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M Harani
Sri Venkateswara Veterinary University, Department of Livestock Farm Complex College of Veterinary Science, Tirupati, Andhra Pradesh, India

MVAN Suryanarayana
Sri Venkateswara Veterinary University, Department of Livestock Farm Complex College of Veterinary Science, Tirupati, Andhra Pradesh, India

B Devasena
Sri Venkateswara Veterinary University, Department of Livestock Farm Complex College of Veterinary Science, Tirupati, Andhra Pradesh, India

Y Ravindra Reddy
Sri Venkateswara Veterinary University, Department of Livestock Farm Complex College of Veterinary Science, Tirupati, Andhra Pradesh, India

Corresponding Author:
M Harani
Sri Venkateswara Veterinary University, Department of Livestock Farm Complex College of Veterinary Science, Tirupati, Andhra Pradesh, India

Effect of probiotic and exogenous fibrolytic enzymes on blood and faecal consistency score

M Harani, MVAN Suryanarayana, B Devasena and Y Ravindra Reddy

Abstract
The objective of the study was to evaluate performance of crossbred calves supplemented with Probiotic and Exogenous Fibrolytic Enzymes under intensive system. In a completely randomized design, 24 growing calves were divided into 3 equal groups of eight each. APBN-1 was fed *ad libitum* and concentrate feed @1% of their body weight to all the calves. A basal diet was fed to one group (T1) and is supplemented with RumEest-ESF@ 10g/animal/day (T2) and 15g/animal/day (T3). All haematological parameters like RBC, WBC, Hb, PCV, MCV, MCH and MCHC were non significantly higher (P>0.05) in Probiotic and EFE groups. Supplementation of Probiotic and EFE did not show any significant difference among the groups pertaining to serum glucose, total protein, albumin, globulin, BUN, creatinine, cholesterol and triglycerides and also on serum enzymes AST and ALT (U/L). The similar trend was observed in the serum macro minerals (Ca, P and Mg). The faecal consistency scores for T1, T2 and T3 were 1.50± 0.27, 2.63 ± 0.18 and 2.88 ± 0.30, respectively and found to be significant (*P*<0.05) among treatment groups. It was concluded that supplementation of EFE and live yeast culture at 15 g/animal/day could improve the and faecal consistency score with no effects on haemogram and blood biochemical parameters. The combination of fibrolytic enzyme and live yeast culture improved rumination capacity of animal which gives a positive indication for reduction of diarrhoea and in turn productive performance.

Keywords: probiotics, EFE, haematology, blood chemical parameters

Introduction
Usage of antibiotics has been banned in many countries including European Union due to the potential to develop antibiotic resistance in microbial populations associated with human and animal diseases (Prieto *et al.*, 2014) [9]. The existence of antibiotic resistance in bacteria is a major health concern worldwide (Beauchemin *et al.*, 2006). Feed additives are the non-nutritional products used in animal diets to improve production efficiency and performance (Jacela *et al.*, 2009). Feed additives can potentially reduce the use of antibiotics and ionophores in commercial cattle production and enhance the production efficiency of cattle. (Beauchemin *et al.*, 2006). Yeast promotes the development of the immune system in young animals (Quigley, 2012) [10]. However, in some studies, the response to supplementation with DFM has been contradictory (Frizzo *et al.*, 2008) [5]. Many reporters demonstrated both positive or negative effects on the use of a combination of probiotics. The present was therefore undertaken to assess synergistic effects of DFM and EFE on blood biochemistry and faecal consistency score.

Materials and Methods
Twenty-four healthy male crossbred calves (HF × Jersey, Jersey × Sahiwal) reared in Livestock Farm Complex, College of Veterinary Science, Tirupati were selected for the study. All the calves were fed with *ad lib* APBN-1 and concentrate feed @1% of their body weight but T2 is supplemented with 10g of RumEest-ESF/animal/day and T3 is fed with 15g of RumEest-ESF/animal/day. The concentrate feed was pre-treated with RumEest-ESF for an hour before feeding for both supplemented groups. The product ‘‘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). The calves were fed according to the experimental diets at 900 and 1500 hours by weighing in electronic balance. The animals were allowed to have clean drinking water throughout the day. All the calves were dewormed before the start of the trail and at a monthly intervals during the experimental period.

