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Effect of Dietary Fish oil and Soybean Oil on Milk Production and Composition of Holstein Cows in Early Lactation

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ABSTRACT : Milk was collected from 20 primiparous Holstein cows that were distributed into four groups and arranged in a completely randomized design with a 35-day period to determine the effect of feeding fish oil, soybean oil, or their combination on milk production and composition. Experimental diets consisted of: 1) control diet; 2) a diet with 3% (DM basis) added fat from menhaden fish oil; 3) a diet with 3% added fat from soybean oil; and 4) a diet with 1.5% added fat from fish oil and 1.5% fat from soybean oil. The dry matter intake (18.5, 18.9, 18.3, and 18.6 kg/d, for control, fish oil, soybean oil and combination diets, respectively) and milk production (30.31, 32.15, 31.19, and 31.59 kg/d, respectively) were higher for cows that consumed the 3% fish oil-containing diet. Milk from cows fed control, fish oil, soybean oil and fish oil with soybean oil diets contained 3.45, 2.72, 2.96, and 2.87% fat, respectively. Concentration of total omega-3 fatty acids (0.87, 1.28, 0.96, and 1.18 g/100 g of fatty acids, respectively) in milk fat were higher for cows that consumed either fish oil-containing diet, especially the 3% fish oil diet. The n-6:n-3 ratio (4.57, 2.62, 6.17, and 4.08, respectively) in milk fat was lower for the fish oil diet. These results showed that fish oil modified fatty acid profile of milk fat and increased the proportion of beneficial fatty acids for human health. (**Key Words :** Milk, Milk Fat, Omega-3 Fatty Acid, Omega-6 Fatty Acid, Dairy Cow)

INTRODUCTION

Positive human health effects caused by food components have been recently recognized. Fat is the major energy component in milk and accounts for many of the physical properties, manufacturing characteristics, and organoleptic qualities of milk and milk products (Bauman and Griinari, 2003). Milk fat, due to its relatively high proportion of myrisitic (C14:0) and palmitic acids (C16:0), has been associated with human cardiovascular health problems (Noakes et al., 1996). Increasing dietary concentrations of unsaturated fatty acids decreases milk C14:0 and C16:0 levels (Palmquist et al., 1993). Increasing specific unsaturated fatty acids such as conjugated linoleic acid (CLA), linoleic acid (C18:2), linolenic acid (C18:3), ecosapentaenoic acid (C20:5), and docosahexaenoic acid (C22:6) in milk would increase consumer interest and acceptance of milk due to health benefits associated with these fatty acids (Ramaswamy et al., 2001). The fatty acid content of the lactation diet of the dairy cow has an effect on the type and the proportion of the fatty acids that make up milk fat (Grummer, 1991). Conjugated linoleic acid is derived in the rumen from incomplete biohydrogenation of C18:2 (Harfoot and Hazelwood, 1988). The oil of some seeds inculding soybean, rapeseed and … are good sources of unsaturated fatty acids. Soybean seed contains approximately 50% linoleic acid (NRC, 2001; Kahrizi et al., 2007). Marine oils derived from fish oil or algae are a rich source of omega-3 (EPA, DHA) fatty acids. The objective of this research was to determine the effect of feeding fish oil, soybean oil, or their combination on milk fatty acid profiles, especially omega-3, omega-6 and omega-3/omega-6 ratio.

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MATERIALS AND METHODS

Experimental design and data collection

Twenty primiparous Holstein cows (47±11 days in milk) were divided into four groups and arranged in a completely randomized design with 35 days period. The first 2 wk of

Table 1. Ingredient and nutrient content of control (C), fish oil (FO), soybean oil (SO), and fish oil with soybean oil (FO+SO) diets

