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2021-10-26

Lamminen , M 2021 , ' Nutritional value of microalgae for ruminants and implications from microalgae production ' , CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources , vol. 16 , 054 . https://doi.org/10.1079/PAVSNNR202116054

http://hdl.handle.net/10138/337006
https://doi.org/10.1079/PAVSNNR202116054

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Received: 28 August 2021
Accepted: 20 September 2021

doi: 10.1079/PAVSNNR202116054

The electronic version of this article is the definitive one. It is located here: http://www.cabi.org/cabreviews

(Online ISSN 1749-8848)

Abstract

Microalgae are a diverse group of microorganisms that are an interesting alternative feed resource for ruminant production. Microalgae species with high protein concentration and adequate amino acid (AA) composition can be used to substitute conventional protein feeds, whereas species with high carbohydrate or lipid concentration can be used to supply energy. Microalgal polyunsaturated acids and short-chain fatty acids have potential to improve the nutritive value of ruminant milk and meat for human consumption and mitigate enteric methane emissions. Microalgae composition is very plastid in comparison to conventional ruminant feeds and it can be influenced relatively easily by environmental conditions, such as nutrient supply. Microalgae also contain many compounds, especially carbohydrates and cell coverings, which are not usually found in ruminant feeds. Standard feed evaluation methods involving the use of crucibles or nylon bags (detergent fibre method, *in vitro* digestibility and *in vivo* rumen incubation) suit poorly to the analysis of microalgae with microscopic particle size. This paper attempts to give a general overview of the nutritive value (protein, lipids and carbohydrates) of microalgae for ruminant feeding applications and the possibilities to tailor microalgae composition for a certain ruminant feeding objectives. In addition, the key knowledge gaps related to the nutritive value of microalgae for ruminant nutrition are identified.

Keywords: microalgae, ruminants, nutrition, nutritive value, microalgae production

Review Methodology: The following databases were used for the literature research: Scopus (Elsevier, Amsterdam, the Netherlands), Web of Science (Thomson Reuters Science, New York, USA) and Google Scholar (Google LLC, Mountain View, USA). Main keywords used in search are: cow, ruminant, microalgae and specific names of different microalgae species. In addition, backward reference searching (i.e. identifying the references cited in the articles obtained by the method described above) and forward reference searching (i.e. identifying articles that cite the articles obtained by the method described above) were used.

Introduction

Microalgae, here understood as both prokaryotic and eukaryotic, mostly photosynthetic unicellular organisms (also known as phytoplankton), are a very interesting alternative feed resource for ruminant production. Microalgae have rapid growth rate [1, 2] and high concentration of interesting nutritive components (e.g. protein, unsaturated fatty acids (FA) and antioxidants) [3]. The protein yields of microalgae have exceeded those of conventional protein feeds by factor of 2–25 in North-Western Europe [4]. Moreover, in microalgae production, it is possible to utilise and recycle production inputs (e.g. nutrients, flue gases, waste and salt water) outside of conventional food and feed production chains [5]. Microalgae can also be produced on marginal or non-arable land, which reduces the competition with edible crops [1]. Microalgae are a highly diverse group of

organisms found in different biological kingdoms (Eubacteria, Plantae, Chromistae, Protists and Fungi) [6] and adapted to various environmental conditions [7, 8]. This is also reflected to the nutritive value of microalgae, which is highly variable between and within species (Table 1). The difficulties with defining what an 'alga' is (e.g. some references only accept photosynthetic eukaryotic species as algae), and complex and constantly changing taxonomy further complicate the picture [89, 90]. For example, fungi-like heterotrophic microorganism *Aurantiochytrium limacinum* may or may not be considered as an microalga, depending on the source of information. The complexity of microalgal components such as cell wall, storage polysaccharides and various organelles also reflects the evolutionary history of these species. The evolutionary oldest microalgae, cyanobacteria, are typically relatively simple in structure, and the complexity has increased with evolution in primary endosymbiosis (resulting in e.g. Chlorophytes and Rhodophytes) and even more in secondary endosymbiosis (resulting in e.g. Euglenophytes and Haptophytes) [91].

Microalgae are cultivated in open (raceway ponds) or closed systems (photobioreactors and fermenters) under autotrophic (with the presence of CO_2 and sunlight) or heterotrophic conditions (with the presence of organic carbon and absence of sunlight) [92]. The chemical composition of microalgae can be manipulated relatively easily by altering the growth medium and growing conditions (e.g. temperature, pH, salinity and availability of light and different nutrients) [93–96]. This is a great advantage, because on terrestrial plants, usually heavier, more laborious and expensive methods, such as breeding, genetic manipulation and processing, are needed in order to trigger major changes in the chemical composition of biomass. This diversity and possibility to influence the composition (i.e. plasticity) of microalgae enables multiple different utilisation possibilities in animal nutrition, but also tailoring microalgae to fit optimally to certain livestock production targets. Locally produced, productive and sustainable protein feeds and feeds with ability to decrease enteric methane emissions are urgently needed in ruminant nutrition. The current mainstream protein feeds (e.g. soybean and rapeseed meals) are well fitted to animal nutrition, but their production and consumption are burdened with various sustainability challenges [97, 98]. Moreover, the low protein self-sufficiency of European Union [99] makes livestock sector vulnerable to trade distortions, availability and price volatility of imported protein feeds [100, 101].

The utilisation of microalgae rich in unsaturated fat as ruminant feed can also generate direct health benefits for humans by decreasing the concentration of saturated fatty acids (SFA) and increasing that of polyunsaturated fatty acids (PUFA) in milk and meat [27, 102]. Diets high in lipids, especially 12:0, 14:0, 18:1 n-9, 18:2 n-6 and 18:3 n-3, have also potential to decrease enteric methane production of ruminants [103]. Microalgae rich in lipids or carbohydrates can be utilised as energy feeds, and species with high concentration of antioxidants or minerals (e.g. iodine, selenium) to supplement dairy cow diets aiming to improve for example animal health or nutritive quality of milk or meat.

The high production cost of microalgae currently hinders their large-scale utilisation in animal nutrition. The estimated biomass cost of microalgae (e.g. 0.42–5.1 €/kg as summarised by [104]) still largely exceeds the world market price of soya bean meal (on average 0.34 €/kg during 2014–2020; [99]). However, microalgae production price compares more favourably to the market price of fishmeal and oil (1–1.9 €/kg in European Union during 2012–2018; [105]); therefore microalgae likely first establishes itself in animal nutrition as a substitute of aquatic protein and lipids. With further technical development and the use of waste resources in microalgae production, it is possible to decrease the production cost of microalgae [106].

The lipid-extracted microalgae biomass derived from production of, for example, biofuels or dietary supplements are high-value co- or by-products for animal nutrition, which can also improve the economic feasibility of microalgae biofuels [107]. Moreover, total mixed ration feeding used in ruminant nutrition allows the utilisation of feed resources with relatively low dry matter (DM) concentration, such as fresh microalgae paste. Also delivering microalgae to ruminants via drinking water is feasible [108]. Thus, the drying and dewatering of microalgae biomass, which contributes significantly to the energy consumption of microalgae production [109], is not always necessary when utilised in ruminant production. However, the hygienic quality of fresh microalgae paste should be ensured with quick consumption after harvesting (i.e. close proximity of microalgae production and consumption sites), or the use of preservatives. In this review, In this review, the research on the nutritive value of microalgae is summarised on the perspective of ruminant nutrition, and the impacts of microalgae production practices on these characteristics are discussed. The composition of industrially most relevant microalgae genus [6] and species commonly studied in animal nutrition are reviewed with emphasis on macronutrients (protein, lipids and carbohydrates). Although microalgae contain also other compounds (e.g. pigments, antioxidants and minerals) potential to generate value-added animal products, they are out of scope of this review.

Protein quantity and quality

The nitrogen (N) concentration of microalgae has varied from 5 to 114 g/kg DM in literature (Table 1). Species highest in N include *Spirulina* spp. and *Chlorella* spp.. The N concentration instead of crude protein (CP) is reported in Table 1 to allow fair comparison between microalgae species and conventional feed resources. The non-protein N (NPN) concentration of microalgae (e.g. 2%–35% of

Table 1. Proximate composition (g/kg dry matter) of some microalgae species.

Phylum (common name), kingdom[a]	Species	Primary growth environment[a]	Ash	Nitrogen	Crude fat	NDF	α-linked glucose	Reference
Bacillariophyta (diatoms), kingdom: Chromista	*Phaeodactylum tricornutum*	Saline water	164[b] (112–251)[c]	64 (29-83)	150 (74–223)	46 (28–64)	0.4 (0.3–0.5)	[9–14]
Bigyra, kingdom: Chromista	*Aurantiochytrium limacinum*, syn. *Schizochytrium limacinum*	Saline water	35 (28–40)	22 (19–26)	667 (608–743)	42	39	[15–20]
Chlorophyta (green algae), kingdom: Plantae	*Botryococcus braunii*	Fresh water	63 (54–72)	75 (57–89)	309 (110–471)	n.a.	n.a.	[21, 22]
	Chlamydomonas reinhardtii	Fresh water	109 (48–128)	54 (15–98)	141 (88–247)	n.a.	332 (73–693)	[12, 23–26]
	Chlorella vulgaris	Fresh water and terrestrial environments	112 (24–287)	68 (31–108)	155 (11–366)	55 (0–128)	115 (2.0–550)	[11, 13, 25, 27–36]
	Chlorella pyrenoidosa	Fresh water and terrestrial environments	80 (46–131)	89 (74–104)	158 (10–254)	75 (4–146)	n.a.	[33, 37–40]
	Dunaliella salina	Saline water	323 (90–720)	39 (12–64)	187 (101–281)	11 (0–21)	n.a.	[37, 41–43]
	Haematococcus pluvialis	Fresh water	54	53 (27–80)	277 (140–370)	n.a.	n.a.	[44–47]
	Parachlorella kessleri, syn. *Chlorella kessleri*	Fresh water and terrestrial environments	64 (56–71)	83 (75–91)	114	n.a.	n.a.	[12, 48]
	Scenedesmus acutus	Fresh water	80 (60–100)	88 (80–96)	130 (120–140)	n.a.	n.a.	[49]
	Scenedesmus quadricauda	Fresh water	48 (27–64)	82 (72–90)	138 (76–199)	n.a.	n.a.	[12, 43, 48]
	Scenedesmus dimorphus	Fresh water	88 (24–182)	83 (43–98)	120 (74–252)	n.a.	n.a.	[43, 50, 51]
	Tetraselmis impellucida	Saline water	173	56	231	n.a.	n.a.	[50]
	Tetraselmis suecica	Saline water	178 (146–207)	62 (27–85)	181 (80–307)	n.a.	n.a.	[12, 13, 52–56]
Cyanobacteria (blue-green algae), kingdom: Eubacteria	*Arthrospira platensis,* syn. *Spirulina platensis*	Fresh water	98 (66–195)	93 (26–114)	58 (8–116)	25 (0–120)	159 (35–609)	[11, 13, 27, 29, 37, 48, 57–68]
	Arthrospira maxima, syn. *Spirulina maxima*	Fresh water	96 (63–115)	99 (83–119)	61 (7–121)	n.a.	286 (52–700)	[49, 50, 63, 69–71]

Continued

Table 1. Continued.

Phylum (common name), kingdom[a]	Species	Primary growth environment[a]	Ash	Nitrogen	Crude fat	NDF	α-linked glucose	Reference
Euglenozoa, kingdom: Protozoa	*Euglena gracilis*	Fresh water	102 (35–140)	32 (18–46)	129 (81–193)	3.3 (0–6.5)	n.a.	[25, 72–75]
Haptophyta, kingdom: Chromista	*Isochrysis galbana*	Saline water	235 (160–310)	51 (32–73)	222 (162–274)	n.a.	0.1	[10, 76, 77]
	Pavlova lutheri	Saline water	n.a.	46	120	n.a.	n.a.	[76]
	Pavlova salina	Saline water	n.a.	42	120	n.a.	n.a.	[76]
Ochrophyta, kingdom: Chromista	*Nannochloropsis gaditana*	Saline water	104 (70–158)	53 (5–95)	118 (15–293)	99 (90–111)	14 (1–26)	[9, 11, 27, 50, 56, 78–81]
	Nannochloropsis oculata	Brackish water	85 (20–245)	56 (28–112)	283 (0–587)	n.a.	n.a.	[11, 25, 65, 82]
	Nannochloropsis oceanica	Saline water	192 (75–350)	55 (21–78)	236 (84–368)	288	0.3	[10, 13, 14, 83]
Rhodophyta (red algae), kingdom: Plantae	*Porphyridium cruentum*	Saline water and terrestrial environments	199 (168–236)	56 (34–90)	65 (58–76)	n.a.	n.a.	[11, 84, 85]
	Porphyridium purpureum	Saline and brackish water, terrestrial environments	204	53	88	n.a.	n.a.	[86]

n.a. = not available.
[a][87, 88].
[b]Average value.
[c]Min – max. Only shown if more than one data point available.

total N; [28, 110]) has in some occasions exceeded those of rapeseed and soya bean meals (3%–17% of total N; [111–115]). Therefore, different N-to-CP conversion factors have sometimes been suggested for microalgae than for conventional feeds (e.g. 4.68–6.35 instead of 6.25; [84, 116, 117]). In contrast to monogastric animals, high dietary NPN concentration is not a problem for ruminants, although rumen fermentation and milk production seem to respond positively for the supply of true protein [118, 119].

Similarly to terrestrial plants, microalgae require C, N, P, S, K and many trace elements for their growth [120]. Nitrogen deprivation in the growth medium decreases the protein synthesis in microalgae [121], leading to decreased protein concentration in the biomass [122, 123]. Some, but not all cyanobacteria are able to fix atmospheric N, which makes the particular species less dependent on N supply from the environment or growth medium [124, 125]. Increasing NPN concentrations in *Chlorella vulgaris* have been reported with decreasing N concentrations in the growth medium, which might be related to the reduction of protein synthesis in microalgal cells [28]. Also, S supply can influence the protein concentration of microalgae [126]. In addition, the protein concentration of microalgae can vary depending on the growth phase; some species reach peak protein concentrations at exponential growth phase [127, 128], others at later phases of growth, that is, in early or late stationary phase [128].

Methionine, histidine and lysine are most interesting essential amino acids (EAA) in dairy cow nutrition, as their dietary supply is often limiting milk production [129–132]. Methionine and cysteine are important amino acids (AA) for wool production, and arginine and citrulline for gestation on ruminants [133]. However, any AA has the potential to limit milk, meat or wool production depending on the diet and its effects on microbial protein synthesis in the rumen [134]. In comparison to conventional feeds, microalgae are generally lower in histidine, but good sources of methionine (Table 2). It has sometimes been reported that cyanobacteria would be higher in valine, leucine and isoleucine and lower in lysine than diatoms and green algae, and lower in histidine and phenylalanine than green algae [149], but this was not observed here based on data reviewed in Table 2. Here we have only reviewed non-toxic *Arthrospira* spp., but the toxicity of cyanobacteria may also have an effect on the AA profile, as the toxins include AA like leucine and arginine [150].

It has been demonstrated with *Chlamydomonas reinhardtii*, a biological model species, that N starvation decreases the total concentration of amino acids [151], but the responses of individual amino acids depend on their physiological function in the algae. The changes in N supply cause no changes in the concentrations of most AA in *Chlamydomonas reinhardtii* [152]. However, the N-rich AA lysine, asparagine and especially arginine are very responsive to N supply; their concentrations decrease during N starvation, sparing N to other functions, and increase rapidly with increasing N supply [152]. Especially free AA respond rapidly to external factors on cyanobacteria, green algae and diatoms [149]. Also on diatom *Navicula* sp. limited N supply has decreased the proportion of arginine whereas the proportion of other amino acids stayed relatively stable [153]. The concentrations of glutamic acid have been reported to increase during both N starvation and N supplementation [152]. Sulphur is taken by algae as sulphate, reduced to sulphide and immediately incorporated into cysteine, which is the precursor of all S-containing cellular compounds, including methionine [154]. Therefore, it is logical that short-term S-starvation has decreased cysteine, but not methionine concentration of *Chlorella sorokiniana* [126].

These physiological responses may explain some of the variation in amino acid composition within species in Table 2. There are also a considerable number of studies where microalgae AA profiles have been reported to be relatively stable in different phases of algae life cycle [155, 156] and under different growth conditions [28, 153, 157]. Different acid hydrolysis conditions during AA analysis can result in significant changes in the concentrations of microalgal AA, but it has not markedly changed the AA profile (proportion of individual AA of total AA) of a single species within an experiment [157]. Nevertheless, microalgal AA profiles deviating remarkably from those in Table 2 have sometimes been reported [84, 158, 159].

In addition to intracellular amino acids, some microalgae, like *Euglena gracilis*, are able to excrete amino acids into the culture medium at least in some cultivation conditions [160]. Excreted amounts of arginine, alanine, leucine, lysine and valine have been reported to exceed 30 mg/L in *Euglena gracilis*, and in terms of arginine, concentrations up to 80 mg/L have been observed. Microalgae also release other proteinaceous compounds to culture medium, like extracellular enzymes (e.g. carbonic anhydrases, proteases and phenolixidases), protease inhibitors and phycoerythrin-like proteins [161]. Microalgae culture medium is currently a poorly utilised source of bioactive compounds [161].

For ruminant nutrition, the degradability of protein in the rumen is a very important characteristic that determines the availability of undegraded AA for intestinal absorption and affects the efficiency of conversion of feed N into milk protein. Generally, protein feeds with low ruminal protein degradability but high intestinal protein degradability are valued, which spares AA from microbial digestion in rumen but allows absorption from intestines for the host ruminant. *In vitro* ruminal protein degradability of different microalgae species is very variable [37, 57]. It has been reported to be 74% for non-cell-disrupted *Arthrospira platensis*, 79% for *Chlorella* spp., 54% *Nannochloropsis* spp. and 77% for *Phaeodactylum tricornitum* [57]. After 48-hour *in vitro* incubation, around 50% of the crude protein in *Chlorella vulgaris* was found rumen undegradable, and around 40%–48% of rumen undegradable protein was estimated to be intestinally digestible with some variation between microalgal cultivation conditions

Table 2. The amino acid composition (g/160 g N)[a] of some conventional feeds and microalgae species.

