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Effect of Supplementation with Calcium Salts of Fish Oil on n-3 Fatty Acids in Milk Fat[1]

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ABSTRACT

Enrichment of milk fat with n-3 fatty acids, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may be advantageous because of their beneficial effects on human health. In addition, these fatty acids play an important role in reproductive processes in dairy cows. Our objective was to evaluate the protection of EPA and DHA against rumen biohydrogenation provided by Ca salts of fish oil. Four Holstein cows were assigned in a Latin square design to the following treatments: 1) ruminal infusion of Ca salts of fish oil and palm fatty acid distillate low dose (CaFO-1), 2) ruminal infusion of Ca salts of fish oil and palm fatty acid distillate high dose (CaFO-2), 3) ruminal infusion of fish oil high dose (RFO), and 4) abomasal infusion of fish oil high dose (AFO). The high dose of fish oil provided ~16 and ~21 g/d of EPA and DHA, respectively, whereas the low dose (CaFO-1) provided 50% of these amounts. A 10-d pretreatment period was used as a baseline, followed by 9-d treatment periods with interceding intervals of 10 d. Supplements were infused every 6 h, milk samples were taken the last 3 d, and plasma samples were collected the last day of baseline and treatment periods. Milk fat content of EPA and DHA were 5 to 6 times greater with AFO, but did not differ among other treatments. Milk and milk protein yield were unaffected by treatment, but milk fat yield and DM intake were reduced by 20 and 15%, respectively, by RFO. Overall, results indicate rumen biohydrogenation of long chain n-3 fatty acids was extensive, averaging >85% for EPA and >75% for DHA for the Ca salts and unprotected fish oil supplements. Thus, Ca salts of fish oil offered no protection against the biohydrogenation of EPA and DHA beyond that observed with unprotected fish oil; however, the Ca salts did provide rumen inertness by preventing the negative effects on DM intake and milk fat yield observed with unprotected fish oil.
Key words: calcium salts of fatty acids, fish oil, n-3 fatty acid, rumen protection

INTRODUCTION

There is growing interest in enhancing the intake of n-3 fatty acids, especially eicosapentaenoic acid (**EPA**; C20:5 n-3) and docosahexaenoic acid (**DHA**; C22:6 n-3), in lactating dairy cows. One reason relates to functional foods and a desire to increase the EPA and DHA content of dairy products; a high intake of these fatty acids was associated with reduced risk of several chronic diseases including coronary heart disease (Lock and Bauman, 2004). Increasing the EPA and DHA intake of dairy cows is also of interest because of possible beneficial effects on reproductive performance; an increase in n-3 fatty acids may amplify the suppression of uterine $PGF_{2\alpha}$ synthesis, thereby potentially decreasing early pregnancy loses (Mattos et al., 2000). Dietary polyunsaturated fatty acids (**PUFA**) are extensively metabolized in the rumen, and this has a major impact on the profile of fatty acids available for absorption (Palmquist et al., 2005). Therefore, minimizing rumen biohydrogenation of PUFA is the major challenge in formulating dietary supplements that will enhance the postruminal supply of PUFA.

Wu and Papas (1997) reviewed the historical development of rumen-stable systems for the delivery of bioactive compounds in ruminant diets. They emphasized that the efficiency of the delivery system was dependent on the extent of the rumen protection and the postruminal bioavailability of the compound of interest. Several technologies are currently used to provide rumen protection for dietary supplements with Ca salts of fatty

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acids being most extensively utilized for lipid supplements. Although Ca salts of fatty acids are often referred to as being protected, Palmquist (2006) emphasized that this technology was not developed to protect unsaturated fatty acids against rumen biohydrogenation, but rather to render the fatty acids "ruminally inert with regard to their effects on the microbial population." Although EPA and DHA have not been specifically examined, studies with 18-carbon unsaturated fatty acids indicate the extent of protection by Ca salts will be affected by a number of factors including ruminal pH and degree of unsaturation and chain length of the fatty acids (Wu et al., 1991; Enjalbert et al., 1997).

The objective of the present study was to use Ca salts of fish oil and examine the protection against rumen biohydrogenation offered to EPA and DHA. Our approach to evaluate efficacy of this rumen protection method was to compare the transfer efficiency of EPA and DHA to milk fat observed when the supplement was added to the rumen vs. when it was abomasally infused. This approach was used recently to evaluate rumen protection methods for conjugated linoleic acid isomers (de Veth et al., 2005) and has the advantage that it provides an evaluation that accounts for both rumen protection and postruminal bioavailability.

