Use of microalgae in ruminant nutrition and implications on milk quality – A Review

Iolanda Altomonte[1], Federica Salari [1], Rosario Licitra [1], Mina Martini [1, 2]

[1]Department of Veterinary Science, University of Pisa, viale delle Piagge 2, Pisa, Italy

[2]Research Center "Nutraceuticals and Food for Health" -Nutrafood, University of Pisa, via del Borghetto 80, Pisa, Italy.

Corresponding author: Mina Martini mina.martini@unipi.it

Abstract

Microalgae are photoautotroph unicellular or multicellular microorganisms which are smaller than 400 µm and can be used as an animal feed source. Ruminants seem to be promising targets of this new feedstuff, as they can also use non-protein nitrogens present in algae and digest the cell walls of algal organisms. Despite the potential for use of microalgae in ruminant feeding, to our knowledge the applications are still limited and there are no reviews in the literature on the effects of microalgae on milk yield and quality. This paper reviews the studies on the use of microalgae for dairy ruminant feeding in order to provide complete information on the state of the art, limitations, and their potential use. The major effects of microalgae on milk production are the changes in the milk fatty acid profile, especially related to the long chain fatty acids and the omega 3 series, in particular DHA and EPA which are beneficial for human health. These results are interesting as to date attempts to increase the omega 3 content in milk by feeding have led to limited results, since PUFA biohydrogenation in the rumen is massive. However, excessive algal supplementation might negatively impacts on palatability, feed intake, the ruminal metabolism and may have negative effecs on milk production and fat. In conclusion, careful attention should be paied in terms of the amount of algae supplemented and ruminoprotected forms should be considered in order to prevent reductions in the feed intake, and a deterioration in milk yield and quality. Further reseach is needed to identify the more appropriate species/feed and the effects of a prolonged supplementation.

Keywords: microalgae, ruminant feeding, milk quality, omega 3 fatty acids

1.Introduction

Ruminant milk is one the most consumed beverage in the world and its importance for human nutrition and health is well known given its protein, sugar, fat, vitamins and mineral content. In the last twenty years, several studies have focused on improving the nutritional and nutraceutical quality of milk, and at providing it with an added nutritional value.

Research on improving milk composition is also of interest to producers given that dairy industries worldwide have instituted penalty and premium programs to provide incentives for dairy producers to improve milk composition and quality (Draaiyer et al., 2009).

In addition to the importance for human health, milk and livestock productions are contributors to global food security, in fact the world population is expected to increase and the demand for foods of animal origin will grow.

At the same time, livestock farming impacts on emissions of pollutants and the degradation of natural resources. For example, livestock farming has an impact in terms of land use, as currently one third of arable land is dedicated to feedstuff production. In this regard, the research on non typical feedstuffs as a substitute for standard ones represents an opportunity, especially in terms of overcoming some of the problems related to the depletion of natural resources, the use of GMO products such as soy, or when the costs of traditionally used feedstuff are very high (Liponi et al., 2007; McAllister et al., 2011).

Microalgae are photoautotroph unicellular or multicellular microorganisms which are smaller than 400 μm. They can be used as an economical unconventional animal feed source, since they are very efficient in converting solar energy, are not dependent on external environmental conditions, and characterized by higher productions per unit area than traditional crops (Priyadarshani and Rath, 2012). Given the above characteristics, microalgae can therefore contribute to reducing the exploitation of natural resources (Holman and Malau-Aduli 2013).

In addition, some species can be grown for biodiesel production (Kovač et al., 2013), and the residual algal mass, partially or totally defatted, can be used as animal feed (Lum et al., 2013; Drewery et al., 2014). Microalgae are also used in the pharmaceutical and cosmetic industries (Christaki et al., 2011, Ribeiro et al., 2017).

In terms of the chemical composition, microalgae are rich in macro-components. Their composition is widely variable due to the algae genus, species and growing conditions (Spalaore et al., 2006; Venckus, et al., 2017). In general, microalgae are composed of (on dry matter): 39-71% of protein, 10-57% of carbohydrates, mainly polysaccharides, cellulose, and starches (Chen et al., 2013); and 6-86% of lipids especially sterols and long chain PUFA fatty acids (Spalaore et al., 2006; Ryckebosch et al., 2014).

Currently in Europe, the microalgae registered as animal feed or ingredients for animal feed (EU regulation 767/2009) are: *Spirulina maxima* and *Spirulina platensis*; genus Schizochytrium. Unlike their common use in feeding aquatic animals, the use of microalgae in feeding terrestrial species is more recent, especially in poultry and pigs. According to Lum et al. (2013) ruminants may be promising users of this new feedstuff, as they can also benefit from the non-protein nitrogens present in algae and digest the cell walls of algal organisms.

Despite their potential use in ruminant feeding, to our knowledge the applications are still limited and there are no reviews in the literature concerning the effects of microalgae on milk yield and quality. This paper reviews the studies on the use of microalgae for dairy ruminant feeding in order to provide complete information on the state of the art, the limitations, and their potential use.

2. Feeding trials including microalgae in ruminants

2.1 Effects on dry matter intake

The characteristics of the diets in the studies on the effects of microalgae supplementation on ruminant milk yield and quality are reported in table 1. The literature has evaluated the integration of three types of microalgae-based products with different raw fat percentages (RF):

a) whole algal meal and defatted algal meal: the latter have an average RF content of about 5% and consist of 57% partially deoiled microalgae and the 43% soyhulls; b) microalgae-based oils contain 55-56% RF, and c) dried or freeze-dried algae biomass with RF ranging from 5-60% whose fat can be encapsulated and rumen protected. Most of the products used for the studies are commercial and are rich in DHA derived from saltwater microalgae.

Microalgae-based feeds in ruminant diets are introduced in order to supplement the ration, as a source of:

a) energy: used in the partial substitution of corn or concentrate (Boeckaert et al., 2008; Da Silva et al., 2016), or added to the lipid supplementation (Toral et al. , 2010; Stamey et al., 2012),
b) protein: in partial replacement of soy (Reynolds et al., 2006; Póti et al., 2015; Stamm, 2015) or rapeseed (Lamminen et al., 2017).
c) enhance the antioxidant defence system and oxidant status of products (Tsiplakou et al., 2018) given the natural content of natural antioxidant compounds.

In table 2 the results of the studies of the effects of microalgae on feed intake, milk yield and quality are shown. When the supplementation of algal products is exceeded, feed ingestion decreases and in cows fed unifeed, the intake decreases from 7% to 45% (Boeckart et al., 2006; Moate et al., 2013). Although without recording a decrease in total feed intake, some authors, have detected qualitative changes in intake. In particular, a reduction in the intake of the concentrate containing microalgae was balanced by a higher intake of silage (Lamminen et al., 2017).

In cows, the maximum amount of microalgae ingested without effects on feed intake varies in different studies in a fairly wide range from 4 to 79 g of microalgae/ kg of dry matter in the diet (Weatherly, 2015; Stamm, 2015). The decrease depends on the type of feedstuff, for example products based on algal meal in dairy cows, are accepted up to inclusions of 10-11 g/ kg of the dry matter intake (Boeckart et al., 2008; Moate et al., 2011), while meal made up of defatted microalgae and soyhulls, appear to be better tolerated, up to 92 g / kg of dry matter (Da Silva et al., 2016). On the other hand studies on algal oil- supplementation have shown that it does not affect the intake in cows if integrated up to 194 g / day per head (Stamey et al., 2012). In sheep, a reduction in the intake of concentrate was observed with an algal biomass supplementation of about 12 g / kg (estimated value) of the ration (Papadopoulos et al., 2002).

