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Effect of fibrolytic enzyme supplementation on rumen fermentation pattern and haemato-biochemical parameters in Gir calves

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Abstract
The present study was conducted to investigate the effect of fibrolytic enzyme on rumen fermentation pattern and haemato-biochemical parameters in Gir calves. Fifteen male Gir calves of around one year age was randomly selected and calves were randomly divided into three group with five calves in each for duration of 90 days. Three treatments were: only TMR (T_1), TMR supplemented with EFEs at 0.025% of DM (T_2) and TMR supplemented with EFEs at 0.050% of DM (T_3). Statistical analysis revealed that pH was significantly ($p<0.01$) decreased in both treatments (6.96±0.04 & 6.89±0.04) as compared to control (7.09±0.03). TVFAs (8.81±0.67 & 8.62±0.64 vs. 7.62±0.40) and total-N (74.71±2.89 & 72.01±3.71 vs. 67.48±2.95) were significantly ($p<0.01$) increased in both treatments as compared to control. TCA-N was significantly ($p<0.01$) improved in T_2 (41.69±2.77) as compared to T_1 (46.53±2.68) and it was at par with the T_3 (44.03±3.24). Supplementation of fibrolytic enzymes had no significant effect on haemoglobin, packed cell volume, glucose, total protein, triglycerides and cholesterol. While, the mean values of various haemato-biochemical parameters during experimental feeding were well within the normal physiological range.

Keywords: Fibrolytic enzyme, rumen fermentation pattern, haemato-biochemical parameter

Introduction
Calves play an important role in development of the dairy sector of country, as the future of the dairy herd solely depends upon the successful raising of young calves. Important aspects in the calf rearing are the health management and proper nutrition. Poor management practices lead to economic losses to the farmers in terms of higher calf mortality, poor growth rate, delayed maturity and poor productivity (Anonymous, 2012) [1]. In order to achieve maximum growth of calf, feed must be balanced nutritionally. In India ruminant feeding system relies mainly on fibrous crop residues because the cost of good quality forages is often high and forage availability is limited (Thammiah *et al.*, 2017) [16]. The constraint in feeding agricultural by products directly to farm animals in general are low protein content, high crude fibre and low digestibility. Increasing the nutrient availability for better utilization of crop residue has been a primary focus of farmer for sustainable profit in farm. One of the most potential methods in improving feedstuff digestibility has been the use of exogenous fibrolytic enzymes (Chandra *et al*., 2010) [6]. Addition of fibrolytic enzymes had a marked effect on increasing the total microbial population in rumen and increased microbial activities (Rode *et al.*, 1999) [12]. Keeping the above facts in view, the effect of supplementing exogenous fibrolytic enzymes (Cellulase and xylanases) on rumen fermentation and haemato-biochemical parameters in male Gir calves were investigated.

Materials and Methods
Experimental animals
Fifteen male Gir calves of around one year age was randomly selected and calves were randomly divided into three group 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 supplemented without EFEs.
T_2 = TMR was supplemented with 0.025% EFEs.
T_3 = TMR was supplemented with 0.050% EFEs.

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) [9] 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 respective TMR are given in Table 1.

Table 1: Parts composition of total mixed ration used in experiment

Sr. No.	Ingredients	TMR
1	Groundnut Haulm	50
2	Maize fodder	10
3	ISI grade-I cattle feed	20
4	Cotton seed cake	13.5
5	Ground maize	05
6	Mineral mixture	01
7	Salt	0.5
	Total	100

Estimation of rumen fermentation parameters

On last day of experiment about 100 ml rumen liquor was collected from three calves from each treatment with the help of stomach tube against negative pressure created by a suction pump. The samples were collected at 0 hour (pre-feeding), 3 hours and 6 hours post feeding to study different rumen parameters. The rumen liquor was immediately brought to laboratory and strained through four layers of muslin cloth referred as strained rumen liquor (SRL). After collection, 0.1 ml of 10 N H_2SO_4 was added except in samples used for pH determination to stop microbial activity. The samples were then stored in deep freezer for estimation of TVFAs and various nitrogen fractions. pH was recorded by using pen-type pH meter, TVFAs were determined by method of Barnett and Reid (1957) [4] by using Markham's steam distillation apparatus and ammonia nitrogen was estimated by Conway's micro-diffusion method (Conway, 1957) [7].

