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Effect of ruminally protected fat on *in vitro* fermentation and apparent nutrient digestibility in buffaloes (*Bubalus bubalis*)

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ABSTRACT

Experiments were conducted to evaluate effects of supplementation of calcium salts of long chain fatty acids (Ca-LCFA) as a rumen inert fat (PF) on *in vitro* fermentation and apparent nutrient digestion in adult buffaloes fed wheat straw based diets. For the *in vitro* fermentation study, five total mixed rations (TMR) consisting of a concentrate mixture (CM), green *Sorghum bicolor*, WS and supplemented without (C) or with 30 g/kg dry matter (DM) rice bran fatty acid oil (RBO) (30 RBO) or 20 g/kg RBO + 10 g/kg PF (20 RBO/10 PF) or 10 g/kg RBO + 20 g/kg PF (10 RBO/20 PF) or 30 g/kg PF in the DM in the ratio of 340:50:580:30 were prepared. The *in vitro* DM degradability (IVDMD), TN, trichloro acetic acid precipitable N (TCA-N), non-protein N (NPN) and ammonia N (NH_3-N) were similar among groups. Within the fat supplemented groups, total volatile fatty acid (TVFA) concentration increased linearly (P=0.025) with PF supplementation. Apparent nutrient digestibility was determined on 20 adult buffaloes divided into five equal groups fed CM supplemented without (C) or with 300 g RBO (30 RBO) or 200 g RBO + 100 g PF (20 RBO/10 PF) or 100 g RBO + 200 g PF (10 RBO/20 PF) or 300 g PF (30 PF) along with limited green *S. bicolor* and WS maintaining forage: concentrate ratio of 650:350.

Abbreviations: ADF, acid detergent fiber expressed inclusive of residual ash; aNDF, neutral detergent fiber assayed with heat stable amylase and expressed inclusive of residual ash; BW, body weight; Ca-LCFA, calcium salts of long chain fatty acids; CM, concentrate mixture; CP, crude protein; DE, digestible energy; DM, dry matter; DMD, DM degradability; EE, ether extract; IVDMD, *in vitro* DM degradability; ME, metabolizable energy; NH_3-N, ammonia N; NPN, non-protein N; OM, organic matter; PF, ruminally protected fat; RBO, rice bran fatty acid oil; RL, rumen liquor; SRL, strained rumen liquor; TA, total, ash; TCA-N, trichloro acetic acid precipitable N; TCHO, total carbohydrate; TMR, total mixed ration; VFA, volatile fatty acids; WS, wheat straw.

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Fat supplementation had no effect on the DM intake and apparent digestibilities of DM, organic matter (OM), crude protein (CP), total carbohydrate (TCHO) and neutral detergent fiber (aNDF). Within fat supplemented groups, inclusion of PF increased digestibilities of DM, OM, ether extract (EE), TCHO, aNDF and ADF. Supplemental fat also increased the digestible energy (DE) and metabolizable energy (ME) content of the diet, which also increased linearly with PF supplementation. All buffaloes were in positive N, Ca and P balances. We conclude that 200–300 g supplemental PF in the form of Ca-LCFA can be included in straw based diets fed to buffaloes to increase its energy density without adversely affecting DM intake and digestibility.

1. Introduction

The energy density of a ruminant ration can be enhanced by incorporating fermentable carbohydrates such as cereal grains or fats. However there is limitation to the use of high levels of cereal grains in the ration as it reduces rumen pH which can cause rumen acidosis. Based on its impacts on rumen metabolism, supplemental fat can be either rumen active or inert (PF). Rumen active fats are extensively hydrolyzed and have the potential to interfere with microbial fermentation in the rumen, while PF are resistant to hydrolysis by rumen microbes (Palmquist and Jenkins, 1980; Jenkins, 1993). Hence, PF can enhance the energy density of the ration without impact on ruminal fermentation. Among the various forms of PF, calcium salts of long chain fatty acids (Ca-LCFA) are relatively less degradable in the rumen (Elmeddah et al., 1991), have high intestinal digestibility and are a source of Ca. 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, is a good source of fat for livestock feeding (Saijpaul et al., 2001). Hence it was used in preparation of PF by a local method (Naik et al., 2007a,b).

