

## Effect of exogenous fibrolytic enzymes supplementation on nutrient intake and digestibility in Black Bengal kids

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Received: 25 August 2022 ; Accepted: 28 July 2023

### ABSTRACT

The present study was carried out to evaluate the effect of supplementation of exogenous fibrolytic enzymes (EFE) to the Total Mixed Ration (TMR) at two different levels on intake, nutrient digestibility and nutrient availability in Black Bengal kids. A digestibility trial was conducted on 15 post-weaned Black Bengal kids divided into three groups. Control ( $T_0$ ) group was fed *ad lib*. TMR was prepared from concentrate mixture and green fodder @ 40: 60 on DM basis.  $T_1$  and  $T_2$  groups were supplemented with EFE cellulase and xylanase @ 8000 and 16000 IU/kg TMR DM and 12000 and 24000 IU/kg TMR DM, respectively. The study revealed significantly higher digestibility of nutrients, i.e. DM, OM, EE, NDF, ADF, hemicellulose, cellulose and total carbohydrates in both enzyme supplemented groups than the control group. However, the digestibility of CP, cellulose and hemicellulose was significantly higher only in  $T_2$  group than the control. There was no significant difference between two enzyme supplemented groups in terms of digestibility of different nutrients except for CP which was significantly higher in  $T_2$  than  $T_1$ . The difference in voluntary intake of DM and OM were non-significant but the intake of CP, DCP and TDN were higher in enzyme supplemented groups. Similarly, TDN, DE and ME content of the diet were also higher in two enzyme supplemented groups than control. However, there was no significant difference between  $T_1$  and  $T_2$  in terms of nutrient availability and intake. Based on the present study, supplementation of EFE @ cellulase 8000 and xylanase 16000 IU/ kg DM was found to be optimum for improving the nutrient digestibility and availability in Black Bengal kids, which may further improve the productive and reproductive performance of the animals.

**Keywords:** Black Bengal kids, Digestibility, Exogenous fibrolytic enzymes, Nutrient intake

Ruminants in the tropics and subtropics largely depend on forage plants as important source of nutrients. The fibre occupies the major portion in the dry matter of forages (Mousa *et al.* 2022). 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). Exogenous fibrolytic enzymes (EFE) added to the ruminant diet can increase fiber digestibility and production efficiency. Cellulases (endo- $\beta$ -glucanases, exo- $\beta$ -glucanases or cellobiohydrolases and  $\beta$ -glucosidases) and xylanases (arabino furosides, acetyl xylan esterases, glucuro-nidases,  $\beta$ -xylosidases, and endo- $\beta$ -xylanases) are enzymes that break the links in cellulose and hemicellulose to release soluble sugars (Tirado-González *et al.* 2016). These enzymes hydrolyze components of the cell wall to produce substrates that favor selected populations of microorganisms (Salem *et al.* 2015). Supplementing the ruminant diet with EFE can increase the availability of energy in fibrous feed by improving ruminal fermentation, fiber and DM

degradability and microbial protein synthesis (Valdes *et al.* 2015, Selzer *et al.* 2021).

In India, goat by virtue of its adaptability in diverse agro-climatic condition plays a pivotal role in the economy of the weaker section and 4.2% employment generation has been accounted in goat farming in the rural sector. The Black Bengal goat is a breed typically found throughout Bangladesh, West Bengal, Assam and Odisha (Eastern region). It produces high-quality meat and skin, and is preferred for high prolificacy rate. The livestock sector in India is currently facing major constraints such as limited availability of fodder crops, high cost and lower nutritional quality of available feed resources. Some studies showed that enzyme addition increases nutrient digestibility and enhanced productive animal performance of ruminants (Gado *et al.* 2014, Tirado-González *et al.* 2018, Anil 2021), but others showed only low effects on animal performance (Bueno *et al.* 2013). In recent years, the use of EFE as feed additives in ruminants has drawn significant interest (Aboul-Fotouh *et al.* 2017, Song *et al.* 2018, Lourenco *et al.* 2020, Anil 2021, Carrillo-Díaz *et al.* 2022). The majority of research on supplementation of EFE in ruminants are restricted to large ruminants (Reddy *et al.*

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2016). The present study was carried out with the main objective to evaluate the effect of supplementation of EFE in combination, namely, cellulase and xylanase to the Total Mixed Ration (TMR) at two different levels on voluntary intake, nutrient digestibility and nutrient availability in Black Bengal kids.

