

Original Research Article

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Influence of Fibrolytic Fungus, Bacteria and Enzyme on Weight Gain and Structural Growth of Crossbred Calves

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ABSTRACT

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An experiment was planned to elucidate the influence of feeding diet (green fodder part) treated with fungi or bacteria or enzyme and 1/3 mixture of all the three on body weight, daily weight gain, and structural growth of crossbred calves. Twenty crossbred (Holstein Friesian x Kankrej) calves were allotted to five treatments (each treatment had one male and three female calves) with similar body weight and sex. Experimental animals of treatment group 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 1/3 dose of fungus + bacteria + enzyme together. The results revealed that average body weight at the end of experiment was 158.15 ± 3.39 kg. Total gain in weight and average daily gain was 72.46 kg and 509.36 ± 14.09 g, respectively with non-significant effect of fibrolytic microbes and enzymes. Feeding of fibrolytic fungus and consortia supplemented diet had reduced ($P < 0.05$) body length whereas effect of feeding fibrolytic bacteria and enzyme resulted in similar body length. Average heart girth significantly reduce ($P < 0.05$) on feeding fibrolytic bacteria, enzymes and consortium supplemented diet of crossbred heifers in comparison to control and heart girth of fibrolytic fungus fed heifers was at par with control. In conclusion feeding hybrid napier treated with fibrolytic fungus, bacteria, enzymes and consortia to crossbred heifers was without improvement in weight gain and structural body growth.

Introduction

Proper management of young stock in terms of optimal nutritional care (Anonymous, 2012) is needed to increase milk production and income. The available feed resources like dry roughages, green fodder, concentrate and crop residue are inadequate and costly to meet nutrient requirement for growing bovines. The

feed shortage could be overcome by use of alternative feed technologies (Walli *et al.*, 2006), which may lead to increase in nutrient supply within the ruminant system. One of the primary factors affecting livestock production is fiber digestibility, with often less than 600 g/kg of dietary fiber being digested by the animal (Van Soest 1994). In recent years, there has been use of microbial products as feed

additives in ruminant diets to resolve feed related problem. Microorganisms produce different groups of enzymes and help to degrade cellulosic material. They have been isolated from rumen fungi and bacteria which can improve feed intake as well as growth and feed efficiency of young ones. Authors reported an increase in the digestion of fibre and improved animal performances by using feed additive (Adesogan *et al.*, 2007; Bala *et al.*, 2009). Direct fed microbes (DFM) with exogenous fibrolytic enzyme (EFE) had considerable beneficial influence on growth performance and feed conversion efficiency of the crossbred calves without causing adverse effect on behavioural traits (Kocyigit *et al.*, 2016). However, many have reported either no effect at all or sometimes negative effect (Baloyi, 2008). Many researchers reported that yeast culture improved feed intake (Ayad *et al.*, 2013); feed conversion efficiency, growth rate (Kumar *et al.*, 2015) and nutrient digestibility (Sehgal *et al.*, 2008) in cost effective manners (Hutjens, 2003). Fibrolytic enzymes are added in ruminant diets to improve nutrient digestibility (Lunagariya, 2019), and growth rate (Dean *et al.*, 2013). Influences of DFM on growth traits of dairy calves have not been adequately studied and the findings reported in literature are inconsistent (Elghandour *et al.*, 2015).

Considering above facts in mind, the present study was planned to observe the influence of feeding green fodder treated with either fungi or bacteria or enzyme or all three mix on total body weight gain, daily weight gain, body length and heart girth of crossbred calves.

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 College of Veterinary science and Animal

Husbandry, Anand Agricultural University, Anand. Twenty crossbred (Holstein Friesian 50% x Kankrej 50%) calves were allotted to five treatments (each treatment had one male and three female calves) with similar body weight (85.70 ± 6.37 kg) and age (167.55 ± 21.70 days).

