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EFFECT OF GRADED SUPPLEMENTATION OF CALCIUM SALTS OF PALM FATTY ACIDS ON LACTATION PERFORMANCE OF NILI RAVI BUFFALOES

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ABSTRACT

The objective of this study was to investigate the effect of graded amount of calcium salts of palm fatty acids (Ca-PFA) supplementation on dry matter intake, milk yield, milk fat, and milk fatty acid profile in lactating buffalo. Twelve multiparous early-lactating buffaloes were arranged in a 4 × 4 Latin-square design with a period length of 21 day. The 4 treatments were basal diet with supplementation of 0, 200, 400, and 600 g of Ca-PFA per day. The 3.5% fat-corrected milk yield, milk fat content, and milk fat yield showed a quadratic function with a transient maximumat 400 g/d of supplemental level. The concentration and yield of C16:0 and C18:0 increased linearly, whereas concentration of C16:1 and C18:2 passed a transient maximum and afterward decreased upon intake of Ca-PFA. The C16:0 yield tended to increase linearly, whereas C16:1 yield showed a quadratic function with increasing Ca-PFA intake. Cumulatively, increasing the Ca-PFA supplementation decreased the content and yield of de novo milk FA by 21.7% and increased preformed milk FA by approximately 10.0%. In conclusion, under the current feeding scheme, the elevated Ca-PFA intake increased milk and milk fat yields and the responses were maximal at 400 g/dof Ca-PFA supplemental level.

Key words: Palm fatty acid, milk yield, milk fatty acid, buffalo.

INTRODUCTION

The water buffalo (*Bubalus bubalis*) is ranked second among livestock because of its contribution to global milk supplies (15% of the total) total amounting to 120 billion liters produced annually (FAOSTAT, 2017). In Pakistan, 13.7 million milking buffaloes produced around 27.3 billion liters of milk with herd average of 5.45kg/buffalo per day (FAOSTAT, 2017), far lower than the milk average of Holstein (28.7 kg/d; FAOSTAT, 2017). Conversely, buffalo milk contains higher fat content (7.8 ± 2.3%; Pegolo *et al.,* 2017) compared with cow milk (3.84%; USDA, 2017). Increased milk fat-to-protein ratio in buffalo milk indicates that the demand of energy for milk production in lactating buffalo might be higher compared to cow. Interestingly, the fatty acid (FA) composition of buffalo milk is similar to cow milk (Pegolo *et al.,* 2017). This similar composition of FA in buffalo and cow milk offers an opportunity to investigate the advantages of fat feeding to increase milk yield and milk composition in buffalo.

Calcium salts of palm fatty acids (Ca-PFA)-based products are the alternate sources of energy that has been shown to increase milk yield from 15 to 20% and milk fat yield from 8.36 to 10.5% in dairy cow (Batistel *et al.,* 2017; de Souza *et al.,* 2017). Likewise, a study conducted in lactating buffalo by feeding 300 g/d of Ca-PFA observed an increase of 22% in milk yield (Polidori *et al.,* 1997). Recently, our research group investigated the effects of feeding Ca-PFA in lactating buffalo and observed an increased milk yield by 7.85% and milk fat yield by 14.3% (Hifzulrahman *et al.,* 2019). Nevertheless, high levels of feeding Ca-FA could decrease the DMI (Schauf and Clark, 1992; Rabiee *et al.,* 2012) and consequently the milk yield (Schauf and Clark, 1992). Previously, Ca-PFA was fed from 100 to 300 g/d in lactating buffalo (Polidori *et al.,* 1997; Ranjan *et al.,* 2012) and from 100 to 1764 g/d in dairy cow (Schauff and Clark, 1992; Piperova *et al.,* 2004; Beaulieu and Palmquist, 1995). To our knowledge, no study has been conducted to investigate the optimal amount of Ca-PFA to supplement in the diets fed to lactating buffalo. Our hypothesis is that the positive impact on milk yield would decrease after passing the optimum supplementation of Ca-PFA. Therefore, the aim of this study was to determine the optimum level of supplementing the Ca-PFA in lactating buffalo by graded increase in its level from 0 to 600 g/d in the diet and observing its effects on DMI, milk yield, milk fat, and milk FA profile.

