Annals of the University of Oradea, Fascicle: Ecotoxicology, Animal Husbandry and Food Science and Technology, Vol. XIX/B 2020
Analele Universitatii din Oradea, Fascicula: Ecotoxicologie, Zootehnie si Tehnologii de Industrie Alimentara, Vol.XIX/B 2020

THE CONTENT OF MILK IN BIOACTIE FATTY ACIDS WITH IMPLICATIONS FOR HUMAN HEALTH

Șánta Anita[1], Mierliță Daniel[2]*, Pop Ioan Mircea[3]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca 400372;
[2]University of Oradea, Department of Animal Science, Oradea City, Romania;
[3]University of Agricultural Science and Veterinary Medicine of Iasi, Faculty of Food and Animal Sciences, 8 Mihail Sadoveanu Alley, Romania; *dadi.mierlita@yahoo.com

Abstract

Milk fat is composed mainly of fatty acids. Cow's milk has a high concentration of saturated fatty acids and trans type fatty acids, which is why the consumption of milk and dairy products is associated with the occurrence of certain diseases, especially those of a cardiovascular nature and those associated with obesity. However, numerous studies have shown that polyunsaturated fatty acids include a special category of fats called omega 3 fatty acids and CLA (conjugated linoleic acid or omega 7 fatty acids), which are essential for the development and maintenance of human health. This bibliographic review aims to collect the main results regarding the content and nutritional quality of fats in cow's milk, with express reference to the structure and content of functional fatty acids with implications on the health of consumers of milk and dairy products.

Key words: milk, omega 3 fatty acids, CLA, atherogenic index (AI) and thrombogenic index (TI)

INTRODUCTION

The nutritional quality of milk and dairy products is a very important parameter, especially in terms of the links between food and human health, a vital area of research today. The international medical scientific world considers that dietary fats, food fats and especially those of animal origin, are responsible for certain diseases, especially those of a cardiovascular nature and those associated with obesity (Abu-Gazaleh et al. 2007; Hu et al., 2001). However, research initiated by Hu (2001) has shown that polyunsaturated fatty acids include a special category of fats called omega 3 fatty acids and CLA (conjugated linoleic acid or omega 7 fatty acids), which are essential for the development and maintenance of human health (ensures the development of nerve cells to children, prevents and treats cardiovascular diseases, protects and preserves the integrity of vascular endothelium, prevents obesity, has anti-cancer and antioxidant potential, etc.). They must be provided by food because they are not synthesized in the human body, especially CLA which is present only in milk and meat from ruminants (cattle, sheep, goats), resulting in the processes of biohydrogenation of linoleic acid (C18:2 c9 , c12) and linolenic acid (C18: 3 c9, c12, c15) from feed, by microorganisms in the rumen (Ip et. al., 2004).

Due to the positive effects that Omega 3 and CLA fatty acids have on the human body, foods enriched in these fatty acids are included in the category of "functional foods".

As a result, the consumer understands by quality, a product safe for his health (unpolluted), which in addition to the intake of nutrients must also contain a series of active biocomponents with sanogenic effect (Omega-3 fatty acids, conjugated linoleic acid (CLA), *trans*-vaccine acid (VA), antioxidants, vitamins, microelements, etc.), with an important role in ensuring the "quality of life". Consequently, a new approach is needed to the concept of quality of agri food products, which should represent as accurately as possible the interests and preferences of consumers.

The lipid fraction of milk and meat has been considered to have negative effects on consumer health, due to its high content of saturated fatty acids and *trans* type fatty acids, which are responsible for cardiovascular and obesity-associated diseases. However, numerous studies have shown that whole milk is more effective in preventing cardiovascular disease in humans than skim milk (Steinmetz et al., 1994). These positive effects are attributed to the functional fatty acids (FA) present in whole milk fat. Functional fatty acids are represented by: omega-3 fatty acids, conjugated linoleic acid (CLA; isomer C18:2 *cis*-9, *trans*-11; also called rumenic acid-RA and isomer C18:2 *trans*-10, *cis*-12) and *trans*-vaccenic acid (C18:1 *trans*-11; VA).

