Milk Production and Composition from Cows Fed Small Amounts of Fish Oil with Extruded Soybeans¹

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ABSTRACT

Eight Holstein (189 ± 57 DIM) and 4 Brown Swiss (126 ± 49 DIM) multiparous cows were used in a replicated 4×4 Latin square with 28-d periods to determine the minimal dietary concentration of fish oil necessary to maximize milk conjugated linoleic acid (CLA) and vaccenic acid (VA). Treatments consisted of a control diet with a 50:50 ratio of forage to concentrate (dry matter basis), and 3 diets with 2% added fat consisting of 0.33% fish oil, 0.67% fish oil, and 1% fish oil with extruded soybeans providing the balance of added fat. Dry matter intake (23.1, 22.6, 22.8, and 22.9 kg/d, for control, low, medium, and high fish oil diets, respectively) was similar for all diets. Milk production (21.5, 23.7, 22.7, and 24.2 kg/d) was higher for cows fed the fat-supplemented diets vs. the control. Milk fat (4.42, 3.81, 3.80, and 4.03%) and true protein (3.71, 3.58, 3.54, and 3.55%) concentrations decreased when cows were fed diets containing supplemental fat. Concentration of milk *cis*-9,*trans*-11 CLA (0.55, 1.17, 1.03, and 1.19 g/100 g of fatty acids) was increased similarly by all diets containing supplemental fat. Milk VA (1.12, 2.47, 2.13, and 2.63 g/100 g of fatty acids) was increased most in milk from cows fed the low and high fish oil diets. Milk total n-3 fatty acids were increased (0.82, 0.96, 0.92, and 1.01 g/100 g of fatty acids) by all fat-supplemented diets. The low fish oil diet was as effective at increasing VA and CLA in milk as the high fish oil diet, showing that only low concentrations of dietary fish oil are necessary for increasing concentrations of VA and CLA in milk.

Key words: milk, fatty acid, fish oil, extruded soybean

INTRODUCTION

The different positional and geometric isomers of conjugated linoleic acid (CLA) confer different health effects on mammals (Belury, 2002). The *cis*-9,*trans*-11 18:2 isomer has been shown to be anticarcinogenic (Ip et al., 1999), whereas the *trans*-10,*cis*-12 18:2 isomer is capable of decreasing body fat and increasing lean body mass (Park et al., 1999). The *trans*-10,*cis*-12 isomer also decreases fat concentrations in the milk of dairy cows in a dose-dependent fashion (Peterson et al., 2002). These effects on mammals have encouraged research efforts to identify methods of increasing the *cis*-9,*trans*-11 CLA isomer and to understand the mechanisms of *trans*-10,*cis*-12 CLA production so that its synthesis will occur only when desired.

Vaccenic acid (VA; *trans*-11 18:1) can be converted to *cis*-9,*trans*-11 CLA by the mammalian enzyme Δ^9 -desaturase; both cattle (Griinari et al., 2000) and humans (Adlof et al., 2000) are capable of synthesizing *cis*-9,*trans*-11 CLA from VA. Recent research also suggests that the extent of VA conversion into CLA in the milk of dairy cattle may depend on the diet and that much, if not most, of the CLA present in milk is synthesized endogenously. Estimates of the total amount of milk CLA that is synthesized endogenously range from 64 to 93% (Griinari et al., 2000; Piperova et al., 2002) of the total CLA present in milk. Increasing VA is therefore an important part of this research aimed at increasing CLA.

Although dietary fish oil dramatically and consistently increases milk VA and CLA concentrations, it can also decrease feed intake, milk production, and milk fat yield or concentration (Donovan et al., 2000; Chouinard et al., 2001; Whitlock et al., 2002). Donovan et al. (2000) fed fish oil to dairy cows at 0, 1, 2, or 3% of diet DM and observed maximum concentrations of milk VA and CLA at 2% fish oil supplementation. Because fish oil contains low amounts of known precursors of VA and CLA, the authors speculated that fish oil enhanced the conversion of linoleic acid or linolenic acid, or both, from other feed sources into VA and CLA, possibly by inhibiting the final step in the biohydrogenation of VA to stearic acid (Shingfield et al., 2003). Recent research by AbuGhazaleh and Jenkins (2004) demonstrated that

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docosahexaenoic acid (22:6n-3) is likely the component of fish oil responsible for this reaction. A subsequent study by Whitlock et al. (2002), in which fish oil was fed in combination with extruded soybeans as a source of linoleic acid, supported that hypothesis. Whitlock et al. (2002) found that milk VA and CLA were increased similarly for cows fed 1% fish oil in combination with 1% fat from extruded soybeans compared with when cows were fed 2% fish oil. When cows were fed a diet containing 2% fat from extruded soybeans, VA and CLA concentrations increased less than half as much as when they were fed the fish oil-supplemented diets. We hypothesized that, when fed in combination with a source of linoleic acid such as extruded soybeans, only a small amount of fish oil is necessary to increase the production of VA and CLA to these high concentrations. The objective of this research was to determine the lowest concentrations of fish oil in combination with a high linoleic acid source, such as extruded soybeans, that would cause the maximal increase of VA and CLA concentrations in milk fat.