Table 1. Total amount of fatty acids provided by the lipid supplements

Fatty acid, g/d	CaFO-2[1]	Fish oil[2]
10:0	0.1	<0.1
12:0	0.5	0.1
14:0	10.5	9.4
14:1	0.1	0.1
15:0	0.9	0.7
16:0	90.4	22.6
16:1	11.7	12.1
17:0	1.2	0.7
18:0	11.1	4.1
18:1 *trans*-6 to 8	0.1	0.1
18:1 *trans*-9	0.1	<0.1
18:1 *trans*-11	1.8	2.2
18:1 *trans*-12	<0.01	0.2
18:1 *cis*-9	61.5	12.2
18:2 *cis*-9, *cis*-12	15.3	1.6
18:3	0.8	0.3
20:0	3.3	3.1
20.4	1.3	1.1
EPA[3]	16.6	16.2
DPA[3]	3.7	3.8
DHA[3]	21.2	22.0
Others	40.0	32.4
Total	292	145

[1]Values are for the Ca salts of fish oil and palm fatty acid distillate high dose (CaFO-2). Values for Ca salts of fish oil and palm fatty acid distillate low dose (CaFO-1) represent one-half of CaFO-2.

[2]Amounts provided by the fish oil supplement given by ruminal or abomasal infusion.

[3]EPA = eicosapentaenoic acid, 20:5 all *cis* 5, 8, 11, 14, 17; DPA = docosapentaenoic acid, 22:5 all *cis* 7, 10, 13, 16, 19; DHA = docosahexaenoic acid, 22:6 all *cis* 4, 7, 10, 13, 16, 19.

MATERIALS AND METHODS

Animals and Experimental Design

All procedures involving cows were approved by the Cornell University Institutional Animal Care and Use Committee. Four multiparous Holstein cows (143 ± 31 DIM) with rumen fistula were assigned to a 4 × 4 Latin square in which treatment supplements were 1) Ca salts of fish oil and palm fatty acid distillate low dose (**CaFO-1**), 2) Ca salts of fish oil and palm fatty acid distillate high dose (**CaFO-2**), 3) ruminal infusion of fish oil high dose (**RFO**), and 4) abomasal infusion of fish oil high dose (**AFO**). The Ca salt formulations and the fish oil were supplied by Virtus Nutrition Inc. (Corcoran, CA). A 10-d period before initiation of the experiment served as the baseline, followed by 9-d treatment periods with a 10-d washout interval between treatments. The amount of fat and fatty acids provided by the treatments is presented in Table 1. The CaFO-2, RFO, and AFO supplements provided similar amounts of EPA (~16 g/d) and DHA (~21 g/d), whereas the CaFO-1 supplement provided 50% of these amounts. Total daily dose was divided in 4 equal portions and infused every 6 h directly into the rumen or the abomasum. Abomasal infusion was via 0.5 cm (i.d.) polyvinyl chloride tubing that passed through the rumen fistula.

Cows were housed in individual stalls and fed a TMR diet formulated using the Cornell Net Carbohydrate and Protein System (Fox et al., 2004) to meet or exceed their nutritional requirements. Cows were fed ad libitum allowing 10% refusals with fresh feed offered twice daily and orts recorded daily. Feed samples were taken the last 3 d of each treatment; composites were formed and analyzed by wet chemistry methods for CP, ADF, NDF and ether extract (Table 2; Dairy One Cooperative Inc., Ithaca, NY). Fresh water was available throughout the study.

Cows were milked at 0700 and 1900 h daily, and milk weights recorded; samples from each milking were stored at 4°C with preservative (bronopol tablet; D&F Control System, San Ramon, CA) until analyzed for fat and true protein (Dairy One Cooperative Inc.) as described previously by Bernal-Santos et al. (2003). During the last 3 d of each treatment period, additional aliquots of milk were collected and immediately stored at –20°C until analyzed for fatty acids.

On the last day of each treatment period following the morning milking, blood (10 mL) was obtained via coccigeal venipuncture and collected in vacuum tubes containing sodium heparin (100 U/mL of blood). Plasma was harvested by centrifugation (2,800 × *g* for 15 min at 4°C) and stored at –20°C until fatty acid analysis.

