Comparison of oil and fat supplementation on lactation performance of Nili Ravi buffaloes

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ABSTRACT

The effects of feeding rumen-inert fat sources on production responses of lactating dairy cows have been well reported but less thoroughly described in lactating dairy buffalo. The objective of this study was to investigate the effect of oil and 2 different rumen-inert fat sources on dry matter intake, milk yield, milk composition, and milk fatty acid (FA) profile in Nili Ravi buffalo. Twelve multiparous mid-lactating Nili Ravi buffaloes received 4 treatments in a 4 × 4 Latin square design with a period length of 21 d. The treatments were (1) the basal diet without supplementation of oil or fats (CTRL), (2) the basal diet supplemented with canola oil (CO), (3) the basal diet supplemented with calcium salts of palm FA (Ca-FA), and (4) the basal diet supplemented with high palmitic acid (PA). Dry matter intake was decreased by 4.4% in the CO compared with Ca-FA and PA. Milk yield and milk fat yield were increased by 7.8 and 14.3%, respectively, in CO, Ca-FA, and PA compared with the CTRL. Milk fat content increased by 7.5%, whereas milk fat yield tended to increase with the supplementation of Ca-FA and PA compared with CO. No effect on milk yield and milk composition was observed in Ca-FA versus PA treatments. The yield of medium-chain FA was increased by Ca-FA and PA versus CO. The CO treatment increased the yield of long-chain FA compared with Ca-FA and PA treatments. Plasma glucose level was higher in CO, Ca-FA, and PA compared with the CTRL. In conclusion, feeding rumen-inert fats in the lactating buffalo diet proved to be a useful strategy to increase the 3.5% fat-corrected milk yield due to the higher milk fat content in this study.

Key words: canola oil, rumen-inert fat, milk yield, buffalo

INTRODUCTION

Buffalo (*Bubalus bubalis*) is the second largest source of milk in the world, contributing 14.4% of total milk produced per annum (FAOSTAT, 2016). India, Pakistan, and China contribute 68, 22, and 6%, respectively, of the total milk produced by buffalo in the world (FAOSTAT, 2016). Buffalo milk contains higher fat content ($7.8 \pm 2.3\%$; Pegolo et al., 2017) compared with cow milk ($3.4 \pm 0.38\%$; Conte et al., 2016); however, the fatty acid (FA) composition of buffalo milk is similar to cow milk, as it contains 70.5 versus 69.6% SFA, 26.0 versus 24.5% MUFA, and 3.5 versus 3.8% PUFA, respectively (Pegolo et al., 2017). The concentrations of short-chain FA (SCFA), medium-chain FA (MCFA), and long-chain FA (LCFA) were also reported similar in buffalo (9.7, 53.7, and 32.7%, respectively) compared with cow milk (10.2, 47.6, and 38.0%, respectively). This similar composition of FA in buffalo and cow milk provides an opportunity to explore the benefits of feeding fats used in dairy cow diets to modify milk fat yield and concentration in buffalo milk. Vegetable oils in the diet of dairy cows may alter the milk fat composition, as vegetable oils increased UFA and decreased SFA (Tanaka, 2005). Canola oil is reported to contain the highest content of UFA (approximately 90%), mainly oleic (C18:1), linoleic (C18:2), and linolenic acids (C18:3), compared with other vegetable sources (Welter et al., 2016). However, higher concentrations of UFA in the diets of dairy cows have been reported to impede DMI and rumen function (Jenkins and Jenny, 1989; Pantoja et al., 1996). Consequently, much work has been focused on methods to include additional fat in the diet without altering rumen fermentation (review by Jenkins and McGuire, 2006). Feeding rumen-inert fat in the form of calcium soap of FA (rumen-protected UFA) or prilled saturated fats is a common practice, and they were reported to have fewer negative effects on ruminal fermentation compared with oil (Palmquist and Jenkins, 1980; Jenkins and Jenny, 1992; Rabiee et al., 2012). The use of these products has been shown

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to improve milk production, milk fat yield, and milk composition in various studies conducted on dairy cow (Mosley et al., 2007; Wang et al., 2010; Lock et al., 2013; de Souza et al., 2017).

To our knowledge, limited studies have been conducted to investigate the effect of feeding calcium soap of FA in lactating buffaloes; however, in those studies an increase in milk yield and modification in milk FA profile was observed (Polidori et al., 1997; Shelke et al., 2012). On the other hand, no study has investigated the effect of feeding prilled fats to lactating buffalo. Interestingly, previous research indicates that the protection of calcium salt from ruminal biohydrogenation is incomplete, as calcium ions partially dissociate in the rumen (Wu et al., 1991; Chouinard et al., 1997; Giesy et al., 2002). Therefore, the objective of the current study was to investigate the effect of dietary FA sources with different FA profile (oil, Ca salts of palm FA, and high palmitic acid) on DMI, milk yield, milk components, and milk FA profile in Nili Ravi buffaloes.