MILK FAT CONTENT AND STRUCTURE

Milk fat has very wide variation limits, between 2.5 and 7.5%. Mainly milk fats are triglycerides, complex lipids and free fatty acids. Triglycerides, which have a share 95% of the lipid fraction, are composed of fatty acids of different chain lengths (between 4 and 24 C atoms) and different degrees of saturation.

The largest share in milk fats is fatty acids, which represent about 90% of their weight. Over 95% of fatty acids are found in the form of triglycerides, the rest in the form of mono- and diglycerides, phospholipids and in the form of cholesterol esters. Free fatty acids are present in a small proportion (Kay et al., 2004).

Cow's milk is composed of an average of 4% fat, of which 97-98% are triacylglycerols (Jensen, 2002). Milk fat usually contains a high proportion of saturated fatty acids (70% of the total identified fatty acids) and monounsaturated fatty acids (MUFA, 25.6%), to which are added small amounts of polyunsaturated fatty acids (PUFA, 3,3%). *Trans* type fatty acids represent for about 4% of total fatty acids (Ferlay and colab., 2008; Shingfield and colab., 2008). However, these average values can be greatly altered by various animal-dependent factors (breed, age, lactation stage) and nutritional factors (supplementation of feed content with fat, nature of feed, mode of preservation of feed, amount of food concentrates, etc.). The effects of genetic and physiological factors are limited, while major changes in the FA composition of milk can be induced by nutritional manipulation (Ferlay et al., 2011).

SATURATED FATTY ACIDS (SFA)

More than half of the fatty acids in milk are saturated (approximately 65-70%), constituting about 19 g / l milk. The most important saturated fatty acid in milk is palmitic acid with a share of 30% of total saturated fatty acids.

SFA from milk has a dual origin: long-chain FA (C16 and above) are taken from plasma lipoproteins (on average 60%), and short and medium chain FA (4: 0 to 16: 0) are synthesized de novo (on average 40%) in the mammary gland from acetic acid and beta-hydroxybutyrate, from rumen fermentation (Chilliard and Ferlay, 2004).

There is evidence that SFA in food leads to increased serum concentrations of low-density cholesterol (LDL-bad cholesterol) (Givens, 2010), a predictor of the risk of cardiovascular disease. However, such effects are related only to lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0), while other saturated fatty acids have neutral or positive effects on human health. (Mensink et al., 2003).

In many European countries, milk and dairy products supply on average approx. 40% of the total SFA contribution. As a result, there has been a great deal of interest in manipulating the FA profile of milk fat to address consumer concerns. The main monounsaturated fatty acid (MUFA) in milk is oleic acid (C18:1 *cis*-9), followed by 18:1 *trans*. Replacing SFA in human food with oleic acid has been shown to reduce the frequency of cardiovascular disease (Lopez-Huertas, 2010). In contrast, *trans* fatty acids if consumed in excess have led to an increase in the frequency of coronary heart disease (Schingfield et al., 2008; Givens, 2010).

However, recent data seem to indicate that in the case of milk trans-18:1 fatty acids may have beneficial effects in reducing the incidence of cardiovascular disease (the positive effects are greater for *trans* 10- than *trans* 11-18:1) (Roy et al., 2007). Several studies have shown that diets containing low-fat dairy products have been associated with favorable changes in serum cholesterol content.

MONOUNSATURATED FATTY ACIDS

Monounsaturated fatty acids represents for about 25% of total milk fatty acids. Oleic acid (18: lc9) is the unsaturated fatty acid with the highest concentration found in milk (23.8% of MUFA), constituting about 8 g / l in the case of cow's milk. Therefore, milk and dairy products contribute substantially to the intake of oleic acid in most countries.

Oleic acid has beneficial effects on health, as diets high in monounsaturated fatty acids can lower LDL plasma cholesterol and triglyceride levels.

POLYUNSATURATED FATTY ACIDS (PUFA)

The content of cow's milk in polyunsaturated fatty acids is on average 2 g /l.

