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Feeding Lactating Holstein Cows a Lipid Source High in Palmitic Acid Changes the Fatty Acid Composition and Thermal Properties of Lipids in Milk and Butter

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Abstract: The aim was to determine the impact of feeding either low or high palmitic acid supplemental fat to lactating Holstein cows on milk yield and composition, as well as changes in thermal properties of the milk fat. This study used a new dietary lipid supplement, Energizer-RP10® (IFFCO, Johor, Malaysia) which was commercially available and a source of unesterified fatty acids. There are numerous studies in the published literature reporting the impact of dietary lipids high in palmitic acid on animal performance but few reports determined the impact on the thermal properties of the milk fat produced. Cows fed the HP diet showed higher fat content and produced more fat-corrected milk. Concentration of palmitic acid in milk was higher for high palmitic than low palmitic. Textural analysis of butter showed that anhydrous milk fat from high palmitic was harder at both ambient and 10°C temperatures compared with low palmitic. Temperatures at onset to melting and peak of melting of butter from high palmitic were higher than low palmitic. Feeding a high palmitic acid supplement to lactating cows changed the FA composition of milk lipids and modified their functional property that was illustrated by a harder butter. The implications are that feeding lipid supplements high in palmitic acid could impact the physicochemical properties of dairy products, butter in particular but also, potentially other dairy products that contain milk fat.

INTRODUCTION

Supplementation with specific dietary lipids was one method used to modify the Fatty Acid (FA) composition of milk fat (Grummer, 1991; Palmquist *et al*., 1993; Ashes *et al*., 1997; Demeyer and Doreau, 1999). Feeding lipids high in palmitic acid to lactating cows had dramatic effects on the FA composition of milk lipids, particularly because palmitic acid undergoes none or very little modification by rumen microbes. Early researchers

(Steele and Moore, 1968) observed that feeding palmitic acid at 100 g kg^{-1} of the concentrate mixture markedly increased C16:0 in milk fat and that the butter produced from the high C16:0 milk was harder. These researchers also reported that for 1 cow, palmitic acid constituted over 70% of the FA portion of milk. Banks *et al*. (1976c) found that feeding FA from a blend of palm oil and palmitic acid increased C16:0 in milk fat compared with feeding tallow or soybean oil. Feeding a blend of palm oil and palmitic acid produced a harder milk fat than either tallow or soybean oil (Banks *et al*., 1976a). These researchers observed that, as the proportion of C16:0 increased in milk fat, melting temperature increased (Banks *et al*., 1976a; Banks *et al*., 1980a). More recent research with palmitic acid in commercially available dietary lipid supplements, calcium salts of fatty acids (Palmquist *et al*., 1993) and unesterified fatty acids of palmitic acid (Mosley *et al*., 2007; Wartjes *et al*., 2008) report information on animal performance but these studies did not look at the impact of the dietary lipid on thermal properties of the milk lipids. The current understanding of how these new commercial dietary lipids high in palmitic acid impact milk lipids is based on research conducted in the 1960s (Steele and Moore, 1968) and 1970s (Banks *et al*., 1976a; Banks *et al*., 1980a). These were excellent studies but additional information on the association between diet and the physicochemical properties of milk lipids improves the understanding of mammary biology. Even though, a harder milk fat is often viewed as negative, particularly for butter consumers, milk fat with a higher melting point can be advantageous to manufacturers of delicate chocolates and the pastry industry (Timms, 1989)

The Phospholipid (PL) fraction of milk plays an important role in Milk Fat Globule Membrane (MFGM) formation. Approximately 410 g kg^{-1} of the total lipid extracted from the MFGM was in the phospholipid fraction (Fong *et al*., 2007). Recent evidence showed that increased amounts of MFGM could act as a nutraceutical and might be linked with prevention of certain types of cancer (Spitsberg, 2005). Milk PL is a rich source of arachidonic acid and n-3 FA (McCaughey *et al*., 2005). The impact of dietary lipids on the FA of the PL fraction of milk lipid was studied (McCaughey *et al*., 2005) but the impact of diet on FA composition of PL is not well defined compared with the knowledge related to the FA composition of the TG fraction of milk lipids. The objective was to determine the effect of a high palmitic acid supplement compared with a low palmitic acid supplement on milk yield and composition in particular the FA composition of milk, buttermilk and butter for the TG and PL fractions and the thermal properties of butter.

MATERIALS AND METHODS

Animal and diets: About 12 lactating Holstein dairy cows (8 multiparous and 4 primiparous cows) were used in a cross-over design. Each period was 14 days and the last 5 days of each period were assigned to data collection. About 2 week periods were successfully used to study changes in FA composition (Banks *et al*., 1990; Shingfield *et al*., 2003; Carroll *et al*., 2006). The treatment variable in the current study was the type of dietary lipid supplement fed (Table 1). The lipid supplement was either a high palmitic acid lipid (free FA; Energizer-RP10®, IFFCO, Johor, Malaysia) or a low palmitic acid lipid (yellow grease, a rendered fat). The lipid supplements were added into a TMR at 2, 100 g^{-1} of the total diet DM to create the High Palmitic acid (HP) and Low Palmitic acid (LP) diets (Table 2). All dietary ingredients were similar with the only exception being the source of lipid supplement. Diets were similar in chemical composition although the LP diet was lower in total FA because the

Table 1: Ingredient and chemical composition of diets supplemented with fat sources containing high and low concentration of palmitic acid (DM basis)

	Treatments[ab]	
Composition	HP	LP
Ingredient (g kg^{-1}):		
Alfalfa hay, sliced	430.00	430.00
Beet pulp	140.00	140.00
Corn, flaked	100.00	100.00
Barley, rolled	100.00	100.00
Cottonseed whole	100.00	100.00
Molasses, cane	50.00	50.00
Almond hulls	40.00	40.00
Mineral[c]	20.00	20.00
Supplemented fat	20.00	20.00
Chemical:		
NEL[d], (MJ kg^{-1})	6.99	6.99
Crude protein (g kg^{-1})	184.50	184.00
Soluble protein (g kg^{-1})	75.50	69.00
Acid detergent fiber (g kg^{-1})	239.00	238.00
Neutral detergent fiber (g kg^{-1})	339.50	336.50
Ash (g kg^{-1})	85.00	86.00
Non fiber carbohydrates (g kg^{-1})	361.00	364.00
Calcium (g kg^{-1})	9.20	9.30
Phosphorus (g kg^{-1})	4.00	3.90
Magnesium (g kg^{-1})	4.70	5.00
Potassium (g kg^{-1})	19.20	18.90
Sodium (g kg^{-1})	4.50	4.10
Iron (mg kg^{-1})	296.50	380.50
Manganese (mg kg^{-1})	64.50	67.50
Zinc (mg kg^{-1})	102.00	93.00
Copper (mg kg^{-1})	11.50	13.00