## MATERIALS AND METHODS

Animal experiment was conducted in the experimental goat unit of Eastern Regional Station of ICAR-National Dairy Research Institute (ICAR-NDRI-ERS), Kalyani, West Bengal, India during the year 2022. ICAR-NDRI-ERS 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 average weather conditions of the study area are hot and humid, with a minimum and maximum ambient temperature being recorded at 7°C and 39°C during winter and summer, respectively. The average annual rainfall was 1500 mm.

**Experimental animals and diets:** Fifteen weaned healthy Black Bengal kids (weight  $9.47 \pm 0.26$  kg; age  $331.1 \pm 12.7$  days) were divided equally into three groups based on their body weight and age; namely, Control ( $T_0$ ), Treatment-1 ( $T_1$ ), Treatment-2 ( $T_2$ ). There were no statistical differences among three groups. All the experimental animals were vaccinated against prevailing diseases like PPR, Goat pox and Enterotoxaemia, and were also dewormed with Ivermectin and Albendazole before starting of the experiment. All the kids were housed individually in well-ventilated experimental shed under uniform management conditions. *Ad lib.* clean and fresh drinking water was provided twice daily to all the animals at 10:00 AM and 4:00 PM. Experimental shed and animals were cleaned regularly throughout the trial period.

**Exogenous fibrolytic enzymes:** Two individual preparations of exogenous fibrolytic enzymes (cellulase and xylanase) in powder forms were procured from Lumis Biotech Pvt. Ltd. The promised activity of enzyme powder cellulase by the manufacturer was 50000 IU/g and that of xylanase powder was 50000 IU/g.

**Feeding of experimental animals:** Animals in each experimental group were fed *ad lib.* Total Mixed Ration (TMR) was provided individually for 70 days at 10:30 AM every day (Table 1). TMR (CP 13%, TDN 63.75%) was prepared for each group separately from concentrate mixture and green fodder at the ratio of 40% and 60% of DM, respectively and the same ratio was maintained throughout the experimental period. No enzymes were added to the TMR of control group, while TMR of  $T_1$  and  $T_2$  were prepared by supplementing low and high level of EFE (cellulase and xylanase) through the concentrate mixture, respectively.

**Control group ( $T_0$ ):** Animals in control group were fed *ad lib.* TMR without enzyme supplementation as per requirements (Nutrient requirements of goat ICAR 2013). **Treatment group-1 ( $T_1$ ):** *ad lib.* TMR supplemented with EFE cellulase and Xylanase @ 8000 and 16000 IU/kg

DM of TMR through the concentrate mixture. **Treatment group-2 ( $T_2$ ):** *ad lib.* TMR supplemented with EFE cellulase and xylanase @ 12000 and 24000 IU/kg DM of TMR through the concentrate mixture.

Table 1. Ingredient composition (% DM) of experimental ration (TMR)

| Ingredient (% of TMR DM)    | Treatment |       |       |
|-----------------------------|-----------|-------|-------|
|                             | $T_0$     | $T_1$ | $T_2$ |
| Maize                       | 14        | 14    | 14    |
| Wheat bran                  | 9.6       | 9.6   | 9.6   |
| Ground nut cake             | 5.6       | 5.6   | 5.6   |
| Mustard oil cake            | 9.6       | 9.6   | 9.6   |
| Mineral mixture             | 0.8       | 0.8   | 0.8   |
| Salt                        | 0.4       | 0.4   | 0.4   |
| Green fodder                | 60        | 60    | 60    |
| Cellulase (IU/kg DM of TMR) | -         | 8000  | 12000 |
| Xylanase (IU/kg DM of TMR)  | -         | 16000 | 24000 |

**Digestion trial:** A digestion trial of 6 days was conducted on 15 animals (5 experimental kids under each group) by total collection method at the mid of the growth trial to study the nutrient intake and digestibility of nutrients. Body weights of animals were recorded before and after digestion trial on two consecutive days before feeding and watering. Proper record of feed consumed, residue left and faeces voided by individual animal in control and treatment groups were maintained during this period. Fresh and adequate drinking water was provided twice a day. 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:

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

**Statistical analysis:** Data related to voluntary intake, digestibility coefficients and nutrient intake were analyzed by one-way ANOVA. Computerized IBM SPSS 20.0 package was used for ANOVA. Duncan's DMRT test was used to measure the differences of means.