Estimation of haemato-biochemical parameters

At the end of experimental period, blood samples from all experimental Gir calves were collected in vacutainer Na-EDTA vial early in the morning before feeding from jugular vein with all aseptic precautions using 20-gauge needle. Haematological studies were performed soon after collection of the blood viz., haemoglobin (Hb) and packed cell volume (PCV). Haemoglobin was estimated by Sahli's acid hematin method and PCV was estimated by micro haematocrit method. For separation of serum, blood was collected in second tube without anticoagulant and kept in slating position. These tubes were incubated for 1 hour at 37°C. Blood clots were broken and tubes were centrifuged at 2500 rpm for 30 minutes. Serum was pipetted out in small pyrex tubes and kept for analysis. Serum glucose, total protein, triglycerides and cholesterol were analysed by serum biochemical analyser (Dia-CHEM 240 Plus) using Diatek's kit. (Tietz *et al.*, 1976) [18].

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) [15]. The significance of mean differences was tested by Duncan's new multiple range test (DNMRT).

Results and Discussion

Effect of fibrolytic enzymes on rumen fermentation parameters of experimental Gir calves before feeding (0 hour) and 3 and 6 hours post feeding are presented in Table 2. Results revealed significant (p<0.01) decrease in ruminal pH at both levels of enzyme treated group (T_2 and T_3) than control group (T_1) but between T_2 and T_3 nonsignificant effect on pH was observed. This decline in pH value on enzyme supplementation might be due to increased TVFAs concentration. Irrespective of treatment significantly (p<0.01) lower value of pH was observed at 3 hour post feeding which then increased gradually upto 6 hours. In support of present findings, Bassiouni *et al.* (2010) [5] and Gaffar *et al.* (2010) [8] reported fibrolytic enzyme supplementation significantly (p<0.05) decrease ruminal pH.

Results revealed significantly (p<0.01) increased TVFAs in T_2 and T_3 than control group (T_1) but difference between T_2 and T_3 was non-significant. Concentration of TVFAs in SRL collected at different time intervals showed peak value at 3 h post feeding which then declined up to 6 h post feeding following the same pattern in both the treatment groups. The higher TVFAs with enzyme addition could be result of higher availability of fermentable soluble carbohydrate due to increased fibrolytic activity in rumen. In accordance to the present findings Sheikh *et al.* (2017) [13] and Yuangklang *et al*. (2017) [19] observed significant (p<0.05) effect of fibrolytic enzyme supplementation on TVFAs concentration.

Results revealed non-significant (p>0.01) effect of exogenous fibrolytic enzyme supplementation on NH_3-N concentration. But concentration of NH_3-N in SRL collected at different time intervals differed significantly (p<0.01) with peak value at 3 h post feeding and then declined at 6 h of post feeding. In support of present findings Singh and Das (2008) [14], Arriola *et al.* (2011) [2] and Barbadikar (2012) [3] reported non-significant (p>0.05) effect of fibrolytic enzyme supplementation on NH_3-N concentration.

Statistical analysis revealed significant (p<0.05) increased TCA-N in 0.025% exogenous fibrolytic enzyme supplemented group (T_2) over control group (T_1). But there was no-significant effect (p>0.05) of 0.05% enzyme supplemented group (T_3) was observed over control (T_1) and T_2. Irrespective of treatments, TCA-N level significantly (p>0.05) increased from 0 h to 6 h and attained peak at 6 h of post feeding. The increased TCA-N concentration in present study is indicative of more synthesis of microbial protein. Rajamma *et al.* (2014) [11] and Thube (2016) [17] reported significantly (p<0.01) increased TCA-N concentration due to enzyme supplementation.

Data revealed significantly (p<0.01) increased total nitrogen in T_2 and T_3 group than control group (T_1) but difference between T_2 and T_3 was non-significant. Concentration of total nitrogen in SRL collected at different time intervals differed significantly (p<0.01), with maximum value at 3 h post feeding then declined at 6 h post feeding following the same pattern in both the treatment groups. In accordance to present finding, Thube (2016) [17] and Sheikh *et al.* (2017) [13] also reported significant (p<0.05) effect of fibrolytic enzyme supplementation on total nitrogen.

Results revealed non-significant (p>0.05) effect of fibrolytic enzyme supplementation on NPN concentration. But concentration of NPN in SRL collected at different time intervals differed significantly (p<0.01), the maximum NPN concentration was observed at 3 h post feeding, which then declined at 6 h of post feeding following the same pattern in

both the treatment groups. In support to present finding, Patel *et al*. (2015) [10] and Sheikh *et al*. (2017) [13] also reported non-significant ($p>0.05$) effect of fibrolytic enzyme supplementation on NPN concentration.