Table 2. Ingredient and chemical composition of the diet

Dietary composition	
Ingredient, % of DM	
Grass hay	40.80
Cottonseed	7.20
Cracked corn	21.58
Bread meal	4.91
Wheat middlings	6.40
Gluten feed	4.80
Soybean meal (48% CP)	6.35
Amino plus[1]	4.00
Urea	0.27
Molasses	0.71
Limestone	1.49
Salt	0.40
Selenium	0.05
Trace mineral mix[2]	0.02
Sodium sesquicarbonate	0.89
Magnesium oxide	0.11
Vitamin supplement[3]	0.02
Chemical analysis, % of DM	
CP	17.43
Available protein	16.68
ADF	24.98
NDF	41.93
Ether extract[4]	4.88
NE_L, Mcal/kg	1.60

[1]Ag Processing Inc., Omaha, NE.

[2]The mix (DM basis) contained: 0.57% Ca, 15.75% sulfur, and 1,360 ppm cobalt, 40,800 ppm copper, 2,700 ppm iodine, 10,200 ppm iron, 122,450 ppm manganese, and 122,450 ppm zinc.

[3]Vitamin supplement contained 165 IU/g of vitamin A, 30 IU/g of vitamin D, and 750 IU/g of vitamin E.

[4]Value does not include treatment supplements.

Fatty Acid Analysis and Transfer Efficiency

The extraction and methylation of milk fatty acids used the method of Hara and Radin (1978) and transmethylation as described by Bernal-Santos et al. (2003). Plasma fatty acids were extracted (Bligh and Dyer, 1959) and transmethylated according to the method described by Christie (1989). The fish oil used for abomasal and ruminal infusions contained the fatty acids esterified in triglycerides; these fatty acids were extracted and transmethylated as described for milk fat. The Ca salt supplements are formulated using free fatty acids; acid hydrolysis and ether extraction was used for these fatty acids (Dairy One Cooperative Inc.) as described by de Veth et al. (2006), and methylation utilized 1% methanolic sulfuric acid (Christie, 1989).

Fatty acid methyl esters were quantified using a gas chromatograph (Hewlett Packard GCD system HP G1800 A, Avondale, PA) equipped with a CP-Sil 88 capillary column (100 m × 0.25 mm i.d. with 0.2-μm film thickness; Varian Instruments, Walnut Creek, CA). The oven temperature was set at 80°C for 3 min, then increased to 190°C and maintained for 10 min, with a final increase to 225°C held for 22 min. Fatty acid peaks were identified using pure methyl ester standards (NuChek Prep, Elysian, MN). A butter oil reference standard (CRM 164; Commission of the European Community Bureau of References, Brussels, Belgium) was analyzed periodically to control for column performance and as a check for the calculation of recoveries and correction factors for individual fatty acids.

Transfer efficiencies of EPA and DHA were calculated on an individual cow basis by subtracting the mean of the milk fat yield for each fatty acid during the last 3 d of baseline period from the mean during the last 3 d of each treatment period, and then dividing this number by the amount of each specific fatty acid provided by the treatment supplement.

Statistical Analysis

Milk production variables and milk fatty acid composition from last 3 d of treatment and plasma fatty acids from the last day of treatment were used for statistical analysis. Data were analyzed with PROC MIXED of SAS (SAS Institute, 2001); treatment and period were included in the model as fixed effects, and cow was included as a random variable. All data for 1 cow during 1 treatment period (RFO) were removed from the analysis because of clinical signs of mastitis.

RESULTS AND DISCUSSION

Milk yield and milk protein content and yield were not different among treatments. The CaFO-2 provided twice the amount of total fat than the other treatments, and this may explain the modest increase in milk fat yield compared with the CaFO-1 and AFO treatments. In contrast, the RFO treatment caused a reduction in milk fat content, milk fat yield, and DMI as compared with the treatments involving Ca salts of fish oil (Table 3). Decreased DMI was reported when diets were supplemented with unprotected fish oil (Shingfield et al., 2006), but DMI was unaffected when Ca salts of fish oil were fed (Allred et al., 2006). Polyunsaturated fatty acids can cause modifications in the rumen environment and changes in the microbial population that result in decreased fiber digestibility and a reduction in DMI (Doreau and Chilliard, 1997). Fotouhi and Jenkins (1992) proposed that feeding Ca salts of unsaturated fatty acids prevents drastic modifications of the rumen environment because the release of unsaturated fatty acids occurs slowly. As free unsaturated fatty acids are removed from the free fatty acid pool by biohydrogenation, Ca salts of PUFA will further dissociate to maintain the balance between dissociated and undissociated unsaturated fatty acids, thereby conferring rumen inertness as to effects on fiber digestion by this more gradual shift.

