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Effect of exogenous fibrolytic enzyme and live yeast culture on the rumen fermentation pattern of crossbred calves

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Abstract
Twenty-four crossbred calves were randomly divided into three groups in a Completely Randomized Design in which the animals in group 1 were fed with APBN-1 and concentrate feed @1% of body weight while the animals in groups T2 and T3 were fed on the same ration as in T1 but were also supplemented with RumEest-ESF @ 10 and 15g/animal/day, respectively. Supplementation of Probiotic and EFE to crossbred calves revealed that rumen pH values were highest at 0 h and declined to minimum by 6 h post feeding, while TVFA, NH_3-N, and N fractions reached peak at 6 h post feeding and later followed a decreasing trend in all the treatments. Among all the VFA molar proportions (moles/100 moles), butyrate was found to be increased ($P<0.05$) at the expense of propionate and acetate. The values were 59.06, 57.96 and 56.60 of Acetic acid, 29.15, 29.03 and 29.36 of Propionic acid and 8.80, 10.35 and 11.28 of Butyric acid for T1, T2 and T3 groups, respectively. It was concluded that supplementation of EFE and live yeast culture @ 15 g/animal/day improved the rumen fermentation patterns, which is shown from the higher Butyric acid production, Total Volatile Fatty Acids, Total Nitrogen, TCA precipitable nitrogen, residual nitrogen, and food and protozoal nitrogen.

Keywords: *Exogenous fibrolytic* enzymes, yeast culture, rumen fermentation. crossbred calves

Introduction
The ruminant production efficiency in India is low because of the nutrient scarcity. According to the IGFRI (2013) [11] estimates, India is facing a deficit of 35.6% of green fodder, 10.9% of dry crop residues and 44% of concentrate feed ingredients. Optimization of energy use efficiency is one of the major solutions in combating the feed scarcity and enhancing the production. Methods that increase the fiber digestion are likely to play a role in improving the energy availability of ruminant diets and reducing feed costs (Hu *et al*., 2021) [10].
Feed additives could potentially increase the usage efficiency of feed, thereby increasing the nutrient digestibility (Elghandour *et al*., 2018) [6]. Among the available feed additives, direct-fed microbial and exogenous fibrolytic enzymes are the potent sources for improving the growth rate and efficiency of utilization. Probiotics increases the feed intake and feed efficiency while fibrolytic enzymes enhance the degradation of fibre. Exogenous Fibrolytic Enzymes (EFE) functions synergistically with ruminal microbes by enhancing the digestibility of the cell wall fractions (Reddy *et al*., 2016a, b) [20, 21]. Besides, EFE increases the attachment of cellobiose- and glucose-utilizing bacteria in the rumen (Saleem *et al*., 2019) [19, 22]. Further, the live yeast culture improves the ruminal gut microbiota and nutrient digestibility contributing to animal productivity (Vyas *et al*., 2014) [26]. Live yeast culture (*Saccharomyces cerevisiae*) combats the decreased pH while EFE functions more efficiently in the pH levels higher than 6.0 (Mavrommatis *et al*., 2020) [14]. Hence, the supplementation of EFE and live yeast culture combination improves the production performance by better rumen fermentation. Rumen fermentation reflects the health and production status of ruminants. Although the synergistic action of EFE and live yeast culture were well documented on rumen fermentation, the results were not consistent and varied with species, composition, and feed offered. Hence, the present study dealt with the effect of EFE and live yeast culture combination on the rumen fermentation pattern of growing crossbred calves.

Materials and Methods
The supplement ‘‘RumEest-ESF’’ used in the present study was procured from Neospark Drugs and Chemicals Private Limited, Hyderabad.

The product is a combination of Probiotics (*Saccharomyces cerevisiae* @ 5 billion CFU/ gram) and fibrolytic enzymes (Cellulase, Xylanase, β- glucanase).

Twenty-four healthy male crossbred calves (HF × Jersey, Jersey × Sahiwal) were used in this study. The calves were adapted for stall feeding conditions, dewormed and vaccinated against Foot and Mouth disease before commencement of the study. The animals were pre-weighed and assigned to three groups and fed dietary treatments viz. Control diet (T1) - Basal ration (*ad libitum* APBN-1 + concentrate pellet feed @ 1% of body weight, basal ration with 10g of RumEest-ESF (T2)(EY10) and basal ration with 15g of RumEest-ESF(T3)(EY15). The concentrate feed was incubated with RumEest-ESF for an hour before feeding for both supplemented groups.

