Performance of Male Crossbred Calves as Influenced by Substitution of Grain by Wheat Bran and the Addition of Lactic Acid Bacteria to Diet

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ABSTRACT : To study the effect of wheat bran and lactic acid producing bacteria (LAB) on the performance of calves, 20 crossbred male cattle calves (day old), distributed into two groups were fed on calf starters containing 50 or 0% maize grain, along with green berseem ad libitum and milk as per body weight. Each group was further divided into two sub groups and one subgroup of each group was supplemented with mixed culture of LAB (*Lactobacillus acidophilus L. casei, L. Jugarti*). Milk feeding was discontinued after 8 weeks of age. The addition of culture increased ($p<0.05$) DM intake in calves receiving grainless diet from eighth week to the thirteenth one. There was about 21% higher body weight gain and 14% lower feed : gain ratio in culture supplemented calves. DM digestibility was significantly lower ($p<0.05$) in calves getting grain without culture. The crude protein NDF and ADF digestibility was higher ($p<0.05$) in grainless than the grain fed group. No major change on rumen fermentation pattern among different treatments was found. The concentration of total volatile fatty acids (TVFA) and protozoa count was higher ($p<0.05$) in grain fed group. However, lactic acid concentration was higher and rumen pH was lower due to culture feeding. The incidence as well as severity of diarrhoea was reduced in culture supplemented group. The results indicate that crossbred calves can be reared successfully on grainless diet and berseen fodder. The performance of calves was also improved by LAB supplementation. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 2 : 188-194)***

Key Words : Grain, Lactic Acid Bacteria, Probiotic, Calves, Growth

INTRODUCTION

In most of the developing countries, due to non-availability of cereal grains for livestock feeding, it is a need of present scenario to search for alternate energy sources. Wheat bran is a major cereal by-product available in abundance in wheat growing regions of country to the tune of 4.75 Million Metric Tones per year (Ranjhan, 1994). Since it is palatable, has adequate nutrients and cheaper, therefore, can be a suitable alternate energy source for animal feeding (Giri et al., 2000; Chaudhary et al., 2001).

Despite searching for alternate feed resources for animals, researchers have been continuously trying to find out suitable measures to harvest maximum energy from the feed of animals. Different microbial feed additives like yeast, bacteria and fungi are used for manipulating the rumen fermentation and the microbial eco-system of gastrointestinal tract of animals (Wallace and Newbold, 1993; Jouany et al., 1998; Kamel et al., 2000; Chiou et al., 2000; Blackman, 2000; Conway and Wang, 2000). Among the different microbial feed additives, *Saccharomyces cerevisiae* and *Asprgillus oryzae* are mainly effective at well functional rumen, however, lactobacillus are effective at preruminant stage. Fuller (1972) reported that lactobacillus colonize the gut and competitively exclude the pathogenic bacteria. Reduction in the coliform number in the faeces and calf scores were also reported due to feeding of lactobacillus (Karmey et al., 1986; Abou-Tarboush et al., 1996; Wenk, 2000). Several reports on the feeding LAB in animals are available but these reports are on high plane of nutrition where cereal grain was one of the major component of diet. The aim of present experiment was to study the effect of substitution of cereal grain by wheat bran in calf starter and supplementation of LAB on growth performance and nutrient utilization in crossbred cattle calves.

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MATERIALS AND METHODS

Animals and treatments

Twenty, one day old crossbred calves (interse *Bos indicus* × *B. taurus*) average body weight (28.0 kg) were distributed into two groups of 10 animals in each. One group of animals was given a calf starter based on cereal grain along with milk and green fodder ad libitum (Group-G). Calves of other group were fed calf starter prepared without grain (grain replaced with wheat bran) along with milk and *ad libitum* green fodder (G0). Each group was further divided into two subgroups i.e. GC and GC0 for group-G and G0C and G0C0 for group-G0. Animals of subgroups GC and G0C were given cultures (*Lactobacillus acidophilus, L. casei and L. jugarti*).