Table 2: Rumen parameters at different intervals in control and different treatment groups

Hours	Treatments			Mean±SE
	T_1	T_2	T_3	
Rumen pH				
0	7.10±0.04	7.00±004	6.93±0.05	7.01^{a}±0.05
3	7.00±0.04	6.83±0.07	6.77±0.03	6.87^{b}±0.07
6	7.17±0.03	7.03±0.07	6.97±0.07	$7.06^{a\pm}$0.06
Mean±SE	7.09^{a}±0.03	6.96^{b}±0.04	6.89^{b}±0.04	
TVFAs (Mmol/dL)				
0	6.78±0.14	7.31±0.19	7.12±0.19	7.07^{c}±0.15
3	8.71^{b}±0.26	10.59^{a}±0.31	10.25^{ab}±0.26	9.85^{a}±0.58
6	7.38^{b}±0.12	8.55^{a}±0.28	8.50^{a}±0.05	8.14^{b}±0.38
Mean±SE	7.62^{b}±0.40	8.81^{a}±0.67	8.62^{a}±0.64	
NH_3-N (mg/dL)				
0	14.79±0.22	15.13±0.23	15.03±0.43	14.98^{c}±0.10
3	19.22±0.40	21.10±0.52	20.37±0.37	20.23^{a}±0.55
6	15.88±0.20	16.27±0.25	15.93±0.34	16.03^{b}±0.12
Mean±SE	16.63±0.94	17.50±1.29	17.11±1.16	
TCA-N (mg/dL)				
0	35.71±0.38	41.45±2.16	37.40±0.60	38.18^{c}±1.70
3	40.27±1.55	44.18±3.26	41.85±0.75	42.10^{b}±1.13
6	49.08±2.17	53.97±2.38	52.83±1.65	51.96^{a}±1.48
Mean±SE	41.69^{b}±2.77	$46.53^{a\pm}$2.68	44.03^{ab}±3.24	
Total nitrogen (mg/dL)				
0	59.88^{b}±1.05	67.65^{a}±1.57	62.50^{ab}±1.04	63.34^{c}±2.28
3	74.28±2.38	81.82±3.03	80.63±1.35	78.91^{a}±2.34
6	68.28±3.35	74.67±2.55	72.91±0.52	71.95^{b}±1.91
Mean±SE	$67.48^{b\pm}$2.95	74.71^{a}±2.89	72.01^{a}±3.71	
NPN (mg/dL)				
0	24.18±1.40	26.20±0.82	25.10±0.65	25.16^{b}±0.59
3	28.01±1.07	30.49±0.97	31.06±1.37	29.85^{a}±0.94
6	25.21±1.21	27.85±1.25	27.80±1.15	26.95^{b}±0.87
Mean±SE	25.80±0.80	28.18±0.88	27.99±1.21	

Note: ^{abc} Means with different superscripts within column and row differ significantly from each other ($p<0.01$).

pH- potential of hydrogen, TVFAs- total volatile fatty acids, NH_3-N- ammonia nitrogen, NPN- non protein nitrogen.

Effect of fibrolytic enzymes on haemato-biochemical parameters of experimental Gir calves are presented in Table 3. Results revealed that fibrolytic enzymes showed non-significant ($p>0.05$) effect on haemato-biochemical parameters.

Table 3: Haemato-biochemical parameters in Gir calves under control and different treatment groups

Parameters	Treatments		
	T_1	T_2	T_3
Hb (%)	11.90±0.44	12.16±0.49	11.84±0.60
PCV (%)	38.94±1.26	36.64±1.91	37.38±2.38
Glucose (mg/dL)	60.00±5.48	57.40±4.61	62.00±7.52
Total protein (g/dL)	6.63±0.56	7.28±0.29	7.12±0.60
Triglycerides (mg/dL)	24.20±2.22	30.00±3.46	29.00±2.93
Cholesterol (mg/dL)	129.00±8.54	148.00±12.74	150.00±6.76

Note: ^{NS} - non significant

Conclusion

Rumen parameters (pH, total volatile fatty acid, total protein and TCA-N) significantly affected at both levels of fibrolytic enzyme supplemented group than control group which indicate improved nutrient utilization and microbial protein synthesis. The mean values of various haemato-biochemical parameters during experimental feeding were well within the normal physiological range and no significant effect of fibrolytic enzyme supplementation was seen. 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 percent level without any adverse effect on animal health.

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