MATERIALS AND METHODS

Experimental Design and Data Collection

All procedures for this study were carried out under approval of the South Dakota State University Animal Care and Use Committee. Eight Holstein [189 ± 57 (SD) DIM] and 4 Brown Swiss (126 ± 49 DIM) multiparous cows were used in a replicated 4×4 Latin square with 28-d periods. Two blocks of Holsteins and one block of Brown Swiss were used because of cow availability, but this also provided an opportunity for limited breed comparisons. Weeks 1 and 2 of each period were used for adjustment to diets and wk 3 and 4 for data collection. Dietary treatments consisted of a control diet (**CONT**) or CONT diet supplemented with 2% fat. The fat-supplemented diets were 0.33% fish oil and 1.67% fat from extruded soybeans (**LFO**, low fish oil), 0.67% fish oil and 1.33% fat from extruded soybeans (**MFO**, medium fish oil), and 1% fish oil and 1% fat from extruded soybeans (**HFO**, high fish oil). Total mixed diets (Table 1) were formulated to be isonitrogenous and contain more than sufficient amounts of the major nutrients (NRC, 2001). Cows were housed in a free-stall barn and individually fed a TMR ad libitum once daily (0700 h) using Calan Broadbent feeder doors (American Calan, Inc., Northwood, NH). The corn silage and alfalfa hay were mixed in the mixer wagon equipped with cutting knives (New Direction Equipment, Sioux Falls, SD) prior to blending forages with concentrates in the Calan Data Ranger (American Calan, Inc.). Amounts fed and refused were recorded daily, with amounts fed adjusted to achieve 5 to 10% refusals. Visual observation of refusals

Table 1. Ingredient and nutrient content of diets containing no fish oil or extruded soybeans (**CONT**), 0.33% fish oil and 1.67% fat from extruded soybeans (**LFO**), 0.67% fish oil and 1.33% fat from extruded soybeans (**MFO**), and 1% fish oil and 1% fat from extruded soybeans (**HFO**)

Item	Diet			
	CONT	LFO	MFO	HFO
	(% of DM)			
Ingredient composition				
Alfalfa hay	25.00	25.00	25.00	25.00
Corn silage	25.00	25.00	25.00	25.00
Cracked corn	30.07	28.17	28.05	27.94
Soybean meal, 44% CP	15.63	8.33	9.89	11.44
Soy hulls	2.00	2.00	2.00	2.00
Fish oil	—	0.33	0.67	1.00
Extruded soybeans	—	8.87	7.09	5.32
High Zn trace mineralized salt	0.25	0.25	0.25	0.25
Magnesium oxide	0.15	0.15	0.15	0.15
Dicalcium phosphate	0.60	0.60	0.60	0.60
Limestone	1.10	1.10	1.10	1.10
Vitamin A, D, and E premix ¹	0.10	0.10	0.10	0.10
Vitamin E premix ²	0.10	0.10	0.10	0.10
Chemical composition				
DM, %	75.6	76.1	76.0	76.2
CP	19.1	19.5	19.1	19.2
RUP, ³ % of CP	32.8	36.1	35.4	34.8
Ether extract	3.2	4.8	4.4	4.8
Total fatty acids	2.8	4.2	3.9	4.3
ADF	21.8	20.8	20.6	20.7
NDF	30.5	28.2	28.5	29.2
Lignin	3.0	3.1	3.0	3.1
NFC ⁴	39.1	39.2	39.8	38.4
Ash	8.1	8.3	8.2	8.4
Ca	1.16	1.02	1.32	1.28
P	0.43	0.43	0.46	0.42
Mg	0.33	0.32	0.31	0.33
NE _L , ³ Mcal/kg	1.65	1.68	1.68	1.69

¹Contains 4,400,000 IU of vitamin A; 880,000 IU of vitamin D, and 440 IU of vitamin E per kilogram.

²Contains 44,000 IU of vitamin E per kilogram.

³Estimated from NRC (2001).

⁴NFC = 100 – (CP + NDF + ether extract + ash).

indicated no apparent sorting of ingredients by the cows.

Dry matter content (105°C for 24 h) of corn silage was determined weekly for adjustment of proportions of ingredients in the TMR. Samples of alfalfa hay, corn silage, and concentrate mixes were collected weekly and stored at –20°C until analyses. Weekly samples were dried at 55°C in a Despatch oven (style V-23; Despatch Oven Co., Minneapolis, MN) for at least 24 h, ground through a 2-mm screen of a Wiley mill (model 3; Arthur H. Thomas Co., Philadelphia, PA), and composited by period. Subsamples of feed composites were dried at 105°C for 24 h to correct to 100% DM. Composites were analyzed for CP, ether extract, and ash according to AOAC methods (1997). Samples were reground (Brinkman ultracentrifuge mill; Brinkman Instruments Co., Westbury, NY) through a 1-mm screen prior to analyses for Ca, P, Mg (AOAC, 1997), and fiber. Neutral deter-

