



Effects of calcium salt of palm fatty acid supplementation on production performance, nutrient utilization and blood metabolites in Surti buffaloes (*Bubalus bubalis*)

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ABSTRACT

Objective of the study was to investigate the effect of dietary supplementation of rumen protected fat on productive performance, nutrient utilization and blood biochemical profile of Surti buffaloes. Eighteen multiparous buffaloes (2–4 lactation) in early lactation were divided in three homogenous groups of CON (control), BF₁₀₀ and BF₂₀₀ with six animals in each group. The animals in CON were fed with a basal diet consisting of concentrate mixture, green sorghum and paddy straw as per ICAR nutrient requirements, while the animals in BF₁₀₀ and BF₂₀₀ group were fed with same ration and supplemented with 0.75% (100 g/d) and 1.5% (200 g/d) bypass fat on DMI basis, respectively for 15 days pre-partum to 90 days post-partum. The dry matter intake, body condition score, milk yield and milk composition parameters like protein, lactose and SNF were not influenced by supplemental bypass fat. Milk fat percentage, production of 4% fat corrected milk (FCM), solid corrected milk (SCM) and energy corrected milk (ECM) increased quadratically with the increasing level of rumen protected fat in the diet. Feed efficiency (FCM/DMI) and energetic efficiency of milk production improved in a quadratic manner. Nutrient intake of DCP, TDN and digestibility of DM, CP, CF and NFE except EE remained statistically non-significant. The serum triglycerides, cholesterol and calcium level were higher in bypass fat-supplemented group. However, serum total protein and glucose level remained statistically at par. Thus, bypass fat supplementation at 0.75% of the DM intake (100 g/d) increased the milk fat percentage, FCM production and feed efficiency along with serum triglycerides and cholesterol level in lactating Surti buffaloes.

Key words: Blood-biochemicals, Bypass fat, Digestibility, Surti Buffaloes

Energy is the major limiting nutrient that affects the production potential of lactating animals and the animals are not able to get sufficient energy from their diets resulting in a lower productive performance (Ranjhan *et al.* 2012). Maximizing energy intake by increasing the energy density of the diet is a logical feeding strategy for early lactating buffaloes. Excessive grain feeding increases energy density of the diet, but rapid fermentation can lead to a suboptimal rumen environment and decline in the concentration of milk fat. Fat supplementation also increases density of energy of the diet but high dietary fat can lead to a reduction in fibre digestion in the rumen and a decline in milk fat percentage depending on the amount and type of fat fed (Purushothaman *et al.* 2008). In order to counter these undesirable effects, dietary supplementation of fat as a salt of long chain fatty acids is a good alternative (Naik *et al.*

2009 and Ranjhan *et al.* 2012). Calcium salts of long chain fatty acids (Ca-LCFA) are relatively less degradable in rumen, high intestinal digestibility and serves as an additional source of calcium. The positive effect of feeding Ca salt of fatty acid was more evident at the early lactation and maximum response was observed with the addition of 2 to 3% (150 to 300 g/d) of bypass fat (Singh *et al.* 2015). Research reports on feeding of bypass fat especially on blood biochemical profile of buffaloes are sparse. Therefore, an attempt was made to assess the effect of dietary supplementation of rumen protected fat on production performance, nutrient utilization and blood biochemical profile of Surti buffaloes.

MATERIALS AND METHODS

Location of the study: The present experiment was conducted at Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat. The experiment was conducted during October 2015 to January 2016.

Experimental animals and rations: Lactating Surti buffaloes (18) in early lactations (2 to 4 lactations) with an average daily milk yield 4.32 ± 0.20 kg and live body weight

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383.55±8.56 kg, were randomly divided into 3 homogenous groups with 6 animals in each group on the basis of parity and previous lactation milk yield. The concentrate and roughage ratio of the diet was tried to be maintained at 40:60 and the animals were fed as per ICAR nutrient requirement (1998). Chaffed roughage (particle size - 2.0 to 2.5 cm) consisted of green fodder (sorghum) and dry fodder (paddy straw) at the ratio of 50:50. Rumen protected fat was additionally supplemented to BF₁₀₀ and BF₂₀₀ groups at 0.75% (100 g/d) and 1.50% (200 g/d) of DM intake after mixing with the concentrate mixture, respectively for 15 days pre-partum to 90 days post-partum and persistence of milk production was monitored up to 90 days of lactation. The commercial bypass fat (84% fat on DM basis) consisted of Ca salts of palm fatty acid having mixture of long-chain saturated and unsaturated fatty acids. The concentrate was offered twice a day in equal parts at 04:00 and 16:00 hours prior to milking. *Ad lib.* chaffed fodder available from 10:00 hours and 17:00 hours onwards. Leftover, if any, was weighed next morning. DM content of forage and leftover was determined to calculate the daily DMI. The animals were kept in well-ventilated byres with free access of fresh clean drinking water.

