



# Milk Fatty Acids Profile and the Impact on Human Health



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## Abstract

Milk fat is the component that shows major sensitivity to modification through animal feed, which allows to manipulate the fatty acids profile. This review approached the milk fatty acids composition, physiological mechanisms and dietary modifications that may lead to changes in the fatty acids profile and their influence on human health. The proportions of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) on milk have variations that are associated with many factors and their interactions. In the rumen, the microorganisms promote changes in the diet fatty acid profile through biohydrogenation, factor that may difficult the study of different nutritional management in the final product composition, and because that, studies should match the lipid profile with the ruminal microbiota arrangement. In the mammary gland, fat formation is a complex process which milk fatty acids are derived from "de novo" synthesis in epithelial cells and from long chain fatty acids arising from diet and adipose tissue mobilization, the last one in negative energy balance. For beneficial effects on human health, a lower n-6: n-3 ratio may reduce the risk of coronary artery disease. Future studies may continue including modifications in the fatty acid profile using different diets, but at a molecular level and with effects on gene expression of enzymes involved in the milk fat formation process. In addition, animals' genetic manipulation and selection to obtain products with desired specific composition may be more deepen in the next studies.

**Keywords:** Biohydrogenation; Nutritional handling; Genetic selection

**Abbreviations:** SFA: Saturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; CLA: Conjugated Linoleic Acid; VFA: Volatile Fatty Acids; NEB: Negative Energy Balance; LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein

## Introduction

The continuous increase of the world population has resulted in higher cow's milk consumption, which explain the annual per capita consumption parameter of 284.5 liters of milk equivalent on USA [1] and 111.4 of world average [2]. In addition to the demand for quantity, the consumers are paying more attention to the organic milk, as well as for milk and derived products with greater concentrations of desirable fatty acids, which would make them healthier [3,4]. In the last 20 to 25 years many researchers have compared the nutrient concentrations of milk, studying the possibilities and limitations resulting of cow's diet manipulation, however, the great majority focused on fat composition [4,5]. This is due to the milk fat sensibility to modifications through diets be higher than the protein or lactose's sensibility, which allow to manipulate the fatty acids profile by feeding the animals with different nutrients sources [5]. According to Jenkins & McGuire [5], the amount of fat which could be modified around 3 percentage points, while the protein content in 0.5 and the lactose, in turn, cannot be manipulated through diet, except when used extreme and not usual diets. Moreover, there are changes in this proportions during the lactation and according to the milk composition of the livestock breeds.

Considering the significant variations on milk fat content and composition due to changes on diet, this review's purpose was to evaluate the milk fatty acids profile, the physiological mechanisms that could lead to changes in this profile and its influence on human health. In order to review scientific articles published in recent years, from authors that, through animals' diet modifications, attempted to manipulate the milk fatty acids profile.

## Overall Composition of Milk Fatty Acids

The milk fat is composed by 98% of triglycerides, 1% phospholipids and 0.5% of sterols, which around 70% are saturated fatty acids (SFA) and the proportion of polyunsaturated (PUFA) and C18:2 and C18:3 range from 3% to 1% in relation to the amount of total lipids [6]. Data that agree with the nutritional composition of fluid milk, according to the Table 1. The milk composition in general follow this pattern, nonetheless, it's necessary ramify this quantity in the main fatty acids (Table 2). The SFA present in higher quantity on milk is the palmitic (C16:0), which represents round 40% of the total SFA. Related to the amount of monounsaturated fatty acids (MUFA) and PUFA, the oleic (C18:1) and the linoleic (C18:2) acids are the most representatives. In this

amount are included the cis and trans fatty acids. The main trans fatty acid of milk is the C18:1 *trans-11*, called vaccenic acid [7]. Furthermore, it contains quantities of conjugated linoleic acid (CLA), with predominance of the isomer CLA *cis-9, trans-11* [8]. However, this fatty acid profile suffers changes associated to many factors, like genetic quality of livestock, stage of lactation, ruminal fermentation or, even, fiber and energy intake and seasonal impacts [9]. This is due to milk fatty acids source, which comes from diet, microbial synthesis, body fat and from the “*de novo*” synthesis on the mammary gland [10-12].