[a]Treatments were HP = High palmitic saturated Fatty Acids (FA) from 20 g kg^{-1} palmitate flaked hydrogenated FA/10 g diet; LP = Low Palmitic saturated FA from yellow grease; [b]Ingredient and nutrient composition of diets sampled each period (n = 2 for each treatment); [c]Mineral-mix contained not <80.3 g kg^{-1} calcium and not >102.3 g kg^{-1} calcium, not >120.5 g kg^{-1} sodium, not <40 g kg^{-1} (%) phosphorus, not <16.16 mg kg^{-1} selenium and not >17.00 mg kg^{-1} selenium.; [dNE]L calculated according to Moe *et al*. (1972): NEL (Mcal kg^{-1}) = 0.0245* TDN-0.12

Table 2: Fatty acid composition (g fatty acid/kg DM) of fat sources containing high (Energizer-RP10®) and low (Yellow grease) levels of palmitic acid

Parameters	Energizer-RP10®	Yellow grease
C4:0-C10:0	<2.2	<0.7
C12:0	21.9	1.1
C14:0	40.6	6.6
C15:0	1.6	0.8
C16:0	793.1	121.2
C16:1 cis	0.9	14.8
C17:0	0.8	2.5
C18:0	25.1	59.1
C18:1 trans 9	00.0	12.4
C18:1 trans 11	00.0	17.7
C18:1cis 9 and 10	35.2	284.9
C18:2	1.2	154.1
C18:3	0.0	12.4
C18:2 cis 9, trans 11	0.0	0.7
Total FA	923.9	691.0

Table 3: Fatty acid composition (g fatty acid/100 g fatty acid) of diets supplemented with fat sources containing high and low levels of palmitic acid

	Treatments[a]		
Parameters	HP	LP	SD
C6:0	0.00	0.00	<0.01
C8:0	0.04	0.15	0.08
C9:0	0.04	0.14	0.07
C10:0	0.03	0.03	<0.01
C11:0	0.36	0.18	0.13
C12:0	0.23	0.27	0.03
C13:0	0.01	0.05	0.03
C14:0	1.87	0.99	0.62
C14:1 cis	0.00	0.00	<0.01
C15:0	0.21	0.28	0.05
C16:0	49.92	30.03	14.06
C16:1 trans	0.05	0.20	0.11
C16:1 cis	0.35	0.67	0.23
C17:0	0.16	0.28	0.08
C18:0	2.84	6.72	2.74
C18:1 trans 6 and 8	0.06	0.51	0.32
C18:1 trans 9	0.09	0.66	0.40
C18:1 trans 10	0.07	1.06	0.70
C18:1 trans 11	0.08	0.77	0.49
C18:1 trans 12	0.06	0.46	0.28
C18:1 trans 13 and 14	0.00	0.00	<0.01
C18:1 cis 9 and 10	13.24	22.73	6.71
C18:1 cis 11	0.57	1.25	0.48
C18:1 cis 12	0.50	1.31	0.57
C18:1 cis 13	0.04	0.10	0.04
C18:1 trans16	0.01	0.09	0.06
C18:2	26.44	26.96	0.37
C18:3	2.41	3.00	0.42
C18:2 trans 8, cis 10	0.00	0.00	<0.01
C18:2 trans 10, cis 12	0.00	0.00	<0.01
C18:2 cis 11, cis 13	0.00	0.00	<0.01
C18:2 all trans	0.26	1.11	0.60
C20:4	0.00	0.00	<0.01
C20:5	0.00	0.00	<0.01
C22:5	0.00	0.00	<0.01
C22:6	0.00	0.00	<0.01

[a]Treatments were HP = High Palmitic saturated Fatty Acids (FA) from 2 (%) palmitate flaked hydrogenated FA; LP = Low Palmitic saturated FA from yellow grease

yellow grease had other non-lipid constituents. The HP diet had higher C16:0 than the LP diet (Table 3) as planned.

Each cow was housed in individual pens 82 m length and 53 m width with an area bedded with rice hulls. Each cow had free access to her ration using an individual feed box and to clean water provided in a trough. All cows received 500 mg of exogenous recombinant bovine somatotropin every 14 days (Posilac, Monsanto Co., St. Louis, MO, USA). This study was conducted from August to September, 2004. The Institutional Animal Care and Use Committee of the University of California at Davis approved care of the cows.

All cows were fed twice daily following milking at approximately 07:30 and 19:30 h. Amounts of feed fed were similar across periods and adjusted for the amount of feed refused to allow <5 g/refusal per 100 g feed offered (as is basis). Samples of each diet were collected daily during the 2nd week of each period and the individual daily diet samples were composited by period. Feed refusals were weighed three times each week and were subsampled and composited for week 2. A portion of the week 2 feed composite samples and refusal composite samples were subsampled, oven dried at 100°C for 16 h and weighted immediately after removal from the oven to determine dry matter content. The remaining portion of feed samples were air dried at room temperature (25°C) for approximately 96 h and then ground through a 1 mm screen using a Wiley mill (Arthur A. Thomas, Philadelphia, PA, USA). Ground samples were stored in sealed plastic containers at room temperature (25°C) until analyzed. Feed samples were later analyzed for total Nitrogen (N), soluble N, Acid Detergent Fiber (ADF), Neutral Detergent Fiber (aNDF), non fiber carbohydrates, ash, minerals and individual FA content.

Milk schedule and milk processing: Cows were milked twice daily at approximately 0700 and 1900 h and milk yield was recorded at each milking using Dairy Planner (Westfalia, Naperville, IL, USA). Milk samples from each cow were collected 2 times during week 2 of each period. Milk collection consisted of sampling from consecutive evening and morning milkings (Sunday PM and Monday A.M, Monday P.M and Tuesday A.M). Westfalia milk meters and samplers (Westfalia, Naperville, IL, USA) were used to measure milk yield and collect proportional milk samples. Milk samples were preserved in 2-bromo-2-nitro-propane-1, 3-diol (Silliker Labs, Inc., Modesto, CA, USA) and kept refrigerated (5°C) until analyzed immediately following the morning collection. Prior to analyses, milk samples were placed in a water bath, warmed to 40°C and the a.m and p.m samples from each cow were gently mixed to create a daily composite. Milk samples were analyzed for their content of fat, protein, solids-not-fat, lactose and FA composition.

During week 2 of each period the total milk yield of each cow (12 h period of milk production) was collected at one milking (0700 h) and the cream separated. On the morning of day 13, the milk output of 6 cows, 3 cows from each dietary treatment was collected for cream collection. On morning of day 14 the milk from the remaining 6 cows was collected. In period 2, the cows collected on Tuesday were those previously collected on the Wednesday during period 1. The cows collected on Wednesday in period 2 were those collected on Tuesday of period 1. For both days the milk was collected in 37.8 L plastic milk buckets and maintained at 39°C in a water bath. Milk from each cow was passed through a cream separator (McCormick, 3-F, Power Washing Cream Separator, International Harvestor Company, Chicago, IL, USA). The initial yield of cream was between 1-3 L. The raw cream was washed with a Phosphate Buffered Saline solution (PBS; 1:10 w:w) and the cream passed through the separator again. The PBS solution was 100 mM NaCl and 50 mM phosphate at pH 7.0. The washed cream was stored at 4°C overnight. In the following day, the washed cream was placed in a mixer (Kitchen Aid, Professional 600, St. Joseph, MI, USA) and the cream churned. Once butter was formed, the buttermilk was separated from the butter using 2 layers of cheesecloth. The butter and buttermilk were stored at -20°C until analyzed.