MATERIALS AND METHODS

Animals

The experiment was conducted from June to August 2015 in a tiestall barn located at the Dairy Animals Training and Research Center, University of Veterinary & Animal Sciences, Ravi Campus (31.02°N, 73.85°E, and 186 m altitude; Pattoki, Pakistan). The entire experiment was conducted in accordance with the regulations approved by the ethical committee for animal welfare at University of Veterinary & Animal Sciences. Twelve lactating multiparous Nili Ravi buffaloes, with (mean $\pm$ SD) 7.04 $\pm$ 0.98 kg/d of milk yield, 6.05 $\pm$ 0.56% milk fat, 452 $\pm$ 32 kg of BW, and 101 $\pm$ 42 DIM were used. The buffaloes were individually tied in a naturally ventilated barn and had free access to automatic drinking bowls.

Experimental Design, Treatments, and Feeding

Buffaloes were randomly assigned to a treatment sequence in a 4 $\times$ 4 Latin square design with 21-d periods. The treatments were (1) the basal diet without supplementation of oil or fats (**CTRL**), (2) the basal diet with canola oil supplementation (**CO**), (3) the basal diet with calcium salts of palm FA (**Ca-FA**; Energizer Gold, IFFCO, Johor, Malaysia), and (4) basal diet with high palmitic acid supplementation (**PA**; Energizer RP-10, IFFCO). 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 (Fox et al., 2003). The diet was composed (on DM basis) of 47.7% oat silage, 19.6% wheat bran, 13.5% wheat straw, 9.97% canola meal, 5.84% ground corn, and 3.29% sugarcane molasses. Chemical composition of the basal diet is presented in Table 1. The forage-to-concentrate ratio was 60:40 as typically fed in commercial buffalo dairy farms located in periurban areas of Pakistan. Feeding rate of CO and PA was 170 g/d and Ca-FA was 200 g/d in the study and was adjusted to provide similar NE_L in these 3 treatments. The FA profiles of oil and rumen-inert fats are presented in Table 2. The diets were offered in fixed amounts per buffalo. This was achieved by feeding an ad libitum diet 7 d before the start of experiment and estimating the DMI of individual buffalo. Buffaloes were relatively similar in BW; hence, they were offered a similar quantity of DM (17 kg/buffalo per day) assuming similar lactation persistency in the entire experiment. The buffaloes were fed once daily at 0900 h.

Experimental Measures, Sample Collection, and Analyses

Diet offered as well as individual orts were weighed daily. Three samples of each feedstuff were collected weekly in each period to evaluate the DM and for further laboratory analysis. These samples were analyzed for DM, CP, NDF, ADF, ether extract, and ash following official methods of 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 the first 2 wk and consecutively on d 15 to 21 of each period. Milk production from morning and evening milking were pooled daily and assayed by infrared analysis using Lactostar 3510 (Funke-Gerber, Berlin, Germany) for fat, protein, and lactose. An aliquot of milk from d 21 of each period was stored at -20°C without preservative until analyzed for FA profile. Blood samples of all the buffaloes were collected on third last day of each period from jugular vein following Haque et al. (2012). Blood samples were collected in heparinized syringes and immediately centrifuged at $2,000 \times g$ for 15 min at 4°C . Plasma was separated, aliquoted, and 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 Biotechnologies AG, Lichtenfels, Germany), and glucose (BioMed-Glucose L.S, Egy-Chem for lab technology, Badar city, Elrubaki, Egypt). Buffaloes were weighed on d 15 of each treatment before feed distribution.

Table 1. Nutrient composition of the treatment¹ diets²

Item	CTRL	CO	Ca-FA	PA
Ingredient (% of DM, unless noted)				
DM (%)	48.5	48.7	48.8	48.7
Forage	61.3	60.6	60.5	60.6
CP	11.8	11.7	11.7	11.7
ADF	30.8	30.4	30.4	30.4
NDF	51.3	50.7	50.6	50.7
NFC	27.9	27.6	27.6	27.7
Ether extract total	3.27	4.34	4.23	4.23
Ash	7.71	7.63	7.81	7.79
Predicted nutritive values				
RUP (% CP)	29.2	28.9	29.3	29.2
ME (Mcal/kg)	2.13	2.21	2.20	2.20
NE _L (Mcal/kg)	1.37	1.42	1.42	1.42

¹Treatments were CTRL = basal diet with no supplementation of oil or rumen-inert fat; CO = basal diet + 170 g of canola oil; Ca-FA = basal diet + 200 g of calcium salts of palm fatty acid (Energizer Gold, IFFCO, Johor, Malaysia); and PA = basal diet + 170 g of palmitic acid (Energizer RP-10, IFFCO).

²The diet contained oat silage (47.7%), wheat bran (19.6%), wheat straw (13.5%), canola meal (9.97%), ground corn (5.84%), and sugarcane molasses (3.29%).

