



# Effects of Feeding Calcium Salts of Palm Fatty Acids on Lactation and Reproduction Performance in Nili Ravi Buffaloes

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## ABSTRACT

The objective of present study was to evaluate the effect of Ca salts of palm fatty acids on milk production, milk fat, plasma metabolites, and reproductive parameters in early-lactating Nili Ravi buffaloes. Sixteen early-lactating Nili Ravi buffaloes received 2 treatments in a completely randomized design with a period length of 120-d. The treatments were 1) Ctrl = basal diet without supplementation of fat, 2) Ca-FA = basal diet with calcium salts of palm fatty acids supplementation. The Ca-FA treatment increased the 3.5% fat-corrected milk yield by 11.0% compared with the Ctrl, whereas, the actual milk yield remained unaffected. The Ca-FA treatment increased the concentration of milk fat and milk fat yield by 3.5 and 11.2%, respectively, compared with the Ctrl. However, the Ca-FA treatment did not change the plasma urea nitrogen, glucose, and triglyceride, compared with the Ctrl. Similarly, the reproductive indices were not different between Ca-FA treatment versus Ctrl. In conclusion, the Ca-FA are useful to increase milk production and milk fat yield without effecting the plasma metabolites and reproductive responses in early-lactating buffalo.

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## Authors' Contribution

Hifzulrahman, MA and MNH designed and conceived the experiments. Hifzulrahman, JAB and MS performed the experiment. Hifzulrahman and MUA analysed the data. Hifzulrahman and MUA wrote the manuscript. MNH and MA reviewed and revised the manuscript.

## Key words

Palm fatty acid, Milk yield, Milk fat, Reproduction, Nili Ravi Buffalo

## INTRODUCTION

At the onset of parturition as well as during early lactation, the decrease in dry matter intake is common in dairy animals that could limit the potential to produce milk production because of negative energy balance as suggested by NRC (2001). This period of negative energy balance leads to higher fat mobilization to support lactation production. One of the possible solutions to minimize the effect of negative energy balance is to increase the energy intake during early lactation (McCarthy *et al.*, 2015). Energy intake is most commonly increased by feeding concentrate, based on cereal grains (Bargo *et al.*, 2003), however, slug feeding of starch-based supplements may result in decreased dry matter intake (Auld *et al.*, 2013) and milk yield (Doyle *et al.*, 2006). Feeding of fats is another possible solution to increase the energy density of a diet. Fat feeding increases energy density and energy intake without the risk of acidosis associated with feeding highly fermentable carbohydrates (Jenkins and McGuire, 2006).

Vegetable oils are often fed in buffalo and cow diet and contains higher concentration of unsaturated fatty acids (FA) compared with the saturated FA. Negative effect of unsaturated FA was more often observed on dry matter intake, milk production, milk fat, and ruminal fermentation (Jenkins and Jenny, 1989; Pantoja *et al.*, 1996; Rabiee *et al.*, 2012). As a result, many studies (review by Jenkins and McGuire, 2006) were performed to investigate the fat feeding strategy without altering the ruminal fermentation. Two strategies were adopted to achieve this target: 1) protecting unsaturated FA by Ca salt formation and 2) feeding highly saturated FA.

Most Ca salts products used in dairy cow diet and available in market are derived from palm FA distillate, a by-product of palm oil processing and these products have balance of saturated and unsaturated FA, mainly C16:0 and 18-C FA (Rico *et al.*, 2014). A recent meta-analysis reported a positive effect of Ca salts of palm FA on production and composition of milk in dairy cows (Rabiee *et al.*, 2012). Fat supplementation increased probability of cows to become pregnant and decreased time to conception and interval to first ovulation during early lactation in dairy cows (de Veth *et al.*, 2009). Other studies also reported improved performance of reproduction on fat feeding, but

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results were inconsistent (Santos *et al.*, 2008).

As per authors' knowledge, few studies evaluated the response of rumen-inert fats on milk production and composition in buffaloes (Polidori *et al.*, 1997; Shelke *et al.*, 2012) and only single study reported the reproductive response of fat feeding in buffalo (Ramteke *et al.*, 2014). Therefore, the fat feeding topic has received so far, less attention for buffalo than for cows and is not much investigated to date. Hence, the current study was planned with the objective to investigate the effects of feeding Ca salts of palm FA on milk production, milk fat, plasma metabolites, and reproductive performance in early-lactating Nili Ravi buffaloes.