All the calves were housed in a well-ventilated animal shed with the provision of individual feeding and watering. The calves were fed according to the experimental diets at 9:00 and 15:00 hours. The animals had free access to fresh and clean drinking water throughout the day. All the calves were dewormed before the start of the trail and at monthly intervals during the experimental period.

After adaptation for 14 days, rumen liquor was collected from each animal for 3 consecutive days by using suction pump. On the day of rumen liquor collection, animals were offered water one hour before starting 0 hour collection and after last collection to eliminate influence of water on the concentration of rumen metabolites. The rumen fluid was strained through four layers of muslin cloth. About 100 ml of the sample was drawn at each collection into a clean and sterile polyethylene bottle. On the day of collection, five collections were made for six animals from each group, the first one before feeding is designated as 0 hour and the other collections after feeding at 3, 6, and 9 hours. Rumen liquor samples were collected at 7:00 AM, 10:00 AM, 1:00 PM, and 4:00 PM for 3 succeeding days after digestibility trail. The collections were preserved by adding 1 ml of 20% H_2SO_4 to prevent further fermentation. The Total Volatile Fatty Acid (TVFA) concentration of Strained Rumen Liquor (SRL) was determined by using the procedure of Barnett and Reid (1957) [1]. The total nitrogen was estimated through Micro-Kjeldahl method. The TCA-insoluble protein nitrogen was measured according to the protocol given by Cline *et al*. (1958) [4]. The residual nitrogen and food and protozoal nitrogen were determined as per Singh *et al*. (1968) [24].

Statistical analysis

Data obtained were subjected to one-way analysis of variance (version 23.0; SPSS, 2015) and the treatment means were ranked using Duncan's multiple range test with a significance at P <0.05 (Duncan, 1955) [5]. All the statistical procedures followed were in accordance with Snedecor and Cochran (1994) [25].

Results and Discussion

Total Volatile Fatty acid

The TVFA concentration in SRL of crossbred calves supplemented with EFE and live yeast culture are presented in Table 1. The mean TVFA concentration (meq/L) was higher (P<0.01) in EY15. In consistent, few authors observed higher TVFA concentration on feeding the combination of EFE and live yeast culture (Can *et al*., 2007; Poonooru *et al*., 2015; Lopuszanska-Rusek and Bilik, 2011) [2, 16, 13]. The higher TVFA concentration could be related to the synergistic effects of EFE and live yeast culture on the rumen microbiome (Poonooru *et al*., 2015) [16]. The higher bacterial population might have increased the production of TVFA (Gurpreet Singh *et al*., 2008) [8].

Table 1: Effect of EFE and YC supplementation on TVFA concentration (mEq/L of SRL)

Treatment	Hour					
	0	3	6	9	Mean**	SEM
T1	87.33	97.17	101.17	95.50	95.29^{A}	1.95
T2	89.67	100.83	106.33	101.83	99.67^{B}	1.17
T3	89.50	103.50	107.17	104.17	101.08^{C}	1.13
Mean**	88.83^{a}	100.50^{b}	104.89^{c}	100.50^{b}		
SEM	0.75	1.84	1.88	2.59		

^{ABC}Values in the columns bearing different superscripts differ significantly (P<0.01)

^{abc}Values in the rows bearing different superscripts differ significantly (P<0.01)

No Diet × Hour Interaction was observed (P=0.503).

The hourly changes in the TVFA concentration are depicted in Fig 1. Time after feeding increased (P<0.01) the TVFA concentration up to 6 h post feeding, irrespective of the treatment, followed by a decline. However, Poonooru *et al*. (2015) [16] recorded a peak concentration of TVFA at 4 h post-feeding. The initial increase in the TVFA concentation might be connected to the rapid breakdown of feed particles immediately after feeding (Rajamma *et al*., 2014) [17]. No diet x hour interactions were observed.