Table 2. Fatty acid composition of concentrate mixes, forages, extruded soybeans, and fish oil from cows fed diets containing no fish oil or extruded soybeans (CONT), 0.33% fish oil and 1.67% fat from extruded soybeans (LFO), 0.67% fish oil and 1.33% fat from extruded soybeans (MFO), and 1% fish oil and 1% fat from extruded soybeans (HFO)

Fatty acid ¹	Concentrate mix				Corn silage	Alfalfa hay	Extruded soybeans	Fish oil
	CONT	LFO	MFO	HFO				
	(g/100 g of fatty acid)							
14:0	0.18	0.72	1.76	2.98	0.21	0.55	0.05	7.49
14:1	ND ²	ND	ND	ND	ND	ND	ND	0.06
16:0	20.47	22.59	24.78	25.24	29.48	37.09	9.86	16.76
16:1	ND	1.25	1.77	3.34	0.12	0.26	0.07	12.66
18:0	4.45	6.53	6.37	5.85	3.45	6.61	3.02	2.48
18:1 <i>t</i> 6	ND	ND	ND	ND	ND	ND	ND	ND
18:1 <i>t</i> 9	0.05	0.03	0.09	0.07	ND	ND	ND	0.30
18:1 <i>c</i> 6	1.43	1.23	1.55	1.14	ND	ND	ND	0.28
18:1 <i>t</i> 11	ND	ND	ND	ND	ND	ND	ND	ND
18:1 <i>c</i> 9	28.98	22.50	17.29	20.16	4.00	3.07	14.64	6.63
18:1 <i>c</i> 11	1.46	2.01	1.76	2.09	0.73	0.46	2.60	3.14
18:2 <i>t</i> 9, <i>t</i> 12	ND	ND	ND	0.06	ND	ND	0.02	0.27
18:2 <i>c</i> 9, <i>c</i> 12	18.50	40.70	31.02	24.60	18.72	10.06	58.29	1.17
18:3 <i>n</i> -6	0.17	0.14	0.18	0.18	ND	ND	0.37	0.13
18:3 <i>n</i> -3	5.87	10.27	11.26	9.95	4.93	0.40	8.97	0.95
20:1	0.67	0.58	0.59	0.62	0.84	ND	ND	0.90
20:5 <i>n</i> -3	ND	0.06	0.12	0.18	ND	0.40	ND	8.95
22:5	ND	ND	ND	ND	ND	ND	ND	1.14
22:6 <i>n</i> -3	ND	0.04	0.07	0.11	ND	ND	ND	5.31

¹Expressed as the number of carbons:number of double bonds.

²ND = Not detectable.

gent fiber (procedure B, Van Soest et al., 1991), ADF (Robertson and Van Soest, 1981), and acid detergent lignin (Lowry et al., 1994) were determined with an Ankom fiber analyzer using the filter bag technique (Ankom Technology Corp., Fairport, NY).

Cows were milked twice daily at 0430 and 1600 h, with individual milk weights recorded at each milking. Milk from individual cows was sampled during two 24-h periods on the last 2 d of wk 3 and 4 of each period and made into daily composites. Composites were divided into 2 aliquots for analyses. One was refrigerated at 4°C and sent to Valley Queen Cheese Factory (Milbank, SD) for analyses of fat, true protein, total solids, and lactose (AOAC, 1997) by mid-infrared spectrophotometry (Multispec; Foss Food Technology Corp., Eden Prairie, MN) and SCC (AOAC, 1997) using a Fossomatic 90 (Foss Food Technology Corp.). The second sample was stored at -20°C for fatty acid analysis as butyl esters by GLC (AbuGhazaleh et al., 2001), with the modification of decreasing the heating time to 30 min. Esters of fatty acids were separated on a 0.25 mm $\times$ 100 m column (SP2560; Supelco, Inc., Bellefonte, PA). The temperature and split ratio in the injector port was 230°C and 75:1 with a column flow of 1.5 mL/min of He. Oven temperature was initially set at 60°C for 5 min, then increased to 165°C at 3°C/min, held at 165°C for 10 min, raised to 230°C at 5°C/min, and finally held at 220°C for 32 min. Standard mixtures of fatty acids

(FIM-FAME-7; Matreya, Inc., Pleasant Gap, PA; and GLC-68D; Nu-Check-Prep, Inc., Elysian, MN) and standards of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA (Matreya, Inc.) were analyzed for identification of the fatty acid composition of milk samples. Fatty acid compositions of feeds were determined as described by AbuGhazaleh et al. (2001).

Body weights and BCS of cows (Wildman et al., 1982) were recorded at the beginning of the trial and at the end of each period to give an indication of the size and BCS of cows used in this trial. Body weights and BCS were the average of 2 observations taken at the beginning and end of each period.