Analytical techniques: The dried samples of concentrate mixture, paddy straw and green sorghum fodder (offered and leftover) were ground to pass through a 1-mm sieve in a Willey grinder and pooled samples were analyzed for proximate composition (AOAC 2000). Buffaloes were milked twice a day at 04:00 and 16:00 hours and milk yield was recorded daily at each milking throughout the trial. Milk samples were collected for two consecutive days at fortnight intervals after thorough mixing of milk and subjected to analysis of various milk constituents like fat, SNF, protein and lactose by pre-calibrated ultrasonic milk analyzer (Lactoscan LA, Milkotronics Ltd, Bulgaria). In the study, 4% fat corrected milk (FCM) was calculated by Gaines and Davidson (1923) while solid corrected milk (SCM), energy corrected milk (ECM) and gross energy of milk were calculated using the equations given by Tyrrell and Reid (1965). The energetic efficiency for milk production was calculated by dividing milk energy by digestible energy (DE) of the feed consumed presuming that one kg TDN is equivalent to 4,400 kcal digestible energy (NRC 2001). Body condition score of individual animal was determined before starting of experiment and completion of experiment as per scores given by Vanessa *et al.* (2009).

Nutrient digestibility: Digestibility trial was conducted on all the animals of groups during protected fat supplementation period for seven days in the same housing system. Total faeces voided per 24 h by each animal were collected manually for 7 days. Aliquots of faeces for each animal were dried in a hot air oven at 80°C and for nitrogen estimation samples were preserved in 10% H₂SO₄. TDN intake for individual animal was calculated based on the intake of digestible nutrients.

Collection and analysis of blood samples: Blood sample

from each animal was collected by puncturing the jugular vein at 30-day intervals during the 90-day experimental period at 3 h post offering of the concentrate mixture at morning. Serum samples were harvested and stored at -40°C for subsequent analysis of biochemical profile. Diagnostic kits were used for analyzing various blood biochemical parameters, i.e., glucose (GOD-PAP method), calcium (Arsenazo method), total protein (Biuret method), total cholesterol (CHOD-POD method) and triglycerides (GPO-POD method).

Statistical analysis: Statistical analysis of the data was performed using SPSS (20.0) statistical package. Data were analyzed by one-way analysis of variance. Linear and quadratic effects of treatment were tested for all data using orthogonal polynomial contrasts. Significance was declared at P<0.05; differences between means were tested using post-hoc with Duncan's multiple range test. All statistical procedures were carried out as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Chemical composition of feeds and forage: The chemical composition of feed ingredients is presented in Table 1. The chemical composition of the concentrate mixture with or without supplemental bypass fat was similar except ether extract and total ash contents which were higher (P<0.05) in groups BF₁₀₀ and BF₂₀₀. The total ash content of bypass fat was comparatively higher than other feeds and fodder due to high level of Ca contributed from bypass fat.

Feed intake and milk production: The results in present study revealed that bypass fat had non-significant (P>0.05) effect on dry matter intake (DMI). DMI through concentrate and roughage was also similar (Table 2). Most of the workers reported that the DMI of dairy animals was not altered (Naik *et al.* 2009, Tyagi *et al.* 2010, Sirohi *et al.* 2010, Mudgal *et al.* 2012, Savsani *et al.* 2016) on supplementation of bypass fat. This might be due to added inert fat was likely to be largely unavailable in the rumen because of its low solubility and high melting point, thereby not impairing rumen fiber digestibility, resulting dry matter intake was not affected on supplementation of rumen protected fat (Purushothaman *et al.* 2008). Body condition scoring is a subjective method of assessing the amount of ME stored in fat and muscle (body reserve) on a live animal

Table 1. Chemical composition of feed offered (as on % DM basis)

Particular	Concentrate	Green sorghum	Paddy straw	Bypass fat
Organic matter	93.20	92.60	89.40	–
Crude protein	19.60	05.40	03.10	–
Crude fibre	11.20	24.60	35.70	–
Ether extract	02.80	01.70	01.10	83.80
NFE	59.60	60.90	49.50	–
Total ash	06.80	07.40	10.60	16.20

NFE, nitrogen free extract.