Three hypotheses have been formulated to explain the changes in feed intake linked to the administration of microalgae. One hypothesis attributes the changes to the low palatability both in sheep and cows (Franklin et al 1999; Papadopoulos et al., 2002; Lamminen et al., 2017). The low acceptability may be due to the taste and odour, to the physical structure of the feed, especially if the microalgae are dry and finely powdered. The palatability could be improved by pelleting the ration (Lamminen et al., 2017). A second explanation is the decrease in fiber digestibility, which is partly linked to the fermentable carbohydrates in the algae and to the small particle size which could have a negative influence on rumen pH (Stokes et al., 2015). A third hypothesis is the disturbance of the rumen fermentation through the PUFA contained in the algae which could have toxic effects on the rumen microflora (Boeckart et al., 2008).

Franklin et al. (1999) ruled out a negative effect of algae fat yield on ruminal metabolism in cows. In fact, in their study, the quantity of fat provided by the experimental diet was comparable with that of the control diet. Toral et al. (2010) also ruled out the negative effects of algal fat yield in sheep. They report that several studies have found that the inclusion of vegetable oils in the diet of dairy sheep does not have apparent negative effects on feed ingestion (Pulina et al., 2006; GómezCortés et al., 2008, Hervás et al., 2008). However, in sheep, only a few studies have analyzed the effects of the inclusion of unprotected lipids of a marine origin.

2.2 Effects on the milk yield

With regard to the effects of microalgae feeding on the quantitative production of milk, it is not straightforward to compare the literature because of the differences in the amount of microalgae supplemented, in the duration of the experiment, and in the composition of the diet.

However, most authors have not found an influence on the milk yield, either in cows or small ruminants, and no effects have also been reported in studies where reductions or changes in the intake were observed (Franklin et al., 1999; Papadopoulos et al., 2002; Moate et al., 2013; Tsiplakou et al., 2017a, 2018; Weatherly, 2015; Lamminen et al., 2017).

Despite reducing feed intake, the dietary addition of algae does not affect milk yield presumably because of the increased feed efficiency (Franklin et al., 1999; Papadopulos et al., 2002). The increased feed efficiency was probably a result of the direct incorporation of fatty acids from algae into milk fat (Goulas, 2000).

However, the literature also reports cases in which production losses have occurred. For example Boeckaert et al. (2008) showed that a 45% lower milk yield was produced in cows fed algae in quantity of 43.0 g/kg of DM of the ration through the rumen fistula (Boeckaert et al., 2008). Production decreases have also been found in sheep with 25 g/kg of algal biomass of DM of the diet, in diets that also included of corn silage and alfalfa hay silage (Reynolds et al., 2006).

On the other hand, the administration of *Spirulina* (200 g per day, about 10-14 g/kg of DM) led to a higher milk yield in cows with a maximum increase of 25% in daily production during a 90-day experimental period (Kulpys et al., 2009). The authors explained that the improvement was due to the chemical composition of the microalga *Spirulina platensis* which influences both the biological activity of the ruminal flora and phisiolocical status of the animal. Moreover, studies found that total daily intake of water was greater in steers receiving *Spirulina platensis* (Panjaitan et al., 2010), this aspect in dairy cows should be further investigate as the increased water intake could affect milk yield and quality.

In addition, beneficial effects of some microalgae species on metabolic status and defence system of animals as well as on oxidant status of products have been reported. Regarding this latter issue Tsiplakou et al. (2018) found higher superoxide dismutase activity in blood and milk and higher catalase activities in the blood plasma in goats that fed *Chlorella vulgaris.* Superoxide dismutase and catalase are among the main components of the intracellular antioxidant defence mechanisms which regulate reactive oxygen species accumulation within tissues, whereas enzyme lactoperoxidase in milk is related to the oxidation of lipids. In the above reported study also a reduction of anoxidative stress biomarker (protein carbonyls) in milk was found.

2.3 Effects on milk composition

2.3.1 Protein and lactos**e**

Regarding the results of algal supplementation on the synthesis of milk proteins, different results have been reported depending on the species, diet, ingestion, and milk yield.

As a result of adding microalgae, some authors reported no changes in milk proteins in the diet in either cows or sheep and goats (Bichi et al., 2013; Moate et al., 2013; da Silva et al., 2016; Tsiplakou et al., 2017a, 2018). In contrast, other studies have reported a decrease in protein yield in cows, mainly followed by a decrease in feed intake and milk yield (Boeckaert et al., 2008). Others have also reported a tendency of milk protein to decrease, although not related to decreased intake or milk yield (Lamminen et al., 2017). According to Lamminen et al., (2017) the decrease in milk protein might to be due to the low presence of histidine in microalgae. This amino acid limits milk production and may become suboptimal in the case of algal administration.

In sheep, decreases in the percentage of proteins have been found (Papadopulos et al., 2002; Toral et al., 2010). Unlike findings reported in cows by Boeckaert et al. (2008), these differences were not associated with changes in the feed intake, or with negative effects on the rumen microflora.

In sheep Reynolds et al. (2006) observed increases in the daily protein yield with a diet based on pelletted alfalfa hay and algae compared to a diet of corn silage and algae. The authors attributed the increases to the higher intake of protein due to the alfalfa hay. In the same study, decreases in the daily protein yield and increases in percentages were observed in animals fed a diet based on alfalfa hay-silage supplemented with microalgae compared to corn silage; in this case the protein changes were linked to a concentration effect due to the decrease in milk yield.

Contrasting results on the effects of algal supplementation on lactose have also been reported. According to some authors, lactose decreases with the addition of microalgae in cows' feed (Boeckaert et al., 2008) mainly linked to decreases in the milk yield; and decreases in lactose percentages have also been observed in sheep (Papadopulos, 2002; Reynolds et al., 2006). In contrast, other authors have reported lactose increases (Moate et al., 2013), while others have reported no variations (Kulpys et al., 2009; Bichi et al., 2013; Poti et al., 2015; Da Silva et al., 2016; Tsiplakou et al., 2017a, 2018).

1.3.2 Fat

In cows receiving microalgae supplementation, there is a reduction in secreted milk fat (Boeckaert, et al., 2008; Moate et al., 2013; Weatherly, 2015); fat yield decreases range from a minimum loss of 22% to a maximum of 59% (Franklin et al 1999; Boeckaert, et al., 2008). In addition, low fat percentages have been recorded in both cows and sheep (Franklin et al., 1999; Boeckaert, et al., 2008; Toral et al., 2010; Bichi et al., 2013; Moate et al., 2013; Poti et al., 2015). The decreases are consistent with other studies that have included marine products, such as fish oil, fish meal, or marine algae.

However the literature results on fat also vary, since no significant changes in milk fat have been reported (Stamey et al., 2012; Da Silva et al., 2016; Lamminen et al., 2017; Tsiplakou et al., 2017a, 2018).

Milk fat decreases could be related either to a higher fat content of experimental diets compared to control (Table 1) (Toral et al., 2010) or to a negative energy balance as a result of the low feed intake or to a low fat syndrome related to the accumulation in the rumen of trans fatty acids intermediate in the biohydrogenation and to the formation in the rumen of C18: 2 isomer inhibitors of lipid synthesis (Boeckaert et al., 2008; Moate et al., 2013). The increase in fat synthesis inhibitors might be related to toxic effects on the ruminal microbiota which did not adapt to the dietary supply of very long chain n-3 polyunsaturated fatty acids (Bichi et al., 2013).

