Effect of Lactic Acid Producing Bacteria on the Performance of Male Crossbred Calves Fed Roughage Based Diet

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ABSTRACT : To study the effect of feeding lactic acid producing bacteria on the performance of cattle calves, twenty four, day old male crossbred cattle calves (*Bos indicus*×*Bos taurus*), were distributed into two groups of 12 animals each. The animals were fed on calf starter containing wheat bran and green berseem *ad libitum* and milk as per requirement upto 8 weeks of age. The diet of calves of Group 2 was supplemented with 500 ml culture of *Lactobacillus acidophilus*-15. Total duration of the experiment was 31 weeks. There was no significant difference in intake and digestibility of dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF) and crude protein (CP) between the groups. The rumen pH, protozoa numbers, concentration of volatile fatty acids (VFA), ammonia nitrogen (NH_3-N), trichloroacetic acid precipitable nitrogen (TCA-ppt N) and activity of microbial enzymes (carboxymethylcellulase, xylanase, amylase and protease) were not affected due to probiotic supplementation. Average live weight gain of the calves was improved (about 10%) and feed:gain ratio was reduced (about 5%) in the animals given *Lactobacillus* culture. The data indicated that crossbred calves could be reared on a diet devoid of cereal grain and addition of *Lactobacillus* culture in the diet resulted in an added advantage in growth performance of the animals. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 8 : 1110-1115)***

Key Words : Crossbred Calves, Lactic Acid Bacteria, Body Weight Gain, DFM

INTRODUCTION

Animals have a natural well stabilized microbial eco-system in their gastrointestinal tract and any deviation from the normal in the microbial environment affects the performance of the animals. The use of lactic acid bacteria as a feed additive influences drastically the microbial ecosystem, especially that of the young calves and helps the animal in establishing the native rumen microbes in the gastro-intestinal tract. This bacterium also helps in maintaining equilibrium among various microbial groups of the eco-system (Morill et al., 1977; Bae et al., 2003; Tanwattana et al., 2003; Vinderola and Reinheimer, 2003; Byun et al., 2004; Saito, 2004). For the improvement in utilization of fibrous feeds by ruminants, the manipulation of microbial ecosystem of the rumen has been tried with antibiotics and many other chemicals. But due to the disadvantages of feeding of antibiotics, like toxicity, allergy and the residues of these feed additives in livestock products, their use is being discouraged. The use of live microbial supplements as probiotic provides a suitable alternative (Wallace and Newbold, 1993; Newbold et al., 1996; Jouany et al., 1998; Ando et al., 2004; Chen et al., 2004; Dey et al., 2004; Sar et al., 2004). The use of live culture of *Lactobacillus* as feed additive resulted in higher feed intake, nutrient digestibility with no change in body weight gain in buffalo calves (Bakshi and Langer 1990), whereas improvement in body weight gain and feed conversion efficiency was reported in buffalo calves (Prahalada et al., 2001). Khuntia and Chaudhary (2002) reported no effect of microbial feed additive on nutrient digestibility in buffalo calves. Diarrhea was controlled with no change in body weight in buffalo calves by *Lactobacillus* feeding as reported by Agarwal et al. (2002) and Das et al. (2002), whereas, Oropeza et al. (1998) could not achieve any impact of feeding various doses of *Lactobacillus* on diarrhea and body weight gain in calves. Looking at the inconsistency in the response of animals to the feeding of *Lactobacillus* culture, the present experiment was conducted to study the effect of *Lactobacillus acidophilus-15* feeding on nutrient digestibility, body weight gain, rumen fermentation pattern and microbial enzyme profile in buffalo calves fed on a grainless diet.

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MATERIALS AND METHODS

Animals

Twenty four, day old male crossbred calves (*Bos indicus*×*Bos taurus*) were procured from the dairy farm of Indian Veterinary Research Institute, Izatnagar, India and were distributed into two groups of 12 animals each. All the animals were given same ration. The calves of group 2 were fed on a diet supplemented with live culture of *Lactobacillus acidophilus*-15 while no supplement was given to group1 (control) animals. The observations were recorded from birth to 31 weeks of age.