## MATERIALS AND METHODS

### *Animals*

The study was conducted from November 2016 to February 2017 in a tie-stall pen located at the Buffalo Research Institute, Livestock and Dairy Development Department, Punjab (31.02°N, 73.85°E, and 186 m altitude; Pattoki, Pakistan). The experiment was performed in accordance with the guidelines by University of Veterinary and Animal Sciences, ethical committee for animal welfare. Sixteen lactating Nili Ravi buffaloes were grouped on the basis of their days in milk (pretrial average days in milk = 18 ± 8.91; mean ± SD) and daily milk yield (pretrial average milk yield = 6.73 ± 2.49 kg/d and milk fat = 5.60 ± 0.56%; mean ± SD). All the buffaloes were kept and fed treatment diets in a naturally ventilated barn and water was available to all animals round the clock.

### *Experimental design, treatments and feedings*

The buffaloes were randomly assigned to two treatment diets in a complete randomized design for the period length of 120-d. The treatments were 1) Ctrl = basal diet without supplementation of fat and 2) Ca-FA = basal diet with supplementation of calcium salts of palm FA (Energizer Gold; IFFCO, Johor, Malaysia). The Ca-FA supplement contained mainly C16:0 (47.0%), C18:1 (38.0%), and C18:2 (8.0%) in FA profile. The Ca-FA supplement was top-dressed to provide 200 g of Ca salts of palm FA/buffalo per day. Diet was composed of oats fodder and wheat straw fed ad-libitum along with a prepared concentrate at the rate of 5 kg per buffalo per day (Table I). The diets were 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 Cornell Net Carbohydrate and Protein System 5.0.2. All the buffaloes were fed as a single herd and feeding time was 0900 h and frequency was once daily.

**Table I. Ingredient and nutrient composition of basal diet.**

Item	Diet
<b>Ingredient (% of DM)</b>	
Wheat bran	35.0
Corn gluten feed	15.0
Sugarcane molasses	13.0
Corn grain	13.0
Rapeseed meal	10.0
Canola meal	7.00
Soybean meal	4.00
Calcium carbonate	0.50
Mineral mixture	2.00
Salt	0.50
<b>Nutrient composition</b>	
Dry matter (%)	86.8
Crude protein (%)	19.7
Neutral detergent Fiber (%)	28.2
Non-fibrous carbohydrate (%)	43.3
Ether extract (%)	3.00
Ash (%)	9.70
Predicted ME (mcal/kg)	2.90

### *Sampling and analysis*

Buffaloes were milked out by hand milking procedure at 0600 and 1800 h and milk yield were recorded daily for each buffalo individually (Mor *et al.*, 2018). Samples of milk were collected after each week to analyze percentage of milk fat through Gerber method (ISO, 1976). Feed samples of basal diet was analyzed for proximate analysis according to the procedure given by the AOAC (2005). Blood samples of all buffaloes were collected on monthly basis from jugular vein. Samples after collection in heparinized syringes were immediately centrifuged at 2000 rpm for 15 minutes. Plasma was separated, aliquoted, and stored at -20 °C to be assayed by enzymatic method and estimated for the analysis of Glucose (BioMed-Glucose L.S, Egy-Chem for lab technology, Badr city, Elrubaki, Egypt), plasma urea nitrogen, and triglyceride (Fluitest Urea and Fluitest TG, respectively, from Analyticon Biotechnologies AG, Litchenfels, Germany). Buffaloes were observed by the same technician for 30 minutes, 2 times daily for signs of estrous, and were artificially inseminated (AI) accordingly. Date and number of services per buffalo was recorded and buffaloes were inseminated even after the trial period of 120-d. Pregnancy

diagnosis was performed by rectal palpation of the uterus, 60 days post AI. Data of pregnancy diagnosis was recorded after the experimental period up to December 2017 in order to observe the confirm pregnancy status of buffaloes. Reproductive parameters were calculated from the recorded data of AI, number of services per buffalo, and confirm pregnancy status of each buffalo. Projected calving interval was calculated by adding 310 days in days open of each buffalo.

#### Calculations and statistics

Data were analyzed using MIXED procedure of SAS University Edition (SAS Institute Inc., Cary, NC). Treatment, treatment week, and their interaction were included as fixed effects. The initial observation in the beginning of experiment of production parameters were used as a covariate to minimize the biasness in the results. Treatment differences were considered significant if  $P \leq 0.05$  and as a trend for  $0.05 < P \leq 0.10$ . All the results are presented as least squares means with standard errors of the mean.

## RESULTS

The treatment into weak interaction was non-significant in the data for any parameter and therefore it is not presented in the results.

#### Milk yield and milk composition

The milk yield and milk composition results are summarized in Table II. The Ca-FA treatment had no effect on milk production compared with the Ctrl ( $P = 0.20$ ), however, the Ca-FA treatment increased the 3.5% fat-corrected milk production by 11.0% compared with the Ctrl ( $P = 0.04$ ). Similarly, the Ca-FA treatment increased the concentration of milk fat by 3.5% ( $P = 0.01$ ) and milk fat yield by 11.2% ( $P = 0.04$ ), compared with the Ctrl.