MATERIALS AND METHODS

Animals: The experiment was conducted from November 2015 to January 2016 in a tie-stall barn located at University of Veterinary & Animal Sciences, Ravi Campus (31.02°N, 73.85°E, and 186 m altitude; Pattoki, Pakistan). Twelve lactating multiparous Nili Ravi buffaloes, with (mean ± SD) 9.30 ± 1.39 kg/d of milk

yield, 5.06 ± 0.91% milk fat, 506 ± 54 kg of BW, and 56 ± 21 DIM were used. The buffaloes were kept individually in a naturally ventilated barn and had free access to automatic drinking bowls.

Treatments and Experimental Design: Dietary treatments were consisted of no supplemental fat and 3 supplemental levels of Ca-PFA (RumiFat Plus, EcolexSdn. Bhd. Selangor, Malaysia) added to the basal diet in a 4 × 4 Latin square design. The duration of each period was 21 day given one week of adaptation before each period. The amount of Ca-PFA was supplemented daily to target intakes of 0, 200, 400, and 600 g/d per buffalo. The diet was formulated using Cornell-Penn-Miner-Dairy 3.0.10 from Cornell University (Ithaca, NY), University of Pennsylvania (Philadelphia, PA), and Miner Institute (Chazy, NY), based on CNCPS 5.0.2 (Fox *et al.,* 2003). The diet was formulated to support 9 kg of milk with 8% milk fat and 4% crude protein in milk. The ME balance and MP balance were supporting this production as per CNCPS model. Ingredient and chemical composition of the diet is presented in Table 1 and 2. The Ca-PFA supplement (RumiFat Plus) consisted of 1.2% myristic acid, 47% palmitic acid, 5.0% stearic acid, 38% oleic acid, and 8.0% linoleic acid. The diets were offered in a fixed amount per buffalo and Ca-PFA supplement was top-dressed individually. Buffaloes were relatively similar in BW; hence, they were offered a similar quantity of DM (13.9 kg/buffalo per day on DM basis) assuming similar lactation persistency in the entire experiment. Separately prepared concentrate was mixed with silage and wheat straw before offering to each group. The buffaloes were fed once daily at 0900 h.

Experimental Measures, Sampling, and Analyses: Feed intake was observed on daily basis. Three samples of each feedstuff were collected weekly in each period to evaluate the DM and for further laboratory analysis. Samples of each feedstuff in triplicate were collected weekly in each period and analyzed for chemical composition following AOAC international (2005). Buffaloes were milked twice daily at 0600 and 1800 h. Milk production was recorded at each milking. Milk samples were taken on alternate days in first 2 weeks and continuously from day 15 to 21 of each period. Milk production from morning and evening milking were pooled daily and analyzed by Gerber method for milk fat content (IDF, 1981). An aliquot of milk from day 21 of each period was stored at −20°C without preservative until analyzed for FA profile as described by Hifzulrahman *et al.* (2019). Samples from all the buffaloes were collected on 3rd last day of each period in heparinized syringes and immediately centrifuged at 2,000 × g for 15 min at 4°C. Plasma was separated, aliquoted, and stored at −20°C to be assayed by enzymatic method as described by Hifzulrahman *et al.* (2019).

Calculations and Statistics: Data were analyzed using the GLIMMIX procedure of SAS University Edition (SAS Institute Inc., Cary, NC), with main effects of period and treatments, whereas buffaloes were designated as random effect in the model. Treatments were compared with linear and quadratic polynomial models to examine the response surface for the level of Ca-PFA. 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

Fatty Acids Intake: The FA intakes are presented in Table 3. The intake of C16:0 was increased by 553% with the supplemental level of 600 g/d of Ca-PFA. However, the intake of C16:1 was linearly decreased ($P<0.01$) with increasing Ca-PFA intake. Similarly, the total FA intake was linearly increased ($P \leq 0.01$) by 173% with 600 g/d of Ca-PFA intake.

Feed Intake, Milk Production, and Milk Composition: Dry matter intake, milk production, and milk fat results are presented in Table 4. A trend for a quadratic decrease ($P=0.08$) was observed on DMI with increasing Ca-PFA intake. The Ca-PFA intake increased the milk yield quadratically ($P=0.04$). Milk fat concentration and yields of 3.5% FCM and milk fat showed a quadratic increase ($P<0.05$) and were maximized with 400 g/d of supplemental level.

