DHA content in milk and biohydrogenation pathway in rumen: A review

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Docosahexaenoic acid (DHA) is an essential human nutrient that may promote neural health and development. DHA occurs naturally in milk in concentrations that are influenced by many factors, including the dietary intake of the cow and the rumen microbiome. We reviewed the literature of milk DHA content and the biohydrogenation pathway in rumen of dairy cows aim to enhance the DHA content. DHA in milk is mainly derived from two sources: α-linolenic acid (ALA) occurring in the liver and consumed as part of the diet, and overall dietary intake. Rumen biohydrogenation, the lymphatic system, and blood circulation influence the movement of dietary intake of DHA into the milk supply. Rumen biohydrogenation reduces DHA in ruminal environmental and limits DHA incorporation into milk. The *fat-1* gene may increase DHA uptake into the body but this lacks experimental confirmation. Additional studies are needed to define the mechanisms by which different dietary sources of DHA are associated with variations of DHA in milk, the pathway of DHA biohydrogenation in the rumen, and the function of the *fat-1*gene on DHA supply in dairy cows.

1 # DHA Content in Milk and Biohydrogenation Pathway in

2 # Rumen: A Review

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ABSTRACT

Docosahexaenoic acid (DHA) is an essential human nutrient that may promote neural health and development. DHA occurs naturally in milk in concentrations that are influenced by many factors, including the dietary intake of the cow and the rumen microbiome. We reviewed the literature of milk DHA content and the biohydrogenation pathway in rumen of dairy cows aim to enhance the DHA content. DHA in milk is mainly derived from two sources: α-linolenic acid (ALA) occurring in the liver and consumed as part of the diet, and overall dietary intake. Rumen biohydrogenation, the lymphatic system, and blood circulation influence the movement of dietary intake of DHA into the milk supply. Rumen biohydrogenation reduces DHA in ruminal environmental and limits DHA incorporation into milk. The *fat-1* gene may increase DHA uptake into the body but this lacks experimental confirmation. Additional studies are needed to define the mechanisms by which different dietary sources of DHA are associated with variations of DHA in milk, the pathway of DHA biohydrogenation in the rumen, and the function of the *fat-1*gene on DHA supply in dairy cows.

Keywords: α-linolenic acid, biohydrogenation, dairy cows, Docosahexaenoic acid, *fat-1* gene, ruminal microorganism.

INTRODUCTION

Some researchers reported that milk fat could increase triglycerides in blood. However, many researches showed milk fat had no adverse effects on the concentrations of fasting blood lipids, glucose, and insulin (Benatar, Sidhu & Stewart, 2013; Engel Elhauge & Tholstrup, 2017). And even some study showed benefit for blood pressure supplement with milk fat (Rietsema et al., 2019). Substances found in enriched milk, including medium and odd chain SFA (saturated fatty acid), globular phospholipids, unsaturated fatty acids, branched-chain fatty acids, natural trans fatty acids, vitamins K1 and K2, and calcium, have been found to have positive health effects (Mozaffarian & Wu, 2018). Among them, conjugated linoleic acid (CLA), which is peculiarly originated from the rumen (Jaglan, 2019), and omega-3 polyunsaturated fatty acids (n-3 PUFA) have been found to show health benefits to humans (Swanson, Block & Mousa, 2012). Docosahexaenoic acid (DHA, C22:6n-3) is an n-3 PUFA found in the mammalian central nervous system (Gázquez, 2017), making up 10% to 15% of the total cerebral fatty acids (about 10 μmol/g brain). Some bacteria and lower eukaryotes can produce DHA *de novo* via a polyketide synthase pathway (Kabeya et al., 2018) but humans lack the key fat desaturase enzyme for synthesizing DHA (especially Δ12 and Δ13/n-3 desaturase). 2008 FAO/WHO (2008) recommended a daily intake of DHA+EPA of 300 mg for lactating women, recent studies shown a daily intake of 100 mg DHA for infants and 250 mg/day for adolescents DHA+ Eicosapentaenoic acid (EPA, C20:5n-3) (Saini and Keum 2018; Saini et al. (2018). The American Heart Association recommends an intake of 2-4 g/day of DHA+EPA for hypertriglyceridemia patients (Michael et al., 2011). Gebauer et al., (2006) recommended an intake of approximately 500 mg/d of EPA+DHA to reduce the risk of cardiovascular disease. However, most populations only get approximately 100 mg of DHA+EPA per day, which is much lower than the recommendations (Afshin et al., 2019).

The human body can synthesize DHA in extremely limited amounts using α-linolenic acid (ALA, C18:3n-3) (Plourde & Cunnane, 2007), and only approximately 0-4% of dietary ALA may be converted to DHA (Burdge & Wootton, 2002), so DHA needs to be supplemented (Hashimoto et al., 2017).

Milk is a possible dietary source of DHA, dietary source of DHA, but its concentration is particularly low (Bai et al., 2018; Ishaq & Nawaz, 2018; Shingfield, Bonnet & Scollan, 2013). The DHA content of milk is influenced by the rumen microbiota, endogenous synthesis, and dietary intakes of DHA by dairy cows. The rumen biohydrogen content limits the efficient dietary incorporation of DHA into milk and the pathway of DHA hydrogen in the rumen is still unclear. Fat-1 gene was also used to increase DHA in milk (Wu et al., 2012), but researches is limited. We mainly analyzed the literature and defined factors affecting the conversion of dietary DHA into milk and explored strategies to increase the DHA content in milk.

Methodology

The scholarly articles in this review were obtained from web of knowledge, google scholar Baidu scholar and subject-specific professional websites, the date from 1999-2019. The keywords "dairy cow",

73 "dairy cattle", "rumen", "bacteria", "biohydrogenation" "DHA", "microalgae" and "fish oil" were used in

74 the search. All of the articles included in this review were peer-reviewed. The article chose in this paper

75 should show the relation between DHA with dairy cow bacteria or rumen biohydrogenation. The qualitative

76 and quantitative articles were reviewed in this paper. The qualitative articles provide insights into problems

77 by helping to understand the reason and opinions. The quantitative articles use measurable data to express

78 facts and discover research patterns.

79 SOURCES OF DHA IN MILK

80 DHA in milk comes from three major sources: those synthesized from endogenous ALA, those synthesized by

81 the microorganisms in the rumen and intestines of cows, and those converted from the diet.

