CHLORELLA VULGARIS MICROALGAE IN RUMINANT NUTRITION: A REVIEW OF THE CHEMICAL COMPOSITION AND NUTRITIVE VALUE

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Abstract

Recently, microalgae, natural marine resources, have gained increasing interests as a feed for animals. *Chlorella vulgaris* microalgae are single-cell microorganisms that have been used to provide nutrition to humans and animals for centuries. In the present review, we unveil the composition and nutritive value of *C. vulgaris* microalgae as a feed for ruminants. Research has shown that inclusion of *C. vulgaris* microalgae in diets improved feed utilization, milk production and quality, growth performance, and meat quality in ruminants, as a result of improved diet nutritive value. Very low doses of *C. vulgaris* in feed enhance growth and lactational performance of ruminants. Additionally, *C. vulgaris* showed very promising results as an alternative to corn and soybean meal; however, it is an expensive protein feed. Therefore, the main constraint to use of *C. vulgaris* as a feedstuff is its high cost of production, making improvement of cultivation technology to reduce the production costs a critical issue in the near future.

Key words: *Chlorella vulgaris*, feed supplements, microalgae, rumen fermentation and microorganisms, unconventional feeds

There is increasing demand for meat and milk. New sustainable raw materials and resources could play an essential role in livestock production systems sustainability (Cedillo et al., 2014; Elghandour et al., 2016 c). Marine microalgae can be one of these resources that can play important roles in the sustainable production of ruminants (Kholif et al., 2021).

Microalgae have a broad use; however, the existing technology for their production is yet inefficient for a cost-effective production (Kotrbaček et al., 2015). *Chlorella vulgaris* is the most researched microalgae species until now (Ru et al., 2020). *C. vulgaris* cell is 2 to 10 µm in diameter, with spherical, subspherical or ellipsoid shape without flagella (Ru et al., 2020). It contains a high concentration of protein and biological value, determined by its essential amino acids content and low rumi-

nal degradability, for ruminants (Kholif et al., 2017 d). The relatively high protein content of *C. vulgaris* is in contrast with the limited nutritive value of its protein for non-ruminants due to its content of non-protein nitrogen that is composed of nucleic acids, nitrogen-containing cell walls, and amines; however, ruminant can utilize it with high efficiency (Madeira et al., 2017; Ru et al., 2020). Ruminant can utilize non-protein nitrogen, by virtue of ruminal bacteria intervention, to synthesize protein with high biological value, evident by balanced amino acids and degradability.

Despite the high nutritional values and health implications of *C. vulgaris*, some cautions should be considered to avoid or minimize possible toxicity of the microalgal biomass (Kovač et al., 2013). However, most experiments showed no apparent toxicological property, not only for *C. vulgaris* but for most of the studied microalgal species (Becker, 2013). High levels of *C. vulgaris* in diets of ruminant showed less successful results due to their low digestibility as a result of the rigid cellulosic cell wall of *C. vulgaris* cells (Kotrbaček et al., 2015). Mechanical breakdown of algal cells with a bead mill increased their digestibility (Kotrbaček et al., 2015).

Nevertheless, in many experiments, it was found that even a very low, economically acceptable addition of *Chlorella* biomass to animal feed can positively influence growth and performance. This is due to the presence of pigments, antioxidants, provitamins, vitamins, and a growth substance known as the *Chlorella* Growth Factor (CGF), which can stimulate or enhance the immune system, increase feed intake and utilization, and promote reproduction (Kotrbaček et al., 2015; Madeira et al., 2017; Ru et al., 2020). The CGF is a growth substance, which can induce the immune system, improve feed consumption and utilization, promote reproduction, and modulate cytokine production (Kotrbaček et al., 2015). Previous studies (Kholif et al., 2017 b; Tsiplakou et al., 2017) observed that *C. vulgaris* improved ruminal fermentation and decreased *in vitro* methane (CH₄) production. However, others (Sucu, 2020) reported unclear effects of *C. vulgaris* on CH₄ production. Additionally, Kholif et al. (2017 d) showed enhanced feed utilization, increased milk production, and improved milk nutritive value with feeding *C. vulgaris* to goats.

Accordingly, this work reviews the composition, nutritive value, and the potential use of *C. vulgaris* microalgae in ruminant diet, particularly its effects on growth performance, meat quality, milk production, and milk composition.

