Enhancing Lactation Performance in Nili Ravi Buffalo: The Impact of Dietary Calcium Salts of Fatty Acids and Starch

Rana Yaser Arafat[1], Saima[1], Hifzulrahman[1], Ehsaan Ullah Khan[1], Maqsood Akhtar[2], Arfan Asghar[2], Muhammad Naveed-ul-Haque[1]

[1] Department of Animal Nutrition, University of Veterinary and Animal Sciences, Outfall Road, Lahore 54000, Pakistan

[2] Livestock Production Research Institute, Bahadurnagar Okara, 56300, Pakistan

Available ORCID

Author name	ORCID ID
Rana Yaser Arafat	0009-0000-4575-7646
Saima	0000-0003-2747-9691
Hifzulrahman	0000-0002-4090-5053
Ehsan Ullah Khan	0000-0003-1062-4223
Maqsood Akhtar	
Arfan Asghar	0009-0005-1375-7998
Muhammad Naveed-ul-Haque	0000-0003-1605-7629

Abstract

This study evaluated the effects of dietary starch and calcium salts of fatty acids on lactation performance, metabolic profiles, and methane emissions in lactating Nili Ravi buffalos. Sixteen lactating multiparous Nili Ravi buffaloes at 7.53 ± 0.76 kg/d of milk yield and 125 ± 45 DIM were randomly assigned to one of four treatments in a 4 × 4 Latin square design: low starch low fat (LSLF), low starch high fat (LSHF), high starch low fat (HSLF), and high starch high fat (HSHF). Each period lasted 21 days, with data collected during the last week. Experimental diets were formulated using the Cornell Penn Miner Dairy software, and all buffaloes received a fixed allowance of 16 kg of dry matter (DM) per day. The high fat treatments included 300 grams of calcium salts of fatty acids (Ca-FA) per animal per day, while starch levels were adjusted using maize grains.

Dry matter intake (DMI), body weight (BW), and milk yield were recorded daily, and milk composition was analyzed for fat, protein, and lactose content. Rumen fluid samples were taken for pH measurement, and blood samples were analyzed for plasma urea nitrogen (PUN), triglycerides, and glucose. Methane emissions were measured and expressed in MJ, Mcal, g/d, and g/kg DMI. The interaction between dietary starch and fat did not significantly affect DMI, BW, rumen pH, milk yield, milk protein percentage, or nitrogen efficiency. However, milk fat percentage and milk lactose percentage were significantly influenced by fat, with no significant interaction. Metabolic parameters, including plasma pH, glucose, cholesterol, urea, PUN, and triglycerides, showed non-significant effects. A notable interaction effect was observed for methane emissions per unit of DMI, suggesting a complex relationship between dietary components and methane emissions efficiency. Overall, the results showed that, while starch and fat had significant individual effects on several parameters, their interaction was non-significant, except for methane emissions per unit of DMI. The findings suggest that dietary starch and fat can be independently adjusted to optimize Nili Ravi lactating buffalo performance and milk composition. This study provides valuable insights for formulating diets aimed at enhancing productivity while mitigating methane emissions.

Keywords: Buffalo, starch, rumen bypass fat, milk yield, methane emissions

Introduction

Buffalo (*Bubalus bubalis)* is an important contributor of milk in Pakistan (60.93%) with a population of 41.2 million heads producing 37.3 metric tons of raw milk (FAOSTAT, 2020). Nili Ravi buffalo is regarded as the world's best milk-producing breed found primarily in Pakistan but the average milk production in buffaloes is still low (Pegolo *et* al. 2017). Buffalo milk is preferred over cow milk due to its high-fat content and organoleptic qualities (Bilal et al. 2006). Buffalo milk contains a high fat content of 7.8 ± 2.3% (Pegolo et al. 2017) compared with cow 3.84% (USDA, 2017) indicating higher energy demands. Fat and carbohydrates are the main energy components of ruminant nutrition (Schroeder et al. 2014; Carmo et al. 2015). Fat may be added as inert sources like calcium salts of long chain fatty acids (Ca-FA) and non-rumen inert sources like full-fat rapeseeds and oil (Bargo et al. 2003). In Pakistan, there is a traditional practice of feeding full-fat cotton seeds with lint as a source of bypass fat with the aim to increase milk fat content but it has not been defined into proper guidelines indicating the extent it may be included safely and cost effectively.