Phylum	Species	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Cys	Reference
	Soybean meal	73[b] (60–91)[c]	27 (24-32)	46 (38–51)	77 (65–87)	62 (52–70)	14 (10–16)	51 (44–57)	38 (34–46)	14 (11–14)	48 (41–58)	16 (8–17)	[135]
	Rapeseed meal	58 (50–64)	27 (24–29)	40 (37–43)	68 (66–75)	53 (38–62)	20 (18–22)	39 (34–43)	43 (38–48)	12 (11–17)	51 (47–55)	24 (18–26)	[136]
	Fishmeal	58 (57–64)	22 (16–35)	43 (32–50)	70 (55–81)	75 (70–81)	28 (23–35)	38 (28–43)	41 (31–46)	11 (8–12)	49 (39–57)	8 (7–9)	[137]
	Wheat	47 (40–54)	23 (20–28)	34 (31–38)	65 (59–70)	29 (25–33)	16 (14–19)	45 (40–50)	29 (26–32)	12 (9–14)	43 (37–49)	22 (19–27)	[138]
Bacillariophyta (diatom)	*Phaeodactylum tricornutum*	42 (29–50)	12 (9–17)	44 (35–63)	71 (54–100)	45 (41–49)	22 (20–28)	59 (37–111)	46 (37–62)	16 (13–20)	50 (39–67)	7 (4–11)	[10, 21, 57, 76]
Bigyra	*Aurantiochytrium limacinum*, syn. *Schizochytrium limacinum*	65 (32–78)	22 (20–22)	41 (23–46)	69 (39–78)	60 (39–66)	26 (12–34)	43 (27–48)	43 (22–51)	n.a.	62 (54–93)	34 (2–48)	[139, 140]
Chlorophyta (green algae)	*Botryococcus braunii*	157	11	26	54	36	19	34	28	17	34	11	[21]
	Chlamydomonas reinhardtii	102	34	74	172	91	44	103	86	0	103	21	[26]
	Chlorella vulgaris	57 (38–79)	20 (14–27)	35 (24–59)	78 (67–91)	54 (46–84)	19 (10–24)	53 (42–116)	38 (23–43)	15 (0.06–24)	49 (40–73)	9 (1–13)	[27–29, 35, 36, 141, 142]
	Chlorella pyrenoidosa	59 (47–69)	18 (14–20)	42 (33–53)	74 (50–87)	57 (47–68)	15 (5–34)	46 (41–54)	45 (39–49)	13 (10–18)	59 (54–67)	12 (8–17)	[142, 144]
	Dunaliella salina	n.a.	22 (21–24)	19 (1–48)	36 (5–80)	40 (30–64)	n.a.	n.a.	13 (4–32)	n.a.	18 (2–55)	n.a.	[145]
	Dunaliella bardawil	79	28	49	108	61	22	70	60	9	86	10	[146]
	Haematococcus pluvialis	44	13	23	55	29	n.a.	32	40	n.a.	32	n.a.	[46]
	Parachlorella kessleri, syn. *Chlorella kessleri*	73 (62–83)	16 (15–16)	34 (34–35)	64 (63–64)	43 (43–44)	37 (37–37)	39 (39–39)	34 (32–37)	n.a.	42 (42–43)	33 (32–33)	[48]
	Scenedesmus acutus	58 (53–63)	16 (15–17)	39 (32–44)	82 (66–93)	54 (50–57)	15 (12–21)	43 (36–50)	52 (48–58)	13 (12–14)	65 (55–72)	10 (7–14)	[49]
	Scenedesmus quadricauda	43 (37–49)	16 (13–19)	24 (20–28)	53 (47–59)	52 (43–61)	40 (39–41)	37 (30–43)	29 (28–29)	n.a.	39 (31–47)	6 (26–27)	[48]
	Scenedesmus dimorphus	51 (50–54)	22 (20–22)	38 (37–42)	85 (81–91)	59 (58–60)	22 (21–24)	55 (52–59)	47 (43–52)	10 (7–21)	54 (52–58)	11 (10–12)	[50, 51]
	Tetraselmis impellucida	44	23	45	91	60	27	62	50	22	59	n.a.	[50]
	Tetraselmis suecica	60 (45–72)	18 (9–29)	39 (31–45)	82 (70–103)	48 (38–62)	24 (13–34)	75 (59–99)	46 (40–51)	10 7–13)	48 (35–65)	10 (8–12)	[52, 55, 56, 76]
Cyanobacteria	*Arthrospira platensis,* syn. *Spirulina platensis*	65 (38–80)	14 (9–18)	45 (25–59)	73 (40–93)	38 (24–49)	22 (11–35)	40 (17–51)	41 (16–51)	10 (5–13)	54 (31–71)	15 (7–28)	[27, 29, 48, 57, 58, 65, 144, 147]
	Arthrospira maxima, syn. *Spirulina maxima*	69 (61–78)	17 (15–19)	59 (56–60)	84 (73–93)	50 (46–52)	21 (13–28)	48 (43–50)	49 (42–55)	14 (12–15)	66 (62–76)	6 (4–8)	[49, 50, 71, 148]
Euglenozoa	*Euglena gracilis*	64	26	39	80	66	20	44	60	17	63	15	[72]

Haptophyta	*Isochrysis galbana*	37 (32–41)	14 (11–17)	57 (51–62)	102 (92–112)	38 (31–45)	28 (25–31)	81 (57–106)	53 (46–60)	19 (14–25)	66 (61–70)	8 (6–10)	[10, 76]
	Pavlova lutheri	42	12	71	117	46	53	124	55	18	85	6	[76]
	Pavlova salina	37	8	57	116	45	22	101	60	9	69	6	[76]
Ochrophyta	*Nannochloropsis gaditana*	59 (56–62)	19 (17–21)	44 (39–49)	82 (69–98)	61 (55–66)	19 (13–24)	49 (45–57)	46 (45–51)	19 (14–23)	53 (50–58)	8 (8–9)	[27, 50, 56]
	Nannochloropsis oculata	42 (33–52)	14 (11–17)	54 (47–62)	96 (91–101)	51 (45–57)	23 (21–24)	80 (55–104)	57 (50–64)	10 (3–17)	67 (60–74)	5	[65, 76]
	Nannochloropsis oceanica	54 (49–58)	16 (15–17)	52 (35–70)	79 (67–90)	71 (48–93)	18	46 (39–52)	46 (36–55)	17	97 (46–149)	7	[10, 83]
Rhodophyta (red algae)	*Porphyridium cruentum*	139	20	94	104	98	50	89	112	25	45	6	[84]
	Porphyridium purpureum	85	7	54	110	62	19	4	48	n.a.	76	3	[86]

n.a., not available.
[a]If no other information was provided, N-to-CP conversion factor of 6.25 was assumed for unit conversion.
[b]Average value.
[c]Min–max. Only shown if more than one data point available.

[28]. *In vivo* data suggests that the ruminal protein degradability of *Arthrospira platensis* might be higher than that of rapeseed meal but lower than that of faba beans [29, 58]. In another *in vivo* study, *Arthrospira platensis* resulted in numerically higher ruminal ammonia concentration than isonitrogenously supplemented soya bean meal, which might indicate higher rumen protein degradability, but the difference did not reach statistical significance [162]. Effective degradability of N in ruminants has been reported to be 63%–71% for soya bean meal [135], 69%–77% for rapeseed meal [136] and 77%–80% for faba beans [163]. Mechanical cell disruption can increase *in vitro* ruminal protein digestibility, especially if the digestibility is low in the initial biomass [57].

Lipids

The lipid concentration (Table 1) and FA composition (Tables 3 and 4) of microalgae is highly variable between phylum and genera, and even within species, reflecting the plasticity and adaptability of microalgae to environmental conditions. Even the proportion of total FA from crude fat can vary greatly between and within species (Table 3); for example, in *Nannochloropsis gaditana* variation of 151–667 g/kg crude fat has been reported [9, 27]. Despite this vast diversity, 16:0 is usually one of the most abundant fatty acids across many microalgae genera (Tables 3 and 4). Typically, microalgae species within the same phyla and class have relatively similar FA composition [177]. High lipid concentration increases the energy density of the diet, which is favourable especially for the high yielding dairy cows. However, there is also limit to dietary lipid concentration in ruminant diets, as concentrations above 6%–7% in DM are associated with decreased DM intake and ruminal fibre digestibility, which may subsequently lead to decreased milk yield [226].

Microalgae contain many FA that are of interest in ruminant nutrition. Forages are typically the main source of omega-3 FA, especially α-linolenic acid (ALA; 18:3 n-3), for ruminants [174, 227, 228]. In contrast, omega-6 FA, mainly linoleic acid (LA; 18:2 n-6; 40%–60% of total FA), are major FA in concentrates like barley, oat, wheat [229], maize grain [230] and soybean meal [135]. Very-long-chain PUFA (>C18) cannot be synthesised in significant amounts in higher plants; in contrast, many algae and especially marine algae species are able to synthesise and accumulate high amounts of these FA, including arachidonic acid (20:4 n-6), eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) [231] (Table 4). Therefore, microalgae and fish oil derived from fish consuming microalgae are the most important sources of omega-3 very-long-chain PUFA for ruminants, although microalgal sources are still rarely used in practice. The use of fishmeal in ruminant nutrition is prohibited in EU (excluding milk replacers for young ruminants; EC No 999/2001) [232], Japan and Australia [233]. Especially, microalgae genera *Isochrysis*, *Nannochloropsis*, *Phaeodactylum*, *Pavlova* and *Thalassiosira* have been identified as potential substitutes for fish oil due to their high concentration of omega-3 long-chain PUFA, mainly EPA [234] (Table 4). Genera *Isochrysis*, *Pavlova* and especially species *Aurantiochytrium limacinum* are known to accumulate high concentrations of DHA (Table 4). Interestingly, some microalgae contain significant amounts of medium-chain SFA, mainly myristic acid (14:0), which are very rarely found in ruminant feeds (Table 4). Fatty acids 12:0 and 14:0 have been identified as very promising inhibitors of ruminal methane production that are reported to decrease ruminal methane emissions (g CH_4/kg energy corrected milk) up to 30% [103]. Conventional lipid supplements rich in 12:0 and 14:0 are typically very expensive making practical applications in commercial dairy farms unlikely [103], which may open an interesting market niche for microalgae production. Another FA rare in conventional ruminant feeds but rich in cyanobacteria (Table 4) is γ-linolenic acid (GLA; 18:3 n-6).

In many microalgae algae species, enhancing CO_2 supply and inducing stressful conditions, such as the limitation or even deprivation of nutrients (N, P, S), can generally cause the accumulation of N-free storage compounds such as lipids and starch or other polysaccharides into the biomass [94, 235, 236]. This is a well-known and largely utilised mechanism to boost microalgal lipid production. However, nutrient deprivation also decreases the growth rate and biomass production of microalgae, especially if long-term [236, 237]. The species, strain and environmental conditions determine whether the given microalga accumulates lipids, carbohydrates or both, and whether the accumulation of these storage compounds happens simultaneously or at different time scales [94, 236]. While N limitation is the most important method boosting microalgae lipid production, other environmental factors, like supply of minerals [238, 239], temperature [95, 240], light intensity [95, 241], production under photosynthetic or heterotrophic growth conditions [242, 243] and the growth phase of microalgae [244] can also affect the quantity and quality of microalgae lipids. The detailed description of complex effects of nutrient supply status and other environmental factors on microalgae composition are beyond the scope of this review, and the interested reader is referred to recent reviews [92, 94, 245] and references therein.

With sufficient N supply, microalgal lipid fractions are usually dominated by polar lipids (e.g. phospholipids, sphingolipids and glycolipids found mainly on membranes) [246, 247]. However, the proportion of neutral lipids (storage lipids such as triacylglycerols and steryl esters) typically increases with decreasing N concentration in the growth medium [231, 237, 246, 247]. The same response has been observed in other environmentally stressful growing conditions, too [235]. This contradicts the response on higher eukaryotes where abundant nutrient

Table 3. Total fatty acid content (TFA; g/kg crude fat) and saturated fatty acid composition (g/100 g total fatty acids) of some conventional feeds and microalgae species. Main fatty acids (>10 g/100 g total fatty acids) are bolded.

Phylum	Species	TFA	C4:0–8:0[a]	10:0	12:0	14:0	16:0	18:0	20:0	22:0	24:0	Reference
	Soybean meal		0.05	0.1	0	0.1[b] (0–0.2)[c]	**15** (13–16)	4.2 (3.8–4.7)	0.2 (0–0.3)	0.4 (0.3–0.5)	0	[164, 166]
	Rapeseed meal		n.a.	n.a.	0.2	0.1 (0–0.2)	7.5 (6.1–9.2)	1.8 (1.4–2.3)	0.3 (0.3–0.4)	0	0.4	[165, 167–169]
	Wheat		n.a.	0	0	0.3	**13**	2.8	0.7	1.8	2.2	[170]
	Maize silage		n.a.	n.a.	0.2 (0.2–0.2)	0.4 (0.4–0.5)	**16** (13–17)	1.4 (0–2.3)	0.8 (0–1.8)	0.5 (0.5–0.6)	0	[171, 173]
	C3 grass silage, pre-wilted, formic acid-treated		n.a.	n.a.	0.4 (0.2–0.6)	0.5 (0.4–0.7)	**16** (15–17)	1.6 (1.2–2.0)	0.5 (0.5–0.6)	0.9 (0.9–0.9)	0.6 (0.6–0.7)	[27, 29, 174]
	Fish oil		n.a.	n.a.	0.1 (0.1–0.1)	6.6 (0.4–14)	**15** (7.0–20)	3.0 (1.0–4.6)	0.4 (0.2–0.6)	0.2 (0.1–0.2)	0.2 (0–0.5)	[171–173,175, 176]
Bacillariophyta	*Phaeodactylum tricornutum*	748 (713–783)	0.0	0.0	0.0	7.6 (3.0–16)	**18** (8.5–26)	1.4 (0–5.5)	0.1 (0–0.4)	0.1 (0–0.5)	0.9 (0–2.6)	[9, 14, 57, 128, 177–180]
Bigyra	*Aurantiochytrium limacinum*, syn. *Schizochytrium limacinum*	810	0.0	0.0	0.1 (0–0.4)	3.2 (0.5–8.7)	**38** (20–57)	1.2 (0.6–1.7)	0.3 (0.2–0.5)	0.2 (0.1–0.4)	2.1	[16, 20, 140, 181, 182]
Chlorophyta	*Botryococcus braunii*		1.3 (0.4–4.0)	n.a.	0.1 (0–0.7)	0.5 (0–1.3)	**11** (6.4–17)	0.6 (0–1.3)	0.0 (0–0)	0.0 (0–0)	0.0 (0–0)	[22, 177]
	Chlamydomonas reinhardtii		n.a.	n.a.	n.a.	0.3 (0–3.2)	**25** (12–37)	2.2 (0–10)	0.0 (0–0.2)	0.0 (0–0.9)	0.0 (0–0.4)	[26, 177, 183–186]
	Chlorella vulgaris	269	3.2	n.a.	0.4 (0–0.9)	0.5 (0–2.0)	**17** (7.7–25)	1.4 (0–6.5)	0.1 (0–0.3)	0.0 (0–0.1)	1.0 (0–4.0)	[27, 28, 33, 35, 36, 177, 187, 188]
	Chlorella pyrenoidosa		8.0 (2.8–12)	n.a.	1.2 (1.0–1.4)	0.8 (0.3–2.2)	**19** (14–26)	1.2 (0.4–3.1)	0 (0–0)	n.a.	0 (0–0)	[33, 188]
	Dunaliella salina		0.1 (0–0.4)	0.0 (0–0.2)	0.1 (0–0.8)	0.5 (0–2.2)	**13** (1.4–27)	0.5 (0–1.5)	0.2 (0–1.2)	0.3 (0–2.4)	0 (0–0)	[43, 177, 180, 189, 190]
	Haematococcus pluvialis		**12** (2.9–21)	n.a.	0.6 (0.3–0.8)	0.7 (0–3.1)	**18** (4.4–23)	3.2 (0–14)	0.4 (0–2.3)	0.1 (0–1.0)	0.3 (0–1.1)	[177, 191, 192]
	Parachlorella kessleri, syn. *Chlorella kessleri*		n.a.	0.0 (0–0.2)	n.a.	0.1 (0–0.8)	**18** (3.0–32)	6.5 (0–72)	0.0 (0–0)	0.0 (0–0)	0.0 (0–0.4)	[177, 193, 194]
	Scenedesmus acutus		n.a.	0.0	0.0 (0–0)	0.4 (0–1.7)	**19** (13–30)	1.6 (0.4–2.3)	0.2 (0–0.7)	0.5	0.3 (0.2–0.4)	[195, 197]
	Scenedesmus quadricauda		n.a.	n.a.	0.0 (0–0.1)	1.5 (0–2.0)	9.7 (7.2–13)	0.5 (0.2–1.1)	0.1 (0–0.2)	0.1 (0–0.2)	0.1 (0–0.4)	[43, 195, 198, 199]
	Scenedesmus dimorphus		n.a.	n.a.	4.2 (0.1–5.3)	1.4 (1.0–1.8)	**16** (12–30)	9.0 (1.2–13)	0.5 (0.4–0.7)	0.3 (0.3–0.4)	0.0	[43, 51, 200]
	Tetraselmis impellucida		n.a.	n.a.	n.a.	1.9	**29**	1.0	n.a.	n.a.	n.a.	[50]
	Tetraselmis suecica		n.a.	n.a.	n.a.	1.5 (0–8.0)	**28** (13–50)	1.2 (0.2–3.3)	n.a.	n.a.	n.a.	[53, 128, 179, 189, 201]

Continued

Table 3. Continued.

Phylum	Species	TFA	C4:0–8:0[a]	10:0	12:0	14:0	16:0	18:0	20:0	22:0	24:0	Reference
Cyanobacteria	*Arthrospira platensis,* syn. *Spirulina platensis*	598 (561–671)	2.3 (0–4.1)	3.9 (0–7.7)	0.4 (0–1.7)	0.3 (0–2.6)	**40** (13–52)	0.9 (0–1.7)	0.0 (0–0.1)	0.0 (0–0)	0.1 (0–0.9)	[27, 57, 162, 177, 179, 188, 202, 203] (Lamminen *et al*., unpublished)
	Arthrospira maxima, syn. *Spirulina maxima*		5.2	n.a.	0.5	0.2 (0–0.7)	**30** (25–36)	1.9 (0–4.8)	0.0 (0–0)	0.0 (0–0)	0.2 (0–0.6)	[50, 177, 188]
Euglenozoa	*Euglena gracilis*		n.a.	0.3	3.2 (0–7.2)	**15** (0–79)	**16** (4.1–33)	2.1 (0–7.4)	0 (0–0)	0.8 (0–14)	0.5 (0–6.1)	[72, 177, 204–206]
Haptophyta	*Isochrysis galbana*		n.a.	n.a.	0.0	**16** (11–23)	**25** (8.8–41)	2.6 (0–7.0)	0 (0–0)	0	0 (0–0)	[128, 177, 189, 207]
	Pavlova lutheri		n.a.	n.a.	n.a.	**14** (10–35)	**26** (15–41)	3.3 (0.7–11)	n.a.	n.a.	n.a.	[128, [208–[210]
	Pavlova salina		n.a.	n.a.	n.a.	**15** (13–18)	**16** (14–19)	0.4 (0.1–1.0)	n.a.	n.a.	n.a.	[180, 210]
Ochrophyta	*Nannochloropsis gaditana*	481 (151–667)	n.a.	0.1	0.3	5.0 (0–12)	**28** (14–57)	3.9 (0–17)	0.1 (0–0.2)	0.3 (0–0.6)	0 (0–0)	[9, 11, 27, 50, 177, 189, 211, 212]
	Nannochloropsis oculata		n.a.	n.a.	n.a.	3.2 (0–8.4)	**20** (8.5–33)	1.1 (0–2.3)	4.3 (0–6.8)	0	0	[177, 180, 189, 213–217]
	Nannochloropsis oceanica		n.a.	n.a.	1.2	4.5 (2.8–17)	**27** (18–41)	9.9 (0.4–29)	0.2 (0–0.8)	0.4 (0.2–0.6)	0.0 (0–0.1)	[14, 178, 207, 218–220]
Rhodophyta	*Porphyridium cruentum*		n.a.	n.a.	0.0	0.7 (0–1.8)	**35** (26–48)	0.7 (0.3–1.3)	n.a.	n.a.	0.0	[180, 207, 221–224]
	Porphyridium purpureum		n.a.	n.a.	n.a.	0.6 (0–5.2)	**26** (1.6–37)	1.3 (0–3.4)	0.0 (0–0)	0.0 (0–0)	0.0 (0–0)	[86, 177, 225]

n.a., not available.
[a]Short-chain fatty acids, sum of 4:0, 6:0 and 8:0 fatty acids.
[b]Average value.
[c]Min–max. Only shown if more than one data point available.

