Through ruminant nutrition to human health: role of fatty acids

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In the last decades, a new awareness on human nutrition has increased and the concept of ‘food’ has changed from ‘source of nutrients for body’s needs’ to ‘health promoter’. Fruits and vegetables have always been considered beneficial for human health. More recent studies have demonstrated that bioactive components are also present in animal-derived foods, such as milk and dairy products. A broader concept of ‘nutritional safety’ implies the knowledge of how the nutrients contained in animal-derived foods positively affect human health, and how to increase their content. The improvement of dairy products fatty acid (FA) composition can involve strategies in animal nutrition. This review aims to discuss the role of FAs supplementation in ameliorating milk fat composition, environmental impact and animal health. In particular, we have focused on the role of n-3 and CLA FAs and how animal nutrition strategies can positively affect both human and animal health. Several studies have demonstrated that through adequate nutritional strategies is possible to manipulate and improve FA composition of milk and derived products (cheese). Moreover, feeding animals with n-3 FAs has proved to reduce emission of methane (CH4), but further nutritional strategies are needed in order to address this crucial environmental issue. In relation to animal health, n-3 FAs have been proved to modulate immune and inflammatory response in dairy ruminants. Recent studies have addressed the potential programming effects of increased maternal n-3 polyunsaturated FAs intake on offspring’s immune functions showing that feeding bioactive FAs to pregnant animals can affect progeny health status.

Keywords: ruminant, dairy products, lipid sources, polyunsaturated fatty acids, human health

Introduction

Recently, a new awareness on human nutrition has increased and the concept of ‘food’ has changed from ‘source of nutrients for body’s needs’ to ‘health promoter’ (Zymon et al., 2014). Consumers consider that food contributes directly to their health (Elsanhoty et al., 2009). Due to this fact, there is an increasing demand of ‘functional foods,’ foods which contain important levels of biological active components (Bhat and Bhat, 2011), which can affect one or a limited number of functions in the body, consequently having positive effects on human health (Bellisle et al., 1998). Fruits and vegetables have been included into this particular category for years. More recent studies have demonstrated that bioactive components are also present in animal-derived foods such as milk and dairy products (Bauman et al., 2006). This leads to change in the idea of food safety to a broader concept of ‘nutritional safety,’ that implies the knowledge of how the nutrients contained in animal-derived foods positively affect human health, and how to increase their content in milk, meat and eggs (Cheli and Dell’Orto, 2015). This new interest is also a consequence of the awareness that, in the last 150 years, the n-6:n-3 fatty acid (FA) ratio in westernized diet has increased drastically, changing from 1 : 1 to 15 : 1, due to the consumption of vegetable oils rich in n-6 FA (McDaniel et al., 2010). This shift in FA ratio is associated with health disorders, such as cardiovascular diseases, arthritis, psoriasis and colitis (Kearns et al., 1999; McDaniel et al., 2010), and various neuroendocrine conditions. In particular, it has been observed that saturated fatty acids (SFA) (specifically C14:0 and C16:0) and trans-fatty acids (TFA) in the diet increase the incidence of these kind of pathologies, with the risk associated with TFA being higher than SFA (Shingfield et al., 2008; Givens, 2010; Shingfield et al., 2013). Moreover, high amount of SFA is also associated with lowered insulin sensitivity, increasing the risk of metabolic syndrome and diabetes (Funaki, 2009; Kennedy et al., 2009). Interestingly, the costs related to cardiovascular diseases have been estimated as €200 billion/annum for European Union (Allender et al., 2008) and as $403 billion/annum in the United States (Thom et al., 2006). The future will be even worse due to longer life and the increasing in obesity in the developed countries (Givens, 2010). Public health policies recommend to decrease the intake of SFA and TFA and increase the amount of long-chain n-3 polyunsaturated fatty acids (PUFA), especially EPA (20:5 n-3) and DHA (22:6 n-3) from marine sources. Regular consumption of n-3 FAs has been shown to protect against cardiovascular morbidity and mortality (Calder, 2004), and reduce inflammatory diseases (Calder, 2012 and 2013) and

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neurodegenerative illnesses (Calon and Cole, 2007). During the past couple of decades, several studies, principally based on in vitro, and then based on clinical trials on humans, have been proving different biological activities of CLA found in food products of ruminant origin. These studies highlighted that CLA can positively affect human health, such as diminishing cancer, atherosclerosis, diabetes and obesity (Benjamin and Spener, 2009).