Statistical Analysis

Data were analyzed as a replicated 4 $\times$ 4 Latin square using the mixed procedures of SAS (SAS Institute, 1996). The statistical model was $Y = \text{treatment} + \text{breed} + \text{period} + \text{cow} (\text{breed}) + \text{treatment} \times \text{breed}$, where cow (breed) is the random effect used to test treatment, breed, and the interaction of treatment $\times$ breed. Treatment $\times$ breed was tested for all variables and was allowed in the model and noted in the tables only when significant ($P < 0.05$). Preplanned contrasts were supplemental fat versus no fat, linear, and quadratic relationships to analyze the linear increase of fish oil and decrease of extruded soybeans in the fat-supplemented

Table 3. Fatty acid composition of diets containing no fish oil or extruded soybeans (CONT), 0.33% fish oil and 1.67% fat from extruded soybeans (LFO), 0.67% fish oil and 1.33% fat from extruded soybeans (MFO), and 1% fish oil and 1% fat from extruded soybeans (HFO)

Fatty acid ¹	Diet			
	CONT	LFO	MFO	HFO
	(g/100 g of fatty acid)			
14:0	0.26	0.55	1.02	1.52
14:1	ND ²	ND	ND	ND
16:0	25.70	26.99	28.78	29.23
16:1	0.08	0.76	0.93	1.58
18:0	4.60	5.74	5.55	5.29
18:1 ω 6	ND	ND	ND	ND
18:1 ω 9	0.03	0.02	0.04	0.03
18:1 ω 6	0.78	0.67	0.72	0.50
18:1 ω 11 ³	0.02	0.02	0.02	0.02
18:1 ω 9	17.42	13.77	9.94	10.85
18:1 ω 11	1.06	1.36	1.14	1.25
18:2 ω 9, ω 12	ND	ND	ND	0.03
18:2 ω 9, ω 12	16.53	32.33	26.75	21.90
18:3 ω -6	0.09	0.08	0.09	0.08
18:3 ω -3	4.43	6.80	6.71	6.31
20:1	0.56	0.51	0.51	0.52
20:5 ω -3	ND	0.04	0.07	0.11
22:5	ND	ND	ND	ND
22:6 ω -3	ND	0.02	0.04	0.06

¹Expressed as number of carbons:number of double bonds.

²ND = Not detectable.

³Vaccenic acid.

diets. Significance was declared at $P < 0.05$, unless otherwise noted.

RESULTS AND DISCUSSION

Diets (Table 1) were formulated to be isonitrogenous (18% CP) but averaged greater than 19% CP, possibly because of higher than anticipated CP in the alfalfa hay. The alfalfa hay also contained more calcium than anticipated. The chemical composition was similar among diets except that ether extract and total fatty acid contents were greater in fat-supplemented diets, as expected. Accordingly, the NE_L concentration was increased in the fat-supplemented diets. Any differences in dietary NDF, ADF, and RUP were minor and would not have affected the studied outcomes.

The fatty acid composition of concentrate mixes, forages, extruded soybeans, and fish oil are presented in Table 2 and for TMR in Table 3. The concentrations of known precursors of CLA, *cis*-9,*cis*-12 18:2 (linoleic acid) and 18:3 ω -3 (linolenic acid), were high in extruded soybeans (58.3 and 9.0 g/100 g of fatty acids, respectively) and low in fish oil (1.2 and 1.0 g/100 g of fatty acids).

DMI, Milk Yield, and Milk Composition

Dry matter intake (Table 4) was similar for all 4 diets in this study. Other studies (Donovan et al., 2000; Whitlock et al., 2002) showed decreased DMI when fish oil was fed at greater concentrations than in the present study. The study by Donovan et al. (2000) showed similar intake for cattle fed fish oil at 1% of diet DM compared with a control diet, but intake decreased by 18 and 29% when fed at 2 and 3% of dietary DM, respectively. Dry matter intake was similar to controls in another study (Whitlock et al., 2002) when cattle were fed 2% fat from extruded soybeans, but decreased 7% when diets contained 1% fish oil and 1% fat from extruded soybeans, and decreased 11% when diets contained 2% fish oil as compared with a control diet. In the present study, DMI was successfully maintained by feeding lower concentrations of fish oil and by providing the balance to 2% added fat with extruded soybeans.

Milk production (Table 4) was greater ($P < 0.05$) for cows fed diets containing added fat compared with CONT. Milk production was similar for cows fed the 3 diets containing fish oil and extruded soybeans, although production by all cows used in this experiment was lower than desired because the cows were in mid-to late lactation. The increased milk production for cows fed fat agreed with research by Dhiman et al. (1999), Schingoethe et al. (1996), and Whitlock et al. (2002), who observed increased milk production in cows fed extruded soybeans. Donovan et al. (2000) observed decreased milk production in cattle fed fish oil at 3% of dietary DM, but similar production when fish oil was fed at 2% and increased production when fish oil was fed at 1% as compared with a control diet. However, a study by Whitlock et al. (2002) showed a 9% decrease in milk production when cattle were fed 2% fish oil, but similar milk production when cows were fed 1% fish oil and 1% fat from extruded soybeans as compared with a control diet. Energy-corrected milk and FCM were similar among diets, in agreement with Donovan et al. (2000) and Whitlock et al. (2002) when fish oil was fed at similar amounts as in the present study.