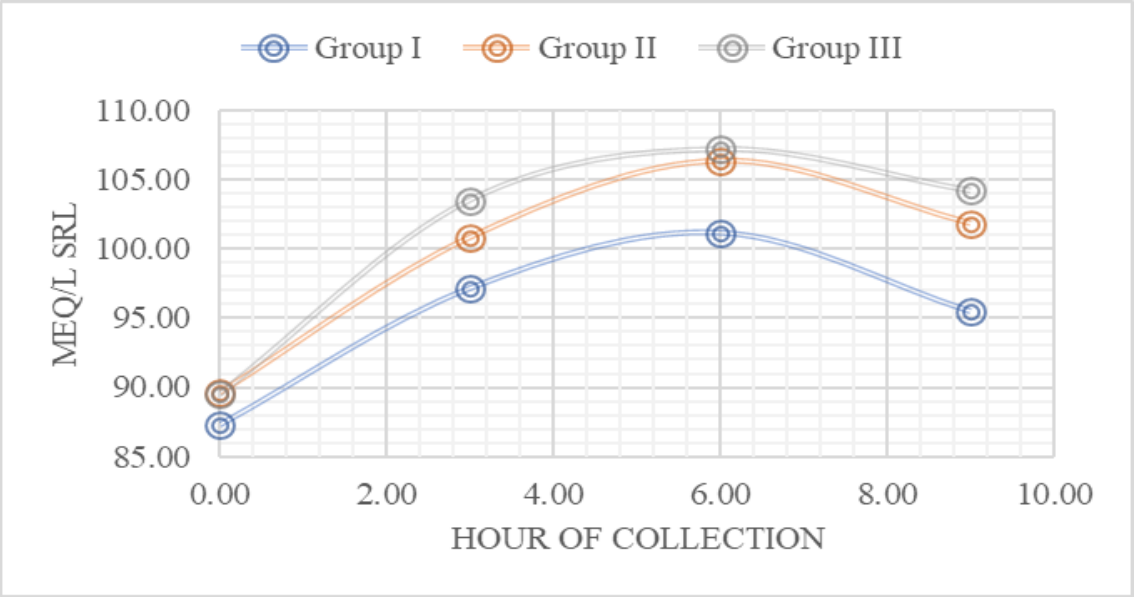

Fig 1: The hourly changes in the TVFA concentration are depicted in

Individual Volatile fatty acids

The VFA concentration in SRL of crossbred calves supplemented with EFE and live yeast culture are presented in Table 2. The production of Acetic, Propionic, Isobutyric, Valerate and Isovaleric acids were non-significant among the treatments. The fractionization of VFA was done at 3rd hr interval with the hypothesis that the production of VFA would reach the peak levels at this stage. The Butyric acid production was highest for the treatment EY15 fed group followed by EY10 and Control respectively. Significant (P<0.05) differences were noticed between the groups pertaining to this parameter. Similarly, few authors reported higher Butyrate fractions on feeding fibrolytic enzymes or live yeast culture (Selzer, 2017; Krueger *et al*., 2008) [23, 12]. In contrast with the above results, few authors reported no effect of either fibrinolytic enzymes (Ran *et al*., 2019; Yang *et al*., 2002) [19, 27] or live yeast culture (Moya *et al*., 2018) [15] on the molar proportions of VFA. The higher Butyrate proportion could be due to increased reactivity of fibrolytic enzyme or yeast causing enhanced colonization of rumen microbiome, thereby increasing the degradability of fiber fractions.

Table 2: Effect of Probiotic and EFE supplementation on VFA fractionization (moles/100 moles)

Parameter	T1	T2	T3
Acetate	59.1 ± 0.73	58.0 ± 0.46	56.6 ± 0.77
Propionate	29.2 ± 0.61	29.0 ± 0.72	29.4 ± 0.69
Butyrate	8.8^{a} ± 0.87	10.4^{b} ± 0.46	11.3^{b} ± 0.35
Valerate	1.2 ± 0.04	1.0 ± 0.06	1.2 ± 0.05
Iso-valerate	1.2 ± 0.11	1.0 ± 0.10	1.0 ± 0.09
Iso-butyrate	0.7 ± 0.04	0.6 ± 0.04	0.6 ± 0.05
A:P	2.1 ± 0.06	2.0 ± 0.06	1.9 ± 0.07

[ab] values in a row bearing different superscripts differ significantly *(P<0.05).

Total Nitrogen

The total nitrogen concentration in SRL of crossbred calves supplemented with EFE and live yeast culture are presented in Table 3. The mean total nitrogen concentration in SRL of crossbred calves was higher (P<0.01) in EY15 group compared to other treatments. The higher total N concentration in supplemented groups could be due to a result of better degradation of protein in the rumen due to efficient fermentation (Poonooru *et al*., 2015) [16]. Similarly, Can *et al*. (2007) [2] observed a higher total nitrogen content on supplementing a combination of EFE and live yeast culture. On contrary, Lopuszanska-Rusek and Bilik (2011) [13] reported that supplementation of both EFEs and live yeast culture in rations of dairy cows had no effect on nitrogen concentration.

