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 2×2 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, β -glucosidase, α -amylase, β -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 β -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 α -amylase (U/dL) increased from 34.3 on d 4 to 87.2 at 8 wk, and then decreased to 56.6 (13 wk). β -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 α -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.

Received August 4, 2004.

Accepted February 11, 2005.

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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 ± 0.7 kg) were separated from their dams following 24 h of colostrum feeding, and reared in individual sheds. In a 2×2 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 ₃	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°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°C for chemical analysis.

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

Table 2. Dry feed intake (DM, g/d) in calves during pre- and postweaning periods.¹

Feed intake ²													SEM
Age (wk)	Calf starter				Oat hay				Total				
	FM		NFM		FM		NFM		FM		NFM		
Prewaning period													
Phase 1 ³													
1	36 ^a		36 ^a		0 ^a		0 ^a		337 ^a		364 ^a		11
2	123 ^b		117 ^b		1 ^a		1 ^{ab}		467 ^b		471 ^b		26
3	193 ^c		246 ^c		3 ^{ab}		5 ^{bc}		542 ^c		616 ^c		35
4	244 ^c		266 ^c		4 ^b		9 ^c		554 ^c		576 ^c		36
SEM	22**		20**		1*		2*		29**		28**		
Phase 2													
5	298 ^a		350 ^a		12 ^a		14 ^a		555 ^a		605 ^a		40
6	399 ^{ab}		403 ^a		32 ^{ab}		31 ^a		671 ^a		654 ^{ab}		44
7	496 ^b		494 ^{ab}		46 ^{bc}		45 ^{ab}		696 ^{ab}		697 ^{ab}		64
8	635 ^c		589 ^b		66 ^c		66 ^b		848 ^b		791 ^b		65
SEM	39**		46**		11**		10**		55*		62*		
Postweaning period													
	Calf starter				Oat hay				Total				
	FFM	DFM	FNFM	DNFM	FFM	DFM	FNFM	DNFM	FFM	DFM	FNFM	DNFM	
9	836	881	925 ^a	685 ^a	84 ^a	90 ^a	67 ^a	111 ^a	920	971	992 ^a	796 ^a	64
10	1029	840	954 ^a	784 ^{ab}	106 ^{ab}	128 ^{ab}	85 ^{ab}	127 ^a	1135	968	1039 ^a	911 ^{ab}	90
11	1132	980	1115 ^{ab}	924 ^{bc}	117 ^{ab}	120 ^{ab}	108 ^{abc}	155 ^{ab}	1249	1100	1223 ^{ab}	1079 ^{bc}	106
12	1137	1099	1233 ^{ab}	1111 ^{cd}	123 ^{ab}	109 ^{ab}	136 ^{bc}	153 ^{ab}	1260	1208	1369 ^{ab}	1264 ^{cd}	102
13	1250	1168	1334 ^b	1225 ^d	173 ^b	174 ^b	151 ^c	189 ^b	1423	1342	1585 ^b	1414 ^d	98
SEM	120	121	112*	48**	22*	19*	14**	20*	138	129	111**	46**	

a,b,c,d Means bearing different superscripts in a column differ significantly (* $P < 0.05$; ** $P < 0.01$).

¹Total DM intake includes DM from milk during preweaning period.

²FM = Group fed calf starter with fish meal; NFM = group fed calf starter without fish meal; F = faunated; D = defaunated (ciliate-free).

³Phase 1 = high milk feeding phase; phase 2 = reduced milk feeding phase.

tration of reducing sugar after incubation with enzyme extract was determined for CM cellulase, xylanase, and α -amylase (Miller, 1959). β -Glucosidase activity was measured from the concentration of reduced p-nitrophenol (Shewale and Sadana, 1981). For the determination of β -galactosidase, the method of McFeters et al. (1967) was followed. The proteases-induced release of amino acids and peptides (expressed in milligrams of protein released) were estimated by Lowry's method (Lowry et al., 1951). The ammonia produced from urease was determined by the method of Weatherburn (1967). Unit of enzyme activity (U) was calculated as the amount of enzyme that produced one unit of reducing product per milliliter per minute under the assay conditions. The acidified ruminal fluid was analyzed for total VFA (Barnett and Reid, 1956) and ammonia N (AOAC, 1980).