Table 3. Production variables by treatment[1]

Variable	Treatment[2]				SEM	*P*-value
	CaFO-1	CaFO-2	RFO	AFO		
DMI, kg/d	21.9[a]	22.1[a]	18.5[b]	21.0[a]	1.36	0.01
Milk yield, kg/d	23.4	26.4	24.4	23.0	1.96	0.22
Milk fat						
%	3.76[a]	3.61[a]	2.76[b]	3.56[a]	0.13	0.002
g/d	868[ab]	948[a]	682[c]	813[b]	58	0.008
Milk protein						
%	3.02	2.90	2.68	3.01	0.16	0.16
g/d	690	758	652	690	46	0.22

[a-c]Different superscripts represent differences among treatments ($P < 0.05$).

[1]Values represent least squares means of last 3 d of treatment.

[2]Cows received 1) ruminal infusion of Ca salts of fish oil and palm fatty acid distillate low dose (CaFO-1), 2) ruminal infusion of Ca salts of fish oil and palm fatty acid distillate high dose (CaFO-2), 3) ruminal infusion of fish oil high dose (RFO), and 4) abomasal infusion of fish oil (AFO) high dose. The high dose provided 16.6 and 21.2 g/d of eicosapentaenoic acid and docosahexaenoic acid, respectively, whereas the low dose provided 50% of these amounts.

The milk fatty acid profile during the last 3 d of the treatment period is presented in Table 4. The EPA and DHA were found in very low concentrations in milk fat; during the baseline period, EPA and DHA averaged only 0.04 and 0.02% of milk fatty acids, respectively. All treatments resulted in an increase in the milk fat content of EPA and DHA, and this was especially dramatic for the AFO treatment (an increase of 12 and 30 times baseline, respectively). The CaFO-1, CaFO-2, and RFO treatments were similar in milk fat content of EPA and DHA with concentrations representing about 20 to 25% of AFO. Changes in the milk fatty acid profile were observed for other fatty acids, especially with the RFO treatment where alterations included a marked increase in the proportion of total trans fatty acids and a reduction in stearic and oleic acids. Among *trans*-18:1 fatty acids, the greatest change was observed in vaccenic acid (*trans*-11 18:1; 8 times greater) followed by *trans*-10 18:1 and *trans*-12 18:1. This is in agreement with reports from in vitro studies (Abughazaleh and Jenkins, 2004a,b) and in vivo studies involving unprotected fish oil supplements (Loor et al., 2005; Shingfield et al., 2006). Milk fat content of *cis*-9, *trans*-11 conjugated linoleic acid (**CLA**) was substantially increased with the RFO treatment and was modestly increased by CaFO-2. This was expected because vaccenic acid was increased in the milk fat when cows received the RFO and CaFO-2 treatments and the majority of *cis*-9, *trans*-11 CLA in milk fat is synthesized endogenously from vaccenic acid as reviewed by Bauman and Lock (2006). Increases in *cis*-9, *trans*-11 CLA were reported with dietary supplements of fish oil and calcium salts of fish oil (e.g., Shingfield et al., 2003; Allred et al., 2006).

Treatment effects on the plasma fatty acid profile paralleled those observed for milk fat (Table 5). Abomasal infusion resulted in an increase in plasma concentrations of EPA and DHA, and ruminal infusion of fish oil caused an increase in the proportion of *trans*-16:1, *trans*-10 18:1 plus *trans*-11 18:1, and *cis*-9, *trans*-11 CLA, and a substantial decrease in stearic acid.

The increase in *trans* isomers in milk fat and plasma fatty acids, and the reduction in milk fat yield observed with the RFO treatment reflect the modification of rumen microbial population and pathways of biohydrogenation typically caused by dietary supplements high in PUFA. These changes promote the formation and accumulation of unique biohydrogenation intermediates, some of which are potent inhibitors of milk fat synthesis (Bauman and Griinari, 2003). Although there was no reduction in milk fat or decrease in DMI when Ca salts of fish oil were fed, a moderate increase in the proportion of *trans* fatty acid isomers occurred in plasma and milk fat consistent with some dissociation and biohydrogenation of PUFA in the rumen.