The effect of supplemental PF on apparent nutrient digestibility varies with its level of inclusion, forage: concentrate ratio and type of forage in the diet. As per NRC (2001), the mixture of cereal grains and forages usually contain about 30 g/100 kg fat and total dietary fat should not exceed 60–70 g/kg of dietary dry matter (DM) intake. Thus, 30–40 g/kg of DM intake can be in the form of supplemental fat. The majority of studies conducted on PF used concentrate and forage based diets. However in developing countries, where, forage availability is limited, farmers use mostly low grade crop residues as the main forage. This study was completed to evaluate effects of supplementation of a locally prepared Ca-LCFA as PF on *in vitro* fermentation and apparent nutrient digestibility in adult buffaloes fed wheat straw (WS) based diets.

2. Materials and methods

2.1. Site of study

The study was completed in the Department of Animal Nutrition, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India, situated 30°54′N, 75°48′E and 247 m above mean sea level. The experiment was conducted in August when ambient temperature, relative humidity and mean annual rainfall were 25.2–33.4 °C, 67–88% and 179 mm, respectively.

2.2. Preparation of ruminally protected fat

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 sulphuric acid (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 l of water was added and boiled for 30 min without cover on medium heat (Naik et al., 2007a). When the PF became granular and non-sticky, it was filtered through muslin cloth with repeated washings under running tap water and then sun dried. The PF was kept in airtight containers in a cool place (*i.e.*, 25 °C) after mixing with butylated hydroxy toluene (BHT) at 0.5 g/kg as an antioxidant.

2.3. In vitro fermentation study

2.3.1. Preparation of total mixed rations

Five total mixed rations (TMR) were prepared containing a concentrate mixture (*i.e.*, maize grain 350, rice bran 200, deoiled rice bran 120, mustard cake 200, soybean meal 100, mineral mixture 20 and salt 10 g/kg weight), green *Sorghum bicolor*, wheat straw (WS) and supplemented without (C) or with 30 g/kg RBO (30 RBO) or 20 g/kg RBO + 10 g/kg PF (20 RBO/10 PF) or 10 g/kg RBO + 20 g/kg PF (10 RBO/20 PF) or 30 g/kg PF (30 PF) in the ratio of 340:50:580:30 on a DM basis.

2.3.2. Preparation for in vitro fermentation

Rumen liquor (RL) was collected 2 h post-feeding from three rumen fistulated buffaloes (455 ± 13.4 kg BW) fed 10 kg TMR (DM basis) containing a concentrate mixture (CM), 2 kg fresh green fodder and WS with a forage: concentrate ratio of 650:350. The RL from the three animals was pooled in equal proportion after straining through four layers of muslin cloth to serve as inoculum. The five TMR samples (each 500 mg) were incubated at 39 °C with 40 ml buffer (McDougall, 1948) and 10 ml strained rumen liquor (SRL) in an *in vitro* fermentation system (Tilley and Terry, 1963) in triplicate. Samples were shaken thrice daily at regular intervals. After 48 h of incubation, the fermentation was arrested using 1 ml mercuric chloride (5 g/100 ml) and 2 ml Na_2CO_3 (1N). The tubes containing samples were centrifuged at 1118 × *g* for 15 min at 25 °C and the supernatant was collected for analysis for total N, trichloro acetic acid precipitable N (TCA-N), ammonia N (NH_3-N) and total volatile fatty acids (VFA). Samples for estimation of N fractions were preserved with two drops of 10N H_2SO_4 in separate plastic vials. All samples were stored frozen (−20 °C) until analyzed. Residues were incubated with 50 ml of 2.0 g/l pepsin in 0.1 N HCl for further 48 h at 39 °C with intermittent shaking. Residues were filtered through pre-weighed sintered glass crucible, dried at 100 °C for 24 h and weighed after cooling in a dessicator to determine the *in vitro* DM degradability (IVDMD).

2.3.3. Analytical procedures

All the feed (*i.e.*, concentrates, *S. bicolor*, WS, TMR) samples were analyzed for DM (procedure 4.1.03) and total ash (TA; procedure 4.1.10) as described by AOAC (2000). The N was estimated by the copper catalyst Kjeldahl method (AOAC, 2000; 4.2.09). Ether extract (EE) of the samples without PF was determined by AOAC (2000; 4.5.01), where as samples containing PF were analyzed by Folch et al. (1957) after acid (6N HCl) hydrolysis. Total carbohydrates (TCHO) were calculated by subtracting the sum of CP and EE from organic matter (OM). Neutral detergent fiber was assayed with a heat stable amylase without sodium sulphite and expressed inclusive of residual ash (aNDF) and acid detergent fiber (ADF) is expressed inclusive of residual ash (Van Soest et al., 1991). The method suggested by Talpatra et al. (1940) was used to determine the Ca content while Ames (1966) method was used for determination of P after wet ashing. The VFA content of the supernatant was determined following the method of Bernett and Reid (1957) in a Markham distillation apparatus. The Conway (1957) method was employed for determination of NH_3-N, and TCA-N was measured by the method of Cline et al. (1958). Non-protein N (NPN) was calculated as the difference between N and TCA-N.