Weekly microscopic examination of ruminal fluid was made to observe the establishment of ciliated protozoa in the rumen; and their identification and counting were according to Kamra et al. (1991).

Statistical Analyses

The experimental data obtained during the pre- and postweaning periods were analyzed differently by AN-

OVA using the model as described in Snedecor and Cochran (1989). Prewaning observations were compiled under the main dietary treatments of FM and NFM, and the postweaning observations were analyzed under the 4 treatments (FFM, DFM, FNFM, and DNFM). The main effects of diet and defaunation were analyzed by orthogonal contrasts. The effect of age and its interaction with different treatment combinations were also analyzed. In all cases, differences among treatment groups were contrasted by Tukey's t -test. Significance was declared at $P < 0.05$ unless otherwise indicated.

$$Y_{ijk} = \mu + T_i + C_{(ij)} + t_k + Tt_{(ik)} + e_{(ijk)}$$

where Y_{ijk} = dependent variables; μ = overall mean; T_i = effect of treatment i , $C_{(ij)}$ = effect of calf j nested within treatment i (or error due to treatment); t_k = effect of time period k ; $Tt_{(ik)}$ = effect of treatment $\times$ period interaction; and $e_{(ijk)}$ = residual.

RESULTS

Feed (DM) Intake and Live Weight Gain

Weekly intakes of milk, calf starter, and oat hay are presented in Table 2. Whole milk was the principal

Table 3. Effects of fish meal and ciliated protozoa on live body weight gains in calves.

Attributes	Group				SEM
	Calf starter with fish meal		Calf starter without fish meal		
Prewaning period					
Initial live weight (kg)	22.7		22.4		1.00
Final live weight (kg)	37.2		36.4		0.97
Postweaning period	Faunated	Defaunated	Faunated	Defaunated	
Initial live weight (kg)	37.5	37.0	36.8	36.0	2.01
Final live weight (kg)	54.8	53.7	54.7	52.3	3.11

component of the diet during the first 2 wk of life. Thereafter, the calves were adapted to dry feed, particularly the calf starter. Refusal to consume calf starter was not seen in any of the calves, even during wk 1. In contrast, few calves (5) consumed oat hay in wk 2, and an appreciable intake was seen only after 5 wk of age in both groups. Rumination was observed as early as 2 wk of age in a few calves (5), which became more pronounced at 4 wk irrespective of dietary treatments. Intake of oat hay before weaning was quite low and averaged <5% of total DM intake and increased to 12% during the postweaning period. Inclusion of fish meal in the calf starter had no effect on feed intake. The average preweaning DM intake was nearly 600 g, with calf starter constituting >50% of the total intake. During the postweaning period, the intake of dry feed increased from 0.82 kg at 8 wk to 1.42 kg at 13 wk of age. The intakes of calf starter and oat hay increased from 612 to 1244 g and from 66 to 172 g, respectively. No significant effect of diet or defaunation was observed during the postweaning period.

Live BW of calves were similar in all treatment groups during the pre- and postweaning periods (Table 3).

Establishment of Ciliated Protozoa in the Rumen

Weekly examinations of ruminal fluid revealed no ciliated protozoa until 8 wk of age. First appearance of ciliate protozoa, with only a low concentration ($0.06 \times 10^4/\text{mL}$) of large entodiniomorphs and no holotrichs, was in the samples collected at 9 wk of age (Table 4) from calves that were allowed contact with adult ani-

mals. The sample collected at 10 wk of age revealed both holotrichs and entodiniomorphs. No significant increase in total ciliate population was observed in ruminal fluid of calves at 12 wk of age, which showed an increase in the number of small entodiniomorphs. Ruminal fluid from the isolated calves did not reveal any protozoa during this period.

