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(54) Title: DAIRY PRODUCTS WITH ENHANCED CLA CONTENT

(57) Abstract

The invention provides a dairy product enriched in conjugated linoleic acids ("CLA") and/or other beneficial unsaturated fatty acids. The dairy product typically has a fat fraction with a fatty acid composition which includes at least about 15 mg/g CLA and preferably also includes at least about 10 mg/g omega–3 fatty acid. The dairy product may be produced by a ruminant which has been fed a diet which includes a fish–derived product such as fish oil or fish meal. The diet typically includes about 0.5 % to about 5 % by dry weight of oil supplied by the fish–derived product. Examples of dairy products of the present invention include milk in raw or processed form and other products derived from the milk. Isolated lipid fractions and fatty acid compositions derived from the raw milk are also provided.

DAIRY PRODUCTS WITH ENHANCED CLA CONTENT

Background of the Invention

Conjugated linoleic acids ("CLA") are a group of octadecadienoic acids with conjugated double bonds that has been shown to have anticarcinogenic properties (see, e.g., Ha et al., Cancer Res., 50:1097 (1990); Ip et al., Cancer Res., 51:6118 (1991); Ip et al., Carcinogenesis, 17:1045 (1996)). Conjugated linoleic acids are found primarily in meat (Ha et al., Carcinogenesis, 8:1881 (1987); Pariza et al., Carcinogenesis, 6:591 (1985)) and dairy products from ruminant animals (Ha et al., J. Agric. Food Chem., 37:75 (1989); Lin et al., J. Dairy Sci., 78:2358 (1995)). The collective term, CLA, incorporates several isomers, with double bonds present at positions 9 and 11, or 10 and 12, or 11 and 13 on the carbon chain of octadecadienoic acid. The double bond at each of the positions can be in the cis- or trans- form (Parodi, Aust. J. Dairy Technol., 49:93 (1994)). The cis-9, trans-11 octadecadienoic acid isomer is considered by some to be the most potent anticarcinogen (see Ha et al., Cancer Res., 50:1097 (1990); Ip et al., Cancer Res., 51:6118 (1991)).

Several studies have provided increasing evidence that CLA is inhibitory toward cancer. Mice fed a diet containing a synthetic form of CLA and subjected to cancer initiation treatments developed 50% fewer forestomach tumors than mice fed control diets (Ha et al., Cancer Res., 50, 1097 (1990)). Rats fed diets supplemented with 0.5, 1, or 1.5% synthetic CLA and subjected to carcinogens developed 32, 56, and 60% fewer mammary adenocarcinomas, respectively, than rats fed control diets (Ip et al., Cancer Res., 51, 6118 (1991)). Another study by Ip et al. (Carcinogenesis, 17, 1045 (1996)) reported that addition of CLA to the diet inhibited mammary tumorigenesis regardless of the level or type of fat in the diet. Also, female rats fed CLA during mammary gland development exhibited approximately 35% fewer tumors than rats fed control diets even though all rats were fed the control diet after carcinogen treatment (Ip et al., Cancer Res., 54:1212 (1994)). These results provide strong evidence that CLA can help decrease the incidence of cancer and suggest that CLA intake during mammary gland development may provide lasting protection against subsequent mammary tumorigenesis.

The amount of CLA humans should consume to provide protection against cancer is not currently known. Studies with rats indicated that 1% of the diet as CLA resulted in maximal inhibition of tumorigenesis. A subsequent study (Ip et al., Cancer Res., 54, 1212 (1994)) reported that as little as 0.1% CLA in the diet of rats inhibited tumorigenesis. Parodi (Aust. J. Dairy Technol., 49:43 (1994)) suggested that Australians may normally consume from 0.5 to 1.5 g of CLA per day which would be equivalent to approximately 0.05 to 0.15 % of the diet, a level shown to cause inhibition of tumorigenesis in rats. Assuming the average CLA content of milk fat is approximately 3 mg/g, increasing the CLA content 10 fold to 30 mg/g would provide approximately the 1% level of CLA in the diet which, in rats, provided maximal inhibition of cancer.

Dairy products are an important source in human diets of naturally occuring CLA. Dairy products typically contain from 2.5 to 7.0 mg CLA/g of fat (Lin et al., J. Dairy Sci., 78:2358 (1995)). Research reported by Riel indicated the total conjugated diene content of milk fat can vary from 0.78 to 1.46% (or approximately 8 to 15 mg/g) of fat depending on season (J. Dairy Sci., 46:102 (1963)). Riel found that cows consuming pasture during the spring have increased amounts (to 1.46%) of conjugated dienoic acid in the milk fat. In contrast, however, Mackle et al. (J. Dairy Sci., 80 (Suppl. 1):154 (1997)) reported little variation in CLA content with season or type of pasture. It has been speculated that the cis-9, trans-11 octadecadienoic acid is produced in the rumen as a first intermediate in the biohydrogenation of linoleic acid by linoleic acid isomerase from *Butyrivibrio fibrisolvens*. (see, e.g., Kepler et al., J. Biol. Chem., 241:1350 (1996)). It is believed that differing CLA contents of various dairy products probably do not represent different milk processing conditions, but are most likely due to fluctuating levels of CLA in raw milk.

Ruminal biohydrogenation of dietary long-chain fatty acids also may result in production of trans C18:1 fatty acids which can be incorporated in milk fat (see, e.g., Loor et al., J. Dairy Sci., 80(Suppl. 1):164 (1997); Piperova et al., J. Dairy Sci., 80(Suppl. 1):164 (1997); Wonsil et al., J. Nutr., 124:556 (1994)). Dairy products typically contain low concentrations of trans C18:1 fatty acids, therefore, few studies have investigated specific trans C18:1 fatty acids. Transvaccenic acid [*trans*

18:1(n-7)] has been shown to inhibit growth of colonic cancer cells in vitro (Awad et al., Cancer Letters, 91:55 (1995)) and decrease fat accumulation in rat adipocytes (Cromer et al., J. Nutr., 125:2394 (1995)). Increased transvaccenic acid in dairy products may have important health benefits for humans. Another C18:1 fatty acid, petroselinic acid, may also be a beneficial fatty acid in human diets. Petroselenic acid [18:1(n-12)] has been shown to decrease the concentration of arachidonic acid in lipids from heart, liver, and blood (Weber et al., J. Nutr., 125:1563 (1995)).