In terms of using vegetable oils in the diet, the fat inhibitor isomers most involved are known and are mainly trans-10, cis-12 C18: 2 and trans-9, cis-11 C18: 2, both in dairy cows and sheep (Shingfield and Griinari, 2007; Sinclair et al., 2010). However, regarding microalgae, the inhibitor isomers are not completely known. Toral et al. (2010) hypothesized the joint action of trans-9, cis-11 C18: 2 and trans-10 C18: 1, together with other unidentified intermediates, whereas according to Boeckaert et al. (2008), the low fat syndrome could be caused by the reduced synthesis of c9 C18: 1. The latter fatty acid is essential to maintain milk fat fluidity, and the synthesis of milk fat is assumed to be inhibited in the case of a c9 C18: 1 reduced secretion (Gama et al., 2008).

On the other hand, some authors have reported increases in the percentage of fat in goats and sheep feeding microalgae (+ 13-20.0%) (Papadopulos et al., 2002; Reynolds et al. 2006; Poti et al., 2015). In some cases the increases were related to a concentration effect linked to the decrease in milk yield (Reynolds et al. 2006). Other authors have described the increase in fat percentage to the increased forage to concentrate ratio or the experimental diet compared with the control, or to the reduced synthesis trans C18: 1 (n- 7) which has impacts negatively on the milk fat content (Griinari et al. 1998; Papadopulos et al., 2002). Another explanation is the beneficial effects of some algal species on ruminal fermentations (Poti et al., 2015). This hypothesis is also supported by Stamm (2015) who reports increases in the percentage of milk fat (+ 9%) in cows, which are linked to the beneficial effects of spirulina on rumen.

1.3.3 Milk fat globules

The influence of milk fat globules on milk quality and the factors influencing their size have been reviewed by Martini et al. (2016). Modifications in the ruminant diet can modify the size of the fat globules, thus modulating the contribution of globule bioactive compounds (e.g. MFGM Spitsberg, 2005) and also affecting the quality characteristics of milk and cheese, as well as the digestibility of milk fat. The diameter of the globules in dairy cows could increase with the increase in the energy supplied by the diet (Carroll et al 2006, Martini et al., 2010) and with the quantity of fat secreted (Wiking et al., 2004; Martini et al., 2016).

To our knowledge only one study has investigated the effects of microalgae on the number and diameter of milk fat globules (Stamm, 2015). In this study the algae Nannochloropsis, Spirulina and Chlorella, used in the partial substitution of soy, did not influence the average diameter, although the cow diet supplemented with Chlorella affected the number of globules compared to the diet based on Spirulina or Chlorella + Nannochloropsis. The Chlorella treatment also led to a decrease in the number of globules ranging from 1 to 3 microns.

1.3.4 Fatty acid profile of milk

Research on animal feeding has focused on modifying the milk fatty acid profile in order to modulate the content of beneficial elements; and the application of microalgae in this field is quite recent.

The results of the studies of the effects of microalgae supplementation on ruminant milk fatty acids profile are summarised in table 3.

Infusions of microalgae by ruminal fistula, as well as dietary administration have resulted in saturated fatty acid reductions and increases in polyunsaturated fatty acids (PUFAs) in ruminant milk (from increments of + 54% to higher than + 100%) (Franklin et al., 1999; Boeckaert et al ., 2008; Moate et al., 2013, Poti et al., 2015). These changes were also found in dairy products derived from PUFA-enriched milk (Papadopoulos et al., 2002). Some authors have also observed increases in monounsaturated fatty acids (MUFAs) in goats and cows (+ 12% and + 4% respectively) (Póti et al, 2015; Boeckaert et al., 2008) and increases in total fatty acids *de novo* synthesized, with a chain length up to C16: 0 (Poti et al., 2015; Moate et al., 2013).

Microalgae are also rich in omega 3, which are efficiently transferred into the milk. Studies on cows show how the transfer efficiency is greater in the case of ruminal infusions (with increases of omega 3 of + 161%) (Boeckaert et al., 2008), lower, but still considerable with the addition of microalgae in the ration (from +19% to increases higher than 100%) (Stamey et al., 2012; Moate et al., 2013; Póti et al, 2015). Increases have also been recorded in goat's milk (+ 19% of omega 3) (Póti et al, 2015). These results are interesting as to date attempts to increase the omega 3 content in milk by feeding have led to limited results, since the PUFA biohydrogenation in the rumen is massive (Lock and Bauman, 2004).

Of the fatty acids belonging to the omega 3 series in milk, studies have almost unequivocally reported increases in C22: 6 (DHA) as a result of microalgae supplementation. DHA is an essential fatty acid and an important component of the nervous system. An increase in DHA has been observed in cows (Boeckaert et al., 2008; Moate et al., 2013; Póti et al., 2015), goats (Póti et al, 2015) and sheep (Papadopoulos et al., 2002; Reynolds et al., 2006; Bichi et al., 2013), with positive variations ranging from 100 to 1000% or more in cows (Boeckaert et al., 2008; Moate et al., 2013; Poti et al., 2015) + 660% in sheep (Bichi et al., 2013) and + 100% in goats (Poti et al., 2015).

However, Weatherly (2015) reported that DHA enrichment occurs at inclusion levels in milk (15 g/kg of dry matter intake) that lead to subacidosis in cows with a reduced intake and low fat secretion in milk. In addition, the percentage of DHA in the milk fat of algae-fed cows - decreases over time. Although the hypothesis is not confirmed by other studies (Bichi et al., 2013), Franklin et al. (1999) suggested that rumen microorganisms may become acclimated to the presence of non ruminoprotected algae in the diet over time, resulting in greater biohydrogenation of DHA with less DHA incorporation into milk fat.

C20: 5 (EPA), which is another omega 3 fatty acid beneficial for health, has been found to increase from + 17% to + 112% in cows (Stamey et al., 2012; Moate et al., 2013; Vahmani et al., 2013) and + 133% in goats (Póti et al., 2015) and from 50 to 100% or more in sheep (Papadopoulos et al., 2000; Toral et al., 2010; Bichi et al. , 2013) with a microalgae supplemented diet.

In addition, some studies have shown that unsaturated fatty acids with an 18-carbon chain such as linolenic acid (Franklin et al., 1999), linoleic acid (Boeckaert et al., 2008; Franklin et al., 1999) and oleic and stearic acid decrease with supplementation both in cows and sheep (Papadopoulos et al., 2002; Reynolds et al., 2006; Toral et al., 2010; Moate et al., 2013). The exception is goat's milk in which linoleic acid increases (Kouřimská et al., 2014; Poti et al., 2015).

The CLA fatty acids, and their main isomer C18:2 cis-9, trans-11 whose beneficial effects on the metabolism and anticancer action have been shown in animal models, increase in cow's (from + 13% to +108 %) (Boeckaert et al., 2008; Stamey et al., 2012; Moate et al., 2013; Póti et al, 2015) in goat's (+ 28%) (Póti et al, 2015) and sheep milk (+39 %) (Reynolds et al., 2006; Bichi et al., 2013). Similarly, increases in vaccenic acid (C18:1 trans 11) have been observed in cow's (from + 11% to + 203%) (Boeckaert et al., 2008; Stamey et al., 2012; Moate et al., 2013; Póti et al, 2015) and in goat's milk. (+ 151%) (Póti et al, 2015). The increase in C18:2 cis-9, trans-11 associated with algal meal feed was probably due to the inhibitory effects of algae on the rumen biohydrogenation, and also to the increased ruminal production of the C18:1 trans-11 substrate.

The shift in ruminal beta hydroxybutyrate pathway towards the formation of trans-C18:1 fatty acid has been observed also by Tsiplakou et al. (2017b) in goats fed *Chlorella vulgaris*. This effect was associated with changes in the Butyrivibrio fibrisolvens population in their rumen liquid

On the other hand the direct effects of algae on animal metabolism have been ruled out, such as on the activity of the Δ9-desaturase enzyme (Boeckaert et al., 2008; Moate et al., 2013).