Ruminal Fermentation Products

The ruminal pH was not significantly different in groups FM and NFM in phases 1 and 2 (Table 5). The pH declined ($P < 0.05$) to 6.00 in wk 4 and then stabilized, showing only a marginal increase from 6 to 8 wk of age. The total VFA concentration (mM/L) in ruminal fluid increased with age from 30.0 to 73.4 in FM and from 27.9 to 80.5 in NFM during 8 wk. No significant effect of fish meal in calf starter was observed. Ammonia N (mg/dL) concentration showed an initial increase from 15.0 on d 4 to 32.9 at 4 wk, and then decreased to 17.6 at 8 wk.

During the postweaning period, ruminal pH was around 6.3. The total VFA concentration (mM/L) varied with lower concentration in faunated than the defaunated calves, i.e., 68.7 in FNFM vs. 98.7 in DNFM at 9 wk, and 79.1 in FFM vs. 111.7 in DFM at 13 wk of age. No significant effect of fish meal was observed in any period. The ammonia N concentration decreased at 9 wk of age in DNFM (12.6 mg/dL) and was generally lower in defaunated than faunated groups.

Ruminal Microbial Enzymes

The concentrations of different enzymes in ruminal fluid as an index of microbial activity are presented in Tables 6 and 7. The results from the postweaning period are presented to show the effects of fish meal and protozoa and their interactions.

Prewaning period – fibrolytic enzymes. There was no cellulolytic or hemicellulolytic activity in the ruminal fluid of 4-d-old calves. The calves did not have cellulolytic activity at 1 wk of age. At 2 wk of age, the activity of CM-cellulase was observed in only 7 of 24

Table 4. Ciliated protozoal counts (mean $\pm$ SE $\times 10^4/\text{mL}$) in the rumen of calves.

Ciliated protozoa	9 wk	10 wk	12 wk
Large holotrichs	0	0.75 ± 0.12	0.64 ± 0.08
Small holotrichs	0	0.28 ± 0.07	0.23 ± 0.05
Large entodiniomorphs	0.06 ± 0.03	2.54 ± 0.26	2.54 ± 0.22
Small entodiniomorphs	1.15 ± 0.20	16.91 ± 1.16	23.06 ± 1.26
Total	1.21 ± 0.17	20.48 ± 1.22	26.47 ± 1.32

Table 5. Effects of fish meal and ciliated protozoa on ruminal pH and total VFA and ammonia N concentrations in calves.¹

Age (wk)	pH		Total VFA (mM/L)		Ammonia N, (mg/dL)							
	FM	NFM	FM	NFM	FM	NFM						
Prewaning period												
Phase 1 ²												
4 d	6.34 ^a	6.36 ^a	30.0 ^c	27.9 ^d	15.6 ^c	14.3 ^b						
1	6.27 ^a	6.18 ^{ab}	44.0 ^b	43.5 ^c	21.3 ^{bc}	22.5 ^{ab}						
2	6.16 ^{ab}	5.75 ^b	44.5 ^b	51.7 ^{bc}	23.9 ^b	27.6 ^a						
3	5.98 ^{ab}	6.00 ^{ab}	63.6 ^a	65.6 ^a	26.4 ^{ab}	28.6 ^a						
4	5.79 ^b	6.07 ^{ab}	69.8 ^a	67.8 ^a	35.0 ^a	29.7 ^a						
SEM	0.17*	0.18*	4.75*	4.30*	2.10**	2.65**						
Phase 2												
5	6.05 ^{ab}	6.07	65.6 ^{ab}	73.1 ^{ab}	26.4	25.4 ^a						
6	6.00 ^b	6.21	62.8 ^{ab}	67.5 ^{ab}	22.6	25.1 ^a						
7	6.12 ^{ab}	6.22	58.3 ^b	58.9 ^b	24.4	19.7 ^{ab}						
8	6.30 ^a	6.17	73.4 ^a	80.5 ^a	19.6	15.6 ^b						
SEM	0.09*	0.11	4.62*	6.9*	2.52	2.19**						
Postweaning period												
	pH				Total VFA (mM/L)				Ammonia N (mg/dL)			
	FFM	DFM	FNFM	DNFM	FFM	DFM	FNFM	DNFM	FFM	DFM	FNFM	DNFM
9	6.38	6.27	6.27	6.18	70.7 ^p	77.0 ^{pq}	68.7 ^p	98.7 ^q	22.0 ^q	17.6 ^{pq}	20.9 ^{pq}	12.6 ^p
11	6.36	6.27	6.35	6.19	72.9 ^p	93.6 ^{pq}	77.3 ^p	113.7 ^q	24.2	18.0	17.3	16.6
13	6.44	6.31	6.22	6.38	79.1 ^p	111.7 ^q	85.9 ^{pq}	88.8 ^{pq}	22.8	14.8	19.4	18.9
SEM	0.12	0.12	0.01	0.11	9.06	13.51	8.40	14.36	2.36	2.07	3.51	2.54