To meet nutrient requirements (ICAR, 1998), all calves were individually fed green hybrid napier (Coimbatore-3 variety), concentrate and sorghum hay. The exogenous fibrolytic microbes and enzymes use in this experiment were isolated and purified from animals of this farm by the Department Animal Biotechnology, Veterinary College, AAU, Anand. The experimental animals were kept let loose for two hours (8:00 to 10:00) in morning for exercise during which wholesome clean drinking water was available. Drinking water was also offered two times in afternoon and night hrs. Measured quantity 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 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 1/3 dose of fungus + bacteria + enzyme to animals of Fungus fed (FF), Bacteria fed (BF), Enzyme fed (EF) and Consortium fed (CF), respectively. Two kilogram of hybrid napier for treatment group calves was thoroughly mixed with fungus, bacteria, enzymes and consortium before 20 hrs of feeding as per treatment and were covered with plastic sheet on cemented floor and again mixed two time before feeding. Treated hybrid napier was equally distributed among the calves of treatment considering moisture loss at time of feeding. Quantity of hybrid napier and concentrate was fed to

calves so as to without left over and sorghum hay was offered *ad libitum* to fulfill remaining nutrient requirement. Leftover of fodder was measured on the next day morning. Feed and fodder was adjusted at biweekly intervals as per body weight of animals. Weekly dry matter and crude protein of feed and fodders were analyzed as per AOAC (1995). The weight of the all experimental animals was recorded for two consecutive days at biweekly interval in morning between 8.00 to 8.30 hrs on weighbridge before feeding and watering after taking left over. Average of the two days was considered as the final weight. The difference in body weight between two biweekly intervals was considered as the weight gain. Body measurement viz., heart girth and length of animals were also measured biweekly using standard measure tape. The body length was taken from point of shoulder to pin bone while the heart girth was measured taking the circumference from behind the hump and behind the brisket. All the measurements were taken by keeping the animals on flat floor while standing in normal position. An experimental design was Completely Randomized Design and the data was analyzed by per Snedecor and Cochran (2002).

Results and Discussion

Body weight and growth rate

Proximate composition of concentrate and fodder is given in table 1. DM and nutrients intake of experimental animals was offered as per requirement (ICAR, 1998). Average bi-weekly body weight of crossbred calves recorded during the experiment is given in table 2. The difference in body weight of experimental animals was statistically non-significant at initiation as well as at the end of the experiment. Average body weight of experimental animals at the end of experiment was 158.15 ± 3.39 kg. Total gain (72.46 kg)

and average daily gain (509.36 ± 14.09) in body weight followed the same trends.

Similar growth rate in calves was reported by Di Francia *et al.*, (2008) using fungus supplement (451 to 529 g/head/day), Bayatkouhsar *et al.*, (2013) using probiotic (549 g/head/day.), Geiger *et al.*, (2014) using milk replacer with DFM (520 g/head/day), Kumar *et al.*, (2015) using anaerobic fungal zoospores (515.52 ± 20.11 g/head/day), Shahzad *et al.*, (2016) using fungus (480 to 560 g/head/day), Sharma *et al.*, (2018) using probiotic (494.6 g/head/day). However, higher growth rate in calves than current study was noted by Dey *et al.*, (2004) using ruminal fungi (709 g/head/day), Lascano *et al.*, (2009) using *Saccharomyces cerevisiae* (800 ± 0.01 g/head/day), Sehgal *et al.*, (2008) using anaerobic fungi *Neocallimastix* sp. (659.81 g/head/day). While Khuntia and Chaudhary (2002) had reported lower growth rate (366 g) of calves on feeding lactic acid producing bacteria (LAB).

Feeding direct-fed microbial supplemented milk replacer in Holstein calves (Geiger *et al.*, 2014) revealed non-significant effect on body weight and average daily gain. Similarly average daily gain of crossbred (zebu x brown swiss) steers was also non-significant on feeding exogenous fibrolytic enzymes @ 2, 4 and 6 ppm in diet containing 88% concentrate and 12% forage (dry basis) for 75 days.