Omega-3 fatty acids also have been shown to provide important health benefits. Two omega-3 fatty acids, eicosapentaenoic acid ("EPA", C20:5) and docosahexaenoic acid ("DHA", C22:6) have been shown to have beneficial effects on human cardiovascular disease (see, e.g., Bang et al., Acta Med. Scand., 200:69 (1976); Hirai et al., Lancet, 2(8204):1132-33 (1976)), rheumatoid arthritis (Kremer et al., Ann. Intern. Med., 104:497 (1987)), dermatitis (Bjornobe et al., Brit. J. Dermatol., 117:463 (1987)), and ulcerative colitis (Ross, Nutr. Rev., 51:47 (1993)). Additionally, intake of omega-3 fatty acids has been linked to inhibition of prostate cancer (Rose et al., Lipids, 27:798 (1992)) and pancreatic cancer (Roebuck, Lipids, 27:804 (1992)). Human dietary sources of long-chain omega-3 fatty acids are generally limited to some cold water, deep sea fish and a few plant sources. Incorporation of omega-3 fatty acids into a widely available and palatable form of food, such as dairy products, would provide greater access to omega-3 fatty acids at a reasonable price.

The fat fraction of milk from ruminant animals contains numerous fatty acids, mainly saturated (circa 66%), but also monounsaturated (circa 30%) and polyunsaturated (circa 4%). All short-chain (C4:0 to C10:0) and half of the medium-chain (C12:0 to C17:0) fatty acids in milk fat are synthesized from acetate and ß-hydroxybutyrate in the mammary gland epithelial cells. The other half of medium-chain and almost all long-chain (C18:0 and longer) fatty acids are derived from blood plasma fatty acids of dietary origin as modified by rumen microbial fermentation or from mobilization of body fat stores.

Several attempts have been made to increase the CLA content of milk fat by feeding diets high in linoleic and linolenic acids. In one report, cows were fed diets containing raw soybeans, roasted soybeans, soybean oil, 2.2% linseed oil, or 4.4%

linseed oil and it was reported that milk fat from cows fed soybean oil contained an average of 21.2 mg CLA/g of fat (Dhiman et al., J. Dairy Sci., 80(Suppl. 1):184 (1997)). In a subsequently conducted study feeding several levels of soybean oil, it was reported a concentration of 20.8 mg CLA/g of fat was achieved with 4% soybean oil. Loor et al. found only minor increases (5 mg/g of fat vs. 2.3 mg/g of fat) in CLA when cows were fed canola oil or canolamide compared to control cows (Loor et al., J. Dairy Sci., 80(Suppl.1):164 (1997)). Similarly, no differences in CLA content of milk fat was observed when cows were fed canola oil, olive oil, or high-oleic sunflower oil compared to control diets (Bandara et al, J. Dairy Sci., 80(Suppl. 1):242 (1997)). Kelly et al., however, reported concentrations of CLA averaged 18.1 mg/g of fat when cows were fed sunflower oil high in linolenic acid (Kelly et al., J. Dairy Sci., 80(Suppl. 1):243 (1997)).

Other studies have addressed alterations in CLA concentrations as influenced by forage to concentrate ratios, season, or pasture feeding. Jiang et al. reported that cows fed a ration containing 35:65 forage to concentrate ratio had CLA contents in milk fat averaging 11.28 mg/g of fat compared to 5.05 mg CLA/g of fat for cows fed a 50:50 forage to concentrate ration (Jiang et al., J. Dairy Sci., 79:438 (1997)). Mackle et al. (1997, *supra*) investigated effects of season and pasture feeding on CLA concentrations and found little variation in CLA content as a result of season or type of pasture. Loor et al (1997, *supra*) reported the content of CLA in milk fat could be increased as a result of abomasal infusion of CLA and the content of CLA in milk fat was directly related to the amount of CLA available in the small intestine.

Fish oil and fish meal have been included in diets for dairy cows as sources of energy (see, e.g., Jones et al., J. Dairy Sci., 80(Suppl. 1):243 (1997)) and bypass protein (see, e.g., Calsamiglia et al., J. Dairy Sci., 78:1999 (1995); and Windschitl, J. Dairy Sci., 74:3475 (1991)). Other studies have investigated effects of feeding fish meal and fish oil on milk fat depression (see, e.g., Pennington et al., J. Dairy Sci., 58:49 (1975); and Spain et al, J. Dairy Sci., 78:1142 (1995)). Pennington et al. (1975, *supra*) reported increases in trans monounsaturated fatty acids in milk fat as a result of feeding cod-liver oil but did not characterize individual C18:1 isomers and did not measure CLA. Fish oil has also been included in rations of dairy cows in an attempt to alter the degree of unsaturation of milk fat (Brumby et al., J. Dairy Res.,

39:167 (1972); Storry et al., J. Dairy Sci., 57:1046 (1974)). Although several studies have examined effects of feeding fish oil and fish meal on milk composition as a part of the effects of feeding these products as energy and protein sources, no previous studies have investigated the effects of feeding fish-derived materials, such as fish oil or fish meal, on CLA content of milk and dairy products.

Summary of the Invention

The invention relates to dairy products enriched in conjugated linoleic acids ("CLA") and other beneficial unsaturated fatty acids. The dairy product typically has a fat fraction with a fatty acid composition which includes at least about 15 mg/g CLA, as well as other beneficial fatty acids such as transvaccenic acid, petroselinic acid and omega-3 fatty acid(s). Milk with this unsaturated fatty acid content may be produced by a ruminant which has been fed a diet which includes a fish-derived product such as fish oil or fish meal. The diet typically includes about 0.25% to about 10% by dry weight of oil supplied by the fish-derived product. The ruminant is an animal such as a cow, sheep, buffalo or goat. Examples of dairy products of the present invention include milk in raw or processed form and other products derived from the milk. The processed milk may be in a variety of forms, e.g., reduced and low fat forms, such as "1% milk," or enriched fat forms, such as cream or "half and half." Other examples of the present dairy products include products obtained by further processing of the milk, such as natural and processed cheeses, cottage cheese, yogurt, butter, ice cream, butter milk, cultured butter milk, sour cream, ghee, frozen yogurt, sherbet, anhydrous butter oil, anhydrous butter fat, powdered milk, condensed milk, evaporated milk and whey products.

For example, the unsaturated fatty acid-enriched milk may be produced by feeding a lactating ruminant animal a diet which includes fish oil at about 0.5% to about 5% by dry weight of the total diet. Typically, the diet is supplemented with about 1% to about 4% by dry weight fish oil. In the method of the invention, milk is collected from the ruminant fed the fish-derived product. The milk can be used as collected ("raw milk") or may be further processed into another dairy product using standard methods. The milk or other dairy product produced by the method of the invention has an enhanced level of CLA relative to milk produced by ruminants fed

a diet not supplemented with such an oil source. Preferably, the dairy product includes a fat fraction with a fatty acid composition which includes at least about 20 mg/g and more preferably, at least about 25 mg/g CLA. In other preferred embodiments of the invention, the dairy product has a fat fraction which contains enhanced levels of unsaturated fatty acids such as omega-3 fatty acid(s), transvacennic acid, and/or petroselinic acid.

