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Fatty Acid Profile of Bovine Milk Naturally Enhanced with Docosahexaenoic Acid

MAMUN M. OR-RASHID, NICHOLAS E. ODONGO, TOM C. WRIGHT, AND BRIAN W. MCBRIDE*

Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Recent studies have shown that the fatty acid profile of dietary lipid has the potential for improving the health of consumers. The present study was conducted to determine the fatty acid composition of commercial milks, namely, Dairy-Oh! Homo-Milk (DOHM), which is naturally enhanced with docosahexaenoic acid (DHA), or regular Homo-Milk (HM). The milk was collected from local supermarkets. The most abundant saturated fatty acids in the milk were butyric (C4:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids. Among unsaturated fatty acids, oleic acid (*cis*-9-C18:1) was also considerably high (502.7 mg/100 mL of milk). The concentration of total *trans*-18:1 was higher ($P < 0.05$) in DOHM than in HM (134.7 vs 107.0 mg/100 mL of milk, respectively), whereas total *cis*-18:1 was higher ($P < 0.05$) in HM than in DOHM (566.4 vs 508.4 mg/100 mL of milk, respectively). The concentration of DHA was 24.0 times higher ($P < 0.05$) in DOHM than in HM. DOHM contained 2.8 times higher ($P < 0.05$) eicosapentaenoic acid (EPA) compared to HM. Milk fat from DOHM contained a greater concentration of *cis*-9,*trans*-11 conjugated linoleic acid (CLA, 16.4 vs 11.6 mg/100 mL of milk, DOHM vs HM, respectively). The total omega-3 polyunsaturated fatty acids content was 2.23 times greater ($P < 0.05$) in DOHM compared with HM, due to an increase in C18:3n-3, EPA, and DHA. The result of the milk fatty acid analyses indicates that milk fat from DOHM had increased contents of EPA, DHA, and *cis*-9,*trans*-11 CLA, which could have a more favorable impact on diet composition and healthfulness.

KEYWORDS: Dairy cow milk; *trans*-18:1; eicosapentaenoic acid; docosahexaenoic acid; conjugated linoleic acid; GLC

INTRODUCTION

Dietary milk fat from dairy cows contains relatively high concentrations of saturated fatty acids, which have been implicated as a factor for a variety of human diseases including heart disease (*1*). However, studies have shown significant alleviation of various risk factors for cardiovascular disease when fish and fish oil containing long-chain polyunsaturated fatty acids, especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are consumed (*2*). Experiments have shown that the enrichment of heart tissue in DHA provides an antiarrhythmic effect (*3*), which may account for the reduction in cardiac arrest and sudden cardiac death in those having a higher DHA status. Additionally, DHA is found in abundance in the brain and retina, where it is required at high levels for optimal mental functioning and visual acuity. Therefore, dietary DHA is regarded as an essential dietary nutrient for infants and children (*4*).

Recommendations have been made by health regulatory agencies to increase the intake of omega-3 polyunsaturated fatty acids (PUFA), such as EPA and DHA, in the human diet (*5*). However, these fatty acids (FA) are present in the diets of most Western countries in less than the optimal daily requirement, due to low consumption of fish products (*6*). Whole milk in the Western diet is one of the major sources of dietary fat and cholesterol intake (*7*). DHA and EPA are usually lacking in whole milk (*8*), because these PUFA cannot be synthesized significantly by ruminant tissues. However, their concentration in milk can be increased by feeding diets rich in these PUFA (*8*). Wright et al. (*9*) fed a custom-designed protein supplement containing fish meal as a source of DHA to dairy cows at different levels, and the FA (i.e., DHA) was increased from 0.26 to 0.72% of total FA. Fish oils (1.1% of total mixed ration as fed) have also been used to increase the DHA from 0.07% (control) to 0.51% of total FA in sheep's milk (*6*).

Recently, Australia's Ministry of Health recognized that the conversion of α-linolenic acid to very-long-chain n-3 PUFA (VLC_n-3_PUFA, >18C) was insufficient to meet physiological requirements (*10*). Accordingly, adequate intake recommendations were made that include a recommendation for adequate intake for VLC_n-3_PUFA, based on the combined intake of EPA plus docosapentaenoic acid (DPA) plus DHA (*10*).

* Author to whom correspondence should be addressed [telephone (519) 824-4120, ext. 53695; fax (519) 836-9873; e-mail bmcbride@uoguelph.ca.