Milk Fatty Acids Concentration: The concentrations of milk FA are presented in Table 5. Concentrations of C6:0-C14:1 were linearly decreased by increasing Ca-PFA supplementation ($P \leq 0.01$). Increasing the Ca-PFA intake showed a linear increase in the concentration of C16:0 ($P \leq 0.01$) and a quadratic increase in C16:1 ($P \leq 0.01$). The C18:0 increased linearly ($P \leq 0.01$) and C18:2 quadratically ($P \leq 0.01$) with increasing Ca-PFA intake, whereas concentration of C18:1 was not affected by the treatments. The Ca-PFA supplementation decreased linearly the content of de novo milk FA by 21.7% ($P \leq 0.01$), whereas increased the concentration of mixed milk FA and preformed milk FA by 7.63 and 10.6%, respectively ($P \leq 0.01$). Increasing the Ca-PFA intake showed a quadratic effect in the concentration of SFA and PUFA ($P \leq 0.01$), whereas MUFA remained unaffected ($P>0.10$).

Milk Fatty Acids Yield: Milk FA yields are presented in Table 6. Increasing the Ca-PFA supplementation decreased the yield of C6:0, C8:0, C10:0, and C12:0 linearly ($P \leq 0.01$). The C14:0 yield showed a decreasing tendency linearly ($P=0.07$), whereas C14:1 yield decreased quadratically ($P \leq 0.01$). The C16:0 yield tended to increase linearly ($P=0.08$), whereas C16:1 yield increased quadratically ($P=0.01$) with increasing Ca-

PFAintake. The Ca-PFA intake increased the yield of C18:0 linearly (P≤0.01) and the yield of C18:2 quadratically (P=0.01). The yield of the C18:1 remained unaffected (P>0.10). The yield of de novo milk FA was decreased by 21.8% (P<0.01). Preformed milk FA were linearly increased by 9.88% (P=0.03) with increasing Ca-PFA intake.

Table 1. Ingredient composition of the basal diet.

Ingredient	% of DM
Corn silage	21.7
Wheat straw	27.4
Wheat bran	12.8
Canola meal	6.50
Ground corn	15.9
Sugarcane molasses	5.27
Soybean meal	9.74
Mineral premix	0.72
Total	100

Table 2. Nutrient composition of the treatment[1] diets.

Item	0	200	400	600
Ingredient, (% of DM, unless noted)				
DM, (%)	62.7	63.0	63.4	63.7
Forage	49.1	48.3	47.6	46.8
CP	12.0	11.8	11.7	11.5
NDF	45.1	44.4	43.7	43.0
ADF	27.1	26.7	26.3	25.8
NFC	34.4	33.9	33.5	32.9
Ether extract total	2.69	3.95	5.21	6.47
Ash	7.61	7.69	7.78	7.86
Predicted nutritive values				
RUP, (% CP)	32.2	32.1	32.1	32.0
ME, (Mcal/kg)	2.23	2.33	2.43	2.53
$NE_{L,}$ (Mcal/kg)	1.44	1.50	1.57	1.63

[1]Treaments consisted of the basal ration (0) plus calcium salts of palm fatty acid (RumiFat Plus, EcolexSdn. Bhd. Selangor, Malaysia) to target intakes of 200, 400, and 600 g/d.

Plasma Metabolites: Results of plasma metabolites are summarized in Table 7. Plasma urea nitrogen was affected quadratically by increasing Ca-PFA intake (P=0.01), whereas blood glucose and triglyceride concentrations were not different across the treatment levels (P>0.10).

Table 3. Fatty acid (FA) intake in different treatments[1].

	Treatment					*P-value*	
FA intake, (g/d)	0	200	400	600	SEM	Linear	Quadratic
C12:0	0.47	0.79	1.11	1.42	0.005	<0.01	0.58
C14:0	2.51	5.07	7.69	10.2	0.04	<0.01	0.39
C16:0	41.2	117	194	269	0.9	<0.01	0.63
C16:1	0.68	0.67	0.66	0.65	0.004	<0.01	0.90
C18:0	5.73	12.3	19.0	25.5	0.09	<0.01	0.53
C18:1	57.5	115	173	229	0.8	<0.01	0.38
C18:2	133	143	153	161	0.8	<0.01	0.35
C18:3	28.8	28.2	27.9	27.4	0.17	<0.01	0.81
Other	4.62	11.7	18.9	25.9	0.09	<0.01	0.64
Total FA	275	433	595	750	2.8	<0.01	0.60

[1]Treaments consisted of the basal ration (0) plus calcium salts of palm fatty acid (RumiFat Plus, EcolexSdn. Bhd. Selangor, Malaysia) to target intakes of 200, 400, and 600 g/d.

Table 4. Response of DMI, milk yield, and milk fat in different treatments[1].

