Rumen Metabolic Development in Crossbred Calves Reared on Animal Protein Free Pre-Starter and Oat Hay

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ABSTRACT : Twenty-four new born crossbred (*Bos indicus×Bos taurus*) calves were distributed in two equal groups and assigned to two different pre-starter diets with (Group 1) and without (Group 2) fish meal to study the effect of replacement of animal protein by vegetable protein in the diet and the age of animals on ruminal metabolic development. All calves were fed colostrum for 24 h and whole milk until weaning at 8 weeks of age. Rumen fluid samples were collected on 4 d, 1 wk, and then weekly interval up to 8 wk of age. Rumen fluid samples were analysed for pH, TVFA, lactic acid and N fractions (total N, total soluble N, trichloro acetic acid (TCA) soluble N, TCA precipitable N and ammonia N). Weekly feed intake and live weight gain pattern showed an increasing trend with the advancement of age, but were similar in both groups. The pH fell steadily during 0-4 wk of age and then stabilized in later period. A close relationship (r=0.80) between starter intake and TVFA concentration was observed in both the groups. Lactic acid (meq/l) and ammonia N (mg/dl) concentration showed initial rise (0.55 and 14.97 on day 4 to 3.38 (7 wk) and 32.85 (4 wk), respectively) to fall (2.74 and 17.60) again during 8 wk of age in response to increase in dry feed consumption (10% initially to 83% of diet dry matter at 8 wk of age). The TCA precipitable fraction of N did not show any change during 0-8 wk of age. Data indicate that the metabolic changes responded rapidly to dry feed intake which did not differ in fish meal and non-fish meal groups, and a poor voluntary consumption of oat hay retards the progressive changes in live weight and rumen microbial development. *(Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 2 : 193-199)*

Key Words : Rumen, Calves, Metabolic Development, Animal Protein

INTRODUCTION

The distinctive and the important role that the ruminants play in the economy of world has been attributed to their peculiar digestive system i.e., the fermentative digestion of fibrous crop residues in the rumino-reticulum. Therefore, dietary adjustments directed towards early development of rumen function may help the animal for early tolerance of fibrous components in their ration. In India, most of the organized farms or farmers have been adopting the practice of weaning calves at or above three months of age and also to include a source of animal protein in the starter diet. But, it has been emphasized to adopt dry feed consumption in calves so as to wean at an early age (Anderson et al., 1987a; Quigley III et al., 1991). This practice is also aimed at sparing the maximum quantity of milk for human consumption. The calves with early developed microbial ecosystem may thus be exposed easily to low cost feeding strategy based on fibrous crop residues. In addition to that the replacement of costly animal protein from the pre-starter diet of young ruminants may have added advantage in reducing the total cost of rearing calves. Fish meal containing diet produced lower total volatile fatty acids (TVFA) and total N concentration in the rumen liquor of growing calves compared to that with vegetable protein (Zerbini and Polan, 1985; Sil et al., 1994). Quigley III et al. (1985) observed no effect on the pattern of essential amino acids in bacterial cell due to age, weaning or ration composition. Warner (1984) emphasized on the total intake of pre-starter diet to its specific protein characteristics. According to Lyford and Huber (1988) the earlier the rumen function is established the calves will become dependent on the products of rumen fermentation and it may be hypothesized that so long as milk is being fed the deficient amino acids in vegetable protein compared to animal protein are supposed to be supplied from milk and later from the ruminal synthesis of microbial protein. The present investigation was therefore, aimed at assessing the rumen metabolic development in pre-ruminant calves on the feeding of fish meal and non-fish meal protein based calf starter and oat hay.