a,b,c,d Means bearing different superscripts in a column differ significantly (* $P < 0.05$; ** $P < 0.01$).

p,q Means bearing different superscripts in a row differ significantly ($P < 0.05$).

¹FM = Group fed on calf starter with fish meal; NFM = group fed on calf starter without fish meal; F = faunated; D = defaunated (ciliate-free).

²Phase 1 = high milk feeding phase; phase 2 = reduced milk feeding phase.

calves (about 30%), which increased to 52% in wk 3, and was present in all calves at 4 wk. Xylanase activity appeared at 1 wk of age in 48% of calves, which increased to 78% at 2 wk, and was present in all calves (17.9 U/dL) at wk 3. Another fibrolytic enzyme, β -glucosidase, showed sporadic activity, i.e., only one animal at 1 wk of age, 4 animals in wk 2, and 16 (67%) in wk 3. All calves showed enzyme activities from 4 wk, which increased with age. No significant effect of fish meal was observed for any of the 3 fibrolytic enzymes.

Prewaning period – amylase and β -galactosidase activities. Amylolytic activity (U/dL) was observed in all calves at 4 d of age (34.6), which then increased ($P < 0.05$) with age (Table 7). β -Galactosidase activity was also present at 1 wk. The enzyme activity (U/dL) was 1.48 and 1.65 on d 4, which increased to 2.06 and 2.83 at 6 wk, and then declined to 1.00 and 0.97 at 8 wk in FM and NFM groups, respectively. The protein source in calf starter had no significant effect on amylolytic or β -galactosidase activity.

Prewaning period – proteolytic and ureolytic enzymes. There was a significant proteolytic activity (U/dL) in ruminal fluid of calves even at d 4 (10.1), which increased to 15.0 at 1 wk, and remained similar up to 8 wk of age. Urease activity (U/dL) showed a

similar increase (8.5 on d 4 to 13.5 at 1 wk), and then reached a steady concentration. No significant difference in protease and urease activity was observed between FM and NFM groups during phases 1 and 2.

Postweaning period. Establishment of ciliated protozoa was observed after 8 wk of age and thus the effects of fish meal and ciliated protozoa on the development of fermentative activity in the rumen were studied.

Postweaning period – fibrolytic enzymes. The cellulolytic activity remained unaffected during 9 to 13 wk of age. Protein source in the calf starter did not have any significant effect on cellulolytic activity. The establishment of ciliates in calves did not contribute to any significant change in cellulolytic activity. Further, no significant protozoa $\times$ protein interaction was seen in any period. The effects of protozoa and protein on xylanase activity were also nonsignificant. The β -glucosidase activity followed a similar pattern and did not show any significant difference between the groups.