**Table 1:** Nutritional composition of fluid milk.

Nutrient	Amount on milk composition (%) <sup>1</sup>
Water	87.690
Proteins	3.280
Carbohydrates (per difference)	4.650
Total lipids	3.660
SFA <sup>2</sup>	2.278
MUFA <sup>3</sup>	1.057
PUFA <sup>4</sup>	0.136
Cholesterol	0.014

Source: USDA National Nutrient Database for Standard Reference [61].

<sup>1</sup>Amount of each nutrient on milk total composition.

<sup>2</sup>Saturated fatty acids.

<sup>3</sup>Monounsaturated fatty acids.

<sup>4</sup>Polyunsaturated fatty acids.

**Table 2:** Profile of main fatty acids of fluid milk.

Component	Amount on milk composition (%) <sup>1</sup>
SFA <sup>2</sup>	2.278
4:0	0.119
6:0	0.070
8:0	0.041
10:0	0.092
12:0	0.103
14:0	0.368
16:0	0.963
18:0	0.444
MUFA <sup>3</sup>	1.057
16:1	0.082
18:1	0.921
PUFA <sup>4</sup>	0.136
18:2	0.083
18:3	0.053

Adapted from USDA National Nutrient Database for Standard Reference [61].

<sup>1</sup>Amount of each fatty acid on milk total composition (SFA, MUFA or PUFA).

<sup>2</sup>Saturated fatty acids.

<sup>3</sup>Monounsaturated fatty acids.

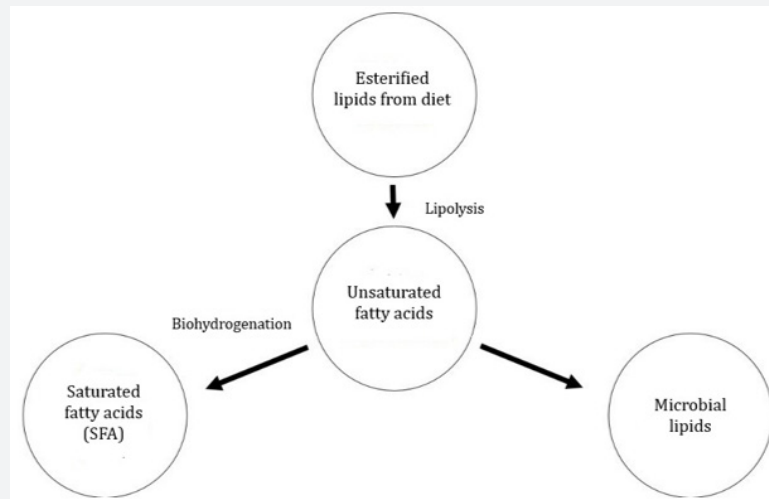
<sup>4</sup>Polyunsaturated fatty acids.

### Fatty Acids Modifications on Rumen

The rumen has a complex microbiota responsible to digest vegetable components, which large part is indigestible for monogastrics, and for these products provision to the ruminant, that, in turn, it transforms them in foodstuff like milk and beef [13]. In this compartment are present several microorganisms like bacteria, fungi and protozoa. The bacteria are in greater quantity and represents around 95% of rumen microbiota [14]. Jami & Mizrahi [13] studied the ruminal bacteria composition of 16 Holstein cows and found that 92.6% were from Phylum *Firmicutes* (41.6%) and *Bacteroidetes* (51%). Other bacteria were found, such as from Phylum *Proteobacteria*, *Actinobacteria* and *Tenericutes*, in proportions of 5.46%, 0.86% and 0.69%, respectively. Another Phylum were grouped and represented 0.37%.