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MATERIALS AND METHODS

Animals and feeding

Twenty-four newly born crossbred calves (*Bos indicus×Bos taurus*) (average body weight, 22.5±0.7 kg) were separated from their dams following 24 h of colostrum feeding and then reared in a shed having individual feeding arrangements. The calves were distributed in two groups (Group 1 and 2) of 12 calves each on staggered basis as and when they were born on the basis of their body weight. Whole milk was fed to all calves in two equally divided doses at

09.00 A.M. and 17.00 P.M. daily after it was warmed (90℃) and cooled to luke warm before feeding the calves. Group 1 was fed with calf starter containing fishmeal (CS 1) and Group 2 with non-fishmeal calf starter (CS 2). The detailed feeding schedule and the composition of calf starter are given in table 1. Oat hay was chaffed (1-2 cm) and offered *ad libitum* to calves. Calf starter and oat hay were offered separately once in a day. Clean drinking water was provided *ad libitum* to all calves.

Strict hygienic and sanitary conditions were maintained in the experimental sheds with the provision of clean drinking water. Proper and timely health care was provided to sick animals. The calves were dewormed periodically as per standard schedule.

Adoption to dry feed consumption and daily intake of dry matter (DM) was recorded from the DM offered and residue left throughout the experimental period. Live weight of calves were recorded at weekly interval before the offer of milk, feed or water.

Table 1. Feeding schedule of calves during preweaning period

Age	Milk (% BW)	Calf starter*+Oat hay
1 d-4 d	10	Nil
5 d-3 wk	10	*Ad libitium*
3-4 wk	8	*Ad libitium*
4-6 wk	6	*Ad libitium*
6-8 wk	3	*Ad libitium*

* Calf starter 1: Maize 50, wheat bran 12, deoiled groundnut cake 27, fish meal 8, mineral mixture 2, salt 1 and vitablend (Vit. A, D_3) 0.01.
Calf starter 2: Fish meal was replaced by deoiled groundnut cake.

Chemical analysis of feeds and fodder

Periodic samples of calf starter, oat hay and residue left were collected, dried for estimation of dry matter (DM) and pooled over the period for the estimation of proximate principles (AOAC, 1980) and fiber fractions (Van Soest et al., 1991). The milk sample was analyzed immediately for total fat and other constituents (AOAC, 1980).

Collection, preservation and analysis of rumen fluid

During the course of experiment about 50 ml of rumen fluid samples were collected with the help of a stomach tube 3 h post feeding from 8 representative animals in each group on 4th day and then weekly up to 8th week of age. Samples were collected in stoppered conical flask and brought to the laboratory for immediate recording of pH. About 30 ml of rumen fluid was strained through muslin cloth, acidified with 2-3 drops of 10 N sulphuric acid and preserved in capped vials in deep freezing for further chemical analysis. The rumen liquor was analysed for total volatile fatty acids (TVFA) (Barnett and Reid, 1956), ammonia nitrogen (NH_3-N), total N (TN), total soluble N (TS-N), TCA soluble N (TCAS-N), TCA precipitable N (TCAP-N) as per AOAC (1980) and lactic acid (Barker and Summerson, 1941).

Statistical analysis

The difference between the treatments was analyzed by student 't'- test, the periodic change, by one way analysis of variance and the effect of age on biochemical parameters and the interaction of age on treatments were analyzed following split-plot design (Snedecor and Cochran, 1989) using the model:

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

Where, Y_{ijk} = Dependent variables
μ = Overall mean
T_i = Effect of treatment I
$C_{(i)j}$ = 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 × period interaction
$e_{(ijk)}$ = Residual

RESULTS AND DISCUSSION

Chemical composition of the diet

The chemical composition of milk, calf starter and oat hay is presented in table 2. The CP, CF and ADF content of oat hay were 4.65, 34.25 and 43.00 percent, respectively and thus can be grouped under mature type (Ranjhan, 1991). An average DM intake of about 60 g oat hay per head per day during 7 and 8 weeks period was indicative of its poor quality and palatability. The nitrogen (N) content of milk, CS 1 and CS 2 was 5.96, 35.20 and 34.48 g kg^{-1}, respectively, which principally contribute to total N availability in calves.

