Two Levels of Palmitic Acid-Enriched Fat Supplement Affect Lactational Performance of Holstein Cows and Feed Utilization of Barki Sheep

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Summary

The effect of feeding palmitic acid-enriched protected fat (PPF) supplement at two levels to increase energy density of diets was tested. In experiment 1, 21 multiparous lactating Holstein cows were fed on a basal diet without PPF supplementation (Control) or supplemented with 250 g (MG250) or 500 g PPF (MG500) for 13 weeks. In experiment 2, 12 adult Barki sheep were fed a basal diet without PAF supplementation (Control), or supplemented with 25 g (ME25), or 50 g of PPF (ME50 treatment) for 1 month. In experiment 1, MG250 treatment increased ($P<0.05$) daily milk production, lactose concentration and yield, and milk efficiency compared with the control and MG500. Feeding PPF did not affect the concentration of fatty acids in milk. Protected fats supplementation increased ($P<0.05$) the concentrations of serum total protein and globulin. In experiment 2, MG50 increased ($P<0.05$) the digestibility of fiber. Both MG25 and MG50 treatments decreased ruminal volatile fatty acids concentration, without affecting the concentration of ruminal ammonia-N or N utilization. Supplementing diets of cows with 250 g PPF enhanced the lactational performance and milk efficiency in mid-lactation. Moreover, supplementing diets of sheep with 50 g PPF enhanced fiber digestion, without affecting N utilization.

Key words

blood metabolites, energy supplements, milk production, nitrogen balance, ruminal protected fat

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Introduction

Rumen capacity limits feeding of high milk producing cow on large amounts of feed to meet their energy requirements. Therefore, increasing the energy density of diet is an alternative solution to meet these requirements (Gomaa et al., 2018; Kholif et al., 2016). Crude oil inclusion in the diet of ruminant is acceptable at low levels; however, increasing the levels of dietary oils and fats, especially those rich in unsaturated fatty acids (UFA) is associated with negative effects on animal and feed efficiency (Gomaa et al., 2018). Moreover, feeding fats which are not protected depress ruminal cellulolytic microbial activity, reduce poly UFA (PUFA), and increase saturated fatty acids (SFA) content in milk fat, making the produced milk less healthy for consumers with cardiovascular disease (Kumar, 2017). Therefore, protecting dietary fats from ruminal biohydrogenation is essential for optimal ruminal microflora activity, especially when needed at high inclusion levels.

Binding dietary fats with calcium salts or other fat binders protects them from extensive ruminal biohydrogenation, protects ruminal microflora from the toxicity of dietary fats, and prevents the fats from interfering with rumen metabolism (Lounglawan et al., 2008). Moreover, protecting UFA from ruminal biohydrogenation reduces their ruminal degradation, allows them to largely bypass the rumen for efficient utilization by the animals (Suksombat, 2009) and remain intact in milk (Kholif et al., 2018; Morsy et al., 2015). Hence, the inclusion of protected fats in the diets of lactating animals can enhance the energy density of the ration, without adverse effects on the ruminal fermentation, intake, or nutrient digestion (Kumar, 2017). Several experiments (Hammon et al., 2008; Onetti and Grummer, 2004; Pramono et al., 2017; Voigt et al., 2005) showed that feeding Holstein cows on diets supplemented with ruminal protected fats increased milk and lactose yields. However, Lohrenz et al. (2010) and Lounglawan et al. (2008) observed unaffected milk production of mid-lactation cows with feeding protected fats. The differences between experiments may be due to fat source, diet components and lactation stage (Onetti and Grummer, 2004).

Because it is difficult to keep cows in metabolic cages, sheep were used as a model to study digestibility and feed utilization. Therefore, the aims of the present study were two: 1) the performance experiment which evaluated the effect of including palmitic acid-enriched protected fat (PPF) at 250 and 500 g daily on milk production, composition, and fatty acid profile of mid-lactation Holstein cows, and 2) the digestibility trial which evaluated the effect of including PPF at 25 and 50 g daily on feed digestion and N utilization on Barki sheep. Our hypothesis was that the efficiency of plasma fatty acids transportation to mammary tissue decreases as lactation progresses; therefore, increasing the concentrations of plasma fatty acids in diet at the mid- or late-lactation is expected to enhance milk production (Grummer, 1988). Additionally, increasing energy density in the diet for replenishing body fat stores during mid to late lactation might be required to help restore body fat stores for the next lactation (Lounglawan et al., 2008). Moreover, Schroeder et al. (2004), in their review, stated that milk response to fat supplementation is higher in mid-lactation cows in a positive energy balance.