3. Conclusions

The literature on the effects of algae on milk production is difficult to compare due to differences in the kinds and amounts of supplementation, type of feed and composition of the diet, the different nutrient profiles among algae feedstuffs, and the duration of the experimental period. The greatest changes have been found in the milk fatty acid profile and are related to the long chain fatty acids and fatty acids of the omega 3 series, especially DHA and EPA. However, excessive algal supplementation seems to have negative effects on palatability, feed intake, the ruminal metabolism, as well as negatively impacting on milk production and fat.

A careful attention should be needed regarding the amount of supplemented algae and rumen-protected forms should be considered in order to prevent reductions in feed intake, and a deterioration in milk yield and quality

Moreover, the following issue should be further clarified: the effects of microalgae on animal metabolic status and welfare; the possible presence of anti-nutritional factors in the various species and the effects of a prolonged supplementation. In addition, the quality and the organoleptic characteristics of dairy products from animals fed microalge should be deepened.

Furthermore, given the effects of the different cultivation conditions on microalgae compositions, and the several points that have yet to be clarified, at the moment it is still too early to clearly define future applications in the dairy sector.

References

Bichi, E., Hervás, G., Toral, P.G., Loor, J.J., Frutos, P., 2013. Milk fat depression induced by dietary marine algae in dairy ewes: Persistency of milk fatty acid composition and animal performance responses. J. Dairy Sci. 96, 524–532.

Boeckaert, C., Vlaeminck. B., Dijkstra. J., Issa-Zacharia. A., Van Nespen. T., Van Straalen. W., Fievez V., 2008. Effect of Dietary Starch or Micro Algae Supplementation on Rumen Fermentation and Milk Fatty Acid Composition of Dairy Cows J. Dairy Sci. 91, 4714–4727.

Carroll, S.M., DePeters, E.J., Taylor, S.J., Rosenberg, M., Perez-Monti, H., Capps, V.A., 2006. Milk composition of Holstein, Jersey, and Brown Swiss cows in response to increasing levels of dietary fat. Anim. Feed Sci. Technol. 131, 451–473.

Chen, C.Y., Zhao, X.-Q. , Yen, H-W, Ho, S.-H., Cheng C.-L.,Lee, D.-J., Bai F.-W. , Chang, J.-S., 2013. Microalgae-based carbohydrates for biofuel production. Biochem Eng J 78, 1–10-

Christaki, E., Florou-Paneri, P., Bonos, E., 2011. Microalgae: a novel ingredient in nutrition. Int. J. Food Sci. Nutr. 62, 794-799.

da Silva, G.G., Ferreira de Jesus, E., Takiya, C.S., Del Valle, T.A., da Silva, T.H., Vendramini ,T.H.A., Yu E.J., Rennó, F.P., 2016. Short communication: Partial replacement of ground corn with algae meal in a dairy cow diet: Milk yield and composition, nutrient digestibility, and metabolic profile J. Dairy Sci. 99, 8880–8884.

Draaiyer, J., Dugdill, B., Bennett, A.,Mounsey, J., 2009. Milk testing and payment systems resource book: a practical guide to assist milk producer groups. Food and Agriculture Organization, Rome, Italy

Drewery, M.L., Sawyer, J.E., Pinchak, W.E., Wickersham, T.A, 2014. Effect of increasing amounts of postextraction algal residue on straw utilization in steersJ. Anim. Sci. 92, 4642-4649.

EU regulation 767/2009..(www.feedmaterialsregister.eu)

Franklin, S.T., Martin, K.R., Baer, R.J., Schingoeth,e D.J., Hippen, A.R., 1999. Dietary marine algae (Schizochytrium sp.) increases concentrations of conjugated linoleic, docosahexaenoic and transvaccenic acids in milk of dairy cows. Journal of Nutrition 129, 2048-2054.

Gama, M.A.S., Garnsworthy, P.C., Griinari, J.M., Leme, P.R., Rodrigues, P.H.M., Souza, L.W.O., Lanna D.P.D., 2008. Diet-induced milk fat depression: Association with changes in milk fatty acid composition and fluidity of milk fat. Livest. Sci. 115, 319–331.

Goulas, C., 2000 The effect of dietary fat on sheep diet digestibility and on lactating ewes performance. Ph.D. Thesis, Agricultural University of Athens

Gómez-Cortés, P., Hervás, G., Mantecón, A.R., Juárez, M., de la Fuente, M.A., Frutos, P., 2008. Milk production, CLA content, and in vitro ruminal fermentation in response to high levels of soybean oil in dairy ewe diet. J. Dairy Sci. 91, 1560–1569.

Griinari, J.M., Dwyer, D.A., McGuire, M.A., Bauman, D.E., Palmquist, D.L., Nurmela, K.V.V., 1998. Trans-octadecenoic acids and milk fat depression in lactating dairy cows. J. Dairy Sci. 81, 1251–1261.

Hervás, G., Luna, P., Mantecón, A.R., Castañares, N., de la Fuente M.A., Juárez, M., Frutos P., 2008. Effect of diet supplementation with sunflower oil in milk production, fatty acid profile and ruminal fermentation in lactating ewes. J. Dairy Res. 75, 399–405.

Holman, B.W.B., Malau-Aduli, A.E.O, 2013. Spirulina as a livestock supplement and animal feed J Anim Physiol Anim Nutr 97, 615-623.

Kouřimská, L., Vondráčková, E., Fantová, M., Nový, P., Nohejlová, L., Michnov, K., 2014. Effect of feeding with algae on fatty acid profile of goat’s milk. Scientia agriculturae bohemica 45, 162–169.

Kovač, D.J., Simeunović, J.B., Babić, O.B., Mišan, A.Č., Milovanović, I.L., 2013. Algae in food and feed. Food and Feed Research 40, 21-31.

Kulpys, J., Paulauskas, E., Pilipaviþius, V., Stankeviþius, R., 2009. Influence of cyanobacteria Arthrospira (Spirulina) platensis biomass additives towards the body condition of lactation cows and biochemical milk indexes. Agronomy Research 7, 823-835.

Lamminen, M., Halmemies-Beauchet-Filleau, A., Kokkonen. T., Simpura, I., Jaakkola, S., Vanhatalo A., 2017.Comparison of microalgae and rapeseed meal as supplementary protein in the grass silage based nutrition of dairy cows. Anim. Feed Sci. Technol. 234, 295–311.

Liponi, G.B., Casini, L., Martini, M., Gatta D., 2007. Faba bean (Vicia faba minor) and pea seeds (Pisum sativum) as protein sources in lactating ewes' diets. Ital. J. Anim. Sci. 6, 309-311.

Lum, K.K., Kim, J., Lei, X.G., 2013. Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. J Anim Sci Biotechnol 4, 53.

Martini, M., Salari, F., Altomonte, I., 2016. The Macrostructure of Milk Lipids: The Fat Globules, Crit Rev Food Sci Nutr 56, 1209-1221.

Martini, M., Liponi, G.B., Salari, F., 2010. Effect of forage: concentrate ratio on the quality of ewe's milk, especially on milk fat globules characteristics and fatty acids composition. J. Dairy Res. 77, 239–244.

McAllister, T.A., Gibb, D., Van Herk, F., McKinnon, J., 2011. Non-traditional sources of energy traditional sources of energy for feedlot cattle for feedlot cattle. Saskatchewan Beef & Forage Symposium January 19th and 20th, 2011.