Glycogenic feedstuffs include cereal grains and their milling byproducts mainly provide fermentable energy (Rearte and Pieroni, 2021; Bargo et al. 2003). Energy based feedstuffs when added to ruminant diets, improve production performance (NRC, 2003). Fats as salts of Ca-FA when added to dairy buffalo rations improved milk yield and fat content (Hifzulrahman et al. 2020; Katiyar et al. 2019). High levels of Ca-FA could decrease dry matter intake (DMI) (Schauf and Clark, 1992; Rabiee et al. 2012) whereas, starch feeding improved DMI, milk yield, and fat content in cows (Sanches-Duarte et al. 2019; Piccicoli-Cappeli et al. 2014; Renolds et al. 2001). High starch diets could improve body condition scores by decreasing mobilization of adipose tissue (Haisan et al. 2021). Milk FAs play specific roles in producing its beneficial and hazardous effects on human health. Those FAs which are derived from the rumen bio hydrogenation process including C18:2 *cis*- 9, *cis*- 12 (C18:2 n-6, linoleic acid) FAs (Lock et al. 2004) are reported to produce cardiovascular ill effects. Feeding of Ca-FA affects de novo synthesis of FAs in milk with a decrease of up to 21.7 percent (Hifzulrahman et al. 2020). The buffalo are typically fed with low starch diets however being a source of glucose the higher level of starch feeding is hypothesized to improve lactation performance. Previous studies on investigating starch with Ca-FA in lactating Nili Ravi buffalo are not available as per author knowledge. However, the higher level of dietary starch in lactating ruminants leads to the synthesis of propionate in the rumen which is directly correlated with the milk volumes (NRC, 2003).

Nevertheless, the nutrient requirements of buffalo have not been properly defined, and the application of currently available data from lactating cow as general guidelines seems not precise is questionable as the data represents different types of species and sets of conditions applied.. The previous studies on feeding of salts of Ca-FA in lactating buffalo indicate improved lactating performance in terms of milk yield and milk components (Naik *et* al. 2009; Ranjan *et* al. 2012; Sharma *et* al. 2016; Hifzulrahman *et* al. 2020) but to our knowledge, no study has been conducted investigating the effects of Ca-FA and starch on production performance in lactating Nili Ravi buffalo. In the current study, we hypothesized that the response to milk yield and composition could be improved to Ca-FA and starch feeding. Therefore, the objectives of the current study were to determine the effects of low and high levels of dietary Ca-FA and starch 0g, 300g and 18.01% and 27.80% respectively, on milk yield, composition, blood metabolites, and methane emissions.

Materials and Methods

Animals

The experiment was conducted in a naturally ventilated tie stall barn located at the dairy sheds of Livestock Production Research Institute, Bahadurnagar Okara (30.801380 ^{0}N and 73.448334 ^{0}E with an altitude of 170 m (570 ft.). The entire experiment was conducted in accordance with regulations approved by the ethical committee for animal welfare at the University of Veterinary & Animal Sciences Lahore. Sixteen lactating multiparous Nili Ravi buffaloes, with (mean ± SD) 7.53 ± 0.76 kg/d of milk yield, 6.13 ± 0.42% milk fat, 512 ± 34 kg of BW, and 125 ± 45 DIM were used. The buffaloes had free access tofresh drinking water.

Experimental design, treatments, and feeding

Buffaloes were randomly assigned to treatment sequences in a 4 × 4 Latin square design, with a 21-day observation and data collecting period. The dietary treatments were silage and wheat straw-based TMR vise (1) low starch low fat (LSLF), (2) low starch high fat (LSHF), (3) high starch low fat (HSLF), and (4) high starch high fat (HSHF). In high fat (HF) TMR, 300 grams of 84% calcium salts of fatty acids (Ca-FA) per animal per day was offered. The starch content of TMR was maintained by the use of maize grains. The experimental diets were formulated using Cornell Penn Miner (CPM) Dairy Beta volume 3 software 3.0.10 from Cornell University (Ithaca, NY), University of Pennsylvania (Philadelphia, PA), and Miner Institute (Chazy, NY). The TMR composition and nutrient analysis on a DM basis is presented in Table 3.1. The Ca-FA feeding rate of 300g per animal per day was adjusted to ensure a consistent NE_L across all treatments. As an adjustment period, animals were fed TMR one week before the experiment began. TMR was offered as a fixed allowance. Because of their uniformity in live body weight, all buffaloes were given the same amount of DM (16 kg/buffalo per day) assuming a similar lactation persistency in the entire experiment. The buffaloes were fed once a day at 0900 h.