The present invention also provides isolated lipid fractions and fatty acid compositions derived from the unsaturated fatty acid-enriched raw milk. The lipid portion can be separated from the raw milk using conventional methods, e.g., supercritical fluid extraction or extraction with organic solvent. The lipid composition obtained in this manner typically has a fatty acid composition which includes at least about 20 mg/g CLA and at least about 10 mg/g omega-3 fatty acid(s). Substantial amounts of triglycerides including transvaccenic acid and petroselinic acid esters are also generally present. If desired, the lipid composition derived from the raw milk (or a process version of the raw milk or lipid composition) may be hydrolysed to convert the triglycerides to free fatty acids or simple corresponding esters (e.g., "lower alkyl" esters - (C(1)-C(6) alkyl esters) of the fatty acids. Alternatively, the triglycerides may be initially converted to free fatty acids which are subsequently esterified. The products of the hydrolysis reaction may be isolated in free acid form or as a salt of the fatty acid(s). The fatty acid salt(s) generally include one or more water soluble monovalent cations as the counterion, e.g., potassium (K^+), sodium (Na^+) and/or ammonium (NH_4^+). Either the lipid composition or the isolated fatty acids (or their corresponding esters) may be further processed to obtain various fractions based on boiling point, melting point, solubility, fractional crystallization or other similar properties.

Detailed Description of the Invention

Definitions

As used herein, "dairy product" includes milk in raw and processed form, natural and processed cheeses, cottage cheese, yogurt, butter, ice cream, butter milk, cultured butter milk, sour cream, ghee, frozen yogurt, sherbet, anhydrous butter oil, anyhdrous butter fat, powdered milk, condensed milk, evaporated milk, whey

products and other milk-based products. The term "dairy product" includes any of the above product having at least about 0.5 wt.% fat content.

As used herein, the terms "raw milk" and "milk produced by a ruminant animal" means isolated milk in the form directly produced by the ruminant without any further processing.

As used herein, the term "milk product" means any product having a fat content of at least about 0.5 wt. % derived from the raw milk by a process which does not alter its liquid state or substantially change the fatty acid composition of the fat fraction. Thus, the term "milk product" includes both raw milk as well as milk which has been processed to enhance its shelf life (e.g., via pasteurization), to alter its physical consistency (e.g., via homogenization), and/or to alter the overall fat content (e.g., via separation). Examples of milk products having an altered overall fat content include enriched fat, full fat, low fat milk (e.g., 1% milk), reduced fat (e.g., 2% milk), half and half, and cream, as well as flavored milk products.

For purposes of definition throughout the application, it is understood herein that a fatty acid is an aliphatic (saturated or unsaturated) monocarboxylic acid. Lipids are understood to be fats or oils including the glyceride esters of fatty acids along with associated phosphatides, sterols, and related compounds. A commonly employed shorthand system is used in this specification to denote the structure of the fatty acids. This system uses the letter "C" accompanied by a number denoting the number of carbons in the hydrocarbon chain, followed by a colon and a number indicating the number of double bonds,e.g., C20:5, eicosapentaenoic acid. Fatty acids are numbered starting at the carboxy carbon. The position of the double bonds may be indicated by adding the Greek letter delta (Δ) followed by the carbon number of the double bond; e.g., C20:5omega-3$\Delta^{5,8,11,14,17}$. The "omega" notation is a shorthand system for unsaturated fatty acids whereby numbering from the CH_3-terminal carbon is used. For convenience, w3 may be used to symbolize "omega-3," when using the numeral shorthand nomenclature described herein. Omega-3 highly unsaturated fatty acids are understood to be polyethylenically unsaturated fatty acids in which the last ethylenic bond is between the third and fourth carbons from the CH_3 end of the fatty acid. Thus, the complete nomenclature for eicosapentaenoic acid, a highly unsaturated omega-3 fatty acid, would be

C20:5$\Delta^{5,8,11,14,17}$. For the sake of brevity, the double bond locations ($\Delta^{5,8,11,14,17}$) will be omitted. Eicosapentaenoic acid is then designated C20:5w3 (also referred to herein as "C20:5"), docosapentaenoic acid (C22:5$\Delta^{7,11,13,16,19}$) is C22:5w3 (also referred to herein as "C22:5"), and docosahexaenoic acid (C22:6$\Delta^{4,7,10,13,16,19}$) is C22:6w3 (also referred to herein as "C22:6").

As used herein, the nomenclature "highly unsaturated fatty acid" ("HUFA") means a fatty acid with 4 or more double bonds.

As used herein, "conjugated linoleic acid" or "CLA" means a fatty acid that is an octadecadienoic acid having conjugated double bonds at positions 9 and 11, 10 and 12, or 11 and 13 on the carbon chain of linoleic acid, wherein the double bond at each of these positions can be in either the cis or trans configuration. The term CLA as used herein refers to a fatty acid composition made up of one or more of the "conjugated linoleic acid" isomers.

As used herein, "omega-3 fatty acid" refers to a fatty acid having a double bond between the third and fourth carbons from the end of the aliphatic chain of the fatty acid. Examples of omega-3 fatty acids include α-linolenic ("C18:3α"), eicosapentaenoic acid (a C20:5 fatty acid) and docosahexaenoic acid ("DHA"; a C22:6 fatty acid).

As used herein, "ruminant" includes a cow, goat, sheep, buffalo, deer or other member of the Ruminantia suborder of hooved mammals.

Components of Fats and Oils

Fat is made up of triacylglycerol molecules (sometimes termed triglycerides). In general, triacylglycerols comprise three long fatty acid chains esterified to glycerol; or, alternatively phrased, glycerol esterified by addition thereto of three long chain fatty acids. Herein, the terms "triacylglycerols" and "triglycerides" are intended to be interchangeable. In general, fats and oils derived from any given plant or animal source comprise a mixture of triacylglycerols, characteristic of the specific source. The mixture of fatty acids isolated from complete hydrolysis of the triacylglycerols in a specific fat or oil are generally referred to as a "fatty acid composition". By the term "fatty acid composition" reference is made to the identifiable fatty acid residues in the various triacylglycerols. The distribution of

specific identifiable fatty acids is typically characterized by the amounts of the individual fatty acids relative to the total mixture of fatty acids obtained from hydrolysis of the particular oil stock. Herein, the amount of an individual fatty acid is stated as the amount in milligrams (mg) of the butyl ester of the specified fatty acid relative to the total weight in grams (g) of the butyl esters of all the fatty acids in the fatty acid composition. Alternatively, the amount of a fatty acid may be stated as mg of the fatty acid per gram of total fat.

For example, typical fatty acid compositions of fish oil are as shown in Table 1 below. The generic fish oil shown in Table 1 was purchased from a pharmacy; the Menhaden fish oil was obtained from Zapata Protein, Inc. (Reedville, VA).