Postweaning period – amylase and β -galactosidase activities. The amylolytic activity (U/dL), showed a declining trend in the postweaning periods (85.8 at 9 wk to 56.6 at 13 wk). The mean amylase activity was higher ($P < 0.01$) in defaunated than faunated calves showing a significant treatment $\times$ period interaction at

Table 6. Effects of fish meal and ciliated protozoa ruminal fibrolytic enzymes in calves.¹

	Carboxymethyl-cellulase (U/dL) ²		Xylanase (U/dL) ²		β -glucosidase (U/dL) ³							
Age (wk)	FM	NFM	FM	NFM	FM	NFM						
Prewaning period												
Phase 1 ⁴												
4d	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a						
1	0 ^a	0 ^a	4.0 ^{ab}	5.4 ^b	0.2 ^{ab}	0 ^a						
2	3.2 ^{ab}	2.7 ^{ab}	10.0 ^b	10.9 ^{bc}	0.5 ^{ab}	0.5 ^{ab}						
3	5.0 ^{bc}	9.6 ^{bc}	18.6 ^c	17.1 ^c	1.8 ^b	1.8 ^b						
4	11.0 ^c	12.8 ^c	20.3 ^c	25.3 ^d	2.4 ^b	3.4 ^c						
SEM	1.7*	2.4*	1.8**	2.2**	0.4*	0.5*						
Phase 2												
5	14.8	13.2 ^a	25.5	33.2	5.13	4.8						
6	18.0	19.5 ^{ab}	25.9	37.7	5.68	4.0						
7	18.6	17.3 ^{ab}	26.6	29.8	5.80	6.0						
8	17.0	22.6 ^b	26.4	33.8	5.01	4.8						
SEM	2.1	2.4*	3.0	4.5	0.9	0.9						
Postweaning period												
	Carboxymethyl-cellulase (U/dL)				Xylanase (U/dL)				β -glucosidase (U/dL)			
	FFM	DFM	FNFM	DNFM	FFM	DFM	FNFM	DNFM	FFM	DFM	FNFM	DNFM
9	18.3	16.6	20.6	21.6	22.7	32.4	33.1	29.3	5.2	5.6	5.1	3.4 ^{ab}
11	18.3	18.8	19.7	16.8	29.4	32.4	24.4	36.8	5.1	4.7	3.5	2.9 ^a
13	14.1	14.3	15.2	20.8	27.2	29.5	28.0	34.7	4.2	5.6	4.7	5.2 ^b
SEM	2.9	3.3	2.7	4.7	2.9	3.3	5.5	5.3	1.0	0.8	0.8	0.6*

a,b,c,d Means bearing different superscripts in a column differ significantly (* $P < 0.05$; ** $P < 0.01$).

¹FM = Group fed on calf starter with fish meal; NFM = group fed on calf starter without fish meal; F = faunated; D = defaunated (ciliate-free).

²Unit of enzyme activity is expressed as μ M reducing sugar released per mL of bacterial extract per min.

³Unit of enzyme activity is expressed as μ M p-nitrophenol released per mL of bacterial extract per min.

⁴Phase 1 = high milk feeding phase; phase 2 = reduced milk feeding phase.

11 wk of age. No significant effect of fish meal on amylase was observed. The declining trend of β -galactosidase activity (U/dL) continued until 13 wk of age, showing a very feeble activity (0.20) compared with its peak activity (2.38) at 6 wk of age. The effects of ciliate protozoa and fish meal were not significant.

Postweaning period – proteolytic and ureolytic enzymes. The proteolytic activity did not change significantly in the postweaning period. No significant effect of protein, protozoa, or their interaction was observed at any period. The activity of urease (U/dL) ranged from 12.5 to 19.2. The effect of protein $\times$ protozoa interaction was also nonsignificant.