Table 4. Unsaturated fatty acid composition (g/100 g total fatty acids) of some conventional feeds and microalgae species. Main fatty acids (>10 g/100 g total fatty acids) are bolded.

Phylum	Species	16:1 n-7	16:4 n-3	18:1 n-9	18:2 n-6 (LA)	18:3 n-3 (ALA)	18:3 n-6 (GLA)	18:4 n-3	20:4 n-6 (ARA)	20:5 n-3 (EPA)	22:6 n-3 (DHA)	Reference
	Soybean meal	0.1[a] (0–0.1)[b]	n.a.	**14** (14–15)	**54** (52–55)	9.4 (8.6–10)	0	n.a.	0 (0–0)	n.a.	n.a.	[164–166]
	Rapeseed meal	1.0 (1.0–1.1)	n.a.	**55** (48–63)	**26** (23–30)	3.9 (2.7–6.0)	0	n.a.	0	n.a.	n.a.	[165, 167–169]
	Wheat	n.a.	n.a.	**12**	**55**	5.0	n.a.	n.a.	0.1	n.a.	n.a.	[170]
	Maize silage	0.3	0	**17** (14–21)	**43** (37–49)	7.7 (5.6–11)	0.3 (0–0.8)	0	0	0 (0–0.1)	0 (0–0)	[171–173]
	C3 grass silage, pre-wilted, formic acid-treated	0.2 (0.2–0.2)	n.a.	4.2 (3.3.-5.1)	**17** (16–19)	**51** (48–55)	0	n.a.	0	0	n.a.	[27, 29, 174]
	Fish oil	4.3 (0.8–7.8)	0.2	8.7 (4.5–13)	1.5 (1.1–2,5)	1.3 (0.6–2.0)	0.7 (0.3–1.8)	3.0	1.4 (0.8–2.1)	**19** (11–36)	**19** (8.8–29)	[171–173, 175, 176]
Bacillariophyta	*Phaeodactylum tricornutum*	**28** (14–42)	0.1	6.9 (0.5–19)	1.7 (0–3.9)	0.8 (0–3.4)	0.4 (0–0.7)	1.1 (0.5–3.6)	0.6 (0–2.4)	**19** (6.3–33)	1.0 (0.2–1.9)	[9, 14, 57, 128, 177–180]
Bigyra	*Aurantiochytrium limacinum*, syn. *Schizochytrium limacinum*	0.1	n.a.	0.7 (0.1–1.3)	0.1 (0–0.4)	0.4 (0–0.6)	0.2 (0–0.3)	0.1	0.8 (0.1–1.5)	0.6 (0.3–1.3)	**43** (27–65)	[16, 20, 140, 181, 182]
Chlorophyta	*Botryococcus braunii*	0.4 (0–1.1)	n.a.	**39** (11–82)	3.8 (0–9.4)	9.8 (0–20)	0.0 (0–0)	0.0 (0–0)	0.2 (0–0.9)	0.8 (0–2.5)	5.3 (0–35)	[22, 177]
	Chlamydomonas reinhardtii	0.4 (0–2.7)	**12** (0–26)	8.2 (0–26)	7.0 (0–18)	**21** (0–42)	1.7 (0–7.1)	9.2 (2.1–29)	0.1 (0–0.9)	0.0 (0–0)	0.0 (0–0.5)	[26, 177, 183–186]
	Chlorella vulgaris	4.9 (0.4–18)	0.5	9.8 (1.5–45)	**15** (0–49)	**25** (2.3–43)	0.9 (0–4.5)	0.0 (0–0.1)	0.3 (0–1.1)	0.1 (0–0.9)	0.2 (0–2.8)	[27, 28, 33, 35, 36, 177, 187, 188]
	Chlorella pyrenoidosa	1.7 (0.9–3.7)	n.a.	**13** (5.3–28)	**13** (9.8–22)	**27** (14–34)	0.2 (0–0.5)	n.a.	0.4 (0.1–1.0)	0.5 (0–1.3)	0.1 (0–0.2)	[33, 188]
	Dunaliella salina	0.5 (0–2.6)	**26** (18–35)	3.2 (0–11)	8.9 (0–20)	**35** (15–63)	1.4 (0–4.3)	0.5 (0–1.4)	1.4 (0–4.2)	0.1 (0–0.2)	0.8 (0–6.9)	[43, 177, 180, 189, 190]
	Haematococcus pluvialis	0.0 (0–0.4)	n.a.	**12** (0–22)	**27** (7.0–39)	**14** (0.4–53)	1.8 (0–4.5)	2.0 (0–3.6)	1.7 (0–4.8)	1.0 (0–3.5)	0.6 (0–5.7)	[177, 191, 192]
	Parachlorella kessleri, syn. *Chlorella kessleri*	0.1 (0–0.9)	0.0 (0–0)	5.5 (0–19)	**33** (0–62)	7.7 (0–21)	0.1 (0–1.4)	0.3 (0–5.9)	0.2 (0–4.4)	0.8 (0–3.6)	0.0 (0–0)	[177, 193, 194]
	Scenedesmus acutus	2.2 (0–3.3)	8.5 (5.1–15)	**22** (6.8–39)	**10** (7.2–12)	**21** (13–35)	0.9 (0–1.5)	2.8 (2.2–3.3)	0.0 (0–0)	0.3 (0–0.8)	0.7 (0–2.1)	[195, 197]
	Scenedesmus quadricauda	1.1 (0–4.6)	**25**	9.1 (5.9–14)	9.8 (3.7–14)	**32** (22–41)	0.9 (0.7–1.4)	2.6 (1.8–3.4)	0.0 (0–0)	0.3 (0–1.0)	0.0 (0–0)	[43, 195, 198, 199]
	Scenedesmus dimorphus	4.7 (3.5–5.9)	**19**	**15** (12–18)	**11** (4.3–18)	**24** (19–29)	1.1 (0.2–2.1)	4.4.	0.0 (0–0.2)	0.1 (0–0.3)	0.2	[43, 51, 200]
	Tetraselmis impellucida	4.8	n.a.	**12**	7.9	**13**	3.7	3.5	n.a.	3.2	n.a.	[50]
	Tetraselmis suecica	2.7 (1.0–5.0)	n.a.	**11** (2.1–36)	4.8 (0–9.5)	**12** (0–21)	0.7	7.1 (3.0–11)	0.8 (0–1.6)	3.7 (0–7.1)	0.0 (0–0)	[53, 128, 179, 189, 201]

Continued

Table 4. Continued.

Phylum	Species	16:1 n-7	16:4 n-3	18:1 n-9	18:2 n-6 (LA)	18:3 n-3 (ALA)	18:3 n-6 (GLA)	18:4 n-3	20:4 n-6 (ARA)	20:5 n-3 (EPA)	22:6 n-3 (DHA)	Reference
Cyanobacteria	*Arthrospira platensis,* syn. *Spirulina platensis*	2.2 (0–6.5)	n.a.	5.0 (0–9.3)	**15** (0–24)	1.4 (0–8.8)	**19** (4.6–28)	0.0 (0–0)	0.0 (0–0)	0.2 (0–1.8)	0.9 (0–5.6)	[27, 57, 162, 177, 179, 188, 202, 203] (Lamminen *et al.*, unpublished)
	Arthrospira maxima, syn. *Spirulina maxima*	5.1 (0.9–9.0)	n.a.	4.2 (1.9–6.1)	**22** (16–33)	1.2 (0–4.9)	**23** (18–25)	0.0 (0–0)	0.0 (0–0)	0.0 (0–0)	0.0 (0–0)	[50, 177, 188]
Euglenozoa	*Euglena gracilis*	0.9 (0–4.9)	n.a.	3.9 (0–8.1)	2.9 (0–16)	4.4 (0–19)	0.2 (0–2.9)	0.3 (0–4.0)	7.0 (0–19)	3.8 (0–14)	2.4 (0–13)	[72, 177, 204–206]
Haptophyta	*Isochrysis galbana*	**15** (0–33)	n.a.	7.6 (2.5–17)	4.8 (1.6–9.6)	5.3 (0–19)	0	**14** (9.8–16)	0.1 (0–0.2)	1.8 (0–6.9)	7.6 (0–20)	[128, 177, 189, 207]
	Pavlova lutheri	**17** (11–33)	n.a.	4.5 (0–10)	1.3 (0–2.5)	0.7 (0–1.9)	0.8 (0.4–1.1)	6.4 (3.3–8.4)	0.2 (0–0.6)	**15** (0–26)	8.8 (0–16)	[128, 208-210]
	Pavlova salina	**11** (4.3–30)	0.0	1.1 (0.2–3.1)	1.8 (1.5–2.0)	1.0 (0–1.4)	1.6 (0.7–2.4)	**11** (4.2–15)	1.4 (0.4–3.7)	**25** (19–28)	8.4 (1.5–11)	[180, 210]
Ochrophyta	*Nannochloropsis gaditana*	**21** (1.7–36)	n.a.	6.5 (0.3–25)	5.9 (0–20)	**10** (0–33)	1.9 (0–3.6)	0.1 (0–0.1)	4.5 (1.7–8.8)	**18** (0.2–38)	0.1 (0–0.3)	[9, 11, 27, 50, 177, 189, 211, 212]
	Nannochloropsis oculata	**23** (6.8–32)	0.0	7.5 (0–37)	2.9 (0–10)	0.5 (0–2.0)	0.7 (0–1.9)	0.0 (0–0.1)	5.5 (0–11)	**26** (13–36)	0.7 (0–3.2)	[177, 180, 189, 213-217]
	Nannochloropsis oceanica	**18** (4.8–27)	0.0 (0–0)	**16** (3.6–32)	3.4 (1.1–9.9)	5.0 (0–12)	n.a.	0.0 (0–0.1)	4.3 (0–8.1)	**16** (2.2–31)	0.8 (0–2.2)	[14, 178, 207, 218–220]
Rhodophyta	*Porphyridium cruentum*	1.1 (0.3–1.9)	0.0	0.7 (0.3–1.3)	6.3 (3.9–10)	0.2 (0–0.4)	0.4 (0.2–1.2)	0.0 (0–0)	**24** (13–35)	**24** (16–41)	0.0 (0–0.1)	[180, 207, 221–224]
	Porphyridium purpureum	1.0 (0–4.1)	n.a.	5.0 (0–18)	**11** (0.3–24)	8.4 (0–23)	0.0 (0–0)	0.0 (0–0)	**19** (0.5–45)	**15** (10–27)	0.0 (0–0)	[86, 177, 225]

n.a., not available.
[a]Average value.
[b]Min–max. Only shown if more than one data point available.

supply increases the concentration of neutral lipids (i.e. storage lipids) [248].

The FA profile of polar and neutral lipids is not identical in microalgae [59, 247, 249]. For example, *Arthrospira platensis* lipids are found mainly in polar fraction as phospholipids and glycolipids, but the major part of some individual FA, like 16:0 and 18:0, is found in neutral lipid fraction [59]. As the solubility of different lipid fractions into various solvents also varies [250], relatively different lipid concentrations and FA profiles for the same microalgae batch might be obtained depending on the solvent used in lipid extraction [59, 218, 251–253]. Even the applicability of selected gas chromatography method can influence the microalgal FA profile [254].

The abovementioned examples highlight the importance of basic understanding of microalgal biology also in animal nutrition, and call for transparency in reporting the origin of microalgae and the analytical methods used in lipid analysis in order to increase the replicability of research. However, they have also direct impacts on animal nutrition, because both microalgae lipid supplements and lipid-extracted biomass can be utilised as feed resources. Solvents can also be used to remove odoriferous compounds in microalgae in order to increase their acceptability for humans and animals [59]. Solvents like hexane, methanol and chloroform are most often used in lipid extraction, but new methods are also being developed, for example, the use of ethanol and cyclopentyl methyl ether as solvents [255]. Ethanol and acetate extraction has triggered only minor changes in the FA profile of *Arthrospira platensis* biomass; however, hexane extraction resulted in complete removal of 16:0 and 18:0 and increased by nearly two-fold the proportion of LA and GLA in total FA [59]. The EPA concentration of *Nannochloropsis* spp. oil has varied up to 15%–20% units depending on the solvent used in lipid extraction [252, 253].

The purpose of microalgae production largely determines the aims of tailoring the FA composition. The high concentration of PUFA is desirable in animal production in order to create potential health effects in animals and humans consuming the animal-derived products. On the other hand, the opposite is true for biodiesel production as the increase of FA unsaturation rate decreases the oxidative stability of the product [256]. For example, the current standards of European Union for biodiesels (EN 14214 and EN 14213) require that the content of FA methyl esters with ≥4 double bonds is less than 1% (mol/mol) and the content of C18:3 (ALA and GLA) in biodiesel intended for vehicle use is lower than 12% (mol/mol) [256, 257].

Another interesting aspect related to the use of microalgae as lipid supplements for ruminants is related to their cell wall and microscopic size. Nutrients in intact microalgae are often protected by cell wall, which can also hinder or prevent the biohydrogenation of microalgal lipids; therefore microalgae biomass and extracted microalgae oil can behave differently in the rumen [258]. It has also been reported that the biohydrogenation of EPA and DHA in microalgae *Schizochytrium* sp. is lower than fish oil [227]. The structure of the microalgal cell wall varies greatly between species [6]. Therefore, it is logical that also the susceptibility of microalgal FA for ruminal biohydrogenation differs between species [178, 259]. Not much is currently known about the ruminal passage kinetics and the biohydrogenation processes of unicellular microalgae; therefore the possible mechanisms can only be speculated. The ruminal passage rate of microalgae might also resemble more that of liquid than regular feed particles due to very small microalgal cell size [179] and easy flowability in liquid. The latter is caused by microalgal cell density close or similar to that of water [260]. In that case, it is possible that the retention time of microalgae in the rumen might be shorter than that for other feed components due to faster passage rate of liquid phase [261]. Subsequently, the digestion process of microalgae might shift to lower parts of gastro-intestinal tract, which also protects FA from ruminal biohydrogenation. Microalgal FA might also be able to escape ruminal biohydrogenation because the efficiency of lipolysis of triglycerides and hydrogenation of FA decreases with decreasing particle size due to declining adherence of bacteria to feed particle surface [262]. Environmental conditions (e.g. N supply) also affect the cell size and cell wall thickness of microalgae [263], which can have direct effects on digestibility of nutrients and ruminal biohydrogenation of lipids in microalgae.

Carbohydrates and fibrous components

Storage carbohydrates

The primary storage carbohydrates found in feeds of non-animal origin are constituted of glucose or fructose subunits with varying bond type that links molecules together. Three main types of storage polysaccharides are found in conventional ruminant feeds: starch (α-(1–4) and α-(1–6) linked glucose) in the majority of higher plants [264] including sub-tropical and tropical grasses [265, 266], fructans (β-linked fructose) in temperate grasses [265, 266] and sucrose (glucose and fructose joined by α-1, β-2 glycosidic linkage) in grasses [265] and some by-products of sugar production [267]. In contrast, there is huge variation in the form of storage carbohydrates between different microalgae genera and it may even vary from species to species within the genera. Sometimes the storage polysaccharides of microalgae are simply stated to contain either α (glycogen or starch) or β-linked (chrysolaminarins and paramylon) glucose molecules (glucan), of which glycogen and chrysolaminarins are water-soluble and starch and paramylon solid crystalline forms [268]. However, as we see in the following section, and which was also demonstrated in [268], microalgal storage polysaccharides are much more complex. The

structural features of storage polysaccharides (e.g. amylose:amylopectin ratio, granule size and gelatinization temperature in starch) affect the physical quality and digestibility of processed feeds [269]; therefore they are important characteristics for the feed industry. Here the storage carbohydrates of microalgae are briefly covered in the sequence of evolutionary history of the phyla, as the complexity of compounds increases with endosymbiotic events. Focus is on the phyla described on Tables 1–4. For more information about the environmental impacts on storage carbohydrate composition, the reader is referred to previously published review [270]. A broad term 'α-linked glucose' is used in Table 1 instead of starch. The starch analysis is typically based on the release of α-(1–4) and α-(1–6) linked glucose from a starch molecule by α-amylases and amyloglucosidases [271]. However, other polysaccharides with similar structure or identical physical behaviour to starch may also be hydrolysed in this process [271]. These include glycogen, maltodextrins, maltose, isomaltose, α-glucan [271] and floridean starch [272]. This challenge is not unique to microalgae, but the comparison of microalgae species is complicated by the diversity of their storage carbohydrates.

In most cyanobacteria, polysaccharides are stored as water-soluble glycogen (α-(1–4) linked glucose) [91, 273–275]. However, a few nitrogen fixing cyanobacteria species store polysaccharides as an insoluble semi-amylopectin, which is an intermediate-type polyglycan between glycogen and amylopectin [274, 275]. Some of the species synthesising semi-amylopectin also contain amylose, forming a starch-like substance [275]. Cyanobacteria are also able to synthesise sucrose, trehalose, glucosylglycerol, glucosylglycerate and glycine betaine, but their production seems to function mainly as a salt acclimation mechanism rather than storage polysaccharide [276]. Sucrose also functions as a carbon-carrier molecule in N-fixing cyanobacteria [276]. The glycogen concentration of *Arthrospira platensis* has varied 35–609 g/kg DM (on average 159 g/kg DM) depending on production conditions (Table 1), and it represents a major part of total carbohydrates found on this species [60, 277]. Especially N depletion increases the glycogen concentration of *Arthrospira* spp. [69, 278].

