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Review

Dietary enrichment of milk and dairy products with n-3 fatty acids: A review

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A B S T R A C T

Milk and dairy products are important sources of nutrients in the human diet; however, they are also the main sources of saturated fatty acids (FAs) that can cause an increase in the risk of cardiometabolic and cardiovascular disease. Unfortunately, the n-3 FAs that are known for their health benefits are found in small amounts in normal ruminant milk fat and dairy products. Dietary supplementation with vegetable seeds or oils rich in α-linolenic acid (ALA) and grass-based feeding can enhance the content of n-3 FAs, particularly ALA, in milk and dairy products. Alternatively, ruminants are often supplemented with fish meal, fish oil and microalgae that are rich in the long-chain n−3 FAs eicosapentaenoic acid and docosahexaenoic acid. In this paper, the latest scientific studies regarding n−3 FA enrichment in milk and dairy products are reviewed, giving an overview of the effectiveness of the different supplementation approaches.

Contents

1. Introduction

Nowadays, the aim of agricultural research is not only to improve yield and productive efficiency but also to improve the nutritional quality of food products. The advancement of research and health concern of consumers led to the concept of functional foods that support creation of claims for foods with better health beneficial micro-components (Oliveira et al., 2015). As a result, researchers and food producers are interested in research and practices that can improve the nutrient profile of food products (Antonacci, Gagliostro, Cano, & Bernal, 2018; Ayana & El-Shabraw, 2015; Tarek, Ashraf, Sobhy, & Walid, 2014). The dairy industry is

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an example that is currently working to modify the fatty acid (FA) composition of milk fat, particularly to enhance health beneficial FAs (Bodkowski et al., 2016; Schettino et al., 2017).

Foods of animal origin are vital sources of nutrients to humans. Milk and dairy products are characterised as important sources of nutrients in human nutrition, offering quality proteins, minerals, vitamins and energy (Niro et al., 2017; Pereira, 2014; Slačanac et al., 2011). Milk and dairy products are also the main sources of saturated FAs in human diet; however, a high intake of saturated FAs may increase the risk of cardiometabolic and cardiovascular disease (Givens, 2015). In addition, excessive intake of saturated FAs have been found to lower insulin sensitivity, an important factor in metabolic disorder and diabetes (Nagao & Yanagita, 2010). In most developed countries, public health policies recommend a reduction in consumption of saturated FAs (SFAs) and an increase in the consumption of n-3 FAs, especially α-linolenic acid (ALA, 18:3n-3) eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), which have health benefits related to cardiovascular diseases, central nervous system and mental health and development, inflammation and immune function (Lock & Bauman, 2004; Russo, 2009).

However, in normal bovine milk fat, small amounts of ALA (0.495 g 100 g $^{-1}$ of fat), EPA (0.067 g 100 g $^{-1}$ of fat) and DHA (<0.002 g 100 g $^{-1}$ fat) are found (Van Valenberg, Hettinga, Dijkstra, Bovenhuis, & Feskens, 2013) because of ruminal biohydrogenation of dietary unsaturated FAs (Frutos, Toral, Belenguer, & Hervás, 2018; Sinclair et al., 2004; Ventto et al., 2017). Hence, modifying the composition of milk and dairy products fat FAs by increasing the amounts of essential FA content (ALA, EPA, DHA or other n-3 FAs) and decreasing the amounts of the less healthy FAs in milk and dairy products seems imperative to the health of consumers.

The composition of FAs in milk and dairy products can extensively be changed by nutritional factors, particularly supplementing with dietary fat sources (Kliem, Humphries, Kirton, Givens, & Reynolds, 2019; Mierlita, 2016; Thanh & Suksombat, 2015; Vafa, Naserian, Moussavi, Valizadeh, & Mesgaran, 2012). In this review, various approaches used to enrich milk and dairy products with n-3 FAs are discussed. Grasses and vegetable seeds or oils such as linseed, hemp seed and rubber seed are primary sources of ALA, while fish meal or fish oil, algal oil or microalgae biomass are recognised sources of EPA and DHA. Many studies have been published on ruminant dietary supplementation with vegetable seeds or oils rich in ALA and fish meal or fish oil that are rich in EPA and DHA. Nowadays, use of microalgae in its different forms (defatted microalgae biomass, full fatted microalgae biomass or algal oil) as a source of EPA and DHA is being explored to increase the EPA and DHA content of milk and dairy products. For each approach, their effects to enhance n-3 FAs, especially ALA, EPA and DHA, and decrease SFAs and the n-6/n-3 ratio in milk and dairy products, particularly cheese and yoghurt, are discussed in detail. To our knowledge, no recent review is available regarding the current development of this field.

2. Effectiveness of unprocessed seeds or vegetable oils in enhancing n-3 fatty acids in milk and dairy products

Linseed oil, rubber seed oil, whole linseed and hemp seed are among the most commonly used dietary supplementary sources of n-3 FAs in ruminants. Inclusion of unprocessed seeds or vegetable oils in the diet of ruminants is an important tool to improve the dietary energy content and the n-3 FAs content of milk and other dairy products (Bianchi et al., 2017; Gómez-Cortés et al., 2018, 2008; Medeiros et al., 2014; Suksombat, Thanh, Meeprom, & Mirattanaphrai, 2014). This feeding strategy is mainly useful when the basal feedstuffs are poor in n-3 FAs content (Pi et al., 2016).