Milk Fat Extraction

Thirty milliliters of raw milk was centrifuged at $17,800 \times g$ for 30 min at 4°C in a Beckman (Fullerton, CA) J2-MC centrifuge. The fat layer was transferred to a microtube and left at room temperature for approximately 30 min before being microcentrifuged at $19,300 \times g$ for 20 min at room temperature. After the second centrifugation, the top layer was removed and was stored at -20°C without preservative until analyzed for FA profile (Luna et al., 2005). Fifty grams of each milk sample was taken in a flask separately and a solvent mixture of 500 mL of chloroform-methanol (2:1) was employed for extraction of pure lipids (Kallio et al., 2006). The process was revised with the same solvent mixture (2×250 mL) for maximum extraction. The oil was dried over anhydrous sodium sulfate and distilled

by rotary thin film evaporator under vacuum to get lipids before treatment with a mixture of chloroform: methanol:0.9% sodium chloride solution (3:48:47 vol/vol) to remove impurities by a separator funnel (Kallio et al., 2006).

Derivatization of Fat and GC Analysis

The extracted lipids were esterified with Boron Trifluoride-Methanol Complex (Merck-Schuchardt, Hohenbrunn, Germany) by following the official method and the FAME were analyzed on GC (AOCS, 2009, 2017a). The GC Shimadzu GC-14A and data processor C-R-4A (Shimadzu Corp., Kyoto, Japan) was used for the identification of methyl esters by standard method Ce 1a-13 (AOCS, 2017b) using a polar column (dimensions: 60 m, 0.25 mm i.d.). The hydrogen flame ionization detector was used with the requisite temperature of detector and injector at 250 and 230°C, respectively. It was programmed to increase the temperature from 160 to 210°C at the rate of 2°C/min. The FAME were identified by the comparison of their corresponding retention times with standard methyl esters (Supelco, Bellefonte, PA) under same conditions.

Statistical Analysis

Data were analyzed using the MIXED 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. The following mathematical model was used for the analysis:

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

Table 2. Fatty acid (FA) profile of different fat sources¹

Item	CO	Ca-FA	PA
Ether extract (% of DM)	100	82.5	95.0
Total FA (% of DM)	88.0	82.5	95.0
Glycerol (% of DM)	9.68		
Pigment (% of DM)	2.32		
% of total FA			
C12:0	0.10	0.20	2.30
C14:0	0.10	1.20	4.20
C16:0	5.90	47.0	86.6
C16:1	0.60		
C18:0	1.90	5.00	2.80
C18:1	53.7	38.0	4.10
C18:2	22.4	8.00	
C18:3	9.80		
Others	5.50	0.60	

¹Sources were CO = basal diet + 170 g of canola oil; Ca-FA = basal diet + 200 g of calcium salts of palm fatty acid (Energizer Gold, IFFCO, Johor, Malaysia); and PA = basal diet + 170 g of palmitic acid (Energizer RP-10, IFFCO).

where Y is the response variable, μ is the overall mean, ε is the random error, Buff_i represents the random effect of buffalo, Per_j represents the 21-d period, and Treat_k represents the treatment diets of experiment. Three preplanned orthogonal contrasts were performed: (1) contrast A, CTRL vs. CO, Ca-FA, and PA to compare the effects of feeding oil and rumen-inert fats; (2) contrast B, CO vs. Ca-FA and PA to compare the feeding of oil versus rumen-inert fats; and (3) contrast C, Ca-FA versus PA to compare the sources of rumen-inert fats. 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

DM and FA Intakes

The DMI and FA intakes are presented in Table 3. The CO, Ca-FA, and PA treatments had no effect on DMI compared with the CTRL (contrast A; $P = 0.80$); however, the CO treatment decreased the DMI by 4.43% compared with Ca-FA and PA treatments (contrast B; $P = 0.04$). As expected, the CO, Ca-FA, and PA treatments increased the intakes of C12:0, C14:0, 16-C, 18-C, and total FA (contrast A; $P \leq 0.01$) compared with the CTRL. The Ca-FA and PA treatments increased the intake of C16:0 by 167% (contrast B; $P < 0.01$) compared with the CO treatment. Contrary to this, the CO treatment increased the intakes of C18:1, C18:2, and C18:3 by 57.7, 15.9, and 15.7%, respectively (contrast B; $P < 0.01$), compared with the Ca-FA and PA treatments. The PA treatment increased the intake of C16:0 by 45.0% compared with the Ca-FA treatment (contrast C; $P < 0.01$).

