research Institute (NDRI), Kalyani, West Bengal, India as per the committee approval of ICAR-NDRI (19-M-AN-06) and accordingly all ethical protocol was followed during the animal trial period. The cattle yard is situated at an altitude of 9.75 m (31.9 feet) above mean sea level, 22°58’30”N latitude and 88°26’04”E longitude. The minimum and maximum ambient temperature of the study area was recorded 7°C and 39°C during winter and summer seasons, respectively. The average annual rainfall was 1500 mm. So, the average weather condition of lower gangetic region of India is hot and humid.

Experimental animals and diets: Fifteen weaned healthy female crossbred Jersey calves (weight 79.73±3.46 kg; age 234.5±11.33 days) were divided equally into three groups based on their body weight and age; namely, Control (T_0), Treatment-1 (T_1) and Treatment-2 (T_2). All the experimental animals were vaccinated against foot and mouth disease, haemorrhagic septicaemia and anthrax, and were also dewormed with ivermectin and albendazole before starting of the experiment. All calves were housed individually in well-ventilated experimental shed under uniform managemental conditions.

Exogenous fibrolytic enzymes: Two individual preparations of Exogenous Fibrolytic Enzymes (EFE, cellulase and xylanase, derived from fungal sp. *Aspergillus niger*) in powder forms (Brisk Bioscience Ltd., Surat, India) were used for the experiment. The activity of enzyme powders was 5,00,000 IU/g for cellulase and 1,00,000 IU/g for xylanase.

Feeding of experimental animals: Animals in each experimental group were fed *ad lib.* total mixed ration (TMR) (CP 12%, TDN 65%) individually for 90 days. The TMR (Table 1) was prepared with concentrate mixture, chaffed paddy straw and oat fodder at the ratio of 40:30:30 (on DM basis).

Control group (T_0): Calves were fed *ad lib.* TMR without EFE supplementation.

Treatment-1 (T_1): Calves were fed *ad lib.* TMR supplemented with EFE cellulase and xylanase @ 8000 and 16000 IU/kg DM of TMR.

Treatment-2 (T_2): Calves were fed *ad lib.* TMR supplemented with EFE cellulase and xylanase @ 12000 and 24000 IU/kg DM of TMR.

Table 1. Ingredient composition (on % DM basis) of experimental total mixed ration (TMR)

Ingredient	Treatment		
	T_0	T_1	T_2
Maize	14.0	14.0	14.0
Wheat bran	8.4	8.4	8.4
GNC	8.4	8.4	8.4
MOC	8.0	8.0	8.0
Mineral mixture	0.8	0.8	0.8
Salt	0.4	0.4	0.4
Oat fodder	30.0	30.0	30.0
Paddy straw	30.0	30.0	30.0
Cellulase (IU/kg DM of TMR)	-	8000	12000
Xylanase (IU/kg DM of TMR)	-	16000	24000

Growth and digestion trial: Growth and intake of calves were recorded during the experimental period following the standard practices. Clean and fresh drinking water was offered *ad lib.* to all the animals during entire experimental period. Intake of feeds was measured on day-to-day basis using electronic balance. Individual calf’s weight before feeding (08.00-09.00 h) was recorded fortnightly using platform electronic balance to measure the body weight gain.

A digestion trial was conducted with 6 days collection period after completion of 75 days of the growth trial to assess the availability of different nutrients. Faces voided by individual calf were collected; weighed daily at 9:00 AM and representative samples of feed offered, residue left and faeces voided were drawn for chemical analysis. The N content in feeds, residues and faeces were analyzed in accordance with Micro-Kjeldahl method (AOAC 2012). Samples of TMR offered and residues left by each animal were analyzed for proximate (AOAC 2012) and cell wall components (Van Soest *et al*. 1991). The digestible energy (DE) value (Mcal/kg DM) of feed was calculated as per the following recommendation of NRC (2001):

$$\text{DE (Mcal/kg of feed)} = 0.04409 \times \text{TDN (\%)}$$

The metabolizable energy (ME) value (Mcal/kg DM) of feed was calculated as per Ibidhi *et al*. (2021) using the following formula,

$$\text{ME (Mcal/kg of feed)} = 0.9215 \times \text{DE} - 0.1434$$

Blood analysis: Blood samples were collected aseptically on 0, 45 and at 90th day of the growth trial at around 9.00 AM before offering feed and water. The plasma obtained after processing of blood was transferred to labelled vials and stored in a Deep Freezer at -20°C for estimation of blood biochemical parameters using commercial kits (Span Cogent Diagnostic Ltd., India). Blood plasma was determined for glucose, total protein, albumin and blood urea. Globulin was calculated by the difference method (total protein-albumin).