2.1. Effectiveness of unprocessed seeds or vegetable oils in enhancing n-3 fatty acids in milk

The maximum differences in n-3 FAs concentrations of ruminant milk due to vegetable oils supplementation are summarised in Table 1. In ruminants, the composition of n-3 FAs in milk mainly reflects that of consumed lipids. Lipids rich in n-3 FAs affect the FA composition of milk not only by direct assimilation of the FAs into the milk, but also indirectly by modulating the expression of lipogenic enzymes (Bodkowski et al., 2016).

Recently, Livingstone et al. (2015) and Kliem et al. (2019) investigated the effects of extruded linseed oil supplementation on FA composition of milk of lactating cows. Milk obtained from cows supplemented with extruded linseed oil had higher ALA and total n-3 FAs content with lower content of total SFA and n-6/n-3 ratio. Other studies (Suksombat et al., 2014; Suksombat, Meeprom, & Mirattanaphrai, 2013; Suksombat, Thanh, Meeprom, & Mirattanaphrai, 2016) conducted to investigate the effects of unextruded linseed oil or whole linseed supplementation on FA

Table 1
Results of different studies of effects of n-3 fatty acid-rich vegetable seed or oil supplementation on n-3 fatty acid content of ruminants' milk.

n-3 Fatty acid	Maximum differences reported (g 100 g $^{-1}$ fatty acids)	Species	Reference
C18:3n-3 (ALA)	+0.36 to +0.67	cow	Kliem et al. (2018); Livingstone et al. (2015)
	+0.60 to +0.90	sheep	Caroprese et al. (2011); Zhang et al. (2006)
	+0.31 to +0.64	goat	Gómez-Cortés et al. (2018); Martínez Marín et al. (2012)
C20:3n-3	−0.03 to +0.01	cow	Kliem et al. (2018); Livingstone et al. (2015)
	+0.01	sheep	Caroprese et al. (2011)
	+0.002 to +0.01	goat	Martínez Marín et al. (2011, 2012)
C20:5n-3 (EPA)	+0.02 to +0.05	cow	Bu et al. (2007); Suksombat et al. (2014)
	+0.03 to +0.22	sheep	Caroprese et al. (2011); Mierlita (2016)
	+0.01 to +0.02	goat	Gómez-Cortés et al. (2018); Martínez Marín et al. (2012)
C22:6n-3 (DHA)	+0.02 to +0.05	cow	Bu et al. (2007); Suksombat et al. (2014)
	+0.01 to +0.17	sheep	Caroprese et al. (2011); Mierlita (2016)
	+0.002 to +0.12	goat	Bernard et al. (2009); Martínez Marín et al. (2011)
C 22:5n-3 (DPA)	−0.01 to +0.04	cow	Livingstone et al. (2015); Suksombat et al. (2014)
	+0.01 to +0.02	goat	Martínez Marín et al. (2011, 2012)
Total n-3	+0.47 to +1.13	cow	Kliem et al. (2018); Livingstone et al. (2015)
	+0.33 to +0.65	sheep	Caroprese et al. (2011); Mierlita (2016)
	+0.43 to +0.67	goat	Martínez Marín et al. (2011, 2012)
n-6/n-3 ratio	−2.40 to −1.56	cow	Suksombat et al. (2014); Kliem et al. (2018)
	−1.10 to −0.04	sheep	Caroprese et al. (2011); Mierlita (2016)
	−4.46 to −4.18	goat	Martínez Marín et al. (2011, 2012)

composition of milk of lactating cows also showed increases in ALA and total n-3 FA content, and decreases in total SFAs and n-6/n-3 ratio. However, the values for ALA and total n-3 FAs content obtained while cows supplemented with extruded linseed oil were higher, which may be attributed to increasing in digestibility, as extrusion helps to enhance digestibility (Kouba & Mourot, 2011).

In most cases, as the supplementation of n-3 FAs rich diets to ruminants is restricted to around 2–3% of dry matter to protect impairment of rumen function, the level of n-3 FAs in milk fat is limited (Dewhurst & Moloney, 2013). Due to extensive rumen biohydrogenation of PUFAs, only 2.2–3.5% of the ingested n-3 FAs are secreted into milk, which in turn limits the level of n-3 FAs in milk fat (Hurtaud, Faucon, Couvreur, & Peyraud, 2010). High level of unprotected linseed oil (5% of DM) supplementation to dairy cows revealed limited increase in ALA (0.53% of total FAs) and decreased milk yield (4.1 kg day $^{-1}$) (Chilliard, Martin, Rouel, & Doreau, 2009) that can be postulated to be due to extensive rumen biohydrogenation and interference with rumen function. Goodridge, Ingalls, and Crow (2001), however, reported similar milk yield but a higher concentration of ALA (6.39% of FAs) compared with control (0.83% of FAs) when casein protected linseed oil was supplemented to lactating cows, suggesting that inclusion of diets rich in n-3 FAs to ruminants' feed as rumen protected form can effectively maximise the incorporation of n-3 FAs in milk fat without affecting milk yield.