DISCUSSION

Establishment of Ciliated Protozoa

The establishment of ciliated protozoa relates to transfaunation from adult faunated animals through contact and the strategic incorporation of dry feed (prestarter, calf starter, hay, etc.) in the dietary regimen of preweaned calves. The acidic condition of rumen with milk/milk replacer and starchy calf starter prevents the establishment of protozoal population (Hun-

gate, 1966). In the present study, acidic conditions (pH around 6.0) prevailed in the rumen up to 6 wk, which showed an increased shift in the following periods, and the establishment of ciliated protozoa after 8 wk of age corresponded to increase in ruminal pH. Bryant et al. (1958) observed ciliated protozoa after 13 wk of age in calves with entodinia, the first to be established followed by diplomodina and holotrichs. Minato et al. (1992) also observed establishment of protozoa in calves at 8 to 10 wk of age. In most of the studies, entodinia in association with holotrichs were the most common ciliates, when the diet was rich in soluble sugars (Bonhomme, 1990). Klapacova and Klapac (1991) observed very small numbers ($\times 10$) of ciliates (1.48/mL of ruminal content) at 3 wk of age, which did not increase significantly until the age of 7 wk in calves on a milk diet. In the present study, the total number of ciliated protozoa increased considerably ($>15\times$) after initial establishment at 10 wk of age, with both holotrichs and entodiniomorphs present. With the change in feed (milk discontinued at 8 wk of age) to a more fibrous type, the pH increased and the protozoal population changed from single (entodinium) to a mixed type (Bonhomme, 1990). Moreover, the interactions between flora and fauna

Table 7. Effects of fish meal and ciliated protozoa on ruminal nonfibrolytic enzymes in calves.¹

Age (wk)	Parameters															
	Amylase (U/dL) ²				β-galactosidase (U/dL) ³				Proteases (U/dL) ⁴				Urease (U/mL) ⁵			
	FM	NFM			FM	NFM			FM	NFM			FM	NFM		
Prewaning period																
Phase 1 ⁶																
4d	36.8	31.8 ^a			0.85 ^a	1.32 ^{ab}			9.7	10.5			8.3 ^a	8.7 ^a		
1	33.9	35.8 ^a			1.32 ^{ab}	1.12 ^{ab}			13.6	16.5			12.7 ^{ab}	14.3 ^b		
2	46.3	42.7 ^{ab}			1.01 ^{ab}	0.98 ^a			14.5	16.1			10.5 ^{ab}	10.4 ^{ab}		
3	48.1	56.6 ^b			1.08 ^{ab}	1.61 ^b			12.7	17.2			12.2 ^{ab}	12.5 ^{ab}		
4	45.1	53.8 ^b			1.48 ^b	1.65 ^b			13.5	15.4			13.9 ^b	13.9 ^{ab}		
SEM	5.7	5.1*			0.15*	0.20*			2.2	2.3			1.5*	1.8*		
Phase 2																
5	76.2	74.1			2.05	1.43 ^a			12.2	13.6			11.5	18.3		
6	71.2	69.4			2.06	2.83 ^b			13.2	16.6			15.2	14.7		
7	72.5	73.8			1.93	1.52 ^a			15.7	16.9			16.8	16.6		
8	89.0	85.4			1.00	0.97 ^a			16.4	17.9			12.2	15.6		
SEM	7.5	9.7			0.33	0.25*			2.0	2.8			2.0	4.0		
Postweaning period																
	Amylase (U/dL)				β-galactosidase (U/dL)				Proteases (U/dL)				Urease (U/mL)			
	FFM	DFM	FNFM	DNFM	FFM	DFM	FNFM	DNFM	FFM	DFM	FNFM	DNFM	FFM	DFM	FNFM	DNFM
9	68.9	102.2 ^b	81.4 ^b	90.5	0.69 ^b	0.54 ^b	0.70 ^c	0.68 ^b	14.0	20.6	18.0	16.5	12.5	13.0	19.2	12.1
11	58.2 ^{pq}	90.1 ^{abq}	51.1 ^{ap}	81.5 ^{pq}	0.54 ^{ab}	0.32 ^{ab}	0.42 ^b	0.42 ^{ab}	21.3	15.0	13.0	16.2	15.9	12.2	16.5	16.1
13	54.7	58.1 ^a	54.3 ^a	59.4	0.20 ^a	0.19 ^a	0.22 ^a	0.21 ^a	16.1	23.8	22.3	24.7	17.6	15.4	17.0	17.5
SEM	4.9	9.9*	8.2	11.8	0.09*	0.07*	0.04*	0.10*	2.7	4.2	4.3	4.2	2.3	2.8	3.0	3.1

^{a,b,c}Means bearing different superscripts in a column differ significantly (* $P < 0.05$).