Green algae include phyla Chlorophyta and Streptophyta, of which land plants have evolved [279]. Due to their shared evolutionary history, Chlorophyta store carbohydrates as starch, similarly to terrestrial plants [91, 275]. In addition, the composition of starch is similar to terrestrial plants constituting of amylopectin and amylose [273]. Even the genes and their functions involved in starch metabolism have remained unchanged during the evolution from ancestral unicellular green algae to complex terrestrial plants [273, 280]. However, there might be some variation in storage carbohydrate composition of Chlrorophyta, as it has been observed that *Scenedesmus obliquus* contained semi-amylopectin instead of amylopectin [281]. Starch concentrations up to 603 g/kg DM have been reported in *Chlorella* sp. [282]. Starch in *Chlorella sorokiniana* was characterised by small granule size (around 1 μm) with narrow size distribution and similar crystallinity pattern (A-type) to cereal starch [283]. Amylose contents of 170 g/kg and 340–380 g/kg have been reported for starch in *Chlorella sorokiniana* [283] and *Chlorella* sp. [284], respectively. This is close to regular wheat and corn, in which amylose content of starch typically ranges 180–300 and 220–330 g/kg, respectively [285]. The gelatinization of *Chlorella sorokiniana* starch has started at 24°C, peaked at 69°C and completed at 97°C [283]. The onset temperature of gelatinization in *Chlorella sorokiniana* starch is much lower, peak temperature similar and final temperature higher than on potato, corn, rice and wheat starch [285]. Resistant starch is the proportion of starch that cannot be degraded by amylolytic hydrolysis, and thus is not digested in small intestines. No resistant starch has been found in *Chlorella* sp. [286], which is beneficial for monogastric nutrition. However, in ruminants, slow degradation rate of starch and starch resisting microbial enzymatic degradation in the forestomachs is beneficial for rumen function as it lowers the risk of acidosis [287].

Rhodophyta (red algae) includes two subclasses, Florideophycidae and Bangiophycidae, which differ slightly in the produced storage carbohydrates [281, 288]. As unicellular (microalgae) species are only found in subclass Bangiophycidae [281], this review focuses on their carbohydrate composition. Traditionally, floridean starch (α-polyglucan consisting only of amylopectin and no amylose) has been considered the main storage carbohydrate in all Rhodophyta, but new research especially with unicellular Bangiophycidae has diversified this conclusion [281, 288, 289]. Storage carbohydrates in unicellular Rhodophyta are diverse, as they contain species that produce glycogen or alternatively amylose and semi-amylopectin as their storage carbohydrates [281, 288, 289]. It has even been suggested that the type of accumulated storage carbohydrate in Rhodophyta may vary depending on the growing conditions [288].

Bacillariophyta, Haptophyta and Ochrophyta store carbohydrates as a chrysolaminarin [91, 268, 290]. Chrysolaminarin is predominantly β-(1,3) linked glucan with occasional β-(1,2), β-(1,4) or β-(1,6) branching, and is easily extractable by dilute acid or hot water [291]. Chrysolaminarin concentrations of 40–300 g/kg DM have been reported during exponential growth phase and up to 810 g/kg DM during the stationary phase [292]. Nitrogen limitation and high light intensities also promote the accumulation of chrysolaminarin (up to 500 g/kg DM) in diatoms [291]. Ochrophyta have no ability to metabolise α-glucans [268]. Some studies have reported small amounts of α-linked glucose in Bigyra (Table 1). However, the storage polysaccharide metabolism of this phylum is largely unknown, as according to phylogenetic analysis of enzymes involved Bigyra have lost the ability to both α- and β-glucan metabolism [268].

Euglenophytes store carbohydrates as an insoluble, highly crystalline fibrillary β-(1,3) linked glucan called

paramylon [91]. The crystallinity of paramylon is comparable to that of cellulose [291] and it is classified as a dietary fibre [293]. Paramylon concentrations of 15–532 g/kg DM have been reported with *Euglena gralicis* [294, 295].

Cell walls and structural carbohydrates

Not much is known about the fibrous fractions of microalgae described as detergent fibre (neutral detergent fibre NDF, acid detergent fibre ADF, acid detergent lignin ADL) (Table 1), which are typically used to evaluate the availability of feed nutrients for livestock. This may relate to the challenges with the analytical methods involving the use of filters when analysing microalgae (crucibles or nylon bags in detergent fibre analysis, *in vitro* digestion method and *in situ* rumen incubation). As unicellular organisms, the microscopic particle size of microalgae is much smaller than the pore size of crucibles (recommendation of 40–90 μm, [296]) or nylon bags (often 17–37 μm, [297, 298]) used in these analytical methods; thus there is a significant risk of washout. Nylon bags with pore size less than 10 μm are not even recommended for incubations involving ruminal liquid, as it can restrict the number of rumen protozoa and bacteria entering the digestion bags [298]. The cell diameter of various spherical microalgae species has been reported to be 2.5–5.6 μm, and while the length of microalgae with spindle, oval or filament cell shape has varied 9–500 μm, their width has been reported to be only 3–10 μm [179]. The change of crucible pore size from 40–100 μm to 16–40 μm resulted in higher NDF concentrations of *Arthrospira platensis* (0 vs. 87 g/kg DM), *Chlorella vulgaris* (0 vs. 15 g/kg DM) and *Nannochloropsis gaditana* (90 vs. 219 g/kg DM) [27]. This highlights the importance of filter pore size in microalgae analysis and warrants further development of the standard analytical methods used in feed evaluation to suit better for the analysis of microalgae. However, regardless of the NDF concentration in microalgae, microalgal NDF likely has no relevance for maintaining rumen function (i.e. microalgae has no physically effective fibre) given the tiny particle size. Even though most of the scarce data on detergent fibre composition of microalgae indicates very low concentrations, very high concentrations are sometimes reported for lipid-extracted microalgae products (e.g. 470 and 47 g/kg DM of NDF and ADF, respectively, for lipid-extracted *Chlorella sorokiniana* residue; [299]).

The unfamiliar algal polysaccharides that are not found in conventional feeds may complicate detergent fibre analysis also when small particle size is not an issue. For example, the ADF fraction (i.e. lignin, cellulose) of brown seaweeds has been reported to be higher than NDF fraction (i.e. hemicellulose, cellulose, lignin) [300], which should be impossible by the definition of detergent fibre method [301]. It is hypothesised that alginate, which is found in high concentrations in brown seaweeds, is accumulated to ADF fraction instead of NDF [300]. Brown algae do not contain any microalgae species; hence, no alginate containing microalgae exists according to current knowledge. Nevertheless, the possibility that other polysaccharides in microalgae may cause challenges with detergent fibre method cannot be ruled out. Situations with ADF higher or very close to NDF have also been reported with microalgae, although the concentrations have been so small that the difference might also be caused by analytical imprecision [37]. Of compounds unique for algae, the storage polysaccharide paramylon (classified as a dietary fibre) seems not to accumulate on detergent fibre fraction, since very low concentrations of NDF (0–6.5 g/kg DM) and ADF (0–2.8 g/kg DM) have been reported with *Euglena gracilis*, species rich in paramylon [72, 73].

Due to the lack of information on detergent fibre composition of microalgae, the chemical composition of microalgal cell walls is reviewed. This knowledge is helpful when narrowing down the species having most potential at animal nutrition applications. In ruminant diets, the digestibility of NDF varies depending on its monosaccharide composition: NDF glucose > NDF xylose and uronic acid > NDF arabinose, galactose and mannose [302]. The understanding of ruminal digestibility of microalgal cell walls is also important from industrial perspective, as microalgae biomass can be pre-treated with ruminal microorganisms in order to increase the yield of gaseous biofuel production [303].

While the cell walls of terrestrial plants constitute mainly of carbohydrates (especially cellulose), phenolic compounds and only minor amounts of protein (up to 10%) [304], the cell wall structure and composition of microalgae can be very variable, as visualised by [6]. However, the cell wall composition of only a limited number of microalgae species is studied and their influence on the accessibility of microalgal nutrients and other valuable compounds is not yet fully understood [89, 305]. The cell wall polysaccharides of microalgae typically form around 100 g/kg of cell DM, although variation is large (from 35 g/kg DM in *Thisochrysis lutea* to 170 g/kg DM in *Tetraselmis chuii*) [30]. So far, the presence of true lignin in green and red macroalgae (seaweeds) could not have been confirmed [306]. Therefore, true lignin will unlikely be found in unicellular microalgae either, which are simpler in structure than multicellular algae. Nevertheless, the enzymes involved in making the simplest lignin monomers have an ancient origin and genes associated in their biosynthesis are found in a variety of eukaryotic microalgae phyla [307]. Indeed, some lignin-like phenolic compounds have been reported on macroalgae, simple multicellular algae [306, 308] and even on some microalgae [309].

The cell walls of cyanobacteria are constituted of peptidoglycan (also called as murein) [310, 311], which is a polymer composed of *N*-acetylglucosamine, *N*-acetylmuramic acid and several AA [311]. Polysaccharides are likely found only as minor components in cell walls of *Arthrospira platensis* [30]. Cyanobacterial cell walls have structural features both of gram-negative and gram-positive bacteria

[310], but they differ in cell wall thickness. Thicknesses of 1.5–10 nm and 20–80 nm have been reported for gram-negative and gram-positive bacteria, respectively [312]. In contrast, the thickness of cyanobacterial cell walls can range from 10 nm to more than 700 nm [310]. Some cyanobacteria also have cellulose-containing minor extracellular components as slime tubes, sheaths and extracellular slime [313].

The diversity of cell wall structures and composition in Chlorophyta is tremendous, not only varying between species and strains, but also in different life stages of the cell [6, 89, 305]. Many different grouping systems have been suggested for Chlorophyta based on the chemical composition of their cell walls. *Chlorella* spp. have been divided into species having glucosamine-rigid cell wall (e.g. *Chlorella vulgaris*) and species with glucose-mannose rigid wall (e.g. *Chlorella pyrenoidosa*) [314, 315]. Chlorophyta also contain species that completely lack a cell wall and have only plasma membrane, like *Dunaliella salina* [6, 89, 316]. In *Chlorella vulgaris,* the rigid cell wall fraction has constituted of 60%–66% of the cell wall DM, and hemicellulose 23%–25% [317]. However, the proportions change during the growth phase, as the mass of rigid fraction stays constant but the mass of hemicellulose increases with cell age [89]. In other type of classification, Chlorophyta have been divided into three groups: (1) species with cell walls constituting of 2-keto-sugar acids (Parasinophytina and Chlorodenrophyceae); (2) species with cell walls constituting of mannans, glucans, chitin-like polysaccharides and algaenan layer (unicellular Trebouxiophyceae and Chlorophyceae) and (3) species with cell walls constituting of xylan, mannan, glucan and sulphated and/or puruvylated polysaccharides (macroalgae species, i.e. seaweed) [89]. Algaenans may pose a challenge for digestion, as they are highly resistant to alkali and acid hydrolysis and aqueous and organic solubilisation [318]. Algaenan has been found on Chlorophyta genera *Scenedesmus*, *Tetraedron*, *Chlorella*, *Botryococcus* and *Haematococcus* as reviewed by [319]. The fibrous material in Chlorophyta can be based on cellulose or chitin-like polysaccharide (β-(1 $\rightarrow$ 3)-xylan or β-(1 $\rightarrow$ 4)-mannan) [89]. *Chlorella* spp. can also contain pectin [320]. Lipid-extracted *Chlorella sorokiniana* residue has contained 420 g/kg DM of hemicellulose, 27 g/kg DM of cellulose and 26 g/kg DM of lignin [299]. The protein concentration of cell walls in Chlorophyta varies largely: from 2% in *Chlorella vulgaris* [317] to 32% in *Neochloris oleoabundans* [321]. Due to their vast diversity, thus far no single industrial process has been found that is applicable to treat with success the cell walls of all Chlorophyta [89]. This is also reflected to *in vitro* rumen DM digestibility of Chlorophyta, which has varied considerably between species (654–797 g/kg DM; [259]). In another study examining the same Chlorophyta species, great differences were found in two-phase gastric/pancreatic *in vitro* digestibility (494–778 g/kg for CP) designed for monogastric applications; in contrast, no or only minor differences were found *in vitro* rumen organic matter digestibility [322].

The cell wall characteristics of unicellular Rhodophyta are less known than the multicellular macroalgae species of the phylum [323]. The cell wall of *Porphyridium purpureum* consists of two layers: the inner cellulose and the outer mucopolysaccharide layer with varying thickness depending on the growth stage [324]. The cell wall of *Porphyridium* spp. and *Dixionella grisea* contains sulphated polysaccharides, whose complete structure is still unknown due to their complex structures and lack of specific degrading enzymes [323, 325]. The main sugars in these polysaccharides are xylose, glucose and galactose with varying ratios [323, 325]. Glycoproteins with novel structures are bound to the cell wall polysaccharides on microalgal Rhodophyta [323].

Very little information is available on the cell wall characteristics of Haptophyta. Many Haptophyta are covered by scales consisting of organic material having cellulose as the main component [291]. Some species with coccoid growth stages have calcified scales (coccoliths) [291] that consist of calcium carbonate [326]. The contribution of cell wall polysaccharides to cell DM is low in *Tisochrysis lutea* [30]. It has also been reported that *Isochrysis galbana* would have only plasma membrane covering and no cell wall [316].

The ornamental cell walls of Bacillariophyta (diatoms) come with many shapes and patterns [327]. The cell walls (frustule) of diatoms have three layers: (1) the innermost organic layer, (2) silicified mineral layer containing also organic matter and (3) external organic coat that is covered with mucilage [290]. The organic matter in diatom cell wall is mainly composed of polysaccharides, proteins and polyamines [290]. The polysaccharides of *Phaeodactulum tricornutum* consist of mannose, glucuronic acid residues and sulphate groups [328]. Genera Cyclotella and Thalassiosira contain also highly crystalline fibres of β-chitin [290]. The rigidity of the diatom cell wall can pose challenges for its own growth and cell division [327], and cell disruption may be beneficial when fed to ruminants as it has been reported to slightly increase organic matter digestibility, intestinal digestibility of rumen undegradable CP and *in vitro* total volatile fatty acid and gas production [28].

The cell walls of Ochrophyta species *Nannochloropsis gaditana* have bilayer structure with cellulosic inner wall (around 750 g/kg of cell wall DM), which is covered by algaenan layer [318]. The algaenan outer wall causes the rigidity of the cell walls of *Nannochloropsis* spp., as it is chemically and enzymatically very resistant material (see above). Around 60 g/kg of cell wall DM of *Nannochloropsis gaditana* is consisted of amino acids [318]. Ochrophyta contain also species with silica scales and species with an envelope called lorica, which is mainly composed of chitin and cellulose [329].

The cell walls of Bigyra species *Schizochytrium aggregatum* and *Thraustochytrium* sp. are reported to contain 210–360 g/kg DM of carbohydrate and 300–430 g/kg DM protein [330]. Cell wall monosaccharides in *Schizochytrium aggregatum* are primarily constituted of L-galactose

(>950 g/kg) and in *Thraustochytrium* of galactose (around 660 g/kg) and xylose (around 330 g/kg), the L-galactose being the predominant form [330].

Euglenophyta have no cell wall, but unusual cell membrane complex, pellicle, which is consisted of proteinaceous strips, microtubules and tubular cisternae of endoplasmic reticulum being located underneath the plasma membrane [329]. The pellicle mainly consists of protein (around 680 g/kg DM, [331]). *Euglena gracilis* has a complex glycan surface consisting of galactan, xylan and aminosugars, but the protein glycosylation is simple with high mannose-type glycans [332].

Conclusion and future research

The tremendous diversity of microalgae between and within species enables multiple utilisation and tailoring opportunities for sustainable ruminant production. Species with high protein concentration have potential to substitute conventional protein feeds in ruminant diets. The knowledge on ruminal degradability of microalgal protein and the influence of different microalgae production conditions on it is still scarce, although ruminal degradability of protein is one of the key characteristics of a feed determining the protein use efficiency of the ruminant. Microalgae are generally rather high in methionine, but lower in histidine than conventional protein feeds. However, it seems possible, at least to some extent, to tailor the AA composition (arginine, asparagine, cysteine, glutamic acid and lysine) of microalgae with N supply on the growth medium. On the other hand, N supply can also have dramatic influence on the lipid:protein or carbohydrate:protein ratio, NPN concentration and lipid profile of microalgae. Therefore, changes of growth medium nutrient supply to the complete composition of microalgae must be carefully evaluated before any specific recommendations can be given for tailoring microalgae production to specific animal production targets. Microalgae with high concentrations of very-long-chain PUFA (e.g. EPA, DHA) can potentially improve the FA profile of milk and meat for human consumption. The possibility to decrease enteric methane emissions with microalgae having high concentrations of 12:0 and 14:0 FA is currently completely underexploited area of research. The production objectives of microalgae for biodiesel and animal production and human consumption may sometimes be in conflict, as the aim is to limit PUFA concentration for biofuel production, but often to maximise it for animal and human nutrition. Very little is known about the NDF and ADF composition of microalgae and the development of the standard feed evaluation methods involving the use of filters (NDF, ADF, ADL, *in vitro* digestion, *in vivo* incubation) is urgently needed in order to suit better for the analysis of unicellular microalgae with microscopic particle size. More knowledge is needed also about the ruminal passage kinetics and the biohydrogenation processes of microalgae, as they directly influence the transfer efficiency of FA from feed to milk and meat and N metabolism of the ruminant. In order to realise the full potential of microalgae in ruminant diets, the economic competitiveness of microalgae needs to be improved with further technical advantages of microalgae production, but also the development of microalgae feeding technique for animals (e.g. via drinking water or as a wet paste) might provide some cost savings for microalgae production. In the future, the possibility to produce added-value products or mitigate climate change via microalgae feeding of ruminants might also provide interesting opportunities for improving economic competitiveness of microalgae.

Acknowledgements

The valuable comments of Anni Halmemies-Beauchet-Filleau (University of Helsinki), Marika Tossavainen (Häme University of Applied Sciences), Wouter Muizelaar (Wageningen University and Research) and Aila Vanhatalo (University of Helsinki) for this paper are highly appreciated.