Reports on the extent of rumen protection offered by Ca salts of 18-carbon unsaturated fatty acids indicate that ruminal biohydrogenation can be extensive, although there are differences among experiments. The degree of rumen protection conferred by Ca salts is correlated with rumen pH and fatty acids pK_d, and decreases as the extent of fatty acid unsaturation increases (Sukhija and Palmquist, 1990). Wu et al. (1991) calculated that the biohydrogenation of 18-carbon unsaturated fatty acids averaged 72% for an unprotected fat supplement compared with 56% for Ca salts of fatty acids for a different fat supplement. As the number of double bonds increased, however, the extent of ruminal biohydrogenation increased and the difference between unprotected and Ca-protected unsaturated fatty acids was reduced; for example, biohydrogenation of 18:3 averaged 84% for the unprotected supplement and 80% for the Ca-protected supplement. In a study with dairy

Table 4. Fatty acid composition of milk fat[1]

Fatty acid, g/100 g of fatty acid	Treatment[2]				SEM	*P*-value
	CaFO-1	CaFO-2	RFO	AFO		
4:0	4.21	4.33	3.83	4.10	0.23	0.22
6:0	1.86^{b}	1.90^{ab}	1.75^{b}	2.05^{a}	0.08	0.02
8:0	0.87^{b}	0.89^{b}	0.89^{b}	1.05^{a}	0.04	0.01
10:0	1.62^{b}	1.61^{b}	1.87^{ab}	2.04^{a}	0.10	0.03
12:0	1.80^{ab}	1.77^{b}	2.10^{ab}	2.19^{a}	0.11	0.05
14:0	7.87	7.65	8.42	8.54	0.42	0.08
14:1 *cis*-9	0.79	0.81	0.69	0.70	0.08	0.41
15:0	0.69^{b}	0.65^{b}	0.87^{a}	0.70^{b}	0.05	0.05
16:0	25.92^{a}	25.92^{a}	23.60^{b}	23.72^{b}	0.90	0.02
16:1 *cis*-9	1.62	1.63	1.56	1.80	0.09	0.30
17:0	0.45^{b}	0.43^{b}	0.50^{a}	0.47^{ab}	0.02	0.03
18:0	11.67^{a}	10.13^{a}	3.10^{b}	13.10^{a}	0.85	0.001
18:1 *trans*-6 to 8	0.43^{cb}	0.53^{b}	0.78^{a}	0.30^{c}	0.06	0.007
18:1 *trans*-9	0.38^{b}	0.48^{b}	0.85^{a}	0.28^{b}	0.07	0.005
18:1 *trans*-10	0.57	0.67	1.27	0.41	0.27	0.19
18:1 *trans*-11	1.54^{b}	2.22^{b}	18.33^{a}	1.28^{b}	0.94	0.001
18:1 *trans*-12	0.64^{c}	0.84^{b}	2.00^{a}	0.42^{d}	0.04	<0.001
18:1 *cis*-9	27.32^{a}	26.40^{a}	9.21^{b}	25.63^{a}	1.18	<0.001
18:2 *cis*-9, *cis*-12	2.66^{b}	2.67^{b}	2.17^{c}	2.95^{a}	0.18	0.002
18:2 *cis*-9, *trans*-11	0.70^{c}	1.04^{b}	6.05^{a}	0.49^{c}	0.11	<0.001
18:3 *cis*-9, *cis*-12, *cis*-15	0.36^{b}	0.37^{b}	0.26^{c}	0.51^{a}	0.03	0.003
20:0	0.16^{a}	0.17^{a}	0.08^{c}	0.12^{b}	0.01	<0.001
EPA[3,4]	0.10^{b}	0.11^{b}	0.14^{b}	0.55^{a}	0.05	0.001
DPA[3]	0.12^{b}	0.12^{b}	0.21^{b}	0.34^{a}	0.03	0.007
DHA[3,4]	0.11^{b}	0.14^{b}	0.20^{b}	0.63^{a}	0.06	0.002
Others	5.40^{b}	6.35^{b}	9.33^{a}	5.41^{b}	0.29	<0.001
Summation[5]						
<16 carbons	19.72^{b}	19.62^{b}	20.47^{ab}	21.37^{a}	0.72	0.04
16 carbons	27.54^{a}	27.55^{a}	25.20^{b}	25.52^{b}	0.97	0.04
>16 carbons	47.35	46.48	44.93	47.70	1.54	0.22

[a-d]Different superscripts represent differences among treatments ($P < 0.05$).