Linoleic (18:2n-6) and α-linolenic (18:3n-3) acids are the main polyunsaturated fatty acids in milk fat. These FA cannot be synthesized in the body and must be obtained from the diet. These FA can be metabolized in the body contributing as precursors to form arachidonic acid (AA, C20:4 n-6) and eicosapentaenoic acid (EPA, C20:5 n-3), which are precursors for prostaglandin synthesis (Palmquist, 2009).

OMEGA-3 FATTY ACIDS

Omega-3 fatty acids are polyunsaturated fatty acids that have in common a carbon-carbon double bond in the catenary position 3. The most important FA n-3 (omega-3) with sanogenic effect are: C18:3n-3 (α-linolenic acid, ALA); C20:5n-3 (eicosapentaenoic acid-EPA) and C22:6n-3 (docosahexaenoic acid-DHA). The importance of functional AF lies in the role they have in the human diet. N-3 fatty acids: reduce the level of LDL (bad cholesterol) in the blood and increase the level of high density lipoproteins (HDL-good cholesterol) which play an important role in preventing cardiovascular disease; reduce high blood pressure; regulates hormonal secretions; intervene in the therapy of arthritis and inflammatory processes (Rubino et. al., 2006); protects and preserves the integrity of vascular endothelium; stimulates the development of nerve cells in children (Hu, 2001).

FA n-3, and especially EPA and docosahexaenoic acid (DHA, C22: 5 n-3), could reduce the risk of cardiovascular disease (Mills et al., 2011).

CONJUGATED LINOLEIC ACID (CLA)

Conjugated linoleic acid (CLA) is in fact a mixture of several isomers of double conjugated linoleic acid, located mainly on carbon atoms 9 and 11 (*cis*-9, *trans*-11 C18:2 = rumenic acid, which represents 80-90% of total CLA isomers).

CLA is provided in human food by agri-food products from ruminants, being synthesized either by rumen biohydrogenation of unsaturated fatty acids in food, or by endogenous transformation of trans-vaccenic acid (C18:1 *trans*-11) in the presence of the enzyme Δ^9-desaturase, in different tissues of the body (mainly the mammary gland). *Trans*-vaccine acid, which is the substrate for the enzymatic synthesis of CLA, comes, as an intermediate, from the rumen biohydrogenation of mono- and polyunsaturated fatty acids in food. Complete rumen biohydrogenation of VA leads to the formation of stearic acid (C18:0). Of the total CLA c9, t11 present in milk, 70-90% comes from the enzymatic desaturation of VA (C18:1 t11) in the mammary gland, while the rest, including the other CLA isomers (especially CLA *cis*-12, *trans*-10) results as intermediates in the process of rumen biohydrogenation of unsaturated fatty acids in food (Chilliard et al., 2007). The level of CLA in milk depends mainly on the level of linoleic acid (C18:2n-6) and linolenic acid (C18:3n-3) in animal feed and on the intensity of the activity of steroil-coenzyme A desaturase, given the value of the desaturation index of substrate (DI 18:2 c9, t11) (Mierlita, 2016). Differences in rumenic acid concentrations in milk fat can also be explained by differences in Δ^9- desaturase activity, caused by a number of animal-dependent or nutritional factors (Griinari and Bauman, 1999; Giorgio et al., 2019). The CLA C18:2 tans-10, *cis*-12 isomer appears to be synthesized exclusively in the rumen because the existence of a Δ^{12}-desaturase in the mammary gland has not been demonstrated (Griinari and Bauman, 1999).

Research on experimental animal models has shown that CLA has an anticancer effect, prevents obesity by reducing the body's lipoforming capacity, has an antioxidant role (reduces oxidative degradation of polyunsaturated fatty acids in the structure of cell membranes) and prevents atherosclerosis (Rubino et al., 2006) has immuno-modulatory action. It is noteworthy that cow's milk contains 9 times less Omega 3 and CLA polyunsaturated fatty acids than women's milk; Rossant (2003) explaining in this way the better development of the brain and the higher level of intelligence found in breastfed infants. A similar conclusion emerges from the research of Andrew (2004) who found that low consumption of PUFA Omega 3 and CLA leads to an increase in the frequency of depressive states (eg in New Zealand 6% of the population suffers from depression compared to only 1% in Japan where consumption of FA Omega 3 and CLA is 4 times higher).