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Recent studies have provided evidence that the fatty acid profile of dietary lipid plays an important role in human health. Several reports on the FA composition of bovine milk have been reported from experimental studies [see the review by Jensen (*11*)]. Although much has been done, research on the FA composition of market milk and dairy products we drink and eat in North America has been almost nonexistent (*11*) to date. The present study was conducted to determine the fatty acid composition of retail milks, naturally enhanced with DHA, using a novel feeding strategy, which was the first innovation of its kind in North America (*9*).

MATERIALS AND METHODS

Samples. Dairy-Oh! Homo-Milk [(DOHM), Neilson Dairy, ON, Canada], naturally enhanced with DHA, was sourced commercially from the local supermarkets of Guelph, ON, Canada. Normal milk [Homo-Milk (HM), Neilson Dairy] was also collected from the same supermarkets to compare with the FA profile of DOHM. Both types of milks were microfiltered and processed similarly in the factory. The results of analyses are given as the mean of 12 samples from each variety. Individual samples were collected once per month over a 12-month period (August 2007−July 2008). Precaution was taken to avoid the possibility of taking samples from the same batch during the collection period from the supermarkets. The samples were transported from the supermarkets to the laboratory and kept at −20 °C for subsequent analyses.

Chemical Analysis. The milk samples were analyzed for fat [method 920.39 (*12*)] at a commercial laboratory (Agri-Food Laboratories, Guelph, ON, Canada).

Analysis of Fatty Acid. Total milk fat was extracted according to the method by Bligh and Dyer (*13*) with minor modifications. A sample of 0.07 mL of milk plus 0.93 mL of water with 2.5 mL of methanol and 1.25 mL of chloroform was mixed into a 15-mL culture tube with a screw-cap Teflon lining. The content of the culture tube was kept for 60 min at room temperature with frequent vortexing. After 1 h, 1.25 mL of chloroform, 1.15 mL of water, and 0.1 mL of 3 M HCl were added, vortex-mixed, and centrifuged. The acid was added to ensure the pH of the extract was acidic. The chloroform layer (bottom phase) containing fat was removed using two Pasteur pipets, one inserted into another. The methanol/water phase was extracted with an additional 1.25 mL of chloroform, and the chloroform phases were combined, dried over anhydrous Na_2SO_4, filtered, and then transferred into a 4-mL vial. Chloroform was removed from the vial under a stream of N_2, and 3 drops of benzene was added and vortexed. The fat content in the vial was methylated as follows (*14*, *15*): 200 μL of $NaOCH_3$ (0.5 M solution in methanol, Sigma-Aldrich, St. Louis, MO) was added for methylation. The vials were kept at room temperature for 25 min. Then, 1 mL of 1 N methanolic sulfuric acid (2.8 mL of 96% sulfuric acid in 100 mL of methanol) was added. After vortexing, the vials were heated at 50 °C for 15 min. After cooling at −20 °C, 1.0 mL of water and 1.0 mL of hexane were added, vortexed, and centrifuged. The upper portion (i.e., hexane layer) containing fatty acid methyl esters (FAME) was transferred into another vial for GLC analysis.

The hexane-containing FAME were analyzed by GLC and subsequently identified as described by Cruz-Hernandez et al. (*14*) and Odongo et al. (*16*) with a different temperature program. Briefly, FAME analysis was performed using an Agilent 6890N GLC (Agilent Technologies, Palo Alto, CA) equipped with a split−splitless injector at 250 °C, a flame ionization detector at 250 °C, and a CP Sil 88 column (100 m × 0.25 mm, 0.2-μm film thickness; Varian Inc., Mississauga, ON, Canada). Hydrogen was used as carrier gas at a constant flow rate of 1 mL/min. The temperature of the GLC oven was set to 45 °C for 4 min, increased at 13 °C/min to 173 °C, held for 28 min, increased at the rate of 4 °C/min to a final temperature of 215 °C, and held for 43 min. Agilent Technologies Chemstation software (rev. B.01.01) was used for data analysis. A 1-μL sample was injected at splitless mode. Peaks were routinely identified by comparison of retention times with fatty acid methyl ester standards (GLC 463, UC-59-M, C21:0, C23:0, and C26:0; NuCheck Prep Inc., Elysian, MN). In addition, some peaks of 18:1 and conjugated linoleic acid (CLA) isomers, for which standard FAME were not available, were identified by comparison to published data as described by Cruz-Hernandez et al. (*14*) and Odongo et al. (*16*).

