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Nutrients for animal health and for improvement of nutritional quality of animal products for human consumption**

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 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. Public health policies recommend to decrease the intake of SFA and TFA and increase the amount of long-chain n-3 polyunsaturated fatty

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acids (PUFA), especially eicosapentaenoic acid (EPA 20:5 n-3) and docosahexaenoic acid (DHA 22:6 n-3) from marine sources.

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 fatty acids in dairy products. Rather, we must consider biological and nutritional values of the individual fatty acids, and this is certainly true for saturated fatty acids. 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 MUFA (monounsaturated FA) 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 fatty acids in ameliorating milk fat composition. In particular, we have focused on the role of n-3 and CLA fatty acids 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 60-70% SFA, 20-35% 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 fatty acids does not occur at positions greater than D9 (Cook, 1996) and the conversion of C18:3 n-3 into its long-chain derivatives (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 FAO/WHO (1993), the optimal n-6: n-3 ratio should be 4:1 to 6:1, and not more than 10:1 (Zymon et al., 2014), but in most countries this theoretical ratio is far from reality, with an excessive intake of omega-6. The omega-3 FA intake in human diet is recommended to be between 250 and 500 mg per day (EFSA J., 2012).

FISH OIL DAIRY COWS	EPA-20:5N-3 (%TOTALFA)	DHA-22:6N-3 (%TOTALFA)	AUTHORS
Control	0.08	0.04	Loor et al., 2005
Treated (270 g/head/d)	0.36	0.17	
Algae dairy cows			
Control	NR	0.09	Boeckaert et al., 2008
Treated (201 g/head/d)	NR	1.10	
Fish oil sheep			
Control	0.03	0.02	Toral et al., 2010
Treated (27.5 g/head/d)	0.15	0.38	
Fish oil goats			
Control	NR	NR	Cattaneo et al., 2006
Treated (45 g /head/d)	0.54	0.37	

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

Interest in manipulating the milk fat content started at the beginning of 80s 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.

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-flavours due to fatty acid 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 Griinari, 2003). There are some aspects to take in consideration when using fish oil as a source of n-3 PUFA. 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 fatty acids or encapsulating the lipids in a matrix of rumen-inert protein (Palmquist, 2009; Jenkins and Bridges, 2007).

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 (SOD), catalase (CAT) and glutathione peroxidase (GPX) (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 for humans, they are present in products of ruminant origin. The most predominant form is rumenic acid (cis-9, trans-11), which represents more than 90% of total CLA in ruminant milk fat. Its origin is attributed to incomplete biohydrogenation of linoleic acid in the rumen (fig. 1) and from endogenous synthesis in the mammary gland (Griinari et al., 2000). Milk usually contains 0.2-0.9% of CLA and its concentration differs among ruminant species, depending also

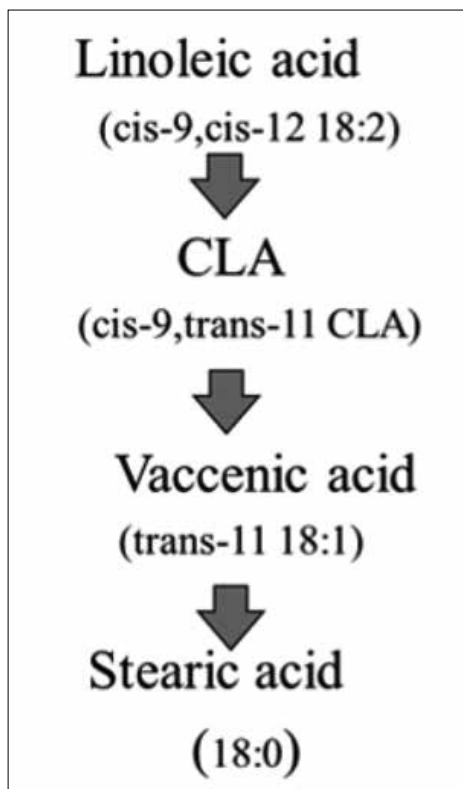


Fig. 1 *Rumen biohydrogenation of fatty acids*

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 (vaccenic acid). 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 towards 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 rumen. 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-Hernandes et al., 2007; Mele et al., 2008; Nudda 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 to 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 200–300 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 et Zervas *et al.* (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 vs 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 cheese-making did not affect the transfer of FA (CLA and VA) from milk to cheese.