Item	Diet[1]			
	C	FO	SO	FO+SO
Ingredient				
Alfalfa hay	20.76	20.79	20.79	20.79
Corn silage	20.76	20.79	20.79	20.79
Ground barley	19.03	9.33	9.33	9.33
Ground corn	9.31	9.33	9.33	9.33
Soybean meal	8.38	9.33	9.33	9.33
Canola meal	5.59	5.60	5.60	5.60
Cottonseed meal	6.99	9.33	9.33	9.33
Beet pulp	6.99	10.36	10.36	10.36
Fish oil	0.00	3.00	0.00	1.50
Soybean oil	0.00	0.00	3.00	1.50
Sodium bicarbonate	0.40	0.40	0.40	0.40
Mineral and vitamin[2]	0.58	0.58	0.58	0.58
Calcium carbonate	0.93	0.93	0.93	0.93
Salt	0.23	0.23	0.23	0.23
Chemical composition				
CP	17.0	17.1	17.3	17.2
Ether extract	2.5	5.4	5.1	5.2
NDF	33.2	33.5	33.9	33.7
NFC[3]	40.7	37.0	37.3	37.1
Ash	6.6	7.0	6.4	6.8
Ca	0.81	0.85	0.85	0.85
P	0.38	0.37	0.37	0.37
Mg	0.22	0.23	0.23	0.23
NE_L (Mcal/kg)[4]	1.57	1.66	1.62	1.64

[1] Control = No supplemental fat; FO = 3% fish oil; SO = 3% soybean oil; FO+SO = 1.5% fish oil+1.5% soybean oil.
[2] Mineral and vitamin mix contained 0.8% Ca, 0.7% P, 0.8% K, 0.4% Mg, 0.3% S, 1.4 mg/kg I, 100 mg/kg Mn, 100 mg/kg Zn, 0.3 mg/kg Co, 0.5 mg/kg Se, 99,450 IU/kg of vitamin A, 13,260 IU/kg of vitamin D, and 497 IU/kg of vitamin E.
[3] NFC = Nonfiber carbohydrates, NFC = 100-(CP+NDF+EE+ash).
[4] Calculated according to NRC (1989) values.

period was used to allow cows to adjust to the experimental diets and wk 3, 4, and 5 for data collection. Dietary treatments consisted of either 0% supplemental fat (control diet; C), the diet with 3% added menhaden fish oil (FO), the diet with 3% added fat from soybean oil (SO), and the diet with 1.5% added fish oil and 1.5% added from soybean oil (FO+SO). Cows were housed in tie stall barn and fed a total mixed ration (TMR) four times daily at approximately 0800, 1000, 1400, and 1600 h in amounts to ensure 5% orts. Total mixed ration (Tabel 1) were formulated to contain more than adequate amounts of major nutrients (NRC, 2001). The TMR offered and orts were measured on a daily basis during the sampling period. Samples were stored at -20°C until analyzed. Feed and ort samples were dried in a forced-air oven at 60°C for 48 h. The dry weights were used to determine feed intake. Subsamples of feed and ort were dried at 105°C for 24 h to correct to 100% DM. Feed samples were ground through a 1-mm screen. The TMR samples were composited by week and analyzed for DM, CP, EE, ADF, NDF, Ca, Phosphorus, and Mg (AOAC, 2000). Cows were milked three times daily at 0830, 1600, and 2400 h, with individual milk weights recorded at each milking. Duplicate subsamples of milk were collected at the regular milking over a 48-h period (six consecutive milkings) in the last week of experiment. One set of samples was stored at 4°C until analyzed for fat, protein, lactose, SNF, and total solids (Milk-O-Scan 133B Foss Electric Denmark). The second set of samples was frozen and kept at -20°C until analyzed for fatty acids. Fatty acids analysis of fish oil, soybean oil, and TMR samples were carried out using the preparation method of Sukhija and palmquist (1988). The internal standard used was 22:1 (erucic acid). Milk fatty acids were analysed according to a modification of this procedure. Milk (20 ml) was centerifuged at 8,000×g for 30 min to form a solid milk fat layer on top of the milk, and 100 mg of milk fat was used for analysis. Two mililiters of hexane were used as a solvent. Methylation occurred by heating samples for 1.5 h at 50°C. After removal of the solvent layer, 1.0 ml of hexane was added to the original culture tube, and samples were again mixed and centrifuged, with the solvent layer being removed and composited with the first solvent layer. Approximately 0.5 g of anhydrous sodium sulfate was added to the composited sample, and the sample was vortexed again and let stand for 0.5 h before the final centrifugation (Qui et al., 2004). The GC was equipped with a 30-m capillary column for analysis of all TMR, oil and milk fatty acids. The injector and detector ports were set at 280°C and 300°C, respectively. The column was held at 160°C for 5 min and then increased at 2°C/min to 180°C and held for 5 min, then increased at 2°C/min to 190°C and held for 5 min.