Inclusion of unprotected rubber seed oil (4%), flaxseed oil (4%) or a mixture of both (2% rubber seed oil and 2% flaxseed oil) to the diet of lactating cows enhanced the concentrations of ALA in milk by 40%, 86% and 51%, respectively (Pi et al., 2016). In addition, these authors reported an increase in the sum of n-3 FAs (from +64% to + 82%) and decrease in SFAs (from −31% to −29%). Another study conducted by Bu, Wang, Dhiman, and Liu (2007) to investigate the effects of soybean seed oil (4%), flaxseed oil (4%) or a mixture of both (2% soybean seed oil and 2% flaxseed oil) on FA composition of milk of lactating cows, however, showed increase in ALA, and sum of n-3 polyunsaturated FAs (PUFAs) when only flaxseed oil was included in the diet, which can be attributed to the low content of ALA in soybean seed oil.

In dairy sheep and goats, interest has increased in the last years in incorporating vegetable oils rich in n-3 FAs to the diet to modify the FA composition, mainly to enhance the content of health beneficial FAs in milk, particularly n-3 FAs. In sheep, increase in ALA and total n-3 FAs and decrease in total SFAs and n-6/n-3 ratio was found when linseed oil, sunflower seed oil or hemp seed were added to the diet of lactating ewes (Caroprese et al., 2011; Mierlita, 2016; Vargas-Bello-Pérez et al., 2014; Zhang, Mustafa, & Zhao, 2006). Compared with the control, supplementation yielded a 1.3–2 fold increase in ALA and a 1.6–2 fold increase in total n-3 FAs, whereas SFAs and n-6/n-3 decreased 1.03–1.2 fold and 1.4–1.5 fold, respectively. In addition to ALA, hemp seed supplementation increased EPA (2.1 fold) and DHA (1.9 fold), which is not observed with linseed or sunflower oils supplementation. Antonacci et al. (2018) also reported an increase in ALA (1.97 versus 5.18 g 100 g $^{-1}$ FAs) and n-3 FAs (2.07 versus 5.33 g 100 g $^{-1}$ FAs), and a decrease in SFAs (66.50 versus 52.29 g 100 g $^{-1}$ FAs) and n-6/n-3 (5.66 versus 1.89 g 100 g $^{-1}$ FAs) ratio as the level of linseed oil to substitute soybean seed oil increased (0, 25, 50, 75 and 100%).

Similar to dairy cows and sheep, dietary vegetable seed oil supplementation significantly modifies the FA composition of the milk of dairy goats (Ayana & El-Shabraw, 2015; Gómez-Cortés et al., 2018). Sunflower oil supplementation of dairy goat feed decreased SFAs and increased total n-3 FAs; however, the contents of individual n-3 FAs were not affected by sunflower oils supplementation (Bernard, Shingfield, Rouel, Ferlay, & Chilliard, 2009; Martínez Marín et al., 2011). On the other hand, linseed oil supplementation enhanced the contents of n-3 FAs (especially ALA) and decreased SFA and n-6/n-3 ratio in goat milk under different feeding conditions (Bernard et al., 2009; Gómez-Cortés et al., 2018; Martínez Marín et al., 2011, 2012); suggesting that incorporation of linseed oil to the diet of dairy goats is an imperative feeding strategy to enhance health beneficial FAs (n-3 FAs) in goat milk.

2.2. Effectiveness of unprocessed seeds or vegetable oils in enhancing n-3 fatty acids in cheese and yoghurt

Unlike milk, few experiments have investigated the effects of n-3 FAs rich vegetable seeds or oils supplementation on the FAs profile of cheese and yoghurt. Cattani, Mantovani, Schiavon, Bittante, and Bailoni (2014) investigated the effects of extruded flaxseed on the FAs composition of cows' milk ripened cheese, and reported decrease in SFAs (especially C16:0; 32.2 versus 27.3% of total FAs) and n-6/n-3 ratio (9.68 versus 5.16% of total FAs), whereas ALA and the sum of n-3 FAs increased 0.28 versus 0.58 and 0.30 versus 0.61% of total FAs, respectively, in cheese produced using milk of supplemented cows compared with that from control cows. Likewise, studies were undertaken by Zhang et al. (2006) and Vargas-Bello-Pérez et al. (2014) to examine the effects of flaxseed, sunflower seed or extruded linseed supplementation on FAs profile of cheese produced from ewes' milk, and showed increased ALA and sum of n-3 FAs, whereas SFAs and n-6/n-3 ratio decreased.