Materials and Methods

The study was conducted at the Laboratory of Animal Nutrition, Department of Animal Production and Agricultural Experimental Station of the Faculty of Agriculture, Alexandria University, Egypt. All procedures were approved and authorized by Institutional Animal Care and Use Committee of the Alexandria University 19ALEXU-IACUC/08-19-05-14-3-25) and performed in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Federation of Animal Science Societies; Champaign, IL, USA). All efforts were made to minimize suffering by animals.

Palmitic Acid-Enriched Protected Fat

Palmitic acid-enriched protected fat (Megalac®, Volac Wilmar, Malaysia) contain palm fatty acid and calcium that produce a rumen-insoluble fat supplement. The product contains 5% moisture, 84% oil, 9% calcium, and 33.3 MJ metabolizable energy per kg DM (27.3 MJ NE_L kg^{-1} DM). The fatty acid profile of the ruminal protected fats product is presented in Table 1.

Table 1. Fatty acids profile (g kg^{-1} total fatty acids) of palmitic acid-enriched protected fat (Megalac®, Volac Wilmar, Malaysia)

Fatty acid	Palmitic acid-enriched protected fat
C6:0	1.9
C8:0	11.1
C10:0	0.3
C11:0	0.5
C12:0	3.5
C13:0	2.6
C14:1	1.3
C14:0	11.5
C15:1	0.5
C15:0	1.2
C16:1	1.1
C16:0	415.0
C17:0	0.8
C18:0	91.9
C18:1	125.6
C18:2	324.6
C20:5	0.7
C20:2	0.7
C20:1	1.8
C20:0	2.8
C22:6	0.6
Total saturated fatty acid	543.2
Total unsaturated fatty acid	456.8

Diets, Cows and Management

Twenty-one multiparous lactating Holstein cows (2.11 ± 1.01 parity) in mid lactation (105 ± 4.2 days in milk), stratified by live body weight (400 ± 3 kg), parity and previous milk production, were assigned randomly in a complete randomized design to three treatments (7 cows per treatment). The cows were housed individually in soil-surfaced tie stall barns (122 × 175 cm2 cow^{-1}), under shade, without any bedding and with free access to water. They were fed individually on concentrates, Egyptian berseem clover (*Trifoluim alexandrium* L.), and corn silage at 70:15:15, respectively, to meet their nutrient requirements according to NRC (2001) recommendations, as a control diet. The control diet was supplemented daily with two levels of PPF: 250 g (MG250 treatment) or 500 g (MG500 treatment), representing 1.2 and 2.4% of total feed intake, and 11.5 and 12.7 g PPF per kg of milk produced. Based on the energy level of the protected fat as indicated by the manufacturer, the energy density of the experimental diets was increased by 6.83 MJ and 13.65 MJ kg^{-1} DM for the MG250 and MG500 treatments, respectively.

The portion of the protected fats was mixed with the concentrate feed mixtures once per day at 06:00 h. Adjustments were made to the diets to ensure collection of orts. The cows were offered feeds three times daily at 06:00 h, 12:00 h, and 18:00 h for 13 weeks. The cows were offered a portion of concentrate feed mixtures, followed by corn silage and berseem clover. The feed intake was recorded daily by weighing the offered diets and refusals from the previous day. The samples of berseem clover and concentrate mixtures were taken daily, composited biweekly, dried at 60°C in a forced-air oven for 48 h (AOAC, 1997) and stored for chemical analyses. The ingredient and chemical compositions of the diets are shown in Table 2.

Dried feed samples were ground to pass a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) and analyzed for DM, ash, nitrogen, and ether extract (EE) according to AOAC (1997) official methods. Neutral detergent fiber (NDF) was determined by the procedure of Van Soest et al. (1991), without use of an alpha amylase but with sodium sulfite and expressed exclusive of residual ash. Acid detergent fiber (ADF) was analyzed and expressed exclusive of residual ash (AOAC, 1997).

Sampling and Analysis of Blood Serum

The cows were sampled for blood monthly, where 10 mL of blood samples were taken 4 h after feeding from the jugular vein of all cows of each treatment into a clean dry tube without anticoagulants. Blood samples were centrifuged at 4,000×g for 20 min. The serum was separated into 2-mL Eppendorf tubes and frozen at -20°C until analysis. Using specific kits (Stanbio Laboratory, Boerne, Texas, USA) and following the manufacturer's instructions, blood serum samples were analyzed for concentrations of total protein, albumin, urea-N, glucose, triglycerides, cholesterol, and creatinine. Globulin concentration was calculated by subtracting albumin values from their corresponding total protein values.

Milk Sampling, Milk Composition, and Fatty Acids Analysis

The cows were machine-milked three times daily at 05:00 h, 11:00 h, and 19:00 h, and samples (100 g kg^{-1} of recorded milk yield) were collected at each milking. A mixed sample of milk (proportional to amounts produced in each milking time) was taken daily.