Statistical analysis

Production variables were reduced to means for each treatment for each cow before statistical analyses. Data were analyzed by least squares ANOVA using the general linear models (GLM) procedure of SAS (1999). The model was:

$$Y_{ij} = \mu+T_i+e_{ij}$$

Where μ = overall population mean, T_i = effect of treatment, and e_{ij} = residual error term. The residual was used as the error term to test for main effects of treatment The Duncan's multiple range test was used for comparing means. Significance was declared at p<0.05.

RESULTS AND DISCUSSION

The chemical composition of the four diets is shown in Table 1. Total mixed diets were relatively similar in NDF.

Table 2. Fatty acid composition of total mixed ration, fish oil, and soybean oil

Fatty acid[2]	TMR[1]				Fish oil	Soybean oil
	Control	FO	SO	FO+SO		
	g/100 g of fatty acids					
C14:0	0.62	2.76	0.92	1.44	8.23	0.11
C16:0	27.48	30.36	25.63	29.04	16.65	11.83
C16:1	1.13	2.68	1.40	1.89	9.56	0.18
C18:0	3.62	4.06	4.64	4.38	3.68	3.75
C18:1c9	20.72	15.66	21.23	18.35	12.96	22.10
C18:2c9,c12	34.44	25.56	35.97	30.50	1.38	53.20
C18:3 (n-3)	5.82	6.41	8.03	7.50	2.93	6.30
C20:0	0.68	1.02	0.72	0.82	0.46	0.32
C20:5 (n-3)	0.00	2.20	0.00	1.63	11.50	0.00
C22:6 (n-3)	0.00	1.76	0.00	0.91	10.30	0.00
Others	5.49	7.53	1.46	3.54	22.35	2.21
Unsaturated	62.26	54.27	66.88	60.78	48.63	81.78
Saturated	32.40	38.20	39.91	35.68	29.02	16.01
n-6/n-3 ratio	5.77	2.38	4.34	3.04	0.06	8.44

[1] Control = No supplemental fat; FO = 3% fish oil; SO = Soybean oil; FO+SO = 1.5% fish oil+1.5% soybean oil.
[2] Expressed as number of carbons:number of double bonds.

The control diet contained 2.5% EE (DM basis), and the EE content of the other diets was 5.4, 5.1, and 5.2% as a result of the addition of soybean oil and fish oil to the diet. Consequently, the diets containing supplemental fat had a higher NE_L concentration (1.62 to 1.66 vs. 1.57 Mcal/kg for control diet). Fatty acid compositions of TMR, fish oil, and soybean oil are presented in Table 2. The lipid of soybean oil contained the high concentration of linoleic acid (53.2 g/100 g fatty acid). Fish oil was characterized by its content of long chain (>C20) fatty acids, being particularly rich in C20:5 n-3 (eicosapentaenoic acid,EPA) and C22:6 n-3 (docosahexaenoic acid, DHA; 11.5 and 10.3 g/100 g of fatty acid, respectively).

DMI, milk yield, and milk composition

DMI was similar among all treatments (p>0.05). The same results were reported by Fan et al. (2004). In contrast, fish oil has shown to have a negative effect on DMI (AbuGhazaleh et al., 2002; Whitlock et al., 2002). In these experiments, heat-treated soybean was used as oil source. The addition of polyunsaturated oils in free form tends to decrease DMI, whereas supplementation of oil through seeds maintains or increases DMI (Allen, 2000).