For the last two to three decades, linseed has been known as a very good source of n-3 FAs, especially ALA. Inclusion of linseed either in the form of linseed oil or linseed extrudate to the diet of dairy goats raised the contents of ALA (from +77.78% to +137.04%) and sum of n-3 FAs (from +65.35% to +114.17%) in goat milk yoghurt drinks (Borková, 2018). According to Borková (2018), goats fed linseed extrudate supplement produced lower concentrations of ALA (1.92 versus 2.56 g 100 g $^{-1}$ total FAs) and total n-3 FAs (2.10 versus 2.72 g 100 g $^{-1}$ total FAs) compared with goats fed linseed oil supplement. This could be because when vegetable fat is given in the form of free oil it is immediately available in the rumen whereas when oil is inside intact seeds it is released gradually (Nudda et al., 2014).

In yoghurt made from milk of sheep fed with palm oil supplement, there was an increase in ALA content (from 0.30 to 0.58 g 100 g $^{-1}$ FA) as the level in the diet increased (2%, 4% and 6%), whereas ALA was not detected in the yoghurt from milk of sheep not fed palm oil (Bianchi et al., 2017). In addition to ALA, health-promoting FAs, such as conjugated linoleic acid (CLA) and arachidonic acid, increased as the level of palm oil increased. Moreover, these authors reported a decrease in short and medium chain saturated FAs in yoghurt samples made from the milk of ewes fed with the supplement, suggesting that incorporation of palm oil into the diet of sheep can result in positive effects on sheep yoghurt.

In general, all the aforementioned studies indicated that the evaluated dairy products (cheese and yoghurt) showed similar ALA and n-3 FA profiles to that of the ALA and total n-3 FA enriched milk, suggesting that the ALA and total n-3 FAs in cheese and yoghurt reflects the FA profile of the raw milk used to produce these products.

3. Effectiveness of fish meal and fish oils in enhancing n-3 fatty acids in dairy products

Fish meal is a source of high quality protein, energy and easily digestible essential FAs and amino acids. Moreover, it contains large quantities of minerals (phosphorus and calcium), vitamins (biotin, choline, B12, A, D and E) and trace elements (iodine and selenium) (Cho & Kim, 2011). The compositional quality of the nutrients found in fish meal distinguishes it from other dietary supplements, particularly its content of long-chain polyunsaturated n-3 FAs and essential amino acids. Fish meal and fish oils are among the major dietary sources of n-3 FAs, particularly DHA, docosapentaenoic acid (DPA) and EPA, and are used as dietary supplements for ruminant and non-ruminant animals (Cho & Kim, 2011). Several studies have reported that fish meal and fish oils supplementation improve the growth and performance of ruminant and non-ruminant animals (Farhoomand & Checaniazer, 2009; Luo et al., 2013; Toral et al., 2010a). In addition, these feed ingredients enhance the quality of animals products (Kouba & Mourot, 2011; Mir, Rafiq, Kumar, Singh, & Shukla, 2017). FAs in fish oil are highly unsaturated and are known to interfere with rumen function, leading to fat depression in milk (Dewhurst & Moloney, 2013). Similarly, Chilliard, Ferlay, and Doreau (2001) indicated that fish oil FAs or intermediate products of their metabolism have inhibitory effects on lipoprotein lipase activity, suggested to cause fat depression in milk despite there appear to be an increase in n-3 FA concentration.

3.1. Effectiveness of fish meal or fish oils in enhancing n-3 fatty acids in milk

The maximum differences in n-3 FA concentrations of ruminants' milk due to fish meal or fish oils supplementation are summarised in Table 2. Incorporation of fish oil at 2% DM to a TMR based feeding in lactating dairy cows elevated DHA (+430%), EPA (+2000%) and ALA (+114%) concentrations in milk (Kupczyński et al., 2010). Vafa et al. (2012) investigated whether inclusion of fish oil either alone (2%) or combined with canola oil (1% fish oil + 1% canola oils) to the diet of early lactating Holstein cows affected the FA profile of milk. Cows fed fish oil alone or combined with canola oil had higher concentrations of DHA (0.05 versus 3.09 g 100 g $^{-1}$ FAs for the former and 0.05 versus 1.12 g 100 g $^{-1}$ FAs for the latter) and EPA (0.08 versus 2.78 g 100 g $^{-1}$ FAs for the former and 0.08 versus 1.57 for the latter). On the other hand, the same authors demonstrated that inclusion of fish oil alone negatively affected the concentration of ALA but positively affected the concentration when combined with canola oil. Shingfield et al. (2006) observed increases in n-3 FAs in milk when fish oil combined with sunflower oil (1:2, w/w) was included in the diet of lactating cows. Thanh and Suksombat (2015) also indicated that feed supplements of fish oil combined with linseed oil (1:1, w/w), sunflower oil (1:1, w/w) or linseed and sunflower oils (1:1:1, w/w/w) increased the n-3 FAs in cows' milk, including ALA.