Housing and management

The calves were housed individually in a well ventilated clean shed having individual feeding and watering arrangements. The calves were let loose in the paddock

Table 1. Feeding schedule of calves

Age of calves (week)	Milk (part of BW)	Calf starter	Green fodder
1	1/10	-	-
2-4	1/10	*ad lib*	*ad lib*
5-6	1/15	*ad lib*	*ad lib*
7-8	1/20	*ad lib*	*ad lib*
9-31	-	50% DM through concentrate	*ad lib*

during morning for a short duration for exercise where calves were having free access to fresh drinking water. The calves were dewormed periodically as per standard schedule and vaccinated against prevalent contagious diseases at appropriate age.

Feeding of animals

All the animals were offered calf starter along with green fodder *ad libitum* and milk was given at the rate of 10% of body weight. The quantity of milk was gradually reduced and was completely withdrawn after 8 weeks of age. The calves were shifted to normal feed as per NRC (1989) requirement for 500 g average daily live weight gain. Details of feeding schedule are given in Table 1. The concentrate mixture comprised of 98.5% wheat bran, 1% chalk and 0.5% common salt. To the animals of group 2, during milk feeding period (up to 8 weeks) out of total required milk/animal, 500 ml was given as fermented culture of *Lactobacillus acidophilus*. After 8 weeks of age instead of fermented milk, culture was given in the form of fermented feed. The animals were first offered fermented feed and only after finishing it, the remaining amount of concentrate mixture was given. Water was made available to the animals twice daily.

Preparation and feeding of direct fed microbials (DFM)

Pure culture of *Lactobacillus acidophilus*-15 procured from National Dairy Research Institute, Karnal, India was grown on skim milk over night at 39°C and was used for the feeding of treatment group till the period of milk feeding (8 weeks of age). For the preparation of fermented feed 3.0 kg concentrate mixture was mixed with 2.8 litre water and 200 ml *Lactobacillus* culture. The *Lactobacillus* counts were 10^7-10^8 cfu g^{-1} fermented product on fresh basis. The contents were mixed and incubated for 24 h at 39°C in an airtight stainless steel container. The animals were fed fresh fermented feed @ 500 g/day/animal.

Measurements and observations

During the experimental period daily feed offered, residue left and weekly change in body weight were recorded. Feed conversion efficiency of the animals was calculated by the dry matter intake and body weight gain of the individual animals. One digestion trial at 12 weeks of age and one metabolism trial at 26 weeks of age were conducted to determine the nutrient utilization by taking 5 representative calves from each group. The animals were adapted in metabolic cages for 2 days before sample collection. Feed offered and residue left during the trials were collected daily. For chemical analyses, samples of individual animals were pooled for 6 days. Faeces voided daily by the individual animal was weighed separately. During metabolism trial urine was also collected in bottles. The amount of faeces and urine collected in 24 h was quantified and representative samples were transported to the laboratory. Suitable aliquots were preserved for further analyses.

For rumen fermentation study 50 ml of rumen liquor was collected through oesophageal tube at 3 h post feeding for 3 consecutive days. The samples were brought to the laboratory and were filtered through 2 layers of muslin cloth for obtaining strained rumen liquor (SRL). Immediately after straining, pH was measured. About 20 ml of SRL was preserved with 2 drops of 10N H_2SO_4 for estimation of NH_3-N. Another 10 ml of SRL was preserved with a few drops of saturated mercuric chloride for the estimation of total volatile fatty acids (TVFA) and lactic acid. The samples for enzyme estimation were preserved at -20°C. Two ml of SRL was mixed with an equal amount of methyl green formalized saline reagent and was kept at room temperature for the counting of ciliate protozoa.