82 **Metabolic Conversion of ALA to DHA**

83 DHA can be synthesized from ALA through metabolic pathways in the liver (Figure 1) (Kabeya et al., 2018;

84 Kim et al., 2014). The process occurs in the endoplasmic reticulum and peroxisome, which are organelles. ALA

85 is desaturated to stearidonic acid in the endoplasmic reticulum (C18:4n-3) catalyzed in a rate-limiting reaction

86 by Δ6 desaturase and then converted to tetracosahexaenoic acid (C24:6n-3). Tetracosahexaenoic acid is then

87 transferred into peroxisome where it undergoes β-oxidation to form DHA. The reaction sequence for converting

88 ALA to eicosatetraenoic acid (C20:4n-3) is as follows: ALA → eicosatrienoic acid (C20:3n-3) → C20:4n-3

89 (Kabeya et al., 2018). Δ6 and Δ5 desaturases are the key enzymes in the metabolic pathways (Missotten et al.,

90 2009) and the activity of these two desaturate enzymes can determine the amount of DHA synthesized. For

91 example, the expressions of Δ6 and Δ5 desaturase in human subjects are positively correlated with SFA and

92 PUFA but negatively correlated with linoleic acid (LA) and ALA in foods (Xiang et al., 2006). Omega-6

93 polyunsaturated fatty acids (n-6 PUFA) are essential in human and animal diets (Saini & Keum, 2018). Changing

94 the ratio of n-3:n-6 PUFA in the diet can influence the expression of Δ6 desaturase enzyme in rats (Missotten et

95 al., 2009; Neuringer, Anderson & Connor, 1988). Missotten et al., (2009) added fish oil and linseed oil to pig

96 diets and determined the expressions of Δ6 and Δ5 desaturase in the liver, subcutaneous fat, and the *longissimus*

97 *dorsi* muscle. The addition of fish oil increased the expression of Δ5 desaturase only in the *longissimus dorsi*

98 muscle, but not in the liver or subcutaneous fat; the addition of linseed oil had no effect on the expression of Δ5

99 desaturase in all three tissues; the expression of Δ6 desaturase in all three tissues was not affected by either type

100 of oil (Missotten et al., 2009). Studies in rats have shown that protein (Narce et al., 1988) and micromineral

101 (Johnson et al., 1989) depletion in the diet reduced the activity of the Δ6 desaturase enzyme. There have been

102 no reports on the effect of dietary fat on the expression of Δ6 and Δ5 desaturases in cows.

103 **Synthesis of DHA by rumen microorganisms**

104 Microorganisms, including microbes in the ocean and environment, are able to synthesize DHA *de novo* via a

105 polyketide synthase pathway (Kabeya et al., 2018; Dongming, Jackson, & Quinn, 2015; Xue et al., 2013).

106 Diverse and interdependent populations of bacteria, protozoa, and fungi inhabit the rumen of dairy cattle (Russell

107 & Rychlik, 2001) but no studies have been conducted to date that show the relationship between rumen microbes

108 and DHA metabolism. Some articles have indicated that microbes in the rumen may synthesize DHA

109 independently. For example, Bianchi et al. (2014) found that *Cellulophaga* can produce DHA. *Cellulophaga*

110 belongs to phylum Bacteroidetes which is the most abundant phylum in the rumen of older dairy cows (Jami et

111 al., 2013), and infers that there may be a kind of microbe (belong to phylum Bacteroidetes) in the rumen that can

112 produce DHA. *Yarrowia lipolytica* is a yeast widely distributed in the natural environment that can also produce

113 DHA (Dongming, Jackson & Quinn, 2015; Damude et al., 2006; Gong et al., 2014), however, it is not clear

114 if yeast living in the rumen can produce DHA (Prakasan et al., 2013). In an *in vivo* experiment with dairy cows,

115 the animals were fed diets containing linseeds or no linseeds, and no DHA at all, but DHA was subsequently

116 found in the duodenal chyme (0.07 g/d and 0.08 g/d, respectively) (Shingfield K J , Lee M R F , Humphries D J

117 , et al. Effect of linseed oil and fish oil alone or as an equal mixture on ruminal fatty acid metabolism in growing

118 steers fed maize silage-based diets[J]. Journal of Animal Science, 2011, 89(11):3728-41) (Kairenius, Toivonen

119 & Shingfield, 2011). The DHA in the chyme came from the rumen and originated from synthesis by rumen

120 microorganisms or from the blood circulation into salivary secretions.

121 Additional research may need to focus on the identification of DHA-producing bacteria in the rumen. Increasing

122 the content of DHA-producing bacteria present may increase DHA synthesis in the rumen, which results in more

123 DHA in milk. In addition, there may exist interrelationships in DHA synthesis between microbial species with

124 a functional network (Moraïs & Mizrahi, 2019), which should be explored.

125 **Dietary DHA**

126 An increase in dietary DHA can significantly increase the bodily content of DHA and the subsequent milk

127 content of DHA (Scollan et al., 2001a; Vahmani, Fredeen & Glover, 2013). Currently, the major dietary DHA

128 sources for cows are fish oil and microalgae products.

129 **Fish oil**

130 Fish oils contain a variety of n-3 PUFA, of which EPA and DHA are the most abundant (Mahla et al., 2017).

131 Studies have been conducted on supplementing the diets of dairy cattle with fish oil (Table 1) and most studies

132 indicate that this practice could reduce milk fat. The study conducted by Pirondini et al. (2015) showed no

133 negative effects of fish oil (0.8% dry matter) on milk fat when cattle were provided a low starch diet. The type

134 of diet fed, including the percentage of forage (Shingfield et al., 2003) and type of forage (Chilliard, Ferlay &

135 Doreau, 2001), plays an important role in milk fat concentrations.

136 The supplementation of fish oil alone or fish oil combined with other oils (such as extruded soybean, canola

137 oil) all resulted in improved DHA concentrations in milk (Vahmani, Fredeen & Glover, 2013; AbuGhazaleh et

138 al., 2002; Ramaswamy et al., 2001; Vafa et al., 2012; Whitlock et al., 2002). Kairenius et al. (2015),

139 supplemented with fish oil at doses of 75, 150 and 300g/day (around 0.4, 0.8 and 1.88% diet) which increased

140 the DHA concentration in milk (0.03, 0.05 and 0.10 g/100g total milk fatty acid or 0.22, 0.39 and 0.67 g/day in

141 milk). Other studies have shown a positive correlation for DHA content between dietary intake and milk