Milk Yield and Milk Composition

Milk yield and milk composition results are presented in Table 4. The actual milk yield and 3.5% FCM yield were increased by 7.8% (contrast A; $P = 0.02$) and 12.2% ($P < 0.01$), respectively, in the CO, Ca-FA, and PA treatment compared with the CTRL, whereas, milk composition remained unaffected ($P > 0.10$). Milk yield was similar between the CO versus Ca-FA and PA treatments (contrast B; $P = 0.70$); however, Ca-FA and PA treatment tended to increase the 3.5% FCM yield by 6.25% ($P = 0.10$) and increased milk fat content by 7.51% ($P = 0.04$) compared with the CO treatment. The Ca-FA treatment had no effect on DMI, milk yield, and milk composition compared with the PA treatment (contrast C; $P > 0.10$).

Milk FA Concentrations

Results of milk FA concentrations of buffaloes fed treatment diets are presented in Table 5. The CO, Ca-FA, and PA treatments tended to increase the concentrations of C8:0 by 7.99% (contrast A; $P = 0.08$) and C16:0 by 4.63% ($P = 0.09$), whereas concentrations of C6:0, C10:0, C12:0, C14:0, C14:1, C16:1, C18:0, C18:1, and C18:2 remained unaffected ($P > 0.10$) compared with the CTRL. The CO, Ca-FA, and PA had no effect on concentrations of SFA, MUFA, PUFA, UFA, SCFA, MCFA, and LCFA compared with the CTRL diet ($P > 0.10$). The Ca-FA and PA treatments increased the concentration of C16:0 by 25.8% (contrast B; $P < 0.01$) compared with the CO treatment. The Ca-FA and PA treatments increased the concentration of MCFA by 18.0% (contrast B; $P < 0.01$) compared with CO. The CO treatment decreased the concentrations of C14:1 by

Table 3. Dry matter and fatty acid (FA) intake (g/d) in different treatments

Item	Treatment ¹				SEM	P-value ²		
	CTRL	CO	Ca-FA	PA		A	B	C
DMI (kg/d)	15.6	15.1	15.9	15.7	0.25	0.80	0.04	0.59
FA intake (g/d)								
C12:0	8.56	8.35	8.95	12.0	0.15	<0.01	<0.01	<0.01
C14:0	1.68	1.77	3.63	8.22	0.072	<0.01	<0.01	<0.01
C16:0	52.4	59.1	129	187	1.9	<0.01	<0.01	<0.01
C16:1	2.45	3.23	2.47	2.41	0.044	<0.01	<0.01	0.32
C18:0	5.28	7.89	13.3	9.57	0.171	<0.01	<0.01	<0.01
C18:1	44.2	122	105	49.7	1.57	<0.01	<0.01	<0.01
C18:2	129	157	144	127	2.3	<0.01	<0.01	<0.01
C18:3	74.6	86.0	75.2	73.5	1.26	0.01	<0.01	0.33
Other	7.48	15.5	8.64	7.36	0.176	<0.01	<0.01	<0.01
Total FA	326	460	489	478	7.4	<0.01	0.01	0.26

¹Treatments were CTRL = basal diet with no supplementation of oil or rumen-inert fat; CO = basal diet + 170 g of canola oil; Ca-FA = basal diet + 200 g of calcium salts of palm fatty acid (Energizer Gold, IFFCO, Johor, Malaysia); and PA = basal diet + 170 g of palmitic acid (Energizer RP-10, IFFCO).

²A = CTRL vs. CO, Ca-FA, and PA. B = CO vs. Ca-FA and PA. C = Ca-FA vs. PA.

Table 4. Milk yield and milk composition

Item	Treatment ¹				SEM	P-value ²		
	CTRL	CO	Ca-FA	PA		A	B	C
Milk yield (kg/d)								
Milk	7.56	8.09	8.35	8.02	1.003	0.02	0.70	0.28
3.5% FCM	10.4	11.2	12.2	11.6	1.28	<0.01	0.10	0.25
Milk composition (%)								
Fat	6.08	5.99	6.47	6.41	0.237	0.29	0.04	0.78
Protein	3.99	3.86	3.84	3.80	0.108	0.21	0.78	0.79
Lactose	4.90	4.69	4.72	4.67	0.117	0.14	0.96	0.77

¹Treatments were CTRL = basal diet with no supplementation of oil or rumen-inert fat; CO = basal diet + 170 g of canola oil; Ca-FA = basal diet + 200 g of calcium salts of palm fatty acid (Energizer Gold, IFFCO, Johor, Malaysia); and PA = basal diet + 170 g of palmitic acid (Energizer RP-10, IFFCO).

²A = CTRL vs. CO, Ca-FA, and PA; B = CO vs. Ca-FA and PA; C = Ca-FA vs. PA.