[1]Values represent least squares means of last 3 d of treatment.

[2]Cows received 1) ruminal infusion of Ca salts of fish oil and palm fatty acid distillate low dose (CaFO-1), 2) ruminal infusion of Ca salts of fish oil and palm fatty acid distillate high dose (CaFO-2), 3) ruminal infusion of fish oil high dose (RFO), and 4) abomasal infusion of fish oil high dose (AFO). The high dose provided 16.6 and 21.2 g/d of EPA and DHA, respectively, whereas the low dose provided half these amounts.

[3]EPA = eicosapentaenoic acid, 20:5 all *cis* 5, 8, 11, 14, 17; DPA = docosapentaenoic acid, 22:5 all *cis* 7, 10, 13, 16, 19; DHA = docosahexaenoic acid, 22:6 all *cis* 4, 7, 10, 13, 16, 19.

[4]Mean for EPA and DHA during baseline period was 0.04 and 0.02 g/100 g of fatty acids, respectively.

[5]Summation does not include fatty acids present at trace levels and listed above as others.

cows, Lundy et al. (2004) reported ruminal biohydrogenation of linoleic acid averaged 95% for unprotected soybean oil and 92% for the Ca salts of soybean oil. Harvatine and Allen (2006) supplemented Ca salts of unsaturated fatty acids and used a kinetic approach to estimate the extent of rumen biohydrogenation in lactating cows. They found that protection of the 18-carbon PUFA from biohydrogenation was minimal in a commercial source of protected fat (Ca salts of fatty acids). Likewise in sheep, Fotouhi and Jenkins (1992) observed the extent of ruminal biohydrogenation of linoleic acid was 93% for free linoleic acid and 95% for Ca salts of linoleic acid.

There have been no in vivo investigations of the protection against ruminal biohydrogenation provided by Ca salts of longer chain PUFA (>18-carbons) or Ca salts of fish oil. In addition, the aforementioned studies estimated the extent of biohydrogenation as the difference between the fatty acids originally supplied and the fatty acids recovered in the outflow from the rumen. To more completely evaluate protection, a comparison against unprotected fatty acids in the same lipid supplement is necessary, and ideally the comparison should account for differences in intestinal absorption to ensure that bioavailability was not altered by the protection method (Wu and Papas, 1997). The present study did this by utilizing EPA and DHA secreted into milk fat and comparing values observed with a rumen-protected supplement and during postruminal infusion of an unprotected supplement. No significant increases in EPA and DHA output in milk fat were observed for CaFO-2 compared with CaFO-1 (Table 6). In vitro studies suggest that increasing the dose of EPA and DHA may reduce the rate of ruminal biohydrogenation (Abughazaleh and

Table 5. Fatty acid composition of plasma[1]

