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Calcium salts of polyunsaturated fatty acids deliver more essential fatty acids to the lactating dairy cow

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ABSTRACT

Recent research has focused on the importance of supplying essential fatty acids to the lactating dairy cow. The addition of essential fatty acids, specifically linoleic and linolenic acid, to dairy cow diets has been investigated as a method to increase reproductive efficiency. Rumen bacteria, however, biohydrogenate polyunsaturated fatty acids (PUFA) to saturated fatty acids. This is an important issue because it can also lead to milk fat depression when unsaturated fatty acids are fed. The formation of Ca salts has previously been shown to partially protect unsaturated fatty acids from rumen biohydrogenation. The objective of this experiment was to evaluate feed intake, milk production, and milk composition of cows fed Ca salts of palm fatty acids (CS) compared with those fed Ca salts of palm fatty acids with an increased content of PUFA (CS+PUFA). Nineteen lactating Holstein cows were used in a switchback experiment to determine any differences between CS and CS+PUFA on milk production and composition. This experiment consisted of 3 consecutive periods of 14 d. Treatments were formulated to provide 450 g/d (dry matter basis) of the Ca salt supplement and were mixed with the same basal ration. Milk weights and feed intakes were recorded daily for each cow. Milk samples were collected the last 2 d of each period and analyzed for milk composition and fatty acids. Dry matter intake [28.0 vs. 27.0 kg/d; standard error of the mean (SEM) = 0.4] and milk production (44.4 vs. 44.0 kg/d; SEM = 0.7) were not different between treatments for CS and CS+PUFA, respectively. Milk fat percentage (3.34 vs. 3.22%; SEM = 0.07) and milk protein percentage (2.78 vs. 2.80%; SEM = 0.01) were not different for CS- and CS+PUFA-fed cows. Feeding CS+PUFA reduced the concentration of palmitic acid in milk fat (28.3 vs. 26.8 wt%; SEM = 0.3). Supplementation of CS+PUFA increased the linoleic acid concentration (3.96 vs. 4.61 wt%; SEM = 0.1) of milk fat, indicating that linoleic acid was partially protected from rumen biohydrogenation. Concentrations of conjugated linoleic acid were also increased (0.44 vs. 0.52 wt%; SEM = 0.02) when cows consumed CS+PUFA, indicating that some biohydrogenation did occur. Supplementing CS+PUFA did not alter milk production, milk fat percentage, or dry matter intake when compared with CS. The CS+PUFA supplement supplied more linoleic acid to the small intestine for milk fat synthesis.
Key words: milk, polyunsaturated fatty acid, milk fat, dry matter intake

INTRODUCTION

Early-lactation dairy cows use body reserves to meet the demands of milk production (Bauman and Currie 1980). The use of Ca salts to supply additional fatty acids (**FA**) to the diet can be beneficial to increase the energy density of the diet and overcome limitations in energy supply. Milk yield (Erickson et al., 1992) and milk fat percentage (Schauff and Clark, 1992) have been increased by the addition of Ca salts of palm FA without negatively affecting the digestibility of other dietary ingredients (Chouinard et al., 1998). In addition to supplying energy, Ca salts are a means of altering FA delivery to the lactating dairy cow. Using Ca salts to supply more unsaturated FA (Chouinard et al., 1998) may be an approach to meeting the requirements for specific FA.

Recent research has focused on the importance of supplying essential FA to the lactating dairy cow. Essential FA are those that cannot be synthesized in the body so they must be supplied by the diet (Mattos et al., 2000). They are important in many cellular functions, such as specific roles in cellular membrane structure, and are required to produce prostaglandins (Grummer and Carroll, 1991). These essential FA belong to a larger group of FA called polyunsaturated FA (**PUFA**). Research suggests that the addition of essential FA, specifically linoleic acid (18:2 *cis*-9, *cis*-12) and linolenic acid (18:3 *cis*-9, *cis*-12, *cis*-15), can improve first-service conception and overall conception rate (Staples et al., 1998).