Sample collection and analyses

The diet offered as well as individual orts were weighed daily. Three samples of composite TMR from each treatment were collected in each period to evaluate DM and further laboratory analysis. These samples were analyzed for DM, CP, NDF, ADF, ether extract, and ash following the official methods of AOAC International (2005). The starch content of diets were analyzed as per protocol published by Anwar et al (2024). Rumen fluids were collected 4-5 hours postprandial for the estimation of rumen pH by oral stomach tubing following Muizelaar et al., and samples were immediately assessed for pH using Cardy Twin Soil and Water pH Meter; Spectrum Technologies, Inc. Plainfield, IL. Buffaloes were milked twice daily at 0200 and 1400 h. Milk production was recorded at each milking. Milk samples were taken on a daily basis. Milk production from morning and evening milking was pooled daily and assayed by infrared analysis using Lactoscan-S Milk Analyzer (Milkotronic Bulgaria) for fat, protein, and lactose. Blood samples were collected at end of each period from jugular vein following Haque et al. (2012). Blood samples were collected in heparinized syringes and immediately centrifuged at 2000 × *g* for 15 min at 4°C. Plasma was separated, aliquot stored at −20°C to be assayed by enzymatic method and estimated for the analysis of plasma urea nitrogen (PUN; Fluitest Urea, Analyticon Biotechnologies AG, Lichtenfels, Germany), triglycerides (Fluitest TG, Analyticon Bio- technologies AG, Lichtenfels, Germany), and glucose (BioMed-Glucose L.S, Egy-Chem for lab technology, Badar city, Elrubaki, Egypt). Buffaloes were weighed on d 21 of each treatment before feed distribution.

Statistical analysis

Data were analyze using the MIXED procedure of SAS University Edition (SAS Institute, Inc., Carry. NC with main effects of period and treatments, whereas buffalo were designed as random effect in this model. The following mathematical model was used for the analysis:

$$Y_{ijk} = \mu + \mathrm{Buff}_i + \mathrm{Per}_j + \mathrm{Treat}_k + \varepsilon_{ijk},$$

where Y is the response variable, μ is the overall mean, ε is the random error, Buff_i represents the random effectof buffalo, Per_j represents the 21-d period, and Treat_k represents the treatment diets of experiment. Four preplanned contrasts were performed: (1) low starch low fat (LSLF), (2) low starch high fat (LSHF), (3) high starch low fat (HSLF) and (4) high starch high fat (HSHF). Standard errors of the mean are reported and treatment differences were considered significant if $P \leq 0.05$ and as a trend for $0.05 < P \leq 0.10$.

Results

DMI, BW, milk yield, and composition

The results on DMI, BW, milk yield, and milk composition are presented in Table 3.2. The interaction between dietary starch and fat did not show significant effects on any of the measured parameters. Furthermore, levels of starch and fat, both had significant ($P \leq 0.05$) impact on the BW of buffaloes. Rumen pH and milk yield remained non-significant for both levels of fat and starch. However, for milk composition, milk fat, milk lactose, and milk fat yield significantly ($P \leq 0.01$) increased with an increase in fat. The HSHF treatment exhibited 17.43%, 6.42%, and 26.47% higher milk fat, milk lactose, and milk fat yield as compared to LSLF, respectively. Milk protein %, milk protein yield (g), and milk lactose yield (g) showed a non-significant impact for either of the treatments. These results suggest that there were no synergistic or antagonistic interactions between dietary starch and fat in influencing the productive and physiological parameters determined in this study.

Production efficiencies

Production efficiencies are presented in Table 3.3. The interaction between dietary starch and fat levels did not significantly affect any of the evaluated parameters in this study. However, 4% FCM, ECM, MEO, and the ratio of 4% FCM: DMI was significantly ($P \leq 0.05$) influenced by increasing fat levels and HSHF treatment exhibited 21.80%, 20.46%, 22.38%, and 25.00% higher output for the respective parameters to that of LSLF treatment. Parameters of PCMT, 3.4% PCM: DM, feed efficiency, and nitrogen efficiency were non-significant for all the treatments. Therefore, the results indicate that the dietary effect of starch and fat appears to operate independently without significant interactive influence on these parameters.