The oil in seeds may have been released slowly during ruminal digestion and had low negative effects on fiber digestibility, passage rate and DMI. Milk production did not differ significantlly (p = 0.94) between cows fed supplemental fat and the control diet averaging 31.31±0.96 kg/d (Table 3). This results is in agreement with the results of previous researchs in which no effect on production was observed when fish oil and soybean oil were added to the diet (AbuGhazaleh et al., 2002; Whitlock et al., 2002). Milk production appeared to be higher when cows were fed fat than when they were fed the control diet. Cows fed fish oil (FO) produced more milk (32.15 kg/d) than cows fed soybean oil (SO) (31.19 kg/d). In contrast to milk production, energy-corrected milk, FCM, and SCM of cows fed diets containing supplemental fat were lower than when cows were fed control diet. The lowered milk fat concentration led to overall lower FCM, ECM, and SCM yields for the three diets containing supplemental fat, which would agree with Whitlock et al. (2002). Milk fat percentages and yield were lowered when cows were fed diets with supplemental fat (p<0.05). Cows fed the soybean oil diet had a higher fat concentration and yield than cows fed the fish oil diet. This decrease in milk fat concentration and yield has been reported before (Ramaswamy et al., 2001; Whitlock et al., 2002). Abughazaleh et al. (2002) did not show a decrease in milk fat percentage and yield of cows were consuming fish oil. In present study, the decrease in milk fat percentage was associated with a specific increase in trans-10, cis-12 CLA. Loor and Herbein (2003) observed the reduction of milk fat percentage by trans-10, cis-12 CLA. Milk protein concentration was lowered (p<0.05) when cows were fed diets with supplemental fat, but protein yield was similar among all treatments. Our results would agree with Chilliard and Doreau (1997). In contrast, Ramaswamy et al. (2001) and Whitlock et al. (2002) did not observe a decrease in protein concentration with fish oil and soybean oil supplementations. Dietary fat has been shown to adversely affect microbial fermentation and microbial protein yield in the rumen, thereby decreasing the supply of protein available for utilization by the cow (Dhiman et al., 2000). Although not investigated in the present study, dietary fat decreased amino acid uptake by the small intestine, decreased mammary blood flow, and insulin resistance (Cant et al., 1993), each of which could potentially

Table 3. Milk yield, milk composition and dry matter intake from cows fed control (C), fish oil (FO), soybean oil (SO), and fish oil with soybean oil (FO+SO) diets

Item	Diet[1]			FO+SO	SE
	C	FO	SO		
DIM (kg/d)	18.5	18.9	18.3	18.6	0.60
Milk (kg/d)	30.31	32.15	31.19	31.59	0.96
4% FCM (kg/d)[2]	27.87	25.91	26.27	26.29	1.16
ECM (kg/d)[3]	30.21	27.75	28.16	28.37	1.20
SCM (kg/d)[4]	26.77	24.28	24.92	25.42	1.23
Fat					
%	3.45[a]	2.72[b]	2.96[ab]	2.87[ab]	0.15
kg/d	1.05	0.87	0.92	0.91	0.06
Protein					
%	3.07[a]	2.60[b]	2.68[ab]	2.77[ab]	0.06
kg/d	0.93	0.83	0.84	0.87	0.04
Lactose					
%	4.54	4.49	4.62	4.74	0.13
kg/d	1.37	1.44	1.45	1.49	0.07
Total solid					
%	11.57	10.32	10.61	10.89	0.27
kg/d	3.51	3.31	3.31	3.44	0.15
SNF[5]					
%	8.11	7.60	7.80	8.01	0.18
kg/d	2.46	2.44	2.43	2.53	0.11
MUN (mg/dl)[6]	20.14	17.73	20.62	19.06	0.80

[a, b] Means in the same row with different letters differ (p<0.05).

[1] Control = No supplemental fat; FO = 3% fish oil; SO = 3% soybean oil; FO+SO = 1.5% fish oil+1.5% soybean oil.

[2] Fat corrected milk.