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Table 1. Ingredient and chemical composition of the diets

Composition	Content
Ingredient, % of DM	
Alfalfa hay, chopped	30.5
Triticale silage	5.8
Rolled barley	22.6
Steam-rolled corn	14.5
Whole cottonseed	9.1
Dried distillers grains	6.9
Soybean meal	4.9
CS or CS+PUFA[1]	1.6
Blood meal	0.9
Sodium bicarbonate	0.9
Fish meal, menhaden	0.8
Trace mineral premix[2]	0.8
Vitamin premix[3]	0.3
Calcium carbonate	0.3
Magnesium oxide	0.2
Dicalcium phosphate	0.1
Chemical composition,[4] % of DM	
CP	19.2
ADF	19.6
NDF	29.8
Lipid	6.0
NFC	39.9
Ca	0.9
P	0.4
K	1.5

[1]CS = Ca salts of palm fatty acids (Megalac, Church and Dwight Inc., Princeton, NJ); CS+PUFA = Ca salts of palm fatty acids with increased content of polyunsaturated fatty acids (Megalac-R, Church and Dwight).

[2]Trace mineral mix (mg/kg of mix) contained: Mn, 3,500; Zn, 3,000; Cu, 750; Fe, 20; I, 85; Co, 15; and Se, 35.

[3]Vitamin premix provided 2,110,000 IU/kg of retinyl acetate, 390,000 IU/kg of cholecalciferol, and 7,550 IU/kg of DL-α-tocopheryl acetate.

[4]The NE_L was estimated by laboratory analysis to be 1.7 Mcal/kg.

Many sources of PUFA are already available to dairy producers as feed ingredients. Rumen bacteria, however, biohydrogenate unsaturated FA to saturated FA (Wu et al., 1991). This is an important issue because it can also lead to milk fat depression when high levels of unsaturated FA are fed (Griinari et al., 1998). Feeding rumen-inert fat is important to avoid negative effects on fiber degradation and rumen microbe populations. Another potential negative factor when feeding Ca salts of unsaturated FA is a reduction in feed intake (Allen, 2000). Depression of feed intake, however, was observed at dietary concentrations (>5% of DM) of FA greater than typically recommended (Allen, 2000).

The objective of this experiment was to evaluate DMI, milk production, and milk composition effects of cows fed Ca salts of palm FA high in PUFA (**CS+PUFA**) compared with those fed Ca salts of palm FA (**CS**). We hypothesized that CS+PUFA would not reduce DMI or cause milk fat depression and that CS+PUFA would increase the concentration of unsaturated FA in milk fat.

MATERIALS AND METHODS

The University of Idaho Animal Care and Use Committee approved all animal procedures before initiation of the experiment. Nineteen lactating Holstein cows (83 ± 9 DIM) were randomly assigned to a treatment sequence and were used in a switchback design. The experiment consisted of 3 periods of 14 d each. All cows were housed in the same pen and were individually fed using Calan gates (American Calan Inc., Northwood, NH). Treatments consisted of 2 commercial fat supplements: CS (Megalac, Church and Dwight Co. Inc., Princeton, NJ) and CS+PUFA (Megalac-R, Church and Dwight Co. Inc.) to provide 450 g/d (DM basis) of Ca salt supplement, and were mixed with the basal diet. The basal diet (Table 1) consisted of alfalfa hay, triticale silage, whole cottonseed, and a corn- and barley-based grain mix. The diet was formulated to meet the nutrient requirements of a 680-kg cow producing 45.5 kg of milk with 3.7% milk fat (NRC, 2001). The FA compositions of supplemental Ca salts are presented in Table 2. Cows were fed once daily to achieve 5 to 10% feed refusals and daily feed intake was measured.

