RESEARCH ARTICLE

Effect of Fibrolytic Microbes and Enzymes on Biochemical Blood Parameters in Crossbred Calves

RI Kapadiya[1]*, SV Shah[2], YG Patel[3], PR Pandya[4]

Abstract

Twenty crossbred (HF x Kankrej) calves were allotted randomly to five groups (each had one male and three female calves) almost with similar body weight (85.70 ± 6.37 kg) and age (167.55 ± 21.70 days). Experimental calves of the control group were offered hybrid napier untreated, and the other four groups were offered hybrid napier treated with (i) fungus- *Aspergillus spp*. (1×10^7 per g feed), (ii) fibrolytic bacteria- *Escherichia spp*. (10^6 CFU per g feed), (iii) xylanase (50 mL/kg having xylanase 1.2 IU/mL), and (iv) consortium of 1/3rd dose of fungus + bacteria + enzyme as treatments for 140 days. The blood samples were collected from each calf at a biweekly intervals in the EDTA vacuttee. Nutrients offered to crossbred calves were sufficient to satisfy the nutrients requirements. Bacteria fed calves had significantly higher ($p < 0.05$) mean hemoglobin levels than control. Blood plasma glucose of calves in fibrolytic microbes and enzyme groups was statistically similar to control. Feeding of fungus, bacteria, enzyme, and consortium was without a significant effect on plasma total proteins. In comparison to control (7.53 mg/dL), the plasma phosphorus was significantly ($p < 0.05$) higher in calves fed enzyme (7.87 ± 0.20 mg/dL) and lower in group fed fungus (7.13 mg/dL). In contrast, in bacteria (7.57 mg/dL) and consortium group (7.60 mg/dL) it was statistically similar to control. Plasma glutamic pyruvic transaminase concentration of crossbred calves was lower ($p < 0.05$) in enzyme and higher ($p < 0.05$) in fungus, bacteria, and consortium groups than in control, while plasma glutamic oxaloacetate transaminase concentration in all groups was statistically similar. In conclusion, all blood parameters of different groups were within normal range and without adverse effect on the overall performance of calves.

Keywords: Blood biochemical parameters, Crossbred calves, Fibrolytic enzyme, Fibrolytic microbes, Haemoglobin.

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Introduction

An increased need for feed and fodder and a decrease in available natural resources has led to new diverse challenges and constraints. The available feed resources are inadequate, hence feeding strategies to enhance nutrients availability by use of alternative feed technologies are the ways to fulfill the requirement for growing cattle and buffaloes. Alternative feed resources using agro by-products is a reasonable strategy in low-input systems (Dayani *et al.,* 2012). Still, crop residues are having low digestibility of lignocellulosic bond, often less than 600 g/kg of dietary fiber is digested by the animal (Van Soest, 1994). Plant cell walls typically consist of about 35-50% cellulose, 20-35% hemicellulose, and 10-25% lignin in the dry mass (Sticklen, 2008). Direct fed microbes (DFM) with exogenous fibrolytic enzyme (EFE) had both improved growth performance (Kocyigit *et al.,* 2016) and without or sometimes even negative effect (Baloyi, 2008). Researchers had also reported that yeast culture improved feed intake, feed conversion efficiency, growth rate (Kumar *et al*., 2015), nutrient digestibility and hematology (Agazzi *et al*., 2014) in cost-effective manner. Fibrolytic enzymes in ruminant diets showed improved nutrient digestibility (Lunagariya *et al*., 2019), growth rate and milk production (Kholif *et al*., 2012). Therefore, this experiment was planned to evaluate the effect of fibrolytic microbes and enzymes on blood biochemical parameters of crossbred calves with the above facts in mind.

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Materials and methods

The study was conducted at Livestock Research Station, AAU, Anand for 140 days as per guideline of Institutional Animal Ethics Committee (Sanction No. 285/LPM/2018) of the College. Twenty crossbred (Holstein Friesian x Kankrej) calves were randomly allotted to five groups (each group had one male and three female calves) with almost similar body weight (85.70 ± 6.37 kg) and age (167.55 ± 21.70 days).