In sheep, supplementation of fish oil, which is rich in DHA and EPA, markedly raised the concentration of these long-chain FAs in milk compared with milk obtained from sheep not fed the supplement (Frutos, Toral, & Hervás, 2017; Nudda et al., 2014; Toral et al., 2010a; Tsiplakou & Zervas, 2013). The concentration of these long-chain FAs and other n-3 FAs varies depending on the manner of supplementation. Higher concentrations of DHA, DPA and EPA have been shown when fish oil was supplemented alone (Toral, Shingfield, Hervás, Toivonen, & Frutos, 2010c) compared with when combined with other plant oils (Toral et al., 2010a; Tsiplakou & Zervas, 2013). In contrast, most studies indicated increased concentrations of ALA when fish oil combined with other plant oils was fed as a supplement to lactating ewes, which has also been observed in dairy cows (Shingfield et al., 2006; Thanh & Suksombat, 2015). The highest levels of the long chain n-3 FAs, EPA and DHA, in milk without affecting milk yield and composition, have also been achieved using fish oil protected from rumen biohydrogenation by the formaldehyde-treated protein method (1.3 versus 2.2%; Gulati, Mcgrath, Wynn, & Scott, 2003). This could be attributed to reduction in ruminal biohydrogenation of n-3 FAs and less effect of polyunsaturated FAs on rumen microbes (Kairenius et al., 2018).

Dietary supplementation of dairy goats with fish oil can be an important feeding strategy to enhance the concentrations of n-3 FAs in milk. For instance, milk obtained from goats supplemented with fish oil combined with sunflower oil (1:2, w/w) contained higher concentrations of DHA (+200%), EPA (+117%) and ALA (+16%) than milk obtained from goats supplemented with sunflower oil alone (Bernard et al., 2010). Bernard, Toral, Rouel, and Chilliard (2016) reported that goats fed with concentrates containing only fish oil (40 g day $^{-1}$) produced milk rich in long-chain FAs (20:4n-3, 20:5n-3, 22:3n-3, 22:5n-3 and 22:6n-3), whereas goats fed with concentrates containing fish oil (40 g day $^{-1}$) and extruded linseed oil (360 g day $^{-1}$) produced milk rich in ALA (18:3n-3), suggesting that fish oil is a rich source of long-chain FAs ($\geq$20 carbon) while linseed oil is a rich source of ALA. Another study by Toral, Rouel, Bernard, and Chilliard (2014) has also shown increases in milk EPA content (from 0.03 to 0.10 g 100 g $^{-1}$), DPA (from 0.09 to 0.15 g 100 g $^{-1}$) and DHA (from 0.02 to 0.08 g 100 g $^{-1}$) as the dose of fish oil in the feed increased (0, 20 and 40 g day $^{-1}$).

Table 2
Results of different studies of effects of fish meal or fish oil supplementation on n-3 fatty acids content of ruminants' milk.

n-3 Fatty acid	Maximum differences reported (g 100 g $^{-1}$ fatty acids)	Species	Reference
C18:3n-3 (ALA)	+0.25	cow	Thanh and Suksombat (2015); Vafa et al. (2012)
	−0.04 to +1.2	sheep	Frutos et al. (2017); Tsiplakou and Zervas (2013)
	+0.10 to +1.45	goat	Bernard et al. (2010, 2016)
18:4 n-3	+0.02	cow	Shingfield et al. (2006)
C20:3n-3	+0.14	cow	Shingfield et al. (2006)
	+0.03 to +0.08	sheep	Frutos et al. (2017); Toral et al. (2010c)
C20:4n-3	+0.17	cow	Shingfield et al. (2006)
	+0.15	sheep	Frutos et al. (2017)
C20:5n-3 (EPA)	+0.19 to +0.21	cow	Kupczyński et al. (2011); Vafa et al. (2012)
	+0.11 to +0.12	sheep	Toral et al. (2010c); Tsiplakou and Zervas (2013)
	+0.07	goat	Bernard et al. (2010)
C22:6n-3 (DHA)	+0.24 to +0.37	cow	Thanh and Suksombat (2015); Vafa et al. (2012)
	+0.36 to +1.08	sheep	Frutos et al. (2017); Toral et al. (2010c)
	+0.06 to +0.08	goat	Bernard et al. (2010, 2016)
C 22:5n-3 (DPA)	+0.10	cow	Shingfield et al. (2006)
	+0.10 to +0.78	sheep	Frutos et al. (2017); Tsiplakou and Zervas (2013)
Total n-3	+0.50 to +0.62	cow	Kupczyński et al. (2011); Thanh and Suksombat (2015)
	+0.14 to +2.56	sheep	Frutos et al. (2017); Tsiplakou and Zervas (2013)
	+1.04	goat	Bernard et al. (2016)
n-6/n-3 ratio	−9.35	cow	Thanh and Suksombat (2015)
	−2.33 to −0.25	sheep	Toral et al. (2010c); Tsiplakou and Zervas (2013)
	−0.48	goat	Bernard et al. (2016)

The other form of fish-related feed source used as dietary supplement for ruminants is fish meal (Carroll, Hossain, & Keller, 1994; Vafa et al., 2012). In sheep, inclusion of fish meal to the diet increased the concentrations of n-3 FAs in milk with the highest level (263% increase) observed for DHA (Or-Rashid, Fisher, Karrow, Alzahal, & McBride, 2010). Similar to lactating ewes, fish meal supplementation to lactating dairy cows was able to noticeably increase the n-3 FAs concentration, which also showed the highest level of increment (+233%) for DHA (Adler & Randby, 2007).