Cows were milked twice daily and milk yield was recorded. Milk samples were pooled daily on the last 2 d of each period and analyzed for fat, protein, lactose, SCC (Washington DHIA, Burlington, WA), and FA. Milk fat was extracted following a modified Folch procedure (Clark et al., 1982). Methyl esters were formed using a methanolic sodium methoxide solution (Christie, 1982). Analysis of the methyl esters was performed on a gas-liquid chromatograph (6890 Series with auto injector, Hewlett-Packard, Wilmington, DE) fitted with a flame-ionization detector. The FA profile was determined by split injection (50:1) onto a CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm, Chrompack, Raritan, NJ) using a programmed tem-

Table 2. Fatty acid composition (wt% of total fatty acids) of Ca salt supplements with varying degrees of saturation

Fatty acid	CS[1]	CS+PUFA[2]
12:0	1.4	1.0
14:0	3.1	1.9
14:1 *cis*-9	0.1	0.1
16:0	47.4	32.4
16:1 *cis*-9	0.3	0.2
17:0	0.1	0.1
18:0	4.6	5.0
18:1 *cis*-9	34.7	23.4
18:2 *cis*-9, *cis*-12	5.5	30.5
18:3 *cis*-9, *cis*-12, *cis*-15	0.2	3.1

[1]CS = Ca salts of palm fatty acids (Megalac, Church and Dwight Inc., Princeton, NJ).

[2]CS+PUFA = Ca salts of palm fatty acids with increased content of polyunsaturated fatty acids (Megalac-R, Church and Dwight Inc.).

Table 3. Least squares means of DMI, milk yield, and composition of milk from lactating dairy cows fed Ca salts of fatty acids with varying degree of saturation

Variable	CS[1]	CS+PUFA[2]	SEM	*P*-value
DMI, kg/d	28.0	27.0	0.4	0.14
Milk				
Yield, kg/d	44.4	44.0	0.7	0.66
Fat, %	3.34	3.22	0.07	0.21
Fat yield, kg/d	1.45	1.41	0.04	0.17
Protein, %	2.78	2.80	0.01	0.50
Protein yield, kg/d	1.24	1.23	0.02	0.78
Lactose, %	4.76	4.76	0.01	0.91
SCS	4.19	4.15	0.09	0.73

[1]CS = Ca salts of palm fatty acids (Megalac, Church and Dwight Inc., Princeton, NJ).

[2]CS+PUFA = Ca salts of palm fatty acids with increased content of polyunsaturated fatty acids (Megalac-R, Church and Dwight Inc.).

perature gradient method. The hydrogen carrier gas pressure was constant, and the injector and detector temperatures were 255°C. Initial oven temperature was 70°C. After injection of a sample, the oven temperature was increased at 4°C/min to 175°C and held for 3 min. The oven temperature was then raised at 1°C/min to 185°C and held for 20 min. The temperature was then increased at 3°C/min to 215°C, followed by an increase at 10°C/min to 240°C and held for 5 min, after which it was returned to 70°C. Individual FA were identified by comparison of retention times with those of pure standards (Matreya Inc., Pleasant Gap, PA). A response correction factor for each FA methyl ester was used to convert peak area percentage to weight percentage. Correction factors were determined by analyzing butter oil of a known FA profile with certified values (CRM 164, European Community Bureau of Reference, Brussels, Belgium).

All cows completed each of the 3 periods. Data were analyzed by ANOVA using the MIXED procedures of SAS (version 9.2, SAS Institute Inc., Cary, NC). The model included the effect of treatment, period, sequence, and cow within sequence. The following model was used:

$$Y_{ijkl} = \mu + T_i + P_j + S_k + C(S)_l + E_{ijkl},$$

where Y_{ijkl} is the observation, μ is the overall mean, T_i is treatment (i = CS or CS+PUFA), P_j is period (j = 1, 2, and 3), S_k is sequence (k = 1 and 2), $C(S)_l$ is cow within sequence (l = 1 to 19), and E_{ijkl} is residual error. Data are reported as least squares means ± SE of the means. Significant difference between treatments was declared at $P < 0.05$.

