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Supplementation of Probiotic and Fibrolytic Enzymes on Growth Performance and Nutrient Utilization in Crossbred Calves

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ABSTRACT

The present study was conducted to evaluate the combined effect of yeast (*Saccharomyces cerevisiae*) and exogenous fibrolytic enzymes (EFE; Cellulase, Xylanase and ß-glucanase) at 2 levels 10 and 15g/animal/day on growth performance and nutrient utilization in cross bred calves. Twenty-four male Jersey × Sahiwal crossbred calves were randomly divided into 3 groups (5-6 months age, 80- 90 kg B.Wt) and were fed with *ad libtum*APBN-1 and concentrate feed @ 1 % of body weight as a basal ration (T_1) which is supplemented with RumEest-ESF at 10 and 15g/animal/day to make groups T_2 and T_3 groups, respectively for a period of 90 days. The average daily gain was higher (P>0.05) in T_3 (599.6 g/d) over T_2 (578.3 g/d) and T_1 (543.6 g/d). No differences were observed for initial and final body weights of the calves. Improved Feed efficiency was observed in yeast and EFE supplemented groups compared to control. The digestibility coefficient of OM, CP, CF, NDF and Hemi-cellulose were found to be significantly (P< 0.05) higher in T_3 among treatments. The DM intakes (kg/day) were 2.46, 2.55 and 2.42 in T_1, T_2 and T_3, respectively. The % DCP and % TDN were significantly (P<0.05) higher in yeast and EFE supplemented group compared to control. Further, the behavioural traits were non-significant, except for total ruminating time (P< 0.05) which was higher in T_2 and T_3 than T_1. It was concluded that supplementation of probiotics and EFE has shown improved feed efficiency, growth rate and rumination time in crossbred calves.

HIGHLIGHTS

- Significantly higher (P<0.05) digestibility of organic matter, Crude protein and crude fiber was observed in probiotics and exogenous fibrolytic enzymes supplemented calves.
- Average daily gain was higher in probitics and EFE supplemented calves.

Keywords: Behavioural traits, Crossbred Calves, Cellulase, Growth, *Saccharomyces cerevisiae*

Ruminant production, especially in developing countries is poor compared to the developed countries. The reason could be attributed to the lower digestibility-related feed resources. In tropics, farmers are forced to feed their animals with poor quality roughages containing high amount of structural carbohydrates with little or no concentrate diets adversely affecting the production potential of calves (Arowolo and He, 2018) and Ruminants (Venkateswarlu *et al.,* 2018).

To improve the utilization of poor-quality roughages nutritionists are evaluating different strategies to manipulate the rumen microbiota. One of such strategy is supplementation of yeast and exogenous fibrolytic enzymes. Several studies had shown that supplementation

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of exogenous fibrolytic enzymes increases the fibre digestion there by increased available energy to ruminants which results in increased nutrient digestibility (Kholif *et al.,* 2012), growth rate (Malik and Bandla, 2010) and rumen fibrolytic bacteria activity (Harini *et al.,* 2021). Further, supplementation of yeast had shown that yeast cell wall products mannooligosaccharides, β-glucans have positive impact on growth of animals (Eicher *et al.,* 2010) and also absorb the pathogens and improves the immune response (Liu *et al.,* 2018 and Ma *et al.,* 2020). However, the response of the yeast was not consistent which will depend on several factors (Patra, 2012). Few studies dealt the synergistic action of combination of roughage-supplemented fibrolytic enzymes and live yeast culture on rumen fermentation (Poonooru *et al.,* 2015). However, the research pertaining to their synergistic effects on the growth and nutrient digestibility of cross bred calves is not clear under Indian conditions. Hence, the current experiment was carried out to study the effect of Exogenous Fibrolytic Enzymes and yeast combination on growth, digestibility and Behavioural traits of cross bred calves.

MATERIALS AND METHODS

The present experiment was conducted at Livestock Farm Complex, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati.

