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Effect of fibrolytic microbes and enzymes on nutrient and energy intake in crossbred calves

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Abstract

The study investigated the impact of dietary supplementation with fibrolytic microbes and enzymes on nutrient and energy intake in crossbred calves. Twenty Holstein Friesian × Kankrej calves were randomly allocated into five groups: control, fungus-fed (*Aspergillus* spp.), bacteria-fed (*Escherichia* spp.), enzyme-fed (xylanase), and consortium-fed (combination of all three additives). Over 140 days, calves received treated or untreated hybrid napier fodder alongside concentrate mixture and sorghum hay. Key nutrient intake parameters measured included dry matter, crude protein, digestible crude protein, ether extract, crude fiber, nitrogen-free extract, total digestible nutrients, and metabolizable energy. Statistical analysis revealed no significant differences ($p > 0.05$) in nutrient or energy intake across treatment groups. Although the fungus-fed group showed a non-significant increase in dry matter intake, this did not translate into significant growth differences. Notably, the enzyme-fed group exhibited higher average daily growth despite consuming less dry matter. These findings suggest that supplementation with fibrolytic microbes and enzymes does not significantly alter nutrient or energy intake but may positively influence growth performance. The results support the use of such feed additives in crossbred calves under tropical feeding conditions without adversely affecting nutrient consumption.

Keywords: Fibrolytic microbes, exogenous fibrolytic enzymes, nutrient intake, energy intake, crossbred calves, rumen function, tropical feeding system

Introduction

In rural regions of our nation, raising livestock is a crucial economic activity. Livestock has long been considered one of humanity's oldest resources. It offers additional income to many families reliant on agriculture and to numerous landless households. Additionally, it supplies protein through milk. Livestock also contributes to income through draught power, organic manure, hides, and skins, providing a steady income throughout the year. To boost milk production and enhance farmers' earnings, a comprehensive strategy is necessary to advance the animal production sector. This involves the effective management of young animals with appropriate nutritional care and oversight (Anonymous, 2012) [3]. However, challenges such as rapid growth and high feed consumption can undermine the advantages and economic viability of these practices. India boasts a diverse agro-ecological landscape. The rising demand for feed and fodder, coupled with diminishing natural resources, presents new and varied challenges. The available feed resources, including dry roughages, green fodder, concentrates, and crop residues (straw and stovers), are insufficient and expensive to meet the nutritional needs of growing cattle and buffaloes. Walli *et al.* (2006) [36] suggested that the issue of feed scarcity could be addressed by adopting alternative feed technologies, potentially enhancing nutrient supply within the ruminant system.

The capacity of young dairy calves to effectively process fibrous feeds significantly impacts nutrient intake, energy availability, and subsequent growth performance. In crossbred calves, the development of rumen functionality occurs gradually, and the early establishment of fibrolytic microbial populations is often inadequate to support optimal breakdown of plant cell wall components. Consequently, the intake and utilization of fiber-rich diets during the early growth phase may be limited, resulting in a reduced energy supply (Suárez *et al.*, 2006) [33].

In India, the poor quality of native grasses, crop residues, and agro-industrial by-products is a common issue in animal feed. Therefore, utilizing alternative feed resources derived from agro by-products is a practical approach in low-input systems (Dayani *et al.*, 2012) [12]. Microorganisms have been isolated from the rumen, and through the use of fungi, bacteria, and enzymes, it is possible to enhance feed intake, growth, and feed efficiency in young animals. Numerous studies have documented improvements in fiber digestion and animal performance with the use of feed additives (Adesogan *et al.*, 2007; Bala *et al.*, 2009) [1, 6]. Feed additives can enhance gut health in animals, potentially leading to a higher digestion rate and improved growth performance (Frizzo *et al.*, 2010; Kawakami *et al.*, 2010; Frizzo *et al.*, 2011) [16, 19, 15]. The combination of direct-fed microbial (DFM) and exogenous fibrolytic enzyme (EFE) has shown significant positive effects on the growth performance and feed conversion efficiency of crossbred calves without negatively impacting their behavior (Kocuyigit *et al.*, 2016) [21]. However, some studies have reported no effect or even negative effects (Bowman *et al.*, 2003, Vicini *et al.*, 2003, Baloyi, 2008) [11, 35, 7].