RESULTS

The early-lactation cows used in this study were high-producing cows averaging more than 40 kg/d of milk for the duration of the experiment (Table 3). Milk fat and protein percentages were low, probably because of stage of lactation and amount of milk produced (Table 3). No significant difference was detected for DMI between cows fed CS and cows fed CS+PUFA (Table 3). No difference in milk production or composition was detected between treatments (Table 3). Fatty acid content of short- and medium-chain FA (<16 carbons) did not differ between treatments (Table 4). Feeding CS+PUFA reduced ($P < 0.001$) the concentration of palmitic acid (16:0) by 5% in milk fat, whereas the concentration of linoleic acid was increased by 16% ($P < 0.001$; Table 4). No increase in linolenic acid was detected when CS+PUFA was fed, although approximately 12 g more of linolenic acid was consumed by cows fed CS+PUFA as compared with cows fed CS. Vaccenic acid (18:1 *trans*-11) tended ($P < 0.06$) to increase in milk fat, and an increase ($P < 0.006$) in conjugated linoleic acid (**CLA**; 18:2 *cis*-9, *trans*-11) concentration was detected when cows consumed CS+PUFA compared with cows fed CS.

DISCUSSION

Decreased DMI and low milk fat may indicate a disruption of rumen fermentation. Negative effects of reduced fermentation include alteration of VFA production and decreased fiber digestibility, which ultimately lead to reduced DMI, decreased milk production, and low milk fat content. It has been suggested (Drackley et al., 1992; Firkins and Eastridge, 1994; Bremmer et al., 1998; Allen, 2000) that DMI is reduced as the degree of unsaturated FA in the diet increases, possibly because of negative effects on rumen fermentation (Jenkins, 1993). Grummer (1988) reported that Ca salts of long-chain FA are rumen inert and do not have any negative impact on rumen fermentation, DMI, or milk fat concentration, further supporting the lack of effect of CS+PUFA on DMI or milk production observed in

Table 4. Fatty acid composition (wt% of total fatty acids) of milk fat from lactating dairy cows fed Ca salts of fatty acids with varying degree of saturation

Fatty acid	CS[1]	CS+PUFA[2]	SEM	*P*-value
4:0	3.51	3.52	0.06	0.96
6:0	2.25	2.21	0.05	0.53
8:0	1.30	1.28	0.03	0.67
10:0	2.85	2.80	0.07	0.64
12:0	3.03	2.99	0.08	0.76
14:0	9.34	9.12	0.22	0.49
14:1 *cis*-9	0.63	0.62	0.02	0.85
15:0	0.78	0.80	0.01	0.42
16:0	28.30	26.80	0.21	<0.001
17:0	0.42	0.43	0.008	0.14
18:0	11.55	11.92	0.21	0.24
18:1 *trans*-10	0.50	0.47	0.06	0.83
18:1 *trans*-11	2.04	2.33	0.10	0.06
18:1 *cis*-9	23.57	22.83	0.38	0.20
18:1 *cis*-11	0.83	1.09	0.12	0.11
18:1 *cis*-12	0.51	0.70	0.05	0.01
18:1 *trans*-16	0.59	0.63	0.01	0.06
18:2 *cis*-9, *cis*-12	3.96	4.61	0.08	<0.001
18:2 *cis*-9, *trans*-11	0.44	0.52	0.02	0.006
18:3 *cis*-9, *cis*-12, *cis*-15	0.39	0.41	0.01	0.34
18:3 *cis*-6, *cis*-9, *cis*-12	0.06	0.05	0.006	0.40
20:0	0.20	0.26	0.02	0.06
20:3 *cis*-11, *cis*-14, *cis*-17	0.30	0.36	0.02	0.01
20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14	0.08	0.06	0.04	0.77
20:4 *cis*-8, *cis*-11, *cis*-14, *cis*-17	0.05	0.34	0.07	0.06
22:1 *cis*-13	0.05	0.08	0.02	0.37

[1]CS = Ca salts of palm fatty acids (Megalac, Church and Dwight Inc., Princeton, NJ).