142   concentrations (Lacasse et al., 2002). However, increased supplementation had no constant linear relationship
143   between dietary DHA intakes and DHA concentrations in milk. Donovan et al. (2000) showed that
144   supplementation with fish oil at 0, 1 and 2 % of total diet increased DHA concentrations in milk, but that the
145   concentration decreased with 3 % of total diet fish oil supplementation. Kairenius et al. (2015) reported no
146   difference between the control group and 75 g/day supplementation group for DHA concentrations (0.03 to 0.03
147   g/100g total milk fatty acid or 0.22 to 0.22 g/day in milk). There may be a liner relationship between fish oil
148   supplementation and milk DHA within certain range, which may be between 0.4% to 3%.
149   The DHA content of milk is also affected by the host's metabolism and the biohydrogenation pathway in
150   the rumen. In theory, minimizing the effects of rumen biohydrogenation in the rumen could increase the DHA
151   content of milk (Casta Eda-Gutiérrez et al., 2007). However, Lacasse et al. (2002) reported that supplementation
152   with fish oil or rumen-protected fish oil at the same doses in the diet made no difference in the DHA
153   concentration of milk. This effect may be due to the reduced digestibility of DHA in rumen-protected fish oil.
154   Dietary supplementation of fish oil can increase the DHA content of milk, but the effect of DHA intake is
155   affected by many factors that need to be quantitatively defined.

156   **Microalgae**
157   Microalgae are microscopic photosynthetic organisms found in marine and fresh waters that are used as an
158   animal feed (Priyadarshani & Rath, 2012). Microalgae are a good source of protein, carbohydrates, and long
159   chain PUFA, some of which are rich in DHA (Ryckebosch et al., 2014). Microalgae have been shown to improve
160   the DHA content in milk when used as an additive to dairy cattle feed (Altomonte et al., 2018). The effects of
161   microalgae supplementation on the fatty acid profile of milk are summarized in Table 2.
162   Supplementation with microalgae has been shown to improve the DHA concentration of milk with a
163   negative effect on the overall fat content of milk. Microalgae supplementation has a liner relationship with the
164   DHA content of milk (Altomonte et al., 2018; Boeckaert et al., 2008; Fougère, Delavaud & Bernard, 2018; Póti
165   et al., 2015) and fish oil has been shown to have the same effect. Three microalgae feeding styles were utilized
166   (microalgae, rumen protect microalgae, and microalgae oil) and each produced unique results. The feeding of
167   rumen-protected microalgae can improve the concentration of milk DHA markedly, compared to feeding
168   microalgae alone (Franklin et al., 1999). Rumen-protected microalgae can reduce the biohydrogenation of DHA
169   in the rumen. Stamey, Shepherd, Veth & Corl (2012) supplemented with 150 g/day of microalgae and 194 g/day
170   of microalgae oil, respectively, and found that the microalgae oil supplementation produced a lower milk fat
171   content, DHA concentration and efficient transport of dietary DHA into milk compared with supplementation
172   of microalgae. The DHA in microalgae oil is able to be biohydrogenated in the rumen more easily than
173   microalgae, although DHA is chosen as a source of dietary DHA more often. Moate et al. (2013) reported that
174   there is an exact linear relationship between microalgae intake and the DHA content of milk. However, no
175   experiments have revealed the range in which microalgae supplementation has a linear relationship with the
176   DHA concentration of milk.
177   An increased concentration of DHA in milk depends on the DHA content in dietary microalgae and is

178 dependent on the species of microalgae and its processing methods. There are many kinds of microalgae that
179 can be used in animal diets with substantially different levels of DHA (Madeira et al., 2017) that can be
180 influenced by the way they are processed. Protecting microalgae from rumen degradation can preserve
181 approximately 45% of the DHA content versus un-protected microalgae (Stamey et al., 2012).

182 **Transport ratio of DHA in milk**

183 The efficiency of DHA incorporation from the feed into milk was low, as showed in table 3. The incorporation
184 efficiency of DHA can be calculated as the ratio of milk DHA content to dietary DHA intake. Fish oil
185 supplementation increased the DHA content in milk by approximately 6.86% (range from 1.35% to 14.4%),
186 while microalgae supplementation increased it approximately 7.08% (range from 1.09% to 14.0%). The
187 efficiency can be influenced by many factors.

TRANSPORTATION OF DIETARY DHA INTO MILK

189 Figure 2 shows how dietary DHA is moved through the body into the milk. The majority of dietary DHA is
190 hydrogenated in the rumen, with 60-98% of DHA transformed in the rumen to the corresponding geometric
191 isomers via cis-trans isomerization of double bonds in DHA (NRC, 2001; Scollanet al. 2001; Kim et al. 2008;
192 Kim et al. 2008; Shingfield et al 2011; Shingfield et al 2012; Kairenius et al 2018). Intact DHA (or by-pass)
193 flows into the small intestine where approximately 70-100% is absorbed (Doreau et al. 1994; Wachira et al.
194 2000; NRC 2001; Scollanet al. 2001; Mattos et al. 2004). It is then absorbed via the lymphatic system into the
195 blood circulation system (Doreau & Ferlay, 1994; Scollan et al., 2001b; USA, 2001; Wachira et al., 2000) and
196 is transported via the blood into various tissues and organs of the body, including the brain (Al-Ghannami, Al-
197 Adawi & Ghebremeskel, 2019), bones (Saini & Keum, 2018), and the reproductive system (Gholami et al.,
198 2010), where it is used for tissue repair or energy supply via the β-oxidative pathway. Only 13-25% of DHA
199 absorbed from the small intestine is transported into milk through the mammary gland cells (Shingfield, Bonnet
200 & Scollan, 2013).

201 The biohydrogenation of DHA in the rumen increases the amount of DHA lost by the body (Figure 2)
202 (Kairenius et al., 2018; Kim et al., 2008; Mattos et al., 2004; Scollan et al., 2001; Shingfield et al., 2003;
203 Shingfield et al., 2012; Shingfield et al., 2011; USA, 2001; Wachira et al., 2000). Therefore, reducing the
204 biohydrogenation of DHA in the rumen is important for improving the DHA concentration of milk.