Table 2. DMI and body condition score of Surti buffaloes fed with different levels of rumen protected fat

Attribute	Treatment			SEM	P values*		
	CON	BF ₁₀₀	BF ₂₀₀		C	L	Q
Initial BW (kg)	388.50	385.50	376.67	8.56	0.858	0.600	0.881
Final BW (kg)	386.33	388.17	379.69	8.33	0.918	0.762	0.786
BW change (kg)	-2.17	+ 2.67	+ 3.02	0.75	0.108	0.520	0.058
Initial BCS	3.62	3.60	3.65	0.02	0.353	0.276	0.343
Final BCS	3.53	3.68	3.77	0.03	0.116	0.420	0.055
BCS change	-0.09	+0.08	+0.12	0.04	0.180	0.680	0.418
DMI (kg/d)	12.50	12.32	11.99	0.23	0.686	0.399	0.888
Concentrate DMI (kg/d)	3.33	3.43	3.07	0.09	0.152	0.675	0.061
Roughage DMI (kg/d)	9.17	8.88	8.92	0.17	0.997	0.980	0.938

^{abc}Mean values with different superscript within a row differ significantly. *C, combined effect; L, linear effect; Q, quadratic effect. BW, body weight; BCS, body condition score (1, thin to 5, obese; Vanessa *et al.* 2009). SEM, standard error of the mean; DMI, dry matter intake.

Table 3. Milk production and composition of Surti buffaloes fed with different levels of rumen protected fat

Attribute	Treatment			SEM	P values*		
	CON	BF ₁₀₀	BF ₂₀₀		C	L	Q
	<i>Yield (kg/d)</i>						
Milk yield	4.02	4.80	4.13	0.20	0.244	0.831	0.101
Fat	0.22 ^a	0.30 ^b	0.26 ^{ab}	0.01	<0.008	<0.074	<0.007
4% FCM ¹	4.91 ^a	6.46 ^b	5.61 ^{ab}	0.25	<0.025	0.186	<0.015
SCM ²	4.90 ^a	6.35 ^b	5.43 ^{ab}	0.24	<0.037	0.323	<0.017
ECM ³	5.25 ^a	6.77 ^b	6.87 ^{ab}	0.25	<0.038	0.280	<0.019
Protein	0.14	0.17	0.15	0.01	0.112	0.998	0.059
Lactose	0.20	0.24	0.20	0.01	0.132	0.949	0.057
	<i>Milk composition (%)</i>						
Milk fat	5.61 ^a	6.35 ^b	6.42 ^b	0.15	<0.047	<0.025	0.261
Solid not fat	9.46	9.48	9.21	0.07	0.205	0.137	0.320
Protein	3.58	3.49	3.43	0.05	0.562	0.294	0.895
Lactose	4.91	5.05	4.77	0.05	0.075	0.218	0.051
Feed efficiency ⁴	0.39 ^a	0.53 ^b	0.47 ^{ab}	0.02	<0.016	<0.050	<0.006
Gross energy ⁵ (kcal/kg of milk)	925.63	994.79	988.60	14.98	0.109	0.081	0.216
Total gross energy (Mcal/d)	3.68 ^a	4.76 ^b	4.07 ^b	0.18	<0.037	0.322	<0.017
Energetic efficiency of milk production (%)	12.51 ^a	16.03 ^{ab}	14.45 ^b	0.54	<0.021	0.100	<0.018

^{abc}Mean values with different superscript within a row differ significantly; FCM, fat corrected milk; SCM, solid corrected milk; ECM, energy corrected milk, *C, combined effect; L, linear effect; Q, quadratic effect. ¹FCM (kg/d) = 0.4 × kg milk + 15 × kg fat. ²SCM (kg/d) = 12.3 × kg fat + 6.56 × kg SNF - 0.0752 × kg milk. ³ECM (kg/d) = 0.327 × kg milk + 12.95 × kg fat + 7.65 × kg protein. ⁴Feed efficiency = FCM/DMI. ⁵Gross energy of milk (kcal/kg) = 92.25 × milk fat% + 49.15 × SNF% - 56.40.

and provides better estimates of body fat distribution than body weight does (Ferguson *et al.* 1994). The initial, final and changes in BW and BCS were similar (P>0.05) amongst the groups (Table 2). Similar to the general pattern, in this experiment also, there was initial fall in both BW and BCS in all the groups followed by gradual recovery; however, the recovery of the BW and BCS in BF₁₀₀ and BF₂₀₀ groups was better than the control group.