[2]CS+PUFA = Ca salts of palm fatty acids with increased content of polyunsaturated fatty acids (Megalac-R, Church and Dwight Inc.).

the present study. Drackley et al. (1992) reported that the total amount of unsaturated FA necessary to reduce DMI was greater than 370 g/d. In the current study, the total supplemental unsaturated FA supplied with the CS+PUFA diet was 255 g/d. Therefore, little effect of additional PUFA on rumen fermentation, DMI, and milk production would be expected.

In 2 of the studies mentioned previously (Drackley et al., 1992; Bremmer et al., 1998) that reported decreased DMI when unsaturated FA in the diet increased, milk production was not affected by degree of unsaturation. Firkins and Eastridge (1994) reviewed 11 studies and determined a slight decrease in FCM expressed as a percentage of the control when increased amounts of unsaturated FA were included in the diet. The data they used, however, did not evaluate studies that included chemically processed fats such as Ca salts, to avoid confounding effects of processing and saturation on digestibility and milk production. Chouinard et al. (1998) evaluated milk production when Ca salts of FA varying in degree of saturation were fed. Calcium salts of unsaturated FA did not alter milk production in early-lactation cows when compared with a control diet containing no supplemental fat. In the current study, degree of saturation of the fat supplement did not affect milk production when CS+PUFA was included in the diet compared with inclusion of CS.

Changes in the FA profile were expected when increased amounts of inert polyunsaturated long-chain FA were supplied in the diet. The degree of saturation of milk fat was decreased in early-lactation cows in response to increasing concentrations of unsaturated FA infused into the abomasum (Christensen et al., 1994). The abomasal infusions provided approximately 400 g/d of FA. The amounts of palmitic and stearic acids in milk fat decreased by approximately 18% when canola, soybean, and sunflower oils were infused compared with infusions of saturated fat. The amounts of the unsaturated FA oleic, linoleic, and linolenic acids increased (12, 105, and 49%, respectively) when compared with infusion of saturated FA (Christensen et al., 1994). In the current study, the amount of linoleic acid in milk fat increased by 16%, whereas oleic and linolenic acids were unchanged when additional PUFA were supplied in the diet. The direct manipulation of specific FA in the supplements used during the current study may partially explain the changes in milk FA composition; however, the lack of change in linolenic acid suggests other factors are associated with the transfer of dietary long-chain FA to milk fat.

The decrease in palmitic acid and increase in linoleic acid in milk fat of cows fed the diet containing CS+PUFA compared with the diet containing CS is likely a direct response to changes in the FA profile of the supplements used. The amount of linolenic acid in milk fat, however, does not reflect the increased amount supplied in the CS+PUFA treatment. In agreement with Christensen et al. (1994), altering the FA composition of milk fat can be achieved by supplying specific FA to the mammary gland via formation of Ca salts (Chouinard et al., 1998). When Ca salts of linseed oil were fed to early-lactation dairy cows, a 29% increase in linolenic acid content of milk fat was observed when compared with a control diet that contained no supplemental fat. The Ca salts of linseed oil supplement supplied approximately 460 g/d of linolenic acid; however, recovery from milk fat was only 4 g/d. The amount of linolenic acid fed (Chouinard et al., 1998) was much greater than the 13.4 g/d supplied by CS+PUFA in the current study. Furthermore, linolenic acid may be metabolized to eicosapentaenoic acid after absorption in the small intestine (Mattos et al., 2000). In addition to the low amount fed and tissue metabolism, the lack of any change in linolenic acid concentration in milk fat may be due to biohydrogenation in the rumen.