205 **Ruminal Biohydrogenation**

206 Ruminal biohydrogenation limits the transportation of dietary DHA into milk and is influenced by rumen
207 microbes. Rumen microorganisms include bacteria, protozoa, and fungi. Bacteria play an important role in the
208 biohydrogenation process (Louren, Ramos-Morales, and Wallace, 2010). DHA has two dietary forms: free fatty
209 acids and triacylglycerols. Triacylglycerols must be converted into free fatty acids and then must undergo
210 biohydrogenation by the rumen microbes. Thus, two kinds of microorganisms exist with lipolytic effects and
211 biohydrogenation properties.

212 The lipolytic effect of triacylglycerols mainly depended on the lipase, *Anaerovibrio lipolyticus,* which is

213   a prominent ruminal lipase-producing bacterium (Hungate, 1966). Three putative lipase genes were identified

214   from the draft genome of *Anaerovibrio lipolyticus* (alipA, alipB, alipC) (Privé et al., 2013) and had greater

215   hydrolytic activity against caprylate (C8:0), laurate (C12:0), and myristate (C14:0). *Butyrivibrio fibrisolvens*,

216   *Propionibacterium* (Edwards et al., 2012) *Clostridium*, *Propionibacterium*, *Staphylococcus* (Edwards et al.,

217   2013), and *Pseudomonas aeruginosa* (Priji et al., 2017) are among the bacteria that have the ability to

218   decompose triacylglycerols. Sargolzehi et al. (2015) showed that pyridostigmine bromide could decrease the

219   lipase activity and the immunization against lipase may also inhibit the decomposition of triacylglycerols, just

220   like the immunization against rumen urease inhibits ureolysis in the rumen (Zhao et al., 2015).

221   *Butyrivibrio sp.* is a genus of an important microbe that hydrogenates PUFA in the rumen; it includes

222   *Butyrivibrio fibrisolvens (B. fibrisolvens)*, and *Butyrivibrio proteoclasticus (B. proteoclasticus)*. *B. fibrisolvens*

223   can produce isomerase and change the PUFA structure (for example, converting LA into CLA) (Kepler et al.,

224   1966). Trans-11 vaccenic acid (C18:1), converted from LA, can be hydrogenated to stearic acid (C18:0) by *B.*

225   *proteoclasticus* (Jenkins et al., 2007). However, some studies show that *B. fibrisolvens* failed to successfully

226   induce DHA hydrogenation in the rumen (Jeyanathan, et al., 2016; Maia et al., 2007). *B. proteoclasticus* could

227   hydrogenate DHA (Jeyanathan et al., 2016) *in vitro* in a growth medium containing autoclaved ruminal fluid.

228   Bacterial species, such as *Acetobacter* (Bainbridge, et al., 2016) and *Bacillus* (Petri et al., 2014), but not

229   *Butyrivibrio sp.,* can affect DHA biohydrogenation. However, an experiment by Sakurama et al. (2014) reported

230   that no bacteria (100 strains of anaerobic bacteria were used, *Acetobacter* was included) metabolized DHA.

231   Dietary PUFA has been shown to strongly influence microbial profiles in the rumen. Many studies have shown

232   that DHA intake in a reduction of *B. fibrisolvens* in the rumen in a dose-dependent manner (Shingfield et al.,

233   2012; Maia et al., 2010; Shinji et al., 2009). Abughazaleh & Ishlak (2014) reported that supplement with DHA

234   could reduce the abundance of *B. proteoclasticus*, but other experiments have shown no effect. Shingfield et al.

235   (2012) proposed that DHA and other unsaturated fatty acids could lengthen the bacteria's lag phase. It is well

236   known that rumen bacteria release hydrogens and secrete isomerases, which may hydrogenate the double bonds

237   in unsaturated fatty acids. The biohydrogenase in the rumen is a major factor regulating the biohydrogenation of

238   PUFA. Further studies should focus on the relationship between rumen microbes, DHA, and biohydrogenase.

239   The formation of DHA can also be influenced by the intake of LA. LA can be converted into highly

240   unsaturated fatty acids (HUFA) in vivo. LA and ALA share the same family of enzymes in the formation of

241   HUFA (Fleming & Kris-Etherton, 2014), and compete with one another for enzyme uptake (Gibson,

242   Muhlhausler & Makrides, 2015). Increasing the intake of LA may reduce the formation of DHA from ALA. A

243   study showed that high LA diet could reduce the content of DHA in milk (Aprianita et al. 2014). However, DHA

244   synthesis by ALA in tissues is very low and yet not reported in dairy cows. Ruminal biohydrogenation process

245   is well-studied and understood. The content of unsaturated fatty acids in the diet can influence the

246   biohydrogenation of DHA in the rumen, to various effects. In an *in vitro* study, Chow et al. (2004) found that

247   adding LA and ALA could reduce the biohydrogenation of DHA, which was confirmed in an *in vitro* experiment

248   (Wasowska et al., 2006). Shingfield et al. (2011) found that dietary supplementation of both fish oil and linseed

249   oil at a ratio of 1:1 reduced the hydrogenation of DHA, but increased the hydrogenation of ALA in the rumen.

250   However, Kairenius et al. (2018) reported that dietary addition of linseed oil or sunflower seed oil could promote

251   the biohydrogenation of DHA, EPA, and ALA compared with fish oil alone. We determined that biohydrogenase

252   is not fatty-acid specific and competition exists among unsaturated fatty acids. Short-chain unsaturated fatty

253   acids may tend to be biohydrogenated more readily than long-chain PUFA. Therefore, understanding the

254   mechanisms of biohydrogenation for unsaturated fatty acids and the interactions among these fatty acids in the

255   rumen will help develop dietary strategies to reduce DHA biohydrogenation.

256   **Biohydrogenation Pathways of DHA in Rumen**

257   Studies on the DHA biohydrogenation pathway in the rumen are limited. In 2007, Jenkins et al. (2007) speculated

258   that the first step in the process of DHA biohydrogenation is to convert DHA to a C22:6 isomer that is then

259   hydrogenated to C22:5 fatty acid. However, Kairenius, Toivonen & Shingfield (2011) showed that the C22:6

260   isomer was not detectable in the DHA hydrogenation process, likely due to its short lifetime or the limitation of

261   the analytic method (Escobar et al., 2016). Aldai et al. (2018) investigated the biohydrogenation process of DHA

262   in *in vitro* fermentation using sheep rumen fluid as the inoculator, and determined the metabolites of DHA at 0,

263   1-, 2-, 3-, and 6- hours after fermentation. They found that DHA was initially transformed into mono *trans*

264   methylene interrupted DHA and monoconjugated DHA. Nevertheless, the DHA hydrogenation process started

265   from the isomer formation.