	Treatment					*P-value*	
Item	0	200	400	600	SEM	Linear	Quadratic
DMI, (kg/d)	12.5	12.6	12.5	12.4	0.07	0.17	0.08
Milk yield, (kg/d)	8.67	9.05	9.39	8.84	0.357	0.39	0.04
3.5% FCM,(kg/d)	11.1	12.3	13.0	11.8	0.51	0.09	<0.01
Milk fat, (%)	5.55	5.72	5.99	5.73	0.115	0.07	0.04
Milk fat yield,(g/d)	465	514	553	503	24.0	0.05	0.01

[1]Treaments consisted of the basal ration (0) plus calcium salts of palm fatty acid (RumiFat Plus, EcolexSdn. Bhd. Selangor, Malaysia) to target intakes of 200, 400, and 600 g/d.

Table 5. Milk fatty acid profile.

	Treatment[1]					*P-value*	
Item	**0**	**200**	**400**	**600**	**SEM**	**Linear**	**Quadratic**
Fatty acid, (g/100 g)							
C6:0	1.85	1.65	1.13	0.93	0.115	<0.01	1.00
C8:0	1.00	0.83	0.68	0.60	0.033	<0.01	0.12
C10:0	1.95	1.55	1.60	1.35	0.064	<0.01	0.10
C12:0	2.38	2.03	1.80	1.70	0.057	<0.01	0.04
C14:0	10.5	9.98	9.25	9.30	0.162	<0.01	0.07
C14:1	1.70	1.98	1.88	1.33	0.097	<0.01	<0.01
C16:0	35.4	37.2	36.7	38.7	0.41	<0.01	0.87
C16:1	3.88	4.35	4.13	3.60	0.166	0.14	<0.01
C18:0	13.9	12.0	14.2	16.0	0.24	<0.01	<0.01
C18:1	18.9	18.2	19.1	19.3	0.61	0.42	0.48
C18:2	2.27	3.82	2.63	3.22	0.148	0.01	<0.01
Summation by source							
∑<C16	19.4	18.0	16.3	15.2	0.29	<0.01	0.64
∑ C16:0 + C16:1	39.3	41.6	40.9	42.3	0.47	<0.01	0.30
∑>C16	35.0	34.0	37.5	38.7	0.75	<0.01	0.13
Summation by saturation							
∑ Saturated	67.0	65.3	65.4	68.6	0.54	0.05	<0.01
∑ Monounsaturated	24.5	24.6	25.1	24.3	0.47	0.98	0.32
∑ Polyunsaturated	2.27	3.82	2.63	3.22	0.148	0.01	<0.01

[1]Treaments consisted of the basal ration (0) plus calcium salts of palm fatty acid (RumiFat Plus, EcolexSdn. Bhd. Selangor, Malaysia) to target intakes of 200, 400, and 600 g/d.

Table 6. Milk fatty acid yield.

	Treatment[1]					*P-value*	
Fatty acid, (g/d)	**0**	**200**	**400**	**600**	**SEM**	**Linear**	**Quadratic**
C6:0	9.17	8.40	5.94	4.53	0.715	<0.01	0.66
C8:0	4.94	4.21	3.56	2.94	0.269	<0.01	0.82
C10:0	9.62	7.90	8.51	6.65	0.548	<0.01	0.86
C12:0	11.7	10.3	9.55	8.35	0.565	<0.01	0.90
C14:0	51.8	50.7	49.2	45.8	2.61	0.07	0.62
C14:1	8.38	10.0	10.0	6.54	0.685	0.04	<0.01
C16:0	174	189	196	190	8.8	0.08	0.16
C16:1	19.0	22.2	21.9	17.8	1.23	0.46	<0.01
C18:0	68.2	61.0	75.5	78.6	3.29	<0.01	0.04
C18:1	92.6	92.6	102	95.3	5.42	0.39	0.46
C18:2	11.2	19.4	14.2	15.5	1.34	0.13	0.01
Summation by source							
∑<C16	95.6	91.5	86.8	74.8	4.70	<0.01	0.34
∑ C16:0 + C16:1	193	211	218	208	9.7	0.14	0.09
∑>C16	172	173	199	189	10.7	0.03	0.51
Summation by saturation							
∑ Saturated	329	331	348	337	15.8	0.46	0.61
∑ Monounsaturated	120	125	134	120	6.3	0.72	0.06
∑ Polyunsaturated	11.2	19.4	14.2	15.5	1.34	0.13	0.01

[1]Treaments consisted of the basal ration (0) plus calcium salts of palm fatty acid (RumiFat Plus, EcolexSdn. Bhd. Selangor, Malaysia) to target intakes of 200, 400, and 600 g/d.