Chemical analyses

The samples of feed offered, residue left and faeces voided were analysed for dry matter by drying at 80°C for 48 h in a hot air oven. The ash was determined by igniting the samples at 550°C for 3 h. Ether extract was estimated as per AOAC (1981) and CP in Kjeltech auto-1030 analyser purchased from Gerlardt, Germany. Determination of NDF and ADF was done as per van Soest et al. (1991). The SRL was analysed for NH_3-N, total volatile fatty acids (Bernett and Reid, 1957), lactic acid (Barker and Summerson, 1941) and counting of protozoa was done microscopically using haemocytometer as described by Kamra et al. (1991). The samples for enzyme estimation were kept in ice bath and sonicated in an ultra-sonicator for 5 min followed by centrifugation at 27,000 g at 4°C for 30 min. The supernatant was used for the estimation of enzyme activities (carboxymethylcellulase, α-amylase, xylanase and protease) as described by Agarwal et al. (2002).

Statistical analyses

The data were analysed and significance of means were tested using t- test as per Snedecor and Cochran (1968).

Table 2. Chemical composition of feed and fodder (% DM basis)

Item	Concentrate mixture	Berseem
OM	92.32	85.58
Crude protein	15.10	19.71
Ether extract	3.17	3.08
NDF	47.17	43.91
ADF	15.13	29.80
Cellulose	11.85	26.12
Hemicellulose	32.04	14.11

Table 3. Nutrient digestibility in crossbred calves at 12 weeks of age fed a diet supplemented with *L. acidophilus*-15 fermented feed (means±SE)

Item	Control	*L. acidophilus*
Digestibility of nutrients (%)		
Dry matter	69.16±1.09	72.75±1.2
Organic matter	71.53±0.88	73.73±1.29
Neutral detergent fibre	61.93±1.51	62.27±1.68
Acid detergent fibre	57.01±1.26	59.08±1.92
Body weight (kg)	60.82±2.54	50.53±2.71
Intake of feeds		
Berseem	0.61±0.14	0.41±0.04
Concentrate	1.55±0.09	1.26±0.12
Total	2.15±0.19	1.67±0.14
Percent of body weight	3.52±0.20	3.29±0.13

RESULTS AND DISCUSSION

The results of microbial feed additives are highly inconsistent as reviewed by Yoon and Stern (1995). This inconsistency was assigned to the tolerance level of microbial cultures to the new environment of gastro-intestinal tract. Different microbes and even different strains of same microbe may differ in their resistance towards various factors prevalent in the gastrointestinal tract (Agarwal et al., 2000). Therefore various cultures of *Lactobacillus* were tested for their tolerance to low pH, bile salts and volatile fatty acids (unpublished data) and *Lactobacillus acidophilus*-15 showing highest resistance was selected for the present study.

Chemical composition of ration and supplementation of DFM culture

The chemical composition of feeds and fodder offered to the animals is given in Table 2. The CP content of the concentrate mixture and berseem fodder was 15.1% and 19.7%, respectively. Variation in the chemical composition of roughage depended on the stage of maturity and climatic changes and was within the normal range as reported by Ranjhan, (1994). Like previous study (Khuntia and Chaudhary, 2002), in present experiment also variability among the calves in adapting to fermented milk was found. Since in present study the quantity of fermented milk fed to the calves was more than our previous experiments, some calves took more time to adjust themselves to the fermented milk but all the calves developed affinity to fermented milk and within a week they started consuming all the fermented milk offered to them.

Table 4. Nutrient digestibility and plane of nutrition in crossbred calves at 26 weeks of age fed a diet supplemented with *L acidophilus*-15 fermented feed (means±SE)