Nowadays, it is easy to misunderstand the big amount of information coming from media and other sources about human health and functional foods, creating public confusion about the effects of fat and FAs in dairy products. Rather, we must consider biological and nutritional values of the individual FAs, and this is certainly true for saturated FAs. In fact, these latter are often associated with risk of coronary diseases, and fat contained in milk are mostly saturated. However, milk SFA are different, and some of them have no effect on plasma cholesterol (Bauman et al., 2006). The improvement of animal product FA composition, with a decrease of SFA and increase of monounsaturated FA (MUFA) and beneficial PUFA contents, can involve strategies in animal nutrition, in order to ameliorate the human diet, without any kind of change in consumer’s eating habits (Savoini et al., 2010; Shingfield et al., 2013).

This review aims to discuss the role of FAs in ameliorating milk fat composition, environmental impact and animal health. In particular, we have focused on the role of n-3 and CLA FAs and how animal nutrition strategies can positively affect both human and animal health.

Dietary manipulation of milk fatty acid composition

According to Hippocrates, milk is ‘the nature’s most perfect and complete food’. In fact, with its bioactive components, it is a complete food with an important role in preventing, and in some cases curing different kind of diseases of modern civilization (Akalin et al., 2006; Givens, 2015). In particular, dairy milk fat contains on average 60% to 70% SFA, 20% to 35% MUFA and only 5% PUFA, and the latter are mainly represented by linoleic (C18:2 n-6) and α-linolenic acids (ALA, C18:3 n-3). Unfortunately, n-3 PUFAs cannot be synthesized by animals because desaturation of FAs does not occur at positions > D9 (Cook, 1996) and the conversion of C18:3 n-3 into its long-chain derivates (EPA and DHA) is limited by metabolic factors, due also to the excessive dietary intake of n-6 FA, in particular of C18:2 n-6 (Zymon et al., 2014). According to Food and Agriculture Organization/World Health Organization (1993), the optimal n-6 : n-3 ratio should be 4 : 1 to 6 : 1, and not > 10 : 1 (Zymon et al., 2014), but in most countries this theoretical ratio is far from reality, with an excessive intake of n-6. The n-3 FA intake in human diet is recommended to be between 250 and 500 mg/day (European Food Safety Authority Journal, 2012).

Interest in manipulating the milk fat content started at the beginning of 80 s and the pressure to reduce total fat content and its saturation has continued until now. Different are the strategies to improve fat composition in milk. For example, it is possible to manipulate animal diet adding long-chain FA, such as EPA and DHA, but it is important to take into account their low transfer rate into milk, due to their ruminal biohydrogenation and low intestinal digestibility (Zymon et al., 2014).

n-3 Fatty acids

Several studies (Table 1) investigated the addition of fish oil and marine algae in ruminant diet as a way to enhance EPA and DHA content in milk (Loor et al., 2005; Cattaneo et al., 2006; Boeckaert et al., 2008; Toral et al., 2010; Shingfield et al., 2013; Toral et al., 2014; Bernard et al., 2015). In dairy cows, fish oil appears to have a toxic effect on ruminal microorganisms, reducing fat content and conferring off-flavors due to FA oxidation (Lock and Bauman, 2004). In particular, the phenomenon of reduction of milk fat content is known as ‘Milk Fat Depression,’ and it is typically observed with diets low in fiber and high in concentrates (Bauman and Grinari, 2003). There are some aspects to take in consideration when using fish oil as a source of n-3 PUFA.

Table 1 Effect of dietary fish oil and marine algae supplements on EPA and DHA content of ruminant milk (Shingfield et al., 2013, mod.)