Statistical analysis: Data related to intake of nutrients, growth performance, feed conversion efficiency and blood parameters were analyzed by a mixed analysis of variance (ANOVA) with repeated measures, with treatment was as fixed factor (between subject effect), time period as (within subject) random factor having subject (calf) nested within it and their (period × treatment) interaction. Data in relation to digestion trial were analyzed by one-way ANOVA. Computerized IBM SPSS 20.0 package was used for ANOVA. Tukey's HSD test was used to measure the differences of means with P<0.05.

RESULTS AND DISCUSSION

Chemical composition of feeds: The values of the chemical composition of different feeds fodders are given in Table 2. CP content (%) in different feeds varied from 3.45 in paddy straw to 20.83 in concentrate mixture (Table 2). The concentration of NDF (%) and ADF (%) in the TMR were 33.34 and 23.74, respectively.

Table 2. Chemical composition (on % DM basis) of feeds and forages

Parameter	Concentrate mixture	Oat fodder	Paddy straw	Total mixed ration
DM	90.18	24.58	90.73	46.35
OM	93.58	92.13	85.08	89.16
CP	20.83	7.12	3.45	11.25
EE	4.78	1.89	1.29	2.94
TCHO	67.97	83.12	80.34	74.97
Total ash	6.42	7.87	14.92	10.84
NDF	32.67	66.33	78.39	57.08
ADF	11.29	38.33	53.34	33.34
Hemicellulose	21.38	28.00	25.05	23.74
Cellulose	8.06	31.67	44.63	28.34
Lignin	3.23	6.67	8.71	5.00

Each value is the average of duplicate analysis on dry matter basis. DM-Dry matter, OM-Organic matter, CP-Crude protein, EE-Ether extract, TCHO-Total carbohydrate, NDF-Neutral detergent fibre, ADF-Acid detergent fibre.

Voluntary intake and availability of nutrients: The results of total DMI (kg/d/calf) revealed no significant (P>0.05) difference among three treatments (Table 3). However, there was significant (P<0.001) variation in TDMI among different fortnights indicating the periodic effect; which was mainly due to body weight gain by growing calves over different fortnights. Furthermore, interaction effect of treatment×period was similar among different groups. The average DMI (kg/100 kg BW) for the entire experimental period was 3.09, 3.25 and 3.26 in control, T_1 and T_2 groups, respectively. Significantly (P<0.05) greater DMI per unit body weight (kg/100 kg BW and g/kg $W^{0.75}$) was recorded in enzyme supplemented groups compared to control group; however, difference between T_1 and T_2 was non-significant. Periodic effect (P) and interaction between T×P was similar for DMI per unit body weight (kg/100 kg BW and g/kg $W^{0.75}$).

Similarly, supplementation of EFE did not alter the OM intake (kg/d/calf) among different groups (Table 3). But, OM intake (g)/kg $W^{0.75}$ was greater (P<0.05) in calves under T_1 and T_2 than T_0. Furthermore, periodic effect (P) and interaction effect (T×P) were non-significant for OM intake (kg/d/calf and g/kg $W^{0.75}$); the trend was similar to DM intake. Total CP intake was similar among three treatments (Table 3). However, CP intake (g)/kg $W^{0.75}$ increased (P<0.05) from 10.98 (T_0) to 11.74 (T_2) due to EFE supplementation in the TMR of calves. Furthermore, periodic (P) and interaction (T×P) effects on CP intake were non-significant (P>0.05).

Supplementation of EFE in the TMR diet of calves increased (P<0.01) DCP intake (g/kg $W^{0.75}$) in T_1 and T_2 than T_0 (Table 4). Similarly, intake (g/kg $W^{0.75}$) of TDN and digestible NDF was also significantly greater (P<0.001) in T_1 and T_2 than T_0. Similar trend was also observed in respect of total TDN and digestible NDF intakes. DE and ME intakes (Mcal/kg $W^{0.75}$) were also increased (P<0.001) due to addition of EFE in T_1 and T_2 than non supplemented T_0. Supplementation of EFE also resulted enhanced TDN (%), DCP (%), DNDF (%), DE (Mcal/kg of feed) and ME (Mcal/kg of feed) value of the diets under T_1 and T_2 than T_0 (Table 4).