Table 2. Ingredient and chemical composition (g kg^{-1} DM) of feedstuffs and basal diet fed to lactating Holstein cows and Barki sheep

	Ingredient					Diet	
	CFM (Cows)[1]	CFM (Sheep)[2]	Berseem clover	Berseem hay	Corn silage	Basal diet[3]	Basal diet[4]
DM	906	895	138	910	299	700	902
OM	897	890	870	895	906	902	893
CP	182	147	135	154	86	134	151
EE	25	21	21	18	21	23	20
NSC	273	288	175	242	319	296	265
NDF	417	434	539	481	480	449	458
ADF	276	191	469	392	396	336	292

Note: ADF, acid detergent fiber; CFM, concentrate feed mixture; CP, crude protein; DM, dry matter; EE, ether extract; NDF, neutral detergent fiber; NSC, non-structural carbohydrates; OM, organic matter

[1] Contained per kg DM: 300 g corn, 250 g wheat bran, 300 g cotton seed meal, 120 g soybean meal, 18 g limestone, 10 g NaCl, 2 g minerals mixture

[2] Contained per kg DM: 400 g corn, 250 g wheat bran, 250 g cotton seed meal, 55 g soybean meal, 20 g limestone, 10 g NaCl, 5 g minerals mixture, and 10 sodium bicarbonate

[3] Fed to lactating cows based on CFM, berseem clover and corn silage at 70:15:15, respectively

[4] Fed to sheep based on CFM and berseem hay at 50:50, respectively

Milk samples were analyzed for total solids, fat, protein, and lactose using infrared spectrophotometry (Foss 120 Milko-Scan, Foss Electric, Hillerød, Denmark). The ash content of milk was determined after heating milk samples in a muffle furnace at 550°C for 8 h. At the end of the experiment, fatty acids in milk were determined as described previously in Kholif et al. (2014), using methyl esters prepared by base-catalyzed methanolysis of glycerides (KOH in methanol) according to International Standards on a Perkin-Elmer chromatograph (model 8420, Beaconsfield, Perkin Elmer, Beaconsfield, UK) equipped with a flame ionization detector.

Average yields (g d^{-1}) of each milk component were calculated for individual cows by multiplying milk yield by the component content (g kg^{-1}) of milk. Energy-corrected milk (ECM) was calculated according to Tyrrell and Reid (1965) as:

ECM (kg d^{-1}) = 0.327 × milk yield + 12.95 × fat yield + 7.2 × protein yield, according to Tyrrell and Reid (1965). Moreover, 4% fat corrected milk (FCM, kg day^{-1}) was calculated according to Gaines and Davidson (1923) as: FCM = 0.4 × milk yield + 15 × fat yield.

Digestion and N Balance Experiments

A digestion trial was conducted for 3 weeks, with 2 weeks as a preliminary period and 1 week for sample collection. Twelve adult Barki male sheep, weighing 41 ± 4.1 kg and randomly divided into three groups of 4 males each, were fed a basal diet containing concentrate mixture and clover hay at 50:50 without supplementation of PPF (Control treatment) and the basal diet supplemented with 25 g of PPF (ME25 treatment), or with 50 g of PPF (ME50 treatment). They were fed individually at two equal portions at 07:00 and 16:00 h. The portion of the PPF was mixed with the concentrate feed mixtures once per day at 07:00 h. The sheep were housed individually in metabolic cages, with free access to fresh water. Individual intakes were recorded daily by subtracting the orts from the offered feed. Feces were completely collected in buckets and 100 g/kg of the total collection was stored at -20°C. All collected samples were mixed and one kilogram of the mixture was dried at 60°C for 72 h in a forced air oven, ground to pass through a 1-mm screen, and stored at room temperature until analysis.

The urine was collected into buckets containing 100 ml of 10% sulfuric acid to reduce pH below 3.0 and prevent bacterial destruction of urine samples. The volume of urine at each sampling was determined and sub-samples of 10 ml/100 ml of the total urine were collected from individual sheep and frozen until analysis of total N.

On the last day of the experiment, rumen fluid was collected via stomach tube before morning feeding to determine pH, ammonia-N (NH_3-N) and volatile fatty acids (VFA). Approximately 100 mL of rumen fluid was collected from individual sheep and strained through 4 layers of cheesecloth. A subsample of 5 mL was preserved in 5 mL of 0.2 *M* hydrochloric acid for NH_3-N analysis (AOAC, 1997), while 0.8 mL of strained ruminal fluid was mixed with 0.2 mL of a solution containing 250 g of metaphosphoric acid L^{-1} for VFA analysis by titration, after steam distillation of a 4-mL sample, using the method of Annison (1954).