Calculation and Statistical Analysis. Peak areas were used for calculating the concentration of FA. After correctiong of the peak areas with theoretical correction factors, as suggested by Torres et al. (*17*) and described by Christie (*18*), the weight percentages of FA (g/100 g of total fatty acids) were calculated for all of the samples. Then, individual FA in milk (mg/100 mL of milk) were calculated as described by Greenfield and Southgate (*19*).

The data were analyzed as a completely randomized design using the PROC MIXED procedure of SAS (v. 9.1; SAS Institute, Inc., Cary, NC) using the model $Y_j = \mu + \beta_j + \varepsilon_j$, where μ = overall mean, β = effect of treatment (j = HM or DOHM), and ε_j = random residual error. Effects were considered to be significant at a probability of $P < 0.05$.

RESULTS AND DISCUSSION

Generally, dairy cows (Holstein), producing HM in southwestern Ontario, are fed corn silage/alfalfa haylage based diets supplemented with mixed grains or corn, a soybean meal based commercial protein supplement, minerals, and vitamins [(on a dry matter basis) corn silage, 31.6%; mixed haylage, 21.2%; high-moisture corn, 18.8%; mixed hay, 4.2%; soybean hulls, 3.3%; and a commercial protein supplement, 20.9%; crude protein, 17.6%; acid detergent fiber, 23.2%; neutral detergent fiber, 36.2%; and crude fat, 3.3%] (*20*). The FA contents of the protein supplement are as follows: C18:2n-6, 43.4 g/100 g of FA; C18:3n-3, 3.6 g/100 g of FA; C20:5n-3, 0.26 g/100 g of FA; C22:6n-3, 0.34 g/100 g of FA; total n-6 PUFA, 43.5 g/100 g of FA; and total n-3 PUFA, 4.42 g/100 g of FA (*20*). In contrast, dairy cows (Holstein) producing DOHM in southwestern Ontario are fed corn silage/alfalfa haylage based diets with a fish meal/feather meal based protein supplement, minerals, and vitamins [(on a dry matter basis) straw, 5.8%; corn silage, 32.3%; high-moisture corn, 39.2%; minerals, 2.8%; and a protein supplement, 19.9%; crude protein, 18.4%; acid detergent fiber, 19.8%; neutral detergent fiber, 32.5%; and crude fat, 3.9%] (*9*). The ingredient composition of the protein supplement is, on a dry matter basis, 23.8% wheat, 15.0% fish meal, and 61.2% feather meal (main FA contents, C18:2n-6, 14.5 g/100 g of FA; C18:3n-3, 5.4 g/100 g of FA; C20:5n-3, 2.3 g/100 g of FA; C22:6n-3, 3.9 g/100 g of FA; total n-6 PUFA, 15.6 g/100 g of FA; and total n-3 PUFA, 12.2 g/100 g of FA) (*9*).

Both types of milks had almost similar fat contents (3.25% for HM and 3.28% for DOHM). Results of fatty acid composition of the commercial milks are presented in **Tables 1−3**. For the saturated fatty acids (SFA), the most abundant in both of the milks were butyric acid (C4:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0). The myristic acid in DOHM was 18.0% higher ($P < 0.05$) compared to HM, whereas the difference in palmitic acid was insignificant. The DOHM had 26.2% lower ($P < 0.05$) stearic acid than HM. It is well-known that the stearic acid is the predominant end product of linoleic and linolenic acid biohydrogenation in rumen. The concentrations of some branched-chain SFA (e.g., *iso*-C13:0, *iso*-C15:0, *anteiso*-C15:0, and *anteiso*-C18:0) were greater ($P < 0.05$) in HM, but DOHM had greater ($P < 0.05$) concentration of *anteiso*-C13:0 and *iso*-C17:0 (**Table 1**). The terms *iso* and *anteiso* designate the position of the branch chain in the FA moiety. For *iso*-methyl FA, the position of the branch chain is located on the penultimate carbon atom, whereas the branch point is positioned on the carbon atom two from the end in *anteiso*-methyl-FA (*1*). These fatty acids are synthesized by chain elongation of branched-chain volatile

