

Effect of supplementation of indigenously prepared rumen protected fat on rumen fermentation in buffaloes

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ABSTRACT

Rumen protected fat (PF) i.e. calcium salts of long chain fatty acids (Ca-LCFA) was prepared by an indigenous method by treating rice bran fatty acid oil (RBO), a byproduct of the rice bran oil refinery industry and technical grade calcium hydroxide under specific conditions and evaluated for its supplemental effect on the rumen metabolism (fermentation pattern). In a 3×3 switch over design, 3 rumen fistulated adult male buffaloes (421.4±10.4 kg BW) were offered randomly a conventional concentrate mixture (control) or concentrate mixture (CM) supplemented with 300 g RBO (RBO₃₀₀) or 300 g PF (PF₃₀₀) along with 6.5 kg wheat straw (WS) and 10 kg green berseem daily (roughage: concentrate ratio 65:35). Rumen liquor was collected at 0, 2, 4, 6 and 8 h post-feeding for 3 consecutive days, pooled and analyzed for rumen metabolites. The mean rumen pH in PF₃₀₀ (6.77) was lower than the RBO₃₀₀ group (6.93), but was similar to the control (6.90). The TVFA concentration (meq/dl) was the highest in PF₃₀₀ (8.08) and the lowest in RBO₃₀₀ (6.44). There was no difference in the TN (78.82 to 83.21; mg/dl), NPN (44.99 to 49.47; mg/dl), NH₃-N (9.80 to 11.34; mg/dl), total bacterial count (5.10 to 6.90; ×10¹¹/ml) and total protozoal count (2.12 to 2.31; ×10⁴/ml) among the groups. However, the TCA-N (mg/dl) in PF₃₀₀ (38.22) was higher than the control (32.57) and RBO₃₀₀ (31.03) groups. Thus, supplementation of indigenously prepared PF (Ca-LCFA) up to 300 g/day had no adverse effect on the rumen metabolism in adult buffaloes fed WS based diets with roughage: concentrate ratio of 65:35.

Key words: Buffaloes, Protected fat, Rumen fermentation

Energy intake of minerals fed particularly on straw based diets can be enhanced by making the diets energy dense by incorporating either concentrate (cereal grains) or fat. Addition of concentrates at higher level decreases fibre intake and leads to acidosis (Palmquist and Jenkins 1980). Based on rumen metabolism, supplemental fat can be either rumen active fat or rumen protected fat (PF). Rumen active fat is extensively hydrolyzed and has the potential to interfere with the microbial fermentation in rumen, while PF are resistant to hydrolysis by rumen microbes (Jenkins 1993). Among various forms of PF, calcium salts of long chain fatty acids (Ca-LCFA) are relatively less degradable in rumen (Elmeddah *et al.* 1991), have high intestinal digestibility and are also sources of Ca. Therefore, dietary supplementation of Ca-LCFA as PF can be used to enhance the energy density of the ruminant ration without adversely affecting the dry

matter intake and digestibility (Naik *et al.* 2009a). In developing countries, although PF are available commercially, they are often out of reach of dairy farmers because of high cost. Rice bran fatty acid oil, a byproduct of the rice bran oil refinery industry can be used in preparation of PF (Ca-LCFA) by a simple economical indigenous method (Naik *et al.* 2007a, b).

The effect of dietary supplementation of PF on rumen metabolism is dependant upon the level of inclusion, roughage: concentrate ratio and type of roughage. The mixture of cereal grains and forages usually contain about 3% fat and the total dietary fat should not exceed 6–7% of the DM intake (NRC 2001). Thus, 3–4% fat in dietary DM can be in the form of supplemental fat. The majority of the studies conducted on use of PF have used concentrate and green fodder based diets. However in developing countries, where fodder availability is limited, farmers use mostly low grade crop residues like wheat straw (WS) as the main roughage. This study was conducted to evaluate the effect of the dietary supplementation of an indigenously prepared Ca-LCFA as PF on rumen fermentation in adult buffaloes fed WS based diets.