Pattern of feed (DM) intake and live weight change in animals

Intake of milk, calf starter (CS) and oat hay and

Table 2. Chemical composition of milk, calf starter and oat hay

Constituents (%)	Milk	Calf starter 1	Calf starter 2	Oat hay
OM	12.20	87.50	90.30	88.70
CP	3.80	22.00	21.55	4.65
EE	4.95	3.40	2.95	3.25
T-CHO	3.45	62.10	65.80	80.80
CF	-	9.30	12.40	34.25
ADF	-	1040	11080	43.00
NDF	-	52.90	54.40	73.50

live weight gain at different weeks of age are presented in table 3. Whole milk, CS and oat hay constituted the total diet in calves up to 8 weeks of age. The whole milk was the principal component of the diet during first two weeks of life. Thereafter, the calves were adopted to dry feed, particularly the calf starter. Refusal to calf starter intake was not seen in any of the calves even during first week of age. In contrast, very few calves (five, about 20%) consumed oat hay in the second week and an appreciable intake was seen only after 5 weeks of age in both groups, but the intake was quite low and averaged less than 5 percent of total DM intake. There was no effect of inclusion of fish meal in the concentrate mixture on the feed intake by the animals. Total DM intake and the proportion of dry feed (calf starter+oat hay) increased ($p<0.01$) with the advancement of age concomitant with the decreased rate of whole milk consumption. The average DM intake during 0 to 8 weeks period was nearly 600 g with the calf starter constituting >50% of the total intake. Similar level of DMI was also observed by earlier workers (Ranjhan et al., 1972; Das et al., 1985; Abe et al., 1987). Gupta et al. (1992) observed depression in DMI with deoiled mustard cake, but not with deoiled groundnut cake compared to fish meal containing calf starter in crossbred calves during birth to 3 months of age.

Calves of both the groups showed a similar trend in live weight gain. The poor gains could be attributed to low DMI during 4 to 8 wk period, which was probably due to decreased voluntary feed intake from matured type oat hay. Other workers also observed no adverse effect on live weight gain due to replacement of fish meal with groundnut cake, but they observed higher gain in calves on green fodder based ration (Gupta et al., 1992; Sahoo and Pathak, 1996).

Rumen biochemical parameters

From the observations of considerable increase in dry feed consumption by calves after five weeks of age (table 1), the rumen biochemical parameters are presented in two phases, phase 1 from 4 d to 4 wk and phase 2, from 5 to 8 wk of age, in table 4 and 5, respectively.

pH: The effect of feeding CS 1 or CS 2 on ruminal changes in pH was not seen in both phases of life of pre-ruminant calves. However, a diet induced change was clearly seen during second week onwards as evidenced from their increased consumption of calf starter. The pH declined ($p<0.05$) to reach at 6.00 in 4 week and then stabilized showing a marginal increase during 6 to 8 weeks of age. This may be attributed to the consumption of oat hay and chewing of cud resulting in increased salivation, which stabilized the pH of rumen even at increased consumption of dry feed in the following weeks. Assane and Dardillat (1994) also observed a decrease in pH due to intake of solid feed in the first month of life compared to control animals on whole milk alone. Gradual withdrawal of milk from the calf ration at increased substitution level with dry feed probably resulted in the shift in pH of rumen, which was not uncommon in preweaned calves (Anderson et al., 1987a).

Total volatile fatty acids (TVFA): The TVFA concentration (meq/l) in the rumen liquor increased with age from 30.0 to 73.4 in CS 1 and 27.9 to 80.5 in CS 2 during 8 weeks time period. No significant effect of protein sources in calf starter was observed. With the increase in consumption of dry feed, the

Table 3. Pattern of mean dry matter intake (g/d) and live weight gain of pre-weaned calves