***C. vulgaris* microalgae characterization and nutritional properties**

C. vulgaris is an autotrophic alga but it can also be produced under heterotrophic conditions (Kotrbaček et al., 2015; Madeira et al., 2017; Ru et al., 2020). *C. vulgaris* is a freshwater, unicellular, green alga belonging to the family Chlorellaceae. It has the ability to utilize carbon dioxide as the carbon source and sunlight or organic carbon instead of sunlight as the energy source, making its cultivation easy and controlled in typical bioreactors (Madeira et al., 2017). Chlorophyll-a and chlorophyll-b are the main pigments in *C. vulgaris*, making it the richest source of chlorophyll available (Kotrbaček et al., 2015; Madeira et al., 2017).

The nutritional composition of *C. vulgaris* (Table 1) is generally well known. The chemical composition of *C. vulgaris* depends on the strain and culturing envi-

ronmental conditions like medium nutrients, temperature, light intensity, pH, and salinity (Ru et al., 2020).

Table 1. Chemical composition of *C. vulgaris* microalgae (g/kg dry weight)

Dry matter	Organic matter	Crude protein	Crude carbohydrates	Fiber	Crude fat	References
722	674	136	NR	NR	153	Sucu (2020)
946	943	608	43 (Starch)	0	95.1	Lamminen et al. (2017)
927	848	677	NR	128 (NDF) and 42 (ADF)	10.5	Tsiplakou et al. (2017)
NR	NR	591	173	121 (NDF)	NR	Kholif et al. (2017 b)
901	931	482	299	171	56	Yusof et al. (2011)

ADF = acid detergent fiber, NDF = neutral detergent fiber, NR = not reported.

Table 2. Amino acids profile of *C. vulgaris* microalgae (g/kg protein)

Amino acid	Concentration					
Arginine	38.8	53.9	69	62	64	68
Histidine	11.5	17.7	20	20	20	12
Isoleucine	19.3	29.2	32	44	38	1
Leucine	59.5	68.8	95	94	88	69
Lysine	42.4	48.5	64	67	84	63
Methionine	10.1	18.8	13	12	22	6
Phenylalanine	30.7	41.8	55	55	50	54
Threonine	29.4	33.1	53	52	48	56
Tryptophan	0.019	9.66	0	23	21	11
Valine	38.6	42.6	70	66	55	29
Alanine	52.1	60	94	83	79	101
Aspartic acid	59	55.4	93	98	90	101
Cysteine	0.069	13.2	0	13	14	5
Glutamic acid	77.6	81.1	137	127	116	84
Glycine	32	46.2	63	61	58	79
Proline	30.7	37.6	50	49	48	27
Serine	25.3	31.6	58	43	41	35
Tyrosine	23.8	33.8	28	31	34	78
References	Kholif et al. (2017 b)	Lamminen et al. (2017)	Safi et al. (2013)	Maruyama et al. (1997)	Becker (2007)	Morris Quevedo et al. (1999)

Generally, about 20% of the microalgal proteins are bound to the cell wall, 50% located inside the cells, and the remaining 30% continually migrate in and out of the cell (Berliner, 1986).

Proteins

C. vulgaris has a high protein concentration of 50–60% on dry matter (DM) and the ability to synthesize all amino acids, making it an excellent source of unconventional protein and essential amino acids (Kholif et al., 2017 b). The quality of protein from *C. vulgaris* is equivalent to or even superior to that of usual plant protein fractions, yeast, soy flour, and milk (Becker, 2007 a, b; Kovač et al., 2013; Madeira et al., 2017). The quality of a protein is nutritionally determined mainly by its amino acid profile (Table 2). The amino acids profile and quality of *C. vulgaris* protein favorably meet the standard proposed for human nutrition by the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) (Safi et al., 2014).

Lipids

C. vulgaris contains neutral and polar lipids. Alpha linolenic, palmitic and linolenic acids, and oleic acid are the major lipidic components of *C. vulgaris* (Kholif et al., 2017 b) (Table 3). The main role of lipids in the microalgae is to serve as an energy and carbon reservoir for their metabolism and growth (Ru et al., 2020). *C. vulgaris* can have up to more than 13% lipid content (Kholif et al., 2017 b). However, others (Safi et al., 2014) reported a lipid content ranging from 5 to 58% of dry weight. Yeh and Chang (2012) observed that cultivating *C. vulgaris* under mixotrophic conditions produces more lipid (60–68%).

Table 3. Fatty acid profile of *C. vulgaris* microalgae (g/kg total fatty acids)

C16:0	C16:1n-7	C18:0	C18:1 cis-9	C18:2n-6	C18:3n-6	References
23.8	1.3	2.7	4.1	37.3	40.9	Kholif et al. (2017 b)
13.9	5.7	3.1	2.2	25.3	24.2	Maruyama et al. (1997)
13.1	0.6	3.0	11.2	9.4	3.1	Yeh and Chang (2012)*
2.2	0.3	2.0	0.5	3.3	1.0	Yeh and Chang (2012)**

*In basal medium, mixotrophic.