Fatty acid, g/100 g of fatty acid	Treatment[2]				SEM	*P*-value
	CaFO-1	CaFO-2	RFO	AFO		
14:0	0.37	0.48	0.41	0.35	0.06	0.49
15:0	0.34[b]	0.33[b]	0.43[a]	0.34[b]	0.01	0.009
16:0	9.66[ab]	9.96[a]	9.28[bc]	9.10[c]	0.19	0.04
16:1 *cis*-9	0.73	0.62	0.92	0.88	0.08	0.10
16:1 *trans*	0.21[b]	0.25[b]	0.66[a]	0.23[b]	0.04	<0.001
17:0	0.35	0.33	0.38	0.33	0.02	0.12
18:0	14.50[ab]	13.02[b]	6.96[c]	15.59[a]	0.52	<0.001
18:1 *trans*-4	<0.01	0.22	0.04	<0.01	0.15	0.55
18:1 *trans*-6 to 8	0.10	0.18	0.18	0.07	0.05	0.26
18:1 *trans*-9	0.13	0.28	0.35	0.08	0.08	0.13
18:1 *trans*-10 to 11	0.70[b]	1.23[b]	9.01[a]	0.61[b]	0.40	<0.001
18:1 *trans*-12	0.23[c]	0.37[b]	0.67[a]	0.18[c]	0.02	<0.001
18:1 *trans*-13	0.54[c]	0.65[b]	0.89[a]	0.37[d]	0.04	<0.001
18:1 *cis*-9	6.52[a]	5.99[ab]	5.19[bc]	4.20[c]	0.42	0.006
18:2 *cis*-9, *cis*-12	49.69	47.50	44.64	44.32	2.15	0.11
18:2 *cis*-9, *trans*-11	0.09[b]	0.06[b]	0.19[a]	0.04[b]	0.02	0.02
18:3 *cis*-9, *cis*-12, *cis*-15	3.80	3.47	3.26	3.41	0.20	0.27
18:3 *cis*-6, *cis*-9, *cis*-12	0.74[a]	0.54[b]	0.46[b]	0.52[b]	0.08	0.01
20:0	0.06	0.07	0.03	0.05	0.01	0.10
EPA[3,4]	1.85[b]	2.28[b]	3.10[b]	7.49[a]	0.76	0.003
DPA[3]	0.59	1.72	0.95	0.88	0.73	0.53
DHA[3,4]	0.58[b]	0.77[b]	0.98[b]	1.79[a]	0.21	0.003
Others	8.14	9.47	11.33	8.88	0.75	0.09
Total *trans*	1.69[b]	2.94[b]	11.15[a]	1.32[b]	0.55	<0.001

[a-d]Different superscripts represent differences among treatments ($P < 0.05$).

[1]Values represent least squares means of last day of treatment.

[2]Cows received 1) ruminal infusion of Ca salts of fish oil and palm fatty acid distillate low dose (CaFO-1), 2) ruminal infusion of Ca salts of fish oil and palm fatty acid distillate, high dose (CaFO-2), 3) ruminal infusion of fish oil high dose (RFO), and 4) abomasal infusion of fish oil high dose (AFO). The high dose provided 16.6 and 21.2 g/d of EPA and DHA, respectively, whereas the low dose provided half these amounts.

[3]EPA = eicosapentaenoic acid, 20:5 all *cis* 5, 8, 11, 14, 17; DPA = docosapentaenoic acid, 22:5 all *cis* 7, 10, 13, 16, 19; DHA = docosahexaenoic acid, 22:6 all *cis* 4, 7, 10, 13, 16, 19.

[4]Mean for EPA and DHA during baseline period was 0.76, and 0.09 g/100 g of fatty acid, respectively.

Table 6. Transfer of eicosapentaenoic (EPA) and docosahexaenoic (DHA) from fish oil to milk fat

Variable	Treatment[1]				SEM	*P*-value
	CaFO-1	CaFO-2	RFO	AFO		
Secretion, g/d						
EPA[2]	0.68[b]	0.83[b]	0.63[b]	3.76[a]	0.32	0.001
DHA[2]	0.78[b]	1.06[b]	0.95[b]	4.30[a]	0.41	0.002
Transfer,[3] %						
EPA	4.38[b]	3.08[b]	1.86[b]	21.27[a]	2.26	0.002
DHA	6.00[b]	4.36[b]	3.30[b]	18.90[a]	2.08	0.005

[a,b]Different superscripts represent differences among treatments ($P < 0.05$).

[1]Cows received 1) ruminal infusion of Ca salts of fish oil and palm fatty acid distillate low dose (CaFO-1), 2) ruminal infusion of Ca salts of fish oil and palm fatty acid distillate high dose (CaFO-2), 3) ruminal infusion of fish oil high dose (RFO), and 4) abomasal infusion of fish oil high dose (AFO). The high dose provided 16.6 and 21.2 g/d of EPA and DHA, respectively, whereas the low dose provided half these amounts.

[2]EPA = 20:5 all *cis* 5, 8, 11, 14, 17; DHA = 22:6 all *cis* 4, 7, 10, 13, 16, 19. Mean for EPA and DHA during the baseline period was 0.31, and 0.14 g/d, respectively.

[3]Calculated as [(fatty acid in milk during last 3 d of treatment) – (fatty acid in milk during baseline)] / [amount of fatty acid provided by supplement] × 100.