[3] ECM = (0.327×milk production (kg/d))+(12.95×fat yield (kg/d))+(7.2×protein yield (kg/d).

[4] SCM = (12.3×fat yield (kg/d))+(6.56×SNF yield (kg/d))-(0.0752×milk production (kg/d)).

[5] Solid not fat. [6] Milk urea nitrogen.

decreased uptake of amono acids by the mammary gland. There was no significant difference (p>0.05) in lactose, total solid, SNF, and milk urea nitrogen (MUN) concentrations and yields in the milks. The lactose and SNF percentages were not affected by dietary fat in other study (Fan et al., 2004).

Milk fatty acid composition

Fatty acid composition of raw milks was altered when FO, FO+SO, or SO was incorporated in the diets (Table 4). There were no treatment effects on milk concentrations of short-chain fatty acid, indicating a similar extent of *de novo* synthesis of these fatty acids among treatments. Similar result was reported previously (Qiu et al., 2004), but in other researches, fish oil or soybean oil were reduced the proportion of short-chain fatty acids in milk fat (Ramaswamy et al., 2001; AbuGhazaleh et al., 2002; Whitlock et al., 2002). The concentration of medium-chain fatty acid decreased (p<0.05) when FO+SO or SO was incorporated in the diets. The proportion of C14:0 was lower (p<0.05) in milk from cows fed the fat containing diets, but the concentration of C16:0 was lower in milk from cows fed FO+SO or SO (specially in SO containing diet). This apparent reduction in *de novo* synthesis of fatty acids (≤C16:0) in the mammary gland has been reported with diets that increase the supply of long-chain fatty acid (Grummer, 1991). The concentration of long-chain fatty acid was higher (p<0.05) in SO and FO+SO milks compared with C and FO milks (Table 3). In this study, the FO milk had a higher concentration of long-chain fatty acids compared with C milk. Similar results were reported in previous studies (Ramaswamy et al., 2001; Abughazaleh et al., 2002; Whitlock et al., 2002). The C18:0 fatty acid content was reduced (p<0.05) in the FO milk and was highest (p<0.05) in the SO milk. Feeding FO, SO, or their combination increased the proportion of unsaturated FA and decreased saturated FA in milk fat. Similar results were observed in previous studies (Ramaswamy et al., 2001; AbuGhazaleh et al., 2002; Whitlock et al., 2002). In terms of human health, these alterations may represent an improvement in the fatty acid profile of milk because medium-chain fatty acid and saturated fatty acid have been reported to constitute the hypercholestrolemic portion of milk fat (Ney, 1991). In this study, one isomer of CLA (trans-10, cis-12 C18:2) was identified. The concentration of trans-10, cis-12 C18:2 in milk produced from the FO diet was higher than in milk produced from the SO diet. Baer et al. (2001) also reported similar increase in CLA (trans-10, cis-12 C18:2) concentration of milk from cows fed FO. The concurrent decreases in milk fat concentration agreed with

Table 4. Fatty acid composition of raw milk from cows fed control (C), fish oil (FO), fish oil with soybean oil (FO+SO), or soybean oil (SO) diets