Moate, P.J., Williams, S.R.O., Grainger, C., Hannah, M.C., Ponnampalam, E.N., Eckard, R.J., 2011. Influence of cold-pressed canola, brewers grains and hominy meal as dietary supplements suitable for reducing enteric methane emissions from lactating dairy cows. Anim. Feed Sci. Technol. 166–167, 254–264.

Panjaitan, T., Quigley, S.P., McLennan, S.R.; Poppi, D.P., 2010: Effect of the concentration of Spirulina (Spirulina platensis) algae in the drinking water on water intake by cattle and the proportion of algae bypassing the rumen. Animal Production Science 50, 405–409.

Papadopoulos., B.G, Goulas, C., Apostolaki, E., Abril, R., 2002. Effects of dietary supplements of algae, containing polyunsaturated fatty acids, on milk yield and the composition of milk products in dairy ewes. J. Dairy Res. 69: 357–365.

Póti, P., Pajor, F., Bodnár, Á., Penksza, K., Köles, P., 2015. Effect of micro-alga supplementation on goat and cow milk fatty acid composition. Chilean Journal of Agricultural Research 75, 259-263.

Priyadarshani, I., Rath, B., 2012. Commercial and industrial applications of micro algae – A review J. Algal Biomass Utln. 3: 89–100.

Pulina, G., Nudda, A., Battacone, G., Cannas, A., 2006. Effects of nutrition on the contents of fat, protein, somatic cells, aromatic compounds, and undesirable substances in sheep milk Anim. Feed Sci. Technol. 131, 255-291.

Reynolds, C.K., Cannon, V.L., Loerch, S.C., 2006. Effects of forage source and supplementation with soybean and marine algal oil on milk fatty acid composition of ewes. Anim. Feed Sci. Technol. 131, 333–357.

Ribeiro, J.E.S., Martini, M., Altomonte, I., Salari, F., Nardoni, S., Sorce, C., da Silva, F.L.H., Andreucci A., 2017. Production of Chlorella protothecoides biomass, chlorophyll and carotenoids using the dairy industry by-product scotta as a substrate. Biocatal Agric Biotechnol 11, 207-213.

Ryckebosch, E., Bruneel, C., Termote-Verhalle, R., Goiris, K., Muylaert, K., Foubert, I., 2014. Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty acids as an alternative for fish oil. Food Chem 160, 393-400.

Panjaitan, T., Quigley, S.P., McLennan, S.R., Poppi, D.P. 2010. Effect of the concentration of Spirulina (*Spirulina platensis*) algae in the drinking water on water intake by cattle and the proportion of algae bypassing the rumen. Animal Production Science, 50, 405–409.

Shingfield, K.J., Griinari, J.M., 2007. Role of biohydrogenation intermediates in milk fat depression. Eur. J. Lipid Sci. Technol. 109, 799–816.

Sinclair, L.A., Weerasinghe, W.M., Wilkinson, R.G., de Veth, M.J., Bauman D.E., 2010. A Supplement Containing Trans-10, Cis-12 Conjugated Linoleic Acid Reduces Milk Fat Yield but Does Not Alter Organ Weight or Body Fat Deposition in Lactating Ewes. J. Nutr. 140, 1949-1955.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. J Biosci Bioeng. 101, 87-96.

Stamey, J.A., Shepherd, D.M., de Veth, M. J., Corl, B.A., 2012. Use of algae or algal oil rich in n-3 fatty acids as a feed supplement for dairy cattle. J. Dairy Sci. 95, 5269–5275.

Stamm, 2015. Effects of different microalgae supplements on fatty acid composition, oxidation stability, milk fat globule size and phospholipid content of bovine milk M.Sc. Thesis ISSN 0355-1180

Stokes, R.S., Van Emon, M.L., Loy, D.D., Hansen, S.L., 2015. Assessment of algae meal as a ruminant feedstuff: Nutrient digestibility in sheep as a model species J. Anim. Sci. 9, 5386–5394.

Toral, P.G., Hervás, G., Gómez-Cortés, P., Frutos, P., Juárez, M., de la Fuente, M.A., 2010. Milk fatty acid profile and dairy sheep performance in response to diet supplementation with sunflower oil plus incremental levels of marine algae. J. Dairy Sci. 93, 1655–1667.

Tsiplakou, E., Abdullah, M. A. M., Mavrommatis, A., Chatzikonstantinou, M., Skliros, D., Sotirakoglou, K., Flemetakis, E., Labrou, N. E., Zervas, G. 2018. The effect of dietary Chlorella vulgaris inclusion on goats milk chemical composition, fatty acids profile and enzymes activities related to oxidation. J Anim Physiol Anim Nutr 102, 142—151.

Tsiplakou, E., Abdullah. M.A.M., Alexandros, M., Chatzikonstantinou, M., Skliros, D., Sotirakoglou, K., Flemetakis, E., Labrou, N.E., Zervas, G. 2017a. The effect of dietary Chlorella pyrenoidosa inclusion on goats milk chemical composition, fatty acids profile and enzymes activities related to oxidation. Livest Sci 197, 106-111.

Tsiplakou, E., Abdullah, M. A. M., Skliros, D., Chatzikonstantinou, M., Flemetakis, E., Labrou, N., Zervas, G. 2017b. The effect of dietary Chlorella vulgaris supplementation on micro-organism community, enzyme activities and fatty acid profile in the rumen liquid of goats J Anim Physiol Anim Nutr 101, 275—283.

Vahmani, P., Fredeen, A.H., Glover, K.E., 2013 Effect of supplementation with fish oil or microalgae on fatty acid composition of milk from cows managed in confinement or pasture systems. J. Dairy Sci. 96 , 6660 – 6670.

Van Emon, M.L., Loy, D.D., Hansen, S.L., 2015. Determining the preference, in vitro digestibility, in situ disappearance, and grower period performance of steers fed a novel algae meal derived from heterotrophic microalgae. J. Anim Sci. 93, 3121–3129.

Venckus, P., Kostkevičienė, J., Bendikienė, V., 2017. Green algae Chlorella vulgaris cultivation in municipal wastewater and biomass composition. Journal of Environmental Engineering and Landscape Management 25, 56-63.

Weatherly, M.E., 2015. Algae or yeast supplementation for lactating dairy cows. Theses and Dissertations--Animal and Food Sciences. Paper 47. http://uknowledge.uky.edu/animalsci_etds/47

Wiking, L., Stagsted, J., Bjørck, L., Nielsen, J.H., 2004. Milk fat globule size is affected by fat production in dairy cows. Int. Dairy J. 14, 909–913.

Table 1 Summary of the characteristics of the diets in the studies of the effects of microalgae supplementation on ruminant milk yield and quality

Feed/Algal species		**Duration of treatment diets**	**Ether estract of the diets (g on kg of DM)**	**Raw protein of the diets (g on kg of DM)**	**NDF of the diets (g on kg of DM)**	**ADF of the diets (g on kg of DM)**	**Animal Species**	**Authors**
Defatted meal of *Prototheca moriformis* (57% microalgae-43% soyhulls)	C=basal diet T=Algae replace 34.2% of ground corn of C	21 days	C=37.6 T=39.5	C=166 T=163	C=333 T=345	C=152 T=169	Cow	**Da Silva et al., 2016**
Market products based on marine algae meal rich in DHA	T1= basal diet , T2, T3, T4= basal plus 125, 250, 375 g/cow per d of algal meal respectivelyy	16 days	T1=28 T2=47 T3=34 T4=38	T1=240 T2=198 T3=226 T4=226	T1=323 T2=373 T3=366 T4=363	T1=291 T2=289 T3=284 T4=280	Cow	**Moate et al., 2013**
	C=Total mixed ration (TMR) plus 25 g of sunflower oil/kg of dry matter T= TMR plus 8 g of microalgae/kg of dry matter.	54 days	C=58 T=57	C=190 T=189	C=267 T=260	C=174 T=166	Sheep	**Bichi et al., 2013**
	C=basal diet T1= basal diet plus 25 g of sunflower oil/kg of DM T2= basal diete plus 25 g of sunflower oil/kg of DM and 8 g of microalgae T3= basal diet plus 25 g of sunflower oil/kg of DM and 16 g of microalgae	28 days	C=26 T1=50 T2=54 T3=57 T4=63	C=161 T1=159 T2=158 T3=159 T4=158	C=308 T1=304 T2=296 T3=300 T4=293	C=198 T1=195 T2=190 T3=191 T4=187	Sheep	**Toral et al., 2010**