Table 7. Plasma metabolites.

	Treatment					*P-value*	
Item, (mg/dL)	**0**	**200**	**400**	**600**	**SEM**	**Linear**	**Quadratic**
Plasma urea nitrogen	22.7	21.9	20.3	23.5	0.77	0.82	0.01
Glucose	92.2	89.9	91.6	95.0	2.72	0.41	0.31
Triglyceride	148	158	163	156	6.3	0.28	0.17

[1]Treaments consisted of the basal ration (0) plus calcium salts of palm fatty acid (RumiFat Plus, EcolexSdn. Bhd. Selangor, Malaysia) to target intakes of 200, 400, and 600 g/d.

DISCUSSION

Feeding of Ca-PFA in dairy cows has been shown to increase the milk yield and milk fat yield through increased energy supplies (Polidori *et al.,* 1997; de Souza *et al.,* 2017; Batistel *et al.,* 2017). Moreover, these positive effects can be achieved without the risk of acidosis associated with feeding high amount of fermentable carbohydrates (Jenkins and McGuire, 2006). However, Ca-FAs need to be used with caution, because, at higher levels of supplementation, it might negatively affect the ruminal fermentation and decrease the performance of dairy cow (Schauf and Clark, 1992; Beaulieu and Palmquist, 1995). Ca-PFA supplements are well-investigated and commonly used fat supplements of dairy cow ration. Yet, no study investigated the effects of graded supplementation of Ca-PFA in lactating buffaloto the best of our knowledge. Therefore, our objective was to determine the optimum feeding level of Ca-PFA on milk yield and milk FA profile in lactating buffaloes.

Slight Decrease in the DMI at High Ca-PFA Supplementation: In literature, variable responses on DMI were reported depending upon the amount of Ca-PFA supplementation. In the study by Schauf and Clark (1992), the DMI started decreasing when Ca-PFA intake was 735 g/d or 3% of the DMI. Contrary to this, in recent studies (de Souza *et al.,* 2017; Batistel *et al.,* 2017), supplementation of Ca-PFA showed no effect on DMI at feeding rate of 400 g/d (2-2.4% of the DMI). However, in the present study, the supplementation of Ca-PFA was increased from 0 to 4.8% of the DM and we observed a quadratic trend toward decrease in the DMI by 0.81% at the feeding level of 600 g/d. It is possible that higher supply of unsaturated FA in the rumen exceeds the microbial capacity to convert them into the saturated FA leading to impeded ruminal fermentation (NRC, 2001; Rabiee *et al.,* 2012). This could be further supported by the fact that the proportions of milk FA in our experiment were altered indicating a change in the ruminal fermentation (Beaulieu and Palmquist, 1995). It is also theorized that the unsaturated FA reaching the small intestine stimulate release of cholecystokinin and glucose-dependent insulinotropic peptide -1, both of which regulate satiety and DMI in the cow (see Choi and Palmquist, 1996; Bradford *et al.,* 2008; Relling and Reynolds, 2007).

3.5% FCM Yield Increased with Ca-PFA Supplementation: Present study showed a quadratic response in production parameters with increasing levels of the Ca-PFA. Previous studies reported increased milk production with Ca-PFA supplementation in lactating buffaloes (Polidori *et al.,* 1997) and cows (Schauf and Clark, 1992; de Souza *et al.,* 2017; Batistel *et al.,* 2017). Polidori *et al.* (1997) observed 22% increase in milk production with the supplementation of 300 g/d of Ca-PFA, whereas it was 8.3% at supplementation of 400 g/d in the current study. The high CP level of the diet (17.2% of DM) used in the study by Polidori *et al.* (1997) compared with the present study (11.7% of the DM) could be a possible explanation to this difference in milk yield as the interaction between concentration of protein and fat level has been reported in literature (Petit *et al.,* 2005; Santillo *et al.,* 2016). A logical explanation to the increased milk yield in our study is the increased supply of energy at high Ca-PFAsupplementation. Besides, the increase in milk yield could also be explained using glucose sparing mechanism proposed in the literature (Palmquist and Jenkins, 1980). Similarly, in present study this mechanism is further supported by lower yields of de novo FA and higher yields of preformed FA. The decrease in de novo FA synthesis spare extra glucose for lactose synthesis (Palmquist and Jenkins, 1980).On the other hand, higher use of preformed FA may also spare glucose for lactose synthesis and consequently the increased milk production (Cant *et al.,* 1993a, b).