3.2. Effectiveness of fish meal and fish oils in enhancing n-3 fatty acids in cheese

Investigations conducted to evaluate the effects of fish meal or fish oil supplementation on FA composition of dairy products other than milk are very limited. To the best of our knowledge, there are only two studies (Allred et al., 2006; Mohan, Anand, Kalscheur, Hassan, & Hippen, 2013) both conducted on Cheddar cheese made from cow milk. Cheddar cheese made from milk cows fed a fish oil supplement contained higher DHA (400%), EPA (66.67%), sum of n-3 FAs (11.29%) and n-3/n-6 ratio (9.32%) than that made from milk of cows not fed with the supplement (Allred et al., 2006). Moreover, Allred et al. (2006) reported that fish oil supplementation resulted in a reduction of short chain saturated FAs ($C_{6:0}$ to $C_{12:0}$), medium chain saturated FAs ($C_{14:0}$ and $C_{16:0}$) and total saturated FAs. Likewise, Mohan et al. (2013) reported increases in total n-3 FAs and decreases in total SFAs in throughout ripening in Cheddar cheese made from the milk of cows fed fish oil supplement.

4. Effectiveness of microalgae in enhancing n-3 fatty acids in milk and dairy products

DHA and EPA are either low in amounts or totally absent in most ruminant feedstuffs (Esteban, Girón, Lucía, Restrepo, & Carulla, 2016). Consequently, they are found in a very low amount or totally absent in dairy products (Medeiros et al., 2014; Sharifi, Bashtani, Naserian, & Farhangfar, 2017). However, the use of microalgae, which is rich in DHA and EPA, in dairy animals diet may provide an opportunity to produce dairy products with increased amounts of DHA and EPA (Lum, Kim, & Lei, 2013). DHA and EPA can efficiently be transferred to dairy products while included in the diet of the animals (Altomonte, Salari, Licitra, & Martini, 2018).

4.1. Effectiveness of microalgae in enhancing n-3 fatty acids in milk

The maximum differences in n-3 FAs concentrations of ruminants' milk due to microalgae supplementation are summarised in Table 3. Full-fatted microalgae biomass, defatted microalgae biomass or algal oil can be included in the diet of ruminants to modify the FA composition of their milk (Boeckaert et al., 2008; Glover et al., 2012; Toral et al., 2010b). A raise in DHA and EPA has been reported in cows supplemented with full-fatted microalgae biomass (Craddock, Neale, Probst, & Peoples, 2017; Lock & Bauman, 2004; Moran, Morlacchini, Keegan, & Fusconi, 2017) with positive differences ranging from 1000 to 1122% for DHA and 24–240% for EPA. These authors found that DHA and EPA transferred efficiently from feed to milk, suggesting that full-fatted microalgae biomass may play a positive role in enhancing the health-promoting n-3 FAs in milk.

Milk obtained from cows supplemented with rumen-protected microalgae showed increased levels of DHA (0.24 versus 0.08 g 100 g $^{-1}$ of FAs; Glover et al., 2012). In addition to the increase in DHA content of milk, Glover et al. (2102) reported that rumen-protected microalgae supplementation reduced the SFA content of milk, whereas it increased PUFA content, particularly ALA, and CLA. An increase in milk DHA (+200%) and EPA (+300%) has also been shown for cows on total mixed ration-based feeding supplemented with algal oil (Stamey, Shepherd, de Veth, & Corl, 2012). In their review, Lum et al. (2013) highlighted that feeding of dairy cows with de-fatted microalgae biomass elevated the DHA and EPA content in milk, whereas the content of SFA was reduced.

Moate et al. (2013) observed that the DHA, DPA and EPA content of milk in dairy cows fed algal supplement varied dependent on supplement dose. These authors reported various effects related to feeding different doses (0, 25, 50 and 75 g cow $^{-1}$ day $^{-1}$) of algal meal, but at the highest dose of algal meal (75 g cow $^{-1}$ day $^{-1}$), in particular, concentrations of DHA, DPA and EPA in milk were increased 23-fold, 7-fold, and 2-fold, respectively. Moreover, a 5-fold increase in C18:2 *cis*- 9,*trans*-11 and 2-fold increase in total FAs containing a chain length greater than 18 carbon atoms and PUFAs was observed in milk from cows fed the diet containing 75 g algal meal cow $^{-1}$ day $^{-1}$.