Feeding Trial

To meet nutrient requirements (ICAR, 1998), all calves were individually fed green hybrid napier (CO_3 variety), concentrate, and sorghum hay. The exogenous fibrolytic microbes and enzymes used in this experiment were isolated from animals of this farm and purified by the Department Animal Biotechnology, Veterinary College, AAU, Anand. The experimental animals were let loose for two hours (8:00 to 10:00) in the morning for exercise during which wholesome clean drinking water was available. Drinking water was also offered in afternoon and night hrs. Measured quantities of hybrid napier (treated and untreated), concentrate and sorghum hay were offered to animals at 10:00, 14:00, and 16:00 hrs, respectively. Experimental animals of control group were offered untreated hybrid napier, and other four groups were offered hybrid napier treated with fungus- *Aspergillus spp*. (1×10^7 per g feed), fibrolytic bacteria- *Escherichia spp*. (10^6 CFU per g feed), xylanase (50 mL/kg having xylanase 1.2 IU/ml), and a consortium of 1/3rd dose of fungus + bacteria + enzyme, respectively. Two kilogram per calf of hybrid napier for the treatment group was thoroughly mixed with fungus, bacteria, enzymes or consortium 20 hrs before feeding as per treatment and was covered with a plastic sheet on cemented floor. It was mixed two times before feeding. Treated hybrid napier was equally distributed among the calves considering moisture loss at the time of feeding. Quantity of hybrid napier and concentrate was fed to calves without leftover, and sorghum hay was offered *ad libitum* to fulfill a nutrient requirement.

Blood profile

The blood samples were collected aseptically at biweekly intervals in the morning from the jugular vein in EDTA vacuttee. Plasma was separated by centrifuging vacuttee for 15 minutes at 3000 rpm and was stored at -20°C until analyzed. Hemoglobin level was estimated on blood analyzer (BC-2800 Vet, Mindray). Plasma glucose, glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, total protein, and phosphorus were estimated by using ready to use kits of Corel Clinical System, Goa on biochemistry analyzer (BS-120, Mindray). Data were analyzed statistically, as per Snedecor and Cochran (2002).

Results and discussion

Proximate nutrients of fodders and feed are given in Table 1. It shows that the nutrients intake of crossbred calves was sufficient to satisfy the nutrients requirements (ICAR, 1998). Blood biochemical parameters of calves evaluated are given in Table 2.

Hemoglobin

Significant ($p < 0.05$) difference was found in biweekly hemoglobin levels in calves of different treatment groups. It was significantly higher in bacteria fed calves and lower in enzyme fed calves than the control (Table 2). In earlier studies, feeding a diet with 1 g/day probiotic to calves for the first month of age significantly ($p \leq 0.05$) increased hemoglobin as 9.29 vs. 8.44 g/dL (Agazzi *et al.,* 2014). Similarly, improved hemoglobin (110.0 vs. 98.0 g/L) was observed at the end of the experiment on 20 ml direct oral fed microbes (*Propionibacterium jensenii*) in Holstein bull-calves (Adams *et al.,* 2008). Contrary to the present study, Maldonado *et al.* (2018) reported non-significantly lower hemoglobin (144 ± 13 vs. 147 ± 42 g/L) in calves fed milk fermented with lactic acid

Table 1: Proximate composition of the ingredients and feeds

Feeds/Fodders	*DM %*	*CP %*	*CF %*	*EE %*	*NFE %*	*Ash %*
Compound concentrate	90.50	25.08	10.52	2.89	56.04	13.28
Sorghum hay	87.69	5.34	30.53	2.09	49.71	9.41
Hybrid napier	23.70	8.46	31.23	2.58	40.95	12.49

Note: DM = dry matter, CP = crude protein, CF = crude fibre, EE = ether extract, NFE = nitrogen-free extract