Statistical Analyses

Data for the apparent nutrient digestibility, nitrogen balance and ruminal fermentation were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc. Cary, NC), with individual sheep as the experimental unit. The model included the effect of treatment. Data for feed intake, blood chemistry, and milk production, composition and efficiency were analyzed using the PROC MIXED procedure of SAS with week as a repeated measure and individual cow as the experimental unit. The model included the effect of treatment, week, and the treatment × week interaction. Two covariance structures were considered in the REPEATED statement in PROC MIXED: compound symmetry (cs) and auto-regressive (AR (1)). The error structure with the lowest Akaike information criteria that fits statistic was selected for the model. When the treatment F-test was significant at $P<0.05$, means were compared by applying the probability of difference option of the least squares means statement. The probability of difference option of the least squares means statement was used for multiple comparisons of means, and polynomial (linear and quadratic) contrasts (for the equal spacing of treatments) were used to examine cow and sheep responses to increasing doses of PPF. The treatment × week interaction was non-significant (i.e., $P>0.05$) for most of the measurements and was thus not reported.

Results

Performance Experiment

Feed Intake and Milk Production, Composition and Efficiency

Supplementation with PPF did not affect feed intake by cows (Table 3). The MG250 treatment showed a quadratic increase in daily milk production ($P = 0.017$), ECM ($P = 0.008$), and 4% FCM ($P = 0.012$) compared with the control and the MG500 treatments. Without affecting the concentrations and yields of other components, MG250 treatment also quadratically increased lactose concentration ($P = 0.023$) and yield ($P = 0.013$).

Quadratic increases in milk efficiency expressed as kg milk kg^{-1} DM intake ($P = 0.019$), kg ECM kg^{-1} DM intake ($P = 0.002$), and kg FCM kg-1 DM intake ($P = 0.007$) were observed with the MG250 treatment compared with the control and MG500 treatments (Table 2).

Milk Fatty Acid Profile

Feeding MG250 and MG500 treatments linearly decreased C6:0 ($P = 0.001$) and C20:5 ($P < 0.001$), without affecting the concentrations of other fatty acids (Table 4). Both MG250 and MG500 treatments did not affect the estimated activity of Δ9-desaturase calculated as C14:1/C14:0 and C16:1/C16:0 ratios.

Blood Chemistry

The MG250 and MG500 treatments linearly increased the concentrations of serum total protein ($P = 0.002$) and globulin ($P = 0.007$); however, they linearly decreased ($P = 0.002$) albumin/globulin ratio (Table 5). No effect was observed for the concentrations of albumin, urea-N, creatinine, glucose, triglyceride, and cholesterol.

Table 3. Feed intake, milk production and composition, and feed efficiency of lactating cows fed diets supplemented with different levels of palmitic acid-enriched protected fat

	Diet[1]			SEM	*P values*	
	Control	MG250	MG500		Linear	Quadratic
Intake (kg d^{-1})	20.9	20.4	20.9	0.16	0.966	0.120
Production (kg d^{-1})						
Milk	19.6^{b}	21.8^{a}	19.7^{b}	0.53	0.959	0.017
ECM[2]	17.1^{b}	19.6^{a}	17.1^{b}	0.71	0.988	0.008
4% FCM[3]	17.1^{b}	19.6^{a}	17.5^{b}	0.45	0.787	0.012
Total solids	2.25	2.54	2.19	0.137	0.785	0.072
Solids-not-fat	1.63	1.81	1.55	0.093	0.546	0.063
Fat	0.62	0.72	0.64	0.045	0.677	0.107
Protein	0.69	0.77	0.71	0.047	0.770	0.206
Lactose	0.79^{ab}	0.88^{a}	0.70^{b}	0.039	0.131	0.013
Milk energy output (MJ d^{-1})	52.6	60.1	52.3	3.36	0.956	0.078
Composition (g kg DM^{-1})						
Total solids	114.8	116.4	111.4	1.40	0.110	0.073
Solids-not-fats	83.6	83.4	78.6	1.12	0.006	0.109
Fat	31.1	32.9	32.8	0.96	0.239	0.426
Protein	35.0	35.4	35.8	0.39	0.160	0.993
Lactose	40.6^{a}	40.4^{a}	35.7^{b}	0.74	0.200	0.023
Milk energy content (MJ kg^{-1})	2.67	2.75	2.66	0.0378	0.773	0.094
Feed (milk) efficiency						
kg milk/kg DM intake	0.94^{b}	1.07^{a}	0.94^{b}	0.059	0.960	0.019
kg ECM/kg DM intake	0.82^{b}	0.96^{a}	0.82^{b}	0.054	0.985	0.002
kg FCM/kg DM intake	0.82^{b}	0.96^{a}	0.84^{b}	0.056	0.794	0.007