Table 1. Fatty Acid Composition[a] (Milligrams per 100 mL of Milk) of Two Commercial Milks

fatty acid	HM	DOHM	SEM
C4:0	124.32a	161.41b	4.14
C6:0	71.62	78.74	2.14
C7:0	1.14	1.34	0.07
C8:0	46.13	51.02	1.26
C9:0	1.48	1.58	0.05
C10:0	101.02a	120.32b	0.85
C11:0	2.35	2.37	0.02
C12:0	112.53a	130.11b	1.45
iso-C13:0	0.97a	0.86b	0.02
anteiso-C13:0	2.18a	2.67b	0.06
C13:0	6.18	6.63	0.06
iso-C14:0	4.09	3.60	0.17
C14:0	357.72a	422.03b	6.01
iso-C15:0	6.91a	5.88b	0.14
anteiso-C15:0	15.03a	12.31b	0.30
cis-9-C14:1	19.62a	27.51b	0.19
C15:0	38.51	37.53	1.17
iso-C16:0	10.42	10.71	0.46
C16:0	1032.51	1004.43	9.45
trans-9-C16:1	11.23	11.41	0.29
iso-C17:0	0.63a	1.72b	0.08
anteiso-C17:0	4.04	4.09	0.18
cis-9-C16:1	47.42	54.21	1.22
C17:0	24.11	24.32	0.72
anteiso-C18:0	6.21a	5.47b	0.18
C18:0	409.51a	302.42b	4.33
cis-9-C18:1	537.54a	474.03b	7.32
cis-11-C18:1	16.03	19.01	0.94
cis-12-C18:1	10.52	11.81	0.64
cis-13-C18:1	2.35a	3.61b	0.28
C19:0	0.92a	1.19b	0.06
trans-9,*cis*-12-C18:2	1.02a	1.49b	0.06
trans-11,*cis*-15-C18:2	1.87a	3.55b	0.11
C18:2n-6	56.62a	45.83b	0.87
C20:0	5.79a	10.41b	0.18
C18:3n-6	0.88a	0.49b	0.06
cis-9-C20:1	2.99a	7.78b	0.28
cis-11-C20:1	1.09a	7.07b	0.49
C18:3n-3	11.04a	17.51b	1.39
C20:2n-6	1.45	1.33	0.09
C22:0	2.20a	4.71b	0.10
C20:3n-6	2.97a	1.62b	0.10
cis-13-C22:1	0.00a	0.39b	0.04
C20:3n-3	0.59a	2.07b	0.07
C20:4n-6	4.27a	2.65b	0.27
C23:0	0.36a	0.55b	0.02
C20:4n-3	0.88a	2.22b	0.14
C22:2n-6	0.28	0.32	0.10
C20:5n-3	1.12a	3.12b	0.11
C24:0	1.52a	1.74b	0.05
cis-15−24:1	0.29a	0.65b	0.04
22:4n-6	0.91a	0.53b	0.08
C22:5n-3	2.35a	4.14b	0.05
C26:0	0.66	0.70	0.02
C22:6n-3	0.30a	7.14b	0.11
total SFA[b]	2390.81	2410.64	6.18
total SC_SFA (C4:0-C9:0)[c]	244.63a	294.02b	7.02
total MC_SFA (C10:0-C16:0)[d]	1690.31a	1759.34b	10.76
total LC_SFA (C17:0-C26:0)[e]	451.54a	351.43b	4.33
total MUFA[f]	756.11	752.02	2.36
total n-6 PUFA[g]	67.43a	52.82b	1.05
total n-3 PUFA[h]	16.24a	36.21b	1.71
n-6:n-3 PUFA	4.24a	1.54b	0.40
total *trans*-18:1	107.03a	134.71b	4.55
total *cis*-18:1	566.41a	508.42b	6.14
total VLC_n3_PUFA[i]	3.77a	14.43b	0.25

[a] Means are based on 12 milks per treatment. Means within a row with different letters differ ($P < 0.05$). [b] Total SFA: all saturated fatty acids (without any double bond, C4:0 to C26:0). [c] Total SC_SFA: all short-chain SFA (from C4:0 to C9:0). [d] Total MC_SFA: all medium-chain SFA (from C10:0 to C16:0). [e] Total LC_SFA: all long-chain SFA (from C17:0 to C26:0). [f] Total MUFA: all monounsaturated fatty acids with a single double bond (from C14:1 to C24:1). [g] Total n-6 polyunsaturated fatty acids (PUFA): C18:2n-6; C18:3n-6; C20:2n-6; 20:3n-6; C20:4n-6; C22:2n-6; and C22:4n-6. [h] Total n-3 PUFA: C18:3n-3; C20:3n-3; C20:4n-3; C20:5n-3; C22:5n-3; and C22:6n-3. [i] Total very-long-chain n-3 PUFA (VLC_n3_PUFA, >18C): C20:5n-3; C22:5n-3; and C22:6n-3.