BIOACTIVE FATTY ACIDS AND ANIMAL HEALTH

Among bioactive fatty acids, 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; Calder, 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 PUFA 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 F₂ α (PGF₂ α) secretion. Uterine synthesis of PGF₂ α is regulated in part by substrate availability, and arachidonic acid (C20:4 n-6) is the precursor for PGF₂ α synthesis. Therefore, it is plausible to suggest that increments of the content in endometrial tissue of C20:4 n-6 should enhance uterine PGF₂ α secretion, which may affect uterine health (Cullens et al., 2004; Silvestre et al., 2011). Feeding fish oil acids could reduce PGF₂ α 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 PUFA and immune and inflammatory response

Fatty acids have a significant role in immune response both in humans and in animals (Ingvarsen 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 signalling cascade (Calder, 2013), thus decreasing the production of inflammatory eicosanoids such as of prostaglandin E₂ (Rees et al., 2006), thromboxane B₂ (Caughey et al., 1996), leukotriene B₄ (Kelley et al., 1999), 5-hydroxyeicosatetraenoic acid (Endres et al., 1989) and leukotriene E₄ (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 (BAEC) to a mixture of fatty acids that reflects the composition of NEFA (Non Esterified Fatty Acids) during the first week of lactation determined an increase of pro-inflammatory responses compared to cells exposed to a mixture of fatty acids enriched in EPA and DHA. Increasing the n-3 FA content of vascular phospholipids could mitigate the expression of cytokines

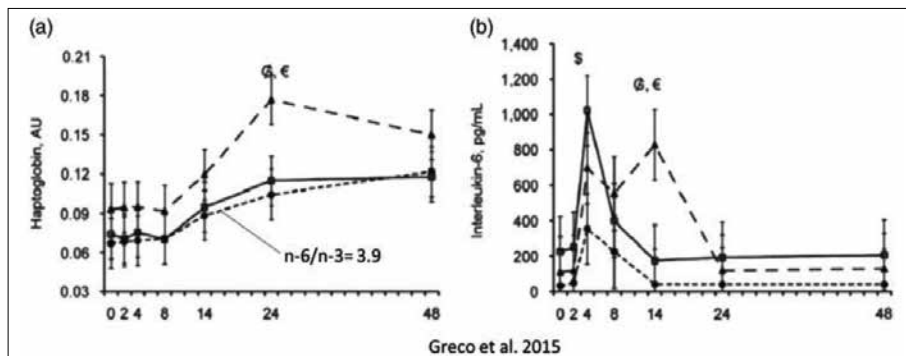


Fig. 2 (a) Lipopolysaccharide (LPS) challenge in the mammary gland in early lactating dairy cows: haptoglobin concentration was higher 24 h after LPS challenge in the mammary gland in cows fed diet n-6/n-3 5.9. (b) Interleukin-6 concentration in plasma increased as the ration of n-6 to n-3 FA increased.

(interleukin-6 and 8), of adhesion molecules (intercellular 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 challenge (LPS). In particular, haptoglobin (Hp) was greatest in the mammary gland of cows fed the highest n-6/n-3 ratio (5.9) (fig. 2a). Moreover, interleukin-6 concentration in plasma increased as the ratio n-6/n-3 FA increased (fig. 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 (fig. 3a and b). 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 affected also goat monocytes activities by up-regulating phagocytic activity and ROS production (fig. 3c and d) (Lecchi et al., 2011) and by interfering with the formation of lipid droplets and by upregulating proteins belonging to the perilipin protein family (PAT) (Lecchi et al., 2013).