The fungi and protozoa are present in lower amount on rumen, nonetheless, it performs the same important role such as the other microorganisms. The fungi zoospores, after adhering to the particles, it attacks, encyst and germinate on the stomas or fractured sites in the vegetal tissue [15]. Nevertheless, also according to Orpin [15] the protozoa adhere to close particles or engulf and degrade plants fragments which contains chlorophyll and starch granules. The bacteria perform the biohydrogenation of fatty acids on rumen, modifying them in order to convert unsaturated fatty acids in others with lower number of unsaturation [16]. The biohydrogenation occurs in various steps of reduction and numerous bacteria are involved, which the active specie may depend of fatty acid unsaturation degree, but the *Butyrivibrio proteoclasticus* can convert PUFA directly to SFA [17].

If the feed remains on rumen, a small quantity of fatty acids is absorbed by microorganisms and catabolized in volatile fatty acids (VFA) and CO<sub>2</sub>, nevertheless, these microorganisms synthetize fatty acids through carbohydrates precursors and hydrogenate unsaturated fatty acids [18]. Thus, as a result of fatty acids modifications caused mainly by ruminal bacteria, the profile ingested by the animal is not the same available for absorption [19]. The unsaturated fatty acids have a short half-life on rumen, because it is quickly hydrogenated to SFA by the microbiota [18]. An amount of 600 to 900 g/kg of PUFA are bio hydrogenated and just 1 to 2% of metabolic hydrogen is used for this purpose [20]. The esterified lipids coming from diet are hydrolyzed to free unsaturated fatty acids, which can be used to synthetize microbial lipids or, through biohydrogenation, turn up to SFA or with lower number of unsaturation (Figure 1). Several enzymes have action in the lipolysis process, such as lipases, galactosidases and phospholipases. In addition, through isomerases and reductases, the compounds are hydrogenated until become SFA [11].



**Figure 1:** Events on rumen that modify the unsaturated fatty acids [11].

Most of bacteria responsible for the biohydrogenation are cellulolytic and the *Butyrivibrio fibrisolvens* has greater relevance [18]. Once the effective fiber in the diet is poor, usually as a result of higher concentrates or lower forage particle size, occurs reduction in these bacteria number on rumen and therefore, higher amount of lipids tends to reach the abomasum without suffer hydrogenation [21,22]. Other factors also have impact in this passage of unsaturated fatty acids from rumen to abomasum, such as passage rate and quantity of PUFA fed through diet, which can be harmful to some microorganisms. The biohydrogenation is associated to the hypothesis that is a mechanism of detoxification enabling the fiber digestion, because in a certain way, the PUFA are toxic to some cellulolytic bacteria [23]. This toxicity may be linked to the modifications that the PUFA cause on bacteria's cell membrane, as the fatty acids with higher number of unsaturation lead to physical changes on the cells' membranes and, for this reason, can change the cells functions [24].

The microorganisms present in the rumen are important for its substantial capacity of digest and absorb plant material [13]. These are also responsible for changes in the profile and feeds composition of the diet, which lead to the theory that what is ingested by the animal is not the same available for absorption. Thus, the ruminal microorganisms stunt the study of different nutritional handlings and its influence in the final products, once it makes difficult and inaccurate estimate what the animal absorb. An alternative would be comparing these studies with the composition and diversity of the ruminal microbiota, that through equations and analysis of this microorganism's pool, the modifications could be quantified and the final product profile could be estimated with higher accuracy.

### Modification of the Milk Fatty Acids Profile

Several studies aimed the modification of milk fatty acids profile to produce a milk with more desirable attributes for human health [25]. In most cases, there was manipulation of cows' diets,

however, some showed that genetic factors also could have influence in this change. Diets composed basically by forages tend to present lower n-6: n-3 ratio. According to Simopoulos [26], ratios of 4:1 have positive effect on human health, nevertheless, the desired would be a 2:1 ratio, or even 1:1. The linoleic and  $\alpha$ -linoleic acids represents, respectively, the fatty acids n-6 e n-3 more abundant on bovine milk and, therefore, have important influence in the n-6:n-3 ratio of this product [25]. While forages are rich in  $\alpha$ -linoleic acid, cereals like corn and soybean are rich in linoleic acid [27]. Thus, the milk fatty acids composition varies according to the production systems and feed adopted, which animals raised grass-fed are exposed to the forage seasonality, not only in quantity but also in quality [25].