Table 2: Haemoglobin and blood plasma biochemical profile of crossbred calves

	Dietary groups				
Attributes	*Control (C)*	*Fungus fed (FF)*	*Bacteria fed (BF)*	*Enzyme fed (EF)*	*Consortium fed (CF)*
Hemoglobin (g/dL)	$9.20^{AB} \pm 0.17$	$9.07^{AB} \pm 0.08$	$9.74^{C} \pm 0.15$	$8.78^{A} \pm 0.14$	$9.28^{B} \pm 0.15$
Plasma glucose (mg/dL)	$80.13^{AB} \pm 1.44$	$83.72^{B} \pm 2.27$	$83.76^{B} \pm 1.82$	$78.92^{A} \pm 1.89$	$78.82^{A} \pm 1.52$
Plasma total protein (g/dL)	5.91 ± 0.12	6.10 ± 0.09	6.20 ± 0.08	6.21 ± 0.14	6.22 ± 0.11
Plasma phosphorus (mg/dL)	7.53B ± 0.15	$7.13^{A} \pm 0.14$	$7.57^{B} \pm 0.20$	$7.87^{C} \pm 0.20$	$7.60^{B} \pm 0.15$
Plasma GPT (U/L)	$36.26^{B} \pm 0.97$	$37.57^{C} \pm 1.11$	$39.32^{D} \pm 1.24$	$32.13^{A} \pm 1.09$	$38.30^{CD} \pm 1.25$
Plasma GOT (U/L)	59.96 ± 1.42	57.85 ± 1.54	61.02 ± 1.66	47.60 ± 1.60	59.13 ± 2.21

Note: GPT = glutamic pyruvic transaminase, GOT= glutamic oxaloacetic transaminase
Mean values bearing different superscript in a row differ significantly ($p < 0.05$).

bacteria at 1×10^9 CFU/calf. Like present finding on enzyme feeding in calves, the feeding of Jersey x Kankrej lactating cows with live yeast and cocktail of enzymes (xylanase, phytase, cellulase, p-glucanase, pectinase, amylase, protease, a-galactosidase, P-galactosidase and lipase) and only cocktail of enzymes for the period of 24 weeks did not alter the haemoglobin level (Vahora, 2004), while Ghazanfar *et al.* (2015) showed improved ($p < 0.05$) hemoglobin (13.26 ± 1.20 vs. 9.76 ± 0.55 g/dL) in dairy heifers fed dietary supplementation of yeast (*Saccharomyces cerevisiae Yea-Sac*[1026]) at 5 g/day.

Plasma Glucose

The plasma glucose content of crossbred calves under all treatment groups was statistically similar to that of the control group. Although apparently, it was lower in enzyme and consortium groups, while higher in fungus and bacteria group in comparison to control. Blood glucose was reported to be numerically higher in Holstein calves fed varying milk replacers (MR) with direct-fed microbial (Geiger *et al.,* 2014). Similarly, the difference in plasma glucose (72.17-74.11 mg/dl) of lactating buffalo was not significant on feeding rations with two types of fibrolytic enzyme at 2 g/kg DM (Azzaz *et al.,* 2013). It was also non-significant in lactating Murrah buffaloes fed fibrolytic enzymes at 1.5 and 3.0 g/kg DM (Shekhar *et al.,* 2010). Contrary to this finding, feeding of two commercial enzymes at 40 g/day in a total mixed ration of lactating Egyptian buffaloes showed higher ($p = 0.011$) serum glucose (74.0 and 73.0 vs. 66.4 mg/dL) (Morsy *et al.,* 2016). The serum glucose was also reported to be significantly ($p < 0.05$) higher (66.92 ± 4.84 and 62.83 ± 5.63 vs. 50.42 ± 3.14 mg/dL) in calves fed diet containing 5 and 10 g/day live yeast (*Saccharomyces cerevisiae*) (Hossain *et al.,* 2012).