Table 3: Effect of EFE and YC supplementation on total N concentration (mg/100 mL)

Treatment	Hour					
	0	3	6	9	Mean**	SEM
T1	72.50	92.50	98.83	94.67	89.63^{A}	1.28
T2	72.67	99.00	109.83	100.00	95.38^{B}	1.65
T3	74.83	103.33	113.17	107.50	99.71^{B}	1.23
Mean**	73.33^{a}	98.28^{b}	107.28^{c}	100.72^{b}		
SEM	0.75	3.15	4.33	3.72		

[ABC]Values in the columns bearing different superscripts differ significantly (P<0.01)
[abc]Values in the rows bearing different superscripts differ significantly (P<0.01)
**Diet × Hour Interaction was observed (P<0.01)

The hourly changes in the Total Nitrogen concentration are showed in Figure 2. The concentration increased ($P<0.01$) up to 6 h post feeding in the crossbred calves followed by a decline. In contrast, Poonooru *et al*. (2015) [16] reported a peak concentration of TVFA at 4 h post-feeding. The hourly changes in Total Nitrogen concentration might be due to active degradation of proteins and hydrolysis of NPN substances in rumen, particularly due to stimulatory effect of EFE and live yeast culture on proteolytic bacteria. The subsequent decline in the Total Nitrogen concentration could be related to changes in the rumen volume through inflow of saliva as well as out flow of digesta (Grubb and Dehority, 1975). The differences in total N concentration due to time of sampling were in agreement with earlier reports (Harikrishna *et al*., 2013; Kiran and Kumar, 2013; Rajamma *et al*., 2014; Poonooru *et al*., 2015) [9, 17, 16]. Diet x hour interaction ($P<0.01$) was observed for Total Nitrogen concentration.