The blood samples were obtained by jugular vein puncture and two blood samples from each animal were collected into vacutainer tubes containing clot activator gel for biochemical studies and EDTA for haematological studies. The samples were transported in a cold environment to prevent denaturation of the blood before analysis. The blood in the tubes containing clot activator gel was centrifuged at 3000 rpm for 15 mins for serum separation. The serum was stored in eppendorf tubes at -20 °C until further analysis. Haematological parameters were determined using Mindray auto-haematology analyser, Model Bc-2800.The blood serum was analyzed for serum glucose, total protein, albumin, globulin, total cholesterol, BUN, triglycerides, ALT, AST, Calcium, Phosphorus and Magnesium were estimated using a commercial kit (Biosystems, Spain) using automated A15 biochemical analyzer, Biosystems, Spain.

According to physical appearance of faeces, faecal fluidity score developed by Di francia *et al*. (2008) [1] was used in this study. The faecal consistency was assessed daily, using a numerical score of 0–4 (i.e., 0 = severe scours, 1 = scours, 2 = soft, 3 = normal, 4 = firm). No treatment for scours was initiated if diarrhoea was assessed to be occurring.

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) [2]. All the statistical procedures followed were in accordance with Snedecor and Cochran (1994) [13].

Results and Discussion

Hemogram

The additives did not show any negative influence on erythrogram parameters and were within the normal range (Table 1). The RBC (10^6/µl), WBC (10^3/µl) and Hb (gram%) of calves fed with/without DFM and EFE were numerically higher in T2, T3 and T1 groups, respectively. All haematological parameters RBC, WBC, Hb, PCV, MCV, MCH and MCHC were found non-significant ($P>0.05$) in calves fed with Probiotic and EFE. The packed cell volume (PCV) (%), mean corpuscular volume (MCV) (fl), mean cell haemoglobin (MCH) (pg) and mean cell haemoglobin concentration (MCHC) (g/dl) values were comparable among treatments and non-significant ($P>0.05$). These results were in agreement with the studies of Hussein (2014) and Milewski and Sobiech (2009) [8] in lambs fed with probiotic-based supplements. Conversely, Sheikh *et al.* (2019) [12] reported that hemoglobin concentration, RBC, WBC and monocyte count in lambs fed Probiotic mix was found significantly ($P<0.01$) higher, with no significant effect on PCV, lymphocyte, eosinophill and neutrophill count.

Table 1: Effect of dietary treatments on Hematological parameters of crossbred calves

Parameter	T1	T2	T3	P-value
RBC (10^6/µl)	8.61 ± 0.25	8.66 ± 0.36	8.49 ± 0.46	0.943
WBC (10^3/µl)	13.36 ± 0.53	14.61 ± 0.51	14.05 ± 0.57	0.279
Hb (g%)	13.25 ± 0.30	13.80 ± 0.26	13.10 ± 0.49	0.379
PCV (%)	36.32 ± 0.85	37.23 ± 0.51	36.14 ± 0.99	0.602
MCV (fl)	42.44 ± 1.44	43.59 ± 2.15	43.50 ± 2.69	0.916
MCH (pg)	15.52 ± 0.66	16.18 ± 0.91	15.91 ± 1.39	0.901
MCHC (g/dl)	36.62 ± 1.12	37.10 ± 0.80	36.51 ± 1.95	0.950

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

Blood biochemical constituents

The serum metabolites, glucose, total protein, albumin, globulin, cholesterol, triglycerides, BUN, creatinine, AST, ALT and serum minerals (Ca, P and Mg) were presented in Table 2. The values were found to be non-significant ($P>0.05$) and were comparable among treatments. These results were in agreement with EL moghazy *et al*. (2015) [4] and Sheikh *et al*. (2019) [12]. On the contrary, Ullah *et al*. (2019) [20] observed a significant (P<0.1) reduction of serum triglyceride levels on supplementing yeast to the diets fed and Sheikh *et al*. (2019) [12] observed that animals fed probiotics showed higher levels of BUN and creatinine in treatment groups.

