RESEARCH ARTICLE

Effect of Varying Levels of Fibrolytic Enzymes Supplementation on Digestibility of Nutrients and Plane of Nutrition in Gir Calves

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Abstract

The present study was conducted to investigate the effect of fibrolytic enzyme on digestibility of nutrients and plane of nutrition in Gir calves. Fifteen male Gir calves of around one year age were randomly divided into three groups with five calves in each and experiment was conducted for 90 days. Three TMR (total mixed ration, roughage: concentrate ratio 60:40) treatments used were: only TMR (T_1), TMR supplemented with exogenous fibrolytic enzymes (EFEs, cellulase 10,00,000 IU/g and xylanase 1,50,000 IU/g in 1:1 ratio), at 0.025% of DM (T_2) and at 0.050% of DM (T_3). Digestibility (%) of dry matter (66.77 vs. 61.95), organic matter (70.18 vs. 66.58) and cellulose (58.54 vs. 53.40) were significantly ($p<0.05$) improved in T_2 as compared to T_1. Digestibility (%) of crude fibre (46.64 & 45.12 vs. 40.11), NDF (55.06 & 52.67 vs. 44.63), ADF (46.60 & 44.24 vs. 35.27) and hemicelluloses (79.69 & 77.22 vs. 71.90) were significantly ($p<0.05$) improved in both treatments (T_2 & T_3) as compared to T_1. Results revealed that no significant effect was observed on DCP intake in both treatments as compared to control. TDN intake (kg/d) was significantly ($p<0.01$) increased in both T_2 (3.51) and T_3 (3.37) as compared to T_1 (3.23). Based on overall results EFEs improved the intake and digestibility of nutrients of feed.

Key words: Digestibility, Exogenous Fibrolytic enzymes, Gir calves, Nutrient intake.

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Introduction

Gujarat is an important state in milk production and marketing in India based on co-operative dairy system**.** Total milk production in Gujarat was around 15.8 million tonnes and per capita milk availability was 633 g/day during 2020-21 (Anonymous, 2023). One of the major constraints for the livestock production in India is the qualitative and quantitative deficiency of feed for livestock (Thammiah *et al.,* 2017). In crop residues, the lignocellulose component is potential source of energy but the utilization of such residues by ruminants is less due to limited lignocellulolytic activity of the rumen microbial population (Thakur *et al.,* 2010). Exogenous fibrolytic enzymes (EFEs) digest the complex fibrous portion to simple components; therefore, these enzymes are essential to animals (Beauchemin *et al.,* 2003). EFEs enhance natural digestive processes to improve the availability of nutrients and feed intake. Supplementation of EFEs improves palatability and forage utilization by ruminants. The net effect of EFEs give rise to increase enzymatic activity within the rumen, which enhances digestibility of the total diet fed. Keeping the above facts in view, the effects of supplementing exogenous fibrolytic enzymes on digestibility of nutrients and plan of nutrition in male Gir calves were investigated.

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Materials and Methods

Experimental Animals

Fifteen male Gir calves of around one year age were randomly selected and divided into three equal groups with five calves in each. Average body weight of calves was around 160 to 162 kg. They were assured for the health and disease. The duration of experiment was 90 days.

T_1 = TMR was without supplemented EFEs (Control).

T_2 = TMR was supplemented with 0.025% EFEs on DM basis.
T_3 = TMR was supplemented with 0.050% EFEs on DM basis.
EFEs used in experiment contained cellulase 10,00,000 IU/g and xylanase 1,50,000 IU/g (1:1 ratio).

Experimental Feeds and Fodders

All the experimental calves were fed with total mixed ration (TMR). The nutrient requirements of growing Gir calves in term of DCP and TDN were met as per ICAR (2013) feeding standards. TMR was prepared by mixing roughage and concentrate in the ratio of 60:40 after grinding/chaffing. The proportions of different ingredients used to prepare TMR T_1 were Groundnut haulm 50%, Maize fodder (chaffed) 10%, ISI grade-I cattle feed 20%, Cotton seed cake 13.5%, Ground maize 5%, Mineral mixture 1% and salt 0.5%.

Digestibility Trial

After 60 days of experimental feeding, a digestion trial was conducted on all the fifteen experimental Gir calves to determine digestibility of the nutrients. The arrangement for quantitative collection of faeces was made during seven days collection period. A proper record of feed consumed, residue left and faeces voided by each animal were maintained during the trial period. In the morning, the residue of each animal was collected and weighed and then a uniform representative sample of the residue was kept in a labelled polythene bag. A representative sample of TMR offered was kept in previously labelled polythene bags. Twenty-gram sample each from TMR and residue was taken in aluminium tray and kept in an oven at 100±5 °C for 24 h for the estimation of dry matter content. The quantity of faeces voided by individual calf was collected and weighed quantitatively after every 24 h at 8.00 A.M. A representative sample ($1/100^{th}$ weight of total faeces) was taken for dry matter estimation. Additionally, another $1/1000^{th}$ part was taken in clean plastic beaker containing 25% Sulphuric acid as preservative for estimation of nitrogen content. Dry matter was estimated each day by placing the sample in hot air oven at 100±5 °C to obtain constant weight.

