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Feed intake and feed conversion efficiency of crossbred calves as an effect of fibrolytic microbes and enzymes

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Abstract
Effect of fibrolytic microbes and enzymes on feed intake and feed efficiency was evaluated in cross-bred 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 (85.70±6.37 kg) and age (167.55± 21.70 days). Experimental calves of control group were offered hybrid napier untreated 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. Nutrients intake of crossbred calves were sufficient to satisfy the nutrients requirements. Dry matter intake of cross-bred calves in bacteria (3.07±0.07) and enzyme (3.07±0.08) fed was statistically at par with control and consortia. Feeding effect of fungus, bacteria, enzyme and consortium on percent dry matter intake, dry matter intake per kg gain, DCP intake, percent DCP intake and DCP intake kg per body weight gain was not significant. While TDN intake was statistically ($P<0.05$) lower in bacterial fed (1.95±0.04) than control and fungus fed but at par with enzyme and consortium. Feed conversion efficiency in terms of DM, DCP and TDN were non-significant among the treatments. To conclude, isolated fibrolytic fungi, bacteria and enzyme were by and large not effective in improving feed intake and feed efficiency of cross-bred calves.

Keywords: Crossbred heifer, fibrolytic microbes, fibrolytic enzymes, feed intake, feed efficiency

Introduction
India has a rich agro-ecological diversity. An increased need of feed & fodder and decrease in available natural resources, leads to new diverse challenges and constraints. The feed resources like dry roughages, green fodder, concentrate and crop residue (straw and stovers) are inadequate to meet nutrient requirement for growing cattle and buffaloes. Walli *et al.* (2006) [28] reported that the problem of feed shortage could be overcome by use of alternative feed technologies. Alternative feed resources using agro by-products is a reasonable strategy in low-input systems (Dayani *et al.*, 2012) [6]. Crop residues has ligno-cellulosic bond with lower degradability in rumen. Wheat straw is a major energy-rich cereal straw in Northern India (109.9 MMT, Saritha and Arora 2012) [20] which is low in protein and minerals. Also lignin is not degraded by rumen microbiota leading to low digestibility (García-Cubero *et al.*, 2012) [9].
Feed additive can improve performance, fibre digestion (Adesogan *et al.*, 2007) [1], and gut health (Kawakami *et al.*, 2010) [12] in animal. Direct fed microbial (DFM) with exogenous fibrolytic enzyme (EFE) had beneficial effect on growth (Dean *et al.*, 2013) [7], feed conversion efficiency (Lunagariya, 2016) [17] and digestibility of nutrients (Lunagariya, 2019) [16]. However, negative or without effect also has been reported by Vinci *et al.* (2003) [27] and Baloyi. (2008) [4]. Yeast culture has improved feed intake (Ayad *et al.*, 2013) [3]; feed conversion efficiency and growth rate (Kumar *et al.*, 2015) [15]
Considering above facts in mind an experiment was planned to evaluate effect of fibrolytic microbes and enzymes on feed intake and feed conversion efficiency in 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 x Kankrej) 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) [11], all calves were individually fed green hybrid napier (CO3 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 morning for exercise during which wholesome clean drinking water was available. Drinking water was also offered 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 cement floor. The grass again mixed two times 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 without left over and sorghum hay was offered *ad libitum* to fulfill 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. Dry matter and crude protein of feed and fodders were analyzed as per AOAC (1995) [2]. 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 electronic weighbridge after taking left over before feeding and watering. The average of two days was considered as the final weight. The difference in body weight between two biweekly intervals has been considered as the weight gain. Feed conversion efficiency was calculated as requirement of dry matter and nutrients needed to gain kilogram of live weight of experimental animals. Data generated were analyzed as per Snedecor and Cochran (2002) [24].

Results and Discussion

Proximate composition is given in table 1. Dry matter and nutrients offered to calves were as per nutrient requirement of ICAR (1998) [11].

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 extracts, NFE= nitrogen free extracts

Dry matter (DM) and nutrient Intake

Daily dry matter (DM) intake (table 2) of crossbred calves of treatment groups was non-significant in comparison to control fed calves. The daily digestible crude protein intake followed the same trend. Daily total digestible nutrients (TDN) intake of crossbred calves of bacteria fed group was significantly (P<0.05) lower in comparison to control, whereas TDN intake in other treatment group of calves differing only non-significantly with control group of calves. DMI (2.53±0.03-2.62±0.04), DCPI (0.215±0.01-0.218±0.01) and TDNI (1.62±0.02-1.66±0.02) on per cent basis was similar and non-significant amongst all treatments. In present study, higher DMI in fungus fed group might be because of higher palatability of feed. However, in spite of increased DM intake with fungus feed, there was no change in average daily gain. Enzyme fed group had lower DMI which contained enzyme, hence average daily gain was higher than other treatments.