Milk production and composition: Effect of bypass fat supplementation on production parameter is shown in Table 3. Average daily milk production (kg/d) was 19.40% higher in BF₁₀₀ than CON and 2.73% higher in BF₂₀₀ than CON, the difference amongst the group was statistically (P>0.05) nonsignificant. Average milk fat percentage was 13.19% higher in BF₁₀₀ than CON and 14.43% higher in BF₂₀₀ than

CON. The supplemental rumen protected fat group showed linear increase (P<0.05) in milk fat percentage as compared to CON while difference between the supplemental protected fat group was statistically non-significant (P>0.05). Similar observations were reported by Ranjhan *et al.* (2012), Mane *et al.* (2016) and Savsani *et al.* (2016). Higher milk fat in BF₁₀₀ and BF₂₀₀ might be due to availability of more fatty acids (SFA and USFA) for absorption in intestine due to protection of fat and these fatty acids are directly incorporated in milk fat after absorption from intestine, leading to increase in milk fat. Both bypass fat supplemented group BF₁₀₀ and BF₂₀₀ showed significantly (P<0.05) higher FCM as compared to CON. The significantly higher (quadratic, P<0.05) FCM in BF₁₀₀ and BF₂₀₀ was attributed to the increase in fat content

and milk yield. Similar body weight throughout the experimental period amongst groups indicated that the extra energy supplied through the feeding of supplemental bypass fat was not utilized by the animals for body fat deposition rather it might be diverted to produce more fat and FCM yield (Thakur and Shelke 2010, Shelke *et al.* 2012). ECM was 27.53% higher in BF₁₀₀ than CON and 30.85% higher in BF₂₀₀ than CON. Higher production of FCM, SCM, and ECM in buffaloes fed supplemental protected fat compared to those fed the control diet was in agreement with Shelke *et al.* (2012).

Milk composition parameters like SNF, protein and lactose remained statistically ($P>0.05$) similar. Generally increase in milk fat decreases milk protein per cent. Slight decrease in milk protein in BF₁₀₀ might be due to inhibition of microbial protein synthesis, absorption of certain fatty acids that may alter the uptake of amino acids by the mammary gland, the dilution effect as a result of increasing milk yield and/or reduction of mammary blood flow rate (Cant *et al.* 1993).

No difference was observed in milk lactose content between buffaloes fed diets containing supplemental fat and those on the control diet. However, an apparent higher milk

lactose percentage noted in buffaloes fed diets containing bypass fat confirmed the findings of a previous study (Singh *et al.* 2015) showing that the inclusion of saturated fatty acids in the concentrate mixture fed to lactating cows resulted in an increase in milk lactose content. Feed efficiency in terms of FCM yield significantly ($P<0.01$) improved in BF₁₀₀ and BF₂₀₀ as compared to CON. Total gross energy of milk was increased quadratically ($P<0.05$) with increasing level of rumen protected fat in experimental buffaloes. Energetic efficiency of milk production was also significantly (quadratic, $P<0.05$) higher in supplemental rumen fat protected group, which could support greater FCM yield with less DM intake.

Nutrient digestibility: Nutrient intake of experimental animals fed different levels of bypass fat is presented in Table 4. Digestible crude protein (DCP) and total digestible nutrient (TDN) intake remained statistically ($P>0.05$) nonsignificant amongst the treatment groups. Sarwar *et al.* (2004) and Purushothaman *et al.* (2008) also observed non-significant effect on DCP and TDN intake of buffaloes fed with varying levels of ruminally protected fat. There was no effect on the digestibility of DM, CP, CF and NFE (Table 4).