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MATERIALS AND METHODS

Preparation of rumen protected fat

Rumen protected fat (PF), i.e. calcium salts of long chain fatty acids (Ca-LCFA) was prepared (Naik *et al.* 2007a, b). Rice bran fatty acid oil (RBO), a byproduct of the rice bran oil refinery industry and technical grade calcium hydroxide were procured from a local market and stored in air tight containers. Concentrate H_2SO_4 (120 ml) in 500 ml tap water was mixed in 4 kg hot RBO. After a few minutes, when effervescence had almost subsided, 1.6 kg of technical grade calcium hydroxide dissolved in 10 liters of water was added and boiled for 30 min without cover on medium heat. When the PF became granular and non-sticky, it was filtered through cloth with repeated washings under running tap water and then sun dried. The PF was kept in air-tight containers in a cool place after mixing with butylated hydroxy toluene @ 0.5 g/kg as an antioxidant (Naik *et al.* 2009a, b).

Experimental design and collection of samples

In a 3×3 switch over design with a feeding interval of 1 month, 3 rumen fistulated adult male buffaloes (421.4±10.4 kg BW) were randomly offered (Kearl 1982) a conventional concentrate mixture (CM) as control or CM supplemented with 300 g RBO (RBO₃₀₀) or 300 g PF (PF₃₀₀) along with 6.5 kg WS and 10 kg green berseem (roughage : concentrate ratio 65 : 35). The control CM contained maize grain 35, rice bran 20, deoiled rice bran 12, mustard cake 20, soybean meal 10, mineral mixture 2 and salt 1 part by weight. The feed was offered once daily immediately after the first collection of rumen liquor (RL) at 9:00 AM, while water was offered twice daily i.e. immediate after the first and last collection of the RL. The RL was collected in a thermos flask from the fistulated animal at 0, 2, 4, 6 and 8 h postfeeding for three consecutive days from various sites of the rumen through a specifically designed probe. The strained rumen liquor (SRL) was obtained by filtering the RL through four layers of muslin cloth.

Analytical procedures and statistical analysis

The samples of feeds and fodder were analyzed for proximate principles (AOAC 2000), cell wall constituents (Goering and Van Soest 1970) and Ca (Talapatra *et al.* 1940). The TCHO was calculated by subtracting the sum of CP and EE from the OM. The pH was measured using an electronic digital pH meter. For the determination of TVFA, 2 drops of saturated solution of mercuric chloride and for nitrogen fractions (TN, TCA-N and NH_3 -N), 2 drops of 1N H_2SO_4 was added in each vial containing 30 ml SRL. All the collected samples were preserved in deep freezer (−20°C) till analyzed. The TN was estimated by the copper catalyst Kjeldahl method (AOAC 2000). The TVFA (Bennett and Reid 1957) and TCA-N (Cline *et al.* 1958) content of the SRL

were measured. The NPN was calculated as the difference between the values of TN and TCA-N. Conway (1957) method was employed for the determination of NH_3 -N. The total bacterial count (TBC) and protozoa count (TPC) were determined at 0 h and 4 h only (Kamra and Agarwal 2003). Data were analyzed for test of significance among different groups (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

The CP content of all the concentrate mixtures (20.56 to 20.78%) met the BIS specification for cattle feed (Table 1). The EE% of the control CM was lower (6.17) than RBO₃₀₀ and PF₃₀₀ (12.10–12.20) CM due to RBO and PF supplementation. The TCHO content of RBO₃₀₀ and PF₃₀₀ CM was lower than the control CM as RBO and PF were supplemented without replacing any specific ingredient. The Ca content of PF₃₀₀ CM was higher than the control CM and RBO₃₀₀ CM, as the PF was in the form of Ca-LCFA (Naik *et al.* 2007b). The chemical composition of berseem fodder and WS were within normal range.