Supplementation of EFE (mixed form of cellulase and xylanase) increased DMI, OMI and CPI per kg $W^{0.75}$ by 6.20 and 6.86; 6.32 and 7.06; and 6.10 and 6.92% in T_1 and T_2 groups, respectively. The increase in feed intake could be due to the improvement in palatability of feeds (Beauchemin *et al*. 2000, Sheikh *et al.* 2017) and increase in the rate of fibre degradation by acting synergistically with rumen microorganisms (Gado *et al*. 2009, Abid *et al*. 2020). Romero *et al.* (2016) also reported that supplementation of xylanase plus @ 1 ml/kg DM of TMR increased the DMI, OMI and CPI (kg/d) in Holstein cows compared to control and cellulase plus xylanase mixture supplemented group (3.4 ml/kg DM of TMR). Similarly, Sheikh *et al*. (2017) observed that addition of exogenous enzyme in the urea molasses treated paddy straw increased DM intake by Corriedale sheep. Whereas, Khademi *et al.* (2022) reported that supplementation of EFE to wheat straw had no positive response on starter feed intake in dairy calves, but increased neutral detergent fber (NDF) digestibility. Higher intakes of TDN and DCP was observed in Barkey lambs fed with wheat straw based ration added with 2.5 g of EFE compared to control (El-Bordeny *et al*. 2017). Lunagariya *et al.* (2019) also recorded greater DCP and TDN intake in lactating HF crossbred cows due to supplementation of commercial EFE (Roxozyme GT®) @ 240 mg/kg TMR. Similarly, Abid *et al*. (2020) observed that feeding olive cake (OC) sprayed with cellulase and xylanase mixture (50:50 by volume) @ 4 (CX04) or 16 (CX16) ml per kg OC DM significantly increased DM, OM and ME intake in both the groups compared to control without showing any significant difference between enzyme treated groups.

In contrast, some studies reported that supplementation of exogenous fibrolytic enzymes resulted no additional benefits on nutrient intake in buffalo male calves (Kady *et al*. 2006), Baladi Friesian steers (Salem *et al*. 2013), Granadina dairy goats (González-Garcia *et al.* 2008) and lambs (Sakita *et al.* 2022).

Table 3. Voluntary nutrients intake, growth performance and blood metabolites as affected by exogenous fibrolytic enzymes supplementation in crossbred calves

Parameter	T_1	T_2	T_3	SEM	P value		
					Treatment (T)	Period (P)	T × P interaction
Dry matter intake (DMI)							
DMI (kg/d)	3.14	3.39	3.45	0.07	P = 0.110	P < 0.001	P = 1.000
DMI (kg/100 kg BW)	3.09^a	3.25^b	3.26^b	0.03	P = 0.036	P = 0.084	P = 1.000
DMI (g/kg $W^{0.75}$)	97.56^a	103.61^b	104.25^b	1.07	P = 0.021	P = 0.060	P = 1.000
Organic matter intake (OMI)							
OMI (kg/d)	2.80	3.03	3.08	0.06	P = 0.101	P < 0.001	P = 1.000
OMI (g/kg $W^{0.75}$)	86.93^a	92.42^b	93.07^b	0.96	P = 0.017	P = 0.060	P = 1.000
Crude protein intake (CPI)							
CPI (g/d)	353.17	381.27	388.42	7.98	P = 0.108	P < 0.001	P = 1.000
CPI (g/kg $W^{0.75}$)	10.98^a	11.65^b	11.74^b	0.12	P = 0.020	P = 0.060	P = 1.000
Body weight (BW) gain and Feed conversion efficiency (FCE)							
Initial BW (kg)	80.15	79.25	79.80	3.46	0.995	-	-
Final BW (kg)	118.31	123.01	124.05	3.89	0.836	-	-
Av. BW (kg)	102.57	104.53	105.30	1.94	P = 0.778	P < 0.001	P = 1.000
BW gain (kg/fortnight)	6.36^a	7.29^b	7.37^b	0.09	P < 0.001	P = 0.964	P = 0.992
ADG (g/d/calf)	424.02^a	486.22^b	491.68^b	6.13	P < 0.001	P = 0.964	P = 0.992
FCE (%)	13.80	14.67	14.87	0.28	P = 0.187	P < 0.001	P = 1.000
Blood metabolites							
Blood glucose (mg/dl)	62.39	64.13	63.33	0.41	P = 0.243	P = 0.282	P = 0.793
Total protein (g/dl)	5.83^a	5.97^b	6.02^b	0.03	P = 0.020	P = 0.002	P = 0.499
Albumin (g/dl)	3.30^a	3.42^b	3.46^b	0.02	P = 0.006	P = 0.152	P = 0.683
Globulin (g/dl)	2.53	2.55	2.56	0.03	P = 0.941	P = 0.077	P = 0.972
Blood urea nitrogen (mg/dl)	13.25	12.91	12.85	0.16	P = 0.578	P = 0.365	P = 0.813