Table 4. Nutrients intake, digestibility coefficient and efficiency of nutrients utilization in Surti buffaloes fed different levels of rumen protected fat

Attribute	Treatment			SEM	P values*		
	CON	BF ₁₀₀	BF ₂₀₀		C	L	Q
<i>Nutrient intake (kg/d)</i>							
DM	12.50	12.32	11.99	0.23	0.686	0.399	0.888
DCP	0.60	0.61	0.57	0.01	0.575	0.527	0.406
TDN	6.63	6.76	6.42	0.14	0.634	0.564	0.454
<i>Nutrient intake (kg/100 kg BW)</i>							
DM	3.23	3.20	3.19	0.04	0.931	0.727	0.828
DCP	0.15	0.16	0.15	0.01	0.766	0.813	0.497
TDN	1.71	1.76	1.71	0.02	0.631	0.957	0.346
<i>Nutrient intake (g/kg W^{0.75})</i>							
DM	143.03	141.68	140.45	1.47	0.795	0.505	0.986
DCP	6.81	6.99	6.69	0.10	0.484	0.628	0.276
TDN	75.85	77.77	75.14	0.92	0.508	0.762	0.268
<i>Digestibility of nutrients (%)</i>							
DM	65.73	66.77	66.64	0.78	0.856	0.658	0.745
CP	57.11	57.80	58.32	0.39	0.466	0.226	0.925
EE	66.97 ^a	77.55 ^b	79.05 ^b	1.80	<0.004	<0.002	0.125
CF	49.59	48.70	48.18	0.29	0.120	0.058	0.738
NFE	63.05	66.49	64.37	0.46	0.402	0.116	0.141
<i>Efficiency of utilization of nutrients</i>							
DMI/milk yield (kg/kg)	3.28	2.68	2.91	0.11	0.147	0.464	0.070
DMI/FCM yield (kg/kg)	2.62 ^b	1.93 ^a	2.14 ^a	0.08	<0.003	<0.022	<0.005
CPI/milk yield (g/kg)	272.86	223.42	237.45	10.45	0.137	0.159	0.146
CPI/FCM yield (g/kg)	218.11 ^b	165.00 ^a	174.22 ^a	7.73	<0.004	<0.006	<0.020
DCPI/milk yield (g/kg)	40.56	33.67	37.00	1.72	0.176	0.226	0.150
DCPI/FCM yield (g/kg)	124.51	95.44	101.68	4.34	<0.007	<0.013	<0.024
TDNI/milk yield (g/kg)	451.18	375.16	416.15	18.91	0.273	0.451	0.156
TDNI/FCM yield (g/kg)	361.24 ^b	277.19 ^a	305.04 ^a	13.92	<0.031	<0.050	<0.040

^{abc}Mean values with different superscript within a row differ significantly. *C, combined effect; L, linear effect; Q, quadratic effect. BW, body weight; DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fibre; NFE, nitrogen free extract; DCPI, digestible crude protein intake; TDNI, total digestible nutrients intake; FCM, fat corrected milk.

However, EE digestibility was higher (linear, $P < 0.05$) in rumen protected fat groups as compared to control group. Similarly, Thakur and Shelke (2010) reported higher EE digestibility in buffaloes supplemented with protected fat prepared from soybean acid oil. Generally, digestibility of fat in ruminant diet is low due to high content of non-fatty material and the small proportion of true fat in the total diet, which causes endogenous secretions to be relatively high. However, added fat is more digestible than the lipid components of the basal diet or that additional fat dilutes endogenous lipid secretions resulting in a more accurate estimate of free lipid digestibility (Palmquist 1991). In the present investigation, digestibility of fibre fractions was not adversely affected on supplementation of Ca salts of palm fatty acids. Calcium soaps of fatty acids remain in intact form in the rumen and unlike direct fat supplementation, do not lead to any toxic effect on rumen microflora thus do not altering the fibre digestion (Harrison *et al.* 1995).

Efficiency of nutrient utilization: There was significant (quadratic, $P < 0.05$) improvement in the efficiency of conversion of DM, CP and TDN to FCM in the BF₁₀₀ and BF₂₀₀ as compared to CON indicating that diet added bypass fat had better energetic efficiency. However, the efficiency of nutrient utilization in comparison to milk yield remained statistically ($P > 0.05$) similar. Similar reports by Sirohi *et al.* (2010) and Tyagi *et al.* (2009) better nutrient utilization per kg of FCM production. There are reports of no effect on CP intake (Tyagi *et al.* 2009, Thakur and Shelke 2010). DCP intake (Naik *et al.* 2007) and TDN intake (Naik *et al.* 2007, Sirohi *et al.* 2010) by the supplementation of bypass fat to dairy animals.