Note: Means in the same row not sharing a common superscript differ ($P<0.05$) according to the probability of difference option of the least squares means statement. SEM= standard error of the mean

[1] The basal diet based on (per kg DM) 700 g of concentrates feed mixture, 150 g berseem clover and 150 g corn silage with no additive (Control treatment) or with daily addition of 250 g palmitic acid-enriched protected fat (MG250 treatment) or 500 g of palmitic acid-enriched protected fat (MG500 treatment)

[2] ECM, energy corrected milk; calculated according to Tyrrell and Reid (1965) as: ECM = 0.327 × milk yield +12.95 × fat yield + 7.2 × protein yield

[3] FCM, fat corrected milk; calculated according to Gaines and Davidson (1923) as: 4% FCM = 0.4 × milk yield + 15 × fat yield

Table 4. Milk fatty acid profile of lactating cows fed diets supplemented with different levels of palmitic acid-enriched protected fat

	Diet[1]			SEM	*P values*	
	Control	MG250	MG500		Linear	Quadratic
C6:0	1.26[a]	0.44[b]	0.39[b]	0.164	0.001	0.071
C8:0	0.56	0.52	0.73	0.117	0.308	0.393
C10:0	1.47	1.39	1.30	0.179	0.521	1.000
C12:0	2.28	2.10	1.80	0.236	0.167	0.834
C14:0	7.86	7.75	7.14	0.389	0.208	0.602
C14:1	0.65	0.59	0.55	0.070	0.312	0.921
C15:0	0.80	0.70	0.68	0.048	0.088	0.491
C15:1	0.45	0.42	0.44	0.036	0.889	0.551
C16:0	27.2	29.1	29.1	0.730	0.092	0.294
C16:1	1.47	1.41	1.34	0.131	0.500	0.982
C17:0	0.45	0.47	0.45	0.027	1.000	0.576
C17:1	0.56	0.62	0.49	0.123	0.697	0.502
C18:0	13.9	15.0	16.1	0.64	0.028	0.970
C18:1C	10.72	8.75	8.20	0.939	0.074	0.544
C18:2C	19.7	20.5	22.3	1.02	0.089	0.693
C18:3 ω-3	1.20	1.26	0.85	0.192	0.203	0.330
C20:5	0.19	0.17	0.13	0.106	0.100	0.285
C22:1 ω-9	0.27	0.37	0.31	0.077	0.727	0.411
C22:6	0.85	0.51	0.65	0.1509	0.368	0.216
Non-identified fatty acids[2]	8.15	7.91	7.11	0.793	0.369	0.774
Total saturated fatty acids (SFA)	55.8	57.5	57.6	0.90	0.165	0.489
Monounsaturated fatty acids	14.1	12.2	11.3	1.90	0.420	0.614
Polyunsaturated fatty acids	21.9	22.4	23.9	1.10	0.218	0.717
Total unsaturated fatty acids (UFA)	36.1	34.6	35.3	1.02	0.582	0.405
UFA/SFA ratio	0.65	0.60	0.61	0.026	0.346	0.400
Athrogenicity index	1.70	1.81	1.70	0.094	0.969	0.344
Estimated Δ^9-desaturase activity						
C14:1/C14:0	0.082	0.074	0.077	0.0074	0.557	0.569
C16:1/C16:0	0.054	0.048	0.046	0.0042	0.206	0.720

Note: Means in the same row not sharing a common superscript differ (P<0.05) according to the probability of difference option of the least squares means statement. SEM= standard error of the mean

[1] The basal diet based on (per kg DM) 700 g of concentrates feed mixture, 150 g berseem clover and 150 g corn silage with no additive (Control treatment) or with daily addition of 250 g palmitic acid-enriched protected fat (MG250 treatment) or 500 g of palmitic acid-enriched protected fat (MG500 treatment)

[2] Unknown fatty acids represent GC peaks not identified as well as values for 13:0, C20:4, C20:3 and C20:0 fatty acids

Table 5. Blood parameters (mg dL^{-1}) of lactating cows fed diets supplemented with different levels of palmitic acid-enriched protected fat

	Diet[1]			SEM	*P values*	
	Control	MG250	MG500		Linear	Quadratic
Total protein	7.46[b]	8.08[a]	8.12[a]	0.139	0.002	0.095
Albumin	4.25	4.13	4.06	0.085	0.097	0.808
Globulin	3.29[b]	3.95[a]	4.06[a]	0.152	0.007	0.139
Albumin/globulin ratio	1.41[a]	1.14[b]	1.04[b]	0.064	0.002	0.263
Urea-N	22.6	25.2	24.0	0.86	0.252	0.078
Creatinine	2.44	2.29	2.05	0.130	0.073	0.815
Creatinine/total protein	0.34[a]	0.29[ab]	0.25[b]	0.021	0.009	0.723
Glucose	58.9	51.2	54.7	1.84	0.086	0.162
Triglycerides	17.4[a]	14.5[b]	14.8[b]	0.82	0.049	0.129
Cholesterol	178	178	184	7.1	0.555	0.746

Note: Means in the same row not sharing a common superscript differ (P<0.05) according to the probability of difference option of the least squares means statement. SEM= standard error of the mean.