^{p,q}Means bearing different superscripts in a row differ significantly ($P < 0.05$).

¹FM = Group fed on calf starter with fish meal; NFM = group fed on calf starter without fish meal; F = faunated; D = defaunated (ciliate-free).

²Unit of enzyme activity is expressed as μM reducing sugar released per mL of bacterial extract per min.

³Unit of enzyme activity is expressed as μM o-nitrophenol released per mL of bacterial extract per min.

⁴Unit of enzyme activity is expressed as mg protein released per mL of bacterial extract per min.

⁵Unit of enzyme activity is expressed as μM ammonia released per mL of bacterial extract per min.

⁶Phase 1 = high milk feeding phase; phase 2 = reduced milk feeding phase.

have an important role in the development of different ciliate population.

Ruminal Fermentative Development

Fermentation products. The initial drop in pH was in response to milk and calf starter intake with no or negligible rumination. Subsequent increase and stabilization may be attributed to the consumption of calf starter and oat hay and chewing of cud resulting in increased salivation. Assane and Dardillat (1994) also observed a decrease in pH due to intake of solid feed in the first month compared with control animals on whole milk alone. With the increase in consumption of dry feed, the total VFA started to increase from the first week and reached a static level as in adult cattle at 4 wk of age. A consistent increase in total VFA in ruminal contents in response to dry feed intake was indicative of ruminal development. The high ammonia N concentration coincided with the period when calves were consuming more milk and then decreased with the increase in dry feed consumption. This result was in agreement with the studies that suggested low absorption and use of ammonia during first 3 wk of age (Godfrey, 1961) and then the concentration decreased, possibly because of increased bacterial assimilation, absorption, and use by ruminal epithelium, and the dilution effect from a larger total rumen volume (Vazquez-Anon et al., 1993). Consequent to increased dry feed consumption, a decrease in pH and concentration of ammonia in the rumen was indicative of increased VFA production, and development of absorptive papillae (Assane and Dardillat, 1994). Diet-induced changes in ruminal fermentation products were clearly seen from the second week onwards as evidenced by the increased consumption of calf starter. Acceleration in adaptation to dry feed consumption appeared to increase the ruminal activity, which was indicated by lower ruminal pH, increased total VFA, and changes in ammonia N concentration.

The postweaning change in fermentation products was more or less in response to solid feed intake, i.e., pH remained static between 6.2 to 6.4, total VFA showed an increasing trend (79 to 91 mM/L), and ammonia N varied between 13 and 24 mg/dL in different treatment groups. Bomba and Zitnan (1992) observed a similar increase in total VFA during 5 to 7 and 9 to 11 wk of age, with a level similar to that of adult animals being reached at 11 wk of age. Luchini et al. (1993) advocated preweaning drenching of calf starter to stimulate postweaning adaptation to dry diets; thus, the postweaning intake would depend more on physiological adaptation. Cozzi et al. (2002) were of the same view that solid feed promoted forestomach development. Although the

general development of the rumen is considered age-dependent, it is principally modulated by VFA produced by microbial fermentation of solid feed (Sutton et al., 1963; Singh et al., 1982). In the present study, calves were weaned at 8 wk of age and an initial boost for dry feed consumption was provided at 3 wk of age by reducing the milk intake to 8% of live weight.

Ruminal microbial enzymes. Many calves showed fibrolytic activity at 1 wk (xylanase), which became prominent at 4 wk of age. The range in values for CM cellulase activity was wide at 3 wk of age, and it narrowed with age. The main fiber-degrading enzymes (CM cellulase and β -glucosidase) were detected when calves started consuming oat hay along with an appreciable amount of calf starter at 4 wk of age. Anderson et al. (1987b) observed cellulolytic bacteria as early as 3 d of age. Amylolytic, proteolytic, and lactate-utilizing bacteria were also found, which increased progressively with age, and in calves weaned early and subjected to an increased intake of prestarter diet. They observed the most significant changes in bacterial population and metabolic activity between 4 and 6 wk of age. In the present study, presence of nonfibrolytic enzymes even at d 4 indicated their probable function at birth. These enzymes, particularly α -amylase, were reflective of an increase in dry feed intake (starch). In response to milk feeding, β -galactosidase activity increased up to 6 wk and then decreased to a nonsignificant level due to weaning at 8 wk of age.