13.8% (contrast B; $P = 0.01$) and increased the concentrations of C18:0, C18:1, and C18:2 by 37.2 ($P = 0.02$), 28.1 ($P < 0.01$), and 49.0% ($P < 0.01$), respectively, compared with the Ca-FA and PA treatments. The CO treatment increased the concentrations of MUFA by 17.8% (contrast B; $P = 0.02$), PUFA by 49.1% ($P < 0.01$), UFA by 19.8% ($P = 0.01$), and LCFA by 32.8% ($P = 0.01$) compared with the Ca-FA and PA treatments. The CO treatment had no effect on concentrations of C6:0, C8:0, C10:0, C12:0, C14:0, C16:1, SFA, and

SCFA compared with the Ca-FA and PA treatments (contrast B; $P > 0.10$). The PA treatment increased the concentrations of C8:0, C14:1, and C16:1 by 11.9 (contrast C; $P = 0.02$), 22.4 ($P < 0.01$), and 32.1% ($P = 0.05$), respectively, whereas it tended to decrease the concentration of C18:2 or PUFA by 15.2% ($P = 0.07$) relative to the Ca-FA. The PA treatment had no effect on concentrations of C6:0, C10:0, C12:0, C14:0, C16:0, C18:0, C18:1, SFA, MUFA, UFA, SCFA, MCFA, and LCFA (contrast C; $P > 0.10$) compared with Ca-FA.

Table 5. Milk fatty acid (FA) concentrations

Item	Treatment ¹				SEM	P-value ²		
	CTRL	CO	Ca-FA	PA		A	B	C
FA (g/100 g)								
C6:0	1.53	1.36	1.49	1.27	0.166	0.41	0.91	0.35
C8:0	1.46	1.53	1.51	1.69	0.053	0.08	0.28	0.02
C10:0	1.53	1.57	1.55	1.73	0.097	0.43	0.53	0.20
C12:0	1.44	1.47	1.57	1.49	0.066	0.37	0.46	0.37
C14:0	9.33	9.08	8.94	9.26	0.206	0.34	0.93	0.29
C14:1	1.63	1.37	1.43	1.75	0.074	0.17	0.01	<0.01
C16:0	31.7	28.3	35.1	36.1	0.72	0.09	<0.01	0.36
C16:1	4.99	4.43	4.27	5.64	0.480	0.71	0.39	0.05
C18:0	13.3	14.2	11.0	9.70	1.359	0.25	0.02	0.48
C18:1	20.1	23.7	18.5	18.5	1.16	0.94	<0.01	0.99
C18:2	2.74	3.80	2.76	2.34	0.159	0.17	<0.01	0.07
Group of fatty acids ³								
SFA	60.3	57.5	61.1	61.2	2.16	0.88	0.14	0.98
MUFA	26.7	29.5	24.2	25.9	1.44	0.90	0.02	0.41
PUFA	2.74	3.80	2.76	2.34	0.159	0.17	<0.01	0.07
UFA	28.4	32.0	26.1	27.3	1.64	0.97	0.01	0.60
SCFA	4.53	4.45	4.56	4.69	0.297	0.91	0.64	0.75
MCFA	49.1	44.6	51.3	54.2	1.28	0.51	<0.01	0.12
LCFA	35.1	40.5	31.4	29.6	2.43	0.64	0.01	0.62

¹Treatments were CTRL = basal diet with no supplementation of oil or rumen-inert fat; CO = basal diet + 170 g of canola oil; Ca-FA = basal diet + 200 g of calcium salts of palm fatty acid (Energizer Gold, IFFCO, Johor, Malaysia); and PA = basal diet + 170 g of palmitic acid (Energizer RP-10, IFFCO).

²A = CTRL vs. CO, Ca-FA, and PA; B = CO vs. Ca-FA and PA; C = Ca-FA vs. PA.

³SCFA = short-chain fatty acids, including C6:0, C8:0, and C10:0; MCFA = medium-chain fatty acids, including C12:0, C14:0, C14:1, C16:0, and C16:1; LCFA = long-chain fatty acids, including C18:0, C18:1, and C18:2.

Table 6. Milk fatty acid (FA) yields

FA yield (g/d)	Treatment ¹					P-value ²		
	CTRL	CO	Ca-FA	PA	SEM	A	B	C
Milk fat yield	440	475	530	504	53.0	<0.01	0.06	0.29
C6:0	6.25	6.67	7.62	6.27	0.962	0.51	0.78	0.24
C8:0	6.36	7.29	8.01	8.37	0.815	0.01	0.07	0.51
C10:0	6.56	7.64	8.26	8.32	0.849	0.02	0.32	0.94
C12:0	6.22	7.08	8.39	7.34	0.824	0.01	0.14	0.09
C14:0	40.8	43.9	48.0	45.9	4.90	0.03	0.19	0.43
C14:1	7.21	6.60	7.69	8.67	0.921	0.31	<0.01	0.07
C16:0	140	134	186	180	17.3	0.01	<0.01	0.58
C16:1	22.4	22.3	23.0	27.2	3.77	0.62	0.45	0.33
C18:0	56.7	74.6	57.3	46.2	10.21	0.78	0.03	0.35
C18:1	88.4	115	95.0	90.8	12.24	0.26	0.05	0.74
C18:2	11.4	18.8	14.1	12.3	2.004	0.02	<0.01	0.40
Group of fatty acids ³								
SFA	263	282	324	302	32.1	0.05	0.14	0.37
MUFA	118	144	126	127	16.1	0.28	0.21	0.95
PUFA	11.4	18.8	14.1	12.3	2.004	0.02	<0.01	0.40
UFA	125	157	136	134	17.7	0.25	0.15	0.91
SCFA	19.2	21.6	23.9	23.0	2.50	0.06	0.36	0.68
MCFA	217	214	273	269	26.8	0.01	<0.01	0.81
LCFA	152	203	162	144	23.2	0.40	0.03	0.47