T4=basal diet plus 25 g of sunflower oil/kg of DM and 24 of microalgae							
Experiment 1 C= basal diet T= h microalgae replaces 17.3% of concentrate of C	11 days	C=30.7 T=30.4	C=152 T=160	C=389 T=385	C=213 T=212	Cow	**Boeckaert et al., 2008**
Isonitrogenus diets Experiment 1: Ca= corn silage Cb=alfalfa pellets Ta, b=C a, b plus soybean oil and micro-algae biomass at 25g/kg of ration DM, in substitution of corn meal Experiment 2: Cc= haylage Cd=Corn silage Tc,d=, Cc, d plus soybean oil and micro-algae 25g/kg of ration DM Ezperiment 3: Ce= corn silage; Te=Ce plus soybean oil and micro-algae at 37g/kg of ration DM	20 days	Experiment 1, 2. 3: :not available	Experiment 1: Ca=139 Ta=136; Cb=152 Tb=145; Experiment 2: Cc=162 Tc=160; Cd=139 Td=133; Experiment 3: Ce=137 Te=136	Experiment 1: Ca=313 Ta=310; Cb=332 Tb=337; Experiment 2: Cc=361 Tc=366; Cd=352 Td=353; Experiment 3: Ce=336 Te=337	Experiment 1: Ca=180 Ta=175; Cb=224 Tb=225; Experiment 2: Cc=271 Tc=272; Cd=200 Td=199; Experiment 3: Ce=191 Te=190	Sheep	**Reynolds et al., 2006**
C=basal diet; T1=C ration with 16.9 g /day of algae; T2= C ration with 27.7 g/day algae ; T3= C ration with 51.7 g/day g algae	42 days	C=53.4 T1=40 T2=42.6 T3=42.6	C=241.8 T1=224.3 T2=198.9 T3=198.9	Fibre= C=206.5 T1=201.2 T2=194 T3=194		Sheep	**Papadopoulos et al., 2002**

Dry biomass, *Spirulina platensis's*	C= basal diet T= C diet plus 200g of 'Spirulina platensis	90 days					Cow	**Kulpys et al., 2009**
i) Spirulina platensis;* *ii) Clorella vulgaris	two experiments tested microalgae feeding compared to diet supplemented with rapeseed meal or without supplementary protein feed Experiment 1 C=basal diet; T1) C plus pelleted rapeseed T2) C plus a mixture of S. platensis and C. vulgaris T3) C plus a mixture of pelleted rapeseed and algae supplement Experiment 2: C= basal diet T1= C plus no protein supplementation T2=C plus pelleted rapeseed T3= C plus Spirulina platensis T4 = C plus mixture of pelleted rapeseed and Spirulina platensis	21 days		Experiment 1: T1=150 T2= 165 T3=162 Experiment 2: T1=125 T2= 146 T3=151 T4=149	Experiment 1: T1=475 T2= 498 T3=490 Experiment 2: T1=421 T2= 413 T3=410 T4=410		Cow	**Lamminen et al., 2017**
Clorella vulgaris	C= basal dieta T=C plus microalgae	30 days	*C=20 *T=19	*C=165 *T=167	*C=486 *T=490	*C=256 *T=269	goat	**Tsiplakou et al., 2018**
			*calculated on the intake					
Chlorella pyrenoidosa	C= basal dieta T=C plus microalgae	28 days	C=68 T=69	C=110 T=115	C=294 T=294	ADF=80 T=79	goat	**Tsiplakou et al., 2017a**
i) Dried *Chlorella kessleri ;*	i) C= basal diet ; T=C diet plus micro-alga	10 days	i) C=20.9 T=20.8; ii)C=22.0	i)C=201.4 T=209.2; ii)C=165.8	*raw fiber=i) C=254.8		i) Goat ii) Cow	**Póti et al., 2015**

ii) Dried *Spirulina platensis*	ii) C= basal diet , T= C diet plus micro-alga		T=21.9	T=165.5	T=253.8; ii)C=259.2 T=258.4			
Powder *Spirulina platensis;* *Chlorella vulgaris;* *Chlorella +Nannochloropsis gaditana* (50:50)	C= basal diet T1= C diet plus soya concentrate T2= C diet plus Spirulina platensis T3= C diet plus Chlorella vulgaris; T4= C diet plus Chlorella vulgaris + Nannochloropsis gaditana	21 days		Not available	Not available	Not available	Cow	**Stamm, 2015**
Spray dried Schizochytrium sp. heterotrophically grown	T1,2,3,4=0, 100 , 300 ,600 , grams of algae per day respectively	28 days	T1=55.3 T2=55.3 plus 60 gr day T3=55.3 plus 120 g/ day T4=55.3 plus 240 g/day	T1, 2,3,4 =158	T1,2,34=370	C, T1,2,3,=234.2	Cow	**Weatherly, 2015**
Commercial products: lipid encapsulated biomass and algal meal	C=basal diet T1=C plus0.5× algal biomass supplement T2= C plus 1× algal biomass supplement T3= C plus 1× algal oil supplement	7 days	C=44 T1=44 plus top dressing 112 g of fat /day T2=44 plus top dressing 244 of fat g/day; T3=44 plus top dressing 145 of fat g/day	C,T1,2,3=146	C, T1,2,3=344	C, T1, T2, T3=207	Cow	**Stamey et al., 2012**

Marine algae Schizochytrium sp rumino procted and non ruminoprotected	C=basal diet diet T1=C plus 910g/ d of protected algae T2= C plus 910g/ d unprotected algae	C=32.1 T1=36.5 T2=38.3	C=170 T1=169.8 T2=169.1	C=266 T1=264 T2=262.6	C=207 T1=212.2 T2=211.1	Cow	**Franklin et al., 1999**

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

1. Da Silva et al., 2016. Basal diet: total mixed ration (TMR). Ingredients (g/kg of dri matter) (DM): corn silage: 501; ground corn: 269; goybean meal: 113; whole raw soybean: 80.1; minerals and vitamins: 16; sodium bicarbonate: 9; dicalcium phosphate: 4.6; urea: 3.80; limestone: 1.4; magnesium oxide: 1.10; salt: 0.90; ammonium sulfate 0.5

2. Moate et al., 2013. Basal diet: 5.9 kg of dry matter per day of concentrates (683 g/kg of cracked wheat (Triticum aestivum), 250 g/kg of cold-pressed canola, 46 g/kg of granulated dried molasses, and 21 g/ kg of mineral mix) and ad libitum alfalfa (Medicago sativa) hay.

3. Bichi et al., 2013. Basal diet: TMR (40:60 forage:concentrate ratio). Ingredients (g/kg of fresh matter): dehydrated alfalfa hay: 392; whole corn grain: 184; soybean meal: 147; whole barley grain: 119; beet pulp: 66; molasses:48; feed supplement: 23; sunflower oil: 21.