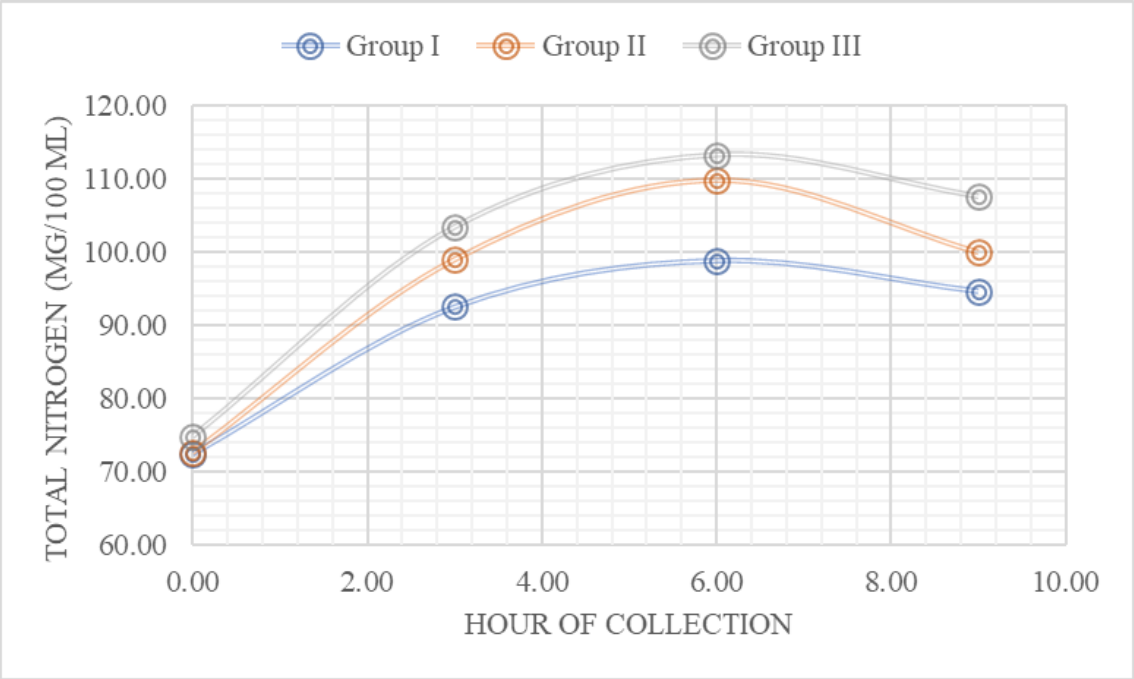

Fig 2: Effect of EFE and YC supplementation on total N concentration

TCA insoluble protein nitrogen

The TCA insoluble protein nitrogen in SRL of crossbred calves supplemented with EFE and live yeast culture are presented in Table 4. The mean TCA insoluble protein nitrogen concentration was higher ($P<0.01$) in EY15 diets compared to other treatments. Likewise, Poonooru *et al*. (2015) [16] reported higher levels of TCA precipitable nitrogen on feeding total mixed rations supplemented with EFE and live yeast culture combination. The higher levels of TCA insoluble nitrogen observed in SRL of crossbred calves supplemented with EFE and live yeast culture might be due to the higher incorporation of ammonia nitrogen into microbial protein (Carro *et al*., 1992) [3].

Table 4: Effect of EFE and YC supplementation on TCA insoluble N concentration (mg/100 mL)

Treatment	Hour					
	0	3	6	9	Mean**	SEM
T1	28.17	34.33	38.58	35.25	34.08^{A}	0.78
T2	28.67	38.58	42.92	38.83	37.25^{B}	1.35
T3	27.83	40.58	43.50	40.75	38.17^{B}	0.75
Mean**	28.22^{a}	37.83^{b}	41.67^{c}	38.28^{b}		
SEM	0.24	1.84	1.55	1.61		

^{AB}Values in the columns bearing different superscripts differ significantly ($P<0.01$)
^{abc} Values in the rows bearing different superscripts differ significantly ($P<0.01$)
No Diet × Hour Interaction was observed (P=0.052).

The hourly changes in the TCA insoluble nitrogen concentration are showed in Fig 3. Time after feeding significantly ($P<0.001$) affected the TCA insoluble protein nitrogen concentration in SRL of crossbred calves. This was in agreement with the findings of earlier researchers (Harikrishna *et al*., 2013; Kiran and Kumar, 2013; Rajamma *et al*., 2014; Poonooru *et al*., 2015) [9, 17, 16]. The TCA insoluble protein nitrogen reached peak concentration at 6 h post feeding beyond which there was a decline. The post-prandially increased TCA insoluble nitrogen is a factor of increased microbial count in all the groups. Diet x hour interaction was not significant (P>0.05) for TCA insoluble protein nitrogen concentration in SRL.

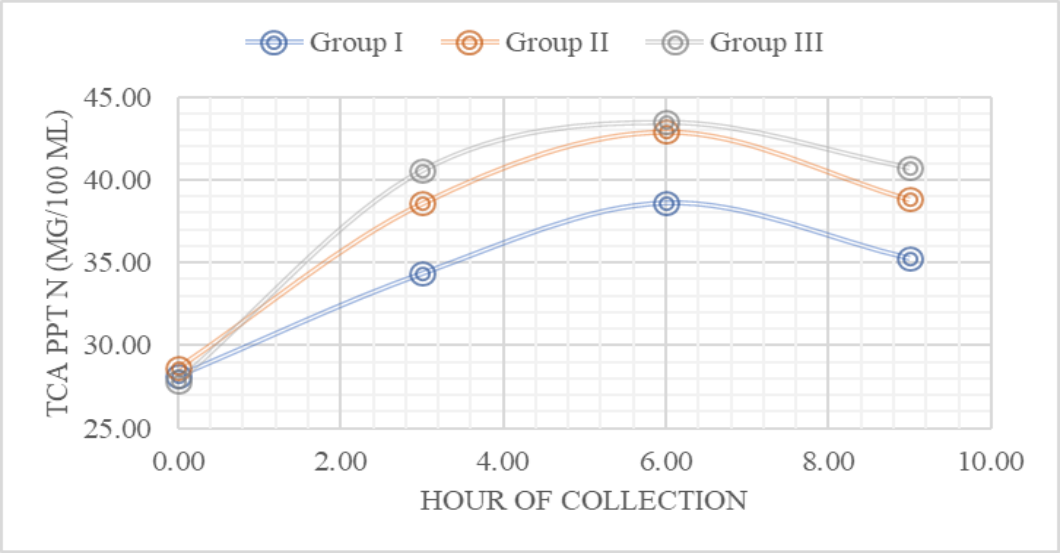

Fig 3: Effect of EFE and YC supplementation on TCA insoluble N concentration

Residual nitrogen

The residual nitrogen in SRL of crossbred calves supplemented with EFE and live yeast culture are presented in Table 5. The residual nitrogen content was higher in EY15 group compared to those fed control and EY10 diets. In corroboration, Poonooru *et al*. (2015) [16] revealed a higher residual nitrogen in buffalo bulls fed diets containing EFE and live yeast culture supplementation. The higher microbial count, ammonia nitrogen, and total nitrogen might have influenced the residual nitrogen in crossbred calves fed EY15 diets.