Analytical procedures: Chemical composition of each diet was determined at a commercial laboratory (Cumberland Valley Analytical Services, Maugansville, MD, USA). Dry matter, ash and minerals in feed were tested using the Association of Official Analytical Chemists (AOAC, 2000; sections 930.15, 942.05 and 985.01, respectively). Acid Detergent Fiber (ADF) was according to 973.18 (AOAC, 1995) with the following modifications: For ADF determination, glass micro-fiber filters with 1.5 μm particle retention were utilized instead of a fritted glass crucible and ash determination was performed on 0.5 g sample for 2 h at 535°C, samples for mineral determination were digested in an open crucible for 20 min in 15 v/v nitric acid on hotplate and then samples were diluted to 50 mL and analyzed on axial view ICP. Neutral Detergent Fiber (aNDF) was determined using Whatman 934-AH glass micro-fiber filters with 1.5 μm particle retention and according to the methods described by Mertens (2002). Soluble protein was measured using a borate-phosphate procedure (Krishnamoorthy *et al*., 1982).

During week 2 of each period a pooled milk sample was collected from each cow. Milk was collected on day 11 p.m. and day 12 a.m. and stored at 4°C. After the morning milking samples were warmed to 40°C in a water bath and the a.m and p.m samples from each cow were gently mixed to create the composite sample. These samples were analyzed for fat, protein, lactose and SNF (AOAC., 1995; Method #972.16) with an infrared milk analyzer (Bentley 150, Bentley Instruments Inc., Chaska, MN, USA) and for urea N by Technicon Auto Analyzer (Technicon Corporation, Emeryville, CA, USA) method N-10a (Marsh *et al*., 1957).

A 10 mL aliquot of milk was obtained and milk fat was isolated and analyzed for total FA of the Triacylglycerol (TG) by Gas Chromatography (GC) according to (Crocker *et al*., 1998; DePeters *et al*., 2001). Fatty acid composition of the milk fat was determined by GC separation of methyl esters a Hewlett Packard 5890 gas chromatograph (Hewlett Packard, Ramsey, MN, USA) equipped with a flame ionization detector and a Supelco 2560, 100 m capillary column containing a 0.25 mm inside diameter and a 0.20 micron film thickness (Supelco Analytical, Bellefonte, PA, USA). Hydrogen was used, as the carrier gas with a linear flow rate of 27 cm sec^{-1} and a column head pressure of 227.53 kpa (33 psi). About 1 μL of the ester mixture was injected using a Hewlett Packard 7673 auto injector and subjected to a split vent flow rate of 100 mL min^{-1}. The injector temperature was set at 210°C and the detector temperature set at 220°C with the column temperature initially set at 70°C for 10 min followed by a programmed increase to 175°C at 20°C per minute for 29 min and finally to 225°C at 5°C per minute for 12 min.

Ester peak areas were acquired from the chromatograph through a Hewlett Packard 's HPIB/RS-232 board and connected to a personal computer using Windows 98, as the operating system (Hewlett Packard, Ramsey, MN, USA). Unknown peak areas were quantified using Chrom Perfect version 3.52 (Justice Innovations, Inc., Palo Alto, CA, USA) and compared with a known quantity of an external standard mixture containing all reported fatty acids. This FA standard mixture was comprised of individual FA obtained from Nu Chek Prep (Nu Chek Prep, Elysian, MN, USA).

A 5 mL aliquot was obtained from the milk collected on Tuesday and Wednesday morning of both periods and analyzed for TG and PL FA , for milk, butter and buttermilk, using methods described by Bitman *et al*., 1984. The milk was aliquoted into a 50 mL (25×150 mm) glass, screw top culture tube and heated rapidly to 80°C and held for 1.5 min to deactivate the lipase enzymes. After cooling, the milk was extracted with 36 mL of chloroform; methanol (2:1) using a Burrell wrist action shaker for 10 min. The lower chloroform layer containing the milk fat was transferred to another glass culture tube and dried down under nitrogen then reconstituted with 2 mL of hexane. A Pasteur pipette was used to transfer the 2 mL of sample to a Water's Sep-Pak silica cartridge (WAT051900) which had been previously prepared by rinsing with 20 mL of chloroform. The

hexane was allowed to drip through by gravity and the sample tube was washed with an additional 1 mL of hexane and added to the cartridge. The TG were eluted by pumping 4×10 mL aliquots of hexane:ethyl ether (1:1) through the cartridge with a glass syringe and saving in 50 mL glass screw top culture tube. The PL were subsequently collected into a 50 mL glass culture tube by pumping 2×10 mL aliquots of methanol followed by 2×10 mL aliquots of chloroform; methanol; water (3:5:2) through the cartridge with a glass syringe. Since, the TG fraction is much more concentrated than the PL fraction only a 2 mL aliquot of the TG fraction eluent was used for analysis while all of the solvent from the PL fraction elution was used. For each fraction the solvents were evaporated under nitrogen at 60°C, followed by a 1 mL wash with iso-octane which was again evaporated under nitrogen. Finally, 2 mL of iso-octane were added to each tube and the milk fat fractions were methylated, isolated and quantified using the procedure described above for milk fat methylation.

Measurements of thermal properties and textural analyses of butter were performed at the Dairy Products Teaching Center at California Polytechnic University, San Luis Obispo, CA, USA. Milk fat crystallization studies were performed on the Anhydrous Milk Fat (AMF) which was separated from the serum fraction by melting the butter at 65°C, then centrifuging at 66.67 Hz while warm. Isothermal turbimetric absorbance was measured on melted (60°C) AMF at 610 nm in a Spectra Max Plus spectrophotometer (Molecular Dynamics, Sunnyvale, CA, U.S.A). Measurements were taken in 30 sec intervals until nucleation occurred which changed drastically the absorbance of the sample. Each sample thus generated a curve of absorbance and time. From this curve researchers measured the slope of the linear part of the curve (absorbance/time) which is proportional to the rate of nucleation.

Texture analysis (butter fat hardness) was performed on a Texture Analyzer TA-XT2 (Texture Technologies. Scarsdale, NY, USA) with a 3 mm diameter cylindrical puncture solid probe attachment at 10°C and ambient temperature. Each sample was made in a 5 cm diameter mold with a 2 cm depth, the probe was lowered at 1 mm sec^{-1} to a depth of 12 mm from the sample surface and then was withdrawn at the same speed. Data acquisition began at 0.049 N of probe force. The penetration force (in N) was reported as hardness, hardness was measured in triplicate on each equilibrated AMF sample and the force in Newtons (N) recorded.