Housing and management

All the animals were housed under similar management conditions in well ventilated, clean and dry pucca shed having individual feeding arrangements. The calves were let loose in the paddock during morning hours for short duration for exercise as well as to facilitate proper washing,

cleaning and drying of the shed. In paddock calves had free access to fresh, clean drinking water. Strict hygienic and sanitary conditions were maintained in the shed throughout the experimental period. Proper and timely health care was extended to sick animals.

Feeds and feeding schedule

The calves weaned at birth were fed individually whole milk, calf starter and berseem fodder. After colostrum feeding the calves of all the groups were given milk, 1/10 of body weight up to 4th week of age then it was reduced to 1/15 of body weight up to 6th week and then 1/20 up to 8 week of age. After 8 week milk feeding was stopped and only calf starter and green berseem was fed. The *ad libitum* feeding of calf starter and green was started by second week age. Two calf starters were compounded. Calf starter-I (CSI), contained crushed maize, wheat bran, groundnut cake (GNC), mineral mixture and salt in the ratio of 50:20:27:2:1 and was fortified with 10 g vitablend (Vit. A and Vit. D_3) per 100 kg of calf starter. In the second calf starter (CSII) maize was completely replaced with wheat bran and the ingredients of this calf starter were wheat bran, GNC, mineral mixture and common salt in the ratio of 76:21:2:1 and fortified with vitablend. The calves of group GC and GC0 were fed CSI and those in G0C and G0C0 were fed CSII.

The calculated amount of whole milk was offered in two divided doses daily at about 08:30 h and 17:00 h up to the age of 8 weeks. Milk was warmed and cooled to lukewarm before feeding. Out of total requirement of milk per day, 100 ml of milk fermented with LAB was fed to group GC and G0C. Calves of subgroups GC0 and G0C0 were given the same quantity of unfermented milk. Weighed amount of calf starter and green berseem was fed daily to all the calves. Clean drinking water was provided twice daily.

Preparation of culture

Pure cultures of LAB (*Lactobacillus acidophilus, L. casei, L. jugarti)* procured from NDRI, Karnal, India were grown on milk for 48 h at 37°C to obtain around 10^7-10^8 cfu ml^{-1} of fermented product. All the above three cultures were prepared separately and were mixed in equal quantity at the time of feeding.

Measurements and observations

During the experimental period daily DMI and weekly change in body weight were recorded. One digestion trial at 4 week of age and another one at 12 week of age, were conducted to determine the nutrients utilisation.

For rumen fermentation study at 12 week of age, 3 calves from each subgroup were selected and 50 ml rumen liquor was collected through stomach tube at 3 h post feeding for 3 consecutive days. The samples were brought to the laboratory under cold condition and were filtered with 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 10 N H_2SO_4 for estimation of NH_3-N. Another 10 ml SRL was preserved with 2-3 drops of mercuric chloride for the estimation of total volatile fatty acids (TVFA) and lactic acid. The samples were preserved at -20°C. Two ml of SRL was mixed with same amount of methyl green formaldehyde formal saline reagent and was kept at room temperature for the counting of ciliate protozoa.

To study the blood biochemical characteristic of calves, blood samples were collected in the morning prior to offering feeds to the calves at 12 weeks of age. The blood was collected through jugular vein in centrifuge tube containing anticoagulant (sodium fluoride and EDTA) and centrifuged at 2,000 rpm for 10 min. to separate plasma and was preserved at -20°C for subsequent analysis of glucose, protein and albumin. Globulin was calculated by difference.

Chemical analysis

The samples of feed offered, residue left and faeces voided were analysed for DM by drying at 100°C for 24 h in 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 crude protein in Kjeltec AUTO-1030 analyser. The NDF and ADF were determined as per van Soest et al. (1991). The SRL was analysed for NH_3-N, TVFA (Bernett and Reid, 1957), lactic acid (Barker and Summersion, 1941) and counting of protozoa was done microscopically using haemocytometer as described by Kamra et al. (1991).

Statistical analysis

The data obtained from the experiment were analysed using 2 × 2 factorial design as per Snedecor and Cochran (1968).