Table 2. *trans*-18:1 Composition[a] (Milligrams per 100 mL of Milk) of Two Commercial Milks

fatty acid	HM	DOHM	SEM
trans-4-C18:1	0.76	1.10	0.17
trans-5-C18:1	0.79	0.85	0.11
trans-6−8-C18:1	10.64	11.31	0.73
trans-9-C18:1	14.71	14.24	0.59
trans-10-C18:1	14.03	15.33	1.15
trans-11-C18:1	28.61a	41.04b	0.95
trans-12-C18:1	11.53a	18.12b	0.79
trans-13−14-C18:1	17.41a	21.82b	0.98
trans-16-C18:1	8.76a	11.13b	0.42

[a] Means are based on 12 milk per treatment. Means within a row with different letters differ ($P < 0.05$).

fatty acids (i.e., branched-chain carboxylic acids) generated by the metabolism of branched-chain amino acid within the rumen (*21*). The main ingredients of the protein supplements of the HM- and DOHM-producing cows' ration are soybean and feather meal, respectively, as we discussed before. Feather meal contains higher amounts of branched-chain amino acids (e.g., valine, leucine, and *iso*-leucine) than soybean (*22*). However, feather meal has lower protein degradability in rumen compared with soybean (*23*), therefore supplying less branched-chain carboxylic acids to the rumen microbes of DOHM-producing cows, hence lowering the supply of some branched-chain SFA in DOHM.

The total SFA was not different ($P > 0.05$) between the two milks. However, the concentrations of total short-chain saturated fatty acids (SC_SFA, C4:0−C9:0) and total medium-chain saturated fatty acids (MC_SFA, C10:0−C16:0) were higher ($P < 0.05$) in DOHM than in HM. The content of total long-chain saturated fatty acids (LC_SFA, C17:0−C26:0) in DOHM was 22.2% lower ($P < 0.05$) compared with HM. It was reported that higher intakes of C12:0, C14:0, and C18:0 were related to a decrease in the total-to-HDL cholesterol ratio (*1*). On the other hand, higher intake of C16:0 was found to be associated with an increase in the total-to-HDL cholesterol ratio in plasma (*24*), which could increase the risk of cardiovascular disease in humans (*1*). Shingfield et al. mentioned in their review (*1*) that butter was predicted to increase the largest ratio in plasma total-to-HDL cholesterol, which could be due to MC_SFA, particularly C16:0, and the reduction in the proportion of this FA in milk and dairy products could be expected to contribute to improved human health.

No difference ($P > 0.05$) in total monounsaturated fatty acids (MUFA) content between the two milks was observed. There was a decrease in total omega-6 PUFA in DOHM compared with HM. The concentration of total omega-3 PUFA in DOHM was 2.23 times higher ($P < 0.05$) than in HM (36.2 vs 16.2 mg/100 mL of milk, respectively). The ration of DOHM-producing cows contains a higher amount of total omega-3 PUFA and a lower amount of omega-6 PUFA compared with the ration of HM-producing cows (as we discussed earlier), which is ultimately reflected in their milks (**Table 1**). The omega-6 PUFA/omega-3 PUFA ratios were 1.46 and 4.16 ($P < 0.05$) for DOHM and HM groups, respectively. The recommended ratio of omega-6 PUFA/omega-3 PUFA in human diet should not be higher than 4.0 (*25*). In the present study, the increases in the content of n-3 PUFA in DOHM resulted in a favorable decline in the n-6/n-3 ratio. Therefore, daily intake of DOHM could decrease the ratio of n-6/n-3 in Western diets to a more desirable level and contribute to higher dietary intake of omega-3 PUFA. The total VLC_n-3_PUFA was 3.8 times higher ($P < 0.05$) in DOHM than that in HM (**Table 1**). Australia's Ministry of Health recommended an increasing dietary intake of total VLC_n-3_PUFA (a combination of EPA + DPA + DHA) to satisfy the physiological requirements of