VACCINE ACID (VA)

Although *trans* type fatty acids have negative effects on human health, *trans*-vaccenic acid (*trans*-11, C18:1), which accounts for 60-80% of all *trans* type fatty acids in milk and meat, has been shown to be (in experiments on human cell lines) that it has beneficial effects on the human body, because in human tissues through a process of elongation and desaturation it is transformed into conjugated linoleic acid (CLA).

To evaluate the nutritional quality of animal fats, in terms of impact on consumer health, Pilarczyk et al., (2015) recommend the calculation of sanogenic lipid indices: PUFA / SFA ratios, n-6 / n-3 FA, atherogenic index (AI), thrombogenic index (TI), desaturation index (DI) and hypocholesterolemic / hypercholesterolemic fatty acid ratio (h/H). Nutritionists believe that the ratio of n-6/n-3 fatty acids should be below 4: 1, and that of PU FA/SFA should be above 0.45, to avoid the occurrence of cardiovascular diseases and those associated with obesity (Simopoulos, 2006). Fats that have a high atherogenic (AI) and thrombogenic index implicitly have a low h/H ratio are recognized as major risk factors for human health (Bucher et. Al., 2002; Sadation-Elahi et al., 2004).

The content of agri-food products in functional fatty acids and implicitly the value of sanogenic lipid indices are influenced on the one hand by genetic and physiological factors and on the other hand by a series of nutritional factors. Most studies have shown that nutritional factors are sovereign in manipulating the fatty acid profile and improving the sanogenic lipid indices of milk and meat fat (Chilliard et al., 2007).

ASSESSMENT OF THE NUTRITIONAL QUALITY OF MILK FATS (LIPID INDICES HEALITY)

PUFA/SFA ratios, n-6/n-3 FA, atherogenic index (AI) and thrombogenic index (TI) are commonly used to assess the nutritional quality of milk fats in terms of impact on consumer health (Pilarczyk et al. , 2015). In general, a PUFA/SFA ratio greater than 0.45 and an n-6/n-3 ratio less than 4.0 are required in the human diet to prevent and even combat "lifestyle diseases" such as coronary heart disease and cancer (Simopoulos, 2002).

The atherogenic and thrombogenic index characterizes dietary fats in terms of the probability of increasing the incidence of pathogenic phenomena, such as the formation of atheroma plaques and/or blood thrombi (Pilarczyk et al., 2015). Addis et al., (2005) demonstrated that there is a direct correlation between the content in saturated FA and the value of these indices (AI and TI) in dietary fats. It is believed that milk fat with high levels of AI and TI may contribute more to the development of atherosclerosis or coronary thrombosis in humans; but milk with a high h/H ratio can have a protective effect against cardiovascular disease (Hanus et al., 2018).

HPI (health promotion index) was proposed by Chen et al. (2004) as an indicator of the sanogenic quality of dietary fats and is based on the evaluation of the effect of some fatty acids in the structure of milk fats on cardiovascular diseases. Milk with a high HPI value is thought to be more beneficial to human health.

The calculation of the sanogenic lipid indices of milk fat is done using the following equations:

- n-6/n-3 = (C18:2n-6 + C18:3n-6 + C20:4n-6) / (C18:3n-3 + C20:5n-3 + C22:3n-3 + C22:5n-3 + C22:6n-3) (Ellis et al. 2006);
- Atherogenic index (AI) = (C12:0 + (C14:0 x 4) + (C16: 0) / MUFA + PUFA) (Chilliard et al, 2003);
- Thrombogenic index (TI) = (12:0 + 16:0 + 18:0) / [(0.5 x MUFA) + (0.5 x n-6FA) + (3 x n-3FA) + (n-3FA/n -6FA)], (Ulbricht & Southgate, 1991);
- Health Promotion Index (HPI) = (n-3 PUFA + n-6 PUFA + MUFA) / [C12: 0 + (4 x C14: 0) + C16: 0], (Chen et al. 2004);
- Hypocholesterolemic / Hypercholesterolemic fatty acid ratio (h/H) = (C18:1 + PUFA) / (C12:0 + C14:0 + C16:0) (Pilarczyk et al., 2015);