Likewise, DHA, DPA and EPA content in sheep and goat milk are affected by nutritional supplementation of microalgae. Supplementation of microalgae to dairy ewes, kept under different feeding regimes caused a large enrichment in DHA content of milk ranging

Table 3
Results of different studies of effects of microalgae supplementation on n-3 fatty acids content of ruminants' milk.

n-3 Fatty acid	Maximum differences reported (g 100 g $^{-1}$ fatty acids)	Species	Reference
C18:3n-3 (ALA)	+0.03	cow	Moate et al. (2013)
	–0.19	sheep	Toral et al. (2010a)
	–0.14 to +0.29	goat	Novotná et al. (2017); Póti et al. (2015)
18:4 n-3	+0.02	sheep	Toral et al. (2010a)
C20:3n-3	+0.02	cow	Moate et al. (2013)
	+0.01 to +0.17	sheep	Reynolds et al. (2006); Toral et al. (2010a)
	+0.10	goat	Póti et al. (2015)
C20:5n-3 (EPA)	+0.10	cow	Moate et al. (2013)
	+0.02 to +0.05	sheep	Bichi et al. (2013); Toral et al. (2010a)
	+0.02 to +0.04	goat	Novotná et al. (2017); Póti et al. (2015)
C22:6n-3 (DHA)	+0.87	cow	Moate et al. (2013)
	+0.33 to +0.54	sheep	Bichi et al. (2013); Toral et al. (2010a)
	+0.02	goat	Novotná et al. (2017); Póti et al. (2015)
C 22:5n-3 (DPA)	+0.52	cow	Moate et al. (2013)
	+0.02 to +0.05	sheep	Bichi et al. (2013); Toral et al. (2010a)
Total n-3	+1.29	cow	Moate et al. (2013)
	+0.40 to +0.48	sheep	Bichi et al. (2013); Toral et al. (2010a)
	+0.33	goat	Póti et al. (2015)
n-6/n-3 ratio	–1.49	cow	Moate et al. (2013)
	–1.97	sheep	Bichi et al. (2013)
	–0.65	goat	Póti et al. (2015)

from 9 to 19-fold (Bichi, Hervás, Toral, Loor, & Frutos, 2013; Reynolds, Cannon, & Loerch, 2006; Toral et al., 2010b). Though comparatively lower than the values reported for DHA, these authors observed elevation in DPA content of milk ranging from 2 to 7-fold and EPA content ranging from 2 to 12-folds when microalgae was included in the diet of lactating ewes. In contrast, inclusion of microalgae to the diet of lactating ewes reduced the ALA content of milk (Toral et al., 2010b).

Compared with those on dairy cows and ewes, studies associated with the effects of feed supplemented with microalgae on the milk of dairy goats are limited. To the best of our knowledge, only two studies investigated the effects of microalgae on the FA composition of goats' milk (Novotná et al., 2017; Póti, Pajor, Bodnár, Penksza, & Köles, 2015). Similar to the situation for cows' and ewes' milk, microalgae supplementation positively affected the concentrations of n-3 FAs in goats' milk, in particular, DHA, EPA and eicosatrienoic acid (Novotná et al., 2017; Póti et al., 2015). However, contradictory results have been reported on the ALA contents of goats' milk. Póti et al. (2015) reported increases in ALA content (0.88 versus 1.17% of total FAs), whereas Novotná et al. (2017) reported decreases (1.18 versus 1.04% of total FAs).

Overall, incorporation of microalgae into the diet of ruminants either in the form of full-fatted biomass, algal oil or de-fatted biomass increased n-3 FAs in milk, especially DHA, DPA and EPA.

4.2. Effectiveness of microalgae in enhancing n-3 fatty acids in cheese and yoghurt

Though numerous experiments have been conducted to evaluate the effects of microalgae supplementation on the FA profile of ruminants' milk, to the best of our knowledge, there has been only one study (Papadopoulos, Goulas, Apostolaki, & Abril, 2002) undertaken to evaluate the effect of full fatted microalgae biomass supplementation on FA profile of Feta cheese and yoghurt made from ewes' milk. Papadopoulos et al. (2002) reported that algal supplementation increased n-3 FA levels, especially DHA, EPA and EDA, and decreased the n-6/n-3 ratio in Feta cheese and yoghurt. DHA, EPA and EDA were not detected in cheese and yoghurt samples made from control milk (from ewes not fed supplement), whereas the concentrations of DHA, EPA and EDA in Feta cheese made from milk from ewes fed microalgae supplement were 7.5, 3.4, and 3.7 g kg $^{-1}$ total FAs, respectively, and 10.4, 1.8 and 1.9 g kg $^{-1}$ total FAs, respectively in yoghurt. The authors also reported a reduction in n-6/n-3 ratio (10.9 versus 2.5) in Feta cheese and (12.1 versus 2.8) in yoghurt, suggesting that Feta cheese and yoghurt made from supplemented milk could be recognised as healthy foods and their incorporation in human diets could increase the ratio of n-6/n-3 FAs to a desirable level.

5. Effectiveness of grass-based feeding in enhancing n-3 fatty acids in milk

The total FA content of grasses ranges from 20 to 50 g kg $^{-1}$ dry matter (Kalac & Samkova, 2010). Though the FA content of grasses is relatively low, grasses have often been the major and also the cheapest source of FAs in ruminant nutrition. The major FA in grasses is ALA, representing 50–75% of the total FAs (Dewhurst & Moloney, 2013).