Milk fat concentrations (Table 4) decreased ($P < 0.01$) 9 to 14% when cattle were fed the diets containing supplemental fat, but milk yield was lower ($P < 0.05$) only for cattle fed MFO as compared with CONT. A decrease in milk fat concentration is common for dairy cows fed fish oil (Cant et al., 1997; Donovan et al., 2000; Whitlock et al., 2002). Other studies showed that milk fat concentration decreased by 6, 20, and 23% when cattle were fed 1, 2, and 3% fish oil (Donovan et al., 2000), and by 7 and 21% when fed 1 and 2% fish oil (Whitlock et al., 2002) compared with control diets. One goal of the current research was to minimize this de-

Table 4. Dry matter intake, milk yield, milk composition, milk SCC, BW, and BCS of cows fed diets containing no fish oil or extruded soybeans (CONT), 0.33% fish oil and 1.67% fat from extruded soybeans (LFO), 0.67% fish oil and 1.33% fat from extruded soybeans (MFO), and 1% fish oil and 1% fat from extruded soybeans (HFO)

Item	Diet				SE	Contrasts, ¹ <i>P</i>		
	CONT	LFO	MFO	HFO		FO	Linear	Quad
DMI, kg/d	23.1	22.6	22.8	22.9	1.5	0.81	0.87	0.99
Milk, kg/d	21.5	23.7	22.7	24.2	2.2	0.01	0.61	0.15
ECM, ² kg/d	24.8	25.4	23.7	26.6	2.2	0.69	0.30	0.03
3.5% FCM, ³ kg/d	24.5	24.8	23.1	26.2	2.2	0.86	0.26	0.03
Milk fat								
%	4.42	3.81	3.80	4.03	0.19	<0.01	0.17	0.40
kg/d	0.94	0.90	0.82	0.97	0.08	0.35	0.21	0.03
Milk protein								
%	3.71	3.58	3.54	3.55	0.13	0.01	0.69	0.70
kg/d	0.79	0.83	0.79	0.85	0.07	0.17	0.71	0.11
Milk lactose								
%	4.51	4.59	4.60	4.61	0.11	0.02	0.79	0.96
kg/d	0.98	1.11	1.07	1.14	0.11	<0.01	0.58	0.24
Milk SCC × 10 ³ /mL	570	117	180	311	255	0.21	0.58	0.91
BW, kg	657	652	659	657	14	0.75	0.44	0.38
BCS ⁴	3.2	3.3	3.3	3.3	0.05	0.05	0.75	0.74

¹FO = Control diets vs. diets containing fish oil and extruded soybeans (CONT vs. 0.33, 0.67, and 1% fish oil diets).

²Orth (1992).

³Parekh (1986).

⁴Scored as 1 = emaciated and 5 = overly fat (Wildman et al., 1982). Effect of breed ($P < 0.05$).

crease in milk fat concentration by lowering the concentration of fish oil in the diet.

Milk protein concentration (Table 4) decreased ($P < 0.01$) when cattle were fed the diets containing supplemental fat, but yield was not affected. This decrease in milk protein concentration differed from the reports of Donovan et al. (2000) and Whitlock et al. (2002), who did not observe a decreased milk protein concentration in response to feeding fish oil. Donovan et al. (2000) and Whitlock et al. (2002) noted a decrease in milk protein yield when cattle were fed fish oil, in contrast to the present study in which milk protein yield was unaffected. The different responses in milk protein yield corresponded more closely with milk production, which was increased in the present study but which decreased in the studies by Donovan et al. (2000) and Whitlock et al. (2002). Jones et al. (2000) also observed no change in milk protein yield when cows were fed fish oil. Milk production was lower and cows were in a later stage of lactation in the present study than in the other reported studies, which may explain why milk fat and protein concentrations were quite high. Milk from the Brown Swiss contained higher fat and protein concentrations than milk from the Holsteins, which is a typical breed difference, but there were no breed × diet interactions.

Changes in BW and body condition (Table 4) in trials with short-term experimental periods such as this trial may not be accurately reflected but are presented to

provide the reader with baseline data. Body weight was not affected by diet in this study. Body condition scores were 0.1 units higher ($P < 0.05$) for cattle fed diets containing supplemental fat. The Brown Swiss cows had a greater ($P < 0.05$) BCS than the Holstein cows (3.53 vs. 3.06, respectively).

Fatty Acid Composition of Milk

The addition of supplemental fat to diets increased ($P < 0.01$) the concentration of long-chain fatty acids and unsaturated fatty acids in milk fat while decreasing ($P < 0.05$) the concentrations of short- and medium-chain fatty acids (Table 5). Supplemental fats that provide long-chain fatty acids commonly decrease de novo synthesis of fatty acids (Grummer, 1991), leading to a greater concentration of long-chain fatty acids. Donovan et al. (2000) observed an increase in long-chain and unsaturated fatty acids when cattle were fed fish oil. The study by Whitlock et al. (2002) found an increase in long-chain and in unsaturated fatty acids when dairy cows were fed 2% fat from extruded soybeans or a diet with 1% fish oil and 1% fat from extruded soybeans.