At the level of the mammary gland, enzymatic desaturation processes performed by Δ^9 - desaturase take place. The activity of this enzyme can be measured indirectly by comparing the product: substrate or product: (substrate + product) ratio of certain fatty acids. Therefore, the C14:1 / C14:1 + C14:0 ratio is the best indicator of this activity, because all the amount of C14:0 in milk fat comes from de novo synthesis in the mammary gland (Lock et al. 2005).

The enzyme Δ^9 - desaturase acts on the mammary gland and other tissues and adds a double bond in the Δ^9 position (between carbons 9 and 10 of saturated fatty acids with a chain length of 10 to 18 carbon atoms) and thus converts myristic acid (14:0) to myristoleic acid (*cis*-9 14:1), palmitic acid (16:0) to palmitoleic acid (*cis*-9 16:1), stearic acid (18:0) to oleic acid (*cis*-9 18:1) and vaccenic acid (VA; *trans*-11 18:1) to the conjugated linoleic acid isomer, respectively rumenic acid (*cis*-9, *trans*-11 18:2). Garnsworthy et al., (2010) suggest that the activity of the enzyme Δ^9 - desaturase, has an important genetic component, which means that the level of CLA *cis*-9, *trans*-11 in milk, is determined on the one hand by race, and on the other. on the other hand it is due to rumen bacteria that favor the formation of acetic acid and promote the production of VA by biohydrogenation especially of linoleic acid (Kucuk et. al. 2001).

Enzymatic desaturation indices are calculated based on mathematical equations; their high values indicating an intensification of the substrate desaturation processes, respectively a higher proportion of unsaturated fatty acids in milk coming from the enzymatic desaturation of saturated fatty acids at the level of the mammary gland:

- DI (18) - index Δ^9-desaturase (18) = 100 [18: 1 / (18:1 + 18:0)] (Pilarczyk et al., 2015);
- DI (18:2 c9,t11) – index Δ^9-desaturase (18:2 c9,t11) = 100 [18:2 c9,t11 / (18:2 c9, t11 + 18:1 t11)], (Pilarczyk et al., 2015).

Although a higher proportion of PUFA in milk fat is desirable from the perspective of the effect on human health, they can influence the technological properties of milk fat in a positive way (butter becomes easily spreadable) or negatively (it increases the susceptibility to oxidation of PUFA, with formation of aldehydes and ketones toxic to the consumer). Thus, indices such as (Hanus et al., 2018) have been proposed:

- the peroxidability index (PI), which represents the degree of unsaturation of food lipids and is used as an indicator of PUFA peroxidation;

PI = 0.025 - Mono + Di + 2 - Tri + 4 - Tetra + 6 - Penta + 8 – Hexa where: Mono, Di, Tri, Tetra, Penta and Hexa represent the percentages of monoenoic, dienoic, trienoic, tetraenoic, pentaenoic and hexaenoic fatty acids.

- the spreading index (SI), for the evaluation of the ratio C16:0 and C18:1 c9, being the most precise indicator of the hardness of the butter (SI = C 18:1 c9/C 16:0).

THE EFFECT OF NUTRITIONAL FACTORS ON BIOACTIVE FATTY ACIDS IN MILK

Nutritional factors (nature of feed, type and structure of feed ration, fat supplement in food and their degree of saturation), in addition to the direct influence they have on bioproductive performance have a major importance in manipulating the structure and content of fatty acids Omega 3 and CLA polyunsaturated fats of animal origin (Khanal et. al., 2004; Palmquist, 2007; Tomasz, 2007).