In a growth trial of 90 days 24 healthy cross bred calves (5-6 months age) were randomly allocated to three dietary treatments and were fed with Control ration (T_1) consisting of ad libitum APBN -1 and concentrate feed, control ration supplemented with RumEest-ESF @ 10 g/ day (T_2) and control ration with RumEest-ESF @ 15 g/ day (T_3). All the animals were offered concentrate mixture @1% body weight and adlibitum quantity of APBN-1 as the basal diet to meet their nutrient requirements as per ICAR (2013). The Concentrate feed was incubated with RumEest-ESF for an hour before feeding. The RumEest-ESF is a combination of Probiotics (*Saccharomyces cerevisiae* @ 5 billion CFU/ gram) and enzymes (Cellulase, Xylanase, β- glucanase) which was procured from the Neospark drugs and chemicals private limited, Hyderabad. The calves were housed in well ventilated experimental sheds where the facilities were available for individual feeding and watering. Calves were offered with experimental diets at 9:00 and 15:00 hours. All the animals have free access to clean drinking water for 24 hrs. All the calves were dewormed before staring the experiment and monthly interval during the experiment.

Growth performance

Growth trial was carried for 90 days. Animals were offered with experimental diets twice a day and left-over feeds were collected next day morning to calculate the dry matter intake. All the animals were weighed individually by using electronic balance at the beginning of the experiment and fortnightly interval during the experimental period in the morning before feeding and watering to know the effect of experimental diets on average daily gain (ADG) and feed efficiency.

Digestion trial

At the end of the growth trial, digestibility trial was carried out by following 7 days collection period to determine the digestibility of nutrients. During the collection period, all the animals were kept in a separate shed where facilities are available for individual feeding, watering and collection of faeces. Total faeces voided during 24hrs was collected manually and recorded every day morning at 9:00 AM for seven consecutive days. The representative samples of faeces voided was taken separately for each animal after through mixing and stored in a deep freezer at –20º C for 7 days. Aliquot of faeces was taken every day for dry matter analysis. To calculate total faecal dry matter output. At the end of the digestion trial pooled faecal samples were thawed to room temperature, mixed thoroughly, dried in a hot air oven at 60ºC and ground to pass through 1 mm screen and preserved in air tight bottles for further analysis.

Chemical analysis

The representative samples of feed offered, left over feed and faeces were collected, dried at 60ºC and ground to pass through 1 mm screen and were analysed for proximate principles (AOAC, 2000) and fibre fractions (Van Soest *et al.*, 1991) respectively.

Behavioural study

Behavioural data recorded in this study was as per the method described by Dias *et al*. (2018). Behaviour of an animal was determined and recorded once a week during 3 months experimental period by walking through the calf barn, at a distance from the calf pen at least 2.0 m, for 12 hours at 5 minutes interval. The behaviour was observed and recorded for each of the following activities - eating (calf's head was in feed manger), drinking water (calf's head in water bucket), lying (calf's body contacted bedding and ground), standing (calf was inactive in upright position) lying and ruminating, standing and ruminating and total ruminating time (Lying and ruminating + standing and ruminating).

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). All the statistical procedures followed were in accordance with Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Chemical composition of APBN-1and Concentrate feed

The chemical composition of APBN-1 and concentrate feed is presented in Table 1. Crude protein content of APBN -1 is 8.5 % which is similar to reports of Singh *et al.* (2018). On contrary to present study more crude protein content was observed by Senthikumar *et al.* (2020). Crude fibre content observed in the present study is higher than that of Jagadeesh *et al.* (2017) and lower than the Senthikumar *et al.* (2020). NDF content observed in the present study was similar to that of Basyble *et al.* (2007). However lower NDF values were reported by reported by Jagadeesh *et al*. (2017), Senthikumar *et al.* (2020) Bora *et al.* (2012). Variation in the chemical composition might be attributed to stage of harvesting. Significant increase in NDF, ADF and Hemicelllulose and decrease in protein content was observed as the plant matures (Basyble *et al.,* 2007). Concentrate mixture was prepared to meet the nutrient requirement of cross bred calves according to ICAR (2013).