¹Treatments were CTRL = basal diet with no supplementation of oil or rumen-inert fat; CO = basal diet + 170 g of canola oil; Ca-FA = basal diet + 200 g of calcium salts of palm fatty acid (Energizer Gold, IFFCO, Johor, Malaysia); and PA = basal diet + 170 g of palmitic acid (Energizer RP-10, IFFCO).

²A = CTRL vs. CO, Ca-FA, and PA; B = CO vs. Ca-FA and PA; C = Ca-FA vs. PA.

³SCFA = short-chain fatty acids, including C6:0, C8:0, and C10:0; MCFA = medium-chain fatty acids, including C12:0, C14:0, C14:1, C16:0, and C16:1; LCFA = long-chain fatty acids, including C18:0, C18:1, and C18:2.

Milk Fat and FA Yields

Milk fat and milk FA yields are presented in Table 6. The CO, Ca-FA, and PA treatments increased milk fat yield by 14.3% compared with the CTRL (contrast A; $P < 0.01$), and the Ca-FA and PA treatment tended to increase the milk fat yield by 8.84% (contrast B; $P = 0.06$) compared with the CO treatment without any difference observed in the Ca-FA versus PA (contrast C; $P = 0.29$). The CO, Ca-FA, and PA treatments increased the yields of C8:0, C10:0, C12:0, C14:0, C16:0, and C18:2 by 24.1% (contrast A; $P = 0.01$), 23.1% ($P = 0.02$), 22.2% ($P = 0.01$), 12.6% ($P = 0.03$), 19.1% ($P = 0.01$), and 32.2% ($P = 0.02$), respectively, compared with the CTRL. Similarly, the CO, Ca-FA, and PA treatments also increased the yields of SFA and MCFA by 15.1% (contrast A; $P = 0.05$) and 16.1% ($P = 0.01$) and tended to increase the yield of SCFA by 18.9% ($P = 0.06$) compared with the CTRL. The yields of C6:0, C14:1, C16:1, C18:0, C18:1, MUFA, UFA, and LCFA were not affected by the CO, Ca-FA, and PA treatments compared with the CTRL (contrast A; $P > 0.10$). The Ca-FA and PA treatments increased the yield of C16:0 by 36.6% (contrast B; $P < 0.01$) and yield of MCFA by 26.6% ($P < 0.01$) compared with CO. The CO treatment decreased the yields of C14:1

by 19.3% (contrast B; $P < 0.01$) and tended to decrease the yield of C8:0 by 10.1% ($P = 0.07$), whereas the yield of C18:0, C18:1, and C18:2 were increased by 44.2% ($P = 0.03$), 23.8% ($P = 0.05$), and 42.4% ($P < 0.01$), respectively, compared with Ca-FA and PA. The CO treatment increased the yields of PUFA by 42.4% (contrast B; $P < 0.01$) and LCFA by 32.7% ($P = 0.03$) compared with the Ca-FA and PA. The yields of C6:0, C10:0, C12:0, C14:0, C16:1, SFA, MUFA, UFA, and SCFA were not different between CO versus Ca-FA and PA (contrast B; $P > 0.10$).

The PA treatment tended to decrease the yield of C12:0 by 12.5% (contrast C; $P = 0.09$), whereas it tended to increase the yield of C14:1 by 12.7% ($P = 0.07$) compared with the Ca-FA. The Ca-FA versus PA treatments had no effect on yields of C6:0, C8:0, C10:0, C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, SFA, MUFA, PUFA, UFA, SCFA, MCFA, LCFA, and milk fat yield (contrast C; $P > 0.10$).

Plasma Metabolites

Response of plasma metabolites is presented in Table 7. The CO, Ca-FA, and PA treatments increased the concentration of plasma glucose by 8.1% (contrast A; $P = 0.05$), whereas PUN and triglyceride concentra-

Table 7. Plasma metabolites

Item	Treatment ¹				SEM	P-value ²		
	CTRL	CO	Ca-FA	PA		A	B	C
Glucose (mg/dL)	71.8	79.8	79.4	73.6	3.12	0.05	0.33	0.16
Plasma urea N (mg/dL)	19.1	19.2	20.7	19.2	1.27	0.63	0.64	0.42
Triglyceride (mg/dL)	189	186	212	200	13.3	0.40	0.15	0.44

¹Treatments were CTRL = basal diet with no supplementation of oil or rumen-inert fat; CO = basal diet + 170 g of canola oil; Ca-FA = basal diet + 200 g of calcium salts of palm fatty acid (Energizer Gold, IFFCO, Johor, Malaysia); and PA = basal diet + 170 g of palmitic acid (Energizer RP-10, IFFCO).