Blood metabolites

The results on plasma metabolites are presented in Table 4. The interaction between dietary starch and fat did not significantly affect any of the plasma metabolites measured in this study. These findings indicate that dietary starch and fat do not interact significantly to alter plasma metabolite profiles in lactating buffaloes for the parameters of plasma pH, plasma glucose, plasma cholesterol, plasma urea, plasma urea nitrogen, or triglycerides.

Methane emission

Results on methane emission are presented in Table 5. The interaction between dietary starch and fat did not significantly affect most of the methane production parameters in this study, although there were trends toward significance. Methane emission per unit of dry matter intake presented a significant interaction and significantly lower (6.02%, $P \leq 0.05$) methane was produced in HSJF treatment as compared to LSLF. Furthermore, individual treatment means showed significance for all the parameters. Methane energy production (CH_4 MJ and CH_4 Mcal), methane emission in grams per day (CH_4, g/d), and methane emission per unit of dry matter intake (CH_4, g/kg DMI) were 6.47%, 6.29%, and 6.48% lower in HSHF as compared to LSLF. These results suggest that higher starch and fat levels tend to reduce methane production and emissions, although these effects are not significantly interactive for most parameters. Though the combination of starch and fat in the diet may have a more complex relationship affecting methane emissions efficiency relative to intake.

Discussion

Our objective was to investigate the effects of feeding low and high levels of Ca-FA fat and starch on milk yield and composition in lactating Nili Ravi buffalo. Four dietary treatments LSLF (low starch low fat), LSHF (low starch high fat), HSLF (high starch low fat) and HSHF (high starch high fat) were offered. Rumen bypass fat; source of both saturated (C16:0) and unsaturated (C18:1) FAs was fed in the form of Ca-FA (calcium-fatty acid) complex to bypass the rumen as the bond of FAs with calcium slows down its degradation in the rumen.

Calcium salts of fatty acids did not affect feed intake

In the current study, we did not find any change in DMI in response to changes in starch and fat levels. The finding agrees with Ranjan et al. (2012) Shelke et al. (2012) and Hifzulrahman et al. (2019) reported no change in DMI. Contrary to our finding Hifzulrahman et al. (2020) reported a quadratic decrease in DMI when Ca-FA was fed 4.32 percent of DM per animal per day. In our study, the Ca-FA was offered 300g per animal per day which was lower as compared to the level offered by Hifzulrahman et al. (2020) therefore, it is possible that the daily allowance of Ca-FA in HF treatments is within rumen handling capacity to successfully bypass the rumen without impacting rumen fermentation as reported by Purushothaman et al. (2008). Fats in the form of Ca-FA did not dissociate and ferment in the rumen, it avoided the satiety effect in the current study. Another possible reason could be the higher starch levels in dietary treatments masked the satiety effect of Ca-FA.

Milk efficiencies improved with Ca-FA

Milk yield did not increase as a result of variation in starch and fat levels. The results are endorsed by Savsani et al. (2015) who reported no increase in milk yield despite feeding Ca-FA in diets of experimental animals. On contrary to our findings, Hifzulrahman et al. (2019), Mubeen et al. (2019), Katiyar et al. (2019) and Hifzulrahman et al. (2020) reported an increase in milk yield at high levels of Ca-FA. Moreover, in the current study plasma glucose levels remained unaffected with varying levels of fat and starch. This may be possible that FA from Ca-FA did not convert into glucose elevating the blood glucose level rather FAs were directly incorporated into milk (NRC, 2001). The difference in results could also be related to climatic conditions as in the current study the animals were stall fed in open sheds during the months of extreme heat that may have affected the milk volumes. Bouraoui et al. (2002), Kekana et al. (2018), and Yadhav et al. (2013) reported low milk yield in lactating animals when kept in a hot climate. However, in the current study, milk fat, 4% FCM, ECM, and MEO were increased with HF treatments as compared to LF. The findings agree with Hifzulrahman et al. (2019), Mubeen et al. (2019), Katiyar et al. (2019) and Hifzulrahman et al. (2020) who reported increased milk fat and lactose when high levels of Ca-FA were fed to the animals. This may be due to FA sparing effects when the animal is mainly deriving energy from starch sources, so the FAs are reserved to be directly incorporated into milk (NRC, 2003). The higher lactose content in