Table 2: Feed intake and feed conversion efficiency of cross-bred calves

Attributes	Dietary groups				
	Control (C)	Fungus fed (FF)	Bacteria fed (BF)	Enzyme fed (EF)	Consortium fed (CF)
Average Daily Gain (g/head/day)	514.28±32.37	512.14±31.03	493.21±30.79	524.64±30.14	521.07±34.69
Intake (kg/head/day)					
DMI	3.17^{AB}±0.07	3.20^{B}±0.07	3.07^{A}±0.07	3.07^{A}±0.08	3.11^{AB}±0.07
DCPI	0.264±0.01	0.265±0.01	0.259±0.01	0.260±0.01	0.263±0.01
TDNI	2.02^{B}±0.04	2.02^{B}±0.04	1.95^{A}±0.04	1.96^{AB}±0.05	1.97^{AB}±0.04
Per cent intake (kg)					
DMI	2.57±0.04	2.62±0.04	2.57±0.05	2.53±0.03	2.58±0.04
DCPI	0.215±0.01	0.217±0.01	0.218±0.01	0.215±0.01	0.218±0.01
TDNI	1.63±0.02	1.66±0.02	1.63±0.03	1.62±0.02	1.64±0.02
Feed conversion efficiency (kg/kg gain in weight)					
DM	6.94±0.30	7.08±0.30	7.04±0.40	6.55±0.30	6.92±0.30
DCP	0.578±0.02	0.586±0.03	0.597±0.03	0.557±0.03	0.587±0.03
TDN	4.40±0.22	4.49±0.24	4.49±0.25	4.17±0.23	4.41±0.22

Note: DMI= dry matter intake, DCPI= digestible crude protein intake, TDNI= total digestible nutrient intake, DCP= digestible crude protein, TDN= total digestible nutrient, **ADG=** Average daily gain Mean values without superscript within a row differ non-significantly (P>0.05)

Result of current findings were in line with Morsy *et al.* (2016) [18] who showed that daily feeding of 40g of two commercial enzyme products were without significant effect on dry matter intake in Egyptian buffaloes. Lunagariya (2016) [17] also indicated that DM and nutrients (crude protein, digestible crude protein and total digestible nutrient) intake of Holstein crossbred cows was without significant (P>0.05) effect on feeding exogenous fibrolytic enzymes supplemented TMR (240 mg/kg TMR) during a trial of 140 days. Barbadikar (2012) [5] had showed that daily DM intake (3.63 vs. 3.69 kg), per cent DM intake (2.77 vs. 2.77 kg), DCP intake (313.51 vs. 320.51 g) and TDN intake (2.07 vs. 2.10 kg) of crossbred calves was non-significant (P>0.05) between control and fibrolytic enzymes fed (0.025%) group of crossbred calves. Kim *et al.* (2012) [13] had indicated non-significant effect of feeding TMR with probiotics (*Lactobacillus acidophilus*: KCTC3140 and *Saccharomyces cerevisiae*: KACC30068) on daily feed intake in Hanwoo steers during experiment day. Dey *et al.* (2004) [8] also report in line with present study as non-significantly/numerically lower DM intake (4.04±0.34 vs. 4.08±0.52kg/day), higher DCP intake (394.18±36.43 vs. 368.65±41.89 g/day) and higher TDN (2.46±0.22 vs. 2.16±0.31 kg/day) on feeding fungal culture (106 CFU/ml) at 160 ml/week to calves.

Similarly, drenching of 250 ml of *Neocallimastix sp*. GR1 culture (≈106 tfu/ml) on every 4th day to Murrah female buffalo calves resulted in similar DM intake (4.12 ± 0.20 vs. 4.14 ± 0.17 kg); whereas DCP (295.39 ± 14.86 vs. 275.13 ± 9.68) and TDN (2.46 ± 0.13 vs. 2.19 ± 0.09) intake increased significantly in Murrah female buffalo calves (Sehgal *et al.*, 2008) [21] for a period of 90 days. Kumar *et al.* (2015) [15] also reported that on feeding anaerobic fungal zoospores of *Neocallimastix sp*. GR-1, incorporated in wheat straw based complete feed blocks (140×10^6 zoospores/block of 14 kg) was without significant effect on DM and DCP intake whereas TDN intake was significantly (P<0.05) higher in 5-7 month old Murrah buffalo male calves and the values were 3.47±0.17 and 3.39±0.09 kg/day; 251.43±14.71 and 277.64±17.76 g/day; 2.14±1.55 and 2.25±1.43 kg/day, respectively for control and treated group.