<table>
<thead>
<tr>
<th></th>
<th>EPA-20:5n-3 (% total FA)</th>
<th>DHA-22:6n-3 (% total FA)</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fish oil dairy cows</strong></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>0.08</td>
<td>0.04</td>
<td>Loor et al. (2005)</td>
</tr>
<tr>
<td>Treated (270 g/head per day)</td>
<td>0.36</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td><strong>Algae dairy cows</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>NR</td>
<td>0.09</td>
<td>Boeckaert et al. (2008)</td>
</tr>
<tr>
<td>Treated (201 g/head per day)</td>
<td>NR</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td><strong>Fish oil sheep</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.03</td>
<td>0.02</td>
<td>Toral et al. (2010)</td>
</tr>
<tr>
<td>Treated (27.5 g/head per day)</td>
<td>0.15</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td><strong>Fish oil goats</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>NR</td>
<td>NR</td>
<td>Cattaneo et al. (2006)</td>
</tr>
<tr>
<td>Treated (45 g /head per day)</td>
<td>0.54</td>
<td>0.37</td>
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FA = fatty acids.
Among them, the economic aspect and the sustainability of fish stocks are the major problems (Zymon et al., 2014), even if fish oil can be obtained from farmed fish. Therefore, it is crucial to consider alternative sources of n-3 PUFA, for example, marine algae rich in DHA (Franklin et al., 1999; Papadopoulos et al., 2002; Toral et al., 2010), linseed (Doreau and Ferlay, 2015) and camelina (Halmemies-Beauchet-Filleau et al., 2011; Pikul et al., 2014), rich in C18:3 n-3, the precursor for EPA and DHA. Another natural dietary source of n-3 PUFA is green pasture. Pasture is able to enrich milk fat in ALA, cis-9 trans-11 CLA and its precursor trans-11 C18:1 (vaccenic acid (VA)) (Dewhurst et al., 2006; Coppa et al., 2011; Shingfield et al., 2013). Interestingly, milk of pasture-fed dairy cows also contain higher levels of EPA and DHA (Hebeisen et al., 1993; Leiber et al., 2005; La Terra et al., 2010). Nevertheless, the possibility to enhance DHA and EPA in milk is limited (Shingfield et al., 2013). Apparent transfer efficiency of EPA and DHA from fish oil to goat milk ranged from 7% to 14% and 7% to 8%, respectively (Cattaneo et al., 2010). A possible solution to limit ruminal biohydrogenation is the use of ruminal protected sources of PUFA, enhancing the transfer efficiency of supplied fat. Doreau and Ferlay (2015) showed the possibility to take advantage of the natural constitution of linseeds, thanks to partially protected lipids when whole seeds are fed. Alternative rumen protection strategies include heating feeds at high temperature, using calcium salts of FAs or encapsulating the lipids in a matrix of rumen-inert protein (Jenkins and Bridges, 2007; Palmquist, 2009).

Milk with a high content of PUFA may benefit human health, but at the same time is more vulnerable to oxidation. In this context, increasing milk vitamin E content may represent a useful tool to protect lipids from peroxidation and to maintain milk nutritional and organoleptic quality (Vagni et al., 2011). Other antioxidants that can be included in the diet are plant extracts rich in polyphenols (Gladine et al., 2007; Gobert et al., 2009), or superoxide dismutase, catalase and glutathione peroxidase (De Marchi et al., 2015).