Table 5: Effect of EFE and YC supplementation on residual N concentration (mg/100 mL)

Treatment	Hour					
	0	**3**	**6**	**9**	**Mean****	**SEM**
T1	21.42	26.33	27.63	27.13	25.63^{A}	0.93
T2	20.00	26.38	32.00	26.33	26.18^{A}	1.21
T3	21.50	30.92	32.21	29.54	28.54^{B}	0.78
Mean**	20.97^{a}	27.88^{b}	30.61^{c}	27.67^{b}		
SEM	0.49	1.52	1.49	0.96		

^{AB}Values in the columns bearing different superscripts differ significantly (P<0.01)
^{abc} Values in the rows bearing different superscripts differ significantly (P<0.01)
No Diet × Hour Interaction was observed (P=0.060)

The hourly changes in the TCA insoluble nitrogen concentration are shown in Figure 4. Time after feeding affected (P<0.01) the residual nitrogen concentration in SRL of crossbred calves. Residual nitrogen concentration increased (P<0.01) up to 6 h post-feeding followed by a steep decline. Higher concentration of total N up to 6 h post-feeding would have influenced the residual N concentration in calves by Poonooru *et al*. (2015) [16]. No significant diet x hour interaction was observed.

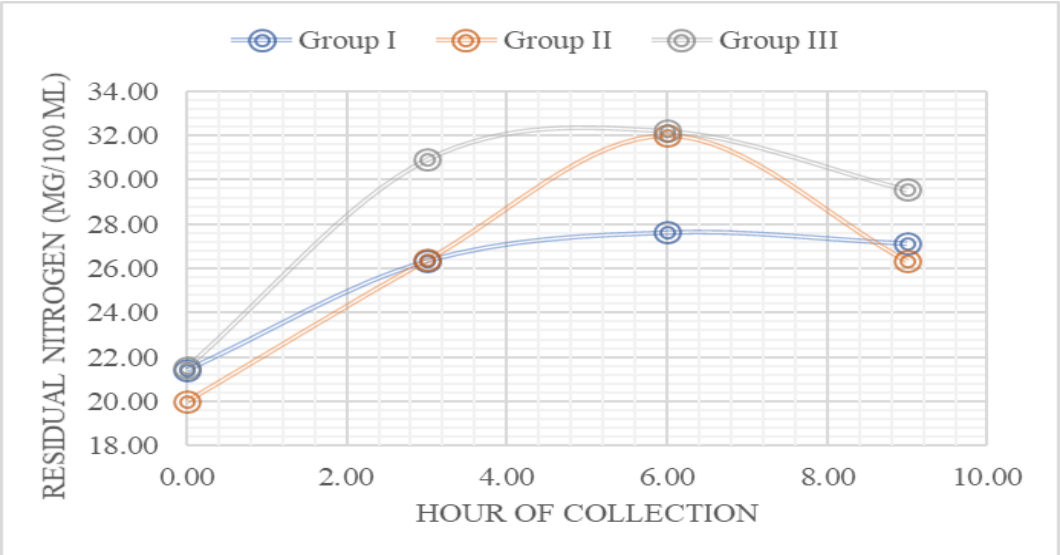

Fig 4: Effect of EFE and YC supplementation on residual N concentration

Food and Protozoal Nitrogen

The food and protozoal nitrogen in SRL of crossbred calves supplemented with EFE and live yeast culture are presented in Table 6. The food and protozoal nitrogen content was higher in EY15 group compared to those fed control and EY10 diets. Conversely, supplementation of yeast culture in TMR had no effect on food and protozoal N concentration (Poonooru *et al*., 2015) [16]. The higher food and protozoal nitrogen could be related to the increased protein degradation and higher microbial count in the calves fed EY10 and EY15 diets.