RESULTS AND DISCUSSION

Chemical composition of feeds

Chemical composition of calf starter, berseem fodder and milk are presented in table 3. Calf starters were computed as per NRC (1989) and had around 22% CP and 90% OM. Due to 100% replacement of maize with wheat bran the fibre fractions of calf starter II were higher than calf starter-I. The CP content of berseem fodder varied between 17.4 to 20.1% which is a normal variation depending on the stage of maturity at the time of harvesting and climatic changes.

Supplementation of culture

There was variability in the acceptance of fermented milk among the calves. Most of the calves relished the fermented milk and thus consumed it easily. However, some of the calves were not having liking for the culture therefore, it was mixed with the calf starter and was fed to the animals.

Feed intake pattern

During early life of calves the milk was the major component of the diet. The calves of all the groups started eating green fodder and calf starter from 2-3 week of age. The intake of calf starter and green fodder increased gradually with the increase in age of the calves. Milk was completely withdrawn from all the calves after 8 weeks of age. The quantity of milk consumed was almost similar in the animals of all the groups. DMI pattern of calves from 0-4 week, 5-8 week, 9-13 week and 0-13 week is given in table 2. There were not either significant differences on concentrate and roughage DMI up to eight weeks of age. Similar results were also reported by Higginbotham and Bath (1993).

Between 9-13 week the intake of calf starter in group G0C was higher ($p<0.05$) where grain was replaced with wheat bran and supplemented with culture than in group G0C0 where neither grain nor culture was given. During this period DMI from berseem was also higher ($p<0.05$) (557 g d^{-1}) in G0C group as compared to minimum (366 g d^{-1}) in G0C0 group. By contrast no difference due to culture additions were found on animals fed grain (group G). The overall DM intake (0-13 week) was significantly higher ($p<0.05$) in the calves of G0C (110 kg) than in G0C0 (75 kg) group. Higher intake was the associative effect of higher calf starter and higher berseem intake in this group but differences were only significant for starter intake was statistically non-significant. However, there are reports where no difference in DMI in control as well as lactobacilli fed calves were found (Abu-Tarboush et al., 1996). The DMI by the calves in different groups was within the feed intake capacity of young calves reared under such feeding systems in an earlier study, (Mondal et al., 1996).

Growth performance and feed conversion ratio

The average initial weight of calves were 25.9±2.8, 30.9±3.6, 27.1±1.1 and 27.9±2.6 kg in groups GC, GC0, G0C and G0C0 respectively and were statistically comparable ($p>0.05$). At the end of 4 week the calves attained a live weight of 30.4±1.8, 35.4±3.2, 34.0±1.7 and 33.8±2.5 kg in respective groups. Although, there was no difference among the groups, at the end of 8 weeks the calves attained a live weight of 40.3±3.3, 44.1±5.6, 44.8±2.4 and 39.7±3.8 kg in group GC, GC0, G0C and G0C0, respectively. The calves of group, G0C0 had the lowest growth rate (275±52 g·d^{-1}) while the highest growth rate (385±71 g·d^{-1}) was found in G0C group, but due to high individual variation differences were non significant (table 3).

From weeks 9-13 when only calf starter and berseem was given the growth rate of all the calves increased irrespective or treatments. This shows that the required

Table 1. Chemical composition of feed and fodder (% DM basis) and milk (as fresh basis) fed to the calves*

Item	Calf starter I	Calf starter II	Berseem	Milk
OM	91.19 - 91.09	90.80 - 89.39	87.64 - 85.82	11.69
Crude protein	20.92 - 22.00	21.05 - 21.56	17.34 - 20.1	3.20
Ether extract	2.80 - 2.79	2.54- 2.12	4.30 - 3.67	4.30
NDF	33.45 -34.12	47.32 - 43.69	49.75 - 51.21	-
ADF	12.83 -10.99	15.24 - 13.44	30.34 - 28.27	-
Cellulose	10.93 -8.07	12.13 - 13.53	24.04 - 23.21	-
Hemicellulose	20.62 -23.13	32.08 - 30.25	19.39 - 24.89	-

* Chemical analysis was done at 4 week and 12 week of feeding trail.