Statistical procedure: Statistical analysis of each variable was performed using PROC GLM of SAS (SAS, 1999). Data were analyzed using a cross-over design that included the effects of cow, period and dietary treatments (Cochran and Cox, 1957). Pearson correlation coefficients were obtained utilizing the JMP 7.0 software (SAS Institute, Cary, NC, USA).

RESULTS

Milk yield and composition: Yields of milk and dry matter intake were not affected by supplementation with HP or LP lipid supplement (Table 4). However, fat-corrected milk yield was higher ($p = 0.01$) when cows were fed the HP lipid supplement than the LP lipid supplement due to the higher concentration of fat in milk. Yield of milk fat was higher ($p<0.01$) for HP (1.63 kg $days^{-1}$) than for LP (1.44 kg day^{-1}) whereas yields of protein, lactose and solids-not-fat were not different. Concentration of fat was higher in milk when cows were fed HP (42.1 g kg^{-1}) than LP (36.8 g kg^{-1}) diets. Lactose and solids-not-fat concentrations were also increased by feeding the HP diet.

Fatty acid composition of the TG fraction: The FA composition of the TG fraction of milk fat from the composite milk sample (Sunday p.m and Monday a.m) collected from all cows was modified by the lipid supplement fed (Table 5). The HP supplement increased ($p<0.01$) the concentration of C16:0 in milk fat (40.91 g/100 g FA) compared with LP (28.14 g/100 g FA). Concentrations of C6:0, C8:0, C10:0, C12:0 and C14:0 were significantly lower in milk fat from cows fed HP than cows fed LP diets, however the proportion of short-chain FA (SCFA; sum of FA with <10 carbons) was similar. The concentration of trans C18:1 isomers in milk TG were all significantly reduced by feeding the HP diet

Table 4: Dry Matter Intake (DMI) and production performance of dairy cows fed diets that were supplemented with fat sources containing high and low levels of palmitic acid

	Treatments[a]			Pr>F
Parameters	HP	LP	SE	Trt[b]
Milk yield, kg d^{-1}	38.70	38.90	0.67	0.82
4% FCM[c]	40.00	37.10	0.60	0.01
DMI	24.80	25.10	0.36	0.61
Fat	1.63	1.44	0.03	<0.01
Protein	1.16	1.14	0.02	0.63
Lactose	1.93	1.88	0.03	0.36
Solids-not-fat	3.47	3.46	0.06	0.91
Milk composition, g kg^{-1}				
Fat	42.10	36.80	0.80	<0.01
Protein	30.00	29.40	0.20	0.09
Lactose	49.70	48.30	0.30	<0.01
Solids-not-fat	89.70	89.30	0.20	0.01

[a]LS means for treatments: HP = High Palmitic saturated Fatty Acids (FA) from 20 g kg^{-1} palmitate flaked hydrogenated FA; LP = Low Palmitic saturated FA from yellow grease; [b]Trt = Probability of F test for the effect of treatment; [c]4 (%) fat-corrected milk = [(kg milk* 0.4)+(kg milk fat*15)]

Table 5: Fatty acid composition (g fatty acid/100 g fatty acid) of triacylglycerol in milk fat

	Treatment[a]			Pr>F
Parameters	HP	LP	SE	Trt[b]
C4:0	3.90	3.84	0.07	0.57
C6:0	1.87	1.98	0.03	0.03
C8:0	0.91	1.01	0.02	0.01
C10:0	1.87	2.09	0.05	0.01
C11:0	0.05	0.04	0.01	0.62
C12:0	2.12	2.35	0.06	0.03
C14:0	8.45	8.90	0.13	0.03
C14:1 cis	0.65	0.66	0.02	0.66
C15:0	0.81	0.87	0.01	<0.01
C16:0	40.91	28.14	0.21	<0.01
C16:1 trans	0.30	0.35	0.01	<0.01
C16:1 cis	1.87	1.27	0.07	<0.01
C17:0	0.51	0.61	0.01	<0.01
C18:0	9.60	14.37	0.15	<0.01
C18:1 trans 5	0.02	0.04	<0.01	<0.01
C18:1 trans 7	0.02	0.04	<0.01	<0.01
C18:1 trans 6 and 8	0.19	0.45	0.01	<0.01
C18:1 trans 9	0.22	0.43	0.01	<0.01
C18:1 trans 10	0.35	0.66	0.01	<0.01
C18:1 trans 11	0.56	0.97	0.02	<0.01
C18:1 trans 12	0.34	0.67	0.01	<0.01
C18:1trans 13 and 14	0.68	1.10	0.04	<0.01
C18:1 cis 9 and 10	19.17	23.41	0.29	<0.01
C18:1 cis 11	0.41	0.46	0.01	<0.01
C18:1 cis 12	0.34	0.68	0.01	<0.01
C18:1 cis 13	0.06	0.08	<0.01	<0.01
C18:1 trans 16	0.29	0.45	0.01	<0.01
C18:2	2.55	2.85	0.03	<0.01
C18:3	0.36	0.42	0.01	0.01
C18:2 cis 9 trans 11	0.26	0.41	0.01	<0.01
C20:4	0.14	0.15	<0.01	0.01
C20:5	0.04	0.04	<0.01	0.96
C22:5	0.06	0.08	<0.01	0.01
C22:6	0.01	0.01	<0.01	0.23
SCFA[c]	6.67	6.83	0.10	0.29
MCFA[d]	57.54	45.28	0.41	<0.01
LCFA[e]	35.67	47.75	0.45	<0.01
PUFA[f]	3.15	3.54	0.05	<0.01
UFA g	23.39	28.58	0.29	<0.01
Total trans C18:1[h]	2.68	4.80	0.08	<0.01

[a]LS means for treatments: HP = High Palmitic saturated Fatty Acids (FA) from 20 g kg^{-1} palmitate flaked hydrogenated FA: LP = Low Palmitic saturated FA from yellow grease; [b]Trt = Probability of F test for the effect of treatment; [c]SCFA = Short-Chain Fatty Acids; sum of C4-C9; [d]MCFA = Medium-Chain Fatty Acids; sum of C10-C17:1cis; [e]LCFA = Long-Chain Fatty Acids; sum of C18-C22:6; [f]PUFA = Poly-Unsaturated Fatty Acids; C18:2, C18:3, C20:4, C20:5, C22:5, C22:6; [g]UFA = Unsaturated Fatty Acids; C18:1cis 9 and 10, C18:1 cis 11, C18:1 cis 12, C18:1 cis 13 and all PUFA FA; [h]Total trans C18:1 = Sum of C18:1 trans 5, C18:1 trans 7, C18:1 trans 6 and 8, C18:1 trans 9, C18:1 trans 10, C18:1 trans 11, C18:1 trans 12, C18:1 trans 13, C18:1 trans 14 and C18:1 trans 16

Table 6: Fatty acid composition (g fatty acid/100 g fatty acid) of milk fat triacylglycerol before washing with a phosphate buffer solution and churning to butter