HF treatments showed that lactose was synthesized by the mammary tissues but it could not be reconstituted with water possibly on account of hot climatic conditions when water was mainly shunted to dissipate the body's heat increment instead of being utilized to reconstitute lactose to make higher milk volumes (NRC, 2003). The dietary treatments did not affect milk protein and lactose yield. The finding agrees with Madan et al. (2013), Savansi et al. (2015) and Rohila et al. (2016). Inconsistent to our finding Zedan et al. (2016), Hifzulrahman et al. (2019), Mubeen et al. (2019) reported an improvement in milk protein content. Milk protein and lactose are more or less constant milk variable and usually do not change much with different dietary regimens. Therefore, milk protein and lactose yield remained unaltered in current study. Milk protein where increased was reported to be on account of better energy and protein utilization leading to AA sparing effects.

High Rumen pH resistance in Nili Ravi buffalo

In the current study, higher levels of fat and starch treatments did not affect rumen pH. The finding agrees with Gao and Oba (2016) who fed high starch diet and Zedan et al. (2016) who fed high Ca-FA diets reported no change in rumen pH. Contrary to our findings, Huntington et al. (2006) and Dijkstra et al. (2012) reported the development of rumen acidosis when high levels of dietary starch were fed to lactating cattle due to rapid fermentation of starch leading to a sudden drop in rumen pH. As per our best knowledge, there are no previous studies on high starch feeding in lactating Nili Ravi buffalo. A possible reason for unchanged rumen pH might be the effective fiber in our dietary treatments as in the current study all the treatments were offered as wheat straw-based TMR providing requisite effective fiber for normal functioning of rumen did not result in sudden fall of pH. The buffalo is considered to be a more efficient converter of feedstuffs as compared to cattle (Marai and Haeeb 2010, Pasha and Zafar 2013). Furthermore, buffalo have stronger rumination behavior than cattle (Vega et al. 2010) which may have resulted in the production of more saliva. The saliva contains bicarbonate ions leading to the neutralization of acids produced as a result of rapid starch fermentation (NRC, 2001).

Ca-FA and starch did not impact the plasma metabolites profile

In the current study, varying levels of starch and fat did not influence the profile of plasma metabolites. The finding agrees with Zedan et al. (2016) who reported no change in blood glucose when fed Ca-FA to lactating buffalo. In agreement with our study, Shelke et al. (2012) also reported no change in plasma triglycerides and cholesterol with high Ca-FA diets. On contrary to that Zedan et al. (2016) reported high plasma cholesterol and total lipids with high Ca-FA diets. Blood glucose is not affected by ground corn grain-based diets (Savari et al. 2018) endorse the finding of the current study. A possible reason for unchanged blood glucose levels may be increased utilization of corn starch in the rumen than in the small intestine as higher availability of glucose in the small intestine elevates blood glucose levels (Plascencia and Zinn, 1996). Plasma urea and plasma urea nitrogen are functions of protein metabolism especially rumen degradable fraction of dietary protein than starch and fat (Roseler et al. 1993) who reported high RDP related to high plasma urea nitrogen.

Ca-FA and starch helped to reduce methane emission in lactating buffalo

Methane emission is reduced in the current study as a result of feeding higher levels of fat and starch and upon the interaction of both. Similar to our results, Machmuller et al. (1998) reported low methane emissions with dietary rumen-protected fat. However, Jeyanathan et al. (2019) and Bougouin et al. (2019) reported no decrease in methane emission as a result of low starch and or fat feeding. Contrary to the results of our study, Borsting et al. (2020) and Darabhigane et al. (2021) reported a decrease in methane emissions as a result of feeding low-starch diets to dairy cattle. Rumen-protected fats indirectly suppress methane emission on account of the exchange of starch content in the diet as starch is directly related to high methane emission (Behan et al. 2019). In the current study addition of Ca-FA, the proportion of methane reduction was only 1.31 percent whereas with LS diets the proportion of methane reduction was higher 5.94 percent. This is possibly on account of low starch substrate availability to rumen starch degrading microflora ultimately leading to a low count of such microflora. Longer rumination time is associated with low methane production in the rumen (Baceninaite et al. 2022) as buffalo are regarded as more proficient in rumination with longer rumination times as compared to cattle (Vega et al. 2010).