Plasma Total Protein

The experiment revealed that feeding of fungus, bacteria, enzyme, and consortium did not affect plasma total protein in crossbred calves. Numerically higher levels (g/dl) were reported in consortium group (6.22 ± 0.11) followed by enzyme (6.21 ± 0.14), bacteria (6.20 ± 0.08), fungus group (6.10 ± 0.09) and least in control group (5.91 ± 0.12) (Table 2). These findings concurred well with Geiger *et al.* (2014) in Holstein calves fed direct microbes, and in female calves of Bulgarian Brown and Black & White Breeds fed milk replacer supplemented with probiotic @ 12 g/day (Dimova *et al.,* 2013). Serum protein was non-significantly higher (7.34 and 6.92 vs. 6.59 g/dL) in multiparous lactating buffaloes fed two exogenous fibrolytic enzymes both at 40 g/day (Kholif *et al.,* 2012). Adams *et al.* (2008) indicated numerically lower serum total protein (64 vs. 68 g/L) in Holstein calves at the end of the experiment on oral administration of 20 mL direct microbes from 0 to 14 weeks. Contrary to present finding, Morsy *et al.* (2016) indicated that an individual application of two commercial enzymes both at 40 g/day in total mixed ration of lactating buffaloes showed higher ($p = 0.091$) serum total protein (8.31 and 8.11 vs. 6.99 g/dL) in comparison to control. El-Bordeny *et al.* (2015) found a significant increase ($p < 0.05$) in total serum proteins (8.83 ± 0.30 and 7.87 ± 0.26 g/dL) of Holstein dairy cows fed 15 g fibrolytic enzyme plus TMR. Similarly, higher ($p < 0.05$) total protein (6.68 ± 0.18 vs. 5.92 ± 0.25 g/dl) of calves was recorded when fed diet containing 5 and 10 g/day live yeast (*Saccharomyces cerevisiae*) as compared to control (Hossain *et al.,* 2012).

Plasma Phosphorus

The mean plasma phosphorus concentration was significantly ($p < 0.05$) higher (7.87 ± 0.20 mg/dL) in calves fed enzymes and lower in calves fed fungus (7.13 ± 0.14 mg/dL) in comparison to control (7.53 ± 0.15 mg/dL). In contrast, in bacteria (7.57 ± 0.20 mg/dL) and consortium group (7.60 ± 0.15 mg/dL) it was statistically similar with control. These findings were in line with Dimova *et al.* (2013) in Bulgarian female calves fed milk replacer supplemented with probiotics. Adams *et al.* (2008) reported similar serum phosphorus (2.7 mmol/L) in Holstein bull calves at 0, 4, 8 and 12 week of experiment on feeding direct microbes (*Propionibacterium jensenii*). Vahora (2004) also showed uniform serum phosphorus (4.63 to 4.67 mg%) under different feeding treatments of live yeast with and without cocktail enzymes for the period of 24 weeks in Jersey x Kankrej lactating cows.

Plasma Transaminases (GPT, GOT)

Blood plasma GPT level of crossbred calves was lower ($p < 0.05$) in enzyme fed and higher ($p < 0.05$) in fungus, bacteria and consortium fed groups than the control. Blood plasma GOT level in all groups however, did not differ statistically. The values of plasma GPT (32.13-39.32 U/l) and GOT (47.60-61.02 U/l) were within normal range indicating fibrolytic microbes and enzymes had no damaging effect on liver and/or muscles of crossbred calves. These findings on effect of treatments were in line with Morsy *et al.* (2016) who indicated similar value of serum ALT (30.1-30.4 units/l) and AST (15.1-15.6 units/l) in Egyptian buffaloes fed two commercial enzymes, and similar were the observations in Holstein dairy cows fed 15 g fibrolytic enzyme (El-Bordeny *et al.,* 2015). Hossain *et al.* (2012) showed non-significantly lower serum AST (94.67 ± 4.99 and 88.67 ± 3.87 vs. 96.67 ± 6.39 µ/L) and higher serum ALT (40.00 ± 3.10 and 38.50 ± 3.49 vs. 37.83 ± 2.14 µ/L) in Kankrej calves fed diet supplemented with live yeast (*Saccharomyces cerevisiae*) at 5 and 10 g/day than the control group. Adams *et al.* (2008) also indicated non-significantly higher serum ALT (11 vs. 10 U/l) and serum AST (64 vs. 63 U/l) of Holstein calves on oral administration of direct microbes @ 20 mL/day from 0 to 14 weeks.

From the study, it was concluded that the concentrations of hemoglobin, plasma glucose, plasma total protein, plasma phosphorus, plasma GPT and plasma GOT in young HF x Kankrej crossbred calves fed fibrolytic fungus, bacteria, enzyme and consortium were within normal range as of control group, and these supplements had no any adverse effect on overall performance of calves.

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