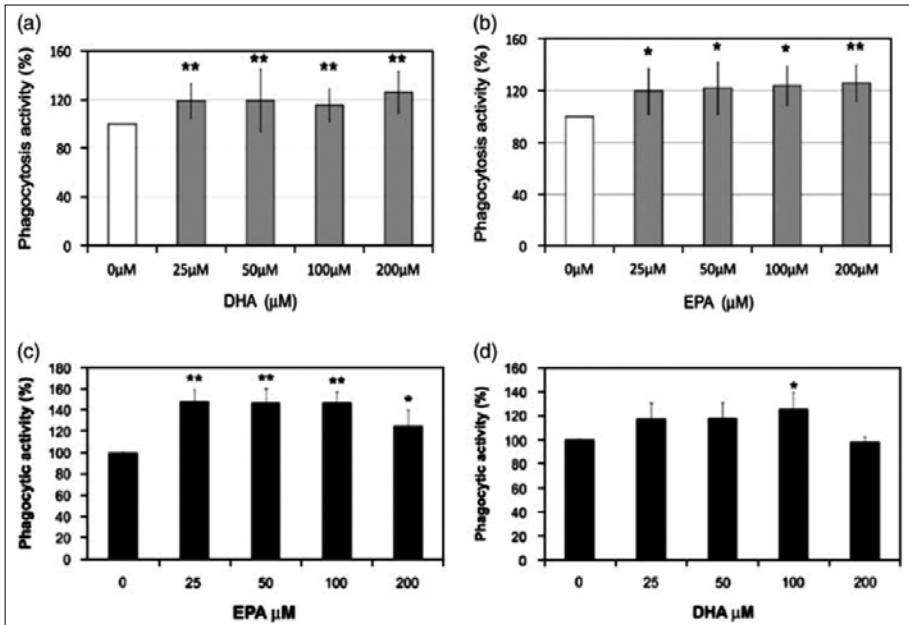


Fig. 3 EPA and DHA may improve the defensive performance of goat neutrophils (a and b) and monocytes (c and d) against bacteria by increasing their phagocytic activity

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 d 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 (HEWL) during lactation.

In conclusion, through ruminant nutrition it is possible to manipulate and improve milk fatty acid composition, with positive effects on human health by the consume of dairy products enriched in bioactive fatty acids.

In relation to animal health, n-3 fatty acids have been proved to modulate immune and inflammatory response in dairy ruminants. Moreover, feeding bioactive fatty acids to pregnant animals can affect progeny health status. At last, dietary long-chain fatty acids 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 (Hosseini and Loo, 2013) and temporal modulation on lipid metabolism (Jacometo et al., 2014).

ABSTRACT

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 composition can involve strategies in animal nutrition. This review aims to discuss the role of fatty acids supplementation in ameliorating milk fat composition and animal health.

RIASSUNTO

Nutrienti per la salute dell’animale e miglioramento delle caratteristiche dietetiche dei prodotti di origine animale. Nel corso degli ultimi decenni si è assistito ad una crescente consapevolezza nel campo della nutrizione e il concetto di “alimento” è passato da “fonte di nutrienti per il soddisfacimento dei fabbisogni dell’organismo” a “promotore di salute”. Gli alimenti di origine vegetale sono da sempre considerati in termini favorevoli per la salute umana. Studi relativamente più recenti hanno messo in luce come componenti bioattivi siano presenti anche negli alimenti di origine animale, come il latte e i prodotti che ne derivano. Un concetto più ampio di “sicurezza nutrizionale” implica quindi la approfondita conoscenza di come i nutrienti presenti degli alimenti di origine animale possano influenzare positivamente la salute umana e di come sia possibile incrementarne il contenuto. Il miglioramento delle qualità nutrizionali del latte e dei prodotti lattiero- caseari, in particolare della composizione lipidica, può essere ottenuto mediante adeguate strategie di alimentazione animale. Nel presente lavoro vengono discussi alcuni aspetti relativi al ruolo e alla somministrazione di specifici acidi grassi ad animali in lattazione al fine di migliorare la composizione lipidica del latte prodotto e la salute degli animali stessi.

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