Table 6: Effect of EFE and YC supplementation on food and protozoal N (mg/100 mL)

Treatment	Hour					
	0	3	6	9	Mean**	SEM
C	10.42	18.08	18.25	18.25	16.25^{A}	1.66
EY10	11.00	21.33	21.58	22.50	19.10^{B}	1.58
EY15	12.50	17.08	21.83	22.67	18.52^{AB}	1.43
Mean**	11.31^{a}	18.83^{b}	20.56^{b}	21.14^{b}		
SEM	0.62	1.28	1.16	1.45		

^{AB}Values in the columns bearing different superscripts differ significantly.

^{ab} Values in the rows bearing different superscripts differ significantly

$^{*}P<0.05$ (n = 6); $^{***}P<0.001$ (n = 6)

No Diet × Hour Interaction was observed (P=0.468)

The hourly changes in the food and protozoal nitrogen are shown in Fig 5. The food and protozoal nitrogen varied with hour with highest concentration in 9 h post-feeding. On contrary, few researchers reported variable peak timings (Poonooru *et al*., 2015; Rajamma *et al*., 2014) [16, 17] The phenomenon of increased food and protozoal nitrogen could be due to the enhanced protein degradation, microbial nitrogen, and total nitrogen after consuming feed. No significant diet x hour interaction was observed.

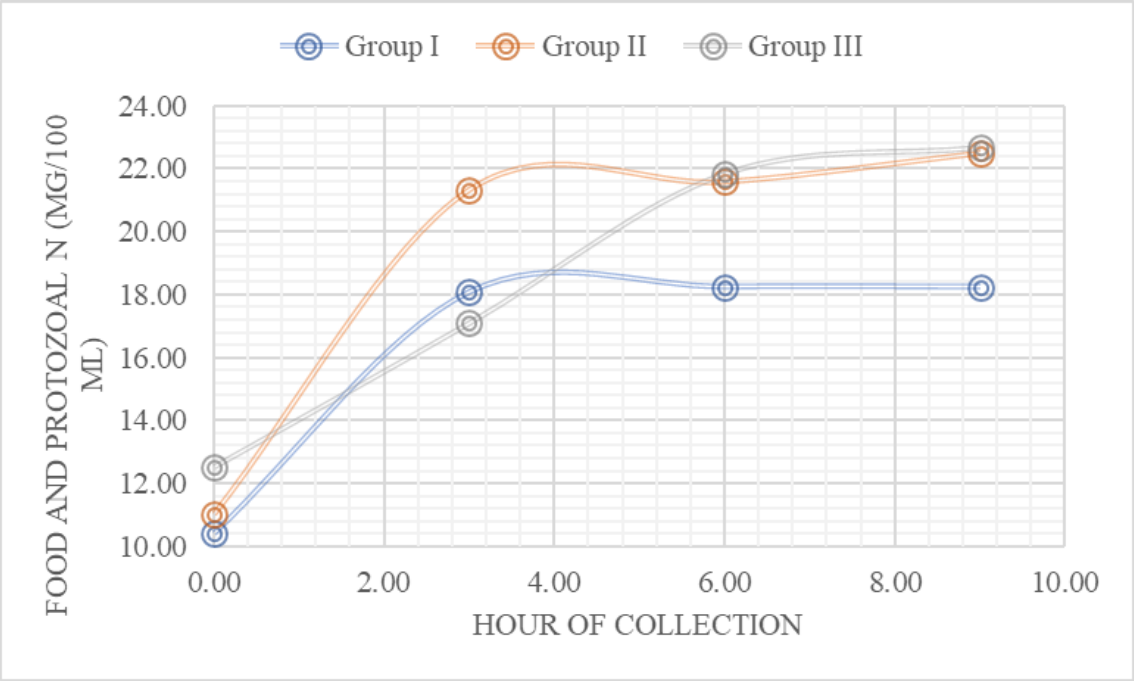

Fig 5: Effect of EFE and YC supplementation on food and protozoal nitrogen

Conclusion

The study concluded that supplementation of EFE and live yeast culture at 15 g/animal/day could improve the rumen fermentation patterns, which is revealed from the higher butyrate levels, total volatile fatty acids, total nitrogen, TCA precipitable nitrogen, residual nitrogen, and food and protozoal nitrogen. The combination of fibrolytic enzyme and live yeast culture in different forage-based diets should be further evaluated in growing calves.

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