4. Toral et al., 2010. Basal diet: TMR. Ingredients (g/kg of fresh matter): dehydrated alfalfa hay: 484; whole corn grain: 136; whole barley grain: 175; soybean meal:: beet pulp: 49; molasses: 37; feed supplement: 21.

5. Boeckaert et al., 2008. Experiment 1 basal diet: TMR. Ingredients (g/kg of DM): grass silage 333; corn silage: 333; standard dairy concentrate: 306; soybean meal: 27.8

6. Reynolds et al., 2006. Ingredients of the basal diets (g/kg of DM): Experiment 1/Control diet a: corn silage: 600; corn meal: 186.6; soybean meal: 173.5; mono-Na phosphate: 10.95; limestone: 20; trace mineral salt: 5; vitamin A: 0.07; vitamin D: 0.18; vitamin E: 0.88; selenium (201 mg/kg): 2.70; zinc oxide (730g Zn/kg): 0.08. Experiment 1/Control diet b: alfalfa meal: 600; corn meal: 381.8; mono-Na phosphate: 10.95; trace mineral salt: 5; vitamin A: 0.07; vitamin D: 0.18; vitamin E: 0.88; selenium (201mg/kg): 1; zinc oxide (730g Zn/kg): 0.08.

Experiment 2/Control diet c: corn silage: 600 corn meal; corn meal: 190.7; soybean meal: 169.4; mono-Na phosphate: 10.95; limestone: 20; trace mineral salt: 5; vitamin A: 0.07; vitamin D: 0.18; vitamin E: 0.88; selenium (201mg/kg): 2.70; zinc oxide (730g Zn/kg): 0.08. Experiment 2/Control diet d: alfalfa haylage: 600; corn meal: 337.3; soybean meal: 44.55; mono-Na phosphate: 10.95; trace mineral salt: 5; vitamin A: 0.07; vitamin D: 0.18; vitamin E: 0.88; selenium (201mg/kg) 1; zinc oxide (730g Zn/kg): 0.08.

Experiment 3/Control diet e: corn silage: 600; corn meal: 124.8; soybean meal: 167.8, mono-Na phosphate: 10.95; limestone: 20; trace mineral salt: 5; vitamin A: 0.07, vitamin D: 0.18; vitamin E: 0.88, selenium (201mg/kg): 2.70; zinc oxide (730g Zn/kg) 0.08.

7. Papadopulos et al. 2002. Basal diets: 600 g pelleted alfalfa hay and concentrate according to milk production at a rate of 1 kg of concentrate for each 1±7 kg milk.

8. Kulpys et al., 2009. Basal diets: 15 kg of silage and haylage, 2 kg of hay and an additional 350 g of combined fodder per 1 litre of milked milk after calving for indoor animal. For animal at pasturethe diet was 60 kg of grass, 100 g vitamin-mineral supplements and 300 g of combined fodder per 1 litre of milked milk.

9. Lamminen et al., 2017. Ingredients of the basal diets (g/kg of DM): Experiment 1: 9.801 kg of DM cereal-sugar beet pulp-based concentrate +silage of primary growth of timothy (Phleum pratense) and meadow fescue (Festuca pratensis) mixture ad libitum. Experiment 2: 10.78 of DM of concentrate cereal-sugar beet pulp-based concentrate +silage of secondary growth of timothy (Phleum pratense) and meadow fescue (Festuca pratensis) mixture ad libitum.

10. Tsiplakou et al., 2018. Basal diet consisted in of alfalfa hay and concentrates (forage/concentrate = 53/47). Ingredients of the concentrate (g/kg as fresh matter): maize grain: 340; barley grain: 380; soybean meal: 150; wheat middlings: 110; calcium phosphate: 15; salt: 3; mineral and vitamin premix.

11. Tsiplakou et al., 2017a. Basal diet consisted in alfalfa hay, wheat straw and concentrates with a forage/concentrate ratio of 50/50. The concentrate (g/ kg as fed) consisted of: maize grain: 340; barley grain: 380; soybean meal: 150; wheat middlings,: 110; calcium phosphate: 15; salt: 3; mineral and vitamin premix: 2.

12. Póti et al., 2015. Ingredients of goat basal diet (g/kg of DM): concentrate: 331; winter wheat: 51; corn: 105; extracted soybean: 33; extracted sunflower: 49; wheat bran: 79; premix: 16; alfalfa hay: 669. Ingredients of cow basal diet (g/kg of DM): concentrate: 146; winter wheat: 22; corn: 46; extracted soybean: 15; extracted sunflower: 21; wheat bran: 35; premix: 7; alfalfa hay: 381; corn silage: 473.

13. Stamm, 2015. Basal diet: Timothy meadow-fescue as grass silage and a concentrate including cereal pulp mixture, molassed sugar beet pulp, minerals and vitamins.

14. Stamey et al., 2012. Basal diet: TMR. Ingredients (g/kg of DM): corn silage: 226; concentrate: 181; ground corn: 35; alfalfa silage: 29; alfalfa hay: 23; barley straw: 5.

15. Franklin et al., 1999. Basal diet: TMR. Ingredients (g/kg of DM): alfalfa hay: 350; corn silage: 125; corn grain: 331; soybean meal: 101; dry distiller’s grains: 44.6; dicalcium phosphate: 10.6; molasses: 7.5; limestone: 8.4; sodium bicarbonate: 7.8; tallow: 4.9; trace minerals: 4.2; magnesium oxide: 1.9, vitamins A, D and E premix: 1.4; vitamin E premix: 0.7.

Table 2. Results of the studies of the effects of microalgae supplementation on ruminant milk yield and quality

Feed/Algal species	**Raw fat of integration (% on DM)**	**Raw protein of integration (% on DM)**	**Animal Species**	**Maximum quantity of microalgae in the diet without affecting the intake**	**Effects on milk yield**	**Effects on milk proteins**	**Effects on milk lactose**	**Effects on milk fat**	**Authors**
Defatted meal of *Prototheca moriformis* (57% microalgae-43% soyhulls)	5.4 %	7.6%	Cow	92 g/kg of the DM of the diet	Not significant	Not significant	Not significant	Not significant	**Da Silva et al., 2016**
Market products based on marine algae meal rich in DHA	Not available	Not available	Cow	Up to 5 g/kg of DMI, the intake (T2) decreases for higher quantities	Not significant	Not significant	Increases starts with supplementations higher than 11 g/kg di DMI (T3 and T4)	Decrease in yield (kg/die) and percentage with supplementations starting from 5g/kg of DMI (T2)	**Moate et al., 2013**
	56%	16.7%	Sheep	8g/kg of the DM of the diet	Not significant	Not significant	Not significant	Decrease in yield (kg/die) and percentagee	**Bichi et al., 2013**
	56.7	17%	Sheep	Up to 24 g/kg of the DM of the diet (T4)	Not significant	Decrease in percentage with supplementtions from 8 g/kg of DM of the diet (T3)	Not available	Decrease of yield (kg/die) and percentages with supplementtions from 8 g/kg of DM of the diet (T3)	**Toral et al., 2010**
	58%		Cow	Decrease with supplementtions of 10g/kg of DMI	Decrease	Decrease in yield kg/die	Decrease in yield kg/die	Decrease in yield kg/die and percentage (with the prolongation of the supplementation)	**Boeckaert et al., 2008**
	39%	17%	Sheep	Decrease with integration up to 25 g/kg of DM of the diet	Decrease from 25g/kg of DM of the diet if the	Increase in concentration (g/kg) from 25g/kg of DM	Increase in concentration (g/kg) from 25g/kg of dry	Increase in concentration (g/kg) from 25g/kg of DM of	**Reynolds et al., 2006**