Palmitic (C16:0) and stearic (C18:0) acids are saturated fatty acids and triacylglycerol acyl chains formed by the esterification of either of these acids do not contain any double carbon-carbon bonds. However, many fatty acids such as oleic acid (a C18:1 fatty acid), linoleic acid (a C18:2 fatty acid), and linolenic acid (a C18:3 fatty acid), are unsaturated. Oleic acid is an 18 carbon fatty acid with a single double bond; linoleic acid is an 18 carbon fatty acid with two double bonds or points of unsaturation; and linolenic is an 18 carbon fatty acid with three double bonds. More specifically,

oleic acid is (Z)-9-octadecanoic acid;

linoleic acid is (Z,Z)-9,12-octadecadienoic acid;

α-linolenic acid is (Z,Z,Z)-9,12,15-octadecatrienoic acid (C18:3α); and

γ-linolenic acid is the (Z,Z,Z)-6,9,12 isomer of octadecatrienoic acid (C18:3γ).

The present dairy products typically also contain other unsaturated fatty acids such as transvaccenic acid, petroselenic acid and/or other omega-3 fatty acid(s).

Dairy Products of the Invention

The invention provides a dairy product enriched in one or more conjugated linoleic acids (collectively refered to as "CLA") and/or other beneficial unsaturated fatty acids. The dairy product typically has a fat fraction with a fatty acid composition which includes at least about 15 mg/g CLA. Preferably, the CLA

content of the dairy product is at least about 20 mg/gram of fat and, more preferably, at least about 25 mg/gram of fat.

The dairy product may be produced by a ruminant which has been fed a diet which includes a fish-derived product such as fish oil or fish meal. Preferably, the diet comprises no more than about 10% by dry weight and more preferably less than about 5% by dry weight of oil supplied by the fish-derived product. Most preferably, the diet comprises about 1% to about 4% by dry weight of a fish oil. Examples of fish oil include, but are not limited to, Menhaden fish oil, cod liver oil, mackerel oil, salmon oil, catfish oil, herring oil, sardine oil or whale oil.

The ruminant is an animal such as a cow, sheep, buffalo, deer or goat. The ruminant is typically fed the diet including a fish-derived product for at least about two days and, preferably, for at least about one week prior to collection of the milk produced.

In addition to being enriched in CLA, milk produced by a ruminant fed a diet including a fish-derived product such as fish oil may also be enriched in omega-3 fatty acids, such as α-linolenic acid (C18:3α), eicosapentaenoic acid ("EPA"; C20:5) and docosahexaenoic acid ("DHA"; C22:6). The milk typically has a fatty acid composition which includes at least about 10 mg/g omega-3 fatty acid(s). Preferably, the omega-3 fatty acid composition includes at least about 8 mg/g α-linolenic acid, at least about 2 mg/g eicosapentaenoic acid, and/or at least about 0.5 mg/g docosahexaenoic acid. More preferably, the omega-3 fatty acid composition includes at least about 12 mg/g α-linolenic acid, at least about 4 mg/g eicosapentaenoic acid, at least about 0.8 mg/g docosahexaenoic acid or any combination thereof.

The dairy product of the invention can be enriched in other fatty acids such as C18:3γ, transvaccenic acid and/or petroselinic acid. Typically, the fatty acid composition includes at least about 3 mg/g C18:3γ, at least about 25 mg/g transvaccenic acid, at least about 8 mg/g petroselinic acid, or any combination thereof. Preferably, the fatty acid composition includes at least about 50 mg/g transvaccenic acid together with a variety of other unsaturated fatty acids.

Examples of dairy products of the present invention include milk in raw or processed form and other products derived from the milk. The milk may be

processed into a variety of forms, e.g., reduced fat such as "2% milk" or enriched fat forms such as cream and "half and half." Other examples of the present dairy products include products obtained by further processing of the milk, such as natural and processed cheeses, cottage cheese, yogurt, butter, ice cream, butter milk, cultured butter milk, sour cream, ghee, frozen yogurt, sherbet, anhydrous butter oil, anyhdrous butter fat, powdered milk, condensed milk, evaporated milk and whey products.

Methods of the Invention

The CLA-enriched milk is produced by feeding a lactating ruminant animal a diet which includes a fish-derived product such as fish oil or fish meal. For example, the diet may include fish oil as about 0.25% to about 10% by dry weight of the total diet. Typically, the diet is supplemented with about 0.5% to about 5% by dry weight of the fish oil (as a percentage of the total diet). Preferably, the fish oil is no more than about 4% by dry weight of the total diet. In the method of the invention, milk is collected from the ruminant fed the fish-derived product. The milk can be used as collected, and may be further processed into another dairy product using standard methods.

The milk or other dairy product produced by the method of the invention includes an enhanced level of CLA relative to milk produced by ruminants fed a diet not supplemented with an oil source. Preferably, the CLA content of the dairy product is at least about 20 mg/gram of fat and, more preferably, at least about 25 mg/gram of fat.

In one embodiment, the ruminant is fed the diet for at least about one week. In another embodiment, the ruminant is fed the diet for at least about one day. Examples of ruminants for use in method of the invention include, but are not limited to, a cow, deer, goat, buffalo or sheep.

Highly unsaturated fatty acids ("HUFAs"), such as those produced by the present process, when exposed to oxidizing conditions can be converted to less desirable unsaturated fatty acids or to saturated fatty acids. Saturation of omega-3 HUFAs can be reduced or prevented by the introduction of synthetic antioxidants or

naturally occurring antioxidants, such as beta-carotene, vitamin E and vitamin C, into the dairy products.

Synthetic antioxidants, such as butylated hydroxytoluene ("BHT"), butylated hydroxyanisole ("BHA"), t-butylhydroquinone ("TBHQ"), and ethoxyquin, or natural antioxidants such as tocopherols, can be incorporated into the dairy products or food or feed products derived therefrom by adding them to the products during processing. The amount of antioxidants incorporated in this manner depends, for example, on subsequent use requirements, such as product formulation, packaging methods, and desired shelf life. Alternatively, compounds which act synergistically with antioxidants to prevent oxidation (e.g., ascorbic acid, citric acid, phosphoric acid) can also be added.