In young calves, limited microbial activity and enzymatic capacity can hinder fiber breakdown. Nutritional strategies aimed at boosting rumen fibrolytic activity, such as supplementing with direct-fed microbes and exogenous fibrolytic enzymes, have been investigated to enhance nutrient and energy intake. Fibrolytic microbial additives are known to maintain rumen microbial balance and encourage the growth of cellulolytic bacteria, while exogenous enzymes may improve fiber hydrolysis through complementary or synergistic actions within the rumen environment (Beauchemin *et al.*, 2003) [10]. Previous research has shown improvements in fiber digestibility and feed intake following such supplementation; however, responses can vary and are influenced by diet composition, enzyme characteristics, and animal age (Beauchemin & Holtshausen, 2010) [9].

Dietary supplementation with feed additives, especially the combined use of direct-fed microbes and exogenous fibrolytic enzymes, has been reported to enhance fiber digestibility, support gut health, and improve growth performance, feed efficiency, and nutrient utilization in ruminants (Adesogan *et al.*, 2007; Dean *et al.*, 2013; Lunagariya, 2019) [1, 13, 23]. Information on the combined effects of fibrolytic microbes and enzymes on nutrient and energy intake in crossbred calves, particularly under tropical feeding systems, remains limited. Therefore, the present study was undertaken to evaluate the influence of dietary supplementation of fibrolytic microbes and enzymes on nutrient and energy intake in crossbred calves.

Many researchers have reported that yeast culture improves feed intake (Robinson and Erasmus, 2009; Ayad *et al.*, 2013); feed conversion efficiency, growth rate (Kumar *et al.*, 2015) [28, 5] and nutrient digestibility (Segal *et al.*, 2008) [29] in cost-effective ways (Hutjens, 2003) [17]. The positive effect of yeast on blood hematology results in improved animal health status (Agazzi *et al.*, 2014) [2]. Dey *et al.* (2004) [14]; Paul *et al.* (2004) [26]; Tripathi *et al.* (2007) [34] used anaerobic fungi in ruminants and observed increased intake and digestibility of lignocellulosic feeds. Considering above facts in mind an experiment was planned to evaluate

effect of fibrolytic microbes and enzymes on Nutrient and Energy Intake in Crossbred Calves.

Materials and Methods

The research took place over 140 days at the Livestock Research Station of Anand Agricultural University (AAU) in Anand. All experimental activities adhered to the ethical guidelines sanctioned by the Institutional Animal Ethics Committee of the College of Veterinary Science and Animal Husbandry, AAU, Anand (Approval No. 285/LPM/2018).

The study involved 20 crossbred calves (Holstein Friesian × Kankrej), which were randomly divided into five dietary treatment groups. Each group consisted of one male and three female calves. At the beginning of the study, the calves had similar body weights (85.70±6.37 kg) and ages (167.55±21.70 days), ensuring consistency across the groups.

The feeding plan was designed to meet the nutrient requirements as recommended by ICAR (1998) [18]. Each calf was individually fed with green hybrid napier fodder (CO-3 variety), a concentrate mixture, and sorghum hay. The fibrolytic fungi, bacteria, and enzymes used for feed treatment were isolated and purified from animals housed at the same research facility by the Department of Animal Biotechnology, Veterinary College, AAU, Anand. The calves were allowed to exercise freely for two hours daily between 08:00 and 10:00, with clean drinking water available at all times. Additional water was provided twice daily at 12:00 and 14:00. Measured amounts of hybrid napier fodder (treated or untreated as per the treatment), concentrate mixture, and sorghum hay were offered at 10:00, 14:00, and 16:00, respectively. The control group received untreated hybrid napier fodder, while the treatment groups were given hybrid napier treated with the fibrolytic fungus *Aspergillus* spp. (1×10^7 per g of feed), fibrolytic bacteria *Escherichia* spp. (1×10^6 CFU per g of feed), xylanase enzyme (50 ml/kg fodder containing 1.2 IU/ml), or a combination of one-third dose of each additive for the Fungus Fed (FF), Bacteria Fed (BF), Enzyme Fed (EF), and Consortium Fed (CF) groups, respectively. For fodder treatment, 2 kg of hybrid napier was thoroughly mixed with the respective microbial or enzyme preparation about 20 hours before feeding. The treated fodder was spread evenly on a cemented floor, covered with a plastic sheet, and mixed twice during the incubation period before being offered to the calves. Moisture loss during treatment was accounted for when distributing the treated fodder equally among animals within each treatment group. Hybrid napier fodder and concentrate mixture were provided in amounts sufficient to minimize leftovers, while sorghum hay was given *ad libitum* to meet the remaining nutrient needs. Any leftover feed was collected and weighed the next morning. Feed allowances were adjusted every two weeks based on changes in body weight.