Table 2. Dry matter intake during experiment by calves

Group	0-4 wk			5-8 wk			9-13 wk		0-13 wk			
	Milk (g d^{-1})	Conc. (g d^{-1})	Berseem (g d^{-1})	Milk (g d^{-1})	Conc. (g d^{-1})	Berseem (g d^{-1})	Conc. (g d^{-1})	Berseem (g d^{-1})	Milk (g d^{-1})	Conc. (g d^{-1})	Berseem (g d^{-1})	Total (kg)
GC	345	116	32	282	431	182	894	389	17.53	47.16^{ab}	19.48	84.34^{ab}
GC0	408	140	53	328	520	202	878	486	20.52	49.12^{ab}	23.00	95.34^{ab}
G0C	363	145	37	322	697	221	1162	557	19.34	64.22^{b}	26.76	109.7^{b}
G0C0	378	71	38	305	394	180	704	366	19.12	38.12^{a}	19.40	75.23^{a}
SEM	27.79	24.70	8.47	20.77	74.41	24.73	118	68.76	1.26	6.13	3.06	8.69

^{a,b} values bearing different superscript in a column differ significantly ($p<0.01$).

GC, Grain with culture; GCO, Grain without culture; GOC, Without grain with culture; GOCO, Without grain and culture.

Table 3. Average daily gain (g) of crossbred calves during experimental period

Group	0-4 wk	5-8 wk	9-13 wk	0-13 wk
GC	157	357	437	325
GC0	161	312	361	288
G0C	246	385	443	366
G0C0	211	275	337	259
SEM	42.62	71.13	86.95	56.28

GC, Grain with culture; GCO, Grain without culture.
GOC, Without grain with culture; GOCO, without grain and culture.

nutrients were consumed and utilized by the calves through calf starter and green berseem fodder. Gupta et al. (1992) reported that restriction of milk had no effect on the growth performance as animal compensates intake by taking calf starter and green berseem. There are reports where no difference in body weight gain in control and lactic acid bacteria supplemented groups were found (Bechman et al., 1977, Jenney et al., 1991 and Higginbotham and Bath, 1993). The substitution of maize by wheat bran had no effect on the growth performance of calves up to 13 week of age. The growth rate in different groups followed similar pattern as observed in earlier studies (Srivastava et al., 1980; Gupta et al., 1992; Mandibaya et al., 1999).

The feed conversion ratio (FCR) at different stages of growth showed no significant effect except during 0-4 week when significantly higher ($p<0.05$) FCR was found in grain than grainless group (table 4). This might be either due to higher growth rate of G0C group as calves at this stage mainly fulfill their DM requirement through milk and contribution of grain was very less or due to higher incidence of diarrhoea observed in GC0 group. Neither grain nor lactobacilli had any significant effect on the FCR among the groups from 5 weeks on, and the differences were not either significant when all the experimental period was considered. No change in FCR was observed by grain feeding in calves (Gupta et al., 1992). Cruywagen et al. (1996) also demonstrated no effect of *Lactobacillus acidophilus* feeding on FCR in calves.

Table 4. Feed conversion ratio of calves during experimental period

Group	0-4 wk	5-8 wk	9-13 wk	0-13 wk
GC	3.84	2.93	3.86	3.34
GC0	5.87	4.15	4.52	4.66
G0C	2.22	3.70	4.06	3.36
G0C0	2.82	4.09	3.65	3.34
SEM	0.97	0.98	0.78	0.61
Significance				
G Vs G0	*	NS	NS	NS
C Vs C0	NS	NS	NS	NS

* Significant at $p<0.05$.
GC, Grain with culture; GCO, Grain without culture.
GOC, Without grain with culture; GOCO, without grain and culture.