Lipids containing the CLA and other beneficial unsaturated fatty acids can also be extracted from the milk products by any suitable means, such as by supercritical fluid extraction, or by extraction with solvents such as chloroform, hexane, methylene chloride, methanol, and the like, and the extract evaporated under reduced pressure to produce a sample of concentrated lipid material. The CLA and other beneficial unsaturated fatty acids in this preparation may be further concentrated by hydrolyzing the lipids and concentrating the unsaturated fraction by employing traditional methods such as urea adduction, fractional distillation or column fractionation. The lipids can also be extracted from the milk products into vegetable or other edible oil. The extracted oils can be refined by well-known processes routinely employed to refine vegetable oils (e.g., chemical refining or physical refining). These refining processes can remove impurities from extracted oils before they are used or sold as edible oils. The refining process consists of a series of processes to degum, bleach, filter, deodorize and polish the extracted oils. Alternatively, vegetable oil which has already been subjected to one or more of these refining processes can be used as the extractant. After refining, the oils can be used directly as a feed or food additive to produce products enriched in CLA and other beneficial unsaturated fatty acids. Alternatively, the oil can be further processed and purified as outlined below and then used in the above applications and also in pharmaceutical applications.

The lipids from the obtained milk products may be extracted by use of a solvent or mixture of solvents such as hexane, chloroform, ether, or methanol. The solvent is removed (for example by a vacuum rotary evaporator, which allows the solvent to be recovered and reused) and the resulting lipid fraction can be hydrolyzed by using any of the well-known methods for converting triglycerides to free fatty acids or esters of fatty acids including base hydrolysis, acid hydrolysis, or enzymatic hydrolysis. The hydrolysis is preferably carried out at as low a temperature as possible (e.g., room temperature to 60°C) and under nitrogen to minimize degradation of the unsaturated fatty acids. After hydrolysis is completed, nonsaponifiable compounds can be extracted into a solvent such as ether, hexane or chloroform and removed. The remaining solution is then acidified by addition of an acid such as HCl and the free fatty acids can be extracted into a solvent such as hexane, ether, or chloroform. The solvent solution containing the free fatty acids could then be cooled to a temperature low enough for the non-HUFAs to crystallize, but not so low that HUFAs crystallize. For example, the solution may be cooled to between about -60°C and about -74°C. The crystallized fatty acids (saturated fatty acids, and mono-, di-, and tri-enoic fatty acids) can then be removed (while keeping the solution cooled) by filtration, centrifugation or settling. The HUFAs remain typically dissolved in the filtrate (or supernatant). The solvent in the filtrate (or supernatant) can then be removed leaving a mixture of fatty acids which are > 90% purity in either omega-3 HUFAs or HUFAs which are greater than or equal to 20 carbons in length. The purified highly unsaturated fatty acids can then be used as a nutritional supplement for humans, as a food additive, or for pharmaceutical applications. For these uses the purified fatty acids can be encapsulated or used directly. Antioxidants are typically added to the fatty acids to improve their stability.

As discussed in detail above, the milk product (or a dry form thereof) can be used directly as a food additive to enhance the content of CLA and other beneficial unsaturated fatty acids of processed foods for human intake or for animal feed. When used as animal feed supplement, CLA and other beneficial unsaturated fatty acids from the present dairy products may be incorporated into the flesh or other products of animals.

The complex lipids containing CLA and other beneficial unsaturated fatty acids can also be extracted from the milk product with solvents and utilized in a more concentrated form (e.g., encapsulated) for pharmaceutical or nutritional purposes and industrial applications. A further aspect of the present invention includes introducing CLA and other beneficial unsaturated fatty acids from the foregoing sources into humans for the treatment of various diseases. As defined herein, "treat" means both the remedial and preventative practice of medicine. The dietary value of CLA and other beneficial unsaturated fatty acids is recognized in the literature as decribed herein, and intake of CLA and other beneficial unsaturated fatty acids produced in accordance with the present invention by humans can be effective for treating cardiovascular diseases, inflammatory and/or immunological diseases and cancer.

Fractionating Milk Fat

Separating milk fat into fractions that vary in melting properties can add value to the milk fat. When such fractions are combined with custom blending and texturization technologies, a range of milk fat fraction ingredients with varying functional properties can be produced.

The most common method used to fractionate milk fat is dry crystallization, a simple, physical process that separates milk fat into fractions with different physical and chemical properties. The process involves heating anhydrous milk fat (99.9% fat) in a crystallizer (e.g., a stainless steel jacketed tank) until fully melted. The milk fat is then allowed to cool and stirred using controlled conditions until the crystallization process is complete. The result is a slurry consisting of solid milk fat crystals suspended in liquid milk fat.

In a final step, the fractions may be separated -- either by vacuum or pressure filtration or centrifugation -- into milk fat ingredients with melting points much lower and higher than milk fat's typical melting point. Fractions with melting points ranging from 43-155°F may be obtained in this manner in contrast to the 95-96°F melting point of ordinary butter. While this type of process can change the physical characteristics of butter, it generally does not alter its characteristic flavor.

Milk fat fractions can be used to improve the texture of low-fat cheese and increase the spreadability of butter at refrigerated temperatures. The functionality of

cold-spreadable butter is a direct result of the functionality of the milk fat ingredients used. The physical characteristics result from the complex interactions between the various phases (solid milk fat, liquid milk fat and aqueous).

One approach for making cold-spreadable butter is to add low-melting fractions to cream during the conventional butter churning process. Another approach is to selectively blend milk fat fractions to achieve a desired melting profile, followed by emulsification with other ingredients and appropriate texturization. A ratio of one-part high-melting fraction to three-part low-melting fraction has proved successful at the pilot level. High-melting fractions can also be used in ultra high temperature-recombined creams to help increase creaming stability during storage and in whipped creams to improve physical and stability properties.

The following examples are presented to illustrate the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention.

Example 1

Two Holstein dairy cows were fed a total mixed ration consisting of 50% concentrate, 25% alfalfa haylage (farm-grown), and 25% corn silage (farm-grown) on a dry matter basis as a basal diet for one week to acclimate them to the ration beginning on day 1. The concentrate contained (expressed as percent of dry matter): corn, 58.9; soybean meal (44% CP), 32.6; dicalcium phosphate, 1.5; limestone, 1.75; sodium bicarbonate, 1.5; magnesium oxide, 0.5; trace mineral salt, 1.0; vitamin A, D, E premix (2,000,000 IU of vitamin A, 400,000 IU of vitamin D, 200 IU of vitamin E per pound), 0.2; vitamin E premix (20,000 IU per pound), 0.1. The cows were housed in a free stall facility at the South Dakota State University Dairy Research and Teaching Facility. The cows were fed using Calan feeding doors (American Calan, Inc., Northwood, NH) and amounts fed and refused were recorded daily. Menhaden fish oil (Zapata Protein, Inc., Reedville, VA) was incorporated in the ration gradually beginning on day 9 until cows were offered 454 g of fish oil

daily. Fish oil was incorporated in the ration through day 29. Milk production, milk composition, feed intake, and milk fat composition were monitored through 4 weeks.