Age (wk)	DM intake (g/d)				Live weight gain (kg/wk)	
	Group 1		Group 2		Group 1	Group 2
	Total DMI	Dry feed (% DM)	Total DMI	Dry feed (% DM)		
1	337^{d}	10.7^{f}	364^{d}	9.9^{e}	0.3^{c}	0.7^{bc}
2	467^{cd}	26.6^{e}	471^{cd}	25.1^{d}	0.9^{bc}	0.4^{c}
3	542^{bc}	36.0^{d}	616^{bc}	40.7^{c}	1.0^{bc}	1.1^{abc}
4	554^{bc}	44.8^{c}	576^{bc}	47.7^{c}	0.8^{bc}	0.7^{bc}
5	555^{bc}	55.9^{b}	605^{bc}	60.2^{b}	1.3^{ab}	1.8^{a}
6	671^{b}	64.2^{b}	654^{ab}	66.4^{b}	1.2^{ab}	1.4^{ab}
7	696^{ab}	80.7^{a}	697^{ab}	77.3^{a}	1.9^{a}	1.3^{ab}
8	848^{a}	82.7^{a}	791^{a}	82.8^{a}	1.2^{ab}	1.3^{ab}
Pooled mean	585	55.6	597	56.1	1.08	1.10
SEM	44	2.55	49	2.60	0.27	0.34
Significance	**	**	**	**	*	*

Means bearing different superscripts in a column differ significantly.
NS, Non-significant; * $p<0.05$; ** $p<0.01$.

Table 4. Changes in rumen biochemical parameters at different stages of growth in preweaned calves (phase 1)

Group	Age					SEM	Significance
	4 d	1 wk	2 wk	3 wk	4 wk		
PH							
1	6.34^{a}	6.27^{a}	6.16^{ab}	5.98^{ab}	5.79^{b}	0.17	*
2	6.36^{a}	6.18^{ab}	5.75^{a}	6.00^{ab}	6.07^{ab}	0.18	*
Total volatile fatty acids (meq/l)							
1	30.0^{c}	44.0^{b}	44.5^{b}	63.6^{a}	69.8^{a}	4.75	*
2	27.9^{d}	43.5^{c}	51.7^{bc}	65.6^{a}	67.8^{a}	4.30	*
Lactic acid (meq/l)							
1	0.64^{b}	0.76^{b}	1.40^{ab}	1.91^{a}	1.69^{a}	0.31	*
2	0.45^{b}	1.48^{a}	1.08^{ab}	2.05^{a}	1.78^{a}	0.38	*
Total N (mg/dl)							
1	170.4	192.3	164.2	197.4	178.7	15.08	NS
2	175.2	187.0	171.8	178.1	170.3	12.34	NS
Total soluble N (mg/dl)							
1	96.2	102.9	94.2	103.7	99.1	7.16	NS
2	91.8	98.3	92.9	102.5	99.5	7.14	NS
TCA soluble N (mg/dl)							
1	49.6	54.0	53.0	54.2	55.7	3.42	NS
2	56.8	63.2	56.4	60.4	62.9	4.65	NS
TCA precipitable N (mg/dl)							
1	56.6	48.9	41.2	47.7	40.7	4.43	NS
2	37.0	35.1	36.5	42.2	36.6	4.24	NS
Ammonia N (mg/dl)							
1	15.62^{c}	21.30^{bc}	23.92^{b}	26.37^{ab}	34.96^{a}	2.10	**
2	14.31^{b}	22.47^{ab}	27.64^{a}	28.60^{a}	29.73^{a}	2.65	**

N, Nitrogen; TCA, Trichloro acetic acid.
Means bearing different superscripts in a row differ significantly.
NS: Non-significant ; * $p<0.05$; ** $p<0.01$.

TVFA started to rise right from first week and was at its peak at the age of 4th week in phase 1, which remained static until 8th week. According to Godfrey (1961) the greatest rate of development of the reticulo-rumen occurred between 2 and 6 weeks of age. The production of VFA from ligno-cellulosic feeds is an indication of the development of rumen bacteria, protozoa and fungi. Therefore with increasing proportion of oat hay in the diet, there was a consistent increase in the level of TVFA in the rumen contents. A close relationship (r=0.80) between calf starter intake and TVFA concentration was also observed. Similar changes in TVFA concentration have also been reported by earlier workers (Singh et al., 1970; Anderson et al., 1987a; Quigley et al., 1991; Vanquez-Anon et al., 1993) in response to dry feed intake.