## RESULTS AND DISCUSSION

**Chemical composition of feeds and fodders:** The values of chemical composition (on per cent DM basis) in terms of DM, OM, CP, EE, TCHO, TA, NDF, ADF, Hemicellulose, Cellulose and ADL (Table 2) were 91.77, 91.40, 20.83, 4.12, 66.45, 8.60, 34.82, 13.16, 21.66, 9.29 and 3.87, respectively for concentrate mixture and 20.56, 90.29, 8.48, 2.37, 78.38,

9.71, 68.08, 42.11, 25.97, 38.32 and 3.79, respectively for mixed green fodder. The average DM, OM, CP, EE, TCHO, TA, NDF, ADF, Hemicellulose, Cellulose and ADL content (on % DM basis) of Total Mixed Ration were 48.58, 90.75, 13.43, 3.05, 73.25, 9.25, 52.35, 30.35, 24.25, 26.71, and 3.83, respectively. Non Fiber Carbohydrate (NFC) content (on % DM basis) of the TMR during the digestibility trial was calculated as 20.90.

Table 2. Chemical composition (%DM basis) of feeds and fodder during digestion trial

| Parameter               | Concentrate Mix. | Green Fodder | Total Mixed Ration |
|-------------------------|------------------|--------------|--------------------|
| Dry matter              | 91.77            | 20.56        | 48.58              |
| Organic matter          | 91.40            | 90.29        | 90.75              |
| Crude protein           | 20.83            | 8.48         | 13.43              |
| Ether extract           | 4.12             | 2.37         | 3.05               |
| Total ash               | 8.60             | 9.71         | 9.25               |
| Acid insoluble ash      | 1.57             | 3.43         | 2.36               |
| Neutral detergent fibre | 34.82            | 68.08        | 52.35              |
| Acid detergent fibre    | 13.16            | 42.11        | 30.35              |
| Hemicellulose           | 21.66            | 25.97        | 24.25              |
| Cellulose               | 9.29             | 38.32        | 26.71              |
| Lignin                  | 3.87             | 3.79         | 3.83               |
| Total carbohydrates     | 66.45            | 78.38        | 73.25              |

\*Each value is the average of triplicate analysis on dry matter basis.

**Voluntary intake of DM, OM and CP:** The voluntary intakes of different nutrients during digestibility trial in groups with or without exogenous fibrolytic enzyme supplementation are presented in Table 3. The average total dry matter intake (DMI g/d/animal) in Control, T<sub>1</sub> and T<sub>2</sub> groups were 254.5, 285.1 and 275.8, respectively. TDMI was non-significantly (P>0.05) higher in enzyme supplemented groups (T<sub>1</sub> and T<sub>2</sub>) as compared to T<sub>0</sub>. The increase in TDMI were 10.70% and 7.75% in EFE supplemented groups T<sub>1</sub> and T<sub>2</sub>, respectively over the control. The average dry matter intake (% of BW) was 2.65,

2.93 and 2.85, respectively. While, the average DMI (g/kg W<sup>0.75</sup>) were 46.54, 51.64 and 50.23 in T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> groups, respectively. The statistical analysis showed that DMI as % of BW and DMI (g/kg W<sup>0.75</sup>) were non-significantly (P>0.05) higher in T<sub>1</sub> and T<sub>2</sub> groups as compared with the control. The organic matter intake was similar as that of average total dry matter intake. The increase in average total OMI were 10.80% and 7.75% in two EFE supplemented groups, i.e. T<sub>1</sub> and T<sub>2</sub>, respectively over the control. Similarly the OMI (kg/100 kg BW) and OMI (g/kg W<sup>0.75</sup>) were higher in T<sub>1</sub> and T<sub>2</sub> groups than the control but the difference was statistically non significant (P>0.05).

Crude Protein Intake (g/d/animal) was 38.19, 42.83 and 40.49 in control, T<sub>1</sub>, and T<sub>2</sub> groups, respectively. The CP intake was significantly (P<0.05) higher in T<sub>1</sub> group than the control, though there was no significant difference between the two enzyme supplemented groups (T<sub>1</sub> and T<sub>2</sub>). Crude Protein Intake (% of BW) was 0.40, 0.44 and 0.42; and Crude Protein Intake (g/kg W<sup>0.75</sup>) were 6.97, 7.75 and 7.36 in control, T<sub>1</sub> and T<sub>2</sub> groups, respectively. The statistical data revealed similar trend as in case of CP intake (g/d).