	Treatment[a]			Pr>F
Parameters	HP	LP	SE	Trt[b]
C4:0	3.81	3.98	0.14	0.40
C6:0	1.88	1.98	0.04	0.09
C8:0	0.95	1.03	0.02	0.02
C10:0	1.98	2.14	0.05	0.06
C11:0	0.04	0.09	0.02	0.12
C12:0	2.25	2.45	0.05	0.02
C14:0	8.54	8.98	0.14	0.05
C14:1 cis	0.62	0.66	0.02	0.13
C15:0	0.80	0.87	0.01	<0.01
C16:0	41.90	28.17	0.33	<0.01
C16:1 trans	0.27	0.28	0.01	0.64
C16:1 cis	1.77	1.36	0.05	<0.01
C17:0	0.49	0.55	0.02	0.10
C18:0	9.26	14.29	0.37	<0.01
C18:1 trans 5	0.02	0.04	<0.01	<0.01
C:18:1 trans 7	0.02	0.04	<0.01	<0.01
C18:1trans 6 and 8	0.20	0.43	0.01	<0.01
C18:1 trans 9	0.21	0.44	0.01	<0.01
C18:1 trans 10	0.34	0.66	0.01	<0.01
C18:1 trans 11	0.54	0.95	0.02	<0.01
C18:1 trans 12	0.33	0.67	0.01	<0.01
C18:1 trans 13 and 14	0.70	1.13	0.01	<0.01
C18:1 cis 9 and 10	18.09	23.07	0.37	<0.01
C18:1 cis 11	0.39	0.45	0.01	<0.01
C18:1 cis 12	0.32	0.70	0.01	<0.01
C18:1 cis 13	0.05	0.09	<0.01	<0.01
C18:1 trans 16	0.28	0.47	0.01	<0.01
C18:2	2.56	2.82	0.03	<0.01
C18:3	0.37	0.42	0.01	<0.01
C18:2 cis 9 trans 11	0.25	0.40	0.01	<0.01
C20:4	0.14	0.15	<0.01	0.40
C20:5	0.04	0.04	<0.01	0.23
C22:5	0.07	0.08	<0.01	0.03
C22:6	0.01	0.01	<0.01	0.23
SCFA[c]	6.64	6.99	0.18	0.20
MCFA[d]	58.67	45.56	0.52	<0.01
LCFA[e]	34.59	47.34	0.66	<0.01
PUFA[f]	3.20	3.52	0.04	<0.01
UFA[g]	22.30	28.23	0.38	<0.01
Total trans C18:1[h]	2.63	4.82	0.07	<0.01

[a]LS means for treatments: HP = High Palmitic saturated Fatty Acids (FA) from 20 g kg^{-1} palmitate flaked hydrogenated FA; LP = Low Palmitic saturated FA from yellow grease; [b]Trt = Probability of F test for the effect of treatment; [c]SCFA = Short-Chain Fatty Acids; sum of C4-C9; [d]MCFA = Medium-Chain Fatty Acids; sum of C10-C17:1 cis; [e]LCFA = Long-Chain Fatty Acids; sum of C18-C22:6; [f]PUFA = Poly-Unsaturated Fatty Acids; C18:2, C18:3, C20:4, C20:5, C22:5, C22:6; [g]UFA = Unsaturated Fatty Acids; C18:1 cis 9 and 10, C18:1 cis 11, C18:1 cis 12, C18:1 cis 13 and all PUFA FA; [h]Total trans C18:1 = Sum of C18:1 trans 5, C18:1 trans 7, C18:1 trans 6 and 8, C18:1 trans 9, C18:1 trans 10, C18:1 trans 11, C18:1 trans 12, C18:1 trans 13, C18:1 trans 14 and C18:1 trans 16

compared with the LP. Milk fat was higher in Polyunsaturated FA (PUFA) when cows consumed the LP supplement. Small but significantly higher concentrations of C18:2, C18:3, C18:2 cis-9 trans-11 (rumenic acid) were found in milk fat from cows fed the LP diet than HP. The proportion of Medium-Chain FA (MCFA; FA with 10-17 carbons) was higher ($p<0.01$) and long-chain (LCFA; FA with 18 and more carbons) was lower ($p<0.01$) for cows fed the HP diet than LP (Table 6). Total Unsaturated FA (UFA) and PUFA were lower ($p<0.01$) in milk TG when cows were fed HP compared with LP (23.39 and 3.15

Table 7: Fatty acid composition (g fatty acid/100 g fatty acid) of triacylglycerols and phospholipids in butter from cows supplemented with fat sources containing high and low levels of palmitic acid

	Triacylglycerol				Phospholipid			
	Treatment[a]			Pr>F[b]	Treatment[a]			Pr>F[b]
Parameters	HP	LP	SE	Trt[b]	HP	LP	SE	Trt[b]
C4:0	4.33	4.30	0.05	0.68	0.00	0.00	<0.01	<0.01
C6:0	2.06	2.14	0.03	0.08	0.00	0.00	<0.01	<0.01
C8:0	0.98	1.08	0.02	<0.01	0.00	0.00	<0.01	<0.01
C10:0	2.08	2.25	0.05	0.04	0.21	0.38	0.12	0.36
C11:0	0.06	0.06	0.02	0.97	0.01	0.01	<0.01	0.90
C12:0	2.24	2.44	0.06	0.04	0.75	0.93	0.17	0.47
C14:0	8.62	9.05	0.13	0.04	5.56	5.95	0.48	0.57
C14:1 cis	0.62	0.67	0.02	0.10	0.32	0.29	0.06	0.72
C15:0	0.81	0.87	0.01	<0.01	0.69	0.71	0.03	0.59
C16:0	41.45	27.66	0.32	<0.01	36.88	25.07	0.93	<0.01
C16:1 trans	0.25	0.27	0.01	0.16	0.25	0.30	0.01	0.01
C16:1 cis	1.75	1.23	0.03	<0.01	1.60	1.13	0.06	<0.01
C17:0	0.47	0.59	0.01	<0.01	0.54	0.60	0.01	<0.01
C18:0	9.41	14.54	0.19	<0.01	14.55	19.14	0.49	<0.01
C18:1 trans 5	0.03	0.04	<0.01	<0.01	0.04	0.04	<0.01	0.34
C:18:1 trans 7	0.02	0.05	<0.01	<0.01	0.02	0.03	<0.01	<0.01
C18:1 trans 6 and 8	0.21	0.45	0.01	<0.01	0.19	0.38	0.02	<0.01
C18:1 trans 9	0.24	0.45	0.01	<0.01	0.34	0.62	0.02	<0.01
C18:1 trans 10	0.36	0.66	0.01	<0.01	0.33	0.61	0.01	<0.01
C18:1 trans 11	0.53	0.95	0.03	<0.01	0.54	0.92	0.04	<0.01
C18:1 trans 12	0.33	0.68	0.01	<0.01	0.37	0.68	0.03	<0.01
C18:1 trans 13 and 14	0.71	1.13	0.03	<0.01	0.76	0.94	0.04	0.01
C18:1 cis 9 and 10	17.95	22.86	0.37	<0.01	24.54	29.01	0.59	<0.01
C18:1 cis 11	0.39	0.46	0.02	0.02	0.48	0.53	0.01	0.03
C18:1 cis 12	0.33	0.69	0.01	<0.01	0.46	0.89	0.02	<0.01
C18:1 cis 13	0.06	0.09	0.01	<0.01	0.06	0.09	<0.01	<0.01
C18:1 trans 16	0.29	0.47	0.01	<0.01	0.30	0.47	0.02	<0.01
C18:2	2.48	2.69	0.03	<0.01	7.57	7.30	0.68	0.78
C18:3	0.35	0.40	0.01	<0.01	0.48	0.50	0.02	0.50
C18:2 cis 9 trans 11	0.23	0.37	0.01	<0.01	0.31	0.50	0.01	<0.01
C20:4	0.15	0.16	0.01	0.23	0.92	0.92	0.12	1.00
C20:5	0.05	0.05	<0.01	0.39	0.15	0.14	0.01	0.71
C22:5	0.07	0.08	<0.01	0.04	0.47	0.45	0.05	0.81
C22:6	0.01	0.01	<0.01	0.24	0.12	0.31	0.10	0.21
SCFA[c]	7.37	7.53	0.07	0.16	0.00	0.00	<0.01	<0.01
MCFA[d]	58.37	45.10	0.51	<0.01	46.78	35.35	1.68	<0.01
LCFA[e]	34.17	47.27	0.56	<0.01	53.02	64.48	1.68	<0.01
PUFA[f]	3.10	3.38	0.04	<0.01	9.71	9.62	0.83	0.94
UFA[g]	21.83	27.49	0.41	<0.01	35.26	40.15	1.29	0.68
Total trans C18:1[h]	2.71	4.86	0.09	<0.01	2.90	4.69	0.10	<0.01