Digestibility of nutrients at 4 week of age

There was no difference in the digestibilities of DM and other nutrients except CP and hemicellulose at 4 weeks of age among the groups (table 5). The probable reason for no effect of grain on the intake and digestibility of nutrients may be either inadequate availability of energy or due to comparable utilisation of wheat bran and grain containing diets or just because most of the diet was milk and only a part of diet was different. Raut et al. (1996) observed similar results in calves. The digestibility of EE remained very close to that of milk because the calf starter contributed very little the total ether extract intake in comparison to crude protein, digestibility of which came down (85-90%) than the values (93-95%) reported for N-digestibility of whole milk (Roy, 1980). From the results it is apparent that NDF, ADF, cellulose and hemicellulose were digested in 4 week which shows fibre degrading capacity of ruminants as early as 4 week of age (Sahoo and Pathak, 1996). Similar to grain, lactobacilli culture also had no effect on the digestibility of nutrients at 4 week.

Digestibility of nutrients at 12 week of age

The digestibility of DM was significantly lower ($p<0.05$) in GC0 than the other groups and an interaction

Table 5. Digestibility of nutrient at 4 week of age of calves

Group	DM	OM	CP	EE	NDF	ADF	Cellulose	Hemicellulose
GC	84.48	85.37	85.06	93.10	61.42	59.53	63.12	74.30
GC0	84.69	85.60	85.96	91.53	66.3	64.7	71.47	63.27
G0C	88.50	88.95	90.01	94.60	69.33	61.83	66.37	82.73
G0C0	84.82	81.96	90.39	93.75	67.36	61.83	67.50	74.28
SEM	1.72	1.96	1.53	1.19	3.96	3.81	4.33	3.34
Significance								
G Vs G0	NS	NS	*	NS	NS	NS	NS	*
C Vs C0	NS	NS	NS	NS	NS	NS	NS	NS

* Significant at $p<0.05$.
GC, Grain with culture; GCO, Grain without culture.
GOC, Without grain with culture; GOCO, without grain and culture.

between culture and grain was found (table 6). The DM digestibility of grain replaced group was higher than the group containing 50% maize in the calf starter which probably helped the calves in grain replaced groups to fulfil their nutrient requirement through grain less calf starter. The difference in digestibility coefficients of OM, ADF and cellulose were statistically significant ($p<0.05$) between grain fed and grain replaced groups. Similar depressed effect of corn on ADF digestibility was also observed by Faulkner et al. (1994). Supplementation of lactobacillus culture had no significant impact on the digestibility of the nutrients except DM, NDF and hemicellulose.

Effect on rumen fermentation

The production of rumen metabolites are given in table 7. Substitution of maize by wheat bran did not significantly affect the rumen pH, which agree with the results found by Panda et al. (1995). However calves fed maize showed a higher ($p<0.05$) concentration of TVFA in the rumen liquor. This might be due to high starch contents of maize which is easily fermented by the microorganisms resulting in high volatile fatty acids production. The lactic acid and NH_3-N of grain and grain less groups did not affected significantly. The pH of culture supplemented group was significantly lower ($p<0.05$) than without culture group (5.87 vs 6.60). This lower pH might be due to higher ($p<0.05$) lactic acid concentration in culture supplemented group than without culture (2.40 vs 2.00 $mg^{-1}100$ ml). Contrary to above, Jonecova et al. (1992) reported increased rumen pH in sheep fed on mixture of lactobacillus and yeast culture although effect was attributed to yeast culture, as yeast increases the uptake of lactate rumen bacteria (Nisbet and Martin, 1991). LAB had no effect on the total volatile fatty acid concentration in rumen. Similar results were reported by Adams et al. (1981), Weidmeier et al. (1987).