Table 1: Chemical composition of feedstuffs (% DM basis)

Nutrient	APBN	Concentrate mixture
DM	89	91
OM	90.6	91.5
TA	9.4	8.5
CP	8.5	20.02
EE	2.2	3.12
CF	32.6	5.25
NFE	47.3	63.11
NDF	79.6	26.2
ADF	46.58	15.3
ADL	8.49	2.62
Hemicellulose	33.02	10.9
Cellulose	37.05	12.32
Calcium	1.58	1.64
Phosphorus	0.68	0.72

Growth performance

Effect of exogenous fibrolytic enzymes (EFE) and yeast supplementation of growth rate and feed efficiency was presented in Table 2. The weight gain of crossbred calves was higher for T_3 group followed by T_2 and T_1. Non significantly higher ($P>0.05$) average daily gain was higher observed in probiotic and EFE supplemented groups might be attributed to the pre-treatment of concentrate feed with enzymes, which enables a stable feed-enzyme complex even before entering rumen (Lourenco *et al.,* 2020). With increased dose of probiotic and EFE, the enzymes attack more cellulose particles paving the way to release more entrapped nutrients including the soluble sugars and the cell bound protein (Malik and Bandla, 2010) improving growth rate. These results are in corroboration with Malik and Bandla (2010), Gallardo *et al*. (2010). On contrary, the dosage did not improve any of the growth parameters in brown swiss and HF calves fed probiotic or EFE combination (Kocyigit *et al.,* 2015). These inconstancies might be related to multiple factors such as method of application, dose of enzyme, contact time of enzyme with substrate, type of substrate, and presence of strong ligno-cellulose bonds.

Table 2: Effect of EFE and yeast supplementation on body weight gain and feed efficiency

Treatment	Initial wt (kg)	Final wt (kg)	Wt gain (kg)*	ADG (g)*	Feed efficiency
T_1	82.3 ± 6.52	131.8 ± 5.98	49.8[a] ± 4.30	543.6[a] ± 47.21	5.30 ± 0.53
T_2	83.4 ± 4.21	136.0 ± 4.59	52.6[b] ± 4.66	578.3[b] ± 51.17	5.07 ± 0.45
T_3	82.6 ± 4.50	137.1 ± 4.16	54.6[c] ± 0.78	599.6[c] ± 44.85	4.88 ± 0.42
P-value	0.911	0.102	0.084	0.084	0.222

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

Nutrient digestibility

The apparent nutrient digestibility of nutrients due to supplementation of EFE and yeast was presented in Table 3.

Table 3: Effect of EFE and yeast supplementation on nutrient digestibility (%) in cross bred calves

Nutrient	T_1	T_2	T_3	P-value
Dry matter	70.24 ± 0.85	72.42 ± 1.34	73.14 ± 1.00	0.168
Organic matter*	73.33[a] ± 0.65	76.11[ab] ± 0.74	76.69[b] ± 0.98	0.017
Crude Protein*	73.01[a] ± 1.01	75.78[ab] ± 1.24	76.66[b] ± 0.72	0.048
Ether extract	64.85 ± 1.57	65.75 ± 0.56	66.72 ± 1.11	0.531
Crude fibre*	58.13[a] ± 0.97	61.89[ab] ± 1.00	62.67[b] ± 1.21	0.015
Nitrogen free extract	79.92 ± 0.95	82.49 ± 0.90	82.45 ± 1.37	0.169
Neutral Detergent Fibre*	59.45[a] ± 1.36	62.26[ab] ± 0.76	63.54[ab] ± 0.98	0.037
Acid Detergent Fibre	54.04 ± 2.20	56.52 ± 0.96	57.13 ± 1.03	0.327
Hemi-cellulose*	67.07[a]± 1.32	70.36[ab]± 1.57	72.57[ab]± 1.51	0.047
Cellulose	58.72 ± 1.10	59.13 ± 1.36	59.60 ± 1.44	0.892

[abc] Values in the rows bearing different superscripts differ significantly *P<0.05 (n=8).