Data were analyzed using the General Linear Model for univerate analysis of SPSS 12.0 for windows (SPSS, 2003). An orthogonal contrast was used to compare the C treatment to all the fat supplemented diets, and polynomial contrasts were used to determine linear and quadratic effects within the supplemental fat treatments (*i.e.*, 30 RBO, 20 RBO/10 PF, 10 RBO/20 PF and 30 PF). Differences among means with $P<0.05$ were accepted as being statistically different.

2.4. Experiment II

2.4.1. Experimental animals and feeding

Twenty adult male buffaloes (451 ± 5.8 kg BW) were divided into five equal groups on the basis of their BW, and kept in a well ventilated, clean, cement floored animal shed. They were offered the CM as prepared in 2.3.1, mixing without (C) or with 300 g RBO (30 RBO) or 200 g RBO + 100 g PF (20 RBO/10 PF) or 100 g RBO + 200 g PF (10 RBO/20 PF) or 300 g PF (30 PF), respectively with 2 kg fresh green fodder (*S. bicolor)* and WS, maintaining a forage: concentrate ratio of 650:350 to meet their nutrient requirements (Kearl, 1982) for a period of 30 d. Clean fresh drinking water was available throughout the experiment *ad libitum*.

2.4.2. Metabolism experiment

At the end of feeding period, a 6-d metabolism study was conducted on all experimental animals in metabolic cages, which had provision for individual feeding and collection of faeces and urine separately. Animals were individually offered clean water *ad libitum*. During the metabolism study, the feeding schedule of the animals remained the same as previously. The feed residues after 24 h consumption of each animal were weighed to determine daily feed intake. Faeces were collected quantitatively from the cages by hand immediately after defecation, while urine was collected continuously into plastic bottles fixed below the metabolic cages. The process of collection was once every 24 h during the entire metabolism study.

2.4.3. Analytical procedures

The DM, TA, N, CP, EE, TCHO, aNDF, ADF, Ca and P content of the feed, faeces, residues and urine samples in quadruplicate were determined as per the procedures provided in Section 2.3.3. The CP (g/kg DM) was calculated by dividing CP intake (g) by DM intake (kg). The digestible energy (DE) and metabolizable energy (ME) values were calculated based on the formulae, DE (MJ) = 17.45 [digestible CP + digestible TCHO + (2.25 × digestible EE)] kg; and ME = DE × 0.82 (NRC, 1989).

Data were analyzed using the General Linear Model for univerate analysis of SPSS 12.0 for windows (SPSS, 2003). An orthogonal contrast was used to compare the C treatment to all the fat supplemented diets, and polynomial contrasts were used to determine linear and quadratic effects within the supplemental fat treatments (*i.e.*, 30 RBO, 20 RBO/10 PF, 10 RBO/20 PF and 30 PF). Differences among means with $P<0.05$ were accepted as being significantly different.

3. Results

3.1. Chemical composition of the feed and fodder

The CP of the various CM ranged from 201 to 209 g/kg and were isonitrogenous (Table 1). The EE of C (62.5 g/kg) was lower ($P<0.001$) than the fat supplemented groups, but, within the fat supplemented groups the EE was similar. The TCHO content in C was higher ($P<0.001$) than the fat supplemented groups and, within supplemental fat levels, the TCHO content was similar. The aNDF, ADF and P content of all CM were similar. The Ca content of C was lower ($P<0.001$) than the fat supplemented concentrates and increased ($P<0.001$) linearly with inclusion of PF.

3.2. Chemical composition of the total mixed rations

The CP of the various TMR ranged from 89.7 to 91.9 g/kg and were isonitrogenous (Table 2). The EE in C (29.0 g/kg) was lowest ($P<0.001$) among the TMR and within the fat supplemented groups, it was similar. The TCHO content in C (811 g/kg) was higher ($P=0.007$) than the fat supplemented groups, which were similar. The aNDF, ADF and P content of all TMR were similar, but the Ca content increased ($P<0.001$) linearly with inclusion of PF.