Two CLA isomers, *cis*-9,*trans*-11 18:2 and *trans*-10,*cis*-12 18:2, as well as their precursors (*trans*-11 18:1 and *trans*-10 18:1), were identified (Table 5). The *cis*-9,*trans*-11 18:2 isomer increased ($P < 0.01$) with the addition of supplemental fat, and concentrations remained similar for all fish oil and extruded soybean

Table 5. Fatty acid composition of milk fat from cows fed diets containing no fish oil or extruded soybeans (CONT), 0.33% fish oil and 1.67% fat from extruded soybeans (LFO), 0.67% fish oil and 1.33% fat from extruded soybeans (MFO), and 1% fish oil and 1% fat from extruded soybeans (HFO)

Fatty acid ¹	Diet				SE	Contrasts, ² <i>P</i>		
	CONT	LFO	MFO	HFO		FO	Linear	Quad
	(g/100 g of fatty acid)							
4:0	3.17	3.14	3.04	3.23	0.10	0.56	0.27	0.05
6:0	2.18	2.02	1.90	2.08	0.08	0.02	0.50	0.05
8:0	1.46	1.29	1.21	1.34	0.06	<0.01	0.44	0.07
10:0	3.53	3.01	2.82	3.08	0.18	<0.01	0.68	0.15
11:0	0.08	0.06	0.06	0.05	0.01	<0.01	0.41	0.59
12:0	4.32	3.54	3.49	3.63	0.23	<0.01	0.65	0.60
13:0	0.13	0.10	0.11	0.09	0.01	<0.01	0.29	0.30
14:0	11.64	10.74	10.62	11.03	0.34	<0.01	0.40	0.39
14:1	1.26	1.27	1.39	1.19	0.13	0.75	0.35	0.05
15:0	1.11	0.96	1.02	0.94	0.04	<0.01	0.64	0.06
16:0	28.56	25.26	26.54	25.52	0.90	<0.01	0.76	0.12
16:1	1.50	1.52	1.79	1.44	0.16	0.41	0.51	<0.01
17:0	0.53	0.48	0.52	0.52	0.02	0.10	0.05	0.10
18:0	8.17	8.34	8.10	8.16	0.56	0.95	0.67	0.68
Total 18:1	20.31	23.76	23.81	22.97	0.89	<0.01	0.44	0.62
18:1 <i>t</i> 6	0.21	0.41	0.40	0.41	0.04	<0.01	0.94	0.91
18:1 <i>t</i> 9	0.25	0.44	0.43	0.44	0.04	<0.01	0.98	0.70
18:1 <i>t</i> 10	0.50	0.99	1.29	0.85	0.25	<0.01	0.57	0.08
18:1 <i>t</i> 11 (VA ³)	1.12	2.47	2.13	2.63	0.23	<0.01	0.49	0.04
18:1 <i>c</i> 9	17.80	18.93	19.00	18.13	0.69	0.17	0.31	0.48
18:1 <i>c</i> 11	0.44	0.53	0.56	0.51	0.03	<0.01	0.50	0.10
18:2 <i>t</i> 9, <i>t</i> 12	0.31	0.47	0.44	0.46	0.03	<0.01	0.82	0.30
18:2 <i>c</i> 9, <i>c</i> 12	2.85	3.41	3.10	3.15	0.19	<0.01	0.07	0.15
CLA ⁴ (c9, <i>t</i> 11)	0.55	1.17	1.03	1.19	0.12	<0.01	0.85	0.11
CLA (t10, <i>c</i> 12)	0.01	0.03	0.03	0.03	0.01	<0.01	0.91	0.88
18:3 <i>n</i> -6	0.04	0.03	0.03	0.03	0.01	<0.01	0.93	0.59
18:3 <i>n</i> -3	0.67	0.78	0.75	0.78	0.04	<0.01	0.94	0.22
20:0	0.17	0.20	0.19	0.23	0.02	0.04	0.07	0.20
20:1	0.11	0.21	0.21	0.24	0.02	<0.01	0.26	0.49
20:2	0.07	0.07	0.07	0.08	0.01	0.65	0.32	0.43
20:3	0.13	0.13	0.10	0.10	0.01	<0.01	0.04	0.07
20:4	0.06	0.06	0.07	0.06	0.01	0.19	0.96	0.27
20:5 <i>n</i> -3	0.06	0.07	0.07	0.10	0.01	0.01	<0.01	0.02
21:0	0.04	0.04	0.05	0.05	0.01	0.38	0.29	0.79
22:0	0.09	0.10	0.09	0.10	0.01	0.05	0.36	0.47
22:1	0.18	0.15	0.13	0.15	0.01	<0.01	0.80	<0.01
22:5	0.11	0.12	0.12	0.13	0.01	0.23	0.03	0.24
22:6 <i>n</i> -3	0.04	0.06	0.06	0.09	0.01	0.01	<0.01	0.04
23:0	0.08	0.08	0.08	0.09	0.01	0.82	0.33	0.26
24:0	0.05	0.05	0.05	0.05	0.01	0.99	0.51	0.38
Unidentified	6.41	7.27	6.94	7.60	0.22	<0.01	0.13	<0.01
Total n-3 FA ⁵	0.82	0.96	0.92	1.01	0.06	<0.01	0.27	0.10
Short	14.87	13.16	12.62	13.51	0.57	<0.01	0.55	0.16
Medium	44.07	39.75	41.36	40.12	1.27	<0.01	0.76	0.18
Long	34.66	39.82	39.08	38.77	1.45	<0.01	0.50	0.87
Unsaturated	28.28	33.32	33.17	32.20	1.17	<0.01	0.39	0.72
Saturated	65.10	59.30	60.14	60.28	1.39	<0.01	0.53	0.80
CLA:VA ⁶	0.49	0.47	0.48	0.45	0.02	0.43	0.15	0.06