**Table II. Milk yield and milk composition of buffaloes fed treatment diets.**

Item	Ctrl <sup>1</sup>	Ca-FA <sup>2</sup>	SEM	P-value
Milk yield, (kg/d)	8.31	8.71	0.235	0.20
Milk fat, (%)	5.68	5.88	0.051	0.01
Milk fat yield, (g/d)	464	516	17.8	0.04
3.5% FCM <sup>3</sup> , (kg/d)	11.0	12.2	0.41	0.04

<sup>1</sup>Ctrl, basal diet without supplementation of fat; <sup>2</sup>Ca-FA, basal diet plus 200 g/d calcium salts of palm fatty acid supplementation (Energizer Gold; IFFCO, Johor, Malaysia); <sup>3</sup>3.5% fat-corrected milk =  $0.432 \times$  milk yield (kg/d) +  $16.23 \times$  fat yield (kg/d).

#### Plasma metabolites

Plasma metabolites are presented in Table III. The Ca-FA treatment had no effect on plasma concentration of glucose ( $P = 0.61$ ), compared with the Ctrl. Similarly, the Ca-FA treatment also had no effect on blood urea nitrogen ( $P = 0.19$ ) and triglyceride ( $P = 0.54$ ) concentrations, compared with the Ctrl.

**Table III. Plasma metabolites of buffaloes fed treatment diets.**

Item	Ctrl <sup>1</sup>	Ca-FA <sup>2</sup>	SEM	P-value
Glucose, (mg/dL)	92.3	91.2	1.55	0.61
Plasma urea nitrogen, (mg/dL)	22.7	21.6	0.51	0.19
Triglyceride, (mg/dL)	156	159	3.5	0.54

<sup>1</sup>Ctrl, basal diet without supplementation of fat; <sup>2</sup>Ca-FA, basal diet plus 200 g/d calcium salts of palm fatty acid supplementation (Energizer Gold; IFFCO, Johor, Malaysia).

#### Reproductive parameters

Reproductive parameters are presented in Table IV. The Ca-FA treatment had no effect on calving to first service interval ( $P = 0.66$ ) and calving to conception interval ( $P = 0.60$ ), compared with the Ctrl. Similarly, the Ca-FA treatment also had no effect on services per conception ( $P = 0.39$ ), days open ( $P = 0.60$ ), and projected calving interval ( $P = 0.60$ ), compared with the Ctrl.

**Table IV. Reproductive performance of buffaloes fed treatment diets.**

Item	Ctrl <sup>1</sup>	Ca-FA <sup>2</sup>	SEM	P-value
Calving-to-first-service interval (days)	74.5	94.4	30.39	0.66
Calving-to-conception interval (days)	169	213	57.34	0.60
Services per conception (no.)	2.63	2.12	0.39	0.39
Days open (days)	169	213	57.34	0.60
Projected calving interval (days)	479	523	57.33	0.60

<sup>1</sup>Ctrl, basal diet without supplementation of fat; <sup>2</sup>Ca-FA, basal diet plus 200 g/d calcium salts of palm fatty acid supplementation (Energizer Gold; IFFCO, Johor, Malaysia).

## DISCUSSION

In this study, our objective was to evaluate the effects of feeding Ca salts of palm FA on reproductive responses, milk production and milk composition in early-lactating buffaloes, compared with the control diet. Formation of calcium salts of unsaturated FA was devised to protect unsaturated FA from ruminal biohydrogenation

(Jenkins and Palmquist, 1984). However, the Ca salt protection of unsaturated FA was incomplete from ruminal biohydrogenation (Wu *et al.*, 1991) due to dissociation of calcium ion within the rumen. Most Ca-salts products used in dairy cow diet and available in market are derived from palm oil processing industry and these products have balance of saturated and unsaturated FA (mainly C16:0 and 18-C FA).