Dean *et al.*, (2013) also reported only numerically increase ($P > 0.05$) in body weight gain of Holstein cows on feeding fibrolytic enzyme applied at ensiling to bermuda grass (1.3 g/kg DM) as well as direct feeding to animal, mixing and then feeding with concentrate, forage or total mixed ration (4 g/head/day). The feeding *Saccharomyces cerevisiae* at 1 g/kg feeding high and low-concentrate diets of 32 Holstein heifers showed similar average daily gain ($0.80 \pm$

0.01 kg/d) at the end of the experiment (Lascano *et al.*, 2009). Contrary to current findings, feeding probiotics and exogenous fibrolytic enzymes in milk or milk replacer at early age of buffalo and crossbred calves had significant effect on body weight and average daily gain.

Sharma *et al.*, (2018) revealed that daily feeding probiotic fermented milk (*Lactobacillus acidophilus*; 10^8 CFU/ml) @ 200 ml to Murrah buffalo calves resulted in higher ($P < 0.05$) final body weight (86.24 vs. 83.63 kg) and daily gain (454.8 vs. 433.9 g) in comparison to control basal diet (concentrate mixture and green fodder) during a 120 days experiment.

Similarly, Sharma *et al.*, (2016) concluded that feeding probiotic (*Saccharomyces cerevisiae*) @ 15g/calf/day in milk for a period of two months resulted in improved ($P < 0.05$) growth rate of buffalo calves under field condition. Feeding of direct fed microbes (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Bacillus subtilis* and *Aspergillus oryzae*) and exogenous fibrolytic enzymes (pectinase, lipase, protease, amylase and cellulase) @ 10 g per animal to Brown Swiss x Eastern Anatolian RedF1 calves resulted higher ($P < 0.05$) weights and growth rate than control group (Kocyigit *et al.*, 2016). Fibrolytic microbes and enzymes feeding had no effect on body weight and average weight gain in crossbred calves after post weaning age.

Tripathi *et al.*, (2007) indicated significant average daily gain and non significant body weight of Murrah buffalo calves (age about 10 months) fed a complete feed mixture consisted of 50% wheat straw and 50% concentrate supplemented with *Orpinomyces sp.* C-14 and *Piromyces sp.* WNG-12 cultures (10^6 thallus forming units per ml). Feeding fungal culture (10^6 CFU/ml) 160 ml per calf per week

supplemented diet (50% wheat straw and 50% concentrate) showed significant ($P < 0.05$) gain in body weight (709.3 ± 59.03 and 614.8 ± 56.92 g/day) in Tharparkar \times Holstein-Friesian calves (Dey *et al.*, 2004).

Body Length and Heart girth

Body length at beginning and end of the experiment was 33.78 ± 0.45 and 43.23 ± 0.51 inch, respectively with 27.97% increase and was without significant effect among treatments. Feeding of fibrolytic fungus and consortium supplemented diet had reduced ($P < 0.05$) body length whereas effect of feeding fibrolytic bacteria and enzyme resulted in similar body length.

During experiment heart girth improved by 17.12% (7.17 inch) from 41.88 ± 0.45 to 49.05 ± 0.42 inch final with non significant effect. Average heart girth of crossbred heifers was significantly reduced ($P < 0.05$) on feeding fibrolytic bacteria, enzymes and consortium supplemented diet in comparison to control, whereas heart girth of fibrolytic fungus fed heifers was statistically at par with control. Similarly Kocyigit *et al.*, (2016) concluded that the feeding of direct fed microbes (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Bacillus subtilis* and *Aspergillus oryzae*) and exogenous fibrolytic enzymes (pectinase, lipase, protease, amylase and cellulase) @ 10 g per animal to Brown Swiss x Eastern Anatolian RedF1 calves were without effect on gain in body measurements like body length and heart girth. Ghazanfar *et al.*, (2015) also reported non-significant reduction in body height (84.13 ± 1.60 vs. 78.34 ± 1.14 cm) and body length (79.36 ± 1.83 vs. 78.53 ± 2.12 cm), whereas heart girth improved (86.38 ± 1.56 vs. 89.13 ± 0.62 cm) non-significant of Sahiwal heifers feeding diet supplemented with yeast (*Saccharomyces cerevisiae*) @ 5 g daily for 120 days feeding trial.

