

## Pre- and Postweaning Attributes in Faunated and Ciliate-Free Calves Fed Calf Starter With or Without Fish Meal

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### ABSTRACT

In a  $2 \times 2$  factorial design, 24 newborn, crossbred (*Bos indicus*  $\times$  *Bos taurus*) calves were distributed in 4 equal groups involving dietary treatments of prestarter diets with (FM) or without fish meal (NFM) in a faunated (F) or ciliate-free (D) ruminal environment to study the ruminal fermentative development in pre- and postweaning periods. Defaunation was achieved by rearing calves in isolation and its effect was studied after first appearance of ciliate protozoa (observed after 8 wk of age) in the faunated animals. Calves were fed colostrum for 24 h and whole milk until weaning at 8 wk of age. Ruminal content samples were collected on d 4, 1 wk, weekly to 8 wk, and then biweekly at 9, 11, and 13 wk of age. The samples were analyzed for fermentation products [pH, total volatile fatty acids (VFA) and ammonia N] and enzyme [carboxymethyl (CM) cellulase, xylanase,  $\beta$ -glucosidase,  $\alpha$ -amylase,  $\beta$ -galactosidase, proteases, and urease] activities. Weekly feed intake increased with age, but was similar in both groups. Ruminal pH declined steadily during 0 to 4 wk of age and then stabilized. The total VFA concentration increased with the age. The ammonia N (mg/dL) concentration increased from 14.9 on d 4 to 32.4 at 4 wk, decreased to 17.6 at 8 wk, and then stabilized during the postweaning period. Samples collected on d 4 had no fibrolytic activity. Xylanase (U/dL) appeared first (1 wk) followed by  $\beta$ -glucosidase (U/dL) and CM cellulase (U/dL), which increased steadily from a low of 4.69, 0.08, and 2.95 to 31.8 (6 wk), 5.92 (7 wk), and 19.8 (8 wk), respectively, and the concentrations showed nonsignificant alterations during postweaning periods. The concentration of  $\alpha$ -amylase (U/dL) increased from 34.3 on d 4 to 87.2 at 8 wk, and then decreased to 56.6 (13 wk).  $\beta$ -Galactosidase increased up to 6 wk then decreased to trace level (0.20 U/dL) at 13 wk of age. The concentrations of proteases and urease reached a steady state after 1 wk of age. The effect of

diet type on ruminal fermentation products and enzyme parameters was nonsignificant. However, a steady and proportional alteration in both parameters in response to dry feed intake with the advancement of age was seen in all calves. Defaunation increased total VFA (97.3 vs. 75.8 mM/L) and  $\alpha$ -amylase activity (80.3 vs. 61.4 U/dL) and decreased ammonia N (16.4 vs. 21.1 mg/dL), whereas the effect on other parameters was nonsignificant. Ruminal fermentative changes responded to dry feed intake, but did not differ in response to animal protein in prestarter diet.

**(Key words:** rumen fermentation, calf starter, defaunation, animal protein)

**Abbreviation key:** CM = carboxymethyl, D = defaunated, F = faunated, FM = fish meal, NFM = no fish meal.

### INTRODUCTION

Dietary adjustments directed toward early development of ruminal function help the animal in early tolerance of fibrous components in their ration. In India, many of the organized farms or farmers have adopted the practice of weaning calves at or above 3 mo of age and including animal protein in the starter diet. Adoption of dry feed consumption in calves at an early age leads to early weaning because of rapid ruminal metabolic development (Anderson et al., 1987a; Quigley et al., 1991). Calves with early-developed microbial ecosystems can thus be exposed to low-cost feeding strategy based on fibrous crop residues. Additionally, the replacement of costly animal protein from the prestarter diet of young ruminants may have the added advantage of reducing the total cost of calf rearing. Quigley et al. (1985) observed no effect on the composition of essential amino acids in bacterial proteins due to age, weaning, or diet composition. According to Warner (1984), the crucial aspect of a calf starter is its total intake rather than its specific protein characteristics. Further, fish meal-containing diets produced lower total VFA and ammonia N concentrations (Zerbini and Polan, 1985; Sil et al., 1994), but no effect on polysaccharide-degrading enzymes (Sil et al., 1994) in the ruminal fluid of growing calves compared with plant protein.

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Calves are generally separated from their dams and reared in separate hutches or in groups. Because of such isolation, those calves generally remain ciliate-free. The role of protozoa was investigated in various defaunation and refaunation studies (Hsu et al., 1991; Williams and Withers, 1993; Koenig et al., 2000; Santra and Karim, 2002). Significant change in microbial activities involved alterations in polysaccharide-degrading enzymes, ruminal ammonia N concentration, bacterial protein synthesis, and ruminal protein outflow.