Bainbridge et al. [28] evaluated the effects of different forage species on milk fatty acids of eight Holstein cows. The traits consisted of grazing pear millet (*Pennisetum glaucum L.*) or grazing mixed winter pastures with more than 15 species. The amount of MUFA and PUFA increased from 2.131 to 2.289 and from 319 to 433 mg/portion of milk with 3.25% of fat, respectively, comparing the grazing periods of pear millet and mixed winter pastures. Varnold et al. [29] showed similar results, which summer pastures proportioned decreasing of unsaturated fatty acids on beef comparing to animals grazing winter pastures. Besides the evaluation of different forage species on cattle feed, some studies used the supplementation with diverse types of oils and diets with higher PUFA levels to attempt modify the milk fatty acids profile. However, the results obtained were just slight changes, as showed in the studies described below. Santos et al. [30] used four Holstein cows to determine the effects of soybean oil utilization as a source of PUFA and coffee hull as a source of antioxidants in the diet. The production and composition of milk did not differ among traits. The milk fatty acids composition was slightly changed, nevertheless, the inclusion of coffee hull increased the total amount of polyphenols and flavonoids, which resulted on higher antioxidant power and, consequently, greater product stability.

In another study, Santos et al. [31] fed four Holstein cows with diet containing citrus pulp pellets as a source of antioxidants and soybean oil as a source of PUFA. The control diet was composed by corn silage, ground corn, soybean meal, urea and mineral supplement, which in the other treatments were added soybean oil and/or citrus pulp pellets. The milk production and concentrations of protein, lactose and urea were similar among traits. In contrast, the milk fat and total solids were higher in the animals fed with the control diet. The animals fed with citrus pulp pellets in the diet presented a milk with greater polyphenols and flavonoids totals, which consequently resulted in increased antioxidant power. The fatty acids composition was barely altered, nevertheless, the cows fed with 18% of citrus pulp pellets showed higher amount of MUFA and lower of SFA comparing to animals of the other traits.

In order to determine whether this limited alterations in the milk fatty acids profile once cows fed with higher PUFA concentrations is caused by biohydrogenation, the study of Lima et al. [32] was developed, with the purpose to compare the results of PUFA infusion directly on the rumen or abomasum. These authors aimed to clarify the role of rumen microbiota in the transfer of antioxidants from citrus pulp into milk while soybean oil used as a source of PUFA. In this regard, were assigned four fistulated Holstein cows. The treatments were: soybean oil administered in the rumen; soybean oil infused in the abomasum; soybean oil and citrus pulp administered in the rumen; and soybean oil and citrus pulp infused in the abomasum. Cows from treatment with soybean oil infused in the abomasum presented lower proportion of short chain fatty acids and higher amounts of PUFA, n-3 and n-6 fatty acids in the milk fat. The treatments with administration of citrus pulp in different places showed similar antioxidants properties, which suggest that the rumen microorganisms have small influence in their metabolism. According to the authors, the results of fatty acids infusion in the abomasum can help to determine the by-pass fat level to be assigned for produce a milk with desirable nutritional aspects.

According to Bilal et al. [33], besides the manipulation of animals' diet to alter the milk fatty acids profile, genetic factors also have effect and can be applied to this purpose. For these authors, the heritability estimates and genetic correlations to unsaturation indexes of milk fat indicate that exist genetic variations, so the fatty acids profile more desirable to human health can be reached by genetic selection. Some studies report that milk from different cows breeds present distinctes proportions of SFA and unsaturated fatty acids, which is related to genetic factors [34-36]. Croissant et al. [34] compared milk samples of Holstein and Jersey cows. The milk from Holstein showed higher amounts of long chain fatty acids, MUFA and PUFA, furthermore, lower proportion of SFA comparing to Jersey. This occurred because were paralleled two different genetic groups and due to the influence of this factors have on the animals' metabolism, which consequently have impact in the mammary gland metabolism.