It is unanimously acknowledged that nutritional factors are sovereign in modulating the fatty acid profile of cow's milk (Rego et al., 2004; Flori et al., 2008; Gomez-Cortes et al., 2008; De La Fuente et al. al., 2009; Nuda et al., 2020). By manipulating the diet, the milk fat content in n-3 FA and CLA can be changed up to five times (Gomez-Cortes et al., 2008, 2009; De La

Fuente et al., 2009). The largest increases in the concentration of n-3 FA and CLA in milk fat are obtained by the use of fresh grass in food, which is rich in PUFA (especially linolenic acid and linoleic acid) and their rumen biohydrogenation results a larger amount of intermediates such as vaccine acid (VA, C18:1, *trans*-11) (Addis et al., 2005; Abu Ghazaleh et al., 2007; Mikolayunas et al., 2008; Herves et al. . al., 2009) or by supplementing the diet with fats (Martini et. al., 2004; Melle et. al., 2007; Pulina et. al., 2006; Sanz-Samplelayo et. al., 2007).

The effect of the season is closely related to the type of diet; the most significant variations were observed in PUFA, with the highest values recorded in spring and summer and the lowest in winter (De La Fuente et. al., 2009). Banni et. al., (1996) noted that sheep's milk is much richer in n-3 FA and CLA compared to cow's milk; One possible reason could be that sheep are fed on pastures, and dairy cows are generally fed preserved and concentrated feed.

CONCLUSIONS

Consumer awareness of the link between dietary fats and health has led to increased demand and intensified research in the field of obtaining foods enriched with bioactive fatty acids. Milk fats and dairy products, contribute significantly to the rational diet of people, containing many beneficial FA (some even unique), such as omega-3 fatty acids (C18:3 c9, c12, c15; C20:5 n-3, C22:5 n-3), linoleic acid conjugated to the two important isomers (CLA: C18:2 c9, t11 and C18:2 t10, c12) and vaccenic acid (VA, C18:1 t11). Increasing these FAs in the structure of milk fats and dairy products, through sustainable cow farm management practices (such as breed, lactation phase) but especially by manipulating nutritional factors (nature of feed, type and structure of feed ration, use fresh green pastures or fodder, feed preservation, feed/concentrate ratio in food, fat supplement in food and their degree of saturation), can improve the health of consumers without a change in diet.

REFERENCES

1. Addis M., Cabiddu A., Pinna G., Decandia M., Piredda G., Pirisi A., Molle G. (2005): Milk and cheese fatty acid composition in sheep fed Mediterranean forages with reference to conjugated linoleic acid *cis*-9, *trans*-11. J. Dairy Sci. 88: 3443-3454.
2. Bauman D.E., Mather I.H., Wall R.J., Lock A.L. (2006): Major advances associated with the biosynthesis of milk. J. Dairy Sci. 89:1235-1243.
3. Chilliard, Y., and A. Ferlay (2004). Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. Reprod. Nutr. Develop. 45:467-492.

