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Effect of Dietary Omega-3 and Omega-6 Fatty Acids Sources on Milk Production and Composition of Holstein Cows in Early Lactation

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Abstract: 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. Milk was collected from 20 primiparous Holstein cows that were distributed into four groups and arranged in a completely randomized design with 35 days 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. Dry matter intake (18.47, 18.87, 18.33 and 18.63 kg day^{-1}, for control, fish oil, soybean oil and combination diets, respectively) and milk production (30.31, 32.15, 31.19 and 31.59 kg day^{-1}) were higher for cows that consumed 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) 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) in milk fat was lower for fish oil diet. These results showed that fish oil modifies fatty acids profile of milk fat and increased the proportion of beneficial fatty acids for human health.

Key words: Milk, Omega-3 fatty acid, Omega-6 fatty acid

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 myristic (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 (Bauman *et al*., 1998; Ramaswamy *et al*., 2001; McGuire *et al*., 1997). 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). Soybean oil is a good source of unsaturated fatty acids and contains approximately 50% linoleic acid (NRC, 2001). Marine oils derived from fish oil or algae are a rich source of omega-3 (EPA, DHA) fatty acids (Kitessa *et al*., 2001). 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.

MATERIALS AND METHODS

Experimental design and data collection: Twenty primiparous Holstein cows (47±11 DIM) were divided into four groups and arranged in a completely randomized design with 35 days period. The first 2 week of 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

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at approximately 0800, 1000, 1400 and 1600 h in amounts to ensure 5% orts. Total mixed ration (Table 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 milk, fish oil, soybean oil and TMR samples were carried out using the preparation method of Sukhija and Palmquist (1988).

Table 1: Ingredient and nutrient content of Control (C), Fish Oil (FO), Soybean Oil (SO) and fish oil with soybean oil (FO+SO) diets

	Diet[1]			
	C	FO	SO	FO+SO
Item	% of DM			
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^{-1})[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^{-1} I, 100 mg kg^{-1} Mn, 100 mg kg^{-1} Zn, 0.3 mg kg^{-1} Co, 0.5 mg kg^{-1} Se, 99, 450 IU kg^{-1} of vitamin A, 13,260 IU kg^{-1} of vitamin D and 497 IU kg^{-1} of vitamin E, [3]NFC = Nonfiber carbohydrates, NFC = 100-(CP + NDF + EE + ash). [4]Calculated according to NRC (1989) values

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

Results regarding chemical composition of the four diets are shown in Table 1. Total mixed diets were relatively similar in NDF. 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^{-1} 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).

Milk yield, milk composition and DMI: Milk production did not differ significantly (p = 0.94) between cows fed supplemental fat and the control diet averaging 31.31±0.96 kg day^{-1} (Table 3).

This results is in agreement with the results of previous researches in which no effect on production was observed when fish oil and soybean oil were added to the diet (Cant *et al*., 1997; Whitlock *et al*., 2002; Abughazaleh *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 day^{-1}) than cows fed Soybean Oil

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

	TMR[1]					
	Control	FO	SO	FO + SO		
Fatty acid[2]	(g/100 g of fatty acids)				Fish oil	Soybean oil
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	5.769	2.382	4.344	3.038	0.056	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

Table 3: Milk yield, milk composition and DMI from cows fed control (C), fish oil (FO), soybean oil (SO) and fish oil with soybean oil (FO+SO) diets

	Diet[1]				
Item	C	FO	SO	FO+SO	SE
Milk (kg day^{-1})	30.31	32.15	31.19	31.59	0.96
4% FCM (kg day^{-1})	27.87	25.91	26.27	26.29	1.16
ECM (kg day^{-1})[2]	30.21	27.75	28.16	28.37	1.20
SCM (kg day^{-1})[3]	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 day^{-1}	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 day^{-1}	0.93	0.83	0.84	0.87	0.04
Lactose (%)	4.54	4.49	4.62	4.74	0.13
kg day^{-1}	1.37	1.44	1.45	1.49	0.07
Total solid (%)	11.57	10.32	10.61	10.89	0.27
kg day^{-1}	3.51	3.31	3.31	3.44	0.15
SNF (%)	8.11	7.60	7.80	8.01	0.18
kg day^{-1}	2.46	2.44	2.43	2.53	0.11
MUN (mg dL^{-1})	20.14	17.73	20.62	19.06	0.80
DMI (kg day^{-1})	18.47	18.87	18.33	18.63	0.60

[ab]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]ECM = (0.327 *milk production (kg day^{-1}))+(12.95 * fat yield (kg day^{-1}))+(7.2 * protein yield (kg day^{-1}). [3]SCM = (12.3 * fat yield (kg day^{-1}))+(6.56 * SNF yield (kg day^{-1}))-0.0752 * milk production (kg day^{-1}))

(SO) (31.19 kg day^{-1}). 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 (p<0.05) when cows were fed diets with supplemental fat (2.85 vs. 3.45% and 0.9 vs. 1.05 kg day^{-1}, for cows fed the fat-supplemented and control diets, respectively). Cows fed the soybean oil diet had a higher fat concentration (2.96%) and yield (0.92 kg day^{-1}) than cows fed the fish oil diet (2.72% and 0.87 kg day^{-1}). This decrease in milk fat concentration and yield has been reported before (Cant *et al*., 1997; Donovan *et al*., 2000; Whitlock *et al*., 2002; Ramaswamy *et al*., 2001). Abughazaleh *et al*. (2002) did not show a decrease in milk fat percentage and yield of cows were consuming fish oil. Milk protein concentration was lowered (p<0.05) when cows were fed diets with supplemental fat (2.68 vs. 3.07%, for cows fed the fat supplemented and control diets, respectively), but protein yield was similar among all treatments. Our results would agree with Chilliard and Doreau (1997) and Cant *et al*. (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. There was no difference (p>0.05) in lactose, total solid, SNF and Milk Urea Nitrogen (MUN) concentrations and yields in the milks. Dry Matter Intake (DMI) was similar among all treatments (p>0.05). In contrast, fish oil has shown to have a negative effect on DMI (Donovan *et al*., 2000; Abughazaleh *et al*., 2002; Whitlock *et al*., 2002).

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 (Qui *et al*., 2004), but in other researches, fish oil or soybean oil were reduced the proportion of short-chain fatty acids in milk fat

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

	Diet[1]				
	C	FO	SO	FO+SO	
Fatty acid[2]	g/100 g fatty acids				SE
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)

(Donovan *et al*., 2000; Abughazaleh *et al*., 2002; Whitlock *et al*., 2002; Ramaswamy *et al*., 2001; Cant *et al*., 1997). 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 (Abughazaleh *et al*., 2002; Ramaswamy *et al*., 2001; Whitlock *et al*., 2002; Cant *et al*., 1997). 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 (Donovan *et al*., 2000; Abughazaleh *et al*., 2002; Whitlock *et al*., 2002; Ramaswamy *et al*., 2001). 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 hypercholesterolemic 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 associations by Baumgard *et al*. (2000) that trans-0, cis-2 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 and C22:6 (docosahexaenoic acid) and have many health benefits including the ability to decrease cardiovascular disease (Hirai *et al*., 1980) and rheumatoid arthritis (Kremer *et al*., 1987). 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; Whitlock *et al*., 2002; Abughazaleh *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 possibility for this low transfer efficiency is that these fatty acids are preferentially partitioned towards other tissues in the body (Ashes *et al*., 1992).

CONCLUSIONS

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 increased 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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