Milk fat composition of samples from the cows fed fish oil was analyzed by packed column gas chromatography (Jones, et al. Quantitative determination of double bond positions in unsaturated fatty acids after oxidative cleavage, J. Amer. Oil Chem. Soc., 42:121 (1965)). Ten ml of each milk sample were placed in individual Mojonnier flasks (Mojonnier, Charleston, SC). An indicator, 1.5 ml of ammonium hydroxide, 10 ml ethanol, 25 ml ethyl ether, and 25 ml of petroleum ether were added to the milk sample followed by centrifugation for one minute. The clear layer was decanted to a flask and the sample was evaporated to dryness on a rotary evaporator. Petroleum ether (approx 3 ml) was added to the flask and the sample was evaporated to dryness again. Boron trifluoride butanol (approximately 3 ml) was added to the sample and the sample was heated for approximately 30 minutes. Samples were allowed to cool while connected to a cold water condensor. Contents of the flask were then transferred to a separatory funnel. Ten ml of distilled water was added followed by 8 ml of saturated sodium bicarbonate solution and 50 ml of half-saturated sodium chloride solution. After allowing the sample to stand for 5 minutes, the bottom layer was discarded. Another 50 ml of half-saturated sodium chloride solution was added and allowed to stand for 5 minutes and again the bottom layer was discarded. This procedure was repeated 3 times. Equal volumes of a portion of the top layer containing the butylated fatty acids and petroleum ether were then transferred to a GC vial for analysis.

A sample of fish oil was analyzed by capillary column gas chromatography using the following procedure. A 6 - 20 mg sample of fish oil was added to a 16 X 150 mm extraction tube, followed by 50 ml of a solution of 0.5% valeric acid and 1.0% 10-undecanoic acid in n-butanol as an internal standard. n-Butanol (750 ml) was added to the sample and vortexed for 4 - 5 seconds. While vortexing, 75 ml of acetyl chloride were slowly added to the sample. Samples were gassed with N_2, sealed, and heated at 100° C for 1.5 h. After cooling, 5 ml of 6% K_2CO_3 was added to the sample followed by addition of 1 ml hexane. The sample was vortexed for 0.5 min, then centrifuged at 250 x g for 20 min. The lower layer was aspirated and the top layer was washed 3 times with distilled, deionized water. The upper layer was

transferred to a GC vial and analyzed using a Hewlett Packard 6890 series gas chromatograph. Individual fatty acids were identified using known standards (Table 1).

Results

Table 2 shows the average milk production and composition of milk from the cows fed 454 g of fish oil daily for 14 days. Milk samples were obtained prior to beginning fish oil supplementation for the pretreatment values. Fish oil in the ration was increased gradually for 1 week, then cows were fed at 454 g of fish oil daily through week 1 and week 2. Milk samples were obtained at the end of week 1 and week 2.

Table 2

Milk production and composition of milk from cows fed 454 g of fish oil daily for 14 days

Milk Production (kg/day)		Milk Composition (wt.%)			
		Fat	Protein	Lactose	SNF
Pretreatment	27.6	4.72	3.52	4.75	8.91
Week 1	27.6	3.90	3.33	4.74	8.70
Week 2	29.1	3.39	3.38	4.91	8.95

Tank milk from the South Dakota State University Dairy Farm was used as a comparison for pretreatment and treatment milk samples. The tank milk was from a herd of cows fed a diet containing the ingredients listed in Table 3. Pretreatment milk samples were obtained from the cows to be fed the fish oil supplemented diet prior to initiation of daily supplementation with 454 gm of Menhaden fish oil. Treatment milk samples were obtained after cows had been receiving 454 gm of Menhaden fish oil daily for 14 days. Samples were analyzed using a butyl esterification method of gas chromatography using a Varian model 3700. Table 4 shows the fatty acid compositions (mg/g of fat) of the tank milk and milk produced by cows before and after supplementation of their feed with 454 gm (1.0 lb) by dry weight fish oil per cow.

Table 3
Contents of Basic Diet Fed to Herd From Which Tank Milk Obtained

Ingredient	Amount (%)
Corn, shelled rolled	54.80
Barley, rolled	9.20
Soybean meal, 44% CP	27.65
Molasses, liquid	5.00
Dicalcium phosphate	1.25
Sodium bicarbonate	0.80
Trace mineral salt	0.50
Magnesium oxide	0.25
Limestone	0.25
Vitamin A, D, & E premix[1]	0.20
Vitamin E premix[2]	0.10

[1]Contains 2,000,000 IU of vitamin A, 400,000 IU of vitamin D, and 200 IU of vitamin E per pound.
[2]Contains 20,000 IU of vitamin E per pound.

Discussion

Inclusion of Menhaden fish oil in diets fed to dairy cows provided long-chain fatty acids that resulted in alterations in milk fat composition. In general, the percent of saturated fatty acids decreased from approximately 71% pretreatment to 61% during treatment with a concurrent increase in unsaturated fatty acids from approximately 27% to 36%. Saturated fats have been implicated as a risk factor in generation of heart disease, therefore health care officials have recommended a shift from saturated fatty acids to unsaturated fatty acids. Feeding fish oil to dairy cows results in decreased percentages of saturated fatty acids in milk fat thereby providing a healthier product for human consumption.

Feeding Menhaden fish oil to dairy cows also resulted in an increase in the concentration of CLA in milk fat. Average pretreatment values (see Table 4) for CLA were 3.1 mg/g of fat compared to 27.2 mg CLA/g of fat in samples from cows fed the fish oil supplemented diet. The increase in CLA varied between animals from 23 mg/g of fat to 31.3 mg/g of fat.

Example 2

Two cows in late lactation were fed a ration containing fish oil (Table 5) and two other cows in late lactation were fed a ration containing fish meal (Table 6).

Table 5
Fish Oil Grain Mix

Ingredient	Percent	Amount (lbs)
Corn, rolled	71.4	714.0
Soybean meal, 44% CP	20.0	200.0
Fish Oil	4.0	40.0
Dicalcium phosphate	1.2	12.0
Trace mineral salt	1.0	10.0
Magnesium oxide	0.5	5.0
Limestone	1.6	16.0
Vitamin A, D, & E premix	0.2	2.0
Vitamin E premix	0.1	1.0

Table 6
Fish Meal Grain Mix

Ingredient	Percent	Amount (lbs)
Corn, rolled	81.0	810.0
Soybean meal, 44% CP	8.5	85.0
Menhaden Fish Meal	7.2	72.0
Dicalcium phosphate	1.0	10.0
Trace mineral salt	1.0	10.0
Magnesium oxide	0.5	5.0
Limestone	0.5	5.0
Vitamin A, D, & E premix	0.2	2.0
Vitamin E premix	0.1	1.0

The total mixed ration (dry matter basis) consisted of:

1) 50% respective grain mix (see Tables 5 and 6);
2) 25% corn silage;
3) 25% alfalfa hay.

The diet was fed individually to cows, ad libitum, with amounts offered and refused recorded daily. Cows were fed the normal herd ration on days 1 through 3.