Jenkins, 2004a). These differences, however, probably reflect the detrimental effects of high doses of PUFA on the rumen microorganisms, and this would be minimized with the use of Ca salts of fatty acids. The 2 doses of Ca salts of fish oil averaged 3.7 and 5.1% transfer of EPA and DHA to milk fat, respectively (Table 6). These values were similar to the 2 and 4% transfer for EPA and DHA observed with dietary supplements of fish oil or ruminal infusion of unprotected fish oil (Jones et al., 2000; Chilliard et al., 2001).

Abomasal infusion of fatty acids is a convenient experimental method to avoid rumen biohydrogenation. Thus, the difference in fatty acid output in milk between abomasal infusion and other treatments can be attributed to metabolism in the rumen, intestinal bioavailability, or both. The transfer of abomasally infused EPA and DHA to milk fatty acids was 21.3 and 18.9%, respectively. These values are similar to those reported by others (Lock and Bauman, 2004). Transfer efficiencies of EPA and DHA are lesser than those observed for 18-carbon unsaturated fatty acids, where transfer in established lactation ranges from 30 to 70% as reviewed by Chilliard et al. (2000). The transfers of EPA and

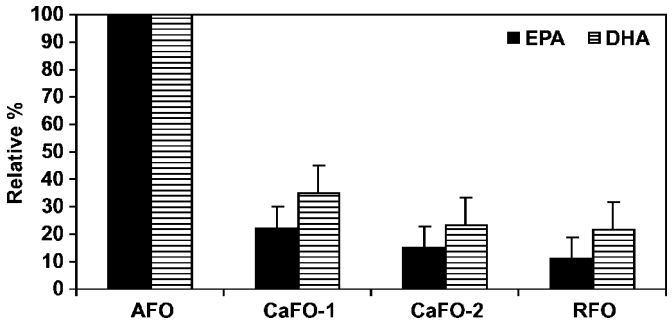

Figure 1. Transfer of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish oil supplements to milk fat as compared with abomasal infusion (corresponding to 100% rumen protection). Cows received 1) ruminal infusion of Ca salts of fish oil and palm fatty acid distillate low dose (CaFO-1), 2) ruminal infusion of Ca salts of fish oil and palm fatty acid distillate high dose (CaFO-2), 3) ruminal infusion of fish oil high dose (RFO), and 4) abomasal infusion of fish oil high dose (AFO). The high dose provided 16.6 and 21.2 g/d of EPA and DHA, respectively, whereas the low dose provided 50% of these amounts.

DHA to milk fat for the Ca salts of fish oil and the rumen infused fish oil treatments are compared relative to abomasal infusion in Figure 1. With the same dose of EPA and DHA supplemented as a Ca salt (CaFO-2), transfer to milk was only 14 and 23%, respectively, of that observed with abomasal infusion. Likewise, transfer efficiency with the ruminal infusion of fish oil was 9% for EPA and 17% for DHA. Thus, the rumen biohydrogenation of EPA and DHA was extensive for the Ca salts and the unprotected fish oil supplements, and the Ca salts of fish oil fatty acids provided no protection of EPA and DHA from rumen biohydrogenation beyond that seen with the unprotected fish oil supplement.

CONCLUSIONS

Supplementation with unprotected fish oil resulted in decreased DMI and a reduction in milk fat yield. These effects were not observed with Ca salts, indicating that this method offers protection from the adverse effects of unsaturated fatty acids on rumen digestion. The benefits of Ca salts, however, did not extend to protection of EPA and DHA from rumen biohydrogenation. Abomasal infusion of fish oil resulted in a considerable increase in the proportion of EPA and DHA in plasma and milk, but there was no difference in secretion of these fatty acids among other treatments indicating a similar degree of biohydrogenation for EPA and DHA in unprotected fish oil and Ca salts of fish oil. The changes observed in the milk fatty acid profile with RFO were indicative of alterations in rumen pathways of biohydrogenation and correspond to those reported by others in situations of diet-induced milk fat depression. Overall, results demonstrate that Ca salts of fish oil provided rumen inertness with regard to adverse effects on DMI and milk fat yield, but provided no protection against biohydrogenation of EPA and DHA in the rumen. Thus, with Ca salts or unprotected fish oil supplements, the postruminal supply of EPA and DHA is a function of the limited proportion of these fatty acids that escapes rumen biohydrogenation.

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