Maurice-van Eijndhoven et al. [35] estimated the difference in the milk fatty acids profile of females Holstein-Friesian, Meuse-

Rhine-Yssel, Dutch-Friesian, Groningen White Headed and Jersey, which are breeds existent on Netherlands. It was used 41,404 samples collected from 24,445 animals. Jersey cows tended to produce higher proportion of SFA, due to the increased amount of C4:0, C6:0, C8:0, C10:0, C12:0, C14:0 and C16:0 comparing to Holstein Friesian, Meuse-Rhine-Yssel and Dutch Friesian. These fatty acids are produced from "de novo" synthesis and the dissimilarity found was related to the distinct milk fat percentage of the breeds. Soyeurt et al. [37] collected data of milk fatty acids and estimated genetic parameters for SFA, MUFA and SFA:unsaturated ratio, besides compare samples from different days in lactation. It was analyzed 100,841 samples from 11,626 primiparous Holstein cows collected between 1991 and 2007. Cows in the first lactation showed milk with lower amount of SFA. Furthermore, the quantity of SFA and the SFA:unsaturated ratio tend to increase until reach the lactation peak and, then, decrease, fact that can be explained due to the occurrence of greater adipose tissue mobilization in the period, which according to Barber et al. [38], is composed in the majority by C16:0 e C18:0. Strong heritability's were reported for SFA and MUFA, both in the beginning and in the end of lactation, however, with some variations.

The great majority of studies that tried to manipulate the milk fatty acids profile kept the focus on the manipulation of the animals' diets. Nonetheless, as a result of the rumen microbiota action and of the animals' physiological mechanisms to form the milk fat in the mammary gland, this modifications barley happens and, because that, a way to alter this profile with greater efficiency and accuracy could be the focus of futures studies. Promising studies in terms of heritability's and positive genetic correlations to select animals with better milk fatty acids profile to human health already exist [39-41]. However, the use of this traits on genetic improvement programs is not frequently yet. Thus, nutritional and genetic strategies should be applied concomitantly.

### Fat Formation in the Mammary Gland

The milk fat is synthesized with fatty acids deriving from the synthesis in the epithelial cells of mammary gland and with long chain fatty acids arising from the bloodstream [42]. This occurs because, in most of the species, the fatty acids produced in the mammary gland have chains no longer than 16 carbons [43]. Fatty acids composed by four to eight carbons are considered short chain, while 10 to 14 are considered medium chain and with more than 16 carbons are considered long chain fatty acids. Short chain fatty acids, medium and a portion of those with 16 carbons are generated in the "de novo" synthesis [44]. This process occurs in the epithelial cells of mammary gland, in which the acetyl-CoA is used to form the malonyl-CoA, reaction catalyzed by the enzyme acetyl-CoA carboxylase and with co-operation of the nicotinamide adenine dinucleotide phosphate (NADPH) in the reduction reactions [43].

Nelson e Cox [45] described that the acetyl-CoA carboxylase contains one biotin molecule and the reaction also depends of one acetyl-CoA molecule and another of bicarbonate ( $\text{HCO}_3^-$ ). The

bicarbonate carboxyl group is transferred to the biotin through energy cost. The biotin, in turn, acts in the transfer of CO<sub>2</sub> to acetyl-CoA, step that result in the formation of malonyl-CoA. The next process is the chain elongation (Figure 2), which all the reactions are catalyzed by the multienzyme complex called fatty acid synthase. The malonyl and acetyl groups of the malonyl-CoA pass through reactions of condensation, reduction, dehydration and

reduction again, which results in an acyl group with two more carbons. Thus, begins the formation of one fatty acids chain, in which with reactions that add acetyl groups, can be expanded until the synthesis of palmitic acid with 16 carbons. Nonetheless, the first four carbons of the “*de novo*” synthesis can be deriving from β-hydroxybutyrate, compound produced by the ruminal epithelium through the absorbed butyrate [46].