Feed and fodder samples were collected weekly and analyzed for dry matter and crude protein content using standard analytical procedures outlined by AOAC (1995) [4]. Nutrient intakes such as CP, DCP, TDN, EE, NFE, CF, and related components were calculated based on dry matter intake. The experimental data were statistically analyzed using a Completely Randomized Design as described by Snedecor and Cochran (2014) [32].

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

Results and Discussion

Table 2: Intake of nutrients and energy by crossbred calves during the experiment

Parameter	T ₁	T ₂	T ₃	T ₄	T ₅	P value
DM (kg/day)	3.29±0.08	3.31±0.08	3.17±0.08	3.17±0.09	3.21±0.08	0.608
DM (kg/100 kg BW)	2.66±0.04	2.71±0.04	2.66±0.05	2.62±0.04	2.66±0.05	0.725
DM (g/kg W ^{0.75})	88.32±1.07	89.81±1.13	87.52±1.36	86.45±1.05	87.83±1.21	0.357
CP (kg/day)	0.41±0.01	0.42±0.01	0.41±0.01	0.41±0.01	0.41±0.01	0.893
CP (kg/100 kg BW)	0.34±0.01	0.34±0.01	0.34±0.01	0.34±0.01	0.34±0.01	0.994
CP (g/kg W ^{0.75})	11.17±0.19	11.27±0.17	11.21±0.18	11.11±0.16	11.24±0.18	0.972
DCP (kg/day)	0.27±0.01	0.27±0.01	0.26±0.01	0.26±0.01	0.27±0.01	0.943
DCP (kg/100 kg BW)	0.22±0.00	0.22±0.00	0.22±0.00	0.22±0.00	0.22±0.00	0.989
DCP (g/kg W ^{0.75})	7.22±0.13	7.29±0.12	7.25±0.12	7.20±0.11	7.28±0.12	0.981
EE (kg/day)	0.08±0.00	0.08±0.00	0.08±0.00	0.08±0.00	0.08±0.00	0.997
EE (kg/100 kg BW)	0.07±0.00	0.07±0.00	0.07±0.00	0.07±0.00	0.07±0.00	0.951
EE (g/kg W ^{0.75})	2.19±0.05	2.21±0.05	2.23±0.05	2.21±0.05	2.23±0.05	0.983
CF (kg/day)	0.77±0.02	0.78±0.02	0.77±0.02	0.77±0.02	0.77±0.02	0.997
CF (kg/100 kg BW)	0.63±0.02	0.64±0.02	0.65±0.02	0.64±0.02	0.65±0.02	0.967
CF (g/kg W ^{0.75})	20.95±0.49	21.11±0.44	21.29±0.43	21.12±0.45	21.28±0.45	0.983
NFE (kg/day)	1.57±0.04	1.57±0.04	1.56±0.04	1.56±0.04	1.57±0.04	0.997
NFE (kg/100 kg BW)	1.28±0.04	1.29±0.03	1.31±0.03	1.30±0.03	1.31±0.03	0.971
NFE (g/kg W ^{0.75})	42.51±1.00	42.84±0.90	43.21±0.88	42.87±0.91	43.18±0.92	0.983
TDN (kg/day)	2.07±0.05	2.09±0.05	2.01±0.05	2.01±0.05	2.03±0.05	0.677
TDN (kg/100 kg BW)	1.68±0.03	1.71±0.03	1.69±0.03	1.66±0.03	1.69±0.03	0.843
TDN (g/kg W ^{0.75})	55.82±0.71	56.70±0.73	55.50±0.85	54.85±0.66	55.66±0.77	0.534
Cal. ME (Mcal/day)	6.85±0.16	6.87±0.16	6.81±0.16	6.80±0.16	6.86±0.16	0.997
Cal. ME (Mcal/100 kg BW)	5.61±0.16	5.66±0.14	5.74±0.15	5.69±0.15	5.72±0.14	0.973
Cal. ME (Kcal/kg W ^{0.75})	185.76±4.37	187.18±3.91	188.80±3.85	187.32±3.96	188.68±4.02	0.984

Where DM = Dry matter; W^{0.75} = Metabolic body weight; CP = Crude protein; DCP = Digestible crude protein; EE = Ether extract; NFE = Nitrogen free extract; Cal. ME = Calculated metabolizable energy; Mcal = Mega calorie; TDN = Total digestible nutrients.