Effect of protein source. A nonsignificant difference between FM and NFM groups with regards to ruminal fermentation products and enzymes, DM intake, and live weight gain suggest no benefit of incorporating fish meal in the prestarter diet. Sil et al. (1994) did not observe any significant change in pH, ammonia N, protease, and urease activities except for a low total VFA concentration in bull calves fed fish meal. The earlier dry feed is introduced to the rumen of calves, the earlier microbial establishment will occur, resulting in higher ruminal fermentative activity and earlier onset of functional rumen. It may thus be hypothesized that as long as milk is fed, it meets the requirement of amino acids lacking in plant protein vis-à-vis animal protein; and later, ruminal microbial protein synthesis makes up the deficit.

Effect of defaunation. Increased total VFA and decreased ammonia N concentrations in defaunated animals were indicative of a shift in microbial population in the rumen due to establishment of ciliated protozoa that occurred after 8 wk of age. Pal et al. (1998) observed no effect on pH and total VFA, but a decreased ammonia N in defaunated animals. However, the observations in the present study were similar to that of Santra and Karim (2002).

A positive shift in α -amylase, xylanase, and urease activities in defaunated animals may be seen in terms of an adaptive increase in bacterial population due to absence of protozoa. Kurihara et al. (1978) found twice the digestion of cellulose with predominant cellulolytic population in faunated rumen compared with major amylolytic population in defaunated animals. Mendoza et al. (1993) also observed increased amylolytic activity by defaunation. Pal et al. (1998) observed higher amylase but lower urease activity with a nonsignificant effect on cellulase and xylanase in defaunated calves. Many reports have suggested protozoan engulfment of feed particles and bacteria, as the cause of decreased degradation of starch and an increased recycling of N (Bird, 1989; Williams and Withers, 1993; Jouany, 1996; Santra and Karim, 2002). This mechanism helps in stabilizing pH, on a high grain diet. Eugene et al. (2004) opined that protozoa prevent the abrupt drop of pH in the rumen showing an interaction with the level of concentrate in the diet. However, there is decreased N use due to continuous recycling of bacterial and feed N and autolysis of heavier protozoa in the rumen. Hsu et al. (1991) observed increased ruminal microbial protease activity and an inconsistent effect on deaminase activity. Fejes and Varady (1996) studied the level of rumen faunation to affect inversely the proportion of bacterial N. Quantitative meta-analysis on the effects of defaunation refers to a lower ammonia N and higher duodenal microbial N flow (Eugene et al., 2004). A lower ammonia N in ciliate-free animals may be attributed to higher microbial synthesis and less bacterial recycling (Koenig et al., 2000). Bird (1989) was of the opinion that the development of a normal microbial population was slower in defaunated (by isolation) compared with faunated animals that were inoculated with a complete package of microorganisms (bacteria, protozoa, and fungi) through rumen inoculum or through mouth-to-mouth contact as practiced in the present experiment. However, the enzyme activities, representing functional microbial population, indicated that both faunated and defaunated calves had similar age- and substrate-dependent changes.

CONCLUSION

The fermentative changes that take place in the rumen are evidence of the gradual development of the rumen, which, may be further stimulated by the inclusion of good calf starter and hay. Inclusion of a fish meal-based calf starter in the diet of preruminant calves seemed to have no advantage over plant (e.g., peanut cake) protein in influencing dry feed consumption or ruminal fermentative development. The presence of various enzyme activities in the rumen at an

early age may be seen as representative of functional microbial communities, and stimulation through dry feed consumption seems to be a useful strategy.

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