References

1. Schenk PM, Thomas-Hall SR, Stephens E, Marx JH, Mussgnug JH, Posten C, et al. Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenergy Research 2008;1:20–43.
2. Singh UB, Ahluwalia AS. Microalgae: a promising tool for carbon sequestration. Mitigation and Adaptation Strategies for Global Change 2013;18:73–95.
3. Becker W. Microalgae for human and animal nutrition. In: Richmond A, Hu Q, editors. Handbook of microalgal culture: applied phycology and biotechnology. 2nd ed. Chicester, United Kingdom: Wiley Blackwell; 2013. p. 461–503.
4. van Krimpen MM, Bikker P, van der Meer IM, van der Peet-Schwering CMC, Vereijken JM. Cultivation, processing and nutritional aspects for pigs and poultry of European protein sources as alternatives for imported soybean products. Report 662. Wageningen, the Netherlands: Wageningen Livestock Research, Wageningen University and Research; 2013. 48 p.
5. Pittman JK, Dean AP, Osundeko O. The potential of sustainable algal biofuel production using wastewater resources. Bioresource Technology 2011;102:17–25.
6. de Carvalho JC, Magalhães Jr AI, de Melo Pereira GV, Medeiros ABP, Sydney EB, Rodrigues C, et al. Microalgal biomass pretreatment for integrated processing into biofuels, food, and feed. Bioresource Technology 2020;300:122719.
7. Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. Renewable and Sustainable Energy Reviews 2010;14:217–32.
8. Sassenhagen I, Wilken S, Godhe A, Rengefors K. Phenotypic plasticity and differentiation in an invasive freshwater microalga. Harmful Algae 2015;41:38–45.
9. Kiani A, Wolf C, Giller K, Eggerschwiler L, Kreuzer M, Schwarm A. *In vitro* ruminal fermentation and methane

inhibitory effect of three species of microalgae. Canadian Journal of Animal Science 2020;100:485–93.

10. Skrede A, Mydland LT, Ahlstrøm Ø, Reitan KI, Gislerød HR, Øverland M. Evaluation of microalgae as sources of digestible nutrients for monogastric animals. Journal of Animal and Feed Sciences 2011;20:131–42.
11. Matos ÂP, Feller R, Moecke EHS, de Oliveira JV, Furigo A, Derner RB, et al. Chemical characterization of six microalgae with potential utility for food application . Journal of the American Oil Chemists' Society 2016;93:963–72.
12. Schulze C, Wetzel M, Reinhardt J, Schmidt M, Felten L, Mundt S. Screening of microalgae for primary metabolites including β-glucans and the influence of nitrate starvation and irradiance on β-glucan production. Journal of Applied Phycology 2016;28:2719–25.
13. Niccolai A, Zittelli GC, Rodolfi L, Biondi N, Tredici MR. Microalgae of interest as food source: biochemical composition and digestibility. Algal Research 2019;42:101617.
14. Sevgili H, Sezen S, Yılayaz A, Aktaş Ö, Pak F, Aasen IM, et al. Apparent nutrient and fatty acid digestibilities of microbial raw materials for rainbow trout (*Oncorhyncus mykiss*) with comparison to conventional ingredients. Algal Research 2019;42:101592.
15. Moran CA, Morlacchini M, Fusconi G. Enhancing the DHA content in milk from dairy cows fed by feeding ALL-G-RICH. Journal of Applied Animal Nutrition 2017;5:E11.
16. Moran CA, Keegan JD, Vienola K, Apajalahti J. Broiler tissue enrichment with docosahexaenoic acid (DHA) through dietary supplementation with *Aurantiochytrium limacinum* algae. Food and Nutrition Sciences 2018;9:1160–73.
17. Moran CA, Morlacchini M, Keegan JD, Fusconi G. The effect of dietary supplementation with *Aurantiochytrium limacinum* on lactating dairy cows in terms of animal health, productivity and milk composition. Journal of Animal Physiology and Animal Nutrition 2018;102:576–90.
18. Marques JA, Del Valle TA, Ghizzi LG, Zilio EMC, Gheller LS, Nunes AT, et al. Increasing dietary levels of docosahexaenoic acid-rich microalgae: ruminal fermentation, animal performance, and milk fatty acid profile of mid-lactating dairy cows. Journal of Dairy Science 2019;102:1–12.
19. Murphy EM, Stanton C, Brien CO, Murphy C, Holden S, Murphy RP, et al. The effect of dietary supplementation of algae rich in docosahexaenoic acid on boar fertility. Theriogenology 2017;90:78–87.
20. Ganuza E, Yang S, Amzquita M, Giraldo-Silva A, Andersen RA. Genomics, biology and phylogeny *Aurantiochytrium acetophilium* sp. nov. (Thraustochytriaceae), including first evidence of sexual reproduction. Protist 2019;170:209–32.
21. Tibbetts SM, Milley JE, Lall SP. Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors. Journal of Applied Phycology 2015;27:1109–19.
22. Cabanelas ITD, Marques SSI, de Souza CO, Druzian JI, Nascimento IA. *Botryococcus*, what to do with it? Effect of nutrient concentration on biorefinery potential. Algal Research 2015;11:43–9.
23. Choi SP, Nguyen MT, Sim SJ. Enzymatic pretreatment of *Chlamydomonas reinhardtii* biomass for ethanol production. Bioresource Technology 2010;101:5330–36.
24. Gardner RD, Lohman E, Gerlach R, Cooksey KE, Peyton BM. Comparison of CO_2 and bicarbonate as inorganic carbon sources for triacylglycerol and starch accumulation in *Chlamydomonas reinhardtii*. Biotechnology and Bioengineering 2013;110:87–96.
25. Metsoviti MN, Papapolymerou G, Karapanagiotidis IT, Katsoulas N. Comparison of growth rate and nutritional content of five microalgae species cultivated in greenhouses. Plants 2019;8:279.
26. Darwish R, Gedi MA, Akapach P, Assaye H, Zaky AS, Gray DA. *Chlamydomonas reinhardtii* is a potential food supplement with the capacity to outperform *Chlorella* and *Spirulina*. Applied Sciences 2020;10:6736.
27. Lamminen M, Halmemies-Beauchet-Filleau A, Kokkonen T, Jaakkola S, Vanhatalo A. Different microalgae species as a substitutive protein feed for soya bean meal in grass silage based dairy cow diets. Animal Feed Science and Technology 2019;247:112–26.
28. Wild KJ, Trautmann A, Katzenmeyer M, Steingaß H, Posten C, Rodehutscord M. Chemical composition and nutritional characteristics for ruminants of the microalgae *Chlorella vulgaris* obtained using different cultivation conditions. Algal Research 2019;38:101385.
29. Lamminen M, Halmemies-Beauchet-Filleau A, Kokkonen T, Simpura I, Vanhatalo A, Jaakkola S. Comparison of microalgae and rapeseed meal as a supplementary protein in the grass silage based nutrition of dairy cows. Animal Feed Science and Technology 2017;234:295–311.
30. Bernaerts TMM, Gheysen L, Kyomugasho C, Jasazzedeh Kermani Z, Vandionant S, Foubert I, et al. Comparison of microalgal biomasses as functional food ingredients: focus on the composition of cell wall related polysaccharides. Algal Research 2018;32:150–61.
31. Kholif A, Morsy T, Matloup O, Anele U, Mohamed A, El-Sayed A. Dietary *Chlorella vulgaris* microalgae improves feed utilization, milk production and concentrations of conjugated linoleic acids in the milk of Damascus goats. The Journal of Agricultural Science 2017;155:508–18
32. Adamakis ID, Lazaridis PA, Terzopoulou E, Torofias S, Valari M, Kalaitzi P, et al. Cultivation, characterization, and properties of *Chlorella vulgaris* microalgae with different lipid contents and effect on fast pyrolysis oil composition. Environmental Science and Pollution Research 2018;25:23018–32.
33. Safafar H, Nørregaard PU, Ljubic A, Møller P, Løvstad Holdt S, Jacobsen C. Enhancement of protein and pigment content in two *Chlorella* species cultivated on industrial process water. Journal of Marine Science and Engineering 2016;4:84.
34. Behrens PW, Bingham SE, Hoeksema SC, Cohoon DL, Cox JC. Studies on the incorporation of CO_2 into starch by *Chlorella vulgaris*. Journal of Applied Phycology 1989;1:123–30.
35. Tibbetts SM, Whitney CG, MacPherson MJ, Bhatti S, Banskota AH, Stefanova R, et al. Biochemical characterization of microalgal biomass from freshwater species isolated in Alberta, Canada for animal feed applications. Algal Research 2015;11:435–47.
36. Tibbetts SM, Mann J, Dumas A. Apparent digestibility of nutrients, energy, essential amino acids and fatty acids of juvenile Atlantic salmon (*Salmo salar* L.) diets containing whole-cell or cell-ruptured *Chlorella vulgaris* meals at five inclusion levels. Aquaculture 2017;481:25–39.

37. Costa DFA, Quigley SP, Isherwood P, McLennan SR, Poppi DP. Supplementation of cattle fed tropical grasses with microalgae increases microbial protein production and average daily gain. Journal of Animal Science 2016;94:2047–58.

38. Yang L, Tan X, Li D, Chu H, Zhou X, Zhang Y, et al. Nutrients removal and lipids production by *Chlorella pyrenoidosa* cultivation using anaerobic digested starch wastewater and alcohol wastewater. Bioresource Technology 2015;181:54–61.

39. Waghmare AG, Salve MK, LeBlanc JG, Arya SS. Concentration and characterization of microalgae proteins from *Chlorella pyrenoidosa*. Bioresources and Bioprocessing 2016;3:16.

40. Tsiplakou E, Abdullah MAM, Alexandros M, Chatzikonstantinou M, Skliros D, Sotirakoglou K, et al. The effect of dietary *Chlorella pyrenoidosa* inclusion on goats milk chemical composition, fatty acids profile and enzymes activities related to oxidation. Livestock Science 2017;197:106–11.

41. Muhaemin M, Kaswadji RF. Biomass nutrient profiles of marine microalgae *Dunaliella salina*. Jurnal Penelitian Sains 2010;13:13313.

42. Ricardo VY, Giffard-Mena I, Cruz-López R, García-Mendoza E, Stephano-Hornedo JL. Characterization of a new *Dunaliella salina* strain isolated from San Quintin, Baja California (México) producer of lipids, pigments and micronutrients. CICIMAR Oceánides 2018;33:1–10.

43. Renaud SM, Parry DL, Thinh LV. Microalgae for use in tropical aquaculture I: Gross chemical and fatty acid composition of twelve species of microalgae from the Northern Territory, Australia. Journal of Applied Phycology 1994;6:337–45.

44. Del Río E, Acién FG, García-Malea MC, Rivas J, Molina-Grima E, Guerrero MG. Efficient one-step production of astaxanthin by the microalga *Haematococcus pluvialis* in continuous culture. Biotechnology and Bioengineering 2005;91:808–15.

45. Grewe CB, Griehl C. The carotenoid astaxanthin from *Haematococcus pluvialis*. In: Posten C, Walter C, editors. Microalgal biotechnology: integration and economy. Berlin, Germany: De Gruyter: 2012. p. 129–43.

46. Ba F, Ursu AV, Laroche C, Djelveh G. *Haematococcus pluvialis* soluble proteins: extraction, characterization, concentration/fractionation and emulsifying properties. Bioresource Technology 2016;200:147–52.

47. Zhu Y, Zhao X, Zhang X, Liu H. Extraction, structural and functional properties of *Haematococcus pluvialis* protein after pigment removal. International Journal of Biological Macromolecules 2019;140:1073–83.

48. Samek D, Mišurcová L, Machů L, Buňka F, Fišera M. Influencing of amino acid composition of green freshwater algae and cyanobacterium by methods of cultivation. Turkish Journal of Biochemistry 2013;38:360–68.

49. Soeder CJ. Massive cultivation of microalgae: Results and prospects. Hydrobiologia 1980;72:197–209.

50. Teuling E, Wierenga PA, Schrama JW, Gruppen H. Comparison of protein extracts from various unicellular green sources. Journal of Agricultural and Food Chemistry 2017;65:7989–8002.

51. Kang J, Wen Z. Use of microalgae for mitigating ammonia and CO_2 emissions from animal production operations—evaluation of gas removal efficiency and algal biomass composition. Algal Research 2015;11:204–10.

52. Tulli F, Chini Zitteli G, Giorgi G, Poli BM, Tibaldi E, Tredici MR. Effect of the inclusion of dried *Tetraselmis suecica* on growth, feed utilization, and fillet composition of European sea bass juveniles fed organic diets. Journal of Aquatic Food Product Technology 2012;21:188–97.

53. Abiusi F, Sampietro G, Marturano G, Biondi N, Rodolfi L, D'Ottavio M, et al. Growth, photosynthetic efficiency, and biochemical composition of *Tetraselmis suecica* F&M-M33 grown with LEDs of different colors. Biotechnology and Bioengineering 2014;111:956–64.

54. Giostri A, Binotti M, Macchi E. Microalgae cofiring in coal power plants: Innovative system layout and energy analysis. Renewable Energy 2016;95:449–64.

55. Gardinaletti G, Messina M, Bruno M, Tulli F, Poli BM, Giorgi G, et al. Effects of graded levels of a blend of *Tisochrysis lutea* and *Tetraselmis suecica* dried biomass on growth and muscle tissue composition of European sea bass (*Dicentrarchus labrax*) fed diets low n fish meal and oil. Aquaculture 2018;485:173–82.

56. Vizcaíno AJ, Sáez MI, Martínez TF, Acién FG, Alarcón FJ. Differential hydrolysis of proteins of four microalgae by the digestive enzymes of gilthead sea bream and Senegalese sole. Algal Research 2019;37:145–53.

57. Wild KJ, Steingaß H, Rodehutscord M. Variability in nutrient composition and *in vitro* crude protein digestibility of 16 microalgae products. Journal of Animal Physiology and Animal Nutrition 2018;102:1306–19.

58. Lamminen M. Halmemies-Beauchet-Filleau A, Kokkonen T, Vanhatalo A, Jaakkola S. The effect of partial substitution of rapeseed meal and faba beans by *Spirulina platensis* microalgae on milk production, nitrogen utilization and amino acid metabolism of lactating dairy cows. Journal of Dairy Science 2019;102:7102–117.

59. Cuellar-Bermudez SP, Barba-Davila B, Serna-Saldivar SO, Parra-Saldivar R, Rodriguez-Rodriguez J, Morales-Davila S. et al. Deodorization of *Arthrospira platensis* biomass for further scale-up food applications. Journal of the Science of Food and Agriculture 2017;97:5123–30.

60. Cheng J, Xia A, Song W, Su H, Zhou J, Cen K. Comparison between heterofermentation and autofermentaton in hydrogen production from *Arthrospira* (*Spirulina*) *platensis* wet biomass. International Journal of Hydrogen Energy 2012;37:6536–44.

61. Panjaitan T, Quigley SP, McLennan SR, Swain AJ, Poppi DP. Spirulina (*Spirulina platensis*) algae supplementation increases microbial protein production and feed intake and decreases retention time of digesta in the rumen of cattle. Animal Production Science 2015;55:535–43.

62. Markou G, Chatzipavlidis I, Georgakakis D. Carbohydrates production and bio-flocculation characteristics in cultures of *Arthrospira* (*Spirulina*) *platensis*: Improvements through phosphorus limitation process. Bioenergy Research 2012;5:915–25.

63. de Oliveira MACL, Monteiro MPC, Robbs PG, Leite SGF. Growth and chemical composition of *Spirulina maxima* and *Spirulina platensis* biomass at different temperatures. Aquaculture International 1999;7:261–75.

64. Dalle Zotte A, Sartori A, Bohatir P, Rémignon H, Ricci R. Effect of dietary supplementation of spirulina (*Arthrospira*

platensis) and thyme (*Thymus vulgaris*) on growth performance, apparent digestibility and health status of companion dwarf rabbits. Livestock Science 2013;152:182–91.

65. Gamboa-Delgado J, Morales-Navarro YI, Nieto-López MG, Villarreal-Cavazos DA, Cruz-Suárez LE. Assimilation of dietary nitrogen supplied by fish meal and microalgal biomass from spirulina (*Arthrospira platensis*) and *Nannochloropsis oculata* in shrimp *Litopenaeus vannamei* fed compound diets. Journal of Applied Phycology 2019;31:2379–89.
66. Alvarenga RR, Rodrigues PB, de Souza Cantarelli V, Zangeronimo MG, da Silva JW, da Silva LR, et al. Energy values and chemical composition of spirulina (*Spirulina platensis*) evaluated with broilers. Revista Brasileira de Zootecnia 2011;40:992–6.
67. Gerencsér ZS, Szendrö ZS, Matics ZS, Radnai I, Kovács M, Nagy I, et al. Effect of dietary supplementation of spirulina (*Arthrospira platensis*) and thyme (*Thymus vulgaris*) on apparent digestibility and productive performance of growing rabbits. World Rabbit Science 2014;22:1–9.
68. Aikawa S, Joseph A, Yamada R, Izumi Y, Yamagishi T, Matsuda F, et al. Direct conversion of spirulina to ethanol without pretreatment or enzymatic hydrolysis processes. Energy and Environmental Science 2013;6:1844.
69. De Philippis R, Sili C, Vincenzini M. Glycogen and poly-β-hydroxybuturate synthesis in *Spirulina maxima*. Journal of General Microbiology 1992;138:1623–28.
70. García-Lopez DA, Olguín EJ, González-Portela RE, Sánchez-Galván G, De Philippis R, Lovitt RW, et al. A novel two-phase bioprocess for the production of *Arthrospira* (*Spirulina*) *maxima* LJGR1 at pilot plant scale during different seasons and for phycocyanin induction under controlled conditions. Bioresource Technology 2020;298:122548.
71. Olvera-Novoa MA, Domínguez-Cen LJ, Olivera-Castillo L, Martínez-Palacios CA. Effect of the use of the microalga *Spirulina maxima* as fish meal replacement in diets for tilapia, *Oreochromis mossambicus* (Peters), fry. Aquaculture Research 1998;29:709–15.
72. Aemiro A, Watanabe S, Suzuki K, Hanada M, Umetsu K, Nishida T. Effects of euglena (*Euglena gracilis*) supplemented to diet (forage:concentrate ratios 60:40) on the basic ruminal fermentation and methane emissions in *in vitro* condition. Animal Feed Science and Technology 2016;212:129–35.
73. Aemiro A, Kiiru P, Watanabe S, Suzuki K, Hanada M, Umetsu K, et al. The effect of euglena (*Euglena gracilis*) supplementation on nutrient intake, digestibility, nitrogen balance and rumen fermentation in sheep. Animal Feed Science and Technology 2017;225:123–33.
74. Tossavainen M, Ilyass U, Ollilainen V, Valkonen K, Ojala A, Romantschuk M. Influence of long term nitrogen limitation on lipid, protein and pigment production of *Euglena gracilis* in photoheterotrophc cultures. PeerJ 2019;7:e6624.
75. Lewis A, Guéguen C. How growth conditions of *Euglena gracilis* cells influence cellular composition as evidenced by Fourier transform infrared spectroscopy and direct infusion high-resolution mass spectrometry. Journal of Applied Phycology 2020;32:153–63.
76. Brown MR. The amino-acid and sugar composition of 16 species of microalgae used in mariculture. Journal of Experimental Marina Biology and Ecology 1991;145:79–99.
77. Bonfanti C, Cardoso C, Afonso C, Matos J, Garcia T, Tanni S, et al. Potential of microalga *Isochrysis galbana*: Bioactivity and bioaccessibility. Algal Research 2018;29:242–48.
78. Fernandes T, Fernandes I, Andrade CAP, Cordeiro N. Marine microalgae growth and carbon partitioning as a function of nutrient availability. Bioresource Technology 2016;214:541–47.
79. Sudasinghe N, Reddy H, Csakan N, Deng S, Lammers P, Schaub T. Temperature-dependent lipid conversion and nonlipid composition of microalgal hydrothermal liquefaction oils monitored by Fourier transform ion cyclotron resonance mass spectrometry. Bioenergy Research 2015;8:1962–72.
80. Molino A, Martino M, Larocca V, Di Sanzo G, Spagnoletta A, Marino T, et al. Eicosapentaenoic acid extraction from *Nannochloropsis gaditana* using carbon dioxide at supercritical conditions. Marine Drugs 2019;17:132.
81. Teuling E, Wierenga PA, Agboola JO, Gruppen H, Schrama JW. Cell wall disruption increases bioavailability of *Nannochloropsis gaditana* nutrients for juvenile Nile tilapia (*Oreochromis niloticus*). Aquaculture 2019;499:269–82.
82. Leow S, Witter JR, Vardon DR, Sharma BK, Guest JS, Strathmann TJ. Prediction of microalgae hydrothermal liquefaction products from feedstock biochemical composition. Green Chemistry 2015;17:3584.
83. Batista S, Pereira R, Oliveira B, Baião LF, Jessen F, Tulli F, et al. Exploring the potential of seaweed *Gracilaria gracilis* and microalga *Nannochloropsis oceanica*, single or blended, as natural dietary ingredients for European seabass *Dicentrarchus labrax*. Journal of Applied Phycology 2020;32:2041–59.
84. Safi C, Charton M, Pignolet O, Silvestre F, Vaca-Garcia C, Pontalier PY. Influence of microalgae cell wall characteristics on protein extractability and determination of nitrogen-to-protein conversion factors. Journal of Applied Phycology 2013;25:523–29.
85. Rebolloso Fuentes MM, Acién Fernández GG, Sánchez Pérez JA, Guil Guerrero JL. Biomass nutrient profiles of the microalga *Porphyridium cruentum*. Food Chemistry 2000;70:345–52.
86. Kavitha MD, Seema Shree MH, Vidyashankar S, Sarada R. Acute and subchronic safety assessment of *Porphyridium purpureum* biomass in the rat model. Journal of Applied Phycology 2016;28:1071–83.
87. Guiry MD, Guiry GM. AlgaeBase. World-wide electronic publication. Galway: National University of Ireland; 2021. Available from: https://www.algaebase.org [Accessed 15 April, 2021].
88. WoRMS Editorial Board. World Register of Marine Species. 2021. Available from: http://www.marinespecies.org [Accessed 1 April 2021].
89. Beaudelet PH, Ricochon G, Linder M, Muniglia L. A new insight into cell walls of Chlorophyta. Algal Research 2017;25:333–71.
90. Guiry MD. How many species of algae are there? Journal of Phycology 2012;48:1057–63.
91. Hildebrand M, Abbriano RM, Polle JEW, Traller JC, Trentacoste EM, Smith SR, et al. Metabolic and cellular organization in evolutionarily diverse microalgae as related to biofuels production. Current Opinion in Chemical Biology 2013;17:506–14.