[a]LS means for treatments: HP = High Palmitic saturated Fatty Acids (FA) from 20 g kg^{-1} palmitate flaked hydrogenated FA; LP = Low Palmitic saturated FA from yellow grease; [b]Trt = Probability of F test for the effect of treatment; [c]SCFA = Short-Chain Fatty Acids; sum of C4-C9; [d]MCFA = Medium-Chain Fatty Acids; sum of C10-C17:1 cis; [e]LCFA = Long-Chain Fatty Acids; sum of C18-C22:6; [f]PUFA = Poly-Unsaturated Fatty Acids; C18:2, C18:3, C20:4, C20:5, C22:5, C22:6; [g]UFA = Unsaturated Fatty Acids; C18:1 cis 9 and 10, C18:1 cis 11, C18:1 cis 12, C18:1 cis 13 and all PUFA FA; [h]Total trans C18:1 = Sum of C18:1 trans 5, C18:1 trans 7, C18:1 trans 6 and 8, C18:1 trans 9, C18:1 trans 10, C18:1 trans 11, C18:1 trans 12, C18:1 trans 13, C18:1 trans 14 and C18:1 trans 16

versus 28.58 and 3.54 g^{-1} 100 g FA, respectively). The concentration of total trans C18:1 isomers was markedly lower for cows fed the HP (2.68, 100 g^{-1} FA) than LP diet (4.80, 100 g^{-1} FA).

Triacylglycerol FA composition of milk lipid collected from the morning milk that was used for cream separation (Table 6) was similar in composition to the composite milk sample (Table 5). Concentration of C16:0 was significantly increased in TG in response to HP supplementation compared with LP while concentrations of C18:2, C18:3, C18:1 cis-9 and 10 and most PUFA were lower. Similar to the composite milk fat, the proportion of MCFA was higher ($p<0.01$) and LCFA lower ($p<0.01$) for HP than LP with no change in SCFA. Total trans C18:1 isomers, UFA and PUFA were significantly lower in milk TG when cows were fed HP than LP.

Fatty acid composition of the butter and buttermilk fractions: The FA composition of butter TG (Table 7)

Table 8: Fatty acid composition (g fatty acid/100 g fatty acid) of triacylglycerols and phospholipids in buttermilk fat from cows supplemented with fat sources containing high and low levels of palmitic acid

	Triacylglycerol				Phospholipid			
	Treatment[a]			Pr>F[b]	Treatment[a]			Pr>F[b]
Parameters	HP	LP	SE	Trt[b]	HP	LP	SE	Trt[b]
C4:0	4.13	4.07	0.05	0.41	0.00	0.00	0.00	0.00
C6:0	1.92	2.02	0.03	0.03	0.00	0.00	0.00	0.00
C8:0	0.92	1.00	0.02	0.01	0.00	0.00	0.00	0.00
C10:0	1.92	2.08	0.05	0.05	0.12	0.17	0.02	0.15
C12:0	2.17	2.29	0.05	0.12	0.24	0.31	0.02	0.09
C14:0	8.59	8.97	0.14	0.07	2.05	2.30	0.09	0.08
C14:1 cis	0.59	0.64	0.02	0.19	0.05	0.07	0.01	0.03
C15:0	0.80	0.87	0.01	<0.01	0.33	0.37	0.04	<0.01
C16:0	42.32	28.40	0.36	<0.01	19.16	13.56	0.31	<0.01
C16:1 trans	0.30	0.36	0.01	<0.01	0.14	0.16	0.01	0.03
C16:1 cis	1.77	1.26	0.04	<0.01	1.38	0.93	0.04	<0.01
C17:0	0.51	0.62	0.01	<0.01	0.33	0.36	0.01	<0.01
C18:0	9.72	15.29	0.19	<0.01	15.15	17.95	0.10	<0.01
C18:1 trans 6 and 8	0.21	0.45	0.01	<0.01	0.11	0.21	0.01	<0.01
C18:1 trans 9	0.21	0.43	<0.01	<0.01	0.33	0.59	0.01	<0.01
C18:1 trans 10	0.35	0.66	0.01	<0.01	0.31	0.56	0.01	<0.01
C18:1 trans 11	0.53	0.95	0.02	<0.01	0.46	0.75	0.02	0.46
C18:1 trans 12	0.34	0.69	0.01	<0.01	0.24	0.44	0.02	<0.01
C18:1 trans 13 and 14	0.59	0.78	0.04	0.01	0.59	0.78	0.02	<0.01
C18:1 cis 9 and 10	17.58	22.47	0.42	<0.01	33.58	36.38	0.41	<0.01
C18:1 cis 11	0.36	0.43	0.01	<0.01	0.54	0.58	0.02	0.13
C18:1 cis 12	0.32	0.68	0.01	<0.01	0.53	1.00	0.02	<0.01
C18:1 trans 16	0.29	0.49	0.01	<0.01	0.22	0.30	0.01	<0.01
C18:2	2.50	2.71	0.03	<0.01	18.34	16.22	0.25	<0.01
C18:3	0.36	0.42	0.01	<0.01	0.85	0.80	0.01	0.03
C18:2 cis 9 trans 11	0.22	0.37	0.01	<0.01	0.49	0.83	0.01	<0.01
C20:4	0.14	0.15	<0.01	0.21	2.69	2.60	0.04	0.18
C20:5	0.04	0.04	<0.01	0.03	0.41	0.35	0.01	<0.01
C22:5	0.07	0.08	<0.01	0.03	0.96	0.96	<0.01	0.97
C22:6	0.01	0.01	<0.01	0.55	0.16	0.13	<0.01	0.64