Table 2: Effect of dietary treatments on biochemical parameters of crossbred calves

Parameter	T1	T2	T3	P value
Glucose	60.13 ± 1.30	62.50 ± 1.30	61.75 ± 1.50	0.468
Total Protein	7.79 ± 0.13	7.72 ± 0.14	7.89 ± 0.18	0.721
Albumin	4.37 ± 0.10	4.55 ± 0.11	4.65 ± 0.19	0.369
Globulin	3.42 ± 0.16	3.17 ± 0.19	3.24 ± 0.07	0.508
Cholesterol	79.90 ± 2.03	78.63 ± 2.05	82.90 ± 2.59	0.399
Triglycerides	25.2±0.33	24.7±0.94	23.7±0.69	0.307
BUN	18.03 ± 0.75	17.55 ± 0.60	17.18 ± 0.66	0.677
Creatinine	1.35 ± 0.12	1.42 ± 0.07	1.54 ± 0.10	0.429
AST	87.17 ± 1.55	84.35 ± 1.82	84.57 ± 2.11	0.495
ALT	33.45 ± 0.75	36.77 ± 0.83	37.01 ± 1.31	0.33
Ca	9.36 ± 0.31	9.92 ± 0.33	9.45 ± 0.38	0.469
P	5.89 ± 0.33	5.12 ± 0.34	5.56 ± 0.37	0.312
Mg	1.9±0.77	2.01±0.76	2.19±0.12	0.272

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

Faecal Consistency Score

The effect of Probiotic and EFE supplementation on faecal consistency score were found to be significant ($P<0.05$) among treatment groups (figure 1). These findings could be

related to the antimicrobial activity and prophylactic role of Probiotic. The results were supported by the previous findings of Jatkauskas and Vrotniakiene (2010) [6] and Le *et al*., (2016) [7], who revealed reduced incidence of diarrhoea by 30-40% with increased average daily gain of calves. Probiotics produce antimicrobial substances and metabolic compounds that suppress the growth of pathogenic microorganisms. In contrast, Riddell *et al*. (2010) [11] found no difference in the incidence of diarrhoea in new born calves.

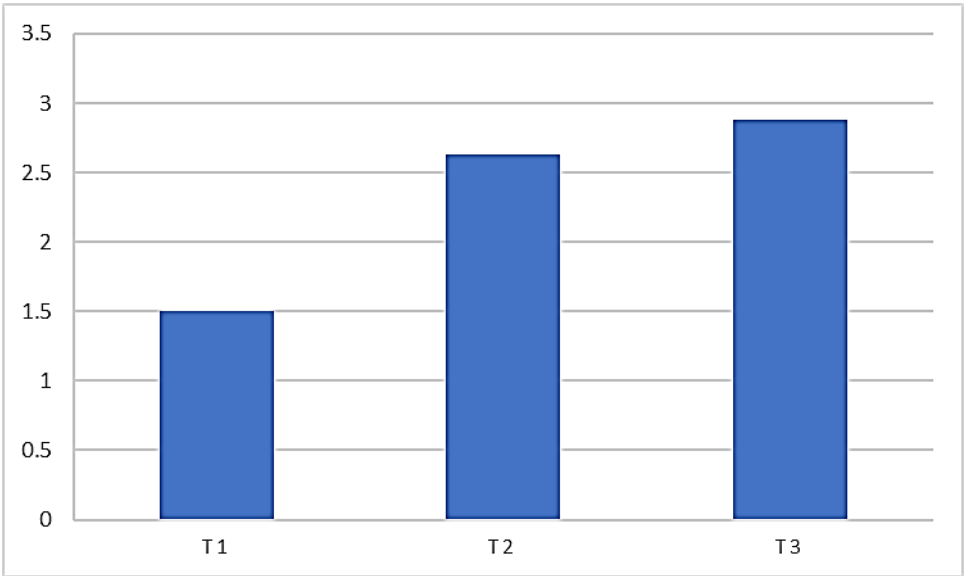

Fig 1: Effect of supplementation on Faecal Consistency Score of crossbred calves (P<0.010)

Conclusion

The study concluded that supplementation of EFE and live yeast culture at 15 g/animal/day could improve the Faecal consistency score with no adverse effects on haemogram and blood biochemical parameters. The combination of fibrolytic enzyme and live yeast culture reduced diarrhoeal condition of animal and enhanced productive performance.

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