Increased Milk Fat Yield with Ca-PFA Supplementation: In our study, the milk fat yield increased by 18.9% with 400 g/d Ca-PFA intake. These results were in agreement with the increase of milk fat yield by 10.5 and 6.56% reported by de Souza *et al.* (2017) and Batistel *et al.* (2017), respectively, by feeding 400 g/d Ca-PFA supplementation in dairy cow. The increase in milk fat yield observed in our study could be explained by the increased concentration and yield of preformed FA (18-C FA), which are preferred for milk fat synthesis during esterification process (Hansen and Knudsen, 1987). Besides, the higher milk C16:0 may further justify the increased milk fat content and yield in our study. It is possible that the dietary C16:0 stimulated

de novo synthesis of milk C16:0 (Rico *et al.,* 2014), thereby, incorporating it directly into triacylglycerol by dispersed mammary gland epithelial cells (Hansen and Knudsen, 1987). Moreover, C16:0 are preferably incorporated into milk fat by the mammary gland, compared to other FA (Loften *et al.,* 2014). This increase of milk C16:0 with increasing dietary intake of C16:0 is also reported by others (Batistel *et al.,* 2017; de Souza *et al.,* 2017; Schauf and Clark, 1992).

Production Responses Decreased by 600 g/d of Ca-PFA Supplementation: In our study, the responses of 3.5% FCM, milk fat content, and yield were maximal with 400 g/d of Ca-PFA and then decreased at 600 g/d. The decrease in milk yield at 600 g/d dose could be a result of over-supplementation of Ca-PFA in our study. The Ca-PFA and prilled fats have been reported to remain inert in the rumen provided they are supplemented below 3.5% or even 2-3% of DM in dairy cows (Grummer, 1988; Harvatine and Allen, 2006). In our study, the feeding of Ca-PFA was 3.8% of the DM at the dose of 600 g/d, consequently may not be rumen-inert to the same extent. Additionally, the protection of fats using calcium soap from ruminal biohydrogenation is also incomplete because unsaturated FA become available in rumen due to dissociation of the calcium ion (Wu *et al.,* 1991), which lead to increased concentration of trans FA (Giesy *et al.,* 2002; Chouinard *et al.,* 1997) and increased formation of biohydrogenation intermediates that negatively affect ruminal bacterial populations and biohydrogenation pathways (Maia *et al.,* 2007). This explanation is further supported by recent biohydrogenation theory, which suggests that under particular dietary conditions, intermediate products resulting from transformed ruminal biohydrogenation act on the mammary gland, which inhibits milk fat synthesis and affect milk FA profile (Bauman *et al.,* 2011).

Milk Fatty Acids Proportion was Altered with Ca-PFA Supplementation: We found a decrease in de novo FA yield (21.7%) and increase in preformed FA yield (~10.0%) with increasing Ca-PFA supplementation from 0 to 600 g/d, which is in agreement with the literature (Schauf and Clark, 1992; de Souza *et al.,* 2017). Counter relation between these two groups of FA (de novo versus preformed) has been observed previously (Glasser *et al.,* 2008). For example, de Souza *et al.* (2017) reported a decrease of 7.88% and increase of 13.0% in de novo and preformed milk FA, respectively, by feeding 400 g/d of Ca-PFA compared to control. In that context, the reduction in de novo milk FA synthesis might be due to greater dissociation of Ca-PFA resulting in increased quantity of some trans FA production as an intermediate of ruminal biohydrogenation, which inhibit milk FA synthesis (Bauman *et al.,* 2011; Bauman and Griinari, 2001).

Conclusions: In the current study, increasing the dietary intake of Ca-PFA (0, 200, 400, and 600 g/d) increased milk yield, milk fat yield, and altered milk FA profile in lactating Nili Ravi buffalo. Increased energy supplies primarily explain the increase in 3.5% FCM yield, whereas the decreased yield of de novo FA in milk and decrease in the transfer of C16:0 and 18-C FA from feed to milk indicate the use of these FA as metabolic fuel rather than export as milk triglycerides; hence, sparing glucose for lactose synthesis and increased milk yield. However, the increase in preformed FA yield and increasing trend of C16:0 yield jointly explained the increase in milk fat yield. In the current feeding scheme, Ca-PFA at the rate of 400 g/d is the optimum to produce positive productive response in lactating dairy buffaloes.

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