[a]LS means for treatments: HP = High Palmitic saturated Fatty Acids (FA) from 2% palmitate flaked hydrogenated FA; LP = Low Palmitic saturated FA from yellow grease; [b]Trt = Probability of F test for the effect of treatment

reflected the TG composition of the source milk (Table 5). Butter produced from milk fat from cows fed HP diet was higher in C16:0 (41.45 vs 27.66 g/100 282 g FA) and lower in C18:0 (9.41 vs 14.54, 100 g^{-1} FA), C18:1 cis-9 and 10 (17.95 vs 22.86, 100 g^{-1} FA) and total trans C18:1 isomers (2.71 vs 4.86, 100 g^{-1} FA), than cows fed LP diet. The proportions of PUFA and UFA were lower for HP compared with LP. The FA composition of the PL fraction of butter fat (Table 7) was similar to the TG fraction. However, there were no differences in C18:2 and C18:3 in the PL fraction in response to lipid supplement whereas the TG fraction differed between diets.

Finally, the buttermilk composition was also analyzed for TG and PL (Table 8). The FA composition of buttermilk also reflected the FA of milk fat. Concentration of C18:2 in PL was higher than in TG and was affected by lipid supplement (18.34 vs. 16.22, 100 g^{-1} FA, $p = 0.01$) for buttermilk from cows fed HP or LP, respectively; however in the TG fraction concentration of C18:2 was decreased by feeding the HP diet (2.50 vs. 2.71, 100 g^{-1} FA, $p<0.01$). Buttermilk PL was a greater source of unsaturated FA compared with the TG fraction.

Table 9: Textural and thermal properties of butter made from anhydrous milk fat produced by lactating cows fed diets supplemented with high (HP) and Low Palmitic acid (LP) fat sources

	Treatment[a]			Pr>F
Items	HP	LP	SE	Trt[b]
Textural properties				
Hardness at 10°C, force (N)	16.84	10.67	0.45	<0.01
Hardness at ambient temperature, force (N)	1.51	0.35	0.15	<0.01
Thermal properties				
Peak melt, °C	38.11	35.96	0.13	<0.01
Onset to peak melt, °C	42.18	40.46	0.10	<0.01
Crystallization upon cooling, °C	15.43	13.73	0.17	<0.01
Temperature to onset of nucleation, °C	22.31	20.16	0.20	<0.01
Time to onset of nucleation, seconds	850.83	1242.50	66.09	<0.01
Nucleation[c]	0.39	0.15	0.04	<0.01

[a]LS means for treatments: HP = High Palmitic saturated Fatty Acids (FA) from 20 g kg^{-1} palmitate flaked hydrogenated FA; LP = Low Palmitic saturated FA from yellow grease; [b]Trt = Probability of F test for the effect of treatment; [c]Nucleation is a measure of the slope, absorbance ÷ time

Thermal properties of butter: Differences in the textural properties of butter were observed between HP and LP (Table 9). Butter was produced from the milk fat of each

cow. Butter produced from milk when cows were fed HP was considerably harder at both 10°C ($p<0.01$) and at ambient temperatures ($p<0.01$) than when cows were fed LP. Force required at 10°C was 16.84 Newtons (N) for butter when cows were fed HP compared with 10.67 N when cows were fed LP. In agreement with thermal properties, both peak melt temperature (38.11 and 35.96°C; Table 9) and onset of melt temperature (42.18 and 40.46°C; Table 9) were higher ($p<0.01$) in butter when cows were fed HP compared with LP, respectively.

Nucleation: Measurements were performed on the Anhydrous Milk Fat (AMF) obtained from each cow. The onset of nucleation was determined as the time at which the first crystals appeared in the spectrophotometer and the first inflection of the sigmoid curve appeared (all samples were maintained at 37°C). Onset of nucleation (Table 9) was shorter for AMF when cows were fed the HP diet (850.8 sec) compared with the LP diet (1242.5 sec). Thus, the temperature upon onset of crystallization was higher (15.43 vs. 13.73°C) when cows were fed HP ($p<0.01$). The speed of nucleation, (measured by slope of the line of absorbance verses time until nucleation) was slower in AMF from cows fed the LP diet (0.15) compared with HP (0.39) (Table 9).

DISCUSSION

Milk yield was not affected by type of lipid supplement (Table 4). This was anticipated since energy density of both diets was similar, it was a short-term study and the assumption was that the apparent digestibility of total dietary FA was similar for the 2 dietary lipid supplements that were used. Therefore, both diets provided sufficient energy to meet the requirements for milk synthesis which was reflected by similar DMI and actual milk yield during the short feeding periods. Previously, Avila *et al*. (2000) observed that adding supplemental lipid to the diet of early lactation cows increased milk yield compared with a diet with no supplemental fat. However, the type of lipid supplement had no effect on production performance. The HP and LP were formulated to be similar in energy density (NEL, Table 1) and unless there was a difference in the digestibility of the lipid supplement, a decrease in DMI or a difference in FA use for milk synthesis versus adipose synthesis, actual milk yield for HP and LP was expected to be similar.

The FA composition of milk fat changes quickly in response to dietary changes when rumen-protected lipids were fed and when milk fat from each milking was analyzed for FA concentration (Carroll *et al*., 2006). The FA in milk TG are derived from at least 2 pools including *de novo* synthesis of FA and preformed FA from blood, the latter accounts for why the FA composition of milk TG changes quickly. Thus, researchers used 14 days periods to study the FA composition of milk. However, the FA in the phospholipids likely change at a different rate so this should be consider when interpreting the data that are presented.

Steele and Moore (1968) observed numerically higher yield of milk fat for dietary palmitic acid compared with stearic acid with no change in milk yield. In contrast, milk yield was numerically lower when cows received palm oil/palmitic acid compared with either soy oil or tallow but milk fat yield was numerically highest for tallow and lowest for soy oil with palm oil/palmitic acid intermediate (Banks *et al*., 1976b). Banks *et al*. (1980a) observed no difference in yield of milk when dietary palm oil/palmitic acid was fed compared with tallow or soy oil but milk fat yield was significantly higher with the diet containing palm oil/palmitic acid. Supplementation with either palmitic acid or palm oil in general showed no response in milk production when compared with a fat supplemented group (Enjalbert *et al*., 2000; Mosley *et al*., 2007; Kliem *et al*., 2009) but milk fat content was increased (Enjalbert *et al*., 2000; Mosley *et al*., 2007).