Table 1
Chemical composition (g/kg) of concentrates and forages on a DM basis.

	C	30 RBO	20 RBO/ 10 PF	10 RBO/ 20 PF	30 PF	S.E.M.	*P*			*Sorghum bicolor*	Wheat straw
							1	2	3		
DM (as fed)	924	923	933	929	928	2.2	0.655	0.615	0.263	252	939
OM	923	924	925	920	914	1.9	0.468	0.159	0.507	900	935
CP	209	203	204	203	201	1.4	0.571	0.430	0.522	81.2	24.5
EE	62.5	133	131	132	132	7.5	<0.001	0.892	0.652	24.5	10.0
TCHO	652	589	590	585	582	7.4	<0.001	0.411	0.655	794	901
aNDF	296	279	319	307	324	6.8	0.218	0.074	0.404	691	851
ADF	123	112	130	118	138	3.8	0.217	0.093	0.900	472	531
Ca	8.8	8.9	10.3	11.7	12.7	0.4	<0.001	<0.001	0.615	04.6	01.3
P	8.8	8.9	8.9	8.1	8.1	0.2	0.086	0.026	1.000	03.5	00.6

CM: concentrate mixture (maize grain 350, rice bran 200, deoiled rice bran 120, mustard cake 200, soybean meal 100, mineral mixture 20 and salt 10 g/kg by weight).
(1) Probability for C *versus* supplemental fat levels (30 RBO, 20 RBO/10 PF, 10 RBO/20 PF and 30 PF).
(2) Probability for the linear effect of supplemental fat levels.
(3) Probability for the quadratic effect of supplemental fat levels.

3.3. In vitro dry matter degradability and fermentation of total mixed rations

The IVDMD values were similar among groups (Table 3), but TVFA (mequiv./dl) concentration in C buffaloes was lower (P=0.022) than the treatment groups and increased linearly (P=0.025) with PF supplementation. The TN, TCA-N, NPN and NH_3-N concentration were similar among groups.

3.4. Voluntary feed intake and digestibility of nutrients

The DM intake was similar among the groups and ranged from 9.25 to 9.89 kg/d (Table 4). Fat supplementation had no effect on DM and OM apparent digestibilities, however both the DM (P=0.018) and OM (P=0.041) digestion increased linearly with inclusion of PF. The apparent digestibility of EE in the fat supplemented groups was higher (P<0.001) than the C group and increased (P<0.001) linearly with inclusion of PF. Linear increases in the TCHO (P=0.023) and aNDF (P=0.028) occurred with inclusion of PF. Within the fat supplemented groups, the apparent digestibility of ADF increased linearly (P<0.001) with PF supplementation. With fat supplementation, the DE (P=0.020) and ME (P=0.018) content of the diet increased. Linear increases also occurred in DE (P=0.024) and ME (P=0.023) with PF supplementation.

Table 2
Chemical composition (g/kg) of total mixed rations (TMR) on a DM basis.

	C	30 RBO	20 RBO/10 PF	10 RBO/20 PF	30 PF	S.E.M.	*P*		
							1	2	3
DM (as fed)	902	901	904	903	903	1.9	0.984	0.845	0.661
OM	930	929	925	923	925	2.2	0.883	0.647	0.618
CP	89.7	91.9	91.9	89.7	89.7	0.7	0.645	0.119	0.989
EE	29.0	53.5	53.1	53.2	53.4	2.6	<0.001	0.907	0.559
TCHO	811	784	780	780	782	3.8	0.007	0.889	0.628
aNDF	650	640	655	638	642	2.7	0.218	0.612	0.376
ADF	365	387	375	366	382	3.5	0.198	0.464	0.080
Ca	4.00	4.00	4.00	5.00	5.00	0.1	<0.001	<0.001	1.000
P	3.60	3.60	3.60	3.60	3.60	0.1	1.000	1.000	1.000

TMR: total mixed ration consisting of concentrate mixture (Table 1), green *S. bicolor*, wheat straw and supplemental fat in the ratio of 340:50:580:30 on a DM basis.
(1) Probability for the C *versus* supplemental fat levels (30 RBO, 20 RBO/10 PF, 10 RBO/20 PF and 30 PF).
(2) Probability for the linear effect of supplemental fat levels.
(3) Probability for quadratic effect of supplemental fat levels.

Table 3
In vitro dry matter degradability (IVDMD) and fermentation of total mixed rations (TMR).