Item	Control	*L. acidophilus*
Digestibility of nutrients (%)		
Dry matter	69.28±1.11	69.69±1.37
Organic matter	72.85±1.03	73.24±1.22
Crude protein	73.23±0.90	70.65±1.06
Ether extract	47.20±3.15	52.75±3.92
Neutral detergent fibre	56.08±1.51	56.84±2.11
Acid detergent fibre	50.02±1.71	50.35±2.56
Hemicellulose	61.90±1.49	62.57±1.68
Cellulose	54.77±1.55	55.22±2.31
Nutritive value of ration and plane of nutrition		
Body weight (kg)	85.25±4.81	87.20±3.44
Nutritive value of ration (%)		
Crude protein	17.44±0.54	16.97±0.18
Total digestible nutrients	67.97±1.46	67.20±1.94
DM intake (kg^{-d})		
Concentrate	1.46±0.10	1.47±0.10
Roughage	1.45±0.13	1.58±0.13
Total	2.91±0.20	3.05±0.20
Nutrient intake (per100 kg body weight)		
DM (kg/d)	3.41±0.08	3.48±0.10
CP (g^{-d})	593±16	592±22
TDN (kg^{-d})	2.31±0.06	2.34±0.09

Digestibility of nutrients

The intake and digestibility of nutrients during digestion and metabolism trials are given in Table 3 and 4. During both trials no significant difference in the intake was observed between the groups. The ratio of DMI through concentrate and roughage was different during both trials. Out of total DMI, about 72% was met through concentrate at 12 weeks of age and its proportion was reduced to about 50% at 26 weeks of age. Addition of *L. acidophilus* culture had no effect ($p>0.05$) on the digestibility of different nutrients (OM, DM, CP, NDF and ADF) during both the trials conducted at 12 and 26 weeks of age. Abu-Tarboush et al. (1996) and Khuntia and Chaudhary, (2002) also found similar results in *Lactobacillus* fed calves. Since the digestibility of nutrients was similar in both the groups, similar was the case for plane of nutrition and nutritive value of the ration given to the calves. The intake of CP was higher in both the groups than NRC (1989) recommendation. The higher CP intake in the calves of both the groups was due to feeding of protein rich green fodder (berseem). The mean intake of DM, and TDN were also similar in both the groups and was sufficient as per the requirements suggested by NRC (1989) for 500 g average daily gain. In the present experiment the digestibility of

Table 5. Effect of feeding of *L. acidophilus*-15 fermented milk or feed on the body weight gain and feed conversion efficiency (FCR) in crossbred calves (means±SE)

Item	Control	*L. acidophilus*
Initial body weight (kg)	25.13±0.79	25.38±0.67
Body weight gain (kg)		
0-98 days	25.66±1.74	28.38±1.69
99-217 days	67.90±4.74	76.86±2.50
0-217 days	93.56±3.73	105.24±5.31
Average daily gain (g)		
0-98 days	262±18	290±17
99-217 days	571±40	646±21
0-217 days	431±36	485±20
Feed conversion efficiency (feed/gain)		
0-98 days	4.13±0.14	3.88±0.17
99-217 days	4.54±0.22	4.27±0.09
0-217 days	4.32±0.19	4.13±0.11

ether extract was lower than the normal value in both the groups. This might be due to presence of higher quantity of carotenoid and chlorophyll pigments in green fodder. which are poorly digestible (Ranjhan. 1994).

Live weight gain and feed conversion efficiency

The growth performance of animals is given in Table 5. During the pre-ruminant stage (upto 8 weeks of age), milk was the major component of the ration of all the animals and they started eating green berseem and calf starter between 2-3 weeks of age. The intake of calf starter and green fodder increased gradually with the increase in age of the calves. Dry matter intake in calves of treatment group tended to be higher than control group throughout the experiment. The DMI by the calves in both groups was within the feed intake capacity of young calves reared under such feeding systems as reported in an earlier study (Panda et al., 1995; Raut et al., 1996; Giri et al., 2000). There are other reports also where no differences in DMI in control as well as lactobacilli fed calves were found (Abu-Tarboush et al., 1996; Khuntia and Chaudhary, 2002).

The increase in live body weight tended to be more in the treatment group throughout the experiment and resulted in about 10% higher average daily gain in body weight. Although differences were statistically non significant but analysis of covariance showed a significant difference ($p<0.05$) between the slopes which was indicative of parallel difference in weight gain between the two groups. Ellinger et al. (1980) and Abu-Tarboush et al. (1996) also reported a non-significant higher body weight gain in calves supplemented with *Lactobacillus* cultures.