92. Singh NK, Dhar DW. Microalgae as second generation biofuel. A review. Agronomy for Sustainable Development 2011;31:605–29.

93. Gu N, Lin Q, Li G, Tan Y, Huang L, Lin J. Effect of salinity on growth, biochemical composition, and lipid productivity of *Nannochloropsis oculata* CS 179. Engineering in Life Sciences 2012;12:631–7.

94. Procházková G, Brányiková I, Zachleder V, Brányik T. Effect of nutrient supply status on biomass composition of eukaryotic green microalgae. Journal of Applied Phycology 2014;26:1359–77.

95. Singh SP, Singh P. Effect of temperature and light on the growth of algae species: A review. Renewable and Sustainable Energy Reviews 2015;50:431–44.

96. Andeden EE, Ozturk S, Aslim B. Effect of alkaline pH and nitrogen starvation on the triacylglycerol (TAG) content, growth, biochemical composition, and fatty acid profile of *Auxenochlorella protothecoides* KP7. Journal of Applied Phycology 2021;33:211–25.

97. Lamminen M. Potential of microalgae to replace conventional protein feeds for sustainable dairy cow nutrition [doctoral thesis]. Dissertationes Schola Doctoralis Scientiae Circumiectalis, Alimentariae, Biologicae, Publication No. 5/2019. Finland: Doctoral Programme in Sustainable Use of Renewable Natural Resources, Doctoral School in Environmental, Food and Biological Sciences, University of Helsinki; 2019. Available from: http://urn.fi/URN:ISBN:978-951-51-5169-8

98. Tzachor A. The future of feed: integrating technologies to decouple feed production from environmental impacts. Industrial Biotechnology 2019;15:52–62.

99. European Commission. Farming markets. 2021. Available from: https://ec.europa.eu/info/food-farming-fisheries/farming/facts-and-figures/markets_en [Accessed: 24 March 2021]

100. Häusling M. The EU protein deficit: what solutions for a long-standing problem? (2010/2011 (INI)). Committee on Agriculture and Rural Development. European Parliament: 2011.

101. de Visser CLM, Schreuder R, Stoddard F. The EU's dependency on soya bean import for the animal feed industry and potential for EU produced alternatives. Oilseeds and Fats, Crops and Lipids 2014;21:D407.

102. Till BE, Huntington JA, Posri W, Early R, Taylor-Pickard J, Sinclair LA. Influence of rate of inclusion of microalgae on the sensory characteristics and fatty acid composition of cheese and performance of dairy cows. Journal of Dairy Science 2019;102:10934–46.

103. Vanhatalo A, Halmemies-Beauchet-Filleau A. Optimising ruminal function: the role of silage and concentrate in dairy cow nutrition to improve feed efficiency and reduce methane and nitrogen emissions. In: McSweeney CS, Mackie RI, editors. Improving rumen function. Cambridge, United Kingdom: Burleigh Dodds Science Publishing; 2020. p. 651–92.

104. Soto-Sierra L, Stoykova P, Nikolov ZL. Extraction and fractionation of microalgae-based protein products. Algal Research 2018;36:175–92.

105. EUMOFA. Monthly Highlights No. 4/2019. European Market Observatory for Fisheries and Aquaculture Products, European Commission: 2019

106. Acién Fernández FG, Fernández Sevilla JM, Molina Grima E. Cost analysis of microalgae production. In: Pandey A, Chang JS, Soccol CR, Lee DJ, Chisti J, editors. Biomass, biofuels and biochemicals: biofuels from microalgae. Amsterdam, the Netherlands: Elsevier; 2019. p. 551–66.

107. Gerber LN, Tester JW, Beal CM, Huntley ME, Sills DL. Target cultivation and financing parameters for sustainable production of fuel and feed from microalgae. Environmental Science and Technology 2016;50:3333–41.

108. Panjaitan T, Quigley SP, McLennan SR, Poppi DP. 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 2010;50:405–9.

109. Slade R, Bauen A. Micro-algae cultivation for biofuels: cost, energy balance, environmental impacts and future prospects. Biomass and Bioenergy 2013;53:29–38.

110. Lourenço SO, Barbarino E, Lavín PL, Lanfer Marquez UM, Aidar E. Distribution of intracellular nitrogen in marine microalgae: calculation of new nitrogen-to-protein conversion factors. European Journal of Phycology 2004;39:17–32.

111. Ahvenjärvi S, Vanhatalo A, Huhtanen P, Varvikko T. Effects of supplementation of grass silage and barley diet with urea, rapeseed meal and heat-moisture-treated rapeseed cake on omasal digesta flow and milk production in lactating dairy cows. Acta Agriculturae Scandinavica, Section A—Animal Science 1999;49:179–89.

112. Choi CW, Vanhatalo A, Ahvenjärvi S, Huhtanen P. Effects of several protein supplements on flow of soluble non-ammonia nitrogen from the forestomach and milk production in dairy cows. Animal Feed Science and Technology 2002;102:15–33.

113. Choi CW, Vanhatalo A, Huhtanen P. Effects of type of grass silage and level of concentrate on the flow of soluble non-ammonia nitrogen entering the omasum of dairy cows. Journal of Animal and Feed Sciences 2003;12:3–22.

114. Fortina R, Malfatto V, Mimosi A, Guo K, Tartari E. The establishment of a database of Italian feeds for the Cornell Net Carbohydrate and Protein System. Italian Journal of Animal Science 2003;2:171–9.

115. Chrenková M, Čerešňáková Z, Weisbjerg MR, Formelová Z, Poláčiková M, Vondráková M. Characterization of proteins in feeds according to the CNCPS and comparison to in situ parameters. Czech Journal of Animal Science 2014;59:288–95.

116. López CVG, del Carmen Cerón García, M, Fernández, FGA, Bustos, CS, Chisti, Y, Sevilla, JMF. Protein measurements of microalgal and cyanobacterial biomass. Bioresource Technology 2010;101:7587–91.

117. Templeton DW, Laurens LML. Nitrogen-to-protein conversion factors revisited for applications of microbial biomass conversion to food, feed and fuel. Algal Research 2015;11:359–67.

118. Russell JB, O'Connor JD, Fox DG, Van Soest PJ, Sniffen CJ. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. Journal of Dairy Science 1992;70:3551–61.

119. Brito AF, Broderick GA. Effect of different protein supplements on milk production and nutrient utilization in lactating dairy cows. Journal of Dairy Science 2007;90:1816–27.

120. Markou G, Vandamme D, Muylaert K. Microalgal and cyanobacterial cultivation: The supply of nutrients. Water Research 2014;65:186–202.

121. Li Y, Fei X, Deng X. Novel molecular insights into nitrogen starvation-induced triacylglycerols accumulation revealed by differential gene expression analysis in green algae *Micractinium pusillum*. Biomass and Bioenergy 2012;42:199–211.

122. Pancha I, Chokshi K, George B, Ghosh T, Paliwal C, Maurya R, et al. Nitrogen stress triggered biochemical and morphological changes in the microalgae *Scenedesmus* sp. CCNM 1077. Bioresource Technology 2014;156:146–54.

123. Fernandes T, Fernandes I, Andrade CAP, Cordeiro N. Changes in fatty acid biosynthesis in marine microalgae as a response to medium nutrient availability. Algal Research 2016;18:314–20.

124. Zehr JP. Nitrogen fixation by marine cyanobacteria. Trends in Microbiology 2011;19:162–73.

125. Peccia J, Haznedaroglu B, Gutierrez J, Zimmerman JB. Nitrogen supply is an important driver of sustainable microalgae biofuel production. Trends in Biotechnology 2013;31:134–8.

126. Carfagna S, Salbitani G, Vona V, Esposito S. Changes in cysteine and O-acetyl-L-serine levels in the microalga *Chlorella sorokiniana* in response to the S-nutritional status. Journal of Plant Physiology 2011;168:2188–95.

127. Basri EM, Maznah WOW. Differential growth and biochemical composition of photoautotrophic and heterotrophic *Isochrysis maritima*: evaluation for use as aquaculture feed. Journal of Applied Phycology 2017;29:1159–70.

128. Fernández-Reiriz MJ, Perez-Camacho A, Ferreiro MJ, Blanco J, Planas M, Campos MJ, et al. Biomass production and variation in the biochemical profile (total protein, carbohydrates, RNA, lipids and fatty acids) of seven species of marine microalgae. Aquaculture 1989;83:17–37.

129. Schwab CG, Satter LD, Clay AB. Response of lactating cows to abomasal infusion of amino acids. Journal of Dairy Science 1976;59:1254–70.

130. Vanhatalo A, Huhtanen P, Toivonen V, Varvikko T. Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. Journal of Dairy Science 1999;82:2674–85.

131. Socha MT, Putnam DE, Garthwaite BD, Whitehouse NL, Kierstead NA, Schwab, et al. Improving intestinal amino acid supply of pre- and postpartum dairy cows with rumen-protected methionine and lysine. Journal of Dairy Science 2005;88:1113—26.

132. Giallongo F, Harper MT, Oh J, Parys C, Shinzato I, Hristov AN. Histidine deficiency has a negative effect on lactational performance on dairy cows. Journal of Dairy Science 2017;100:2784–800.

133. Cao Y, Yao J, Sun X, Liu S, Martin GB. Amino acids in the nutrition and production of sheep and goats. In: Wu G, editor. Amino acids in nutrition and health. Advances in experimental medicine and biology. Vol 1285. Cham: Springer; 2021. p. 63–79.

134. Patton RA, Hristov AN, Lapierre H. Protein feeding and balancing for amino acids in lactating dairy cattle. Veterinary Clinics of North America—Food Animal Practice 2014;30:599–621.

135. Heuzé V, Tran G, Kaushik S. Soybean meal. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO: 2020. Available from: https://www.feedipedia.org/node/674 [Accessed: 15 December 2020].

136. Heuzé V, Tran G, Sauvant D, Lessire M, Lebas F. Rapeseed meal. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO: 2020. Available from: https://www.feedipedia.org/node/52 [Accessed: 15 December 2020].

137. Heuzé V, Tran G, Kaushik S. Fish meal. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO: 2015. Available from: https://www.feedipedia.org/node/208 [Accessed: 15 December 2020].

138. Heuzé V, Tran G, Renaudeau D, Lessire M, Lebas F. Wheat grain. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO: 2015. Available from: https://www.feedipedia.org/node/223 [Accessed: 15 December 2020].

139. Pyle DJ, Garcia RA, Wen Z. Producing docosahexaenoic acid (DHA)-rich algae from biodiesel-derived crude glycerol: effects of impurities on DHA production and algal biomass composition. Journal of Agricultural and Food Chemistry 2008;56:3933–9.

140. Song X, Zang X, Zhang X. Production of high docosahexaenoic acid by *Schizochytrium* sp. using low-cost raw materials from food industry. Journal of Oleo Science 2015;64:197–204.

141. Fowden L. The composition of the bulk proteins of *Chlorella*. Biochemical Journal 1952;50:355–8.

142. Schieler L, McClure L, Dunn M. The amino acid composition of *Chlorella*. Journal of Food Science 1953;18:377–80.

143. Combs GF. Algae (*Chlorella*) as a source of nutrients for the chick. Science 1952;116:453–4.

144. Mišurçova L, Buňka F, Ambrožová JV, Machů L, Samek D, Kráčmar S. Amino acid composition of algal products and its contribution to RDI. Food Chemistry 2014;151:120–5.

145. Sui Y, Muys M, Van de Waal D, D'Adamo S, Vermeir P, Fernandes TV, et al. Enhancement of co-production of nutritional protein and carotenoids in *Dunaliella salina* using a two-phase cultivation assisted by nitrogen level and light intensity. Bioresource Technology 2019;287:121398.

146. Mokady S, Cogan U. Nutritional evaluation of a protein concentrate and of carotenes derived from *Dunaliella bardawil*. Journal of the Science of Food and Agriculture 1988;42:249–54.

147. Burr GS, Barrows FT, Gaylord G, Wolters WR. Apparent digestibility of macro-nutrients and phosphorus in plant-derived ingredients for Atlantic salmon, *Salmo salar* and Arctic charr, *Salvelinus alpinus*. Aquaculture Nutrition 2011;17:570–7.

148. Clément G, Giddey C, Menzi R. Amino acid composition and nutritive value of the alga *Spirulina maxima*. Journal of the Science of Food and Agriculture 1967;18:497–501.

149. Kolmakova AA, Kolmakov VI. Amino acid composition of green microalgae and diatoms, cyanobacteria, and zooplankton (review). Inland Water Biology 2019;12:452–61.

150. Rinehart KL, Namikoshi M, Choi BW. Structure and biosynthesis of toxins from blue-green algae (cyanobacteria). Journal of Applied Phycology 1994;6:159–76.

151. Park JJ, Wang H, Gargouri M, Deshpande RR, Skepper JN, Holguin FO, et al. The response of *Chlamydomonas*

reinhardtii to nitrogen deprivation: a systems biology analysis. The Plant Journal 2015;81:661–24.

152. Himanshu S, Manish RS, Basuthkar JR, Kandala VRC. Regulation of starch, lipids and amino acids upon nitrogen sensing in *Chlamydomonas reinhardtii*. Algal Research 2016;18:33–44.

153. Daume S, Long BM, Crouch P. Changes in amino acid content of an algal feed species (*Navicula* sp.) and their effect on growth and survival of juvenile abalone (*Haliotis rubra*). Journal of Applied Phycology 2003;15:201–7.

154. Giordano M, Norici A, Hell R. Sulfur and phytoplankton: acquisition, metabolism and impact on the environment. New Phytologist 2005;166:371–82.

155. Kanazawa T. Changes of amino acid composition of *Chlorella* cells during their life cycle. Plant and Cell Physiology 1964;5:333–54.

156. Brown MR, Garland CD, Jeffrey SW, Jameson ID, Leroi JM. The gross and amino acid compositions of batch and semi-continuous cultures of *Isochrysis* sp. (clone T.ISO), *Pavlova lutheri* and *Nannochloropsis oculata*. Journal of Applied Phycology 1993;5:285–96.

157. Campanella L, Russo MV, Avino P. Free and total amino acid composition in blue-green algae. Annali di Chimica 2002;92:343–52.

158. Chia MA, Lombardi AT, da Graça Gama Melão M, Parrish CC. Combined nitrogen limitation and cadmium stress stimulate total carbohydrates, lipids, protein and amino acid accumulation in *Chlorella vulgaris* (Trebouxiophyceae). Aquatic Toxicology 2015;160:87–95.

159. Raji AA, Jimoh WA, Abu Bakar NH, Mohd Taufek NH, Muin H, Alias Z, et al. Dietary use of *Spirulina* (*Arthrospira*) and *Chlorella* instead of fish meal on growth and digestibility of nutrients, amino acids and fatty acids by African catfish. Journal of Applied Phycology 2020;32:1763–70.

160. Tomita Y, Takeya M, Suzuki K, Nitta N, Higuchi C, Marukawa-Hashimoto Y, et al. Amino acid excretion from *Euglena gracilis* cells in dark and anaerobic conditions. Algal Research 2019;37:169–77.

161. Liu L, Phnert G, Wei D. Extracellular metabolites from industrial microalgae and their biotechnological potential. Marine Drugs 2016;14:191.

162. Manzocchi E, Guggenbühl B, Kreuzer M, Giller K. Effects of the substitution of soybean meal by spirulina in a hay-based diet for dairy cows on milk composition and sensory perception. Journal of Dairy Science 2020;103:11349–62.