[1] The basal diet based on (per kg DM) 700 g of concentrates feed mixture, 150 g berseem clover and 150 g corn silage with no additive (Control treatment) or with daily addition of 250 g palmitic acid-enriched protected fat (MG250 treatment), or 500 g of palmitic acid-enriched protected fat (MG500 treatment)

Digestion Experiment

Feed Intake and Nutrient Digestibility

No effects were observed with feeding MG25 or MG50 treatment on feed intake, and digestibility of DM, OM, crude protein (CP) and non-structural carbohydrates (NSC) (Table 6). Feeding MG50 treatment linearly increased the digestibility of NDF (P = 0.024) and ADF (P = 0.019), and decreased ether extract (EE) digestibility (quadratic effect, P = 0.019) compared with the control and MG25 treatments. The MG25 treatment increased EE digestibility.

Ruminal Fermentation and N Utilization

Both MG25 and MG50 treatments linearly decreased ruminal VFA concentration, without affecting the concentration of ruminal ammonia-N (Table 7). No treatment effects were observed for N intake, fecal N, urinary N, N balance, or nitrogen balance/nitrogen intake ratio (Table 7).

Table 6. Intake and nutrient digestibility in Barki rams (n = 4 rams per treatment) fed a basal diet supplemented with different levels of palmitic acid-enriched protected fat

	Diet[1]			SEM	*P values*	
	Control	MG25	MG50		Linear	Quadratic
Intake (g d^{-1})	908	947	923	24.0	0.656	0.289
Digestibility (g kg^{-1})						
Dry matter	725	723	743	18.3	0.489	0.647
Organic matter	743	754	776	14.1	0.103	0.791
Crude protein	749	767	781	14.0	0.110	0.909
Ether extract	716[b]	755[a]	668[c]	10.3	0.138	0.019
Non-structural carbohydrates	789	791	807	11.6	0.280	0.597
Neutral detergent fiber	715b	720b	770a	16.7	0.024	0.262
Acid detergent fiber	590b	619b	677a	15.5	0.019	0.646

Note: Means in the same row not sharing a common superscript differ (P<0.05) according to the probability of difference option of the least squares means statement. SEM= standard error of the mean.

[1] The basal diet based on (per kg DM) 500 g of concentrates feed mixture and 500 g berseem hay with no additive (Control treatment) or with daily addition of 25 g palmitic acid-enriched protected fat (MG25 treatment), or 50 g of palmitic acid-enriched protected fat (MG50 treatment).

Table 7. Ruminal fermentation and nitrogen utilization in Barki rams (n = 4 rams per treatment) fed a basal diet supplemented with different levels of palmitic acid-enriched protected fat

	Diet[1]			SEM	*P values*	
	Control	MG25	MG50		Linear	Quadratic
Ruminal fermentation						
pH	6.8	6.7	6.7	0.82	0.453	0.333
Volatile fatty acids (mmol L^{-1})	107.3a	75.0b	88.9b	9.73	0.019	0.066
Ammonia-N (g L^{-1})	13.7	13.1	13.7	1.32	1.000	0.748
Nitrogen utilization (g d^{-1})						
Nitrogen intake	21.5	22.5	22.0	0.68	0.647	0.386
Fecal nitrogen	2.08	2.01	2.06	0.048	0.773	0.392
Urinary nitrogen	4.26	4.89	3.53	0.543	0.368	0.168
Nitrogen balance	15.2	15.6	16.8	0.95	0.250	0.737
N balance/N intake	0.70	0.70	0.77	0.032	0.203	0.351

Note: Means in the same row not sharing a common superscript differ (P<0.05) according to the probability of difference option of the least squares means statement. SEM= standard error of the mean.