**In basal medium, photoheterotrophic.

Polysaccharides

Table 4 shows simple sugars composition of the cell wall polysaccharides in *C. vulgaris*. *C. vulgaris* is rich in carbohydrates and uses them as energy and carbon sources. Additionally, *C. vulgaris* carbohydrates comprise mostly of non-fiber carbohydrates (Kovač et al., 2013; Kholif et al., 2017 b). β -1,3-glucan is one of the major components in *C. vulgaris* and can act as an antioxidant, immunostimulator, and blood lipid reducer (Spolaore et al., 2006). *C. vulgaris* total carbohydrate concentration can range from 12–55% of dry weight when grown under unfavorable environments, particularly with inadequate nitrogen (Choix et al., 2012).

Pigments

Pigments are the substances responsible for the absorption and reflection of certain wavelengths of visible light during photosynthesis. *C. vulgaris* is rich in chlorophyll (Table 5). Chlorophyll is an important pigment for healing of sores, ulcers,

hemorrhoids, hemophilia, diabetes, and asthma and regulation of menstruation. In addition to chlorophyll comprising about 1–3% dry algal cell weight (Kotrbaček et al., 2015), *Chlorella* contains a wide range of orange and yellow carotenoids that contribute to photosynthesis as well as to the protection of cellular structures against damage by free radicals and ultraviolet radiation. The structure of chlorophyll closely resembles hemin (important for hemoglobin formation), making it useful for increasing the red blood cells (Rani et al., 2018).

Table 4. Simple sugars composition of the cell wall polysaccharides (Takeda, 1991)

Neutral sugars	%
Rhamnose	45–54
Arabinose	2–9
Xylose	7–19
Mannose	2–7
Galactose	14–26
Glucose	1–4

Table 5. Potential pigments content in *C. vulgaris*

Pigments	µg/g (DM basis)	References
β-Carotene	7–12,000	Gonzalez and Bashan (2000), Kwang et al. (2010), Kong et al. (2014)
Astaxanthin	550,000	Singh and Gu (2010)
Cantaxanthin	362,000	Singh and Gu (2010), Kong et al. (2014)
Lutein	52–3830	Gonzalez and Bashan (2000), Kwang et al. (2010), Kong et al. (2014)
Chlorophyll-a	250–9630	Gonzalez and Bashan (2000)
Chlorophyll-b	72–5770	Gonzalez and Bashan (2000), Kong et al. (2014)
Pheophytin-a	2310–640	Kwang et al. (2010)
Violoxanthin	10–37	Gonzalez and Bashan (2000)

β-carotene is the most valuable pigment after chlorophyll, and mammalian cells cannot synthesize it. The concentration of β-carotene is in the range of 0.1–0.2% (Becker, 2007 a, b). β-carotene plays an important role in animal health.

Vitamins and minerals

Table 6 shows mineral concentration in *C. vulgaris*. *C. vulgaris* is a rich source of almost all important minerals (Ca, K, Mg, and Zn) and vitamins (A, B₁, B₂, B₆, B₁₂, C, E, niacin, and nicotinate). Additionally, *C. vulgaris* contains minor quantities of folic acid, biotin, inositol, and choline (Rani et al., 2018). *C. vulgaris* contains more vitamins than most cultivated plants (Kovač et al., 2013).

C. vulgaris has selenium and iodine in enough quantities, which play important roles in human and animal health due to their effect on the formation of glutathione-peroxidase that is important for buffering the destructive effect of peroxidation reactions on living cells (Rayman, 2000). Moreover, both of these elements are important for the production of thyroid hormones, which regulate metabolism level in the tis-

sues of virtually all mammals (Trávníček et al., 2010). Such content of minerals and vitamins makes *C. vulgaris* an excellent source of essential micronutrients.

Table 6. Minerals concentration in *C. vulgaris* (mg/100 g DM)

Ca	Fe	K	Mg	Mn	Na	Cu	P	Zn	References
278	82	1197	851	NR	101	48	NR	293	Yusof et al. (2011)
0.94	5.40	132.9	12.36	1.27	16.45	NR	27.08	0.53	Sucu (2020)
0.16	0.20	1.13	0.36	NR	NR	NR	NR	NR	Maruyama et al. (1997)
0.59	0.26	0.05	0.34	NR	1.35	NR	1.76	NR	Tokuşoglu and Ünal (2003)
0.27	0.68	2.15	0.44	0.40	NR	0.19	0.96	0.55	Panahi et al. (2012)

NR = not reported.