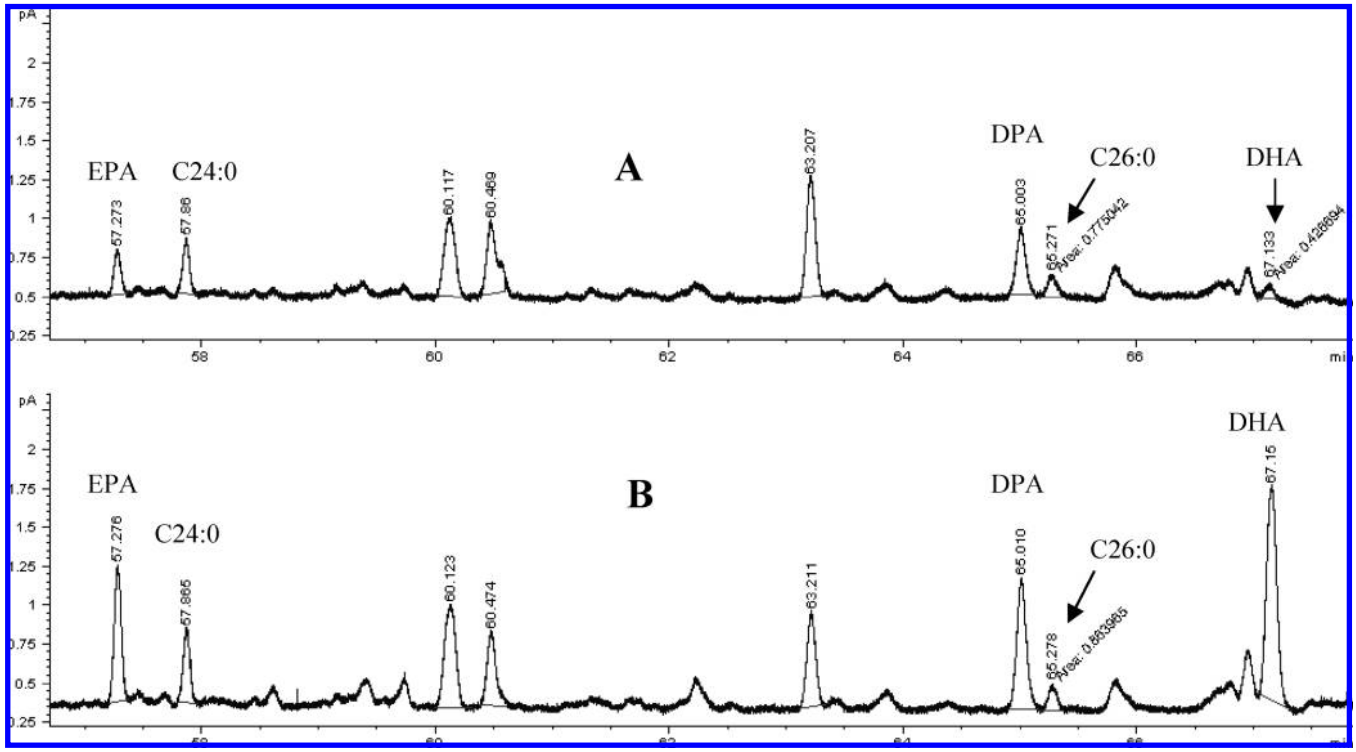

Figure 1. GLC chromatogram showing the relative peak heights of docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), and eicosapentaenoic acid (EPA) in milk fat of HM (**A**) and DOHM (**B**).

growing children, as the linolenic acid content in diets was inadequate to meet their requirements (*10*). The recommended level of total VLC_n-3_PUFA for children aged 1−3 years was 40 mg/day (*10*). In the present study, one serving (250 mL) of DOHM would supply 36.0 mg of VLC_n-3_PUFA. Therefore, one serving a day of DOHM would supply >90% of the recommended daily intake for children aged 1−3 years.