163. Heuzé V, Tran G, Delagarde R, Lessire M, Lebas F. Faba bean (*Vicia faba*). Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO: 2021. Available from: https://www.feedipedia.org/node/4926 [Accessed: 3 July 2021].

164. Bailoni L, Bortolozzo A, Mantovani R, Simonetto A, Schiavon S, Bitante G. Feeding dairy cows with full fat extruded or toasted soybean seeds as replacement of soybean meal and effects on milk yield, fatty acid profile and CLA content. Italian Journal of Animal Science 2004;3:243–58.

165. Thacker P, Widyaratne G. Effects of expeller pressed camelina meal and/or canola meal on digestibility, performance and fatty acid composition of broiler chickens fed wheat-soybean meal-based diets. Archives of Animal Nutrition 2012;66:402–15.

166. Lee JW, Kil DY, Keever BD, Killefer J, McKeith FK, Sulabo RC, et al. Carcass fat quality of pigs is not improved by adding corn germ, beef tallow, palm kernel oil, or glycerol to finishing diets containing distillers dried grains with solubles. Journal of Animal Science 2013;91:2426–37.

167. Hristov AN, Domitrovich C, Wachter A, Cassidy T, Lee C, Shingfield KJ, et al. Effect of replacing solvent-extracted canola meal eith high-oil traditional canola, high-oleid acid canola, or high-erucic acid rapeseed meals on rumen fermentation, digestibility, milk production, and milk fatty acid composition in lactating dairy cows. Journal of Dairy Science 2011;94:4057–74.

168. Chibisa GE, Christensen DA, Mutsvangwa T. Replacing canola meal as the major protein source with wheat distillers' grains alters omasal fatty acid flow and milk fatty acid composition in dairy cows. Canadian Journal of Animal Science 2013;93:137–47.

169. He ML, Gibb D, McKinnon JJ, McAllister TA. Effect of high dietary levels of canola meal on growth performance, carcass quality and meat fatty acid profiles of feedlot cattle. Canadian Journal of Animal Science 2013;93:269–80.

170. Stuper-Szablewska K, Buśko M, Góral T, Perkowski J. The fatty acid profile in different wheat cultivars depending on the level of contamination with microscopic fungi. Food Chemistry 2014;153:216–23.

171. AbuGhazaleh AA, Schingoethe DJ, Hippen AR, Kalscheur KF, Whitlock LA. Fatty acid profiles of milk and rumen digesta from cows fed fish oil, extruded soybeans or their blend. Journal of Dairy Science 2002;85:2266–76.

172. Bharathan M, Schingoethe AA, Hippen AR, Kalscheur KF, Gibson ML, Karges K. Conjugated linoleic acid increases in milk from cows fed condensed corn distillers solubles and fish oil. Journal of Dairy Science 2008;91:2796–807.

173. Shingfield KJ, Lee MRF, Humphries DJ, Scollan ND, Toivonen V, Beever DE et al. Effect of linseed oil and fish oil alone or as an equal mixture on ruminal fatty acid metabolism in growing steers fed maize silage-based diets. Journal of Animal Science 2011;89:3728–41.

174. Halmemies-Beauchet-Filleau A, Kairenius P, Ahvenjärvi S, Crosley LK, Muetzel S, Huhtanen P, et al. Effect of forage conservation method on ruminal lipid metabolism and microbial ecology in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. Journal of Dairy Science 2013;96:2428–47.

175. Mattos R, Staples CR, Arteche A, Wiltbank MC, Diaz FJ, Jenkins TC, et al. The effects of feeding fish oil on uterine secretion of $PGF_{2\alpha}$, milk composition, and metabolic status of periparturient Holstein cows. Journal of Dairy Science 2004;87:921–32.

176. Hosomi R, Fukunaga K, Arai H, Kanda S, Nishiyama T, Yoshida M. Effect of combination of dietary fish protein and fish oil on lipid metabolism in rats. Journal of Food Science and Technlogy 2013;50:266–74.

177. Lang I, Hodac L, Friedl T, Feussner I. Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biology 2011;11:124.

178. Alves SP, Mendonça SH, Silva JL, Bessa RJB. *Nannochloropsis oceanica*, a novel natural source of rumen-protected eicosapentaenoic acid (EPA) for ruminants. Scientific Reports 2018;8:10269.

179. Griffiths MJ, van Hille RP, Harrison STL. Lipid productivity, settling potential and fatty acid profile of 11 microalgal

species grown under nitrogen replete and limited conditions. Journal of Applied Phycology 2012;24:989–1001.

180. Zhukova NV, Aizdaicher NA. Fatty acid composition of 15 species of marine microalgae. Phytochemistry 1995;39:351–6.

181. Taoka Y, Nagano N, Okita Y, Izumida H, Sugimoto S, Hayashi M. Effects of cold shok treatment on total lipid content and fatty acid composition of *Aurantiochytrium limacinum* strain mh0186. Journal of Oleo Science 2011;60:217–20.

182. Dillon GP, Keegan JD, Moran CA. Toxicological evaluation of an untreated *Aurantiochytrium limacinum* biomass, a novel docosahexaenoic acid rich feed ingredient. Food and Chemical Toxicology 2020;141:111397.

183. James GO, Hocart CH, Hillier W, Chen H, Kordbacheh F, Price GD, et al. Fatty acid profiling of *Chlamydomonas reinhardtii* under nitrogen deprivation. Bioresource Technology 2011;102:3343–51.

184. James GO, Hocart CH, Hillier W, Price GD, Djordjevic MA. Temperature modulation of fatty acid profiles for biofuel production in nitrogen deprived *Chlamydomonas reinhardtii*. Bioresource Technology 2013;127:441–7.

185. Weers PMM, Gulati RD. Growth and reproduction of *Daphnia galeata* in response to changes in fatty acids, phosphorus, and nitrogen in *Chlamydomonas reinhardtii*. Limnology and Oceanography 1997;42:1584–9.

186. Ochoa-Alfaro AE, Gaytán-Luna DE, González-Ortega O, Zavala-Arias KG, Paz-Maldonado LMT, Rocha-Uribe A, et al. pH effects on the lipid and fatty acids accumulation in *Chlamydomonas reinhardtii*. Biotechnology Progress 2019;35:e2891.

187. Khoeyi ZA, Seyfabadi J, Ramezanpour Z. Effect of light intensity and photoperiod on biomass and fatty acid composition of the microalgae, *Chlorella vulgaris*. Aquaculture International 2012;20:41–9.

188. Ötleş S, Pire R. Fatty acid composition of *Chlorella* and *Spirulina* microalgae species. Journal of AOAC International 2001;84:1708–14

189. Mourente G, Lubián LM, Odriozola JM. Total fatty acid composition as a taxonomic index of some marine microalgae used as food in marine aquaculture. Hydrobiologia 1990;203:147–54.

190. El-Baky HH, El Baz FK, El-Baroty GS. Production of lipids rich in omega 3 fatty acids from the halotolerant alga *Dunaliella salina*. Biotechnology 2004;3:102–8.

191. Bilbao PGS, Damiani C, Salvador GA, Leonardi P. *Haematococcus pluvialis* as a source of fatty acids and phytosterols: Potential nutritional and biological implications. Journal of Applied Phycology 2016;28:3283–94.

192. Lei A, Chen H, Shen G, Hu Z, Chen L, Wang J. Expression of fatty acid synthesis genes and fatty acid accumulation in *Haematococcus pluvialis* under different stressors. Biotechnology for Biofuels 2012;5:18.

193. Koutra E, Kopsahelis A, Maltezou M, Grammatikopoulos G, Kornaros M. Effect of organic carbon and nutrient supplementation on the digestate-grown microalga, *Parachlorella kessleri*. Bioresource Technology 2019;294:122232.

194. Li X, Přibyl P, Bišova K, Kawano S, Cepák V, Zachleder V, et al. The microalga *Parachlorella kessleri*—a novel highly efficient lipid producer. Biotechnology and Bioengineering 2013;110:97–107.

195. Ahlgren G. Fatty acid content and chemical composition of freshwater microalgae. Journal of Phycology 1992;28:37–50.

196. El-Sheekh M, Abomohra AEF, El-Azim MA, Abou-Shanab R. Effect of temperature on growth and fatty acids profile of the biodiesel producing microalga *Scenedesmus acutus*. Biotechnology, Agronomy, Society and Environment 2017;21:233–9.

197. Demott WR, Müller-Navarra DC. The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia*, a cyanobacterium and lipid emulsions. Freshwater Biology 1997;38:649–64.

198. Ahlgren G, Hyenstrand P. Nitrogen limitation effects of different nitrogen sources on nutritional quality of two freshwater organisms, *Scenedesmus quadricauda* (Chlorophyceae) and *Synechococcus* sp. (Cyanophyceae). Journal of Phycology 2003;39:906–17.

199. Tadesse Z, Boberg M, Sonesten L, Ahlgren G. Effects of algal diets and temperature on the growth and fatty acid content of the cichlid fish *Oreochromis niloticus* L.—a laboratory study. Aquatic Ecology 2003;37:169–82.

200. Ruangsomboon S, Ganmanee M, Choochote S. Effects of different nitrogen, phosphorus, and iron concentrations and salinity on lipid production in newly isolated strain of the tropical green microalga, *Scenedesmus dimorphus* KMITL. Journal of Applied Phycology 2013;25:867–74.

201. Montaini E, Zittelli C, Tredici MR, Molina Grima E, Fernández Sevilla JM. Sánchez Pérez JA. Long-term preservation of *Tetraselmis suecica*: influence of storage on viability and fatty acid profile. Aquaculture 1995;134:81–90.

202. Jafari SMA, Rabbani M, Emtyazjoo M, Piryaei F. Effect of dietary *Spirulina platensis* on fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) fillet. 2014. Aquaculture International 2014;22:1307–15.

203. Colla LM, Bertolin TE, Costa JAV. Fatty acids profile of *Spirulina platensis* grown under different temperatures and nitrogen concentrations. Zeitschrift für Naturforschung C 2014;59:55–9.

204. Rochetta I, Mazzuca M, Conforti V, Ruiz L, Balzaretti V, de Molina MCR. Effect of chromium on the fatty acid composition of two strains of *Euglena gracilis*. Environmental Pollution 2006;141:353–8.

205. Schwarzhans JP, Cholewa D, Grimm P, Beshay U, Risse JM, Friehs K, et al. Dependency of the fatty acid composition of *Euglena gracilis* on growth phase and culture conditions. Journal of Applied Phycology 2015;27:1389–99.

206. Zeng M, Hao W, Zou Y, Shi M, Jiang Y, Xiao P, et al. Fatty acid and metabolomics profiling approaches differentiate heterotrophic and mixotrophic culture conditions in a microalgal food supplement 'Euglena'. BMC Biotechnology 2016;16:49.

207. Patil V, Källqvist T, Olsen E, Vogt G, Gislerød HR. Fatty acid composition of 12 microalgae for possible use in aquaculture. Aquaculture International 2007;15:1–9.

208. Tonon T, Harvey D, Larson TR, Graham IA. Identification of a very long chain polyunsaturated fatty acid Δ4-desaturase from the microalga *Pavlova lutheri*. FEBS Letters 2003;553:440–44.

209. Guihéneuf F, Fourqueray M, Mimouni V, Ulmann L, Jacquette B, Tremblin G. Effect of UV stress on the fatty acid and lipid class composition in two marine microalgae *Pavlova lutheri* (Pavlovophyceae) and *Odontella aurita* (Bacillariophyceae). Journal of Applied Phycology 2010;22:629–38.

210. Volkman JK, Dunstan GA, Jeffrey SW, Kearney PS. Fatty acids from microalgae of the genus *Pavlova.* Phytochemistry 1991;30:1855–59.

211. Mitra M, Patidar SK, George B, Shah F, Mishra S. A euryhaline *Nannochloropsis gaditana* with potential for nutraceutical (EPA) and biodiesel production. Algal Research 2015;8:161–7.

212. San Pedro A, González-López CV, Acién FG, Molina-Grima E. Outdoor pilot production of *Nannochloropsis gaditana*: Influence of culture parameters and lipid production rates in raceway ponds. Algal Research 2015;8:205–13.

213. Babuskin S, Krishnan KR, Babu PAS, Sivarajan M, Sukumar M. Functional foods enriched with marine microalga *Nannochloropsis oculata* as a source of ω-3 fatty acids. Food Technology and Biotechnology 2014;52:292–9.

214. Gomaa AS, Kholif AE, Kholif AM, Salama R, El-Alamy HA, Olafadehan OA. Sunflower oil and *Nannochloropsis oculata* microalgae as sources of unsaturated fatty acids for mitigation of methane production and enhancing diets' nutritive value. Journal of Agricultural and Food Chemistry 2018;66:1751–9.

215. Huang X, Huang Z, Wen W, Yan J. Effects of nitrogen supplementation of the culture medium on the growth, total lipid content and fatty acid profiles of three microalgae (*Tetraselmis subcordiformis*, *Nannochloropsis oculata* and *Pavlova viridis*). Journal of Applied Phycology 2013;25:129–37.

216. Roncarati A, Meluzzi A, Acciarri S, Tallarico N, Melotti P. Fatty acid composition of different microalgae strains (*Nannochloropsis* sp, *Nannochloropsis oculata* (Droop) Hibberd, *Nannochloris atomus* Butcher and *Isochrysis* sp.) according to the culture phase and the carbon dioxide concentration. Journal of the World Aquaculture Society 2004;35:401–11.

217. Hodgson PA, Henderson RJ, Sargent JR, Leftley JW. Patterns of variation in the lipid class and fatty acid composition of *Nannochloropsis oculata* (Eustigmatophyceae) during batch culture I. The growth cycle. Journal of Applied Phycology 1991;3:169–81.

218. Figueiredo ARP, da Costa E, Silva J, Domingues MR, Domingues P. The effects of different extraction methods of lipids from *Nannochloropsis oceanica* on the contents of omega-3 fatty acids. Algal Research 2019;41:101556.

219. Xiao Y, Zhang J, Cui J, Feng Y, Cui Q. Metabolic profiles of *Nannochloropsis oceanica* IMET1 under nitrogen-deficiency. Bioresource Technology 2013;130:731–8.

220. Sirisuk P, Sunwoo I, Kim SH, Awah CC, Ra CH, Kim JM, et al. Enhancement of biomass, lipids, and polyunsaturated fatty acid (PUFA) production in *Nannochloropsis oceanica* with a combination of single wavelength emitting diodes (LEDs) and low temperature in a three-phase culture system. Bioresource Technology 2018;270:504–11.

221. Durmaz Y, Monteiro M, Bandarra N, Gökpinar Ş, Işik O. The effect of low temperature on fatty acid composition and tocopherols of the red microalga, *Porphyridium cruentum*. Journal of Applied Phycology 2007;19:223–7.

222. Khozin-Goldberg I, Yu HZ, Adlerstein D, Didi-Cohen S, Heimer YM, Cohen Z. Triacylglycerols of the red microalga *Porphyridium cruentum* can contribute to the biosynthesis of eukaryotic galactolipids. Lipids 2000;35:881–9.

223. Guil-Guerro JL, Nelarbi EH, Robelloso-Fuentes MM. Eicosapentaenoic and arachidonic acids purification from the red microalga *Porphyridium cruentum*. Bioseparation 2001;9:299–306.

224. Klyachko-Gurvich GL, Doucha J, Kopezkii J, Ryabykh IE, Semenenko VE. Comparative investigation of fatty acid composition in lipids of various strains of *Porphyridium cruentum* and *Porphyridium aerugineum*. Russian Journal of Plant Physiology 1994;41:248–55.

225. Guihéneuf F, Stengel DB. Towards the biorefinery concept: Interaction of light, temperature and nitrogen for optimizing the co-production of high-value compounds in *Porphyridium purpureum*. Algal Research 2015;10:152–63.

226. Beauchemin KA, Kreuzer M, O'Mara F, McAllister TA. Nutritional management for enteric methane abatement: a review. Australian Journal of Experimental Agriculture 2008;48:21–7.

227. Sinclair LA, Cooper SL, Huntington JA, Wilkinson RG, Hallett KG, Enser E, et al. In vitro biohydrogenation of n − 3 polyunsaturated fatty acids protected against ruminal microbial metabolism. Animal Feed Science and Technology 2005;123–24:579–96.

228. Kalač P, Samková E. The effects of feeding various forages on fatty acid composition of bovine milk fat: a review. Czech Journal of Animal Science 2010;55:521–37.

229. Liu K. Comparison of lipid content and fatty acid composition and their distribution within seeds of 5 small grain species. Journal of Food Science 2011;76:C334–42.

230. Ryan E, Galvin K, O'Connor TP, Maguire AR, O'Brien NM. Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. Plant Foods for Human Nutrition 2007;62:85–91.

231. Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, et al. Microalgal triacylglycerols as feedstock for biofuel production: perspectives and advances. The Plant Journal 2008;54:621–39.

232. European Commission. Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. Official Journal of the European Communities 2001;L 147/1.

233. Iborra Martín J. Regulations in Australia, Canada, Japan and United States of America on fishmeal use for feedstuff (IPOL/B/PECH/N/2005_01). Policy Department Structural and Cohesion Policies, Directorate-General Internal Policies of the Union, European Parliament: 2005.

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

235. Hu Q. Environmental effects on cell composition. In: Richmond A, Hu Q, editors. Handbook of microalgal culture: applied phycology and biotechnology. 2nd ed. Chicester, United Kingdom: Wiley Blackwell: 2014. p. 114–22.

236. Ran W, Wang H, Liu Y, Qi M, Xiang Q, Yao C, et al. Storage of starch and lipids in microalgae: biosynthesis and

manipulation by nutrients. Bioresource Technology 2019;291:121894.

237. Fan J, Cui Y, Wan M, Li Y. Lipid accumulation and biosynthesis genes response of the oleaginous *Chlorella pyrenoidosa* under three nutrition stressors. Biotechnology for Biofuels 2014;7:17.

238. Gorain PC, Bagchi SK, Mallick N. Effects of calcium, magnesium and sodium chloride in enhancing lipid accumulation in two green microalgae. Environmental Technology 2013;34:1887–94.

239. Janchot K, Rauytanapanit M, Honda M, Hibino T, Sirisattha S, Praneenararat T, et al. Effects of potassium chloride-induced stress on the carotenoids canthaxanthin, astaxanthin, and lipid accumulations in the green chlorococcal microalga strain TISTR 9500. The Journal Eukaryotic Microbiology 2019;66:778–87.

240. Willette S, Gill SS, Dungan B, Schaub TM, Jarvis, JM, St. Hilaire, R. et al. Alterations in lipidome and metabolome profiles of *Nannochloropsis salina* in response to reduced culture temperature during sinusoidal temperature and light. Algal Research 2018;32:79–92.