In the present study, addition of mixed fibrolytic enzymes enhanced voluntary nutrients intake (DM, OM and CP) and availability of different nutrients (DCP, TDN, digestible NDF, DE and ME) per kg $W^{0.75}$ of crossbred calves fed with TMR containing roughage: concentrate (60:40) in the present study. However, higher dose of these enzymes (T_2) has no additional benefit on voluntary intake and availability of different nutrients.

Growth performance: The final body weight (kg) of calves at the end of 90 days experimentation was 118.31, 123.01 and 124.05 kg in control (T_0), T_1 and T_2 groups, respectively; the difference among treatments was non-significant (Table 3 and Fig. 1). Average fortnightly body weight gain of calves (kg/fortnight) increased significantly (P<0.001) in T_1 (7.29) and T_2 (7.37) groups compared to control T_0 (6.36). Similarly, average daily body weight gain (ADG, g/d/calf) were estimated greater (P<0.001) in T_1 (486.22) and T_2 (491.68) than T_0 (424.02). However, no difference was recorded between T_1 and T_2. Weight of the animals increased as the age was advancing, so periodic (P) effect for total body weight gain was significant (P<0.001) among three groups. However, periodic (P) and interaction (P×T) effects for fortnightly weight gain and ADG were similar among different treatments.

The feed conversion efficiency (FCE, kg live weight gain/100 kg DMI) was 13.80, 14.67 and 14.87 in control (T_0), T_1 and T_2 groups, respectively. However, the difference among three treatments was non-significant (Table 3).

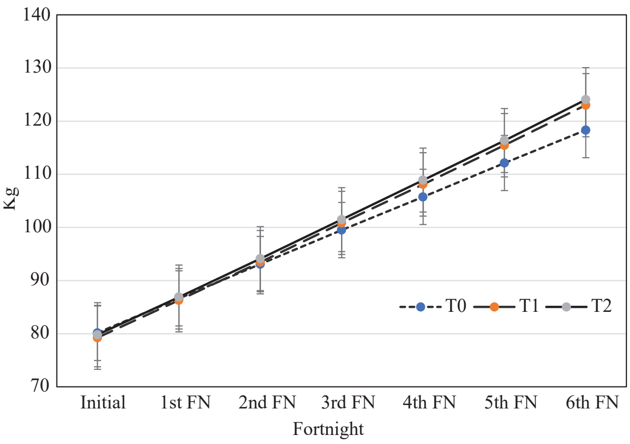

Fig. 1. Effect of exogenous fibrolytic enzymes on growth of crossbred calves.

Although, the periodic effect was significant (P<0.001); but the interaction effect between treatment and period (T×P) was similar.

Supplementation of EFE to calves marginally improved final body weight in T_1 (3.97%) and T_2 (4.85%) than T_0; however, the difference was non-significant. Whereas, calves increased their body weight gain (kg/fortnightly) by 14.62 and 15.88% in T_1 and T_2 groups compared to the control group. Similarly, ADG (g/d/calf) was greater by 14.67 and 15.96% due to supplementation of mixed EFE to calves in T_1 and T_2 compared to T_0. Added EFE (in TMR)

Table 4. Availabilities of different nutrients in crossbred calves under different treatments