Grass-based feeding, in its different forms, fresh or conserved, increased total n-3 FAs, particularly ALA content (from 0.10 to 0.33 g 100 g $^{-1}$ FAs) and decreased SFA content (from 2.00 to 6.66 g 100 g $^{-1}$ FAs) in ruminants' milk compared with total mixed ration-based feeding (Domínguez-Vara et al., 2010; Kalac & Samkova, 2010; Martin, Ferlay, Pradel, Chilliard, & Coulon, 2010; Rego et al., 2016). In addition to an increase in ALA content, Kalac and Samkova (2010) reported an increase in EPA content (0.06 g 100 g $^{-1}$ FAs) and DHA content (0.01 g 100 g $^{-1}$ FAs) of milk obtained from cows kept under grass-based feeding. Compared with conserved grasses, fresh grasses are more effective in increasing n-3 FA content of milk. Mohammed et al. (2009) and Baumgard, Thomson, Kay, Roche, and Kolver (2005) reported an increase in ALA content of 0.48 and 0.63 g 100 g $^{-1}$ FAs in milk from cows fed fresh grasses and cows fed conserved grasses, respectively.

Patel, Wredle, and Bertilsson (2012) investigated the effects of grass-silage proportion on milk FA composition of lactating dairy cows fed grass-silage and grain based concentrate. These authors reported increases in milk ALA (from 0.65 to 0.90 g 100 g $^{-1}$ of FAs), EPA (from 0.06 to 0.15 g 100 g $^{-1}$ FAs) and DPA (from 0.02 to 0.04 g 100 g $^{-1}$ FAs) and decrease in n-6/n-3 (from 2.64 to 1.23 g 100 g $^{-1}$ FAs) as the proportion of grass-silage increased from 50 to 85% of the diet. Likewise, Ward, Malcolmson, Froebe, Wittenberg, and Przybylski (2003) reported an increase in ALA content of milk from 0.37 to 0.52 g 100 g $^{-1}$ FAs as the proportion of fresh forage increased from 50 to 80% of the diet. On the other hand, decrease in total n-3 FAs, ALA, EPA and increase in n-6/n-3 content was observed as grass-silage was replaced with maize-silage (Kliem, Morgan, Humphries, Shingfield, & Givens, 2008).

Overall, grass-based feeding improved n-3 FA content of milk, especially ALA content. Except for the study of Kalac and Samkova (2010) in which a slight increase in DHA content of the milk was reported, all the cited studies did not detect DHA as grasses were incorporated to the diet.

6. Oxidative stability and sensory characteristics of n-3 enriched milk and its products

Oxidation reduces the sensory and nutritional quality of milk and its products (Salles et al., 2019). Oxidative stability of milk and its products is also influenced by FA composition of milk fat. The more unsaturated a FA, the more susceptible to oxidation. For instance, milk obtained from cows fed grass silage showed higher n-3 FA concentration and lipid oxidation compared with that in milk from cows fed corn silage (Havemose, Weisbjerg, Bredie, & Nielsen, 2004), Moreover, Timmons, Weiss, Palmquist, and Harper (2001) reported a positive correlation between oxidative flavour of milk and the concentrations of total PUFAs, linoleic acid and ALA in milk. Hurtaud et al. (2010) reported a rancid aroma of butter as the level of ALA increased (0.23 versus 0.67% of FAs) due to extruded linseed oil supplementation, despite it can be reduced by incorporating antioxidants in the animals' diet (Salles et al., 2019).

In contrast, milk and butter obtained from cows supplemented with marine algae had similar oxidative stability with the control milk and butter, even though the supplemented milk and butter contained a higher n-3 FA content (MacLaren et al., 2012). Similarly, Jones et al. (2005) observed no differences in flavour of cheese and butter produced from milk with a threefold enrichment of EPA and DHA due to fish oil supplementation, compared with control milk. Likewise, Kitessa et al. (2004) and Nelson and Martini (2009) reported that milk obtained from cows fed with fish oil supplemented had oxidative stability and organoleptic properties similar to that of milk obtained from cows not fed the supplement.

7. Conclusions

Significant progress has been made to characterise the effects of n-3 FAs rich sources of feeds on milk FA composition of ruminants compared with other dairy products. Supplementation with a long chain n-3 FA-rich diet positively influences the FA composition of milk and other dairy products. Dietary supplementation with fish meal or fish oil and microalgae are more interesting in terms of increasing the contents of long-chain n-3 FAs (≥20 carbon) especially DHA (+1.08 g 100 g $^{-1}$ FAs) and EPA (+0.21 g 100 g $^{-1}$ FAs). In contrast, grasses-based feeding and supplementation with vegetable seeds or oils such as linseed, rubber seed and hempseed effectively enhance the contents of ALA (+0.90 g 100 g $^{-1}$ FAs) in milk and dairy products. Though it is possible to considerably increase the n-3 FAs content of milk and dairy products under all supplementation conditions, the levels of increase are more effective while supplementation is made in the form of rumen protected, extrudate and free oils. Considering the high consumption of milk and dairy products, substantial increase in n-3 FA content of milk and dairy products by dietary manipulation would be an important approach to increase the intake of n-3 FAs and reduce the risk of cardiometabolic and cardiovascular diseases.

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