In contrast to present study dry matter intake (1426.9 vs. 1402.9 g/day) of Murrah buffalo calves improved (P<0.05) in the probiotics (*Lactobacillus acidophilus*) fermented milk feeding (200 ml daily having 108 CFU/ml) groups compared to control as reported by Sharma *et al.* (2018). Similarly, Nili Ravi buffalo male calves had higher (P<0.05) dry matter intake (2.95 kg/day) on feeding total mixed ration (TMR) having 33% wheat straw treated with fungus (*Arachniotus sp.*) in comparison to control TMR or 67 and 100% fungus treated wheat straw (2.88, 2.80 and 2.71 kg/day) as studied by Shahzad *et al.* (2016) [22].

Feed Conversion efficiency

Average value of feed conversion efficiency in terms of DM, DCP and TDN required to gain one kg body weight of cross bred calves are presented in table 2.The data revealed that there was no significant difference in DMI (6.55±0.30-7.08±0.30) per kg gain of body weight by calves in different treatments. DMI per kg gain by the calves was numerically highest in fungus fed followed by bacterial fed, control, consortium then Enzyme, suggesting enzyme feeding has better feed efficiency. DCP (0.557±0.03-0.597±0.03) and TDN (4.17±0.23-4.49±0.25) required to gain one kilogram body weight followed same trends.

In line with current finding Kocyigit *et al.* (2016) [14] had indicated non-significant improvement in feed conversion efficiency (3.64±0.22 vs. 4.11±0.20 kg feed/kg gain) of Brown Swiss x Eastern Anatolian Red F1 calves on feeding milk treated with microorganisms (*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, Bacillus subtilis* and *Aspergillus oryzae*) plus exogenous fibrolytic enzymes (pectinase, lipase, protease, amylase and cellulase) @ 10 g per animal and offered to the calves every morning in the pre-weaning period. Dietary supplementation of yeast (*saccharomyces cerevisiaeYea-Sac*1026) at 5 g/day resulted in only numerically improved feed conversion efficiency (5.41±0.52 and 6.19±0.49 kg feed/kg gain) of dairy cattle heifer (Ghazanfar*et al.*, 2015) [10]. Vargas *et al.* (2013) [26] had also observed numerically higher feed conversion efficiency (6.68, 7.37, 7.12 and 7.74 kg DM/kg gain) of Zebu x Brown Swiss crossbred steers on feeding diet supplemented 0, 2, 4 and 6 ppm exogenous fibrolytic enzymes, respectively. Barbadikar (2012) [5] also reported only numerical improvement in feed conversion efficiency (6.29 ± 0.31 and 6.78 ± 0.26 kg DM/kg gain) of crossbred calves fed diet supplemented fibrolytic enzymes at 0.025% for 14 weeks. Similarly, Utilization efficiency of protein (556.04±23.66 vs. 601.51±33.43 g DCP/kg gain); energy (3.47±0.11 vs. 3.51±0.32 kg TDN/kg gain) and DM (5.69±0.11 vs. 6.62±0.42 kg/kg gain) was only numerically higher on feeding fungal culture (106 CFU/ml) at 160 ml/week in calves (Dey *et al.*, 2004) [8]. Ramaswami *et al.* (2005) [19] fed roughage based diet with 500 ml culture of *Lactobacillus acidophilus*-15 to male crossbred calves; results showed feed conversion efficiency (4.13±0.11 and 4.32±0.19) improved only numerically.

In contrast to current finding Tripathi *et al.* (2007) [25] had indicated significant improved feed conversion efficiency of Murrah buffalo calves as 8.6±0.3, 7.9±0.3 and 10.5±0.7 kg DM/kg gainon feeding diet supplemented with *Orpinomyces sp*. C-14, *Piromyces sp*. WNG-12 cultures, and without culture, respectively. A significant (P<0.05) improved feed conversion efficiency in term of DCP (485.88±11.82 and 532.99±34.92 g/kg gain) and TDN (4.01±1.88 and 4.21±1.22 kg/kg gain) was observed by Kumar *et al.* (2015) [15] on feeding anaerobic fungal zoospores of *Neocallimastix sp*. GR-1, incorporated in wheat straw based complete feed blocks (140×10^6 zoospores/block of 14 kg) to 5-7 month old Murrah buffalo male calves. Similarly, feed conversion efficiency in terms of DCP (490.83 ± 10.80 vs. 538.95 ± 36.92 g/kg gain) and TDN (4.02 ± 0.07 vs. 4.26 ± 0.24 kg/kg gain) significantly improved in Murrah female buffalo calves for a period of 90 days (Sehgal *et al.*, 2008) [21] on Drenching of 250 ml of *Neocallimastix sp*. GR1 culture (≈106 tfu/ml) on every 4th day.

Overall results indicated that feeding laboratory produce fibrolytic microbes and enzymes was without effect on feed intake and feed conversion efficiency of crossbred calves. Fibrolytic microbes and enzymes need further refinement to be effective in growing crossbred calves.

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