	C	30 RBO	20 RBO/10 PF	10 RBO/20 PF	30 PF	S.E.M.	*P*		
							1	2	3
In vitro DM degradability									
IVDMD	0.53	0.52	0.53	0.54	0.54	0.005	0.555	0.152	0.792
In vitro fermentation									
TVFA (mequiv./dl)	3.23	3.66	3.98	4.80	4.77	0.205	0.022	0.025	0.619
Total N (mg/dl)	15.40	14.00	15.87	16.80	17.73	0.623	0.431	0.074	0.733
TCA-N (mg/dl)	7.93	5.13	6.77	7.47	8.87	0.699	0.584	0.158	0.947
NPN (mg/dl)	7.47	8.87	9.10	9.33	8.87	0.334	0.479	0.930	0.557
NH_3-N (mg/dl)	3.41	4.29	4.57	5.27	4.85	0.222	0.057	0.196	0.381

TMR: total mixed ration consisting of concentrate mixture (Table 1), green *S. bicolor*, wheat straw and supplemental fat in the ratio of 340:50:580:30 on a DM basis.
(1) Probability for C *versus* supplemental fat levels (30 RBO, 20 RBO/10 PF, 10 RBO/20 PF and 30 PF).
(2) Probability for the linear effect of supplemental fat levels.
(3) Probability for the quadratic effect of supplemental fat levels.

Table 4
Voluntary feed intake and digestibility of nutrients in buffaloes.

	C	30 RBO	20 RBO/10 PF	10 RBO/20 PF	30 PF	S.E.M.	*P*		
							1	2	3
Average body weight (kg)	451.75	449.88	463.25	463.5	462.63	5.597	0.907	0.481	0.558
Dry matter intake									
Total (kg/d)	9.71	9.72	9.89	9.25	9.63	0.123	0.602	0.506	0.732
Total (g/kg $W_{kg}^{0.75}$/d)	99.36	99.75	99.05	92.59	96.45	1.282	0.380	0.230	0.446
Digestibility of nutrients									
DM	0.53	0.51	0.54	0.55	0.54	0.005	0.124	0.018	0.107
OM	0.56	0.54	0.56	0.58	0.57	0.005	0.168	0.041	0.114
CP	0.73	0.73	0.73	0.74	0.74	0.006	0.961	0.535	0.805
EE	0.75	0.81	0.84	0.87	0.89	0.012	<0.001	<0.001	0.653
TCHO	0.53	0.50	0.52	0.54	0.53	0.005	0.101	0.023	0.076
aNDF	0.51	0.50	0.51	0.51	0.53	0.005	0.288	0.028	1.000
ADF	0.39	0.36	0.41	0.41	0.44	0.008	0.004	<0.001	0.551
Nutritive value of diets									
DE (MJ/kg DM)	9.60	9.80	10.16	10.53	10.37	0.110	0.020	0.024	0.168
ME (MJ/kg DM)	7.87	8.04	8.33	8.63	8.50	0.090	0.018	0.023	0.172
DE intake (MJ/d)	93.10	95.11	100.41	97.14	99.66	0.988	0.084	0.299	0.528
ME intake (MJ/d)	76.34	77.99	82.33	79.65	81.72	0.810	0.084	0.299	0.528

(1) Probability for C *versus* supplemental fat levels (30 RBO, 20 RBO/10 PF, 10 RBO/20 PF and 30 PF).
(2) Probability for the linear effect of supplemental fat levels.
(3) Probability for the quadratic effect of fat supplemental fat levels.

3.5. Nitrogen, calcium and phosphorus balances

There was no effect of supplemental fat on the N balances, but Ca intake increased at a decreasing rate (linear $P<0.001$; quadratic $P=0.003$) with the increase in the level of PF (Table 5). The Ca excreted through faeces increased ($P=0.004$) linearly with inclusion of PF while, urinary Ca excretion was similar among groups. All animals were in positive Ca and P balances, and values were similar among groups.

4. Discussion

4.1. In vitro dry matter degradability and fermentation of total mixed rations

The similar IVDMD values among our treatment groups are consistent with findings of the earlier workers (Reddy et al., 2001). The linear increase in TVFA concentration with inclusion of PF supports

Table 5
Nitrogen, calcium and phosphorus balances in buffaloes.