4. Chilliard, Y., C. Martin, J. Rouel, and M. Doreau (2009). Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed or linseed oil, and their relationship with methane output. J. Dairy Sci. 92:5199-5211.
5. Chilliard, Y., F. Glasser, A. Ferlay, L. Bernard, J. Rouel, and M. Doreau (2007). Diet, rumen biohydrogenation, cow and goat milk fat nutritional quality: a review. Eur. J. Lipid Sci. Technol. 109:828-855.
6. Collomb, M., U. Bütikofer, R. Sieber, B. Jeangros, and O. Bosset (2002). Composition of fatty acids in cow's milk fat produced in the lowlands, moutains and highlands of Switzerland using high-resolution gas chromatography. Int. Dairy J. 12:649-659.
7. Coppa, M., A. Ferlay, F. Monsallier, I. Verdier-Metz, P. Pradel, R. Didienne, and A. Farruggia (2011). Milk fatty acid composition and cheese texture and appearance from cows fed hay or different grazing systems on upland pastures. J. Dairy Sci. 94:1132–1145.
8. Drackley, J.K., A.D. Beaulieu, and J.P. Elliott (2001). Responses of milk fat composition to dietary fat or nonstructural carbohydrates in Holstein and Jersey cows. J. Dairy Sci. 84:1231-1237.
9. Ellis K.A., Innocent G., Grove-White D., Cripps P., McLean G.W., Hovard C.V., Mihm M. (2006): Comparing the fatty acid composition of organic and conventional milk. J. Dairy Sci. 89: 1938-1950.
10. Falchero, L., G. Lombardi, A. Gorlier, Lonati M., M Odoardi, and A. Cavallero (2010). Variation in fatty acid composition of milk and cheese from cows grazed on two alpine pastures. Dairy Sci. Technol. 90:657-672.
11. Ferlay A., Martin B., Pradel Ph., Coulon J.B., Chilliard Y. (2006): Fatty acid composition and milk lipolytic system in Tarentaise and Montbeliarde cow breeds. J. Dairy Sci. 89: 4026-4041.
12. Ferlay, A., B. Martin, S. Lerch, Ph. Pradel, and Y. Chilliard (2011). Effects of supplementation of maize silage diets with extruded linseed, vitamin E and plant extracts rich in polyphenols, and morning vs. evening milking on milk fatty acid profiles in Holstein and Montbéliarde cows. Animal 4:627-640.
13. Ferlay, A., C. Agabriel, C. Sibra, C. Journal, B. Martin, and Y. Chilliard (2008). Tanker milk variability of fatty acids according to farm feeding and husbandry practices in a French semi-mountain area. Dairy Sci. Technol. 88:193-215.
14. Garnsworthy P.C., Feng S., Lock A.L., Royal M.D. (2010): Short communication: Heritability of milk fatty acid composition and stearoyl-CaA desaturase indices in dairy cows. J. Dairy Sci. 93(4): 1743-1748.
15. Glasser, F., A. Ferlay, and Y. Chilliard (2008). Oilseed lipid supplements and fatty acid composition of cow milk: a meta-analysis. J. Dairy Sci. 91:4687-4703.
16. Hurtaud, C., F. Faucon, S. Couvreur, and J.L. Peyraud (2010). Linear relationship between increasing amounts of extruded linseed in dairy cow diet and milk fatty acid composition and butter properties. J. Dairy Sci. 93:1429-1443.
17. Jenkins T.C., 1993, Lipid metabolism in the rumen. J. Daity Sci. 76, 3851-3863.
18. Jensen, R.G. (2002). The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85:295-350.
19. Kay J.K., Weber W.J., Moore C.E., Bauman D.E., Hansen L.E., Hansen L.B., Chester-Jones H., Crooker B.A., Baumgard L.H. (2005) : Effects of week of lactation and genetic selection for milk yield on milk fatty acid composition in Holstein cows. J. Dairy Sci. 88:3886-3893.
20. Kelsey J.A., Corl B.A., Collier R.A., Bauman D.M. (2003): The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. J. Dairy Sci. 86:2588-2597.