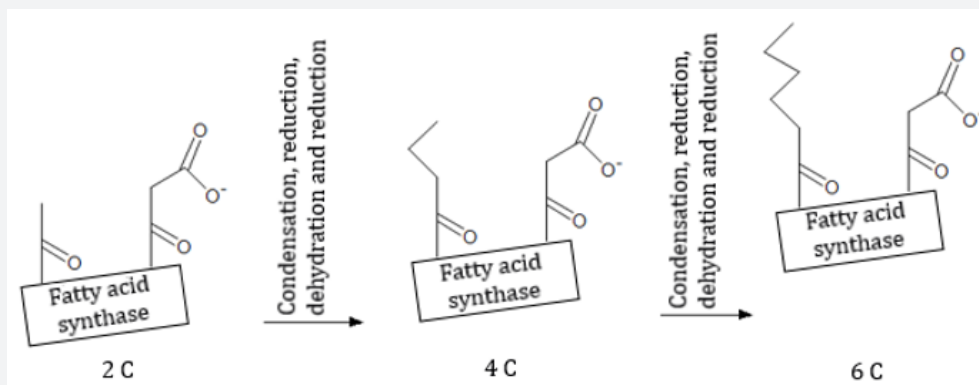


Figure 2: Fatty acids synthesis process.[45]

Fatty acids with 16 carbons and more than that are derived from animal’s bloodstream, however, those with 16 also can be originating from “*de novo*” synthesis [44]. Furthermore, the serum fatty acids can be originated from diet or be products of adipose tissue mobilization, mechanism that take place with more intensity when the animal is in negative energy balance (NEB).

In addition to the unsaturated fatty acids modifications in the rumen, these are exposed to other alterations in the mammary gland alveoli. In these cells the Δ<sup>9</sup>-desaturase converts the stearic acid (C18:0) in oleic acid (C18:1) and, due to this role, studies were conducted aiming to increase its activity [5]. This enzyme performances in the production of the main CLA isomer, reaction

that the oleic acid C18:1 *trans*-11 is changed to C18:2 *cis*-9, *cis*-12 [47] and it’s also contributed to more than 50% of C18:1 *cis*-9 secretion on milk [37]. Corl et al. [48] noted that about 80% of C18:2 *cis*-9, *trans*-11 is produced endogenously, in other words, with the desaturase action. Strategies to increase the CLA content on milk are based in raise the vaccenic acid quantity which pass through the rumen and raising the Δ<sup>9</sup>-desaturase activity [49] (Figure 3). The milk amount of CLA is also affected by physiological factors and variations among animals [50]. Nevertheless, the Δ<sup>9</sup>-desaturase present negative correlations with the milk fat synthesis [37]. Once it operates in reactions of CLA isomers production, which the CLA *trans*-9, *cis*-11 and the CLA *trans*-10, *cis*-12 could reduce the fat synthesis, the later in higher magnitude [51].

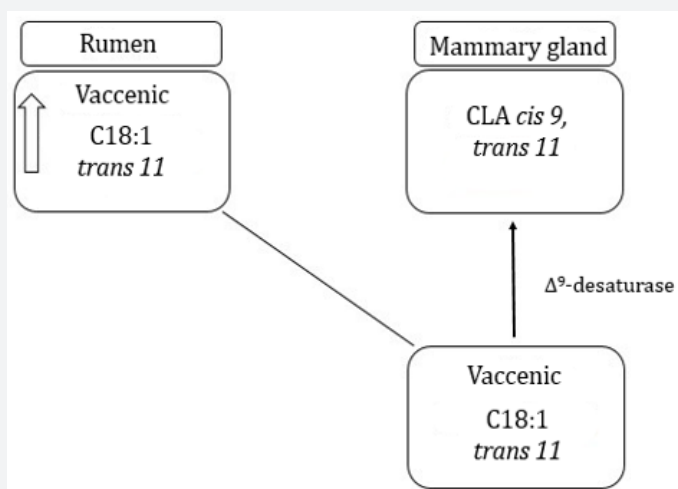


Figure 3: Process of CLA endogenous synthesis in the mammary gland through vaccenic acid.[50]

Perfield et al. [51] concluded that the increase of CLA *trans-9, cis-11* is associated with the milk fat depression (MFD). This conclusion came through the abomasal infusion of 5 g/day of this isomer in four lactating Holstein cows, which this process reduced in 15% the fat production, once there is a decrease of lipogenic enzymes expression and the inhibition of fatty acids desaturation [52]. To reach a fatty acids profile considered healthier to human nutrition is inevitable the search for higher numbers of unsaturation in these, however, some isomers in high amounts can impact negatively, causing a decline in the desaturase activity and, consequently, the MFD.