**Digestibility coefficient (%) of various nutrients:** The mean digestibility coefficients (%) of various nutrients such as DM, OM, EE, CP, TCHO, NDF, ADF, Hemicellulose and Cellulose in growing Black Bengal kids fed TMR containing concentrate and mixed green fodder are presented in Table 4. The digestibility coefficients (%) of DM and OM were 64.47 and 68.09 for control; 66.98 and 70.64 for T<sub>1</sub> and 68.40 and 71.50 for T<sub>2</sub> groups, respectively. Statistical analysis of data demonstrated that the digestibility (%) of DM and OM were significantly (P<0.01) higher in enzyme supplemented groups (T<sub>1</sub> and T<sub>2</sub>) in comparison to control group. However, there was no significant difference (P>0.05) between T<sub>1</sub> and T<sub>2</sub> groups. The digestibility coefficients of EE in Control, T<sub>1</sub> and T<sub>2</sub> groups were 76.65, 80.05 and 81.10, respectively. The statistical analysis showed that there was significantly (P<0.01) higher digestibility in both enzyme treated

Table 3. Intake of DM, OM and CP in Black Bengal kids during digestion trial

| Attribute                           | Groups             |                    |                     | S.E.M. | P-value |
|-------------------------------------|--------------------|--------------------|---------------------|--------|---------|
|                                     | T <sub>0</sub>     | T <sub>1</sub>     | T <sub>2</sub>      |        |         |
| <i>Dry Matter Intake ( DMI)</i>     |                    |                    |                     |        |         |
| DMI(g/d/animal)                     | 254.5              | 285.1              | 275.8               | 5.64   | 0.073   |
| DMI(% of BW)                        | 2.65               | 2.93               | 2.85                | 0.05   | 0.095   |
| DMI( g/kg W <sup>0.75</sup> )       | 46.54              | 51.64              | 50.23               | 0.94   | 0.072   |
| <i>Organic Matter Intake ( OMI)</i> |                    |                    |                     |        |         |
| OMI(g/d/animal)                     | 231.8 <sup>a</sup> | 259.7 <sup>b</sup> | 251.2 <sup>ab</sup> | 5.13   | 0.073   |
| OMI(% of BW)                        | 2.41 <sup>a</sup>  | 2.66 <sup>b</sup>  | 2.59 <sup>ab</sup>  | 0.05   | 0.089   |
| OMI(g/Kg W <sup>0.75</sup> )        | 42.31 <sup>a</sup> | 46.86 <sup>b</sup> | 45.75 <sup>ab</sup> | 0.84   | 0.067   |
| <i>Crude Protein Intake ( CPI)</i>  |                    |                    |                     |        |         |
| CPI (g/d/ animal)                   | 38.19 <sup>a</sup> | 42.83 <sup>b</sup> | 40.49 <sup>ab</sup> | 0.73   | 0.032   |
| CPI (% of BW)                       | 0.40 <sup>a</sup>  | 0.44 <sup>b</sup>  | 0.42 <sup>ab</sup>  | 0.01   | 0.013   |
| CPI ( g/kg W <sup>0.75</sup> )      | 6.97 <sup>a</sup>  | 7.75 <sup>b</sup>  | 7.36 <sup>ab</sup>  | 0.11   | 0.011   |

<sup>a, b</sup> values with different superscripts in a row are significantly (P<0.05) different from other.

groups ( $T_1$  and  $T_2$ ) as compared to control. There was no significant difference ( $P>0.05$ ) between two treatment groups ( $T_1$  and  $T_2$ ). The digestibility coefficients of CP for control,  $T_1$  and  $T_2$  groups were 66.57, 68.99 and 72.02, respectively. The statistical analysis showed that there was significantly ( $P<0.01$ ) higher CP digestibility was found in  $T_2$  as compared to Control and  $T_1$  groups. The digestibility coefficient for TCHO was 67.68, 70.71 and 71.16 for control,  $T_1$  and  $T_2$  groups, respectively. There was significantly ( $P<0.01$ ) positive effect on digestibility of TCHO in both enzyme supplemented groups ( $T_1$  and  $T_2$ ) over the control. However, there was non-significant ( $P>0.01$ ) difference between  $T_1$  and  $T_2$  groups.

Table 4. Nutrient digestibility coefficients (%) in Black Bengal kids during digestibility trial