Concentrate containing fish oil or fish meal was added to rations in increasing amounts beginning on day 4 with full allotments beginning on day 10.

Milk samples (a.m. and p.m.) were composited for analysis of milk composition (fat %, protein %, lactose %, solids, and somatic cell count) and milk fat composition by gas chromatography. Milk weights are recorded for each milking. Milk samples were obtained on day 3 prior to initiation of feeding fish oil, on days 7 and 10 after starting incorporation of fish oil (fish meal), and on day 14 after cows were receiving full allotments of fish oil (fish meal). Samples were analyzed for fatty acid composition using a butyl esterification method by gas chromatography (Hewlett-Packard model 6890). The results are shown in Tables 7-9.

In addition to decreased percentages of saturated fatty acids in milk fat, supplementing the feed of dairy cows with fish oil resulted in an increase in the concentration of CLA in milk fat (see Table 7). Average pretreatment values (see day 3 in Table 7) for CLA were 4.3 mg/g of fat compared to 24.7-27.0 mg CLA/g of fat in samples from cows fed the full allotment of fish oil supplemented diet. The total omega-3 content of the milk produced increased to as high as 11.6 mg/g of fat in contrast to a pretreatment level of 6.3 mg/g of fat. The levels of transvacceniic acid and petroselinic acid also increased in the milk produced by the cows fed the full allotment of fish oil supplemented diet (to 61.1-68.8 mg/g of fat and 10.1-12.1 mg/g of fat respectively).

Supplementing the feed of dairy cows with fish meal also resulted in an increase in the concentration of CLA in milk fat (see Table 8). Average pretreatment values (see day 3 in Table 8) for CLA were 4.7 mg/g of fat compared to 5.8-9.5 mg CLA/g of fat in samples from cows fed the full allotment of fish meal supplemented diet (days 10 and 14). The total omega-3 content of the milk produced was increased up to about 8.3 mg/g of fat in contrast to a pretreatment level of 6.4 mg/g of fat.

To confirm the presence of c9.t11/t9.c11 CLA, one of the milk samples from day 14 of the fish oil supplemented feeding trials was examined by an independent laboratory. The analysis was carried out by gas chromatography on duplicate runs of material which had been hydrolysed and esterified to produce fatty acid methyl

esters (Ha et al., J. Agric. Food Chem., 37:75 (1989)). This analysis determined the CLA content to be 2.8-2.9 wt.% of the total milk fat but was unable to establish between whether the CLA was present as the c9.t11 or t9.c11 isomer. By spiking a sample with standard compounds, it was established that the t9.t11 or t10.t12 CLA isomers were not present in the milk samples.

Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

The invention has been described with reference to various specific and illustrative embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

Table 1

Typical Fish Oil Fatty Acid Composition

Fatty Acid	Generic (mg/g)	Menhaden (mg/g)
C4:0-C12:0	0	0
C14:0	73.4	117.5
C14:1	0	0
C16:0	158.9	146.2
C16:1	77.0	128.9
C18:0	32.5	26.4
C18:1 isomers		
petroselaidic	14.2	11.7
elaidic	1.4	0
petroselinic	1.2	0
transvaccenic	0	0
oleic	90.8	90.0
vaccenic	29.2	34.2
linoelaidic (18:2trans)	1.0	1.0
linoleic (18:2cis)	12.0	9.0
γ lineolenic (18:3)	2.1	4.0
α linolenic (18:3)	8.6	5.4
CLA	1.6	3.2
C20:1	3.3	2.6
C20:4	13.6	5.4
C20:5	180.7	176.1
C24:1	7.1	7.4
C22:5	23.0	28.0
C22:6	118.4	58.4

Table 4
Fatty acid composition (mg/g of fat) of tank milk, pretreatment milk, and posttreatment milk

Fatty Acid	Tank Milk	Pretreatment	Treated
C 4:0	39.6	38.9	42.1
C 6:0	25.8	25.6	23.7
C 8:0	15.6	15.6	13.0
C10:0	36.6	37.4	28.2
C12:0	43.5	45.4	32.8
C14:0	117.5	125.5	118.1
C14:1	17.1	17.0	16.7
C15:0	13.9	12.6	10.0
C15:1	03.4	3.5	3.6
C16:0	282.0	321.9	279.8
C16:1	28.6	31.4	39.9
C17:0	8.2	7.3	7.0
C17:1	4.1	3.6	4.1
C18:0	84.5	81.5	52.1
C18:1	216.4	175.9	196.8
C18:2	22.9	20.9	30.0
C18:3γ	1.8	1.9	3.5
C18:3α	6.1	5.7	13.8
CLA	5.0	3.1	27.2
C20:4	8.8	7.3	17.5
C20:5	0.8	0.7	4.3
C22:5	0.5	0.5	2.0
C22:6	0.0	0.0	0.9
Tot. Omega-3 (mg/g)	6.9	6.4	21.0
Saturated (%)	66.7	71.2	60.7
Unsaturated (%)	31.6	27.2	36.0
Polyunsat'd (%)	5.8	5.1	9.9
Unidentified (%)	1.7	1.7	3.3

Table 7
Fatty acid composition (mg/g of fat) of milk from cows fed fish oil

Fatty Acid	Day 3	Day 7	Day 10	Day 14
C 4:0	29.3	29.4	28.2	29.6
C 6:0	18.4	18.4	19.7	18.6
C 8:0	11.2	11.0	12.8	11.6
C10:0	24.7	24.1	29.9	25.6
C12:0	29.2	28.2	36.4	31.0
C14:0	93.2	97.7	113.4	113.3
C14:1	8.8	8.5	14.4	15.5
C15:0	10.4	10.5	11.3	12.3
C15:1	4.4	4.0	3.6	1.8
C16:0	262.0	271.8	272.1	257.3
C16:1	11.9	12.4	20.9	24.5
C17:0	4.4	4.1	4.2	4.6
C17:1	5.9	6.1	5.8	6.2
C18:0	139.6	129.1	58.0	56.1
C18:1 Petroselaidic	3.3	4.7	7.1	6.9
C18:1 Elaidic	3.7	4.6	7.5	8.3
C18:1 Petroselinic	6.8	8.1	12.1	10.1
C18:1 Transvaccenic	12.7	20.6	68.8	61.1
C18:1 Oleic	221.7	196.6	143.1	143.0
C18:2	36.7	34.5	33.6	26.1
C18:3γ	1.9	3.5	2.2	2.6
C18:3α	6.3	7.0	6.5	8.1
CLA	4.3	6.9	27.0	24.7
C20:3	0	0	0	2.2
C20:4	2.4	2.3	2.9	2.8
C20:5	0	0	0	2.1
C22:5	0	0	0	1.4
C22:6	0	0	0	0
Tot. Omega-3 (mg/g)	6.3	7.0	6.5	11.6
Saturated (%)	65.0	65.2	61.2	58.5
Unsaturated (%)	35.0	34.0	37.8	36.6
Polyunsaturated (%)	5.4	5.7	7.5	7.3
Unidentified (%)	4.3	5.1	5.2	8.8