Antioxidants

Generally, microalgae are considered as a multicomponent of antioxidant system (Kovač et al., 2013). Polyphenols, phycobiliproteins, and vitamins are the most powerful water-soluble antioxidants found in algae. Being a photosynthetic organism, *C. vulgaris* can utilize light and high oxygen concentrations in a medium to form highly effective antioxidative scavenger complexes to protect cells (Kovač et al., 2013).

Applications of *C. vulgaris* microalgae in ruminant feeding

The use of microalgae as animal feed has been evaluated since the 1950s. Nowadays, up to 30% of the present world algal production is purchased for the livestock feed production (Madeira et al., 2017), owing to its extraordinary nutritive value and ease of digestibility at certain levels attributable to its marginal structural carbohydrates content. Feeding small amounts of microalgae is beneficial as it boosts the animals' immunity and resistance to diseases due to its antibacterial and antiviral action, resulting in improved animal productivity and feed utilization efficiency (Madeira et al., 2017; Kholif et al., 2020). Ruminants, by virtue of their rumen, degrade the cell wall of unprocessed microalgae (Madeira et al., 2017; Kholif et al., 2020).

Table 7 summarizes the influence of feeding dietary *C. vulgaris* microalgae on growth performance parameters of ruminant species. Several experiments showed improved growth performance in farm animals, with *C. vulgaris* microalgae supplementation or as a feed ingredient. However, response of animals to *C. vulgaris* differed between experiments. Differences between experiments may be related to the levels of supplementation and lipid in the microalgae. Generally, the lipid extraction temperature during processing has been reported to affect the quality of microalgae protein, its digestibility, and utilization by livestock (Tibbetts et al., 2015).

In vitro evaluation

In vitro evaluation of feeds is a technique that can be used for predicting feed quality (Elghandour et al., 2014, 2016 b). Before the administration of *C. vulgaris* in the diet of animals, it should be preliminarily assessed for its nutritive value and effects on *in vitro* ruminal fermentation (Marrez et al., 2017). Lodge-Ivey et al.

(2014) observed increased *in vitro* volatile fatty acids (VFA) production with the supplementation of concentrate diet with lipid-extracted *C. vulgaris* at 15% compared to soybean. Anele et al. (2016) evaluated the digestibility of 5 non-disrupted microalgae, using rumen batch culture system, and observed that dry matter (DM) digestibility of *C. vulgaris* was 76%. In another experiment, Kholif et al. (2017 b) evaluated the effect of different levels of *C. vulgaris* (0, 20, 40, and 80 mg/g DM) on *in vitro* ruminal fermentation and CH₄ production of three rations containing 25, 50, and 50% corn silage. *C. vulgaris* showed better results with fibrous rations than concentrate-rich diets. Recently, Sucu (2020) evaluated the efficacy of *C. vulgaris* and *C. variabilis* and their combination on *in vitro* ruminal fermentation of wheat and corn silages. Algal treatments decreased gas production (GP) and VFA concentration compared to wheat and corn silages without algal supplementation, with strongest effect for *C. vulgaris* (by 34%) and increased ammonia-N concentration. Additionally, *C. vulgaris* increased propionate with the corn silage substrate. They (Sucu, 2020) reported that the high content of fat in the evaluated microalgae (15.3% for *C. vulgaris*) may inhibit cellulolytic bacteria in the rumen and lead to a reduction of total VFA and GP.

Feed intake

The performance of animals is a function of feed intake because it affords the animal access to energy, protein, minerals, and other factors in the feed needed for growth and milk synthesis and production (Ahmed et al., 2015; Morsy et al., 2015). Feeding *C. vulgaris* at low levels did not affect feed palatability (Kholif et al., 2021). However, feeding large amounts of *C. vulgaris* may decrease feed palatability. Generally, the lower acceptability of microalgae by livestock may be due to the taste and odor properties, nutritive characteristics, or physical structure of the dry powdery form. Lamminen et al. (2017) observed that using pelleted rapeseed and a combination of *Spirulina platensis* and *C. vulgaris* microalgae (1:1 on DM basis) as supplementary protein sources in the diets of Finnish Ayrshire cows did not affect total feed intake.

In some experiments, *C. vulgaris* increased feed intake. Supplementation of *C. vulgaris* enhanced nutrient digestibility, with special concerns for ruminal fiber degradability and metabolic effects, such as enhanced amino acids to metabolizable energy ratio at the tissue level, resulting in increased feed intake (Kholif et al., 2017 d). *C. vulgaris* supplementation in ruminant diet did not compromise palatability, acceptance and consumption. Since animal performance is largely a function of feed intake, *C. vulgaris* could be used as a supplement to enhance performance of ruminant stock. Kholif et al. (2017 d) observed that feeding Damascus goats on diets supplemented with *C. vulgaris* microalgae at 5 and 10 g/goat/day improved feed consumption and apparent digestibility, concentration of ruminal total VFA and propionic acid, and milk production, with no additional response to increasing the level of microalgae in the diet from 5 to 10 g daily. Recently, Kholif et al. (2020) fed lactating Boer goats on a diet supplemented with 10 g *C. vulgaris*/goat/day and observed no effect on feed intake, though feed efficiency was improved.