| Nutrient      | Groups             |                     |                    | S.E.M | P-value |
|---------------|--------------------|---------------------|--------------------|-------|---------|
|               | $T_0$              | $T_1$               | $T_2$              |       |         |
| DM            | 64.47 <sup>a</sup> | 66.98 <sup>b</sup>  | 68.40 <sup>b</sup> | 0.50  | 0.004   |
| OM            | 68.09 <sup>a</sup> | 70.64 <sup>b</sup>  | 71.50 <sup>b</sup> | 0.44  | 0.004   |
| EE            | 76.65 <sup>a</sup> | 80.05 <sup>b</sup>  | 81.10 <sup>b</sup> | 0.42  | < 0.01  |
| CP            | 66.57 <sup>a</sup> | 68.99 <sup>a</sup>  | 72.02 <sup>b</sup> | 0.57  | <0.01   |
| TCHO          | 67.68 <sup>a</sup> | 70.71 <sup>b</sup>  | 71.16 <sup>b</sup> | 0.47  | 0.003   |
| NDF           | 56.95 <sup>a</sup> | 63.67 <sup>b</sup>  | 66.42 <sup>b</sup> | 0.87  | <0.01   |
| ADF           | 36.65 <sup>a</sup> | 47.38 <sup>b</sup>  | 50.15 <sup>b</sup> | 1.64  | <0.01   |
| Hemicellulose | 74.03 <sup>a</sup> | 76.80 <sup>ab</sup> | 79.58 <sup>b</sup> | 0.81  | 0.018   |
| Cellulose     | 33.26 <sup>a</sup> | 45.79 <sup>ab</sup> | 46.85 <sup>b</sup> | 1.82  | 0.002   |

<sup>a, b</sup> values with different superscripts in a row are significantly ( $P<0.05$ ) different from other.

The digestibility coefficients of NDF and ADF were 56.95 and 36.65; 63.67 and 47.38; 66.42 and 50.15 in control,  $T_1$  and  $T_2$  groups, respectively. The statistical data analysis showed that NDF digestibility was significantly ( $P<0.01$ ) higher in both enzyme treated groups ( $T_1$  as well as  $T_2$ ) as compared to control group. Similar trend was observed for ADF digestibility. However there was no significant difference ( $P>0.05$ ) between two treatment groups ( $T_1$  and  $T_2$ ) for both NDF and ADF digestibility. The digestibility coefficient for hemicellulose and cellulose were 74.03, 33.26; 76.80, 45.79; 79.58, 46.85 for control,  $T_1$  and  $T_2$  groups, respectively. The statistical analysis of data revealed that the digestibility coefficient values for hemicellulose was significantly higher ( $P<0.01$ ) in  $T_2$  group than the control. Cellulose digestibility were also significantly ( $P<0.01$ ) higher in both  $T_1$  and  $T_2$  (EFE mixture supplemented groups) than control group. However there was no significant difference between two enzyme supplemented groups.

The results found in the present study were in agreement with earlier studies by different researchers. Selzer *et al.* (2021) conducted an experiment on six merino sheep supplemented with six different levels of cellulase plus and xylanase plus along with smut finger hay and TMR based diet. The different levels of cellulase plus and xylanase plus for six rams were  $T_1$  (0.4 ml and 0 ml),  $T_2$  (0.3 ml and 0.1 ml),  $T_3$  (0.2 ml and 0.2 ml),  $T_4$  (0.1 ml and 0.3 ml),

$T_5$  (0 ml and 0.4 ml) and  $T_6$  (no enzyme). There was significantly ( $P<0.05$ ) higher digestibility of NDF and ADF in  $T_3$  group (25% NDF and 55% ADF higher than  $T_6$  group) compared with all the groups. Furthermore DM, OM and CP were also non-significantly ( $P>0.05$ ) higher in all enzyme treated groups over the control. An experiment on Jersey crossbred calves was demonstrated by Anil (2021) to evaluate the impact of EFE supplementation. The animals were fed TMR without EFE ( $T_0$  or control), TMR with cellulase and xylanase @ 8000 and 16000 IU/kg DM ( $T_1$ ) and TMR with cellulase and xylanase @ 12000 and 24000 IU /kg DM ( $T_2$ ). There was significant ( $P<0.05$ ) increase in DM, OM, ADF, NDF, TCHO, hemicellulose and cellulose digestibility (%) by around 8, 6.5, 12, 10, 7.5, 8 and 10%, respectively in both EFE supplemented groups ( $T_1$  and  $T_2$ ). Moreover Mousa *et al.* (2022) conducted a study on a combination of exogenous fibrolytic enzymes and probiotics (Calfo Care®) supplemented with TMR on male Ossimi fattening lambs assigned into four dietary treatments named as G1 (Control), G2, G3, and G4 which were fed control ration and supplemented with Calfo Care® at concentrations of 0.5, 1, and 2 kg/tonne diet of DM, respectively. There were significant ( $P<0.5$ ) enhancement in digestibility of DM, CP and NFE in groups G2 and G3 as compared to control. The OM digestibility (%) were 6.40, 9, and 2.25% higher in enzyme treated groups than control. The crude fibre and ether extract digestibility (%) were also significantly ( $P<0.05$ ), higher by 12 and 11% in G3 group.