²A = CTRL vs. CO, Ca-FA, and PA; B = CO vs. Ca-FA and PA; C = Ca-FA vs. PA.

tions were not affected compared with the CTRL ($P > 0.10$). Similarly, CO treatment had no effect on concentrations of plasma glucose, PUN, and triglyceride compared with Ca-FA and PA (contrast B; $P > 0.10$). We found no effect of Ca-FA versus PA treatments on plasma glucose, PUN, and triglyceride concentrations (contrast C; $P > 0.10$).

DISCUSSION

Our objective was to investigate the effects of feeding rumen-inert fats on milk production and milk composition in lactating dairy buffaloes compared with a diet without any supplemental fat or oil source (CTRL) as well as with a diet containing oil (CO). Canola oil was high in UFA contents (mainly C18:1 and C18:2) and was potentially exposed to rumen biohydrogenation (DePeters et al., 2001). The PA supplement was concentrated in C16:0, designed to minimize negative effects on rumen fermentation. In contrast, the Ca-FA supplement was a source of both SFA (mainly C16:0) and UFA (mainly C18:1) complexes with a calcium ion to slow down rumen availability of UFA for microbial biohydrogenation.

Canola Oil Supplementation Decreased the DMI Compared with the Rumen-Inert Fats

In the current study, we observed a decrease in the DMI due to the CO treatment compared with the feeding of rumen-inert fats (Ca-FA and PA). This result was in agreement with the studies conducted on dairy cows, where feeding the UFA decreased the DMI compared with the SFA (Jenkins and Jenny, 1989; Harvatine and Allen, 2006; Rabiee et al., 2012). However, no difference was observed between the Ca-FA versus PA treatments, in agreement with the studies conducted on dairy cows (Rico et al., 2014a). It is possible that the higher supply of UFA in the CO treatment exceeds the microbial capacity to convert them into the SFA, leading to an impede ruminal fermentation (NRC, 2001; Rabiee et al., 2012).

Milk Yield Increased Similarly with Oil and Rumen-Inert Fats

As expected, the additional intake of energy provided by the CO, Ca-FA, and PA treatments increased the milk yield by 7.8% compared with the CTRL, in agreement with the studies conducted on lactating buffaloes (Polidori et al., 1997) and cows (de Souza et al., 2017; Batistel et al., 2017). Fat supplementation may spare glucose that could be used for the lactose synthesis (Palmquist and Jenkins, 1980). We observed an increase in the plasma glucose by the CO, Ca-FA, and PA treatments compared with the CTRL. Moreover, a large proportion of the absorbed LCFA could be oxidized by the extrahepatic tissues, thereby decreasing its export in milk and sparing other fuels (FA) for milk synthesis (Piantoni et al., 2015). Interestingly, in the current study, milk yield in the CO treatment was similar to Ca-FA and PA despite a decrease in the DMI. Feeding of oil has been shown to decrease the DMI more rapidly compared with milk yield, as shown by Rico and Harvatine (2013). In their study, feeding of soybean oil to lactating dairy cows in a 21-d period decreased the DMI between d 6 to 21, whereas the average milk yield remained unaffected due to treatment and was only decreased at d 20 (Rico and Harvatine, 2013). It is also possible that the slight decrease in protein intake (82 g) in the CO treatment compared with Ca-FA and PA was compensated through recycling of nitrogen, as proposed by Reynolds and Kristensen (2008). Nevertheless, the 3.5% FCM showed a tendency toward increase in Ca-FA and PA compared with the CO treatment in our study.

Milk Fat Content Increased by Feeding of Rumen-Inert Fat

Milk fat concentration was increased by 7.5% with the addition of Ca-FA and PA compared with the CO treatment. This increase was mainly related to the increase in the concentration of C16:0 by 25.6%, as observed in previous studies (Jenkins and Jenny, 1992;

Baumann et al., 2016). On the other hand, the concentrations of C18:0, C18:1, and C18:2 were higher in the CO treatment, in agreement with the literature (Jenkins and Jenny, 1992; DePeters et al., 2001; Baumann et al., 2016). Higher C18:1 concentration in milk has been associated with higher ruminal biohydrogenation of canola oil and, as a consequence, decreased the milk fat percentage (DePeters et al., 2001), as observed in the CO treatment of our study. The effect of feeding calcium soaps of FA in lactating buffalo was investigated by Polidori et al. (1997), but despite feeding 300 g/d of calcium soaps of FA (2% of the DM), they found no effect on milk fat content. The response of feeding fats is related to the stage of lactation (Chilliard, 1993). The buffaloes in the study of Polidori et al. (1997) were in mid to late lactation, whereas in our study they were in mid lactation, which could possibly explain the differences in milk fat content response between the 2 studies. Nevertheless, feeding unsaturated plant oils, such as canola oil, led to the production of *trans* FA as an intermediate of ruminal biohydrogenation, inhibiting de novo FA synthesis (Baumgard et al., 2000; Bauman and Grinari, 2001; Shingfield and Grinari, 2007). However, feeding of CO did not create any milk fat depression in our study, contrary to the studies where they fed canola oil (DePeters et al., 2001) or canola seed (Chichlowski et al., 2005). This could be due to the low level of canola oil feeding in the current study (i.e., 1.1% of the DM) compared with 1.6% in the study by DePeters et al. (2001).