Attribute	Group			SEM	P value
	T_0	T_1	T_2		
DCP intake (DCPI)					
DCPI (g/d)	284.73[a]	324.94[b]	328.56[b]	7.22	0.021
DCPI (g/kg $W^{0.75}$)	8.20[a]	9.21[b]	9.18[b]	0.14	0.003
TDN intake (TDNI)					
TDNI (kg/d)	2.09[a]	2.48[b]	2.53[b]	0.06	0.002
TDNI (g/kg $W^{0.75}$)	60.18[a]	70.15[b]	70.79[b]	1.15	<0.001
Digestible NDF intake (DNDFI)					
DNDFI (kg/d)	0.99[a]	1.20[b]	1.22[b]	0.04	0.014
DNDFI (g/d/kg $W^{0.75}$)	28.32[a]	33.92[b]	34.08[b]	0.85	0.006
DE intake (Mcal/d/calf)					
DE intake (Mcal/d)	9.22[a]	10.92[b]	11.17[b]	0.26	0.002
DE intake (Mcal/kg $W^{0.75}$)	0.27[a]	0.31[b]	0.31[b]	0.01	P<0.001
ME intake (Mcal/d/calf)					
ME intake (Mcal/d)	8.35[a]	9.92[b]	10.15[b]	0.24	0.002
ME intake (Mcal/kg $W^{0.75}$)	0.24[a]	0.28[b]	0.28[b]	0.01	<0.001
Nutritive value of diets					
TDN%	57.51[a]	61.22[b]	61.97[c]	0.21	<0.001
DCP%	7.85[a]	8.04[b]	8.05[b]	0.02	<0.001
DNDF%	26.74[a]	29.39[b]	29.57[b]	0.42	0.007
DE (Mcal/kg feed)	2.54[a]	2.70[b]	2.73[b]	0.01	P<0.001
ME (Mcal/kg Feed)	2.29[a]	2.45[b]	2.48[b]	0.01	P<0.001

DCP, Digestible crude protein; TDN, Total digestible nutrients; DNDF, Digestible neutral detergent fibre; DE, Digestible energy; ME, Metabolizable energy. Values with different superscripts (a, b, c) in a row are significantly different.

resulted marginal improvement in FCE by 6.30 and 7.70% in T_1 and T_2 groups compared to the control group; however, variation among treatments was similar. However, higher doses of cellulase (12000 IU/kg DM of TMR) and xylanase (24000 IU/kg DM of TMR) did not translate any additional benefit on growth performance in calves.

Significant increase in live weight gain of crossbred calves fed with mixed EFE supplemented TMR in the present study could be due to increased feed intake and digestibility of nutrients (Salem *et al*. 2013, Marwan *et al*. 2019, Abid *et al.* 2020). Voluntary intake (DM, OM and CP) and availability of different nutrients (TDN, DCP, DE and ME) were also increased in the present study. Hence, greater growth performance was recorded in the treated calves. Results of the present study are in agreement with the findings of earlier researchers. Addition of commercially available enzyme (ZADOs) @ 40 g/d/crossbred Baladi Friesian steers increased the live weight gain (kg/d) and feed conversion ratio (FCR) in treated group compared to control (Salem *et al*. 2013). Supplementation of Calfo Care® (Probiotics and enzymes) to Ossimi lambs @ 0.5, 1, and 2 kg/tonne diet DM significantly enhanced total weight gain compared to control animals (Mousa *et al.* 2022). Similarly, Sheikh *et al*. (2017) reported greater ADG (g/d/animal) and final body weight in Corriedale sheep when supplemented with exogenous enzyme (9 g/kg DM) through urea molasses treated paddy straw. Supplementation of endo-1, 4-β-xylanase enzyme to cow-calf herd @ 13,800 fungal xylanase units/kg DM significantly improved ADG and final body weight gain (Lourenco *et al.* 2020). Likewise, feeding tifton-85 hay treated with fibrolytic enzymes extract before 24 h resulted in higher body weight gain in lambs (Sakita *et al*. 2022). Marwan *et al*. (2019) revealed significantly greater FCR (kg DM/kg gain of buffalo calf) in Zymogen (EFE) supplemented group than the control group. Lourenco *et al*. (2020) also reported marginal increase in feed conversion (gain: feed ratio) in beef cattle due to xylanase supplementation to the creep feed.