Table 7. Effect of *C. vulgaris* microalgae on ruminant performance

Level	Animal	Main results	References
10 g <i>C. vulgaris</i> /doe daily	Lactating Boer goats	↑ Feed intake ↑ Nutrient digestibility ↑ Ruminal pH, ammonia-N or propionate ↑ Ruminal total VFA and acetate ↓ CH ₄ /VFA ratio ↑ Serum glucose ↓ Serum total lipids ↑ Daily milk production and milk efficiency ↓ Milk contents of SFA ↑ Milk C18:1n9C, total CLA and total UFA	Kholif et al. (2020)
<i>C. vulgaris</i> as incubation substrates	<i>In vitro</i> experiment	↓ Gas production, ruminal VFA, and ruminal protein degradation	Wild et al. (2019)
Experiment 1 on heifers: 1–30th day – 1.51 g, 31–60th day – 1.82 g, 61–90th day – 2.01 g, 91–120th day – 2.28 g daily.	Yaroslavl breed cows	Heifers: ↑ Basophils in the blood. ↑ Relative number of band neutrophils on d 90th to 120th. ↑ Total number of leukocytes ↓ Number of erythrocytes on the 30th and 60th days of the experiment ↑ Number of erythrocytes on the 90th and 120th days of the experiment by 7.95% and 8.93% respectively ↑ Hemoglobin content on the 90th and 120th days of the experiment ↑ Activity of superoxide dismutase	Bogdanova and Flerova (2018)
Experiment 2 on lactating cows: 6 g daily for 60 days		Lactating cows: ↑ Relative number of band and neutrophils with segmented nuclei ↑ Number of eosinophils by 2.2% on the 60th day ↑ Number of monocytes by 2% on the 90th day ↑ Number of erythrocytes by 15.3% on the 30th day ↑ Number of erythrocytes and the content of hemoglobin ↑ Activity of superoxide dismutase and catalase	

<i>S. platensis</i> and <i>C. vulgaris</i> , in 1:1 on DM basis at 0.23 kg/cow daily or at 0.47 kg/cow daily	Finnish Ayrshire cows	<ul style="list-style-type: none"> ↑ Silage and diet DM intake ↑ CP and essential amino acids intakes ↑ Apparent digestibility of DM, OM, NDF or CP ↑ Milk production ↑ Energy corrected milk yield ↑ Arterial concentrations of plasma metabolites and amino acids ↓ Arterial BHBA concentrations 	Lamminen et al. (2017)
10 g lyophilized <i>C. vulgaris</i> /kg concentrate	Crossbred dairy goats	<ul style="list-style-type: none"> ↑ <i>M. stadtmanae</i>, <i>M. ruminantium</i> and methanogens ↑ Ruminal gas production ↓ Ruminal cellulase and protease activity ↓ Lipase and α-amylase activities ↑ Ruminal <i>C. sticklandii</i>, <i>B. fibrisolvens</i> and <i>R. albus</i> population ↑ Ruminal <i>P. anaerobius</i>, <i>R. flavefaciens</i> and <i>F. succinogenes</i> ↓ Ruminal protozoa population 	Tsiplakou et al. (2017)
<i>C. vulgaris</i> was added at 0, 20, 40 and 80 mg/g DM of three total mixed rations containing maize silage at 25, 50 and 75% and concentrates at the rest percentage	<i>In vitro</i> experiment	<ul style="list-style-type: none"> ↓ Gas production from the 50% silage ration ↑ Gas production from the 25% silage ration ↓ CH₄ production (<i>C. vulgaris</i> at 80 mg/g DM of the 75% silage ration) ↓ CH₄ production (50% silage ration) 	Kholif et al. (2017 b)
<i>C. vulgaris</i> at 0, 5 and 10 g/goat/day for 12 weeks	Lactating Damascus goats	<ul style="list-style-type: none"> ↑ Intake (<i>C. vulgaris</i> at 5 g/doe) ↑ Apparent digestibility (<i>C. vulgaris</i> at 5 and 10 g/doe) ↑ Total VFA and propionic acid ↓ <i>C. vulgaris</i> decreased serum GPT, GOT and cholesterol concentrations ↑ Milk production and total solids, solids not fat and lactose contents (<i>C. vulgaris</i> at 5 g/doe) ↓ <i>C. vulgaris</i> decreased milk contents from total SFA ↑ Milk total CLA 	Kholif et al. (2017 d)

BHBA = β -hydroxybutyric acid, CH₄ = methane, CLA = conjugated linoleic acids, CP = crude protein, DM = dry matter, GOT = glutamic pyruvic transaminase, GPT = glutamic oxaloacetic transaminase, NDF = neutral detergent fiber, OM = organic matter, SFA = saturated fatty acids, UFA = unsaturated fatty acids, VFA = volatile fatty acids.