¹Expressed as number of carbons:number of double bonds.²FO = Control diets vs. diets containing fish oil and extruded soybeans (CONT vs. 0.33, 0.67, and 1% fish oil diets).³Vaccenic acid.⁴Conjugated linoleic acid.⁵n-3 fatty acids: 18:3*n*-3, 20:5*n*-3, 22:6*n*-3; short-chain fatty acids: 4:0 to 12:0; medium-chain fatty acids: 14:0 to 16:1; long-chain fatty acids: 17:0 to 22:6.⁶Ratio of *cis*-9,*trans*-11 conjugated linoleic acid to vaccenic acid.

combinations evaluated. The lack of a linear or quadratic effect indicated that the smallest amount of fish oil (0.33% of diet DM) was sufficient to maximize the changes observed. Previous research by Donovan et al. (2000) showed the maximum *cis-9,trans-11* CLA in milk from cows fed 2% fish oil as the only supplemental fat source. That research was followed with an experiment feeding of 2% fat from fish oil, extruded soybeans, or a combination of 1% fat from each, fish oil and extruded soybeans (Whitlock et al., 2002). Results showed similar milk *cis-9,trans-11* CLA concentrations for cows fed the 2% fish oil diet as those fed the diet with 1% fish oil and 1% fat from extruded soybeans. That amount was greater than the concentration of milk *cis-9,trans-11* CLA present from cows fed 2% extruded soybeans without fish oil. This result demonstrated the necessity of having at least a small amount of fish oil to maximize milk *cis-9,trans-11* CLA concentrations and led to the design of the present study. Previous research (AbuGhazaleh et al., 2002; Whitlock et al., 2002) demonstrated that the addition of fish oil with a high linoleic acid source such as extruded soybeans increased milk CLA and VA more than feeding only the high linoleic source; thus, a diet with only extruded soybeans was not included in the present experiment. The study by AbuGhazaleh et al. (2002) likewise showed that only a small amount of fish oil (0.45% of diet DM, supplied as fish meal) with extruded soybeans caused more than twice as great an increase in milk *cis-9,trans-11* CLA as that caused by feeding the extruded soybeans without the fish meal. The present study did not show as great an increase in milk *cis-9,trans-11* CLA as have previous studies, possibly because cows were in a later stage of lactation and milk production was lower than in previous studies.

A diet $\times$ breed interaction was observed in the study by Whitlock et al. (2002), in which Brown Swiss had inherently higher concentrations of milk *cis-9,trans-11* CLA than Holsteins, but the Brown Swiss were less responsive to dietary manipulation. In that study, the Holsteins actually had a higher concentration of milk *cis-9,trans-11* CLA when fed the combination diet of 1% fish oil and 1% fat from extruded soybeans. No diet $\times$ breed interaction occurred in the present study, but the data did show the same tendency ($P < 0.15$); that is, milk *cis-9,trans-11* CLA was numerically higher in milk from Brown Swiss than from Holsteins when the CONT diet (0.62 vs. 0.50 g/100 g of fatty acids) was fed. Milk *cis-9,trans-11* CLA increased by 2.38-fold to 1.19 g/100 g of fatty acids for Holsteins fed the LFO diet, and by 1.95-fold (1.21 g/100 g of fatty acids) for Brown Swiss fed the HFO diet. Previously, Kelsey et al. (2003) were unable to demonstrate much of a difference in milk concentrations of CLA and their precursors between the

2 breeds because of substantial individual cow variation in milk composition. However, that study included only one diet fed to all cows, and milk was sampled on only one day. In the present study and in the study by Whitlock et al. (2002), the Latin square design allowed each cow to serve as her own control, thus correcting for inherent variability and allowing for the opportunity to detect differences caused by diet without data being confounded by inherent variations.

The *trans-10,cis-12* 18:2 isomer of CLA was present in very small concentrations in milk from cows fed all 4 diets (Table 5) and was elevated ($P < 0.01$) when cows were fed fish oil. This CLA isomer has been shown to decrease milk fat (Peterson et al., 2002), and in the present study milk fat was similarly decreased for all 3 diets that showed elevated milk *trans-10,cis-12* CLA. Whitlock et al. (2002) also showed elevated milk *trans-10,cis-12* CLA from cows fed fish oil.