266   Jeyanathan, et al. (2016) used *in vitro* anaerobic fermentation with a single strain of *Butyrivibrio*

267   *proteoclasticus* P18 to explore the biohydrogenation process of DHA during fermentation and showed the

268   product in the DHA biohydrogenation pathway. This experiment showed that 12 kinds of DHA intermediates

269   (C22:5, C22:4, C22:3 and C22:2 isomers) were transformed in 48h. Toral et al. (2018) found that

270   Docosapentaenoic acid may be a major DHA intermediate product. DHA intermediates and the hydrogenation

271   pathway in the rumen are illustrated in Figure 3.

272   The literature is lacking for enzymes that may regulate the DHA hydrogenation pathway in the rumen.

273   According to a report by Toral et al. (2018), some enzymes may exist relating to hydrogenation, isomerization,

274   and migration in the EPA hydrogenation pathway. However, the specific enzymes have not been identified yet,

275   and further studies should focus on the enzymes that regulate the DHA hydrogenation pathway in the rumen.

276   # OTHER FACTOR

277   ### *Fat-1* gene modification

278   The Fat-1 gene is present in *Caenorhabditis elegans*, a free-living nematode. Spychalla, Kinney & Browse

279   (1997) first reported the *fat-1* gene in *Caenorhabditis elegans*, and specifically expressed the gene in

280   Arabidopsis, confirming the cDNA (complementary DNA) sequence of the *fat-1* gene. The translation product

281   of the *fat-1* gene is n-3 PUFA dehydrogenase, which can catalyze the formation of the corresponding n-3 PUFA

282   using 18-20 carbon n-6 PUFA as the substrate (Kang, 2005). The expression of the *fat-1* gene can promote the

283   synthesis of n-3 PUFA in nematodes. Liu et al. (2017) constructed the eukaryotic expression vector *pef-gfp-*

284   *fat-1*, then transfected *pef-gfp-fat-1* into cow fetal fibroblast cells and determined the fatty acid profile. They
285   found that the expression of *fat-1* gene could increase the DHA concentration in the cells. The birth of transgenic
286   cows that carried and expressed the mammalianized *fat-1* gene (*mfat-1*) (Wu et al., 2012) and a transgenic cow
287   showed increased n-3 PUFA profiles and reduced n-6 PUFA in their tissues and milk (Liu et al., 2017; Wu et
288   al., 2012). The effect of the *fat-1* gene on the conversion of n-6 to n-3 PUFA was also confirmed in a transgenic-
289   pig model (Kang et al., 2004; Li et al., 2018). These findings need to be validated in a large cohort of transgenic
290   animals to support these conclusions.

291   **CONCLUSIONS**

292   The literature is limited regarding the conversion of ALA to DHA in tissues and its effect on DHA content in
293   milk. Many studies have focused on increasing the DHA concentration in milk by manipulation of the DHA
294   supply in the diet. Many dietary factors can influence DHA's passage into milk and their effects need to be
295   quantitated. The majority of dietary DHA is biohydrogenated in the rumen. It is extremely important to reduce
296   our reliance on rumen biohydrogenation and find alternative means for synthesizing DHA.

297   The *fat-1* gene from nematodes is highly effective in converting n-6 PUFA to n-3 PUFA. Since the gene does
298   not exist in mammals, transgenic techniques have been applied, which have been successful in cows, pigs and
299   mice. Thus, it may be worthwhile to examine enlarging the transgenic population.

300

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Figure 1

Biosynthetic conversion pathway of ALA to DHA

Biosynthetic conversion pathway of ALA to DHA. Abbreviations: ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. The first step that ALA is converted to stearidonic acid (C18:4-3n) is a rate-limiting reaction. The Figure is adapted from Kabeya et al. (2018) and Kim et al., (2014).