Estimation of Proximate Composition and Fibre Fractions

All the seven days dried samples of TMR, faeces and acid treated faeces were pooled. Pooled samples of faeces and TMR were ground and stored in air tight polythene containers at room temperature for proximate analysis (AOAC, 2005) and fibre fractions (Van Soest *et al.,* 1991).

Statistical Analysis

The data generated during this experiment were subjected to statistical analysis using one-way and two-way ANOVA as suggested by Snedecor and Cochran (1994). The significance of mean differences was tested by Duncan's new multiple range test.

Results and Discussion

The feeds and fodder used to prepare the total mixed ration (TMR) for feeding of Gir calves during the experimental period were analysed for proximate composition and cell wall fractions at Department of Animal Nutrition, COVSAH, KU, Junagadh and the results are presented in Table 1. Mineral

Table. 1: Proximate composition of different feed ingredients and total mixed ration (TMR) (% DM basis)

Ingredients Attributes	Groundnut Haulms	Green Maize	BSI grade-I Cattle Feed	Cotton Seed Cake	Ground Maize	TMR
Dry matter	95.00	32.85	86.95	95.65	92.30	85.70
Organic matter	84.00	90.79	91.65	92.62	98.42	86.83
Crude protein	10.99	8.00	22.10	25.82	11.44	14.77
Ether extract	1.55	1.34	3.97	9.65	3.20	3.16
Crude fibre	22.31	28.58	11.63	30.77	2.47	20.61
Nitrogen free extract	49.15	52.87	53.95	26.38	81.31	48.27
Total ash	16.00	9.21	8.35	7.38	1.58	13.17
Acid detergent fibre	30.20	39.42	17.39	35.62	3.75	27.52
Neutral detergent fibre	38.11	58.42	26.77	44.62	12.45	36.90
Cellulose	18.57	30.19	10.23	22 .72	2.20	17.52
Hemicelluloses	7.91	19.00	9.38	9.00	8.70	9.38
Lignin	7.87	5.52	5.61	10.92	0.80	7.13
Calcium	1.80	0.51	1.5	0.54	0.05	1.83
Phosphorus	0.43	0.20	0.8	0.84	0.47	0.67

mixture used in TMR contained 25.4% calcium and 12.72% phosphorus on DM basis.

Effects of fibrolytic enzymes on apparent digestibility (%) of nutrients are presented in Table 2. Dry matter (DM) digestibility was significantly ($p<0.05$) higher in 0.025% enzyme treated group (T_2) than control group. The enhanced effect of fibrolytic enzyme on DM digestibility in the current study might be due to improved microbial colonisation or due to the positive synergy effect of fibrolytic enzymes with the ruminal microorganisms which stimulate the endogenous enzyme activity within the rumen. Salem *et al.* (2013), Yuangklang *et al.* (2017) and Refat *et al.* (2018) also reported significant effect ($p<0.05$) of fibrolytic enzyme supplementation on DM digestibility. Organic matter (OM) digestibility was significantly ($p<0.05$) higher in 0.025% enzyme treated group (T_2) than control group. Similar were the findings of Gado *et al.* (2009, 2011), and Gaafar *et al.* (2010). Digestibility of crude protein (CP), ether extract (EE) and NFE had shown no significant effect in both enzymes treated groups (T_2 and T_3) as compared to control group (T_1). Similar findings were also reported by Miachieo and Thakur (2007) and Barbadikar (2012). CF digestibility was significantly ($p<0.05$) increased in both enzymes treated groups (T_2 and T_3) than control group. The fibrolytic enzyme-mediated increased fibre digestibility in this study might have occurred via enhanced attachment and microbial colonization to plant cell wall by ruminal microbes and/or by synergism with enzymes in rumen fluid of feed particles or by direct cell wall hydrolysis. Likewise, Gaafar *et al.* (2010), Shekhar *et al.* (2010) and Thube (2016) reported significant ($p<0.05$) effect of fibrolytic enzymes on digestibilitiy of CF.

NDF and ADF digestibility were significantly ($p<0.05$) increased in both enzymes treated groups (T_2 and T_3) than control group (T_1). Similar findings were also reported by many workers (Shekhar *et al.,* 2010; Gado *et al*., 2011; Salem *et al.,* 2013). Digestibility of hemicellulose was significantly ($p<0.05$) increased in both enzymes treated groups (T_2 and T_3) than control group (T_1). Similar finding was also reported by Shekhar *et al.* (2010) and Thube (2016). Cellulose digestibility was significantly ($p<0.05$) higher in 0.025% enzyme treated group (T_2) than control group. In support of present finding, Shekhar *et al.* (2010), and Sheikh *et al.* (2017) reported significant ($p<0.05$) effect of fibrolytic enzyme on digestibility of cellulose. In the present study higher level of EFEs (0.05%, T_3) in fact suppressed the digestibility of all nutrients than in 0.025% EFEs supplemented group (T_2), though it was better than T_1 control (Table 2).