Effect on protozoal population

The entodinomorphs were dominating in all the groups (table 7). Of the total protozoal population, entodinomorphs were >98% and only <2% were holotrichs. Almost similar protozoal populations were reported earlier by Kamra et al. (1991). There was no effect of culture supplementation on protozoal population but grain feeding resulted in increased ciliate population ($p<0.05$) as compared to grain replaced group. This increase may be due to higher starch and other soluble sugars in cereal grains. Increase in protozoal

Table 6. Digestibility of nutrient at 12 week of age of calves

Group	DM	OM	CP	EE	NDF	ADF	Cellulose	Hemicellulose
GC	72.04	71.41	74.69	67.68	61.35	54.08	63.74	65.98
GC0	66.65	67.64	73.09	65.83	53.63	53.67	54.86	59.01
G0C	72.21	73.46	75.00	69.52	63.25	60.49	66.38	69.51
G0C0	71.12	72.99	74.44	60.23	58.48	61.04	65.45	60.02
SEM	0.86	1.67	2.08	3.66	1.70	2.77	2.69	2.16
Significance								
G Vs G0	*	*	NS	*	NS	*	*	NS
C Vs C0	*	NS	NS	NS	*	NS	NS	**

[a,b] values bearing different superscript in a column differ significantly (* $p<0.05$; ** $p<0.01$).
GC, Grain with culture; GCO, Grain without culture.
GOC, Without grain with culture; GOCO, without grain and culture.
SEM, Standard error of mean.

Table 7. Production of rumen metabolites and ciliate count ($\times 10^5$/ml) in calves at 3 h of post feeding

Group	pH	TVFA ($meq \cdot l^{-1}$)	NH_3-N ($mg \cdot dl^{-1}$)	Lactic acid ($mg \cdot dl^{-1}$)	Holotrich	Enlodinomorph	Total
GC	5.55	90.40	7.91	2.43	0.11	8.13	8.24
GC0	6.58	83.25	8.16	2.10	0.01	8.41	8.52
G0C	6.20	80.11	7.65	2.37	0.09	7.06	7.14
G0C0	6.61	79.61	7.89	1.90	0.10	6.88	6.97
SEM	0.29	2.57	0.20	0.20	0.03	0.54	0.54
Significance level							
G Vs G0	NS	**	NS	NS	NS	NS	*
C Vs C0	*	NS	NS	*	NS	NS	NS

* $p<0.05$, ** $p<0.01$.
GC, Grain with culture; GCO, Grain without culture.
GOC, Without grain with culture; GOCO, without grain and culture.

Table 8. Mortality and incidence of diarrhoea in calves

Group	Mortality (%)	Calves suffered from diarrhoea (%)				Duration of diarrhoea (d)
		0-4 wk	5-8 wk	9-13 wk	0-13 wk	
GC	0	20	0	0	20	2
GC0	20	60	20	0	60	14
G0C	0	0	0	0	0	0
G0C0	0	40	0	0	40	10

GC, Grain with culture; GCO, Grain without culture.

population due to grain supplementation was also reported by Mondal et al. (1996).

Effect on incidence of diarrhoea

It was observed throughout the experiment that out of 10 calves in culture fed groups only one calf suffered with diarrhoea resulting in only 10% cases of diarrhoea (table 8). In contrast to this, the other groups where no culture was given, out of 10 calves, 5 suffered diarrhoea leading the incidence to 50%. Severity and duration of diarrhoea was also high in the calves without culture supplementation and one animal died due to severe diarrhoea at 6 week of age. The incidence of diarrhoea was more up to 4 week and thereafter reduces with the advancement of age and after 8 week the incidence of diarrhoea was almost nil. The calves fed on diet supplemented with lactobacilli had lower coliform bacteria count in rumen liquor and faeces (Kamra et al., 1997). Reduction in coliform count by feeding LAB was reported (Thomas et al., 1974, Ellinger et al., 1980, Jenney et al., 1991, Mandibaya, et al., 1999.). Abe et al. (1995) also observed decrease in incidences of diarrhoea by lactobacillus feeding, however, Morrill et al. (1977) and Cruywagen et al. (1996) found no effect of culture supplementation on the incidence of diarrhoea in animals.

The results of the present experiment indicate that the cereal grain can be replaced with wheat bran from the diet of crossbred calves without any adverse effect on their performance. Further supplementation of mixture of lactobacillus cultures though showed numerical improvement in the health and performance of the calves.

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