Blood biochemical profile: The blood glucose, total

protein, triglycerides, cholesterol and Ca were within the physiological range (Table 5). In the present study, serum glucose (mg/dl) remained statistically ($P > 0.05$) similar amongst treatment group. This may be due to a high metabolic rate of utilization of glucose and homeostatic mechanism of animal body that does not allow appreciable change in glucose level. Serum total protein (g/dl) also showed non-significant difference due to supplementation of bypass fat. However, supplementation of bypass fat significantly (quadratic, $P < 0.01$) increased serum cholesterol and triglyceride level as compared to CON (Table 5). In the present study, increased serum triglycerides and cholesterol levels with the advance in feeding of supplemental bypass fat might be due to enhanced uptake of dietary fatty acid (Fahey *et al.* 2002, Grewal *et al.* 2014). The serum Ca level (mg/dl) increased (linear, $P < 0.01$) by feeding supplemental bypass fat both in BF₁₀₀ and BF₂₀₀ along with the rise in different periodic intervals. Higher ($P < 0.01$) serum Ca level in BF₁₀₀ and BF₂₀₀ than that of CON might be due to rumen bypass fat (Ca salt of fatty acid).

Economics of feeding different levels of bypass fat is presented in Table 6. Total feed cost was calculated by considering concentrate (kg), green fodder (kg), dry fodder (kg) and bypass fat (100 g) at ₹ 14, 2, 4 and 10, respectively. Total feed cost (₹/d/animal) was significantly (linear, $P < 0.01$) increased on supplementation of bypass fat at both the levels. Milk selling price was calculated on the basis of regional dairy cooperative price of ₹ 590/kg fat during the period. Due to significant increase in FCM yield, the selling price was (quadratic, $P < 0.01$) higher in BF₁₀₀ and BF₂₀₀ as compared to CON. Higher milk selling price in BF₁₀₀

Table 5. Blood metabolites of Surti buffaloes fed with different levels of rumen protected fat

Attribute	Treatment			SEM	P values*		
	CON	BF ₁₀₀	BF ₂₀₀		C	L	Q
Glucose (mg/dl)	54.83	54.94	55.19	0.08	0.198	0.085	0.633
Total protein (g/dl)	8.04	8.10	8.11	0.05	0.857	0.616	0.825
Triglycerides (mg/dl)	31.47 ^a	35.33 ^b	36.36 ^c	0.51	<0.000	0.323	<0.003
Cholesterol (mg/dl)	141.81 ^a	151.49 ^b	154.16 ^c	1.30	<0.000	<0.052	<0.001
Calcium (mg/dl)	7.74 ^a	8.66 ^b	9.16 ^c	0.14	<0.000	<0.000	<0.031

^{abc}Mean values with different superscript within a row differ significantly. *C, combined effect; L, linear effect; Q, quadratic effect.

Table 6. Economics of feeding different levels of rumen protected fat to Surti buffaloes

Attribute	Treatment			SEM	P values*		
	CON	BF ₁₀₀	BF ₂₀₀		C	L	Q
Total feed cost (₹/d/animal)	114.06 ^a	124.19 ^b	128.24 ^b	2.48	<0.045	<0.017	0.515
Milk selling price (₹/d/animal)	130.00 ^a	178.83 ^b	155.83 ^{ab}	6.93	<0.007	<0.064	<0.006
ROFC (%)	16.46 ^a	44.01 ^b	21.66 ^{ab}	4.17	<0.007	0.517	<0.002
Feed cost/FCM (₹/kg)	23.94 ^b	19.49 ^a	22.85 ^{ab}	0.76	<0.036	0.508	<0.013
Feed cost/milk (₹/kg)	29.96	26.38	31.15	1.16	0.228	0.670	0.100

^{abc}Means with different superscript in a row differ significantly; ROFC, return over feed cost, *C, combined effect; L, linear effect; Q, quadratic effect.

significantly (quadratic, $P < 0.01$) increased return over feed cost (₹/d/animal) which was 23.36% higher than CON. Similar results were observed by Naik *et al.* (2009) and Grewal *et al.* (2014).

The results of the present study showed that the supplementation of rumen protected fat causes energy enrichment of diets for buffaloes at early stage of lactation, which in turn manifests in higher milk fat and FCM production. Rumen protected fat supplementation also improved serum triglycerides, cholesterol and calcium levels. Supplementation of bypass fat @ 0.75% of DMI (100 g/d) in Surti buffaloes provides promising results as it increased the overall net profit and return over feed cost.

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