Protection of Ca salts of FA from biohydrogenation by rumen microorganisms is not complete. Wu et al. (1991) reported that Ca salts of palm FA were biohydrogenated 57% in vivo, which was substantially lower than for an animal-vegetable blend (87%). Calcium salts of PUFA were not completely protected from biohydrogenation in the current study because intermediates (CLA and vaccenic acid) of the biohydrogenation pathway were increased in milk when cows were fed the CS+PUFA diet. Biohydrogenation of 18-carbon unsaturated FA was approximately 33% when Ca salts of palm FA were fed (Klusmeyer and Clark, 1991). In the current study, however, some protection from biohydrogenation for linoleic acid occurred because no difference in stearic or oleic acid was detected, indicating that biohydrogenation was not complete, and linoleic acid concentration in milk was increased. Protection of linolenic acid, however, was not apparent because milk concentrations were not different. The amount of stearic acid consumed from the CS and CS+PUFA supplements was 20.6 and 21.7 g/d, whereas output in milk fat was 167.5 and 168.1 g/d for CS and CS+PUFA, respectively. The amounts of oleic acid consumed from the CS and CS+PUFA supplements were 155.5 and 101.1 g/d, whereas the output in milk fat was 341.8 and 321.9 g/d, respectively. Sukhija and Palmquist (1990) showed that the dissociation of the Ca ion from FA was dependent on the degree of saturation. The dissociation of FA from Ca salts of palm FA, tallow, and stearic acid was less than 10% at pH 5.5 compared with greater than 40% for soybean oil. The Ca salts of soybean oil had a greater amount of PUFA than the other 3 treatments. Calcium salts of soybean oil required the least amount of HCl for complete dissociation, which indicates a higher p*K*a value or dissociation at higher pH than for the other Ca salts of FA. Therefore, it is probable that the protection provided by Ca salts was less effective for linolenic acid than for linoleic acid.

Rumen biohydrogenation of dietary FA affects the saturation of milk fat. Wu et al. (1991) reported higher rates of biohydrogenation for linolenic acid than for other C_{18} unsaturated FA supplied by Ca salts of palm FA. Linolenic acid is converted to many metabolites in the rumen by microorganisms; the first process is isomerization to *cis*-9, *trans*-11, *cis*-15 18:3. *Cis*-9, *trans*-11, *cis*-15 18:3 is then hydrogenated to yield *trans*-11, *cis*-15 18:2, with further hydrogenation producing vaccenic acid (Harfoot and Hazlewood, 1988). Linoleic acid is isomerized by rumen microorganisms to yield an increase of CLA 18:2 *cis*-9, *trans*-11 (Bauman and Griinari, 2000). Additionally, CLA can then be hydrogenated to vaccenic acid. Vaccenic acid can also be converted to CLA in mammary tissue by the Δ^9-desaturase enzyme (Griinari et al., 2000; Mosley et al., 2006). Feeding CS+PUFA resulted in a 19% increase of CLA and a 14% increase in vaccenic acid in milk fat in the current experiment. The increase in milk CLA may be attributed to incomplete biohydrogenation of linoleic and linolenic acid to vaccenic acid and synthesis of CLA from vaccenic acid in the mammary gland. Increasing the CLA concentration of milk fat may provide beneficial anticarcinogenic effects to dairy product consumers (Lock and Bauman, 2004).

One possible means of increasing the supply of essential FA to tissues within the body is by increasing the flow of these FA to the small intestine for absorption. Supply of linoleic acid to the mammary gland was increased with CS+PUFA because the linoleic acid content of milk fat was greater than in cows fed CS. Therefore, CS+PUFA was able to enhance the delivery of linoleic acid to the mammary gland. Increasing the linolenic acid content of diet with CS+PUFA, however, did not increase the content of linolenic acid in milk fat. This suggests that the relatively low amount (13 g/d) of linolenic acid provided by the CS-PUFA supplement was either inadequately protected from biohydrogenation or was metabolized by other tissues. Alternatively, certain long-chain FA are preferentially partitioned into phospholipids in circulation, and use of FA from this

lipid fraction may be limited compared with FA in the triglyceride fraction (Loor et al., 2002; Mosley et al., 2006).

CONCLUSIONS

Feeding CS+PUFA did not alter milk production or DMI when compared with CS. No evidence for milk fat depression during feeding of CS+PUFA was found. An increase in linoleic acid content of milk indicated that CS+PUFA did supply more polyunsaturated FA to the small intestine that was available for milk fat synthesis. The increased supply of PUFA may also be utilized elsewhere in the body for other metabolic processes. The increase in PUFA in milk fat may present consumers with a healthier FA content.

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