↑ = increased, ↓ = decreased, ↑ = no effect.

Nutrient digestibility

Digestibility of feeds determines its utilization in ruminant feeding (Khattab et al., 2013; Kholif et al., 2017 a; Matloup et al., 2017; Kholif et al., 2018 a). *C. vulgaris* has an inflexible cellulose cell wall, which makes the cell contents inaccessible to the animals' gastrointestinal tract (Kotrbaček et al., 2015). Compared to cyanobacteria, *C. vulgaris* has different cell wall composition, which may be less fermentable and digestible. Researchers (Wild et al., 2019) reported increased net protein utilization of *C. vulgaris* after the rigid cell wall was partially ruptured by drying in a drum. Additionally, Janczyk et al. (2007) observed that ultrasonication increased nutritional value of *C. vulgaris*. The exact constituents of *C. vulgaris* cell wall is inexplicit, but it is opined that the proteinaceous polymers, instead of the carbohydrates, predominantly determine the digestibility (Mahdy et al., 2016).

Recently, Wild et al. (2019) observed that mechanical cell disruption improved the degree of fermentation of *C. vulgaris* in the rumen; however, the disruption is redundant when microalgae are used as a source of protein for ruminants (Wild et al., 2019). In another experiment, Kholif et al. (2020) observed improved nutrient digestibility, especially crude protein (CP) and neutral detergent fiber (NDF) digestibilities with *C. vulgaris* supplementation. The improved nutrient digestibility with *C. vulgaris* indicates that the rigid cell wall may not pose any limitation to its utilization in feeding ruminants, unlike as it does in the non-ruminant counterparts. Ruminants, by virtue of the microbial intervention in the rumen, can reasonably degrade cell wall carbohydrates except for a highly lignified feed, which requires some form of biodegradation before feeding or application of feed additives such as exogenous enzymes.

Ruminal microflora and fermentation

Ruminal fermentation, brought about by the action of microbial populations, degrades and converts ingesta (feed) into water, gases (CH_4 and carbon dioxide) and VFA, the main energy source for ruminants (Elghandour et al., 2016 d; Kholif et al., 2016, 2018 b). *C. vulgaris* supplementation showed great effects on ruminal microorganisms, ruminal fermentation, and ruminal biohydrogenation. Tsiplakou et al. (2017) observed abundance of *Ruminococcus albus* and *Clostridium sticklandii*, a reduced cellulase activity, and increased CH_4 -producing bacteria and protozoa and protease activity, with feeding goats on diet supplemented with *C. vulgaris*.

Regarding the effect of *C. vulgaris* supplementation on ruminal biohydrogenation, Tsiplakou et al. (2017) reported a changed ruminal biohydrogenation with *C. vulgaris* supplementation to goats. As earlier said, they observed that supplementation of *C. vulgaris* increased CH_4 -producing bacteria and protozoa. The changes in ruminal microorganisms involved in biohydrogenation process and CH_4 and ammonia production due to dietary supplementation of microalgae is not yet fully elucidated. They also observed that *C. vulgaris* enhanced the synthesis of trans C 18:1, trans-11 C 18:1, and monounsaturated fatty acids, while it reduced the concentrations of C 18:0 and long-chain fatty acids.

C. vulgaris supplementation was reported to negatively affect ruminal fermentation due its high content of rumen undegradable protein (RUP) and low dietary poly-

saccharides, like cellulose, hemicellulose, pectin, and starch concentrations (Wild et al., 2019). High RUP affects the quantity of dietary protein available for ruminal degradation and hence microbial protein synthesis. Since fiber is needed for efficient ruminal microbes' activity and fermentation, the low polysaccharides content of *C. vulgaris* may impair ruminal fermentation. According to Wild et al. (2019), reduced overall GP, ruminal VFA, and ruminal protein degradation were the consequences of the impaired ruminal fermentation with *C. vulgaris* supplementation. The authors further reported a high RUP concentration in *C. vulgaris*, indicating that the microalgae may be a promising protein source for high-performing ruminants. For high-producing cows, microbial protein may be insufficient to supply the protein need of the animals; hence, it has been advocated that the dietary crude protein of such cows should contain a significant proportion of RUP (Erickson and Kalscheur, 2019). Han and McCormick (2014) reported lower degradability of microalgae carbohydrates than those of soybean meal. This implies that soybean meal may enhance ruminal fermentation better than *C. vulgaris* because ruminal fermentation efficiency partly depends on the degree of fermentability of the dietary carbohydrate for ruminal VFA and thus energy production for the microbes (Olafadehan et al., 2016).