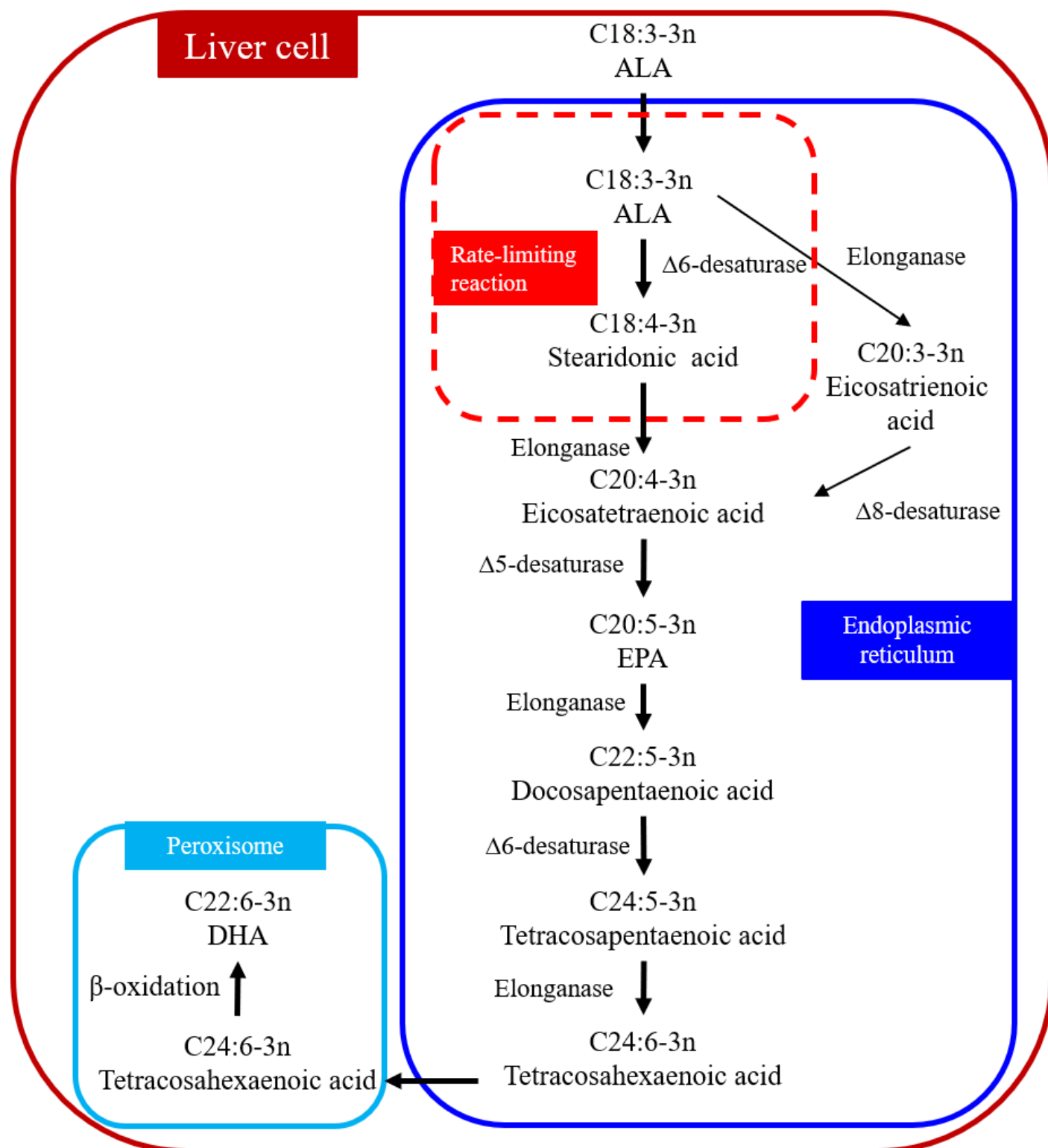



Figure 2

The pathway of DHA transportation into milk

The pathway of DHA transportation into milk. Abbreviations: DHA, docosahexaenoic acid. The Figure is adapted from Shingfield et al. (2003), NRC (USA, 2001), Wachira et al. (2000) and Mattos et al. (2004).

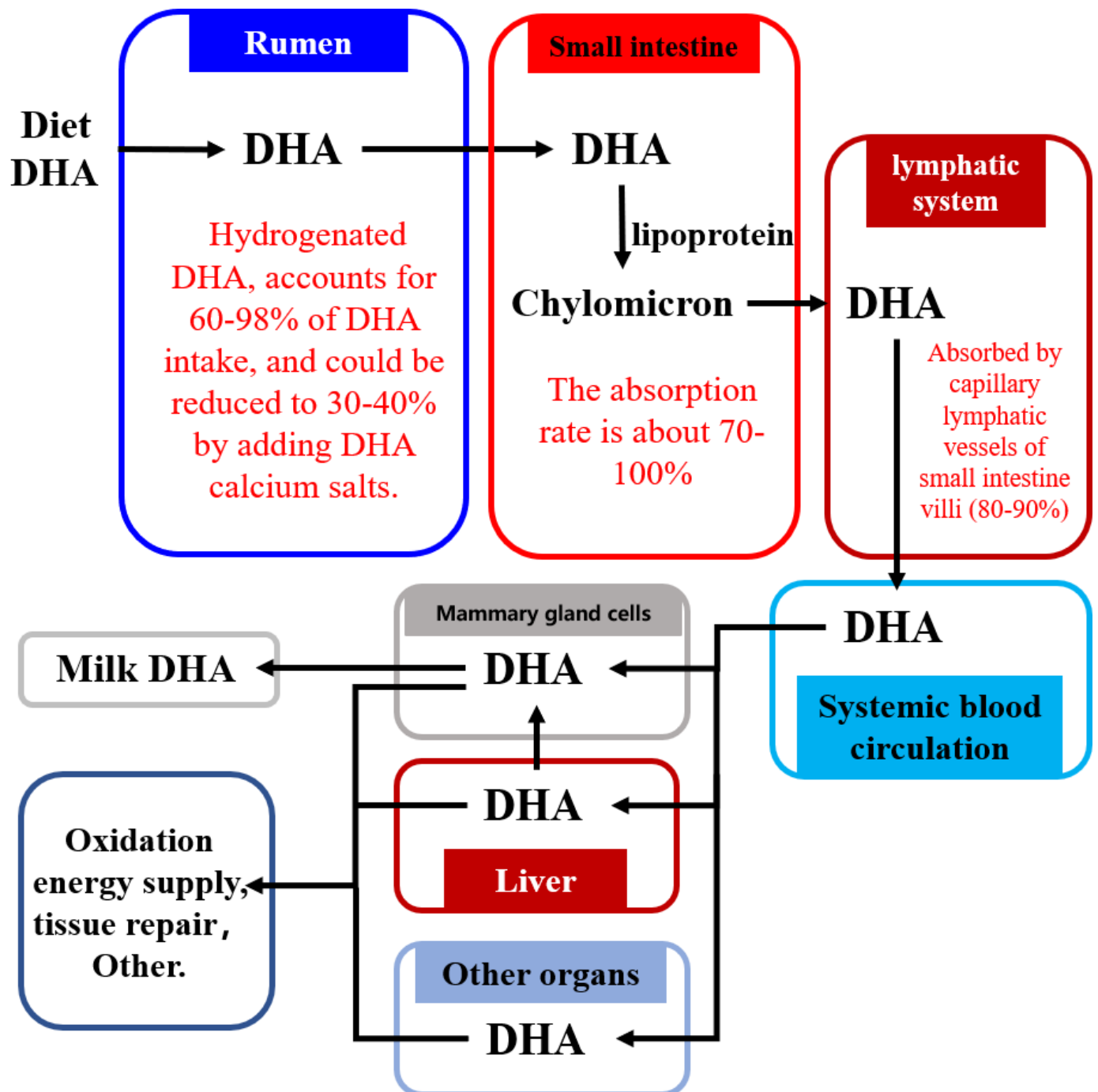
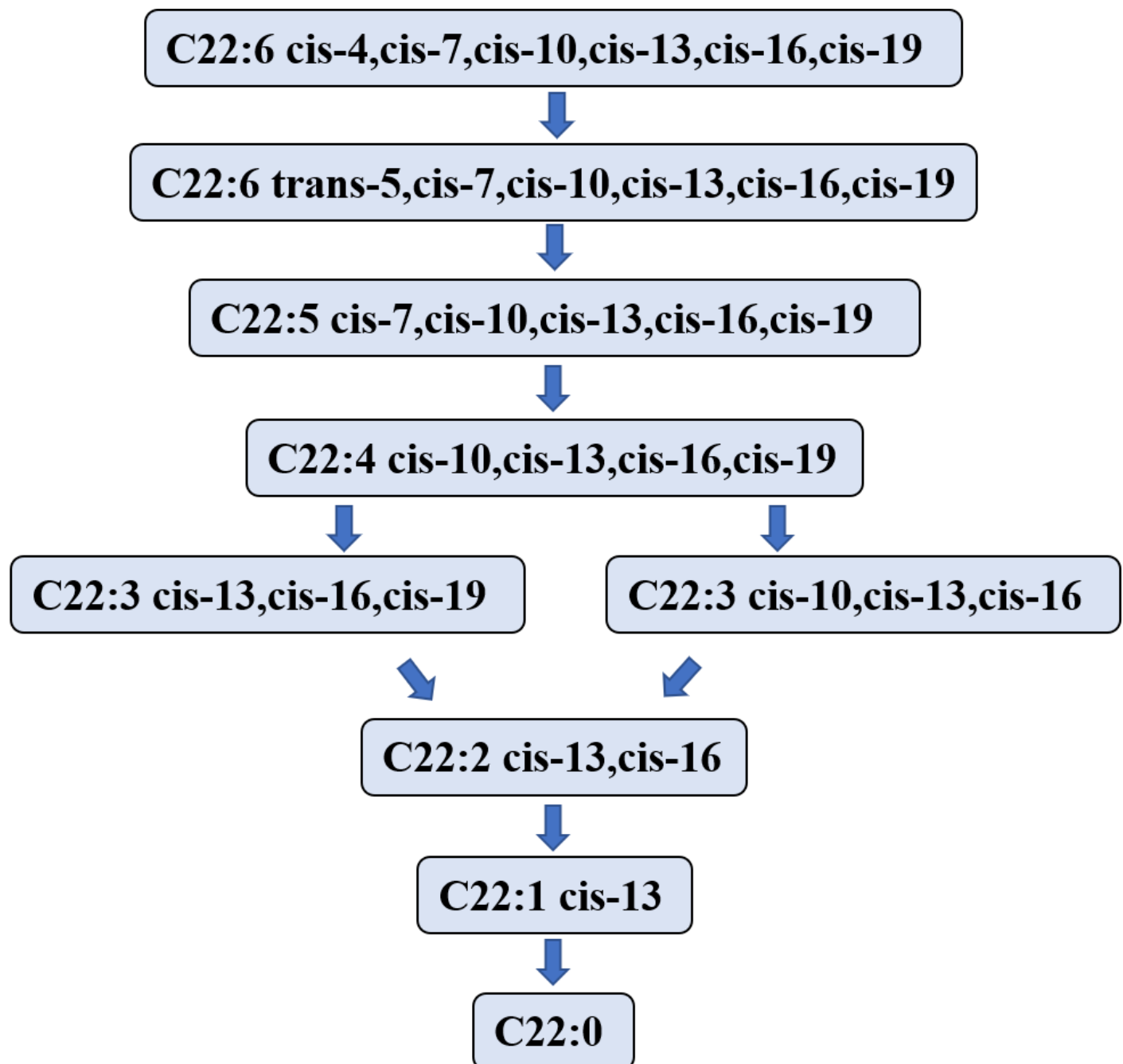