Figure 1 shows the GLC chromatogram of some very long chain n-3 PUFA (>18C). The largest peak of this region was DHA (C22:6n-3) in DOHM (**Figure 1B**). The concentration of DHA was found to be 24.0 times higher ($P < 0.05$) in DOHM than in HM (7.14 vs 0.30 mg/100 mL of milk, respectively; **Table 1**). The content of DHA in DOHM recorded in the present study was similar to those reported by Donovan et al. (*26*). The concentrations of DPA (C22:5n-3) and EPA (C20:5n-3) were greater ($P < 0.05$) in DOHM compared with HM. Compared to the ration of HM-producing cows, the contents of DHA and EPA are higher in the DOHM-producing cows' ration containing fish meal and results in a higher amount of DHA and EPA in DOHM. In another study, supplementation of algae to the diet of dairy cow rations has been used as an effective method to increase the content of DHA and EPA in milk (*8*). DHA is considered to be physiologically essential for the development and maintenance of the brain, retina, and nerves (*27*). Studies have shown that humans convert very little α-linolenic acid to EPA (<0.2% of plasma α-linolenic acid) and DHA (<0.07% of plasma α-linolenic acid) (*28*), and the activities of the responsible enzymes, such as elongases and desaturases, for these conversions depend on gender, age, and physiological and nutritional status. Therefore, these fatty acids have to be supplied through the diet including milk and other dairy products. The National Academy of Sciences (*5*) states the recommended daily intake of DHA plus EPA should be 110 mg for women, 160 mg for men, and 70 mg for boys and girls (9−13 years). In the present study, one serving (250 mL) of DOHM contained 25.6 mg of DHA plus EPA. Therefore, two servings a day of DOHM would supply more than 46.0, 32.0, and 73.0% of the recommended daily intake for women, men, and boys and girls, respectively.

The present study also focused on analyzing the content of linoleic acid (C18:2n-6), linolenic acid (C18:3n-3), 18:1 monoenes, and CLA in commercial milks. The concentration of linoleic acid was lower ($P < 0.05$) in DOHM than in HM (45.8 vs 56.6 mg/100 mL of milk, respectively). The content of linolenic acid also differs significantly between the two milks. Linoleic and linolenic acids are of dietary origin; they are not synthesized by ruminant tissue, and their concentration in milk is dependent on the amount that flows out of the rumen (*29*). In our study, higher amounts of linoleic acid are present in the diet of HM-producing cows largely supplied by soybean products within the diet, which contributes to increase the linoleic acid content in HM. In contrast, the DOHM-producing cows' ration contains a higher amount of linolenic acid.

On average, 76.8% of the total 18:1 monoenes in both milks was *cis*-9-C18:1 and HM had a higher ($P < 0.05$) concentration of this fatty acid in the present study (**Table 1**). The content of *cis*-9-C18:1 was also the highest MUFA of both milks. The concentrations of other *cis* isomers of 18:1 monoene, such as *cis*-11,*cis*-12, were not significantly different between the two milks. However, the concentration of *cis*-13 was higher ($P < 0.05$) in DOHM. The concentrations of *trans*-4, *trans*-5, *trans*-6−8, *trans*-9, and *trans*-10 in both milks did not differ significantly (**Table 2**). On the other hand, *trans*-11, *trans*-12, *trans*-13−14, and *trans*-16 were greater ($P < 0.05$) in DOHM than in HM. High intakes of *trans* FA are associated with a substantial increase in chronic heart disease (CHD) in humans [see the review by Shingfield et al. (*1*)]; however, no study has yet found a significant positive relationship between the intake of ruminant-derived *trans* FA and CHD risk (*1, 30*). Most of the evidence to date suggests that increased milk consumption is associated with a reduction in CHD risk [see the review by Elwood et al. (*31*)]. The lack of increase in CHD risk with the

Table 3. Conjugated Linoleic Acid Composition[a] (Milligrams per 100 mL of Milk) of Two Commercial Milks

fatty acid	HM	DOHM	SEM
total CLA[b]	17.31a	22.42b	0.38
cis-9,*trans*-11 CLA	11.63a	16.43b	0.35
trans-9,*cis*-11 CLA	0.62a	1.14b	0.11
trans-10,*cis*-12 CLA	0.36	0.33	0.06
trans-11,*trans*-13 CLA	0.89	1.06	0.05
trans-9,*trans*-11 + *trans*-10,*trans*-12 CLA	3.80	3.44	0.47

[a] Means are based on 12 milk per treatment. Means within a row with different superscripts differ ($P < 0.05$). [b] Total conjugated linoleic acid (CLA): *cis*-9, *trans*-11 18:2; *trans*-9,*cis*-11 18:2; *trans*-10,*cis*-12 18:2; *trans*-11,*trans*-13 18:2; and *trans*-9,*trans*-11 18:2 plus *trans*-10,*trans*-12 18:2.