The better growth rate in the animals fed *L. acidophilus* fermented feed might be due to cumulative effect of apparently higher dry matter intake and better utilization of nutrients. As it is known that lactobacilli compete with other pathogenic and harmful bacteria in gastro-intestinal tract and probably this competitive exclusion ability of lactobacilli (Fuller and Turvey, 1971) might have played some role in better metabolizable energy utilization by the host animals. The feed conversion efficiency was also similar ($p>0.05$) between the groups though a slightly better efficiency (about 5%) was observed in *L. acidophilus* group and this was again persistent throughout total experimental period. A non-significantly improved feed conversion efficiency was also observed in pre-ruminant calves by Cruywagen et al. (1996) and Khuntia and Chaudhary, (2002). No significant difference in feed conversion efficiency was reported in pre-ruminant crossbred calves fed on diet supplemented with a mixture of three (*L. acidophilus, L. jugarti* and *L. casei*) lactic acid bacteria and the digestibility of NDF and ADF and OM was significantly improved (Das et al., 2002).

Table 6. Effect of feeding of *L. acidophilus*-15 fermented feed on rumen fermentation, enzyme activity and ciliate protozoa in the rumen of crossbred calves (means±SE)

Item	Control	*L.acidophilus*
Ruminal metabolites		
pH	6.61±0.09	6.55±0.09
Lactic acid (meq l^{-1})	3.81±0.75	3.92±1.00
Total nitrogen (g l^{-1})	10.4±0.09	11.00±0.10
TCA precipitable N (g l^{-1})	0.56±0.08	0.68±0.08
TCA soluble N (g l^{-1})	0.48±0.04	0.42±0.06
Ammonia N (g l^{-1})	12.52±0.92	12.56±1.62
TVFA (mmol dl^{-1})	9.42±0.82	9.96±0.72
Molar proportion of VFA (%)		
Acetate	66.97±0.84	64.23±1.09
Propionate	25.35±1.08	22.99±0.69
Butyrate*	7.68±0.86	12.58±0.79
Enzyme activities		
CM cellulase (μmol glucose h^{-1} ml^{-1})	5.41±0.32	5.27±0.26
Amylase (μmol glucose h^{-1} ml^{-1})	110.99±9.98	114.29±12.07
Xylanase (μmol xylose h^{-1} ml^{-1})	14.05±0.70	13.66±0.56
Protease (μg hydrolysed protein h^{-1} ml^{-1})	1,109± 51	1,002± 81
Protozoa count ($\times 10^4$ ml^{-1})		
Holotrich	0.33±0.09	0.62±0.16
Entodinomorphs	33.07±5.81	38.61±5.11
Total	33.41±5.80	39.17±5.00

* Significant at $p<0.05$.

Rumen environment

The results of rumen fermentation studies presented in Table 6 shows that the supplementation of *L. acidophilus* in the diet of crossbred calves had no effect on rumen pH. rumen metabolites (TVFA, lactic acid. TCA ppt-N. ammonia N), microbial enzymes (CM cellulase, xylanase, α-amylase and protease) and ciliate population in the rumen and the values were more or less similar to earlier studies (Khuntia and Chaudhary. 2002). The molar proportion of

acetate and propionate also remained unchanged while butyrate proportion was significantly higher ($p<0.05$) due to *L. acidophilus* feeding. With the feeding of bacterial culture, acetate:propionate ratio increased (2.76 vs. 2.64), due to a relative increase in acetate production. Acetate and butyrate levels were increased at the cost of propionate when *L. acidophilus* was fed to the calves. The entodiniomorphid protozoa were dominating in both the groups and holotrichs were less than 2% of total numbers. The protozoal population was within normal range of 10^4-10^6ml^{-1} (Williams and Coleman, 1992) and were not affected by feeding of *Lactobacillus*.

The results of the present experiment indicate that supplementation of *L. acidophilus*-15 culture in the diet has a positive influence on performance of the calves in terms of growth rate and feed conversion efficiency.

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