The concentration of C16:0 in the TG and PL fractions of milk fat dramatically increased when cows were fed HP compared with LP. Significantly higher C16:0 in milk fat occurred for dietary palm oil/palmitic acid than either soy oil or tallow (Banks *et al*., 1976a) and the same response can be concluded from the results of others (Banks *et al*., 1980a; Steele and Moore, 1968; Banks *et al*., 1976c).

Concentrations of C18:0 and C18:1 were lower for palm oil than for tallow, soy oil and stearic acid. The higher concentration of C18:0 for LP (Table 5) reflects diet FA composition, biohydrogenation of unsaturated FA with 18 carbons which ultimately results in higher supply of preformed stearic acid to the mammary gland. The higher concentration of C18:1 cis-9 and 10 for LP (Table 5) likely reflects mammary stearoyl-CoA desaturase activity. Noble *et al*. (1969) fed various concentrates including ones that contained either 100 g stearic acid or 100 g palmitic acid kg^{-1}. Although, the milk data obtained from feeding these 2 concentrates were not statistically compared with each other, adding 100 g palmitic acid kg^{-1} decreased the concentrations of C6:0, C8:0, C10:0, 363 C12:0, C14:0, C14:1, C18:0, C18:1, C18:2 and C18:3 while concentrations of C16:0 and C16:1 were increased in milk fat compared with 100 g stearic acid kg^{-1} in agreement to findings in the present study. Concentration of C18:1 trans was lower for palm oil/palmitic acid compared with either soy oil or tallow (Noble *et al*., 1969) similar to changes observed for HP and LP (Table 5). The C18:1 trans FA isomers are intermediates of rumen biohydrogenation of unsaturated

FA with 18 carbons provided through the diet (Harfoot, 1978). Increasing the proportion of soybean oil in the diet increased the concentration of C18:1 trans in milk fat (Banks *et al*., 1980b). The LP supplement used in the current study provided a higher amount of polyunsaturated FA containing 18 carbons in the diet than HP including some C18:1 trans isomers and therefore a higher concentration of C18:1 trans FA isomers was observed in the milk fat of cows fed the LP diet, mostly as a consequence of intermediate FA from biohydrogenation of UFA available in the LP supplement, such as C18:2 and C18:1 cis-9.

Supplementing the diet with tallow or soy oil increased the proportion of milk fat that was liquid at 5°C compared with a control diet (Banks *et al*., 1980b). In early research, Banks *et al*. (1976a) studied the melting profiles of milk fat from cows fed tallow, palm oil and palmitic acid blend, or soy oil and they concluded that the proportion of C16:0 in milk fat was a major determinant of melting properties (melting properties is a result of all FA). Approximately 500 g of the crystalline fat kg^{-1} from the soy oil treatment occurred at 8°C. In contrast, 500 g of the tallow milk fat kg^{-1} melted at 15.9°C while 500 g of the palm oil and palmitic acid blend milk fat kg^{-1} melted at 18.2°C. The lower the concentration of C16:0 in milk fat that was observed, the lower the temperature required for melting (Banks *et al*., 1976a; Banks *et al*., 1983). In the current study, the butter produced when cows were fed Hp was harder (Table 9) than butter produced when cows were fed LP when sampled for FA analysis at room temperature. Both the temperature at peak melt and onset to melt were lower for LP than HP. These changes in thermal properties of the butter agree with previous observations previously mentioned (Banks *et al*., 1976a; Banks *et al*., 1983). Enjalbert *et al*. (2000) found that solid fat content of lipid within the temperature range of -10 to 30°C was lowest from butter produced when cows were infused in the duodenum with predominately oleic acid compared with infusion of either stearate or palmitate. Numerically the highest proportion of solid fat in butter occurred with palmitate infusion.

Pearson correlation coefficients (Fig. 1) showed that concentration of C16:0 in the butter was positively correlated to melting point, the higher the concentration of C16:0 the higher was the melting point, similar to observations reported previously (Banks *et al*., 1976a). In contrast, increased concentration of either C18:0 or C18:1 was associated with lower melting point. However, because of the composition of the dietary lipids fed in the current study, C16:0 and C18:0 in milk fat were negatively correlated (-0.898; $p<0.01$) and therefore, although C18:0 has a higher melting point than C16:0 (70 vs. 63°C), C18:0 was negatively correlated to melting point because higher concentrations of C18:0 in the butter

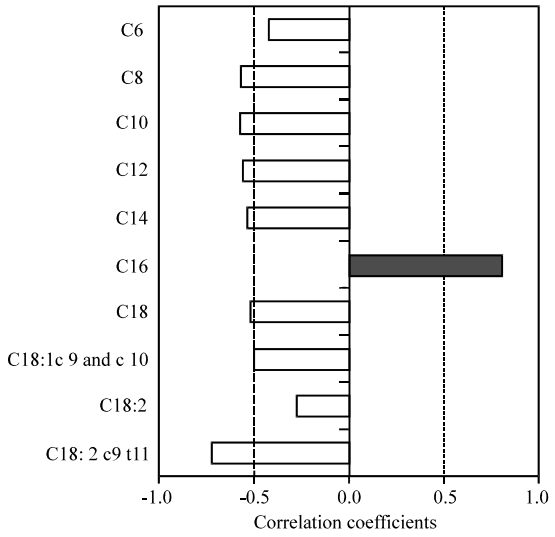

Fig. 1: Pearson correlation coefficients for selected fatty acids in the butter fat and melting point of the butter fat. All correlations differ for $p<0.01$, except for C18:1 c9 and c10 ($p<0.05$) and C18:2 ($p>0.05$)

were also associated with higher content of unsaturated FA, particularly C18:1 cis-9 and 10 and associated with a much lower content of C16:0 (41.4 vs. 27.7 g/100 g FA). Banks *et al*. (1980b) realized this confounding nature associated with FA and diet when they reported the effect of C18:0-C16:0 impacting the portion of milk fat that was liquid milk at 5°C. Thus, the total FA concentration of milk fat alone cannot predict melting properties and must include some knowledge of TG structure and stereospecific distribution of FA to adequately describe melting properties of TG (Banks *et al*., 1989).

CONCLUSION

Feeding a commercially available lipid supplement rich in palmitic acid did not affect DM intake but yields of milk fat and fat-corrected milk were higher when cows were fed the high compared with the low palmitic acid lipid supplement. The FA composition of the TG fraction of milk lipid was dramatically influenced by dietary palmitic acid. These changes in FA of milk lipids impacted the textural and thermal properties of the butter produced. Butter from cows fed the high palmitic acid supplement (Hp) was harder at 10°C and ambient temperature. Temperature at peak melt and onset of melt were also higher for HP. The measurements of nucleation were conducted with AMF and these data will not reflect normal dairy manufacturing practices where nucleation

will occur in individual milk fat globules. However, these data do provide insight that physicochemical properties were altered.

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