### Influence of Fatty Acids Profile on Human Health

Food with better lipidic profile and, consequently, fatty acids, have beneficial effects on human health. Studies show that the use of PUFA and lower n-6: n-3 ratios on human diets can impact the metabolism and physiological mechanisms, in order to prevent or even assist the diseases treatment [53,54]. Guida et al. [54] showed promising results testing diets with energy restriction and lower PUFA n-6: n-3 ratio. It has positive effect in obese patients with plaque-type psoriasis, which improved the clinic response to pharmacological treatment. The patients were dividing in two groups, which one kept the usual diet and the other had an energy restriction of 20 kcal/kg/ideal body weight/day and the diet enhanced with an average of 2.6 g/day with n-3 PUFA. As results, the group submitted to energy restriction, designed to reduce the ingestion of n-6 PUFA and raise the n-3 showed improvement of the metabolic profile and positive effects against the disease. Bell et al. [53] studied the nephrotic range proteinuria, disease characterized by the protein lost in the urine in variable quantity according to the clinical severity, associated to several primary and systemic diseases [55]. The patients were treated for eight weeks with 4 g/day of omega 3. The treatment reduced the remaining low-density lipoprotein (LDL) type III as well as the lipoproteins rich in triglycerides. Thus, according to the authors, the patients' potential cardiovascular risk was minimized.

Although the PUFA n-3 and n-6 are essential to human beings [56], it excess or the high n-6: n-3 ratio may be harmful to health, along with the trans fatty acids. The *trans* fatty acids can be produced by both industrial hydrogenation and biohydrogenation, which the latter occurs only in the ruminants and has the CLA *cis-9, trans-11* as one of the main products [7]. Furthermore, the fatty acids originating from industrial process cause the high-density lipoprotein (HDL) decrease and raise the LDL, which, to a certain extent, can increase the arterial coronary disease risk. The biohydrogenation fatty acids has not clear effects and, for this reason, wanders et al. [7] lead a study with 61 men and women that consumed three diets. One of them was the control and the other two with approximately 20 g/day of oleic acid originating from industrial hydrogenation or with an 80% CLA *cis-9, trans-11* and 20% CLA *trans-10, cis-12* mix. In healthy volunteers, high intakes of the 80:20 CLA *cis-9, trans-11* e 20% CLA *trans-10, cis-12* mix raised the

total HDL. Comparing all the individuals, the CLA effect could be considered less detrimental than the industrial *trans* fatty acids. Therefore, in healthy persons and in the quantity applied in this study, the CLA has positive effects in the serum cholesterol levels.

Other fatty acids deserve attention to human health, because the SFA tend to raise the serum LDL concentrations and, hence, are linked to cardiovascular diseases risk [57]. One of the main SFA sources are the dairy products [58], however, studies have been carried out to produce a milk with better lipidic profile. Organic milks or from cows that had alterations in the diet could have higher n-3 PUFA and a lower n-6: n-3 ratio, on the other hand, it's hard to evaluate the benefits that the different fatty acids profile have on human health [4]. According to Mozaffarian et al. [59], once in a population level, a 5% change in the diet energy level coming from SFA to PUFA would reduce about 10% the risk of arterial coronary disease occurrence. Then, FAO & WHO [60] suggested that some SFA have the effect of raise the LDL serum concentrations, such as, for example, the lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids. Upon a recommendation of these health organizations, the SFA human consumption could not exceed 10% of the total energy intake and, if it's possible, the SFA should be replaced by n-6 e n-3 PUFA, if this change could reduce the risk of arterial coronary diseases [61].

### Conclusion

Many factors affect the milk fatty acids composition and the knowledge of the interactions between them is limited. Tendencies to manipulate animals genetically and select them to obtain products with desirable specific composition already exist. The market's acceptance to milk with a higher number of unsaturation in the fatty acids profile must be evaluated, once it could be beneficial to human health.

### Conflict of Interest

There is no conflict of Interest.

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