	C	30 RBO	20 RBO/10 PF	10 RBO/20 PF	30 PF	S.E.M.	*P*		
							1	2	3
N balance									
Intake (g/d)	154.08	148.97	150.80	146.74	148.39	0.663	<0.001	0.140	0.912
Output (g/d)									
In faeces	41.26	40.09	40.82	37.90	38.00	1.035	0.802	0.387	0.894
In urine	30.32	30.08	21.22	28.45	29.52	2.140	0.683	0.788	0.295
N retention (g/d)	82.50	78.80	88.76	80.38	80.86	2.553	0.805	0.924	0.362
Proportion of absorbed N retained	0.727	0.723	0.806	0.739	0.733	0.0203	0.725	0.847	0.305
Proportion of intake N retained	0.536	0.529	0.589	0.548	0.545	0.0170	0.859	0.960	0.378
Calcium									
Intake (g/d)	42.32	42.80	48.33	53.50	57.08	1.55	<0.001	<0.001	0.003
Output (g/d)									
In faeces	21.60	17.63	31.87	30.08	34.24	2.037	0.011	0.004	0.094
In urine	5.88	7.19	5.06	6.19	4.23	0.416	0.209	0.097	0.929
Retention (g/d)	14.83	17.98	11.40	17.23	18.60	1.474	0.586	0.600	0.243
Phosphorus									
Intake (g/d)	37.38	37.93	38.14	35.06	35.09	0.372	<0.001	<0.001	0.603
Output (g/d)									
In faeces	22.62	23.97	23.67	21.18	20.84	0.773	0.677	0.188	0.992
In urine	0.24	0.29	0.31	0.30	0.30	0.019	0.805	0.943	0.822
Retention (g/d)	14.52	13.67	14.16	13.58	13.95	0.725	0.996	0.978	0.975

(1) Probability for C *versus* supplemental fat levels (30 RBO, 20 RBO/10 PF, 10 RBO/20 PF and 30 PF).
(2) Probability for the linear effect of supplemental fat levels.
(3) Probability for the quadratic effect of supplemental fat levels.

the hypothesis that oil in the form of Ca soaps had no deleterious effects on ruminal fermentation (Ngidi et al., 1990). Earlier workers also reported similarity in TN, TCA-N, NPN (Mishra et al., 2005) and NH_3-N (Alexander et al., 2002b) concentration with or without fat supplementation. Tangendjaja et al. (1993) also observed similar NH_3-N level in the supernatant after fermentation of elephant grass with ruminally unprotected and protected fat.

4.2. Voluntary feed intake and digestibility of nutrients

The DM intake paralleled findings of earlier workers (Srivastava and Mugal, 1987; Naik et al., 2007b) and the non-interference of the supplemental fat on apparent digestibilities paralleled findings of Garcia-Bojalil et al. (1998), Reddy et al. (2003) and Naik et al. (2007b). The linear increase in the apparent digestibilities of the DM, OM, TCHO and aNDF with inclusion of PF may be due to its relatively stable nature causing minimal dissociation in rumen (Sukhija and Palmquist, 1990), or modification in the rumen, leading to improved fiber digestion (Schauff and Clark, 1992). The higher apparent digestibility of the EE in the fat supplemented groups *versus* C (Naik et al., 2007b) indicates that the added fat was more digestible than that of the basal diet (Grummer, 1988) and the linear increase in EE digestibility with inclusion of PF was similar to findings of Alexander et al. (2002a). Within fat supplemented groups, the higher ADF digestibility with higher inclusion of PF may be due to physical coating of the fiber by RBO thereby preventing microbial attachment and/or inhibition of microbial enzyme activity on the surface due to effects of fatty acids on cell membranes (Palmquist and Jenkins, 1980). The increase in the DE and ME content with fat supplementation is almost certain due to the higher EE content of the diets. The linear increase in the DE and ME content with PF supplementation may be due to the higher EE content and digestibility in the treatment groups (Sklan et al., 1990; Reddy et al., 2003).

4.3. Nitrogen calcium and phosphorus balances

Boggs et al. (1987) also did not observe any effect of supplemental fat on the N balances of the buffaloes. The quadratic increase in Ca intake with increasing PF levels as Ca-LCFA may be due to the higher Ca content of the diets. The linear increase in Ca excreted in faeces with inclusion of PF (Alexander et al., 2002a) may be attributed to the higher Ca intake as Ca-LCFA in which excess Ca might have reformed insoluble soaps (Palmquist et al., 1986) in the large intestine and was excreted in faeces.

5. Conclusion

Results show that 200–300 g of supplemental PF in the form of Ca-LCFA can be included in straw based diets fed to buffaloes to increase its energy density without adversely affecting DM intake and digestibility.

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