Mean with different superscripts within a row differ significantly ($p < 0.05$)

Nutrient and Energy Intake

The information regarding dry matter and nutrient intake is detailed in Table 2. There was no significant difference ($p > 0.05$) in dry matter intake (kg/day, kg/100 kg BW, g/kg W^{0.75}) among Crossbred Calves fed with various Fibrolytic Microbes and Enzymes. Although the T₂ group exhibited a higher dry matter intake (kg/day, kg/100 kg BW, g/kg W^{0.75}) compared to the other group, it did not significantly impact the crossbred calves. The increased dry matter intake in the fungus-fed group might be attributed to the enhanced palatability of the feed. However, despite the rise in dry matter consumption with fungal feed, there was no variation in average daily gain. The enzyme-fed group showed a higher average daily growth than the other treatments, as the enzyme-containing dry matter intake was lower.

The current findings align with Morsy *et al.* (2016) [25], who found that daily feeding of 40 g of two commercial enzyme products did not significantly affect dry matter intake in Egyptian buffaloes. Lunagariya (2016) [24] also reported that the intake of dry matter and nutrients (crude protein, digestible crude protein, and total digestible nutrient) in Holstein crossbred cows was not significantly ($p > 0.05$) affected by feeding exogenous fibrolytic enzymes supplemented TMR (240 mg/kg TMR) over a 140-day trial. Barbadikar (2012) [8] demonstrated that daily dry matter intake (3.63 vs. 3.69 kg), percentage dry matter intake (2.77

vs. 2.77 kg), DCP intake (313.51 vs. 320.51 g), and TDN intake (2.07 vs. 2.10 kg) in crossbred calves were not significantly different ($P > 0.05$) between the control and fibrolytic enzymes (0.025%) group of crossbred calves. Kim *et al.* (2012) [20] indicated that feeding TMR with probiotics (*Lactobacillus acidophilus*: KCTC3140 and *Saccharomyces cerevisiae*: KACC30068) had no significant effect on daily feed intake in Hanwoo steers during the experiment. Dey *et al.* (2004) [14] also reported findings consistent with the present study, showing non-significantly/numerically lower dry matter intake (4.04±0.34 vs. 4.08±0.52 kg/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) when feeding fungal culture (106 CFU/ml) at 160 ml/week to calves.

Similarly, administering 250 ml of *Neocallimastix* sp. GR1 culture (≈106 tfu/ml) every fourth day to Murrah female buffalo calves resulted in similar dry matter intake (4.12±0.20 vs. 4.14±0.17 kg); however, 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) [29] over a 90-day period. Kumar *et al.* (2015) [22] also noted that feeding anaerobic fungal zoospores of *Neocallimastix* sp. GR-1, incorporated in wheat straw-based complete feed blocks (140×106 zoospores/block of 14 kg), did not significantly affect dry matter and DCP intake, whereas TDN intake was

significantly ($p < 0.05$) higher in 5-7 month old Murrah buffalo male calves, with values of 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 the control and treated group.

According to Sharma *et al.* (2018) [31], in contrast to the current study, dry matter intake (1426.9 vs. 1402.9 g/day) of Murrah buffalo calves was improved ($p < 0.05$) in the probiotics (*Lactobacillus acidophilus*) fermented milk feeding (200 ml daily with 108 CFU/ml) groups compared to the control. According to Shahzad *et al.* (2016) [30], male Nili Ravi buffalo calves fed total mixed ration (TMR) containing 33% fungus-treated wheat straw (*Arachniotus* sp.) consumed more dry matter (2.95 kg/day) than control TMR or 67 and 100% fungus-treated wheat straw (2.88 , 2.80 , and 2.71 kg/day).

Crossbred calves fed various feeds Fibrolytic Microbes and Enzymes did not significantly ($p > 0.05$) differ in their intake of crude protein and digestible crude protein (kg/day, kg/100 kg BW, g/kg W^{0.75}). In comparison to another group, the T₂ group consumed more crude protein (kg/day, g/kg W^{0.75}) and digestible crude protein (kg/day), albeit this difference was not statistically significant in crossbred calves.