Feeding Rumen-Inert Fats Increased Milk C16:0 Content and Yield

As expected, in the current experiment, concentration and yield of C16:0 were increased in Ca-FA and PA treatments by 26 and 37%, respectively, compared with CO treatment, in agreement with the literature on dairy cows (Jenkins and Jenny, 1992; Baumann et al., 2016). The concentration of C16:0 in milk has been reported to vary from 5 to 48% by feeding of rumen-inert fats (Mosley et al., 2007; Batistel et al., 2017). Similarly, the C16:0 yield in milk was reported to vary from 14.3 to 63% by feeding rumen-inert fats (Mosley et al., 2007; Rico et al., 2014b; Batistel et al., 2017). The mammary gland appears to prefer the dietary C16:0 for incorporation into milk fat over other FA (Loften et al., 2014), and additional C16:0 consumed is partitioned to milk fat directly (Warntjes et al., 2008). Kinsella and Gross (1973) reported that C16:0 was the main FA used for initiation of glycerolipid synthesis by acylation at the sn-1 position. In addition, Hansen and Knudsen (1987) reported that C16:0 stimulated de novo FA synthesis

and incorporation into the milk triglycerides. The de novo synthesis of C8:0, C10:0, C12:0, and C14:0 in our study (as shown by their yields) could also be related to the stimulatory effect of C16:0, as proposed by Rico et al. (2014a), leading to an increase in milk fat yield in the current experiment. Increased de novo synthesis of C8:0, C10:0, C12:0, and C14:0 in CTRL versus CO, Ca-FA, and PA might reflect the increase in the extent of ruminal biohydrogenation, leading to increased milk fat yield, as proposed by Mosley et al. (2007).

Similar Milk Fat Yield in the Ca-FA and PA Treatments

We found no difference in milk fat yield, mainly due to the similar milk C16:0 in Ca-FA versus PA treatments. This could be due to the decrease in de novo synthesis of C16:0 in PA treatment. The de novo synthesis of C16:0 followed the law of diminishing returns, as it decreased with the increase in the dietary C16:0 concentration, in agreement with the literature in dairy cattle (Grummer, 1991; Mosley et al., 2007). In our study, the FA intake-to-milk output ratios were 2.67, 2.28, 1.45, and 0.96 in CTRL, CO, Ca-FA, and PA treatments, respectively. Mosley et al. (2007) showed a decrease in FA intake-to-milk output ratio from 2.66 to 0.53 in dairy cows where the targeted C16:0 intake was increased from 112 to 1140 g/d. This decrease in the transfer of C16:0 from feed to milk with the increase in supply could be related to its oxidation as a metabolic fuel rather than export as milk triglycerides, as suggested by Piantoni et al. (2013).

A Higher Concentration of Milk C18:0 in the Oil Treatment

The canola oil treatment increased the milk LCFA (C18:0, C18:1, and C18:2) content compared with Ca-FA and PA treatments, similar to the observations by DePeters et al. (2001). This increase in LCFA tended to decrease the de novo synthesis of C8:0 in our study, in agreement with the findings of Santillo et al. (2016), where feeding of flaxseed to the lactating buffaloes increased the concentration of LCFA and decreased C8:0 and C10:0. Furthermore, the ratio between LCFA to MCFA was higher in CO (0.95) compared with Ca-FA and PA (0.56) treatments, indicating the changes in carbon number for triglyceride structure, as reported by DePeters et al. (2001). Supplemental C18:0 was preferentially incorporated into the milk fat triglyceride at the expense of C16:0, as has been previously reported (DePeters et al., 2001), effectively lowering its content and increasing C18:0.

CONCLUSIONS

In the current study, oil and rumen-inert fats increased milk yield, milk fat yield, and altered milk FA profile compared with the control, in lactating dairy buffalo. Feeding Ca-FA and PA increased milk fat content and tended to increase the milk fat yield compared with oil. We observed no difference in lactation performance of buffaloes with the feeding of Ca-FA and PA under the current experimental conditions. The findings of our study showed that feeding of rumen-inert fat is a useful strategy to increase the 3.5% FCM yield due to higher milk fat content in lactating buffalo compared with oil.

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