higher intakes of *trans* FA of ruminant food products (e.g., milk, butter, cheese, meat, etc.), relative to the substantial risk associated with *trans* FA from industrial sources (e.g., hydrogenated vegetable oil, margarine, etc.) may be due to an isomer-specific bioactivity (e.g., *trans*-11-C18:1 from the ruminant food products) or the activity of other compounds that mitigate any adverse effects of *trans* FA [see the review by Shingfield et al. (*1*)]. In a recent study Chardigny et al. (*32*) demonstrated that *trans* FA from industrial sources in the diet of healthy humans resulted in lower plasma HDL cholesterol concentrations than did *trans* FA from natural sources. In the present study, the average percentage of *trans*-11-C18:1 in milk was 28.6% of total *trans*-18:1. On the other hand, the average percentage of *trans*-10-C18:1 was only 12.2% of the total *trans*-18:1 FA. The *trans*-11-C18:1 content was higher ($P < 0.05$) in DOHM than in HM; however, there was no difference in *trans*-10-C18:1 between the two commercial milks (**Table 2**). Production of *trans*-18:1 FA is the result of incomplete biohydrogenation of dietary PUFA (*1*). Increased *trans* FA is associated with a decreased milk fat content (*11*). It was confirmed by Griinari et al. (*33*) that a low-fiber and high-unsaturated-FA diet increased the percentage of *trans*-10-C18:1 in milk and was associated with a significant decrease in milk yield and milk fat content.

The concentrations of total CLA and its isomers in the milk are shown in **Table 3**. Total CLA was 29.5% higher ($P < 0.05$) in DOHM compared with HM. It is apparent from our study that higher amounts of DHA (from the fish meal) released in the rumen of DOHM-producing cows inhibit the microbial metabolism of *trans*-18:1 and thus result in an increased supply of *trans*-11-18:1 subsequently available for endogenous conversion to *cis*-9,*trans*-11 CLA (*11*). It was also reported by others (*8*) that DHA-enriched milk contained a higher percentage of CLA compared to controls. AbuGhazaleh and Jacobson (*34*) also state the possible mechanisms that DHA and/or its derivatives inhibit the reductase enzyme within certain rumen microorganisms, causing the increased accumulation of *trans*-18:1 and CLA within the rumen. The concentration of *cis*-9,*trans*-11 CLA (rumenic acid) in DOHM was 41.4% higher ($P < 0.05$) than that of HM (16.4 vs 11.6 mg/100 mL of milk, respectively). Rumenic acid is an intermediate in rumen biohydrogenation of linoleic acid, whereas *trans*-11-C18:1 is a common intermediate in the biohydrogenation of linoleic and linolenic acids (*29*). Most of the CLA found in foods derived from ruminant products is *cis*-9,*trans*-11 CLA (*11*). The *trans*-9,*cis*-11 CLA isomer was also higher ($P < 0.05$) in DOHM compared with HM. The *trans*-10,*cis*-12 CLA isomer was present in both milks, but the difference was not significant. Some other minor isomers of CLA were also detected by GLC (**Table 3**). Isomers of *cis*-9,*trans*-11 CLA have been shown to have potential health benefits in humans, such as lowering the LDL/HDL cholesterol ratio, reducing body fat, inhibiting growth of cancerous cells, and inducing positive cardioprotective effects (*1, 11*).

In summary, the fatty acid content of naturally enhanced DHA-enriched milk was determined in this study. The most abundant fatty acids were butyric, lauric, myristic, palmitic, oleic, and stearic acids. The DHA level in milk fat of DOHM was 24.0 times higher than that of HM. DOHM contained 25.6 mg of DHA plus EPA per serving (i.e., 250 mL); from a consumer health viewpoint, two servings of this milk would contribute more than 73% of the recommended daily intake for growing children. Also, *cis*-9,*trans*-11 CLA was 41.4% higher in DOHM than in HM. This CLA isomer is a naturally occurring fatty acid found in dairy fats that imparts a number of potential health benefits. The total VLC_n-3_PUFA was 3.8 times higher in DOHM than in HM. One serving of DOHM supplied 36.0 mg of VLC_n-3_PUFA, and one serving a day could meet >90% of the recommended daily intake for children aged 1−3 years. The results of the present milk fatty acid analyses indicate that milk fat from DOHM had increased contents of EPA, DPA, DHA, and *cis*-9,*trans*-11 CLA, all of which could have a favorable impact on diet composition and healthfulness.

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