21. Lerch, S., A. Ferlay, D. Pomiès, B. Martin, J.A.A Pires, and Y. Chilliard (2011). Rapeseed or linseed supplementation of grass-based diets: effects on dairy performance of Holstein cows over two consecutive lactations. J. Dairy Sci. In press.
22. Lopez-Huertas, E. (2010). Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies. Pharmacol. Res. 61:200-207.
23. Mills, S., R.P. Ross, C. Hill, G.F. Fitzgerald, and C. Stanton (2011). Milk intelligence: mining milk for bioactive substances associated with human health. Int. Dairy Sci. 377-401.
24. Palladino R.A., Buckley F., Prendiville R., Murphy J.J., Callan J., Kenny D.A. (2010): A comparasion between Holstein-Friesian and Jersey dairy cows and their F1 hybrid on milk fatty acid composition under grazing conditions. J. Dairy Sci. 93(5): 2176-2184.
25. Palladino, R.A., F. Buckley, R. Prendiville, J.J. Murphy, J. Callan, and D.A. Kenny (2010). A comparison between Holstein-Friesian and Jersey dairy cows and their F(1) hybrid on milk fatty acid composition under grazing conditions. J. Dairy Sci. 93:2176-84.
26. Palmquist, D.L. (2009). Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. The Professional Animal Scientist, 25:207-249.
27. Peterson D.G., Kelsey J.A., Bauman D.E. (2002): Analysis of variation in *cis*-9, *trans*-11 conjugated linoleic acid (CLA) in milk fat dairy cows. J. Dairy Sci. 85:2164-2172.
28. Petit, H.V. (2010). Review: feed intake, milk production and milk composition of dairy cows fed flaxseed. Can. J. Anim. Sci. 90: 115-127.
29. Pottier, J., M. Focant, C. Debier, G. De Buysser, C. Goffe, E. Mignolet, E. Froidmont and Y. Larondelle (2006). Effect of dietary vitamin E on rumen biohydrogenation pathways and milk fat depression in dairy cows fed high-fat diets. J. Dairy Sci. 89:685-692.
30. Rego O.A., Alves S.P., Antunes L.M.S., Rosa H.J.D., Alfaia C.F.M., Prates J.A.M., Carita A.R.J., Fonseca A.J.M., Bessa R.J.B. (2009): Rumen biohydrogenation-derived fatty acids in milk fat from grazing dairy cows supplemented with rapeseed, sunflower, or linseed oils. J. Dairy Sci. 92: 4530-4540.
31. Roy, A., A. Ferlay, K.J. Shingfield, and Y. Chilliard (2006). Examination of the persistency of milk fatty acid composition responses to plant oils in cows fed different basal diets, with particular emphasis on trans-C18:1 fatty acids and isomers of conjugated linoleic acid. Animal Sci. 82:479-492.
32. Roy, A., J.M. Chardigny, D. Bauchart, A. Ferlay, S. Lorenz, D. Durand, D. Gruffat, Y. Faulconnier, J.L. Sébédio, and Y. Chilliard (2007). Butters rich either in *trans*-10-C18:1 or in *trans*-11-C18:1 plus *cis*-9, *trans*-11 CLA differentially affect plasma lipids and aortic fatty streak in experimental atherosclerosis in rabbits. Animal. 1:467-476.
33. Shingfield, K.J., L. Bernard, C. Leroux, and Y. Chilliard (2010). Role of *trans* fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal 4:1140-1166.
34. Shingfield, K.J., Y. Chilliard, V. Toivonen, P. Kairenius, and D.I. Givens (2008). *Trans* fatty acids and bioactive lipids in ruminant milk. in: Bioactive components

of milk (Ed. Z. Bösze, Springer, USA) Advances in Experimental Medicine and Biology. 606:3-65.

35. Simopoulos A.P. (1999): Essential fatty acids in health and chronic disease. Am. J. Clin. Nutr. 70: 560-569.
36. Simopoulos A.P. (2008): The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp. Biol. Med. 233:674-688.
37. Soyeurt, H., F. Dehareng, N. Gengler, S. McParland, E. Wall, D.P. Berry, M. Coffey, and P. Dardenne (2011). Mid-infrared prediction of bovine milk fatty acids across multiple breeds, production systems, and countries. J. Dairy Sci. 94:1657-1667.
38. Soyeurt, H., P. Dardenne, F. Dehareng, G. Lognay, D. Veselko, M. Marlier, C. Bertozzi, P. Mayeres, and N. Gengler (2006). Estimating fatty acid content in cow milk using mid-infrared spectrometry. J. Dairy Sci. 89:3690-3695.
39. Thomson N.A., Chand A., Kay J.K. (2003): Predicting Δ^9 – desaturase activity and the association with conjugated linoleic acid (CLA) concentration in bovine milk. Proc New Zealand Soc Anim Prod, Queenstown, New Zealand: 25-30.
40. Thorsdottir I., Hill J., Ramel A. (2004): Seasonal variation in *cis*-9, *trans*-11 conjugated linoleic acid (CLA) content in milk fat from the Nordic Countries. J. Dairy Sci. 87:2800-2802.
41. White, S.L., J.A. Bertrand, M.R. Wade, S.P. Washburn, J.T. Jr Green, and T.C. Jenkins (2001). Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 84:2295-2301.
42. Yassir M.A., Arifah A.K., Yaakub H., Zuraini A., Zakaria Z.A. (2010): Comparison of conjugated linoleic acid and other fatty acid content of milk fat of Mafriwal and Jersey cows. J. of Anim. And Vet. Advances 9 (9): 1318-1323.