Figure 3

The putative biohydrogenation pathway of DHA in the rumen

The putative biohydrogenation pathway of DHA in the rumen. Abbreviations: DHA, docosahexaenoic acid, C22:6 cis-4,cis-7,cis-10,cis-13,cis-16,cis-19. The arrows represent possible major pathways. Neither all putative fatty acids nor the numerous interconversions among C22:6 isomers are presented. The Figure is adapted from Kairenius et al. (2018), Shingfield et al. (2012), Jenkins et al., (2007) and Aldai et al., (2018) and Jeyanathan et al., (2016).

The effects of dietary supplementation of fish oil on milk fat content

Fatty acid≈triacylglycerols + diacylglycerols + monoacylglycerols + free fatty acids. Milk fatty acid =milk fat content×99.13% (MacGibbon & Taylor 2006) . C=control; Ca,b= controls in article; Ta,b= treatments in article; T1, 2, 3=treatments. RPFO: rumen protected fish oil; RUFO: rumen unprotected fish oil. ND: Not detected.

1 Table 1. The effects of dietary supplementation of fish oil on milk fat content

Treatment	Fish oil supplement	Diet DHA intake (g/d)	Milk fatty acid conten (%)	DHA content in milk (g/d)	Increase of DHA content in milk (compared with control group) (g/d)	Reference
C=basal diet	-	-	3.52	0.26	-	Vahmani, Fredeen & Glover, 2013
T=basal diet + RUFO	200 g/d	24.7	3.37	1.68	1.42	
Ca=basal diet	-	0.00	4.30	0.00	-	Pirondini et al., 2015
Ta=basal diet + RUFO	0.80 %	17.6	4.51	0.40	0.40	
C=basal diet	-	0.21	3.46	0.40	-	AbuGhazaleh et al., 2002
T1=basal diet +RUFO	2.00 %	21.3	3.22	2.49	2.09	
T2=basal diet +RUFO	1.00 %	12.2	3.45	1.46	1.06	
C = basal diet	-	ND	3.28	ND	-	Ramaswamy et al., 2001
T = basal diet + RUFO	2.00 %	ND	2.56	ND	-	
C=basal diet	-	0.35	3.40	0.47	-	Vafa et al., 2012
T1=basal diet + RUFO	2.00 %	31.7	2.30	2.18	1.71	
T2=basal diet + RUFO	1.00 %	12.9	2.45	0.84	0.37	
Ca=basal diet	-	-	3.48	0.56	-	Whitlock et al., 2002
Ta1=basal diet +RUFO	2.00 %	11.1	2.87	1.59	1.03	
Ta2=basal diet +RUFO	1.00 %	9.07	3.11	0.97	0.41	
Cb = basal diet	-	-	4.36	0.12	-	
Tb = basal diet + RUFO	0.80 %	18.5	3.87	0.34	0.22	
C = basal diet	-	ND	3.27	0.30	-	Kairenius et al., 2015
T1 = basal diet + RUFO	75 g/d	ND	3.23	0.28	-0.02	
T2 = basal diet + RUFO	150 g/d	ND	3.14	0.42	0.12	
T3 = basal diet + RUFO	300 g/d	ND	3.33	0.59	0.29	
C = basal diet	-	ND	ND	0.30	-	Lacasse et al., 2002
T1 = basal diet + RUFO	3.70 %	ND	ND	1.03	0.73	
T2 = basal diet + RPFO	1.80 %	ND	ND	0.89	0.59	