Conclusions and recommendations

This study investigated the impact of dietary starch and protected fat levels on various production, metabolic, and methane emission parameters in dairy cows. Results showed that while starch and fat independently influenced several parameters, their interaction was generally not significant. Milk fat and lactose percentages were significantly affected by fat but without interaction effects. Metabolic parameters also showed non-significant effects and interactions. These findings imply that starch and fat levels in lactating Nili Ravi buffalo diets can be adjusted separately to optimize performance and milk composition, with particular consideration concerning methane emissions.

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Data availability The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable

Declarations

Ethics approval The entire experiment was conducted in accordance with regulations by the ethical committee for animal welfare at University of Veterinary and Animal Sciences, Lahore, Pakistan.

Consent to participate Not applicable

Consent for publication Not applicable

Conflict of Interest The authors declare no conflicts of interest.

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Table 1 Ingredients and nutrient composition of dietary treatments

	Treatments[a]			
Item	LSLF	LSHF	HSLF	HSHF
Ingredient (% of DM)				
Wheat straw	34	33.3	34	33.32
Corn grain	13.3	13.03	26.6	26.08
Wheat bran	23	22.5	23	22.5
Canola meal	5.33	5.22	5.34	5.23
Soybean hulls	16.8	16.5	3.37	3.3
Molasses cane	2.7	2.64	2.59	2.54
Corn gluten meal 60%	1.71	1.68	1.72	1.68
Soybean meal	1.33	1.3	1.33	1.3
Calcium carbonate	0.74	0.72	0.74	0.72
Urea	0.37	0.36	0.51	0.5
Salt	0.37	0.36	0.37	0.36
Mineral premix	0.37	0.36	0.37	0.36
Bypass fat	0	0.3	0	0.3
Analyzed nutrient composition				
%DM	90.2	90.3	90.1	90.2
OM, % of DM	93.3	93.1	94	93.8
CP, % of DM	12	11.7	12	11.7
NDF, % of DM	51.1	50.1	43.5	42.5
ADF, % of DM	30	29.3	24.2	23.7
Forage NDF, % of DM	26.8	26.3	26.9	26.3
NFC, % of DM	29.4	28.8	37.3	36.5
Ether extract, % of DM	2.95	4.66	3.16	4.87
Starch, % of DM	18.01	17.6	27.8	27.3
Ash, % of DM	6.7	6.88	5.97	6.17
Predicted nutrient value[2]				
MP, g/kg of DM	90.2	90.2	92.2	92.2
RUP, % of CP	34.2	34.4	33.3	33.5
RDP, % of CP	65.8	65.6	66.7	66.5
ME, Mcal/kg of DM	2.22	2.35	2.35	2.47
NE_L, Mcal/kg of DM	1.43	1.51	1.51	1.59
Total	100	100.3	99.7	100

[a] The treatments LSLF = low starch low fat with starch 18.01% and fat 2.95%, LSHF = low starch high fat with starch 17.76% and fat 4.66%, HSLF = high starch low fat with starch 27.8% and fat 3.16%, HSHF = high starch high fat with starch 27.3% and fat 4.87% of DM

[2] Bypass fat, 300 g of 84% bypass fat commercial product

[3] *MP* metabolizable protein, *RUP* rumen un-degradable protein, *RDP* rumen degradable protein, *ME* metabolizable energy, NE_L net energy of lactation: values predicted using CNCPS evaluations.

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Table 2 Effects of Ca-FA and starch on rumen pH, milk yield and composition in lactating Nili Ravi buffalo

	Treatment[a]				SEM	P-value		
Item	LSLF	LSHF	HSLF	HSHF		Starch	Fat	starch × fat
DMI (kg/d)	13.3	13.2	13.2	13.2	0.03	0.15	0.37	0.17
BW (kg)	582	590	589	595	12.06	<0.01	0.02	0.21
Rumen pH	6.73	6.73	6.73	6.69	0.015	0.28	0.18	0.18
Milk yield (kg/d)	7.35	7.65	7.92	8.01	0.376	0.20	0.59	0.77
Milk fat (%)	7.17	8.27	7.28	8.42	0.174	0.43	<0.01	0.9
Milk protein (%)	3.53	3.57	3.52	3.69	0.072	0.45	0.16	0.35
Milk lactose (%)	4.36	4.58	4.39	4.64	0.043	0.32	<0.01	0.72
Milk fat yield (g)	529	627	575	669	28.9	0.13	<0.01	0.93
Milk protein yield (g)	260	273	279	295	14	0.13	0.29	0.92
Milk lactose yield (g)	320	349	348	373	16.9	0.12	0.11	0.93