CLA

CLA belong to a series of positional and geometric isomers of linoleic acid, with conjugated double bonds. Important for their benefits in humans, they are present in products of ruminant origin. The most predominant form is rumenic acid (cis-9, trans-11), which represents >90% of total CLA in ruminant milk fat. Its origin is attributed to incomplete biohydrogenation of linoleic acid in the rumen (Figure 1) and from endogenous synthesis in the mammary gland (Grinari et al., 2000). Milk usually contains 0.2% to 0.9% of CLA and its concentration differs among ruminant species, depending also on stage of lactation (Cattaneo et al., 2012; Tudisco et al., 2013; Wang et al., 2013). Diet is the most significant factor affecting the milk content of cis 9-trans 11CLA and of its precursor, trans-11 C18:1 (VA). Milk CLA can be enhanced by feeding sources rich in PUFA, such as pasture, plant oils, oilseeds, fish oil, marine algae and rumen protected CLA. Nutritional strategies have been directed toward enhancing ruminal production of trans-11 C18:1, in particular formulating diets providing PUFA C18:2 n-6 and C18:3 n-3, which are precursors for VA formation in the rumin. Linseed, sunflower oil and soybean oil all proved to be effective in increasing secretion of cis-9, trans-11 C18:2 in milk fat (Bernard et al., 2005; Cruz-Hernandez et al., 2007; Mele et al., 2008; Nuñez et al., 2014; Toral et al., 2014; Buccioni et al., 2015). Numerous studies have also evidenced that fresh pasture feeding can increase milk CLA content compared with diets based on conserved forages (Leiber et al., 2005; Dewhurst et al., 2006; Coppa et al., 2011; Buccioni et al., 2012). Marine oils, rich in EPA and DHA, have been shown to be more effective than vegetable oils at increasing CLA concentration in ruminant milk. In dairy cows CLA proportion increased from 0.2%–0.6% to 1.5%–2.7% with diets supplemented with 200 to 300 g/d fish oil (Shingfield et al., 2013) and in dairy goats supplementation with 47 g/d fish oil enhanced milk fat CLA content from 0.6% to 1.93% (Savoini et al., 2010). Recently, Tsiplakou and Zervas (2013) showed that the inclusion of soybean oil in combination with fish oil in goat’s diet is also effective, resulting in an enhancement of CLA content in milk (4.04% v. 0.57%).

Dietary manipulation of milk FA can imply changes also in cheese composition. Pintus et al. (2013) showed that the consumption of Pecorino sheep cheese, naturally enriched in ALA, CLA and VA, obtained by feeding dairy ewes with extruded linseed, could lower plasma cholesterol in hypercholesterolaemic patients. Also, plasma contents of CLA, VA and n-3 ALA and EPA were increased and that of the endocannabinoid anandamide, that is linked to adipogenesis, was lowered when the enriched cheese was fed to the same subjects. Another study was performed by Serra et al. (2015), who observed FA modification in buffalo milk by supplying sources of linoleic acid. The FA composition of mozzarella cheese reflected that of milk, showing that cheesemaking did not affect the transfer of FA (CLA and VA) from milk to cheese.

Figure 1 Rumen biohydrogenation of fatty acids.
n-3 and Production of methane

Reducing emissions of greenhouse gases is a social and environmental priority. CH₄ is a potent greenhouse gas produced in the rumen. The production occurs due to the metabolic activity of bacteria, the methanogenic archaea, which use hydrogen (H₂) and carbon dioxide (CO₂) as substrates (Martin et al., 2010). The major part of CH₄ is produced in the digestive tract of ruminants, especially in the rumen (Doreau and Ferlay, 2015). High concentrations of H₂ are toxic for microbial enzymes, affecting their activity and ruminal fermentation, so the formation of CH₄ sustains the efficiency of ruminal degradation with the dissipation of H₂ (Morgavi et al., 2010). Many dietary interventions are aimed to reduce CH₄ emissions and dietary lipids are considered the best way for enteric CH₄ mitigation (Martin et al., 2010; Hristov et al., 2013). Substitution of dietary carbohydrates with lipids can reduce CH₄ emission and can decrease rumen protozoa, which are producers of H₂, the precursor of CH₄ (Doreau and Ferlay, 2015). It has been shown that linseeds rich in n-3 PUFA can reduce CH₄ yield more efficiently than saturated sources (calcium salts of palm oil, tallow), and unsaturated sources containing oleic acid (rapeseed) or linoleic acid (sunflower, cottonseed) (Martin et al., 2010; Doreau and Ferlay, 2015). Other authors demonstrated that it is possible to reduce CH₄ production by adding fish oil in low-starch diets (Piorindini et al., 2015). Also increasing doses of coconut and fish oil quadratically decreased concentration of CH₄ in vitro (Patra and Yu 2013). On the contrary, camelina oil had no effect on ruminal CH₄ emission intensity (Bayat et al., 2015). However, studies in this field are very limited and more investigations are needed in order to find the best way to reduce CH₄ emission with a systematic strategy including animal nutrition.