Contrary to our findings, Patel (2012) and Bueno *et al*. (2013) revealed that supplementation of exogenous fibrolytic enzyme had no additional advantage on final body weight (kg) or ADG in sheep. Similar results were also recorded by Wang *et al*. (2018) and Hassan and Almaamory (2019) who supplemented fibrolytic enzyme (FE) to Holstein bull calves or to Awassi lambs. Likewise, supplementation of fibrolytic enzyme (@ 0.75 ml/kg DM of feed) did not alter ADG in steers (Williams *et al*. 2021).

Blood metabolites: Blood glucose (mg/dl) concentration ranged from 62.39 (T0) to 64.13 (T1); however, the difference was non-significant (Table 3). Whereas, plasma total protein (P<0.05) and albumin (P<0.001) concentration increased significantly due to supplementation of EFE in the diet of calves. The values of plasma total protein and albumin ranged from 5.83 (T_0) to 6.02 (T_2) and 3.30 (T_0) to 3.46 (T_2). Globulin and blood urea nitrogen (BUN) concentration (mg/dl) were similar among three treatments. However, total protein and albumin contents were not affected by the level of EFE supplemented in TMR. Periodic effect was significant ($P<0.001$) only for plasma total protein. However, interaction (T×P) effect was non-significant for all plasma parameters studied.

Supplementation of mixed EFE with two doses did not alter the blood glucose (mg/dl) concentration in crossbred calves. The reason for no changes in blood glucose level in the present study could be due to higher metabolic rate of glucose utilization for growth of animals and also due to homeostatic mechanism of the animal's body. Similar to our findings, Shekhar *et al*. (2010) observed no significant difference in blood glucose concentration of lactating Murrah buffaloes supplemented with exogenous fibrolytic enzymes @ 1.5 and 3.0 g/kg DM of the concentrate.

Blood total protein and albumin concentration were greater in EFE supplemented calves; whereas, blood globulin (g/dl) and blood urea nitrogen concentration remained unaffected due to such enzyme addition in the diet. The increase in blood protein (total protein and albumin) level could be due to greater availability of different nutrients (DCP, TDN and ME) in EFE supplemented calves. The results of the present study corroborated with the findings of earlier workers. Kholif *et al*. (2022) observed that both enzyme and probiotics treatments increased the levels of serum total protein and albumin in lactating Farafra ewes. Feeding of complete feed prepared with urea molasses treated paddy straw and exogenous enzyme (9 g/kg DM) resulted greater total blood protein (g/dl) concentration in Corriedale sheep compared with control, but had no effect on blood glucose (Sheikh *et al*. 2017). Similarly, supplementation of enzyme mixture Zymogen liquid @ 12 ml/100kg body weight resulted significant increase in total protein (g/dl) and albumin (g/dl) level, but had no effect on globulin level in buffalo calves (Marwan *et al*. 2019).

Contrary to our findings, Mohamed *et al*. (2013) revealed that supplementation of EFE @ 15 g/d/animal to Holstein dairy cows significantly decreased globulin level, but there was no significant difference on blood glucose level. Supplementation of EFE did not alter BUN concentration in Sheep (El-Bordeny *et al*. 2017, Sheikh *et al*. 2017), buffalo male calves (Kady *et al*. 2006), Holstein dairy cows (Mohamed *et al*. 2013) and lactating Murrah buffaloes (Shekhar *et al*. 2010).

Supplementation of exogenous fibrolytic enzymes (cellulase and xylanase @ 8000 and 16000 IU/kg TMR DM; and12000 and 24000 IU/kg TMR DM) increased voluntary intake (DM, OM and CP), availability of different nutrients (TDN, DCP, Digestible NDF, DE and ME), nutritive value of feed (TDN%, DCP%, Digestible NDF%, DE (Mcal/kg feed) and ME (Mcal/kg feed)), blood total protein and albumin concentration, and ADG (g/d/calf) in weaned Jersey crossbred calves. However, such mixed EFE did not alter the feed conversion efficiency in calves. Higher level of EFE (12000 and 24000 IU/kg TMR DM) had no added advantage on voluntary intake, digestible nutrients intake, blood parameters and growth performance in calves. Therefore, it may be concluded that supplementation of exogenous fibrolytic enzymes (cellulase and xylanase @ 8000 and 16000 IU/kg TMR DM) may boost growth potential of finisher Jersey crossbred calves with enhanced nutrients intake.

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