The present investigation was aimed at assessing the developmental changes in ruminal fermentative attributes of preruminant calves before and after establishment of ciliated protozoa to feeding of prestarter diet with or without fish meal.

## MATERIALS AND METHODS

### Experimental Design

Twenty-four newborn crossbred calves (*Bos indicus* × *Bos taurus*; average BW,  $22.5 \pm 0.7$  kg) were separated from their dams following 24 h of colostrum feeding, and reared in individual sheds. In a  $2 \times 2$  factorial design, calves were equally distributed on a staggered basis when they were born into 4 treatment groups, involving feeding of calf starter with (FM) or without (NFM) fish meal, and faunated (F) or ciliate-free (defaunated; D) calves. Ciliate-free calves were raised in isolation, whereas the faunated group was allowed daily access (particularly during cleaning and drying of calf hutches) to adult animals in a separate enclosure adjacent to the calf running space, and had common watering facilities. The 4 treatment groups were faunated and fed fish meal (FFM), defaunated and fed fish meal (DFM), faunated and not fed fish meal (FNFM), and defaunated and not fed fish meal (DNFM).

### Feeding Management

Lukewarm whole milk was fed to all calves in 2 equally divided doses at 0900 and 1700 h daily. The milk feeding schedule was as follows: 10% of BW up to 3 wk, followed by 8% during wk 4, 6% during wk 5 and 6, and 3% of BW to wean at 8 wk of age. Calves in groups FM and NFM were fed calf starter with or without fish meal, respectively. Both calf starters were isonitrogenous. The composition of calf starter and nutrient analysis are shown in Table 1. The roughage source was oat hay (chaffed to 1 to 2 cm). Calf starter and oat hay were offered ad libitum. Clean drinking water was available at all times. The feeding trial was for a period of 13 wk comprising a preweaning period (0 to 8 wk) that included high (0 to 4 wk, phase 1) and reduced (5 to 8

**Table 1.** Ingredient composition and nutrient analysis of calf starter and oat hay.

Ingredients (%)	Calf starter 1	Calf starter 2	Oat hay
Ground corn	50	50	...
Wheat bran	12	12	...
Fish meal	8	0	...
De-oiled peanut cake	27	35	...
Mineral mixture	2	2	...
Salt	1	1	...
Vitamin A, D <sub>3</sub>	0.01	0.01	...
Nutrient analysis (% of DM)			
OM	89.5	90.3	88.7
CP	22.0	21.8	4.7
Ether extract	3.2	3.0	3.2
NDF	35.2	35.4	73.5
ADF	10.4	11.1	43.0

wk, phase 2) milk feeding phases, and a postweaning period (9 to 13 wk).

Daily intake of DM was recorded from the DM offered and residue left. Periodic samples of milk, calf starter, oat hay, and residual feed were collected and oven-dried ( $100 \pm 5^\circ\text{C}$ ) for estimation of DM. Body weights were recorded weekly before feeding and watering/milk feeding.

### Collection and Analysis of Ruminal Fluid Samples

Approximately 50 mL of ruminal fluid was collected via a stomach tube at 3 h postfeeding from 4 of 6 calves in each group on d 4, then weekly up to wk 9, and then biweekly up to wk 13. Ruminal fluid was collected from all calves every week for observation of ciliate protozoa. Samples were collected in stoppered conical flasks and brought to the laboratory for immediate recording of pH. Approximately 20 mL of the sample was then frozen (below  $-20^\circ\text{C}$ ) for enzyme analysis. The ruminal fluid was sonicated twice at 80 W for 10 min each, and then centrifuged at  $24,000 \times g$  for 20 min to collect supernatant for enzyme analysis. The rest (about 30 mL) was strained through muslin cloth. One milliliter of ruminal fluid was preserved with 1 mL of formal saline (10% formalin in 0.9% sodium chloride solution) with methylene green indicator in a capped vial for counting of ciliate protozoa; the remainder was acidified with 2 to 3 drops of 10 N sulfuric acid and stored in capped vials in a freezer at  $-20^\circ\text{C}$  for chemical analysis.

Carboxymethyl (CM) cellulase (EC 3.2.1.4), xylanase (EC 3.2.1.6),  $\beta$ -glucosidase (EC 3.2.1.2),  $\alpha$ -amylase (EC 3.2.1.1),  $\beta$ -galactosidase (EC 3.2.1.23), proteases, and urease (EC 3.5.1.5) were assayed using CM cellulose, oat spelt xylan, p-nitrophenyl  $\beta$ -D-glucopyranoside, soluble starch, o-nitrophenyl  $\beta$ -D-galactopyranoside, casein, and urea as substrates, respectively. The concen-

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