Lactic acid: The concentration of lactic acid in rumen liquor increased significantly ($p<0.05$) in 1st and 2nd week in both the groups, which might be attributed to increased intake of calf starter containing about 50% cereal grain. The increased level of starch in the diet stimulated the lactic acid producing bacteria as also reported by Eadie et al. (1959) that a much higher number of lactobacilli get stimulated in calves older than 4 wk. This is probably due to the feeding

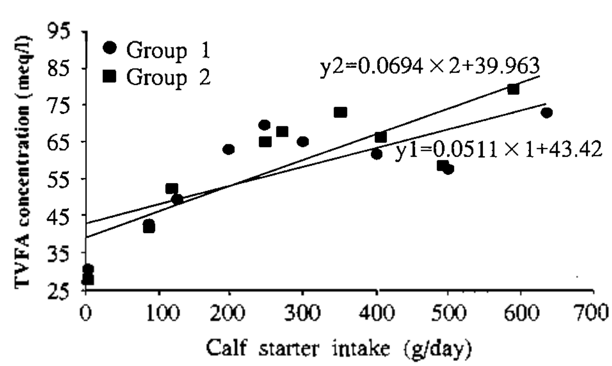

Figure 1. Regression line showing relationship between calf starter intake and TVFA concentration in rumen liquor of calves

Table 5. Changes in rumen biochemical parameters at different stages of growth in preweaned calves (phase 2)

Group	Age				SEM	Significance
	5 wk	6 wk	7 wk	8 wk		
PH						
1	6.05^{ab}	6.00^{b}	6.12^{ab}	6.30^{a}	0.09	*
2	6.07	6.21	6.22	6.17	0.11	NS
Total volatile fatty acids (meq/l)						
1	65.6^{ab}	62.8^{ab}	58.3^{b}	73.4^{a}	4.62	*
2	73.1^{ab}	67.5^{ab}	58.9^{b}	80.5^{a}	6.9	*
Lactic acid (meq/l)						
1	2.07^{b}	2.24^{ab}	4.06^{a}	3.14^{ab}	0.63	*
2	1.14	2.15	2.69	2.33	0.68	NS
Total N (mg/dl)						
1	177.8	173.7	156.1	143.3	12.05	NS
2	174.2	169.2	156.3	153.9	11.34	NS
Total soluble N (mg/dl)						
1	97.9	94.8	90.3	84.2	6.85	NS
2	101.5^{a}	93.5^{ab}	83.4^{b}	81.6^{b}	5.81	*
TCA soluble N (mg/dl)						
1	51.1	54.2	54.9	52.5	3.89	NS
2	59.7	62.6	53.9	53.7	4.06	NS
TCA precipitable N (mg/dl)						
1	42.3	38.1	35.4	31.8	3.74	NS
2	39.3	30.6	29.5	27.9	3.96	NS
Ammonia N (mg/dl)						
1	26.4	22.6	24.4	19.6	2.52	NS
2	25.4^{a}	25.1^{a}	19.7^{ab}	15.6^{b}	2.19	**

N, Nitrogen; TCA, Trichloro acetic acid.
Means bearing different superscripts in a row differ significantly.
NS: Non-significant ; * $p<0.05$; ** $p<0.01$.