In the present study, the digestibility (%) of DM, OM, EE, CP, TCHO, NDF, ADF, Hemicellulose, Cellulose were increased by 3.75, 3.60, 4.75, 3.50, 4.30, 10.55, 22.65, 3.60, 27.35% in  $T_1$  group and 5.75, 4.80, 5.50, 7.60, 4.90, 14.25, 26.90, 7.00, 29.00% in  $T_2$  group, respectively, over the control group. This may be due to the exogenous fibrolytic enzymes breaking off the cross linkages between lignin and cell wall components (cellulose and hemicelluloses) and solubilizing cell wall contents (mainly hemicelluloses) (Kholif *et al.* 2022) and also the EFE can change the rate of ruminal degradability of the potentially digestible NDF (Togtokhbayar *et al.* 2017) and increase the activity and number of non-fibrolytic and fibrolytic bacteria population in rumen fluid (Wang *et al.* 2012). There was synergism effect shown between ruminal and exogenous fibrolytic enzymes such that in the rumen, net combined hydrolytic effect was much higher than that measured from the individual enzyme activity (Morgavi *et al.* 2004). The enhanced nutrient digestibility in enzyme treated diets could be ascribed to the additive effects of enzymatic action and ruminal micro-flora (Morgavi *et al.* 2001). According to Beauchemin *et al.* (2003), synergism with ruminal microbes, stimulation of bacterial colonization, stimulation of ruminal microbial populations, stimulation of bacterial attachment, and improvement in ruminal hydrolytic capacity were some of the main factors in improving feed efficiency and digestion in response to EFE supplementation.

In contrast, Dean *et al.* (2005) observed no effect of



EFE on DM, CP, NDF, and ADF digestibility. Kung *et al.* (2001) reported that excessive use of EFE in diets results in binding of EFE to substrates and secretion of anti-nutritional factors such as phenolic compounds that might affect microbial growth in the rumen and decrease fiber digestion. It is also reported that the use of higher doses of EFE could cause lower saliva production and subsequently result in lower rumen pH and fiber degradation. The present results differ from their results because they used higher doses of enzymes (8800 units carboxyl cellulase and 40,000 xylanase per kg of forage on DM basis) and their method of application was also different.

**Availability of nutrients:** The availability of nutrients (DCP, TDN, Carbohydrates and Energy) for three experimental groups in the present study have been presented in Table 5. The DCP Intake (g/d/animal) was 25.50, 29.71 and 29.16 in control, T<sub>1</sub> and T<sub>2</sub> groups, respectively. Significant ( $P > 0.05$ ) effect was seen in T<sub>1</sub> and T<sub>2</sub> groups over the control. Although non-significant difference was observed in T<sub>1</sub> and T<sub>2</sub> groups. The DCP Intake (g/d/animal) was significantly higher in T<sub>1</sub> (14.80%) and T<sub>2</sub> (12.55%) groups than the control. The DCP Intake (% of BW and g/kg W<sup>0.75</sup>) were also significantly ( $P < 0.05$ ) higher in T<sub>1</sub> and T<sub>2</sub> groups as compared to control group. The values for DCP Intake (g/100 kg BW) were 0.26,

0.30 and 0.30 in T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> groups, respectively and for DCPI (g/kg W<sup>0.75</sup>) were 4.65, 5.36 and 5.30 in T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> groups, respectively.

The TDN intake also followed the similar pattern and was significantly ( $P < 0.05$ ) higher in both enzyme supplemented groups (T<sub>1</sub> and T<sub>2</sub>) as compared to control. However there was no significant difference found in both enzyme supplemented groups (T<sub>1</sub> and T<sub>2</sub>). The TDN intake (g/d/animal) was 172.4, 199.3, 196.0 in control, T<sub>1</sub> and T<sub>2</sub> groups, respectively. An increase of 13.5% and 12.05%, respectively were seen in T<sub>1</sub> and T<sub>2</sub> groups as compared to control. Similarly TDN intake (kg/100 kg BW and g/kg W<sup>0.75</sup>) were also significantly higher in T<sub>1</sub> and T<sub>2</sub> groups compared to control. The values for TDN intake (kg/100 kg BW) were 1.79, 2.05 and 2.02 in control, T<sub>1</sub> and T<sub>2</sub> groups, and for TDN intake (g/kg W<sup>0.75</sup>) were 31.53, 36.10 and 35.66 for control, T<sub>1</sub> and T<sub>2</sub> groups, respectively. Mousa *et al.* (2022) also found significantly higher TDN and DCP intake (7.5% and 1.4% respectively) in EFE supplemented lambs than the control group. Anil (2021) also reported higher intake of nutrients in Jersey crossbred calves supplemented with EFE (cellulase + xylanase) levels similar to the present study.