The mean values of various parameters of rumen metabolites are provided in Table 2 and fermentation pattern are depicted in Figs 1–8. The rumen pH values were within normal range and lowest pH values were observed at 4 h post-feeding in all groups. The mean pH value in PF₃₀₀ group

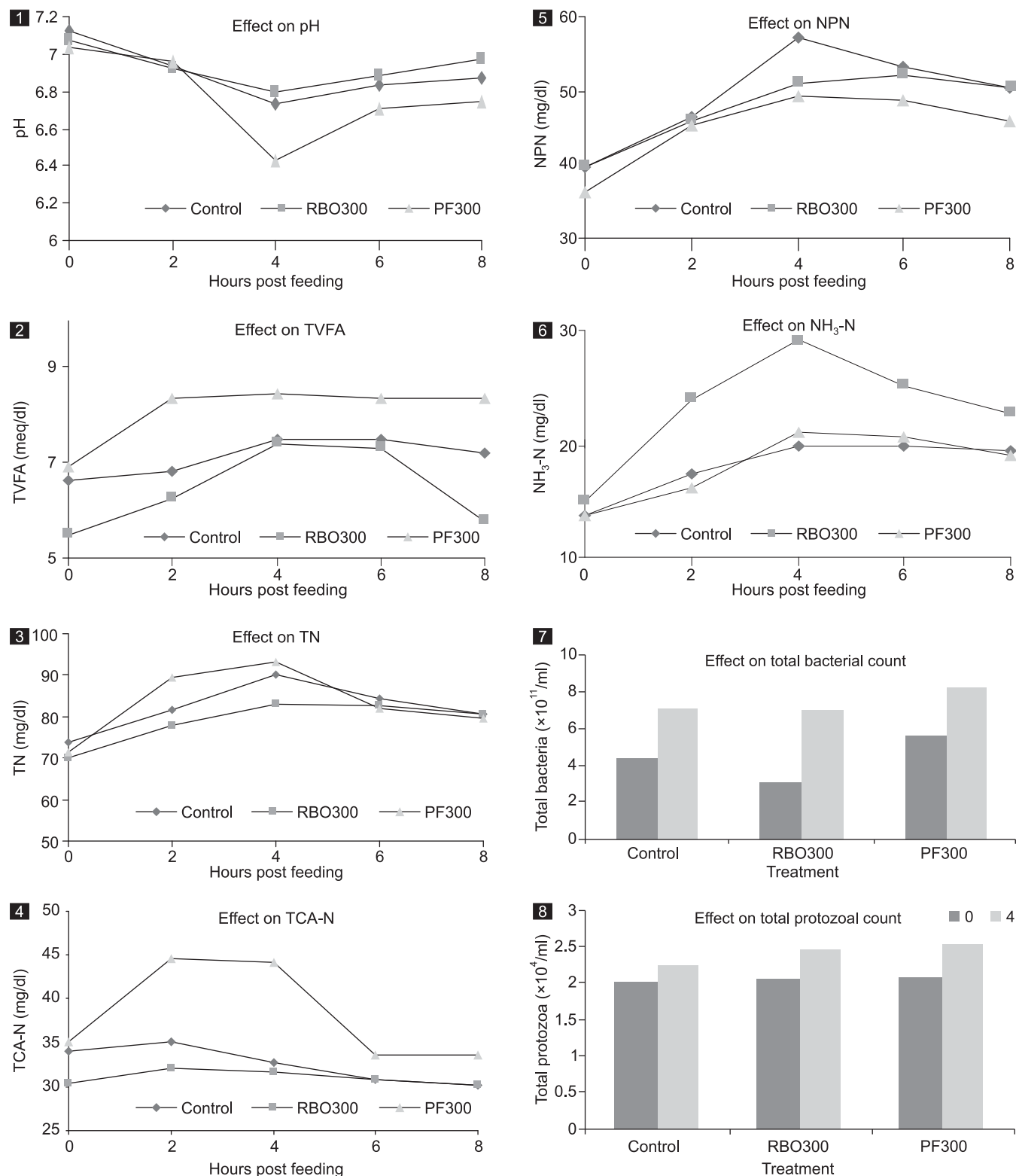
Table 1. Chemical composition (% DM basis) of the feeds