Bioactive fatty acids and animal health

Among bioactive FAs, n-3 PUFA (EPA and DHA) have been shown to be essential for normal growth and development in mammals, explicating several nutritional and health beneficial actions (Innis, 2007; Calder, 2012 and 2013). There is increasing evidence that feeding ruminants with n-3 PUFA may affect fertility, modulate immune and inflammatory response and affect maternal and progeny health.

n-3 Polynsaturated fatty acid and fertility

Feeding fats to dairy cows can improve fertility by the increment of dietary energy density, alteration in the follicle development (Staples and Thatcher, 2005), improvement in embryo quality (Cerri et al., 2004) and other positive effects (Santos et al., 2008). Several studies showed that n-3 PUFA supplementation can positively affect fertility traits (Mattos et al., 2004; Santos et al., 2008; Dirandeh et al., 2014; Otto et al., 2014). n-3 and n-6 PUFA can affect fertility by regulating prostaglandin F₂α (PGF₂α) secretion. Uterine synthesis of PGF₂α is regulated in part by substrate availability, and arachidonic acid (C20:4 n-6) is the precursor for PGF₂α synthesis. Therefore, it is plausible to suggest that increments of the content in endometrial tissue of C20:4 n-6 should enhance uterine PGF₂α secretion, which may affect uterine health (Cullens et al., 2004; Silvestre et al., 2011). Feeding fish oil could reduce PGF₂α secretion, increasing fertility and reducing pregnancy losses (Mattos et al., 2004). In a recent study, Dirandeh et al. (2014) showed that feeding n-6 PUFA after calving to the first estrous cycle and shifting to n-3 PUFA after the first estrous cycle could be a nutritional strategy for improving fertility in lactating dairy cows.

n-3 Polyunsaturated fatty acid and immune and inflammatory response

FAs have a significant role in immune response both in humans and in animals (Ingwartsen and Moyes, 2013). Among them, n-3 PUFA (EPA and DHA) are the most effective, and their influence on the cell types involved in inflammation and on the production of some chemical mediators has been studied for many years.

Long-chain n-3 PUFA modulate immune functions in several ways by replacing, for example, arachidonic acid during the eicosanoid signaling cascade (Calder, 2013), thus decreasing the production of inflammatory eicosanoids such as of prostaglandin E₂ (PGE₂) (Rees et al., 2006), thromboxane B₂ (TXB₂) (Caughey et al., 1996), leukotriene B₄ (LTB₄) (Kelley et al., 1999), 5-hydroxyeicosatetraenoic acid (Endres et al., 1989) and leukotriene E₄ (LTE₄) (Von Schacky et al., 1993). EPA and DHA can also directly interfere with cytokine gene expression (Weldon et al., 2007). Further regulatory pathways include regulation of cell surface expression of adhesion molecules (De Caterina and Libby 1996), membrane fluidity and apoptosis rates (Sweeney et al., 2001). In addition, both EPA and DHA give rise to family of anti-inflammatory mediators called resolvins (Serhan et al., 2002). Most of these activities directly target leukocyte function (Sijben and Calder, 2007).

Contreras et al. (2012) showed that the exposition of Bovine Aortic Endothelial Cells to a mixture of FAs that reflects the composition of non esterified fatty acids (NEFA) during the 1st week of lactation determined an increase of pro-inflammatory responses compared with cells exposed to a mixture of FAs enriched in EPA and DHA. Increasing the n-3 FA content of vascular phospholipids could mitigate the expression of cytokines (interleukin-6 and 8), of adhesion molecules (intracellular and vascular adhesion molecules) associated to an increase of inflammatory response, of reactive oxygen species (ROS), and of pro-inflammatory metabolites of linoleic acid.