The carbohydrates, i.e. digestible neutral detergent fibre intake (NDFI g/d/animal) and digestible total carbohydrates

Table 5. Intake of DCP, TDN, Carbohydrates and Energy in Black Bengal kids of different treatment groups during digestion trial

| Attribute                                      | Groups              |                     |                     | S.E.M. | P-value |
|------------------------------------------------|---------------------|---------------------|---------------------|--------|---------|
|                                                | T <sub>0</sub>      | T <sub>1</sub>      | T <sub>2</sub>      |        |         |
| Average Body Weight (kg)                       | 9.64                | 9.75                | 9.73                | 0.26   | ----    |
| <i>Digestible Crude Protein Intake (DCPI)</i>  |                     |                     |                     |        |         |
| DCPI (g/d/animal)                              | 25.50 <sup>a</sup>  | 29.71 <sup>b</sup>  | 29.16 <sup>b</sup>  | 0.60   | 0.007   |
| DCP(kg/100 kg BW)                              | 0.26 <sup>a</sup>   | 0.30 <sup>b</sup>   | 0.30 <sup>b</sup>   | 0.01   | <0.01   |
| DCPI(g/kg W <sup>0.75</sup> )                  | 4.65 <sup>a</sup>   | 5.36 <sup>b</sup>   | 5.30 <sup>b</sup>   | 0.09   | <0.01   |
| <i>Total Digestible Nutrient Intake (TDNI)</i> |                     |                     |                     |        |         |
| TDNI (g/d/animal)                              | 172.4 <sup>a</sup>  | 199.3 <sup>b</sup>  | 196.0 <sup>b</sup>  | 4.20   | 0.015   |
| TDNI (kg/ 100 kg BW)                           | 1.79 <sup>a</sup>   | 2.05 <sup>b</sup>   | 2.02 <sup>b</sup>   | 0.04   | 0.017   |
| TDNI(g/kg W <sup>0.75</sup> )                  | 31.53 <sup>a</sup>  | 36.10 <sup>b</sup>  | 35.66 <sup>b</sup>  | 0.70   | 0.012   |
| <i>DE Intake</i>                               |                     |                     |                     |        |         |
| DE intake (Mcal/d)                             | 0.760 <sup>a</sup>  | 0.879 <sup>b</sup>  | 0.864 <sup>b</sup>  | 0.019  | 0.015   |
| DE intake (Mcal/ kg W <sup>0.75</sup> )        | 0.139 <sup>a</sup>  | 0.159 <sup>b</sup>  | 0.157 <sup>b</sup>  | 0.003  | 0.012   |
| <i>ME Intake</i>                               |                     |                     |                     |        |         |
| ME intake (Mcal/d)                             | 0.664 <sup>a</sup>  | 0.769 <sup>b</sup>  | 0.757 <sup>b</sup>  | 0.016  | 0.014   |
| ME intake (Mcal/ kg W <sup>0.75</sup> )        | 0.121 <sup>a</sup>  | 0.139 <sup>b</sup>  | 0.138 <sup>b</sup>  | 0.003  | 0.011   |
| <i>Digestible Carbohydrates Intake</i>         |                     |                     |                     |        |         |
| DNDFI(g/d/animal)                              | 72.26 <sup>a</sup>  | 92.99 <sup>b</sup>  | 93.26 <sup>b</sup>  | 2.87   | 0.002   |
| DTCHOI (g/d/animal)                            | 124.01 <sup>a</sup> | 146.73 <sup>b</sup> | 144.20 <sup>b</sup> | 3.39   | 0.009   |
| DNFCI (g/d/animal)                             | 56.76               | 60.56               | 61.53               | 0.95   | 0.097   |
| <i>Nutritive Value of Diets</i>                |                     |                     |                     |        |         |
| DCP % of diet                                  | 10.04               | 10.42               | 10.66               | 0.11   | 0.063   |
| TDN % of diet                                  | 67.52 <sup>a</sup>  | 69.90 <sup>b</sup>  | 71.14 <sup>b</sup>  | 0.40   | <0.01   |
| DE (Mcal/kg)                                   | 2.98 <sup>a</sup>   | 3.08 <sup>b</sup>   | 3.14 <sup>b</sup>   | 0.02   | <0.01   |
| ME (Mcal/kg)                                   | 2.66 <sup>a</sup>   | 2.70 <sup>b</sup>   | 2.75 <sup>b</sup>   | 0.02   | <0.01   |