#### *CA-FA supplementation improved production responses*

As expected, the additional intake of energy provided by Ca-FA treatment increased the 3.5% fat-corrected milk yield, milk fat content, and milk fat yield, compared with the Ctrl, in agreement with the studies conducted on lactating buffaloes (Shelke *et al.*, 2012) and dairy cows (Scott *et al.*, 1995; Batistel *et al.*, 2017). Fat supplementation may spare glucose that could be utilized for the lactose and milk fat synthesis (Rabiee *et al.*, 2012). On the contrary, some studies have observed no effect of fat supplementation on milk yield and milk fat (Rabiee *et al.*, 2012; Polidori *et al.*, 1997; Santillo *et al.*, 2016) in dairy cow and buffalo. We observed 11.0% increase in 3.5% fat-corrected milk yield by Ca-FA feeding in agreement with the previous study in buffalo (Polidori *et al.*, 1997), where the increase was 22% in 4% fat-corrected milk yield by 300 g/d supplementation of Ca salts of palm FA. Higher increase in Polidori *et al.* (1997) study might be due to higher supplementation level of fat, high crude protein intake in diet and different stage of lactation (Rabiee *et al.*, 2012; Loften *et al.*, 2014). In present study, milk fat content and milk fat yield were also increased by 3.5 and 11.2%, respectively, with Ca-FA feeding, which is also in agreement with the observations of Shelke *et al.* (2012) and they reported 5.4% increase in milk fat content in response to rumen protected fat supplementation in buffalo (2.5% of diet dry matter). Similarly, Batistel *et al.* (2017) reported 20% increase in milk fat yield in response to 400 g/d supplementation of Ca salts of palm FA in dairy cow and this higher response also could be due to higher supplementation level of fat feeding compared with the present study. However, Santillo *et al.* (2016) and Polidori *et al.* (1997) reported no effect of fat feeding on milk fat content in buffalo. Moreover, Scott *et al.* (1995) summarized the data of 220 cows from calving until 200 days in milk in response to 450 g/d Ca salts of palm FA addition compared with the control. They reported that fat feeding increased 4% fat-corrected milk yield and milk fat yield by 8.2 and 10.6% in multiparous cows and their findings are also in line with the results of current experiment. Increase in milk fat concentration and yield could be due to dietary incorporation of C16:0 into milk fat because mammary gland appears to prefer the dietary C16:0 for incorporation into milk fat over other

FA (Hutchinson *et al.*, 2012). The Ca-FA source contained approximately 47% C16:0, which can be incorporated into milk fat and can support the proposed mechanism for increased response.

#### *CA-FA supplementation unaffected reproductive indices*

Calving interval is the key of success in commercial dairy farming. We found no effect of Ca-FA feeding on calving to first service interval, calving to conception interval, services per conception, days open, and projected calving interval in agreement to findings reported by Scott *et al.* (1995) on summarizing the data of 220 Holstein cows on Ca salts of palm FA supplementation, compared with the Ctrl. Similarly, Hutchinson *et al.* (2012) fed 51 g/d of lipid-encapsulated conjugated linoleic acid in individual cow and reported that this fat supplement had no substantial effect on calving to first service interval, calving to conception interval, and overall pregnancy rate. Contrary to this, Ramteke *et al.* (2014) observed a significant decrease in postpartum heat days, service period, and No. of AI per conception by feeding of Ca salts of FA in buffalo, compared with the control. Ramteke *et al.* (2014) fed rumen protected fat by 100 g/d 30 days prepartum and 15 g/d per kg of milk yield until 120 days postpartum in buffaloes. Similarly, Tyagi *et al.* (2010) also reported a decreased service period and AI per conception by Ca salts of FA supplementation (2.5% of diet dry matter), compared with the control group in crossbred cows. It is quite possible that any energy spared by fat supplementation is partitioned toward increased milk production rather than improving the energy status and subsequent reproductive performance of dairy cows (Hutchinson *et al.*, 2012), as observed in the present study by increased fat-corrected milk production and milk fat yield. However, the opposite mechanism proposed in literature for improved reproductive performance by fat supplementation could be: fat feeding increases blood progesterone in ruminants by increasing cholesterol content, which is the precursor of progesterone (Staples *et al.*, 1998). Progesterone, maintain pregnancy and support implantation of the embryo by preparing uterus and it may be due to provision of nourishment to conceptus (Ramteke *et al.*, 2014).

#### *No response of CA-FA feeding on plasma metabolites*

CA-FA feeding did not significantly change plasma glucose and triglyceride level in agreement to findings reported in buffalo (Shelke *et al.*, 2012; Ramteke *et al.*, 2014) and cow (Piantoni *et al.*, 2013), compared with the Ctrl group. No change in glucose level might be due to homeostatic mechanism of body that not allowed significant change in glucose level (Shelke *et al.*, 2012).

Similarly, Boken *et al.* (2005) reported no effect of fat supplementation on blood urea nitrogen in agreement to results observed in current experiment.

## CONCLUSIONS

In conclusion, Ca salts of palm FA supplementation increased 3.5% fat-corrected milk yield, milk fat content, and milk fat yield compared with the control, in early-lactating buffalo in the current study. However, the Ca-FA feeding had no effect on plasma metabolites and reproductive responses observed in the current experiment, compared with no supplementation. The findings of our study showed that the fat feeding had positive effect on milk production and milk composition without significantly changing the reproductive performance in early-lactating buffalo, which could be due to partitioning of extra energy toward production rather than reproduction.

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### Statement of conflict of interest

The authors declare no conflict of interest.

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