[1] The basal diet based on (per kg DM) 500 g of concentrates feed mixture and 500 g berseem hay with no additive (Control treatment) or with daily addition of 25 g palmitic acid-enriched protected fat (MG25 treatment), or 50 g of palmitic acid-enriched protected fat (MG50 treatment)

Discussion

Performance Experiment

Feed Intake, and Milk Production, Composition and Efficiency

The similar feed intake by the cows is an evidence of unaffected palatability with feeding of PPF because the odor of soap (fatty acid calcium soap) can negatively affect feed intake. The result also indicates that the levels of PPF in the current study were within the acceptable range that did not impair feed intake in lactating dairy cows. Feed intake determines the nutrient and energy supply to meet the requirements for maintenance and milk production. Therefore, the increased milk production without corresponding increased feed intake is the reason for the enhanced milk (feed) efficiency and utilization by about 14 to 17% with the MG250.

The improved daily milk production with the MG250 (about 11.2, 14.6, and 14.6% for milk, ECM, and FCM, respectively) compared with the control indicates an improvement in the efficiency of energy utilization attributable to a lower energy loss as heat and methane, a direct use of long-chain fatty acids for milk fat secretion and a higher efficiency of ATP production from long-chain fatty acids than acetate (Chilliard, 1993; Garnsworthy, 1997). In addition, the increased milk production with the MG250 treatment may be due to the higher energy intake and more efficient use of fat by mammary gland (Naik, 2013; Schroeder et al., 2004). Efficient lipid metabolism and digestion increase milk production (Pramono et al., 2017; Titi, 2011). Moreover, feeding PPF increased PUFA concentrations, which is a strategy to improve milk composition (Kholif et al., 2018, 2016). The increased milk production with MG250 compared with MG500 reveals the high level of PPF had no advantage over the low level. The result also indicates that the low level of PPF was enough to meet the energy requirements of the cows, and the excess PPF (i.e., MG500 treatment) was not necessary for cows' energy requirement because cows showed their maximum capacity for milk production with the low level of PPF supplementation. Schauff and Clark (1992) observed that 6% protected fats of dietary DM intake quadratically increased milk production and decreased it when the protected fats level was increased to 9% of the dietary DM. In the present experiment, the protected fat additives were about 1.2 and 2.4% of total feed intake. Pramono et al. (2017) observed that feeding lactating cows on ruminal protected fats at 3% of diet DM increased milk production. Moreover, Tyagi et al. (2009), Sirohi et al. (2010), and Gowda et al. (2012) observed increased milk production with supplementation of diets of lactating cows with protected fats by about 5 to 24%.

The increased lactose concentration and yield with MG250, without affecting blood glucose concentration, suggest more glucose synthesis in the liver and utilization by the mammary gland for lactose synthesis, in consistence with Hammon et al. (2008) who reported increased milk and lactose production with increased blood glucose clearance rate when dairy cows' diet was supplemented with ruminal protected fats.

Unexpectedly, PPF supplementation did not affect milk fat or protein concentration. Increased milk production with PPF supplementation was expected to decrease milk protein concentration because enhanced milk production is not synchronized with uptake of amino acids by the mammary gland to synthesis more protein (DePeters and Cant, 1992). Moreover, dietary fat impairs amino acids transport to mammary gland and induces insulin resistance (Palmquist and Moser, 1981). The reason may be related to the level and fatty acids profile of the PPF (Kumar, 2017; Naik, 2013).

The fat to protein ratios ranged between 0.89 and 0.93, which is not normal for healthy cows, and indicate a metabolic disorder of experimental animals (e.g., subclinical ruminal acidosis). However, in the present experiment, we did not observe any metabolic disorders. As explained later, supplementation decreased ruminal VFA concentrations, which indicates that ruminal pH did not decline with fat supplementation. More experiments are required to establish or refute the current findings with more efforts to study this effect on ruminal fermentation.

Milk Fatty Acid Profile

Protected fats supplementation almost did not significantly alter milk fatty acids profile. It was expected that dietary fats supplementation would affect milk fatty acids concentrations and proportions. The reason for this observation is not clear; however, it may be due to the profile of fatty acids in the fed PPF, the amounts of fed fats, and also the stage of lactation of the cows (Schroeder et al., 2004). Unchanged fatty acid profile indicates unchanged physical, organoleptic, and nutritional characteristics of produced milk and dairy products (Chilliard et al., 2001; Grummer, 1991).

The unaffected activity of Δ^9-desaturase supports the data of minimally changed milk fatty acid profile because the synthesis of UFA in general and some monounsaturated fatty acids and nearly all conjugated linoleic acids in particular are regulated by the Δ^9-desaturase activity (Soyeurt et al., 2008).