Recently, Greco et al. (2015) have proved that reducing the n-6/n-3 FA ratio in the diet of early lactation dairy cows can attenuate inflammatory response to lipopolysaccharide (LPS) challenge. In particular, haptoglobin (Hp) was greatest in the mammary gland of cows fed the highest n-6/n-3 ratio (5.9) (Figure 2a). Moreover, interleukin-6 concentration in plasma increased as the n-6/n-3 FA increased (Figure 2b).

In a study by Agazzi et al. (2004), dietary fish oil fed to transition dairy goats was found to be effective on
cell-mediated immune response, with modified mononuclear and polymorphonuclear (PMN) cells ratio as result. Treating cells with DHA (Pisani et al., 2009) exerted an increased PMN leukocytes phagocytic activity and lower ROS production after in vitro challenged with EPA and DHA (Figure 3a and 3b). A subsequent validation in vivo of the obtained results demonstrated that both EPA and DHA have beneficial effects on goats health by improving the defensive performances of neutrophils (Bronzo et al., 2010) avoiding cellular and tissue damages by ROS. EPA and DHA also affected goat monocyte activities by up-regulating phagocytic activity and ROS production (Figure 3c and 3d) (Lecchi et al., 2011) and by interfering with the formation of lipid droplets and by up-regulating proteins belonging to PAT protein family, perilipin family, namely PLIN1 (perilipin), PLIN2 (adipophilin) and PLIN3 (Lecchi et al., 2013).

Another study by Stryker et al. (2013) demonstrated that supplementation of fish meal to pregnant and lactating ewes could alter both innate and acquired immune response.

Specifically, after a LPS challenge at 135 days of pregnancy ewes fed fish meal showed an attenuated febrile response compared to soybean meal, and the basal Hp concentration was lower after a sensitization with hen egg white lysozyme during lactation.

**Maternal diet and progeny health**

A specific role for DHA during fetal and neonatal development has been recognized (Innis, 2007), but DHA status of the mother and the newborn may be sub-optimal if maternal intake is insufficient. Dietary supply of long-chain n-3 PUFA during late pregnancy, and nursing period can improve EPA and DHA content in ruminant colostrum and milk (Cattaneo et al., 2006) and in plasma of the suckling newborn lambs, calves and kids (Moallem and Zachut, 2012; Or-Rashid et al., 2012; Ferroni et al., 2014). The increased supply of EPA and DHA from maternal circulation during fetal development and from colostrum during early neonatal period may affect health progeny status, but only few studies have addressed

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**Figure 2** (a) Lipopolysaccharide (LPS) challenge in the mammary gland in early lactating dairy cows: haptoglobin concentration was higher 24 h after LPS challenge in the mammary gland in cows fed n-6/n-3 5.9. (b) Interleukin-6 concentration in plasma increased as the ration of n-6 to n-3 fatty acid increased.

**Figure 3** EPA and DHA may improve the defensive performance of goat neutrophils (a) and (b) and monocytes (c) and (d) against bacteria by increasing their phagocytic activity.
the potential programming effects of increased maternal n-3 PUFA intake on offspring’s immune functions (Lauritzen et al., 2011; Myles et al., 2014; Ferroni et al., 2015).

Conclusions

Through ruminant nutrition, it is possible to manipulate and improve milk FA composition, with positive effects on human health by the consumption of dairy products enriched in bioactive n-3 and CLA FAs.

Results on reduced emission of CH₄ when feeding animals with n-3 FAs are not conclusive, therefore further nutritional strategies are needed in order to address this crucial environmental issue. In relation to animal health, n-3 FAs have been proved to modulate immune and inflammatory response in dairy ruminants. Moreover, feeding bioactive FAs to pregnant animals can affect progeny health status. At last, dietary long-chain FAs may affect the direction and dimension of changes in lipid metabolism gene network in key physiological organs such as liver (Agazzi et al., 2010), adipose tissue and mammary gland (Hosseiniet al., 2013) and temporal modulation on lipid metabolism (Jacometo et al., 2014).

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Role of fatty acids


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