				based on alfalfa pellets or alfalfa haylage (Tb and Tc); no effect with 37g/kg of DM if the diet is based on insilate(Te)	diet is based on alfalfa pellets or alfalfa haylage (Tb and Tc); no effects with higher supplements in the diet based on corn silage (Te)	of the the diet with alfalfa hay and alfalfa haylage (Tb and Tc), and decreases in daily yield; no significant effects with corn silage diet (Ta, Te)	matter when alfalfa hay is fed (Tb), and decreases in daily yield	the diet when alfalfa haylage is fed (Tb and Tc); no significant effects on daily yield	
		Not available	Sheep	Decrease in concentrate intake with 12 g/kg of DM of the diet (T2) (estimated value)	Not significant	Increase in percentage from 12 g/kg of DM of the diet (T2) (estimated value)	Decrease in percentage with 42g/kg of DM of the diet (T4) (estimated value)	Increase in percentages with 42g/kg of DM of the diet (T4) (estimated value)	**Papadopoulos et al., 2002**
Dry biomass, *Spirulina platensis's*	5%	65%	Cow	From 10-14g /kg of DM (estimated value)	Increase	Not significant	Not significant	Not significant	**Kulpys et al., 2009**
iii) Spirulina platensis; ***iv) Clorella vulgaris***	i) 5.2 % ii) 5.7%	i) 68-70% ii) 61%	Cow	From 20-50g/kg of DM	Not significant	Tendency to decrease milk protein yield	Tendency to decrease	Not significant	**Lamminen et al., 2017**
Lyophilized *Chlorella vulgaris*	1.05%	67.7%	goat	5.15 g/kg DM	Not significant	Not significant	Not significant	Not significant	**Tsiplakou et al., 2018**
Chlorella pyrenoidosa	1.03%	57.4%	goat	5 g/kg DMI	Not significant	Not significant	Not significant	Not significant	**Tsiplakou et al., 2017a**
iii) Dried *Chlorella kessleri* ; **iv) Dried *Spirulina platensis***		Not available	iii) Goat iv) Cow	i) 10 g/kg of DMI ii) 7.4 g/kg of DMI	Not significant	Not significant	Not significant	i) Increase in percentage **ii)** Decrease in percentage	**Póti et al., 2015**
Powder	i) 5.2% ii) 12%	Not available	Cow	i) 50 g ii) 70g	Not significant	Not available	Not available	Increase in percentage	**Stamm, 2015**

iv) Spirulina platensis; ***v) Chlorella vulgaris;*** **vi) *Chlorella +Nannochloropsis gaditana* (50:50)**	iii) 19.2% (*Nannochloropsis*)			iii) 79g of DM of the diet				with *Spirulina vs Chlorella*	
Spray dried Schizochytrium sp. heterotrophically grown	60%	Not available	Cow	Up to 4g/kg of DMI (T2) Decrease from higher integration	Not significant	Not significant	Not available	Decrease with 15 g/kg of DMI (Fat corrected milk yield) (T3)	**Weatherly, 2015**
Commercial products: **lipid encapsulated biomass and algal meal**	l.		Cow	Up to 300 g/day of biomass (T2) and 194g/day of oil (T3)	Not significant	Not significant	Not available	Not significant	**Stamey et al., 2012**
Marine algae Schizochytrium sp rumino procted and non ruminoprotected	i) 19% unprotected ii) 25% protected	Not available	Cow	Decrease with 39.7 g/kg of DM of the diet (T1, T2)	Not significant	Not significant	Not available	Decrease of the percentage	**Franklin et al., 1999**

DM dry matter; DMI dry matter intake

Table 3. Results of the studies of the effects of microalgae supplementation on ruminant milk fatty acids

Fatty acid	**Maxium variations reported**	**Species**	**Authors**
C4:0	i) +19% ii) -27% iii) +22%	i) Goat ii) cow iii) cow	i) Poti et al., 2015 ii) Poti et al., 2015 iii) Moate et al., 2013
C6:0	i) -19% ii) -35%	i) Cow ii) sheep	i) Poti et al., 2015 ii) Papadopoulos et al., 2002
C8:0	i) -10% ii) +12%	cow	i) Poti et al., 2015 ii) Moate et al., 2013
C10:0	i) +11 ii) -25%	i) Cow ii) sheep	i) Moate et al., 2013 ii) Papadopoulos et al., 2002
C14:0	i) +7% ii) +28 iii) +160	i) Cow ii) Sheep iii) sheep	i) Moate et al., 2013 ii) Papadopoulos et al., 2002 iii) Toral et al., 2012
C16:0	i) -5% ii) +21 iii) -26% iv) +7%	i) Cow ii) Sheep iii) Sheep iv) Goats	i) Moate et al., 2013 ii) Papadopoulos et al., 2002; iii) Total et al., 2012 iv) Tsiplakou et al., 2017a
De novo up C16*	+4%	Cow	Moate et al., 2013
C18:0	i) -79% ii) From -64% to 91%	i) cows ii) sheep	i) Moate et al., 2013 ii) Toral et al., 2010; Reynolds et al., 2006; Papadopoulos et al., 2002
t11-C18:1	i) from + 11% to + 203% ii) + 151%	i) cow's ii) goat's	i) Boeckaert et al., 2008; Stamey et al., 2012; Moate et al., 2013; Póti et al, 2015 ii) Póti et al, 2015
CLA isomers	i) from + 13% to +108 % ii) + 28% iii) +39%	i) cow's ii) in goat's iii) sheep milk	i) Boeckaert et al., 2008; Stamey et al., 2012; Moate et al., 2013; Póti et al, 2015 ii) Póti et al, 2015; iii) Reynolds et al., 2006; Bichi et al., 2013
c9-C18:1	i) +44%	i) cow ii) sheep	i) Franklin et al., 1999

	ii) From -6% to -52%		ii) Papadopoulos et al., 2002; Reynolds et al., 2006
c9,12-C18:2	i) From -27% to -10% ii) +26% iii) +10%	i) cow ii) cow iii) goat	i) Boeckaert et al., 2008; Franklin et al., 1999; ii) Moate et al., 2013 iii) Kouřimská et al., 2014; Poti et al., 2015
n-3 C18:3	i) -13% ii) -24%	cow	i) Franklin et al., 1999; ii) Moate et al., 2013
C20: 5	i) From + 17% to + 112% ii) + 133% iii) from +50 to 100% or more	i) cows ii) goats iii) sheep	i) Stamey et al., 2012; Moate et al., 2013; Vahmani et al., 2013 ii) Póti et al., 2015 iii) Papadopoulos et al., 2000; Toral et al., 2010; Bichi et al. , 2013
C22:6	i) from 100 to 1000% or more ii) + 660% iii) + 100%.	i) cows ii) sheep iii) goats	i) Boeckaert et al., 2008; Moate et al., 2013; Poti et al., 2015 ii) Bichi et al., 2013 iii) Poti et al., 2015
MUFA	i) + 12% ii) + 4%	i) goats ii) cows	i) Póti et al, 2015; ii) Boeckaert et al., 2008
PUFA	i) + 54% -higher than + 100% ii) +13%	i) cow ii) goat	i) Franklin et al., 1999; Boeckaert et al ., 2008; Moate et al., 2013, ii) Poti et al., 2015
Omega 3	i) + 161% ii) +19% higher than 100% iii) + 19%	i) Milk of cow ruminal infusions ii) Milk of cow feeeding of microalgae iii) goat's milk	i) Boeckaert et al., 2008 ii) Stamey et al., 2012; Moate et al., 2013; Póti et al, 2015 iii) Póti et al, 2015

De novo = Sum (C4.0 to C15:0) + 0.5*(C16:0 + C16:1).