Blood Chemistry

All measured blood parameters were within the reported reference ranges (Etim et al., 2013). The increased concentrations of serum total protein and globulin with feeding MG250 and MG500 treatments are evidence of enhanced nutritional status of the cows fed PPF (Miner et al., 1990). At the same time, the minimal effects on the concentrations of albumin, urea-N, and creatinine are indicators of unaffected glomerular filtration in the kidney and kidney function (Hosten, 1990; Olafadehan, 2011). Moreover, results of serum urea-N indicate a satisfactory metabolic status and an adequate provision of amounts of N and energy by the diets (Molina et al., 2015).

The unchanged concentration of serum glucose with feeding MG250 and MG500 treatments was not expected since both milk yield and milk lactose were increased in the present experiment. Glucose supply to mammary glands is important for adequate milk and lactose production (Kholif et al., 2016; Morsy et al., 2015). The results of unchanged milk fat concentration with feeding PPF confirm the assumption that feeding of PPF decreases glucose availability for oxidation and milk fat synthesis, making more glucose available for lactose synthesis in the mammary gland (Hammon et al., 2008; Lohrenz et al., 2010; Voigt et al., 2005). Feeding Ca soaps based on C16:0 and C18:1 fatty acids did not affect glucose turnover in dairy cows, but decreased plasma glucose concentrations (Hammon et al., 2008). Singh et al. (2014) observed that supplementing diets of crossbreed dairy cows with protected fats did not affect serum glucose levels during mid-lactation.

The unaffected cholesterol and triglycerides concentrations with feeding PPF during mid-lactation were previously reported by Singh et al. (2014). Both are indirect evidence of minimal effects of the additives on reproductive hormones (Singh et al., 2015).

Digestion Experiment

Feed Intake and Nutrient Digestibility

The effect of PPF on feed intake in Barki sheep was similar to that in cows; minimal effects were observed. We have already explained this observation above.

The enhanced fiber digestibility with MG25 and MG50 treatments confirms the assumption that protecting fat from ruminal biohydrogenation does not depress fiber digestion and cellulolytic activity. These results are consistent with Ngidi et al. (1990) who observed that increasing the level of Ca soap in diet of cows linearly increased NDF digestibility. The increase in the apparent total tract digestibility of NDF in cows fed Ca-soap may be due to an increase in the post-ruminal degradation (Chouinard et al., 1998).

The increased and decreased EE digestibility with MG25 and MG50, respectively, are logical results, in consonance with those reported by Sirohi et al. (2010). The increased EE digestibility indicates more digestibility for added fat than for the basal diet fat (Naik, 2013). The dilution of supplemented fats with the endogenous lipid secretions is another reason for the increased EE digestibility (Grummer, 1988). The decreased EE digestibility with the high level of bypass fat may be due to the limited capacity of the small intestine to absorb dietary fat (Jenkins and Palmquist, 1984) or due to the masking effect of endogenous fecal fat (Ngidi et al., 1990).

Supplementation with PPF did not affect the digestibility of other nutrients which may be due to the non-interference and relatively stable nature of protected fat in the rumen (Naik, 2013). Tyagi et al. (2009) observed unaffected nutrient digestibility with feeding bypass fats to lactating cows.

Ruminal Fermentation and N Utilization

The decreased ruminal VFA concentration with feeding PPF may be considered as an evidence of changed ruminal microflora profile, especially those involved in the production of ruminal VFA. The results of increased fiber digestibility reveal that the microbial cellulolytic numbers and activities were increased with supplementation of PPF; also, the productions of ruminal VFA and ammonia-N were increased and utilized in the production of ruminal microbial protein or were directly absorbed through rumen wall (Fiorentini et al., 2015). The concentration of NH_3-N and VFA in the rumen is a consequence of the balance between their production, absorption, and utilization by microorganisms, as their utilization efficiency by the microorganisms for the microbial protein synthesis depends on the availability of energy in the rumen (Russell et al., 1992).

The concentrations of ruminal ammonia-N ranged from 13.1 to 13.7 g L^{-1}, which is considered suitable for ruminal microbial synthesis and activity (Satter and Slyter, 1974). Unchanged digestibility of CP, ruminal ammonia-N, and blood serum urea-N are indicators of unaltered dietary protein metabolism with feeding PPF.

The minimal effect of feeding PPF on N intake, fecal N, urinary N, N balance, and N balance as a proportion of N intake may be due to the unchanged feed intake (i.e., protein intake) and CP digestibility.

Conclusion

In summary, supplementing diets of lactating Holsten cows with 250 g PPF enhanced the lactational performance and milk efficiency in mid-lactation and did not compromise their welfare. Because no major milk yield and composition responses were obtained with increasing the dose of PPF to 500 g per cow, we recommend the 250 g dose for use in practice; however, repeating the experiment with different levels of PPF and at different stages of lactation is recommended to validate results. Moreover, supplementing diets of sheep with 50 g PPF enhanced fiber digestion without affecting N utilization.

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