Table. 2: Apparent digestibility (%) of nutrients of different treatments

Nutrients (%)	Treatments / TMR		
	T_1	T_2	T_3
Dry matter	$61.95^{b} \pm 0.94$	$66.77^{a} \pm 1.24$	$65.38^{ab} \pm 1.27$
Organic matter	$66.58^{b} \pm 0.65$	$70.18^{a} \pm 0.88$	$68.45^{ab} \pm 1.03$
Crude protein	76.38 ± 0.68	74.62 ± 2.71	72.54 ± 1.16
Ether extract	75.90 ± 1.51	78.21 ± 1.10	77.37 ± 1.33
Nitrogen free extract	74.27 ± 0.93	78.35 ± 1.07	76.58 ± 1.22
Crude fibre	$40.11^{b} \pm 1.17$	$46.64^{a} \pm 1.77$	$45.12^{a} \pm 1.56$
Neutral detergent fibre	$44.63^{b} \pm 1.91$	$55.06^{a} \pm 2.51$	$52.67^{a} \pm 1.99$
Acid detergent fibre	$35.27^{b} \pm 2.62$	$46.60^{a} \pm 3.03$	$44.24^{a} \pm 2.30$
Hemi-cellulose	$71.90^{b} \pm 0.74$	$79.69^{a} \pm 1.39$	$77.22^{a} \pm 2.32$
Cellulose	$53.40^{b} \pm 1.32$	$58.54^{a} \pm 1.41$	$56.34^{ab} \pm 0.87$

Means with different superscripts (a, b) within row differ significantly from each other ($p<0.05$).

Effect of fibrolytic enzymes on plane of nutrition is present in Table 3. Fibrolytic enzymes had no significant ($p>0.05$) effect on DCPI. The higher level of EFEs (0.05%, T_3) in fact apparently suppressed the DCP and TDN intake at most intervals as compared to 0.025% EFEs supplemented group (T_2), though it was better than T_1 control (Table 3). Similar finding was reported by Barbadikar (2012) and Patel *et al.* (2015). TDNI was significantly ($p<0.01$) increased in both enzymes treated groups (T_2 and T_3) than control group (T_1) particularly on 75th and 90th day of experiment. Shekhar *et al.* (2010) also found higher value of TDNI for enzyme treated groups than control group. Major significance of DCP and TDN intake enhance the growth and production of individual animal.

Table. 3: Plane of nutrition of different treatments

Days	T_1	T_2	T_3
Digestible crude protein intake - DCPI (g/d)			
15	499.70 ± 14.04	493.70 ± 28.06	475.52 ± 13.77
30	577.54 ± 21.57	578.55 ± 36.24	551.57 ± 22.67
45	622.66 ± 24.59	618.22 ± 40.16	609.40 ± 15.46
60	629.42 ± 21.58	647.98 ± 37.11	619.04 ± 13.62
75	642.96 ± 19.24	657.89 ± 29.39	622.25 ± 13.57
90	619.27 ± 20.26	630.34 ± 25.92	587.98 ± 16.14
Total digestible nutrient intake - TDNI (g/d)			
15	2.69 ± 0.06	2.87 ± 0.09	2.77 ± 0.05
30	3.11 ± 0.10	3.36 ± 0.12	3.22 ± 0.10
45	3.36 ± 0.12	3.59 ± 0.11	3.56 ± 0.07
60	3.39 ± 0.09	3.76 ± 0.10	3.61 ± 0.07
75	$3.47^{b} \pm 0.09$	$3.82^{a} \pm 0.08$	$3.63^{ab} \pm 0.07$
90	$3.34^{b} \pm 0.10$	$3.66^{a} \pm 0.05$	$3.43^{ab} \pm 0.05$

Means with different superscripts (a, b, c) within row differ significantly ($p<0.05$).

Conclusion

Supplementation of 0.025% fibrolytic enzymes (cellulase 10,00,000 IU/g and xylanase 1,50,000 IU/g in 1:1 ratio) in TMR (60:40 roughage: concentrate) significantly improved the digestibility of DM, OM and cellulose, whereas

supplementation at both levels (0.025% and 0.05%) significantly improved the digestibility of CF, ADF, NDF and hemi-cellulose. Total digestible nutrient (TDN) intake was increased significantly on enzyme supplementation at both levels, *i.e.,* 0.025% and 0.05%. Based on the overall results of study it could be inferred that utilization of fibre rich feed could be increased by fibrolytic enzyme supplementation at 0.025% level without any adverse effect on animal health.

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