The study's findings are consistent with those of Dey *et al.* (2004) [14], who found that ruminal fungus had no discernible impact on the DCP intake of 13-month-old calves. For the control and fungal culture (*Orpinomyces* sp.), the daily DCP consumption was 368.65 ± 41.89 and 394.18 ± 36.43 g/head, respectively. Sehgal *et al.* (2008) [29] reported 275.13 ± 9.68 and 295.39 ± 14.86 g/head/day in the control and fungus groups, respectively, for Murrah buffalo calves (8-11 months old) fed anaerobic fungus *Neocallimastix* sp. However, in buffalo calves (15 months old), Tripathi *et al.* (2007) [34] discovered a substantial impact on DCP intake from a complete feed mixture based on wheat straw, both with and without *Orpinomyces* sp. or *Piromyces* sp. culture administration. For the Control, *Orpinomyces*, and *Piromyces* groups, they reported DCP intake of 415 ± 23.8 , 464 ± 24.2 , and 453 ± 10.9 g/head/day, respectively.

Barbadikar (2012) [8] found that crossbred calves in T₁ [concentrate combination (60%) + wheat straw (40%)] and T₂ [concentrate mixture (60%) + wheat straw (40%) with fibrolytic enzymes @ 0.025%] consumed more DCP (313.51 ± 16.33 and 320.51 ± 12.23 g), respectively. The outcome indicated that fibrolytic enzyme had no discernible impact on crossbred calves' consumption of DCP.

The ether extract, crude fibre, and nitrogen free extract intake (kg/day, kg/100 kg BW, g/kg W^{0.75}) were not significantly ($p > 0.05$) differed in Crossbred Calves fed different feeding Fibrolytic Microbes and Enzymes.

The energy intake includes total digestible nutrient intake (kg/day, kg/100 kg BW, g/kg W^{0.75}) and calculated metabolizable energy intake (Mcal/day, Mcal/100 kg BW, Kcal/kg W^{0.75}) were not significantly ($p > 0.05$) differed in Crossbred Calves fed different feeding Fibrolytic Microbes and Enzymes. In line with present investigation, Dey *et al.* (2004) [14] discovered non-significant effect of ruminal fungus on TDN intake of 13 month old calves and the value were 2.16 ± 0.31 and 2.46 ± 0.22 kg, respectively for control and fungal cultures given (*Orpinomyces* sp.). Higher TDN consumption levels of 2.07 ± 0.11 and 2.10 ± 0.08 kg/head/day were observed by Barbadikar (2012) [8] for T₁ [concentrate

combination (60%) + wheat straw (40%)] and T₂ [concentrate mixture (60%) + wheat straw (40%) with fibrolytic enzymes @ 0.025%] in crossbred calves. This finding indicated that fibrolytic enzyme had no discernible impact on TDN intake in crossbred calves. According to Ramaswami *et al.* (2005) [27], TDN consumption for 216-day-old crossbred calves fed diets supplemented with or without *L. acidophilus* was 2.31 ± 0.06 and 2.34 ± 0.09 kilogram/100 kg body weight, respectively. The variations, however, were not statistically significant. The reports and the current study are comparable.

In contrast with the present study Tripathi *et al.* (2007) [34] showed substantial effect of a complete feed mixture based on wheat straw without and with *Orpinomyces* sp. or *Piromyces* sp. culture administration in buffalo calves (15-month age) on TDN consumption. For Group I (Control), Group II (*Orpinomyces*), and Group III (*Piromyces*), their calculated TDN intake was 2.5 ± 0.1 , 2.9 ± 0.2 , and 3.0 ± 1.0 kg/head/day, respectively. According to Sehgal *et al.* (2008) [29], the anaerobic fungus *Neocallimastix* sp. had a substantial impact on the TDN consumption (kg/head/day) of Murrah buffalo calves aged 8 to 11 months. The TDN intake values in the control and treatment groups were 2.19 ± 0.09 and 2.46 ± 0.13 kg, respectively. The value of TDN intake was higher than the present study.

Conclusion

The present study indicated that supplementation of fibrolytic microbes, exogenous fibrolytic enzymes, or their consortium did not significantly affect dry matter, nutrient, or energy intake in crossbred calves. Intake of CP, DCP, EE, CF, NFE, TDN, and metabolizable energy remained comparable among all treatment and control groups. Although the fungus-fed group showed a numerically higher dry matter intake, it did not result in improved growth performance. The enzyme-fed group exhibited better growth efficiency despite relatively lower dry matter intake, suggesting improved nutrient utilization. Overall, fibrolytic additives did not adversely influence feed intake. These findings support the safe use of fibrolytic microbes and enzymes in calf diets under tropical feeding systems.

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