Conversely, Kholif et al. (2017 d) noted that supplementation of Damascus goats diets with *C. vulgaris* microalgae at 5 and 10 g/goat/day increased the concentrations of ruminal total VFA and propionic acid. However, increasing the doses of microalgae from 5 g to 10 g daily showed minimal effects on ruminal fermentation. In a recent experiment, Kholif et al. (2020) noted increased ruminal VFA, acetate, and acetate/propionate and decreased CH₄/VFA ratio with feeding goats on diet supplemented with 10 g *C. vulgaris* daily.

C. vulgaris increased the proportion of *C. sticklandii*, the most important hyperammonia bacteria (Tsiplakou et al., 2017). Therefore, *C. vulgaris* supplementation increased ruminal ammonia production in cows. Feeding goats on 10 g *C. vulgaris* daily did not affect ruminal ammonia-N concentration (Kholif et al., 2020). The composition of diets, particularly the protein, may be the main reason for the inconsistency between experiments. Diets that are high rumen degradable protein (RDP), particularly non-protein nitrogen such as urea, usually results in higher ammonia N production compared to those high in RUP due to the rapid dietary protein proteolysis.

Methane production

Ruminants are one of the major producers of CH₄ (Elghandour et al., 2017; Hernández et al., 2017). Supplementation of microalgae with high-fat feed showed positive effects as possible candidates to reduce CH₄ production in ruminants (Gomaa et al., 2018) due to lipids, especially polyunsaturated fatty acids (PUFA), which have antimicrobial effects on ruminal methanogens and protozoa (Ebeid et al., 2020). However, the situation is not clear with microalgae, such as *C. vulgaris*, which are rich in protein and low in fat content (Kholif et al., 2017 b). According to Wild et al. (2019) CH₄ volume was positively correlated with CP concentration ($r = 0.70$) but negatively correlated with RUP ($r = -0.75$), implying that feeding RUP

reduces ruminal protein solubility and hence conversion to ammonia, which is accompanied by release of H^+ ions used for ruminal methanogenesis. *C. vulgaris* affects ruminal methanogenesis through a direct impact on H_2 production (Tsiplakou et al., 2017). In the rumen, conversion of protein and amino acids to ammonia and α -keto acids produce H^+ ions, contributing to the ruminal CH_4 pool. Vanegas et al. (2017) opined that excessive RDP increases CH_4 emission. The composition, especially the lipids, proteins, and carbohydrates contents, of microalgae determines its effect on ruminal CH_4 production (Elghandour et al., 2016 a). The cell wall disruption of microalgae plays the most important role in determining the actual CH_4 yield from microalgae (González-Fernández et al., 2012). Ruminal proteases may be a useful biological catalyst for *C. vulgaris* cell wall disruption in order to double CH_4 production compared to the raw biomass. Additionally, Chakraborty et al. (2013) observed that *C. vulgaris* contains α -cellulase, representing about 89.8% of glucose. Tsiplakou et al. (2017) fed goats on a diet supplemented with 10 g lyophilized *C. vulgaris*/kg concentrate and observed increased ruminal *Methanobrevibacter ruminantium*, *Methanospaera stadtmannae*, protozoa, and methanogens bacteria. From the findings above, *C. vulgaris* can be regarded as a promising feedstuff for ruminal CH_4 production and emission abatement. It can thus be used as a feedstuff for a cleaner environment to mitigate the problem of environmental degradation arising from CH_4 emissions from ruminant production.