[a] The treatments LSLF = low starch low fat with starch 18.01% and fat 2.95%, LSHF = low starch high fat with starch 17.76% and fat 4.66%, HSLF = high starch low fat with starch 27.8% and fat 3.16%, HSHF = high starch high fat with starch 27.3% and fat 4.87% of DM

Table 3 Effect of Ca-FA and starch on production efficiencies in lactating Nili Ravi buffalo

Item	Treatment[a]				SEM	*P*-value		
	LSLF	LSHF	HSLF	HSHF		Starch	Fat	starch × fat
4%FCM	10.87	12.47	11.8	13.24	0.57	0.14	0.01	0.89
ECM	11.24	12.71	12.18	13.54	0.579	0.13	0.02	0.92
MEO	7.64	8.74	8.29	9.35	0.397	0.12	0.01	0.96
PCMT	5.86	6.46	6.7	7.21	0.626	0.19	0.36	0.94
4%FCM:DMI	0.8	0.9	0.9	1.0	0.043	0.13	0.01	0.85
3.4%PCM:DMI	0.57	0.61	0.62	0.66	0.031	0.12	0.29	0.96
Feed Efficiency	0.55	0.58	0.6	0.61	0.029	0.18	0.57	0.74
N Efficiency	16.33	17.23	17.24	18.2	0.877	0.28	0.29	0.29

[a] The treatments LSLF = low starch low fat with starch 18.01% and fat 2.95%, LSHF = low starch high fat with starch 17.76% and fat 4.66%, HSLF = high starch low fat with starch 27.8% and fat 3.16%, HSHF = high starch high fat with starch 27.3% and fat 4.87% of DM

FCM (4%) fat corrected milk = (0.4×milk yield)+15×(milk fat content/1000)×milk yield, ECM energy corrected milk = (0.327×milk yield+12.95×milk fat yield/1000)+(7.65×milk protein yield/1000), MEO milk energy output = 0.00929×milk fat yield+0.00563×milk protein yield+.00395×milk lactose yield, 3.4% PCM protein corrected milk = milk yield × 0.294 × milk protein content

Table 4 Effect of Ca-FA and starch on blood metabolites in lactating Nili Ravi buffalo

	Treatment[a]				SEM	*P*-value		
Item	LSLF	LSHF	HSLF	HSHF		Starch	Fat	starch × fat
Plasma pH	7.59	7.58	7.59	7.58	0.006	0.48	0.76	1
Plasma glucose (mg/dL)	53.1	48.1	45.4	47.1	3.05	0.14	0.56	0.26
Plasma cholesterol (mg/dL)	85.8	87.4	86.9	87.6	6.96	0.9	0.82	0.93
Plasma urea	40.7	41.9	39.1	41.1	1.84	0.5	0.36	0.8
PUN[b] (mg/dL)	18.1	19.6	18.3	19.2	0.73	0.93	0.11	0.67
Triglycerides (mg/dL)	6.75	6.81	7.31	7.63	0.617	0.19	0.72	0.81

PUN plasma urea nitrogen

[a] The treatments LSLF = low starch low fat with starch 18.01% and fat 2.95%, LSHF = low starch high fat with starch 17.76% and fat 4.66%, HSLF = high starch low fat with starch 27.8% and fat 3.16%, HSHF = high starch high fat with starch 27.3% and fat 4.87% of DM

Table 5 Effect of Ca-FA and starch on methane emission

	Treatment[*]					*P*-value		
Item	LSLF	LSHF	HSLF	HSHF	SEM	Starch	Fat	starch × fat
CH4, MJ	13.3	13.13	12.51	12.44	0.026	<0.01	<0.01	0.07
CH4, Mcal	3.18	3.14	2.99	2.98	0.006	<0.01	<0.01	0.07
CH4, g/d	223	220	210	209	0.431	<0.01	<0.01	0.07
CH4, g/kg DMI	16.8	16.67	15.9	15.79	0.001	<0.01	<0.01	<0.01

* The treatments LSLF = low starch low fat with starch 18.01% and fat 2.95%, LSHF = low starch high fat with starch 17.76% and fat 4.66%, HSLF = high starch low fat with starch 27.8% and fat 3.16%, HSHF = high starch high fat with starch 27.3% and fat 4.87