<sup>a, b</sup> values with different superscripts in a row are significantly ( $P < 0.05$ ) different from other. DCP, Digestible Crude Protein; TDN, Total Digestible Nutrients; DNDF, Digestible Neutral Detergent Fibre; DTCHO, Digestible total carbohydrates; DNFC, Digestible Non Fiber Carbohydrates; DDE, Digestible Energy; ME, Metabolizable Energy.

intake (TCHOI g/d/animal) were 72.26 and 124.0 in control, 92.99 and 146.7 in  $T_1$ , 93.26 and 144.2 in  $T_2$  group. There was significantly ( $P < 0.01$ ) higher intake of both of the nutrients (TCHO and NDF) in enzyme supplemented groups ( $T_1$  and  $T_2$ ) as compared with the control group. On the other hand, there was non-significant difference ( $P > 0.01$ ) between  $T_1$  and  $T_2$  groups. The dietary energy intake in terms of digestible energy (DE) and metabolizable energy (ME) intake were also significantly ( $P < 0.05$ ) higher in both enzyme supplemented groups ( $T_1$  and  $T_2$  groups). The DE intake (Mcal/day/animal) were 0.760, 0.879 and 0.864, while ME intake (Mcal /day/animal) were 0.664, 0.769 and 0.757 in control,  $T_1$  and  $T_2$  groups, respectively. The DE and ME intake (Mcal/ kg  $W^{0.75}$ ) also followed the similar trend and were significantly ( $P < 0.05$ ) higher in two enzyme supplemented groups than the control. The Digestible Neutral Detergent Fibre intake (DNDFI) and Digestible Total Carbohydrates Intake (DTCHOI) were also significantly ( $P < 0.01$ ) higher in  $T_1$  and  $T_2$  groups than the control group. However there was no significant difference between the two enzyme supplemented groups for all these nutrient intake parameters.

**Nutritive value of diets:** The nutrients percentage of diet among all three different groups (control,  $T_1$  and  $T_2$ ) were also measured during digestibility trial. The DCP% of diet were 10.04, 10.42 and 10.66 in control,  $T_1$  and  $T_2$  groups, respectively. There was higher significant ( $P < 0.01$ ) difference noted in  $T_2$  group as compared to control but the  $T_1$  group showed statistically similar value with both the control and  $T_2$  groups. The TDN (%) were 67.52, 69.90 and 71.14 in control,  $T_1$  and  $T_2$  groups, respectively. The dietary TDN (%) was significantly ( $P < 0.01$ ) higher in both enzyme supplemented groups ( $T_1$  and  $T_2$ ). The DE content (Mcal/kg) was 2.98, 3.08 and 3.14, while ME intake (Mcal/kg) was 2.66, 2.70 and 2.75 for control,  $T_1$  and  $T_2$  groups, respectively. Both DE and ME content were significantly ( $P < 0.01$ ) higher in two enzyme supplemented groups than the control group.

In the present study there was linear increase in digestibility coefficient with the feeding of higher enzyme level. However, though the digestibility of most of the nutrients were numerically higher in  $T_2$  group than  $T_1$ , the difference was statistically non-significant ( $P > 0.05$ ) for most of the nutrients except for CP digestibility, in case of which, the value for  $T_2$  group was significantly higher than both control and  $T_1$  groups ( $P < 0.05$ ). Similarly, in case of DCP and TDN intake also, there was no significant difference ( $P > 0.05$ ) between the two enzyme supplemented groups, which means increase in enzyme level had no further significant effect on digestibility of most of the nutrients and also the intake of DCP and TDN. This may be attributed to the negative feedback action of the high levels of fibrolytic enzymes. This feedback mechanism occurs when enzyme action is inhibited by the production of a critical concentration of a product of the enzyme-substrate interaction. For instance, the fermentation of sugars produced by cell wall hydrolysis may reduce ruminal pH to

levels that inhibit the digestion of the cell wall.

Supplementation of exogenous fibrolytic enzymes (EFE) had significant positive effect on nutrient intake and nutrient digestibility in Black Bengal kids. Hence, it is thereby concluded that the supplementation of EFE @ cellulase 8000 and xylanase 16000 IU/kg DM could improve the feed intake and digestible nutrient availability in Black Bengal kids.

#### ACKNOWLEDGEMENT

The study was supported and funded by ICAR-National Dairy Research Institute, Karnal, Haryana, India.

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