Blood biochemistry

Though *C. vulgaris* affected blood parameters of animals, the values were within normal ranges for healthy animals, implying unaffected health of animals consuming *C. vulgaris* (Kholif et al., 2017 d). Blood serum metabolic profiles have been reported by Olafadehan (2011) as good indicators of the nutritional and health status of animals for diagnosis and prognosis of diseases as well as understanding if some changes in animals' diet affect their physiology (Kholif et al., 2017 c; Morsy et al., 2018). Bogdanova and Flerova (2018) evaluated the effect of feeding of *C. vulgaris* at 1.51, 1.82, 2.01, and 2.28 g daily to cows on blood biochemical and hematological parameters. Treatments improved the body's antioxidant system due to enhanced activity of superoxide dismutase and catalase, indicating the absence of any changes in the antioxidant system. Additionally, *C. vulgaris* increased the concentrations of biochemical, hematological and metabolic indicators of the blood (Bogdanova and Flerova, 2018). Increase in hemoglobin concentration indicates improved speed of redox reactions and availability of oxygen for the tissues, thus strengthening metabolic processes in the organs of animals (Bogdanova and Flerova, 2018). Kholif et al. (2017 d) noted that supplementing Damascus goats diets with *C. vulgaris* microalgae at 5 and 10 g/goat/day decreased serum glutamic oxaloacetic transaminase (GPO), glutamic pyruvic transaminase (GPT), and cholesterol concentrations and increased total protein without affecting serum albumin and globulin concentrations. Lamminen et al. (2017) observed using pelleted rapeseed and a combination of *Spirulina platensis* and *C. vulgaris* microalgae (1:1 on DM basis) as supplementary protein sources in the diets of Finnish Ayrshire cows had minor effect on plasma metabolites. Recently, Kholif et al. (2020) observed minimal effects with feeding *C. vulgaris* to

lactating goats on the concentrations of serum total protein, albumin, globulin, urea-N, GOT, or GPT but noted increased serum glucose and decreased serum total lipids. In summary, results of feeding *C. vulgaris* in ruminants show that it did not induce any intoxication in the animals, thus affirming its safety as ingredient in ruminant nutrition.

Performance

Enhancing the performance of animal is the main objective of feed additives supplementation (Azzaz et al., 2020; Sallam et al., 2020). Supplementation with *C. vulgaris* microalgae showed inconsistent results in ruminants. Lamminen et al. (2017) observed that feeding Finnish Ayrshire cows on diets containing pelleted rapeseed supplement and a mixture of *Spirulina platensis* and *C. vulgaris* microalgae (1:1 on DM basis) did not affect total feed intake or milk production. Kholif et al. (2017 d) observed that feeding Damascus goats on diets supplemented with *C. vulgaris* microalgae at 5 and 10 g/goat/day improved milk production, with no additional response to increasing the level of microalgae in the diet from 5 to 10 g daily. Tsiplakou et al. (2018) evaluated the impact of dietary supplementation with microalgae on goat's milk and observed minimal impact on milk yield, chemical composition, and fatty acid profile. Additionally, they observed increased superoxide dismutase (by 10.31%) and catalase (by 18.66%) activities in the blood plasma of goats fed *C. vulgaris* compared with the control, resulting in improved antioxidant status of the animals and their milk. Recently, Kholif et al. (2020) reported that *C. vulgaris* increased daily milk production of goats with greater concentrations of fat and lactose. Moreover, *C. vulgaris* increased milk content of C18:1n9C, total conjugated linoleic acids (CLA) and total unsaturated fatty acids (UFA), while it decreased the concentrations of C11:0, C15:0, C17:0, C18:1n9T and saturated fatty acids (SFA).

Animal health

C. vulgaris contains an active component called CGF that contains peptides, free amino acids, polyamines, glycoproteins, phytohormones, minerals, vitamins, and other yet undefined components that have the ability to promote cell division and growth and tissue renewal (Kotrbaček et al., 2015). Additionally, CGF can stimulate white blood cells production, phagocytic activity, and antibody-producing lymphocytes formation (Kotrbaček et al., 2015). The activity of the CGF is primarily due to its content of polysaccharides and protein complexes. The mechanism of the action of CGF is through the modulation of cytokine formation.

C. vulgaris contains about 0.1–0.2% of its dry weight as β -carotene, which plays important roles in preventing tumor malignancies, protect cells and tissues against the adverse effects of free radicals that disrupt the homeostasis of cell structures, and lower resistance to disease due to its antioxidative effects.

Conclusions and future perspectives

From a nutritional standpoint, *C. vulgaris* microalgae offer a wide range of excellent compounds such as pigments, antioxidants, provitamins, vitamins, and growth factors that affect ruminant performance and enhance animal physiology and health.

Supplementation with *C. vulgaris* microalgae can enhance livestock productivity by improving their body weight gain and milk production and composition. Repeated research should be conducted on *C. vulgaris* in ruminant rations to establish or refute some of current findings due to some conflicting reports in the literature. Most of the information available on the effect *C. vulgaris* feeding in ruminants focuses majorly on productive performance with little emphasis on reproductive response of the animals. Further investigations are needed on the effect of *C. vulgaris* feeding on the reproductive performance of wool or fiber-producing, meat, and dairy ruminants.

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