Table 8
Fatty acid composition (mg/g of fat) of milk from cows fed fish meal

Fatty Acid	Day 3	Day 7	Day 10	Day 14
C 4:0	30.5	32.0	33.3	32.6
C 6:0	15.2	20.6	21.3	20.7
C 8:0	11.2	12.6	12.8	12.6
C10:0	24.1	26.6	27.3	26.5
C12:0	27.8	30.0	31.8	31.1
C14:0	92.6	97.1	101.1	100.8
C14:1	6.9	9.2	9.9	11.7
C15:0	10.3	10.0	10.6	11.9
C15:1	3.8	3.6	4.2	4.0
C16:0	266.2	273.1	276.9	266.6
C16:1	11.7	15.3	16.4	19.7
C17:0	4.7	4.3	4.5	4.7
C17:1	5.9	5.3	5.7	5.8
C18:0	144.0	122.5	114.9	105.4
C18:1 Petroselaidic	3.0	3.2	2.7	2.9
C18:1 Elaidic	3.4	3.4	1.7	3.5
C18:1 Petroselinic	6.8	6.8	5.8	5.4
C18:1 Transvaccenic	13.1	14.2	13.5	14.3
C18:1 Oleic	220.6	215.5	215.1	218.1
C18:2	32.6	34.8	30.5	24.3
C18:3γ	2.0	2.4	3.1	3.8
C18:3α	6.4	6.0	6.6	8.3
CLA	4.7	5.7	5.8	9.5
C20:3	0	.6	.7	0
C20:4	2.2	2.3	2.4	2.2
C20:5	0	0	0	0
C22:5	0	0	0	0
C22:6	0	0	0	0
Tot. Omega-3 (mg/g)	6.4	6.0	6.6	8.3
Saturated (%)	65.5	65.7	66.3	64.0
Unsaturated (%)	34.5	35.1	34.7	35.8
Polyunsaturated (%)	5.3	5.7	5.5	5.5
Unidentified (%)	4.3	3.5	3.3	4.4

Table 9
Milk production and composition and feed intake of cows fed fish oil/fish meal diet

	Fish Oil						Fish Meal					
	Cow 5492			Cow 5436			Cow 5000			Cow 5535		
	Pre	Step-up	Full	Pre	Step-up	Fill	Pre	Step-up	Fill	Pre	Step-up	Fill
Milk Prod. (kg/d)	15	14.9	12.6	17.7	18.1	18.3	17.3	17.2	16.2	19.8	18.0	17.0
% Fat	4.5	---	3.4	4.0	---	3.6	4.4	---	4.4	4.6	---	4.5
% Protein	3.9	---	3.8	3.6	---	3.5	3.4	---	3.6	3.8	---	3.7
% Solids not Fat	13.8	---	12.6	13.2	---	12.6	13.1	---	13.3	14.1	---	13.8
Somatic Cell (x1000)	4220	---	486	1118	---	72	109	---	234	3332	---	1197
DMI (kg/d)[1]	26.5	27.6	30.7	22.9	25.0	21.1	22.2	22.1	19.9	19.7	19.9	17.3
Fish Oil Intake (kg/d)[2]	---	---	.61	---	---	.42	---	---	.07	---	---	.06

[1] Dry matter intake (DMI) is the average daily intake for day 1 through 3 (Pre), day 4 through 9 (Step-up), and day 10 through 16 (Full).

[2] Fish Oil Intake is the average daily intake for day 10 through 16 (Full).

WHAT IS CLAIMED IS:

1) A dairy product derived from raw milk produced comprising a fat fraction having a fatty acid composition including at least about 15 mg/g CLA and at least about 10 mg/g omega-3 fatty acid.

2) The dairy product of claim 1 wherein fatty acid composition further comprises at least about 25 mg/g transvaccennic acid.

3) The dairy product of claim 1 wherein fatty acid composition comprises at least about 20 mg/g CLA and at least about 50 mg/g transvaccennic acid.

4) The dairy product of claim 1 wherein said dairy product is milk, cheese, yogurt, butter, ice cream, buttermilk, sour cream, ghee, sherbert, anhydrous butter oil, anhydrous butter fat, powdered milk, condensed milk, evaporated milk, whey products, half & half, or cream.

5) The dairy product of claim 1 wherein said dairy product has a fat content of at least about 10 wt.%.

6) The dairy product of claim 1 wherein the fatty acid composition further comprises at least about 2.0 mg/g C20:5 fatty acid.

7) The dairy product of claim 1 wherein the fatty acid composition further comprises at least about 8 mg/g petroselinic acid.

8) Raw milk comprising at least about 15 mg CLA per gram of fat and at least about 10 mg omega-3 fatty acid per gram of fat, wherein said raw milk is produced by a ruminant fed a diet which includes fish oil.

9) The raw milk of claim 8 wherein the ruminant is fed a diet comprising about 1 % to about 4 % by dry weight of the fish oil.

10) The raw milk of claim 8 wherein the ruminant is fed the diet for at least two days.

11) The raw milk of claim 8 wherein the ruminant is a cow, deer, sheep, buffalo, or goat.

12) The raw milk of claim 8 wherein the fish oil comprises Menhaden fish oil, cod liver oil, mackerel oil, salmon oil, catfish oil, herring oil, or a mixture thereof.

13) The raw milk of claim 8 comprising at least about 20 mg CLA per gram of fat.

14) The raw milk of claim 8 further comprising at least about about 25 mg transvaccennic acid per gram of fat.

15) The raw milk of claim 8 further comprising at least about 8 mg/g petroselinic acid.

16) The raw milk of claim 8 further comprising at least about 8 mg/g α-linolenic acid.

17) A lipid composition derived from raw milk, wherein said raw milk comprises a fat fraction having a fatty acid composition including at least about 15 mg/g CLA and at least about 10 mg/g omega-3 fatty acid.

18) The lipid composition of claim 17 wherein the fatty acid composition further comprises at least about 25 mg/g transvaccennic acid.

19) The lipid composition of claim 17 wherein the fatty acid composition further comprises at least about 10 mg/g petroselinic acid.

20) The lipid composition of claim 17 wherein said lipid composition is a low-melting milk fat fraction.

21) A purified fatty acid composition derived from raw milk comprising a fat fraction having a raw fatty acid composition including at least about 15 mg/g CLA and at least about 10 mg/g omega-3 fatty acid.

22) The fatty acid composition of claim 21 wherein the raw fatty acid composition further comprises at least about 25 mg/g transvaccennic acid.