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 (CO3)	23.70	8.46	31.23	2.58	40.95	12.49

Note: DM= dry matter, CP= crude protein, CF= crude fibre, EE= ether extracts, NFE= nitrogen free extracts, CO3= Coimbatore 3

Table.2 Body weight and growth of crossbred calves

Attributes	Dietary groups				
	Control (C)	Fungus fed (FF)	Bacteria fed (BF)	Enzyme fed (EF)	Consortium fed (CF)
Body weight (kg)					
Initial BW	85.60±8.78	85.95±5.05	85.35±6.17	85.60±7.39	85.95±4.48
Final BW	159.15±10.32	158.65±6.24	155.75±9.16	158.35±10.45	158.85±4.66
Total gain	73.55±3.05	72.70±2.43	70.40±4.23	72.75±3.27	72.90±0.33
Daily Growth					
ADG (g/head/day)	514.28±32.37	512.14±31.03	493.21±30.79	524.64±30.14	521.07±34.69
Body length (inch)					
Initial	35.25±0.75	32.75±0.85	33.62±0.94	34.50±1.19	32.75±1.13
Final	44.00±0.84	42.75±1.49	43.50±0.54	43.65±1.84	42.25±0.97
Average	39.68 ^B ± 0.49	38.55 ^A ± 0.57	39.89 ^B ± 0.53	39.91 ^B ± 0.59	38.66 ^A ± 0.56
Total gain	8.75±0.92	10.00±0.72	9.88±0.43	9.15±1.16	9.50±0.35
Heart girth (inch)					
Initial	41.62±1.77	42.37±0.55	41.37±1.16	42.25±0.85	41.75±0.83
Final	50.12±1.33	49.12±0.43	48.12±1.43	49.00±0.71	48.87±0.59
Average	46.24 ^C ± 0.56	45.57 ^{BC} ± 0.40	44.59 ^A ± 0.49	45.19 ^{AB} ± 0.44	44.88 ^{AB} ± 0.40
Total gain	8.50±0.84	6.75±0.14	6.75±0.60	6.75±0.48	7.12±0.52

Mean values bearing different superscript in a row differ significantly (P<0.05)

Note: ADG= Average daily gain

Mean values with different superscript within a row differ significantly (P>0.05)

A trial reported that heart girth (81.16 cm and 78.49 cm) was significantly (P≤0.05) higher and body length (65.27 and 66.54 cm) was non-significantly higher of Holstein calves fed with a standard milk replacer/concentrate diet supplemented with 1 g probiotic powder (*Lactobacillus animalis*, *Lactobacillus paracasei*, *Bacillus coagulans*, having 1.8 ×

10¹⁰ CFU/g) during the first month of life as reported by Agazzi *et al.*, (2014). Bayatkouhsar *et al.*, (2013) also showed non-significant (P > 0.05) effect on the final body length and heart girth of Holstein calves on feeding laboratory and commercial produced probiotic both @ 2.0 × 10⁸ CFU per day in morning milk feeding.

Lascano *et al.*, (2009) also indicated that there was no effect of *Saccharomyces cerevisiae* addition @ 1 g /kg feed on structural measurement (heart girth and body length) of Holstein heifers for period of 133 days.

Contrary to present experiment, Sharma *et al.*, (2018) revealed that daily feeding of probiotic fermented milk (*Lactobacillus acidophilus*; 10⁸ CFU/ml)@ 200 ml for 120 days to Murrah buffalo calves lead to significant(P < 0.05)increase in heart girth (104.26 vs. 98.54 cm) in comparison to control basal diet (concentrate mixture and green fodder).

Crossbred heifers fed diets with hybrid napier which treated with fungus- *Aspergillus*spp. (1× 10⁷ per g feed), fibrolytic bacteria- *Escherichia* spp. (10⁶ CFU per g feed), xylanase (50 ml/kg having xylanase 1.2 IU/ml), and 1/3 dose of fungus + bacteria + enzyme was without improvement in total weight gain, growth rate, body length and heart girth.

Laboratory produced fibrolytic microbes and enzymes needs improvement to be effective on growth rate and structure improvement in crossbred heifers.

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