Parameters	Concentrate mixture			Green fodder	Wheat straw
	Control	RBO ₃₀₀	PF ₃₀₀		
TA	07.18	06.66	07.18	12.78	08.39
OM	92.82	93.34	92.82	87.22	91.61
CP	20.78	20.56	20.56	15.61	03.21
EE	06.17	12.10	12.20	03.36	01.35
TCHO	65.87	60.68	60.06	68.25	87.05
NDF	37.80	35.30	34.90	48.22	71.90
ADF	16.60	17.50	16.30	30.60	51.13
Hemicellulose	21.20	17.8	18.60	17.62	20.77
Cellulose	13.40	14.4	12.50	24.33	40.18
Ca	0.80	0.75	1.40	1.30	0.25

Table 2. Effect of supplemental fat on the rumen metabolites and microbial count

Parameters	Control	RBO ₃₀₀	PF ₃₀₀	SEM
pH	06.90 ^{ab}	06.93 ^b	06.77 ^a	0.03
TVFA (meq/dl)	07.13 ^b	06.44 ^a	08.08 ^c	0.15
TN (mg/dl)	82.04	78.82	83.21	0.98
TCA-N (mg/dl)	32.57 ^a	31.03 ^a	38.22 ^b	0.82
NPN (mg/dl)	49.47	47.79	44.99	0.93
NH_3 -N (mg/dl)	10.03	11.34	09.80	0.37
TBC (× 10 ¹¹ /ml)	5.80	5.10	6.90	0.50
TPC (× 10 ⁴ /ml)	2.12	2.26	2.31	0.08

Means within a row bearing different superscripts differ significantly (P<0.05).



Figs 1–8. Effect of rumen protected fat feeding on: 1. pH; 2. TVFA; 3. TN; 4. TCA-N; 5. NPN; 6. NH₃-N; 7. total bacterial count; 8. total protozoal count.

was lower ($P < 0.05$) than the RBO₃₀₀ group, but was similar to control. In general, pH is inversely proportional to TVFA concentration in RL (Phillipson 1982). The concentration of

TVFA increased ($P < 0.05$) 2 h post feeding showing peak at 4 h postfeeding and reduced to the level similar to that of 2 h by 6–8 h postfeeding (Fig. 2). This may be attributed to the

succession of event that takes place with regard to carbohydrate fermentation in rumen (Prins and Clarke 1979). There was difference ($P < 0.05$) in the mean TVFA concentration (meq/dl) among the groups, being highest in PF₃₀₀ and lowest in RBO₃₀₀. The lowest pH values at 4 h post-feeding in all 3 groups and the lowest mean pH value in PF₃₀₀ coincided well with the maximum concentration of TVFA. There was no difference in the TN (Fig. 3) and NPN (Fig. 4) values among the groups. The TCA-N in PF₃₀₀ group was higher ($P < 0.05$) than the control and RBO₃₀₀ groups, which were similar (Fig. 5). The NH₃-N concentration was similar among the groups and was close to the minimum threshold level of 5–8 mg % as proposed by Maynard *et al.* (1979) for optimum microbial growth (Fig. 6). There was no difference ($P > 0.05$) in the TBC (Fig. 7) and TPC (Fig. 8) count among the groups. There was no adverse effect on the rumen fermentation of the animals on supplementation of Ca-LCFA. Similar observations were recorded for PF, when fed at 5 to 15% (Chalupa *et al.* 1985), 3.5% (Grummer 1988) and 6–8% (Chalupa *et al.* 1986) of the dietary DM. Ruminal pH, ammonia nitrogen and VFA level were not affected adversely by the supplementation of Ca soaps up to 5% of the dietary DM in lactating cows containing 60% forages (Ohajuruka *et al.* 1991). With increase in the level of dietary Ca-LCFA, ruminal pH and TVFA concentration were not changed, but molar percentage of acetate and acetate to propionate ratio increased linearly (Schauff and Clark 1992).

It was concluded that dietary supplementation of indigenously prepared PF (Ca-LCFA) up to 300 g/day had no adverse effect on the rumen fermentation in adult buffaloes fed WS based diets with roughage: concentrate ratio of 65:35.

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