The study indicated that supplementation of Probiotics and EFE (T_2 and T_3) had significantly higher (P<0.05) digestibility of OM, CP, CF, NDF and Hemicellulose as compared to T_1. Similar to these results Beauchemin and Holtshausen (2010), Kumar *et al*. (2010) Marwan *et al.* (2019) also reported higher digestibility coefficient in calves supplemented with exogenous fibrolytic enzymes or live yeast. Increased digestibility might be due to capacity of exogenous enzymes to release reducing sugars from feedstuffs prior to consumption (Ran *et al.,* 2019). Pre-treatment of enzymes with diet can release the sugars from feeds due to the partial solubilization of NDF and ADF (Lynch *et al.,* 2014) or live yeast might have promoted the growth of cellulolytic bacteria, buffering the ruminal fluid and increasing rumen lactic acid utilizing bacteria (Rossow *et al.,* 2018).

Plane of Nutrition

The Effect of EFE and yeast supplementation on nutritive value was presented in Table 4. The dry matter intake was almost similar among the treatments. The present study revealed that supplementation of both EFE and Probiotic had shown significantly (P<0.05) higher % DCP and %TDN inT_2 and T_3compared to control. Even though, the DCP and TDN content expressed as % in the diet consumed or kg/d increased linearly with increased level of enzymes and yeast supplementation, but the differences were not statistically significant and were these are corroborated with the findings of Poonooru *et al*. (2015). Similarly, some researchers reported increased DCP and TDN content with EFE (Marwan *et al*., 2019; Bhasker *et al.*, 2013) and yeast culture (Mahender *et al*., 2005; Raj Kiran *et al*., 2014) supplementation in the diets.

Table 4: Effect of EFE and yeast supplementation on nutritive value in crossbred calves

Parameter	T_1	T_2	T_3
DM intake kg /100 Kg B.Wt	2.47	2.52	2.42
DMI g /Kg $W^{0.75}$	78.0	79.9	76.4
DCP intake g /Kg $W^{0.75}$	7.10	7.45	7.31
TDN intake g /Kg $W^{0.75}$	53.2	56.7	54.3
DCP%	9.1[a]	9.32[ab]	9.56[b]
TDN %	68.2[a]	70.8[ab]	71.1[b]

[ab] Values in the rows bearing different superscripts differ significantly *P<0.05 (n=8).

Behavioural traits

The Lying time, standing time, lying and ruminating time, standing and ruminating time, eating time, chewing time, drinking time and idleness time were non-significant (P>0.05) and total ruminating time was significantly (P<0.05) different among treatments (Table 5). The chewing time was recorded highest in T_2 followed by T_3 and T_1. This finding might be an evidence of the role of the yeast on stimulating ruminative behaviour in bulls as reported by Magrin *et al*. (2018), DeVries and Chevaux (2014) in dairy cows. On contrary, Kocyigit *et al*. (2016) reported an unaltered rumination time and other behavioural traits.

Table 5: Effect of EFE and yeast supplementation on behavioural changes (minutes) of crossbred calves

Parameter	T_1	T_2	T_3	P value
Lying time	265.63 ± 8.99	274.38 ± 12.23	275.63 ± 10.33	0.77
Standing time	454.38 ± 9.02	445.63 ± 12.54	444.38 ± 10.21	0.77
Lying & Ruminating time	63.75 ± 5.57	69.38 ± 6.37	73.75 ± 2.63	0.399
Standing & Ruminating time	188.75 ± 7.43	201.88 ± 4.62	200.00 ± 5.59	0.268
Total ruminating time*	252.50^{a} ± 5.75	271.25^{b} ± 4.30	273.75^{c} ± 4.30	0.010
Eating time	239.38 ± 10.54	240.00 ± 10.00	230.00 ± 9.96	0.741
Chewing time	491.88 ± 10.56	511.25 ± 9.44	503.75 ± 8.44	0.366
Drinking time	8.13 ± 1.32	6.88 ± 0.91	7.50 ± 0.94	0.717
Idleness time	228.13 ± 10.56	208.75 ± 9.44	216.25 ± 8.44	0.366

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

CONCLUSION

Supplementation of crossbred calves with probiotics and exogenous fibrolytic enzymes has resulted in significant improvement in average daily gain, nutrient digestibility and total rumination time compared to non-supplemented groups. Hence, it can be concluded that supplementation of probiotic and exogenous fibrolytic enzymes (RumEest -ESF) @ 10g and 15 g per day has better growth rates. However, higher level of supplementation has better performance over the lower levels.

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