T3 = basal diet + RPFO	3.70 %	ND	ND	1.04	0.74	
C=basal diet	-	-	2.94	0.19	-	Donovan et al., 2000
T1=basal diet +RUFO	1.00 %	14.30	2.77	0.57	0.38	
T2=basal diet +RUFO	2.00 %	50.66	2.35	1.97	1.78	
T3=basal diet +RUFO	3.00 %	92.63	2.28	1.25	1.06	
C = basal diet	-	ND	3.34	ND	-	Baer et al., 2001
T = basal diet + RUFO	2.00 %	ND	2.27	ND	-	
C = basal diet	-	ND	4.56	1	-	Shingfield et al.,2006
T = basal diet + RUFO	1.50 %	ND	2.87	0.54	0.54	

2 Fatty acid≈triacylglycerols + diacylglycerols + monoacylglycerols + free fatty acids.

3 Milk fatty acid =milk fat content×99.13% (MacGibbon & Taylor 2006).

4 C=control; Ca,b= controls in article; Ta,b= treatments in article; T1, 2, 3=treatments.

5 RPFO: rumen protected fish oil; RUFO: rumen unprotected fish oil.

6 ND: Not detected.

The effects of dietary supplementation of microalgae on milk fat content

Fatty acid≈triacylglycerols + diacylglycerols + monoacylglycerols + free fatty acids. Milk fatty acid =milk fat content×99.13% (MacGibbon & Taylor 2006) . C=control. T1, 2, 3=treatments. RPA: rumen protected algae; RUA: rumen unprotected algae. ND: Not detected.

1   Table 2. The effects of dietary supplementation of microalgae on milk fat content

Treatment	Microalgae supplement (g/d)	Diet DHA intake (g/d)	Milk fatty acid (%)	DHA content in milk g/d	Increase of DHA content in milk (compared with control group) (g/d)	Reference
C=basal diet	-	-	4.75	0.13	-	Boeckaert et al., 2008
T=basal diet + RPA	899	43.7	2.18	1.45	1.32	
C=basal diet	-	0.05	3.36	0.70	-	Fougère, Delavaud & Bernard, 2018
T=basal diet + RUA	310	115	2.62	13.6	12.9	
C=basal diet	-	ND	4.75	0.10	-	Póti et al., 2015
T=basal diet + RUA	150	ND	3.46	0.14	0.04	
C=basal diet	-	-	3.67	0.00	-	Franklin, et al., 1999
T1=basal diet + RPA	910	29.2	2.92	5.15	5.15	
T2=basal diet + RUA	910	35.9	2.92	3.23	3.23	
C=basal diet	-	-	3.47	0.10	-	Stamey et al., 2012
T1= basal diet + 0.5×RUA	150	21.6	3.97	0.50	0.40	
T2= basal diet + 1×RUA	300	43.2	3.27	0.59	0.49	
T3= basal diet + 1×RUA oil	194	27.4	3.27	0.30	0.20	
C=basal diet	-	-	4.93	0.44	-	Moate et al. 2013
T1=basal diet + RUA	125	25.0	3.75	3.51	3.07	
T2=basal diet + RUA	250	50.0	3.67	5.09	4.65	
T3=basal diet + RUA	375	75.0	3.80	7.70	7.26	
C=basal (diet + Hydrogenated palm oil fat	-	-	3.90	-	-	Moran et al., 2018
T=basal diet + RUA	100	17.8	3.81	1.32	1.32	

2   Fatty acid≈triacylglycerols + diacylglycerols + monoacylglycerols + free fatty acids.

3   Milk fatty acid =milk fat content×99.13% (MacGibbon & Taylor 2006).

4   C=control. T1, 2, 3=treatments.

5   RPA: rumen protected algae; RUA: rumen unprotected algae.

6   ND: Not detected.

Efficiency of dietary incorporation of DHA into milk

DHA intake: reported in the article or calculation: dry matter intake×fatty content of diet×DHA content. Fatty acid≈triacylglycerols + diacylglycerols + monoacylglycerols + free fatty acids. Milk fatty acid =milk fat content×99.13% (MacGibbon & Taylor 2006) . Milk DHA yield: reported in the article or calculation: milk fatty yield×DHA content. Efficiency: milk DHA/ diet DHA

1   Table 3. Efficiency of dietary incorporation of DHA into milk

DHA source	DHA in take (g/d)	Milk DHA yield (g/d)	Efficiency (%)	Reference
Fish oil	14.30	0.57	3.99	Donovan et al., 2000
Fish oil	50.66	1.97	3.89	Donovan et al., 2000
Fish oil	92.63	1.25	1.35	Donovan et al., 2000
Fish oil	21.27	2.49	11.7	Abughazaleh et al., 2002
Fish oil	12.20	1.46	12.0	Abughazaleh et al., 2002
Fish oil	11.07	1.59	14.4	Whitlock et al., 2002
Fish oil	9.07	0.97	10.7	Whitlock et al., 2002
Fish oil	31.71	2.18	6.87	Vafa et al., 2012
Fish oil	12.87	0.84	6.53	Vafa et al., 2012
Fish oil	24.66	1.68	6.81	Vahmani et al., 2013
Fish oil	17.55	0.40	2.28	Pirondini et al., 2015
Fish oil	18.53	0.34	1.83	Pirondini et al., 2015
Microalgae	35.94	3.23	8.99	Franklin et al., 1999
Microalgae	43.68	1.45	3.32	Boeckaert et al., 2009
Microalgae	25.00	3.51	14.0	Moate et al., 2013
Microalgae	50.00	5.09	10.2	Moate et al., 2013
Microalgae	75.00	7.70	10.3	Moate et al., 2013
Microalgae	21.61	0.50	2.31	Stamey et al,. 2012
Microalgae	43.23	0.59	1.36	Stamey et al,. 2012
Microalgae oil	27.41	0.30	1.09	Stamey et al,. 2012
Microalgae	17.82	1.32	7.41	Moran et al,. 2017
Microalgae	115.5	13.6	11.8	Fougère et al. 2018

2   DHA intake: reported in the article or calculation: dry matter intake×fatty content of diet×DHA

3   content.

4   Fatty acid≈triacylglycerols + diacylglycerols + monoacylglycerols + free fatty acids.

5   Milk fatty acid =milk fat content×99.13% (MacGibbon & Taylor 2006).

6   Milk DHA yield: reported in the article or calculation: milk fatty yield×DHA content.

7   Efficiency: milk DHA/ diet DHA.