241. Guedes AC, Meireles LA, Amaro HM, Malcata FX. Changes in lipid class and fatty acid composition of cultures of *Pavlova lutheri*, in response to light intensity. Journal of the American Oil Chemists' Society 2010;87:791–801.

242. Gladue RM, Maxey JE. Microalgae feeds for aquaculture. Journal of Applied Phycology 1994;6:131–41.

243. Kim DG, Hur SB. Growth and fatty acid composition of three heterotrophic *Chlorella* species. Algae 2013;28:101–109.

244. Boelen P, van Mastrigt A, van de Bovenkamp HH, Heeres HJ, Buma AGJ. Growth phase significantly decreases the DHA-to-EPA ratio in marine microalgae. Aquaculture International 2017;25:577–87.

245. Daneshvar E, Ok YS, Tavakoli S, Sarkar B, Shaheen SM, Hong H, et al. Insights into upstream processing of microalgae: a review. Bioresource Technology 2021;329:124870.

246. Piorreck M, Baasch KH, Pohl P. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. Phytochemistry 1984;23:207–16.

247. Olmstead ILD, Hill DRA, Dias DA, Jayasinghe NS, Callahan DL, Kentish SE, et al. A quantitative analysis of microalgal lipids for optimization of biodiesel and omega-3 production. Biotechnology and Bioengineering 2013;110:2096–104.

248. Athenstaedt K. Neutral lipids in yeast: synthesis, storage and degradation. In: Timmis KN, editor. Handbook of hydrocarbon and lipid microbiology. Berlin, Heidelberg: Springer; 2010. p. 471–80.

249. Guihéneuf F, Mimouni V, Ulmann L, Tremblin G. Combined effects of irradiance level and carbon source on fatty acid and lipid class composition in the microalga *Pavlova lutheri* commonly used in mariculture. Journal of Experimental Marine Biology and Ecology 2009;369:136–43.

250. Shadidi F. Extraction and measurement of total lipids. Current Protocols in Food Analytical Chemistry 2001;7: D1.1.1–11.

251. Laurens LM, Dempster TA, Jones HDT, Wolfrum EJ, Van Wychen S, McAllister JSP, et al. Algal biomass constituent analysis: Method uncertainties and investigation of the underlying measuring chemistries. Analytical Chemistry 2012;84:1879–87.

252. Castejón N, Señoráns FJ. Simultaneous extraction and fractionation of omega-3 acylglycerols and glycolipids from wet microalgal biomass of *Nannochloropsis gaditana* using pressurized liquids. Algal Research 2019;37:74–82.

253. Mitra M, Mishra S. A comparative analysis of different extraction solvent systems on the extractability of eicosapentaenoic acid from the marine eustigmatophyte *Nannochloropsis oceanica*. Algal Research 2019;38:01387.

254. Woo S.-G, Yoo K, Lee J, Bang S, Lee M, On K, et al. Comparison of fatty acid analysis methods for assessing biorefinery applicability of wastewater cultivated microalgae. Talanta 2012;97:103–10.

255. Santoro I, Nardi M, Benincasa C, Costanzo P, Giordano G, Procopio A, et al. Sustainable and selective extraction of lipids and bioactive compounds from microalgae. Molecules 2019;24:4347.

256. Knothe G. Analyzing biofuel: standards and other methods. Journal of the American Oil Chemists' Society 2006;83:823–33.

257. Chisti Y. Biodiesel from microalgae. Biotechnology Advances 2007;25:294–306.

258. Stamey JA, Shepherd DM, de Veth MJ, Corl BA. Use of algae or algal oil rich in n-3 fatty acids as a feed supplement for dairy cattle. Journal of Dairy Science 2012;95:5269–75.

259. Anele UY, Yang WZ, McGinn PJ, Tibbetts SM, McAllister TA. Ruminal *in vitro* gas production, dry matter digestibility, methane abatement potential, and fatty acid biohydrogenation of six species of microalgae. Canadian Journal of Animal Science 2016;96:354–63.

260. Henderson R, Parsons SA, Jefferson B. The impact of algal properties and pre-oxidation on solid-liquid separation of algae. Water Research 2008;42:1827–45.

261. Van Soest PJ. Nutritional ecology of the ruminant. 2nd ed. Ithaca, United States: Cornell University Press: 2008. 476 p.

262. Gerson T, King ASD, Kelly KE, Kelly WJ. Influence of particle size and surface area on in vitro rates of gas production, lipolysis of triacylglycerol and hydrogenation of linoleic acid by sheep rumen digesta or *Ruminococcus flavefaciens*. The Journal of Agricultural Science 1988;110:31–7.

263. Yap BHJ, Crawford SA, Dagastine RR, Scales PJ, Martin GJO. Nitrogen deprivation of microalgae: effect on cell size, cell wall thickness, cell strength, and resistance to mechanical disruption. Journal of Industrial Microbiology 2016;43:1671–80.

264. MacNeill GJ, Mehrpouyan S, Minow MAA, Patterson JA, Tetlow IJ, Emes MJ. Starch as a source, starch as a sink: the bifunctional role of starch in carbon allocation. Journal of Experimental Botany 2017;68:4433–53.

265. White LM. Carbohydrate reserves of grasses: A review. Journal of Range Management 1973;26:13–8.

266. Moore KJ, Hatfield RD. Carbohydrates and forage quality. In: Fahey Jr GC, editor. Forage quality, evaluation, and utilization. Madison, Wisconsin, USA: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America; 1994. p. 229–80.

267. Palmonari A, Cavallini D, Sniffen CJ, Fernandes L, Holder P, Fagioli L, et al. Characterization of molasses chemical composition. Journal of Dairy Science 2020;103:6244–49.

268. Chabi M, Leleu M, Fermont L, Colpaert M, Colleoni C, Ball SG, et al. Retracing storage polysaccharide evolution in Stramenopila. Frontiers in Plant Science 2021;12:629045.

269. Svihus B, Uhlen AK, Harstad OM. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. Animal Feed Science and Technology 2005;122:303–20.

270. Markou G, Angelidaki I, Georgakakis D. Microalgal carbohydrates: an overview of the factors influencing carbohydrates production, and of main bioconversion technologies for production of biofuels. Applied Microbiology and Biotechnology 2012;96:631–45.

271. McCleary BV, Charmier LMJ, McKie VA. Measurement of starch: critical evaluation of current methodology. Starch 2019;71:1800146.

272. Ozaki H, Maeda M, Nisizawa K. Floridean starch of a calcareous red alga, *Joculator maximus*. The Journal of Biochemistry 1967;61:497–503.

273. Ball SG, Morell MK. From bacterial glycogen to starch: understanding the biogenesis of the plant starch granule. Annual Review of Plant Biology 2003;54:207–33.

274. Nakamura Y, Takahashi JI, Sakurai A, Inaba Y, Suzuki E, Nihei S, et al. Some cyanobacteria synthesize semi-amylopectin type α-polyglucans instead of glycogen. Plant and Cell Physiology 2005;46:539–45.

275. Suzuki E, Suzuki R. Variation of storage polysaccharides in phototrophic microorganisms. Journal of Applied Glycoscience 2013;60:21–7.

276. Kolman MA, Nishi CN, Perez-Cenci M, Salerno GS. Sucrose in cyanobacteria: From a salt-response molecule to play a key role in nitrogen fixation. Life 2015;5:102–26.

277. Phélippé M, Gonçalves O, Thouand G, Cogne G, Laroche C. Characterization of the polysaccharides chemical diversity of the cyanobacteria *Arthrospira platensis*. Algal Research 2019;38:101426.

278. Hasunuma T, Kikuyama F, Matsuda M, Aikawa S, Izumi Y, Kondo A. Dynamic metabolic profiling of cyanobacterial glycogen biosynthesis under conditions of nitrate depletion. Journal of Experimental Botany 2013;64:2943–54.

279. Leliaert F, Verbruggen H, Zechman FW. Into the deep: new discoveries at the base of the green plant phylogeny. BioEssays 2011;33:683–92.

280. Ral JP, Derelle E, Ferraz C, Wattebled F, Farinas B, Corellou F, et al. Starch division and partitioning. A mechanism for granule propagation and maintenance in the picophytoplanktonic green alga *Ostreococcus tauri*. Plant Physiology 2004;136:3333–40.

281. Shimonaga T, Konishi M, Oyama Y, Fujiwara S, Satoh A, Fujita N, et al. Variation in storage α-glucans of the Porphyridiales (Rhodophyta). Plant and Cell Physiology 2008;49:103–16.

282. Cheng D, Li D, Yuan Y, Zhou L, Li X, Wu T, et al. Improving carbohydrate and starch accumulation in *Chlorella* sp. AE10 by a novel two-stage process with cell dilution. Biotechnology for Biofuels 2017;10:75.

283. Gifuni I, Olivieri G, Russo Kraus I, D'Errico G, Pollio A, Marzocchella A. Microalgae as a new sources of starch: Isolation and characterization of microalgal starch granules. Chemical Engineering Transactions 2017;57:1423–8.

284. Maršálková B, Širmerová M, Kuřec M, Brányik T, Brányiková I, Melzoch K, et al. Microalgae *Chlorella* sp. as an alternative source of fermentable sugars. Chemical Engineering Transactions 2010;21:1279–84.

285. Singh N, Singh J, Kaur L, Sodhi NS, Gill BS. Morphological, thermal and rheological properties of starches from different botanical sources. Food Chemistry 2003;81:219–31.

286. Fernandes B, Dragone G, Abreu AP, Geada P, Teixeira J, Vicente A. Starch determination in *Chlorella vulgaris*—a comparison between acid and enzymatic methods. Journal of Applied Phycology 2012;24:1203–8.

287. Deckardt K, Khol-Parisini A, Zebeli Q. Pecularities of enhancing resistant starch in ruminants using chemical methods: Opportunities and challenges. Nutrients 2013;5:1970–88.

288. Shimonaga T, Fujiwara S, Kaneko M, Izumo A, Nihei S, Francisco PB, et al. Variation in storage α-polyglucans of red algae: Amylose and semi-amylopectin types in *Porphyridium* and glycogen type in *Cyanidium*. Marine Biotechnology 2007;9:192–202.

289. McCraken DA, Cain JR. Amylose in floridean starch. New Phytologist 1981;88:67–71.

290. Gügi B, Le Costaouec T, Burel C, Lerouge P, Helbert W, Bardor M. Diatom-specific oligosaccharide and polysaccharide structures help to unravel biosynthetic capabilities in diatoms. Marine Drugs 2015;13:5993–6018.

291. Myklestad SM, Granum E. Biology of (1,3)-ß-glucans and related glucans in protozoans and chromistans. In: Bacic A, Fincher GB, Stone BA, editors. Chemistry, biochemistry, and biology of 1–3 beta glucans and related polysaccharides. Burlington, USA: Academic Press; 2009. p. 353–85.

292. Myklestad S. Production of carbohydrates by marine planktonic diatoms. I. Comparison of nine different species in culture. Journal of Experimental Marine Biology and Ecology 1974;15:261–74.

293. Okouchi RES, Yamamoto K, Ota T, Seki K, Imai M, Ota R, et al. Simultaneous intake of *Euglena gracilis* and vegetables exerts synergistic anti-obesity and anti-inflammatory effects by modulating the gut microbiota in diet-induced obese mice. Nutrients 2019;11:204.

294. Amundsen MR. Study of carbohydrates in *Euglena gracilis* [master's thesis]. Finland: Department of Food Science and Nutrition, Faculty of Agriculture and Forestry, University of Helsinki; 2018. Available from: http://urn.fi/URN:NBN:fi:hulib-201806122411

295. Wang Y, Seppänen-Laakso T, Rischer H, Wiebe MG. *Euglena gracilis* growth and cell composition under different temperature, light and trophic conditions. PLoS One 2018;13:e0195329.

296. Mertens DR. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing beakers or crucibles: collaborative study. Journal of AOAC International 2002;85:1217–40.

297. Lund P, Weisbjerg MR, Ahvenjärvi S, Huhtanen P, Udén P, Olafsson B, Volden H. Nordic ringtest on INDF content and NDF degradation characteristics in three feeds. Journal of Animal and Feed Sciences, 2004;13S1:139–42.

298. Tassone S, Fortina R, Peiretti PG. In vitro techniques using the Daisy^{II} incubator for the assessment of digestibility: a review. Animals 2020;10:775.

299. Li S, Liu L, Cheng JJ, Yang X. Comparison study on potential syngas produced by mild thermoconversion of microalgal residues through proton nuclear magnetic resonance and thermogravimetric analysis-fourier thransform infrared spectroscopy. Biomass Conversion and Biorefinery 2021;11:1875–83. In press. DOI: 10.1007/s13399-019-00591-2

300. Muizelaar W, Krooneman J, Van Duinkerken G. Polysaccharides in seaweeds: necessary steps in order to use seaweeds in animal feed. In: Strandberg E, Savoini G, Spoolder HAM, Sauerwein H, Lee M, Hocquette JF, et al. editors. 70th Annual Meeting of the European Federation of Animal Science; 26–30 August 2019; Ghent, Belgium. EAAP Book of Abstract No. 25; 2019. p. 330.

301. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent biber, and nonstrach polysaccharides in relation to animal nutrition. Journal of Dairy Science 1991;74:3583–97.

302. Miron J, Ben-Ghedalia D. Monosaccharide digestibility by dairy cows fed high in concentrate and containing alfalfa silages. Journal of Dairy Science 1994;77:3624–30.

303. Carrillo-Reyes J, Barragán-Trinidad M, Buitrón G. Biological pretreatments of microalgal biomass for gaseous biofuel production and the potential use of rumen microorganisms: A review. Algal Research 2016;18:341–51.

304. Höfte H, Voxeur A. Plant cell walls. Current Biology 2017;27:R865–70.

305. Spain O, Plöhn M, Funk C. The cell wall of green microalgae and its role in heavy metal removal. Physiologia Plantarum 2021;173:526–35. In press. DOI: 10.1111/ppl.13405

306. Espiñeira JM, Novo Uzal E, Gómez Ros LV, Carrión JS, Merino F, Ros Barceló A, et al. Distribution of lignin monomers and the evolution of lignification among lower plants. Plant Biology 2011;13:59–68.

307. Labeeuw L, Martone PT, Boucher Y, Case RJ. Ancient origin of the biosynthesis of lignin precursors. Biology Direct 2015;10:23.

308. Sørensen I, Pettolino FA, Bacic A, Ralph J, Lu F, O'Neill MA, et al. The charophycean green algae provide insights into the early origins of plant cell walls. The Plant Journal 2011;68:201–11.

309. Gunnison D, Alexander M. Basis for the resistance of several algae to microbial decomposition. Applied Microbiology 1975;29:729–38.

310. Hoiczyk E, Hansel A. Cyanobacterial cell walls: news from an unusual prokaryotic envelope. Journal of Bacteriology 2000;182:1191–9.

311. Lee RE. Cyanobacteria. In: Lee RE, editor. Phycology. 4th ed. Cambridge, United Kingdom: Cambridge University Press: 2008. p. 33–80.

312. Mai-Prochnow A, Clauson M, Hong J, Murphy AB. Gram positive and Gram negative bacteria differ in their sensitivity to cold plasma. Scientific Reports 2016;6:38610.

313. Nobles DR, Romanovicz DK, Brown RM. Cellulose in cyanobacteria. Origin of vascular plant cellulose synthase? Plant Physiology 2001;127:529–42.

314. Takeda H. Classification of *Chlorella* strains by cell wall sugar composition. Phytochemistry 1988;27:3823–6.

315. Takeda H. Sugar composition of the cell wall and the taxonomy of Chlorella (Chlorophyceae). Journal of Phycology 1991;27:224–32.

316. Zhu CJ, Lee YK. Determination of biomass dry weight of marine microalgae. Journal of Applied Phycology 1997;9:189–94.

317. Abo-Shady AM, Mohamed YA, Lasheen T. Chemical composition of the cell wall in some green algae species. Biologia Plantarum 1993;35:629–32.

318. Scholz MJ, Weiss TL, Jinkerson RE, Jing J, Roth R, Goodenough U, et al. Ultrastructure and composition of the *Nannochloropsis gaditana* cell wall. Eukaryotic Cell 2014;13:1450–64.

319. Kodner RB, Summons RE, Knoll AH. Phylogenetic investigation of the aliphatic, non-hydrolyzable biopolymer algaenan, with a focus on green algae. Organic Geochemistry 2009;40:854–62.

320. Yamada T, Sakaguchi K. Comparative studies on *Chlorella* cell walls: Induction of protoplast formation. Archives of Microbiology 1982;132:10–3.

321. Rashidi B, Trindade LM. Detailed biochemical and morphologic characteristics of green microalga *Neochloris oleoabundans* cell wall. Algal Research 2018;35:152–9.

322. Tibbetts SM, MacPherson T, McGinn PJ, Fredeen AH. *In vitro* digestion of microalgal biomass from freshwater species isolated in Alberta, Canada for monogastric and ruminant animal feed applications. Algal Research 2016;19:324–32.

323. Arad SM, Levy-Ontman O. Red microalgal cell-wall polysaccharides: biotechnological aspects. Current Opinion in Biotechnology 2010;21:358–64.

324. Orlova TY, Sabutskaya MA, Markina ZV. Ultrastructural changes in marine microalgae from different taxonomic groups during batch cultivation. Russian Journal of Marine Biology 2019;43:202–10.

325. Liberman GN, Ochbauom G, Mejubovsky-Mikhelis M, Bitton R, Arad SM. Physio-chemical characteristics of the sulfated polysaccharides of the red microalgae *Dixionella grisea* and *Porphyridium aerugineum*. International Journal of Biological Macromolecules 2020;145:1171–9.

326. Müller MN. On the genesis and function of coccolithophore calcification. Frontiers in Marine Science 2019;6:49

327. Kröger N, Poulsen N. Diatoms—from cell wall biogenesis to nanotechnology. Annual Reviews of Genetics 2008;42:83–107.

328. Ford CW, Percival E. Carbohydrates of *Phaeodactylum tricornutum*. Part II. A sulphated glucuromannan. Journal of the Chemical Society 1965:7042–6.

329. Gonçalves CA, Figueredo CC. What we really know about the composition and function of microalgae cell coverings?—an overview. Acta Botanica Brasilica 2020;34:599–614.

330. Darley WM, Porter D, Fuller MS. Cell wall composition and synthesis via golgi-directed scale formation in the marine eucaryote, *Schizochytrium aggregatum*, with a note on *Thraustochytrium* sp. Archiv für Mikrobiologie 1973;90:89–106.

331. Hofmann C, Bouck M. Immunological and structural evidence for patterned intussuspective surface growth in a unicellular organism. A postulated role for submembranous proteins and microtubules. The Journal of Cell Biology 1976;69:693–715.

332. O'Neill EC, Kuhaudomlarp S, Rejzek M, Fangel JU, Alagesan K, Kolarich D, et al. Exploring the glycans of *Euglena gracilis*. Biology 2017;6:45.