Mammals, including humans (Adlof et al., 2000), are capable of converting VA into CLA by the enzyme Δ^9 -desaturase; thus, an increase in milk VA as well as an increase in milk CLA is desirable. The milk concentration of VA (Table 5) was increased ($P < 0.01$) by all 3 diets containing supplemental fat. Cows fed the LFO and HFO diets produced similar amounts of VA, but cows fed the HFO diet produced more VA ($P < 0.05$) than those fed the MFO diet. One possible explanation for this apparent discrepancy, as well as for the numerically lower *cis-9,trans-11* CLA concentration, is that the MFO diet contained a slightly lower concentration of total fatty acids (Table 1) than was expected. Similarly to *cis-9,trans-11* CLA, milk VA concentration was affected by fish oil diets in trials by Donovan et al. (2000) and Whitlock et al. (2002). Whitlock et al. (2002) showed that milk VA concentrations were similar for cows fed a diet containing 2% fish oil, or a diet containing 1% fish oil and 1% fat from extruded soybeans, and Holsteins had numerically higher milk VA concentrations when fed the combination diet than when fed fish oil and extruded soybeans separately. The essentially constant ratio of *cis-9,trans-11* CLA to VA with all diets supports the concept that much of the VA is converted to CLA endogenously by Δ^9 -desaturase (Griinari et al., 2000).

The current trial had no diet $\times$ breed effect on milk VA concentrations, but the numerical trends were similar to the previous study (Whitlock et al., 2002). In the current study, milk VA concentration when fed the CONT diet was slightly higher ($P < 0.15$) for Brown Swiss than for Holsteins. Milk VA increased in Holsteins to 2.69 g/100 g of fatty acids when fed the HFO diet, and Brown Swiss increased slightly less (2.53 g/100 g of fatty acids). Holsteins also produced 2.67 g of VA/100 g of fatty acids when fed the LFO diet, whereas

Brown Swiss produced only 2.11 g of VA/100 g of fatty acids. This tended to agree with research by Whitlock et al. (2002) showing that Brown Swiss have inherently higher milk VA but are less responsive to fish oil for increasing milk VA.

Other milk fatty acids were changed with the addition of supplemental fat (Table 5). Milk concentrations of total 18:1 fatty acids were greater ($P < 0.05$) for cows fed all fat-supplemented diets. All measured 18:1 fatty acids except oleic acid (*cis*-9 18:1) were present in milk fat in greater ($P < 0.05$) concentrations when cows were fed the fat-supplemented diets than when fed the CONT diet, and oleic acid appeared numerically higher when cows were fed the LFO and MFO diets compared with CONT. Donovan et al. (2000) and Whitlock et al. (2002) also reported higher concentrations of all milk 18:1 fatty acids except for oleic acid when fish oil diets were fed. When extruded soybeans were fed with fish oil in the study by Whitlock et al. (2002), the milk oleic acid concentration was similar to that of the control diet, and when extruded soybeans were fed without fish oil, the milk oleic acid concentration was slightly ($P > 0.10$) greater than that of the control diet.

The total n-3 fatty acid content of milk (Table 5) was also greater ($P < 0.05$) for all fat-supplemented diets compared with CONT. α -Linolenic acid (18:3n-3) made up the majority of total n-3 fatty acids measured and was present in greater ($P < 0.05$) concentrations in milk from cows fed the diets containing supplemental fat. Eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) were also present in greater ($P < 0.05$) concentrations in milk from cows fed the fat-supplemented diets. These results agreed with those of Donovan et al. (2000), who observed increases in all three n-3 fatty acids measured in milk as the fish oil concentration increased. Whitlock et al. (2002) observed a slightly lower concentration of milk α -linolenic acid for cattle fed 2% fish oil but increased α -linolenic acid for cattle fed extruded soybeans. They also noted a slight decrease in milk eicosapentaenoic acid and docosahexaenoic acid from cattle fed 2% fat from extruded soybeans but increased concentrations of both fatty acids when the diets containing fish oil were fed. Other research with feeding marine oils has shown increases in n-3 fatty acids, especially docosahexaenoic acid (Cant et al., 1997; Franklin et al., 1999). Recent research (AbuGhazaleh and Jenkins, 2004) confirmed that docosahexaenoic acid is likely the component in fish oil that promotes VA accumulation in the presence of linoleic acid.

CONCLUSIONS

Feeding low levels of fish oil in combination with a source of linoleic acid, such as extruded soybeans,

increased milk CLA and VA without decreasing feed intake and milk production. Milk CLA and VA were increased similarly for cattle fed the lowest amounts of fish oil tested (0.33% of diet DM) compared with when they were fed greater amounts of fish oil. Data from this study support the hypothesis that only a small amount of fish oil, possibly as little as 0.33% of dietary DM, is necessary to increase the concentrations of VA and CLA in milk.

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