Fatty acid[2]	Diet[1]				SE
	C	FO	SO	FO+SO	
	g/100 g fatty acids				
C10:0	3.95	3.09	3.23	3.31	0.20
C12:0	3.24	3.39	3.32	3.38	0.18
C14:0	13.82[a]	9.88[b]	9.84[b]	9.90[b]	0.53
C15:0	0.92	0.75	0.68	0.74	0.08
C16:0	30.92[a]	30.91[a]	25.80[b]	27.07[ab]	0.74
C16:1	1.19	2.16	1.83	1.88	0.18
C17:0	0.52	0.29	0.64	0.61	0.07
C18:0	10.07[b]	10.90[a]	14.44[a]	12.16[ab]	0.38
C18:1 (c9)	16.82	17.45	20.63	20.72	0.99
C18:1 (t9)	0.19	0.18	0.23	0.57	0.18
C18:2 (c9,c12)	4.00	3.36	5.92	4.81	0.63
18:2 (t10,c12)	0.08	0.23	0.14	0.09	0.04
18:3 (n-3)	0.77	0.82	0.79	0.78	0.02
20:0	0.39[ab]	1.02[ab]	0.19[b]	1.24[a]	0.14
20:3	0.21	0.30	0.12	0.18	0.04
20:5 (n-3)(EPA)	0.07[c]	0.24[a]	0.09[c]	0.16[b]	0.01
22:6 (n-3)(DHA)	0.04[c]	0.22[a]	0.08[bc]	0.15[ab]	0.02
Others[3]	12.81[b]	14.81[a]	12.03[b]	12.25[b]	0.38
Short[4]	7.19	6.48	6.55	6.69	0.38
Medium[5]	47.37[a]	43.99[ab]	38.79[b]	40.20[b]	0.91
Long[6]	32.63[c]	34.72[bc]	42.63[a]	40.86[ab]	1.90
Unsaturated	23.26	24.96	29.83	29.34	1.20
Saturated	63.83	60.23	58.14	58.41	1.30
Total n-3 FA[7]	0.87[b]	1.28[a]	0.96[b]	1.18[ab]	0.04
n-6/n-3 ratio	4.57[a]	2.62[b]	6.17[a]	4.08[ab]	0.89

[a, b, c] Means within a row without common superscripts differ ($p<0.05$).
[1] Control = No supplemental fat; FO = 3% fish oil; SO = 3% soybean oil; FO+SO = 1.5% fish oil+1.5% soybean oil.
[2] Expressed as number of carbons:number of double bonds. [3] Unidentifiable peaks. [4] Short-chain fatty acids (C10:0-C12:0).
[5] Medium-chain fatty acids (C14:0-C17:0). [6] Long-chain fatty acids (≥C18:0). [7] Total n-3 fatty acids: C18:3 (n-3), C20:5 (n-3), C22:6 (n-3).

associations by Baumgard et al. (2000) that trans-10, cis-12 C18:2 isomer is partially responsible for the decrease in milk fat concentration and yield observed when supplemental fat is fed. The concentration of total n-3 fatty acids increased ($p<0.05$) in the FO milk compared with the other milks (Table 3). Omega-3 fatty acids consist of three isomers, namely α-C18:3 (α-linolenic acid), C20:5 (eicosapentaenoic acid), and C22:6 (docosahexaenoic acid), and have many health benefits. The α-C18:3 did not increase ($p>0.05$) in any of the milks; however, the C20:5 and C22:6 increased ($p<0.05$) in FO and FO+SO milks. Similar results were observed in previous researches (Ramaswamy et al., 2001; AbuGhazaleh et al., 2002; Whitlock et al., 2002). The magnitude of increased concentration of EPA and DHA in milk fat was lower than the concentration of these fatty acids in the diet. This low transfer efficiency has been attributed to both their biohydrogenation in the rumen and their association with plasma lipoproteins which are not desired substrates for mammary lipoprotein lipase (Mansbridge and Blake, 1997). Another possiblity for this low transfer efficiency is that these fatty acids are preferentially partitioned towards other tissues in the body (Ashes et al., 1992).

IMPLICATIONS

The addition of fish oil, soybean oil, or their combination to diets of dairy cows influenced the composition of milk, especially the milk fat composition. Milk produced from the fish oil diet had less fat and protein than that produced from the other diets. Milk fatty acid composition was altered due to the dietary fish oil, soybean oil, or their combination. The concentration of n-3 fatty acids increaded in fish oil milk compared with control, soybean oil, and fish oil with soybean oil milks. The concentration of medium-chain fatty acids decreased in soybean oil and fish oil with soybean oil milks compared with control and fish oil milks. Long-chain fatty acid concentrations were higher in soybean oil and fish oil with soybean oil milks compared with control and fish oil milks. Unsaturated fatty acid concentrations increased in soybean oil and fish oil with soybean oil milks compared with fish oil and control milks. The incorporation of dietary unsaturated fats resulted in milk with improved nutritional value and possible human health benefits.

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