Table 6. Nitrogen fractions in the rumen liquor of pre-weaned calves

Week	IN (g/d)		RL-N (% IN)		NH_3-N (% RL-N)		TCAP-N (% RL-N)	
Group	1	2	1	2	1	2	1	2
1	3.06^{e}	3.22^{e}	5.57^{a}	5.44^{a}	11.08^{d}	12.02^{de}	25.43	18.76^{bc}
2	6.34^{de}	6.2^{de}	2.81^{b}	2.89^{b}	14.57^{c}	16.09^{ab}	25.09	21.25^{abc}
3	8.91^{cd}	10.74^{cd}	2.03^{c}	1.63^{c}	13.36^{bcd}	16.06^{ab}	24.15	23.69^{a}
4	10.48^{cd}	11.09^{cd}	1.79^{cd}	1.57^{c}	19.56^{a}	17.46^{a}	22.76	21.49^{abc}
5	11.81^{c}	13.62^{bc}	1.51^{d}	1.26^{d}	14.83^{ab}	14.55^{bcd}	23.78	22.53^{ab}
6	15.73^{b}	15.45^{abc}	1.12^{e}	1.11^{de}	13.02^{cd}	14.86^{abc}	21.90	18.11^{c}
7	18.72^{b}	17.26^{ab}	0.88^{ef}	0.94^{ef}	15.61^{b}	12.61^{cde}	22.67	18.89^{bc}
8	23.74^{a}	19.91^{a}	0.63^{f}	0.78^{f}	13.67^{cd}	10.11^{e}	22.18	18.16^{c}
SEM	1.73	1.92	0.0093	0.091	0.94	1.00	1.89	1.51
Significance	**	**	*	*	*	*	NS	*

Means bearing different superscripts in a column differ significantly.
NS, Non-significant; * $p<0.05$; ** $p<0.01$.
IN, Intake N; RL-N, Rumen liquor N; NH_3-N, Ammonia N, TCAP-N, Trichloro acetic acid precipitable N.

of high concentrate diet and the availability of fermentable substrate reflected in high ruminal lactate concentration (Anderson et al., 1987a).

N-fractions: The N-fractions include total N, ammonia N, total soluble N, TCA soluble N and TCA precipitable N, which may undergo interconversion from one form to the other in the process of rumen development. The concentration of total N in the rumen liquor remained more or less constant with the advancement of age during first 8 weeks of life. Therefore, a decreasing trend in rumen liquor N (RL-N) as percent of intake N (table 6) indicated an increase in absorption and utilization of feed N in the rumen. The level of ammonia N increased up to 4 wk of age and then declined during 5 to 8 wk of age. Similar trend was also reflected when expressed as percentage of RL-N. The decrease in ammonia N concentration is indicative of increased bacterial utilization (Bryant and Robinson, 1963) and ruminal absorption (Lewis, 1955). 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 other studies that suggested low ammonia absorption and its utilisation during first 3 weeks of age (Godfrey, 1961; Leibholz, 1975) and then the concentration decreased, possibly because of dilution effect from a larger total rumen volume (Vanquez-Anon et al., 1993). Consequent with increased dry feed consumption, a decrease in pH and concentration of ammonia in the rumen was indicative of increased VFA production, increase in forestomach weight and development of absorptive papillae (Assane and Dardillat, 1994).

Total soluble N, which represents the soluble protein N, non-protein N and the microbial N remained almost constant (90 mg/dl) during both phase 1 and 2. According to Anderson et al. (1987b), bacterial population appears to be adequately present very early in the rumen of calves, and subsequent development is stimulated by increased dry feed consumption. Puri et al. (1983) also observed the influence of age and diet on increased microbial protein concentration in buffalo calves. According to Minato et al. (1992), the rumen is rapidly colonized by microbes immediately after birth and then the constitution changes with age and diet without any significant effect on total population. In the present study, a non-significant change in TCAP-N concentration with the advancement of age was indicative of poor dry feed consumption, which probably failed to provide adequate metabolites for increased bacterial population.

Acceleration in adoption to dry feed consumption appeared to increase their ruminal activity, which was indicated by lower ruminal pH, increased TVFA and lactate concentration and changes in ruminal N concentration. The metabolic and physical changes that take place in the rumen are evidence of the gradual development of the rumen which, however, may further be increased with the inclusion of good starter hay. Inclusion of animal protein (fish meal) based calf starter in the diet of pre-ruminant calves seemed to be of no advantage over vegetable protein (groundnut cake) based calf starter in influencing the dry feed consumption or rumen metabolic development.

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