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CHLORELLA VULGARIS MICROALGAE AND COPPER MIXTURE SUPPLEMENTATION ENHANCED THE NUTRIENT DIGESTIBILITY AND MILK ATTRIBUTES IN LACTATING BOER GOATS

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Abstract

Thirty-two lactating Boer goats (35.2 ± 1.4 kg body weight) were grouped into control and three treatment groups in completely randomised design. In treatment groups, supplementation was done as a mixture of 5 g *Chlorella vulgaris* + 4.5 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg5 treatment), 10 g *C. vulgaris* + 9 mg of supplemental Cu/kg diet (Alg10 treatment), or a mixture of 15 g *C. vulgaris* + 13.5 mg of supplemental Cu/kg diet (Alg15 treatment). Treatments did not affect feed intake; however, Alg10 treatment increased (P<0.001) nutrient digestibility. Treatments did not affect ruminal pH, ammonia-N, butyrate; however, the Alg10 treatment increased (P<0.01) ruminal total volatile fatty acids, propionate and acetate concentrations. Without affecting other blood measurements, the Alg10 treatment quadratically increased (P<0.001) serum glucose and Cu. The Alg10 treatments increased (P<0.001) daily milk production and the concentration of fat, and enhanced milk (feed) efficiency. The Alg10 treatment decreased (P<0.05) milk saturated fatty acids and the atherogenic index, and increased the proportions of total conjugated linoleic acids, C18:1n9t, odd fatty acids and total unsaturated fatty acids compared with the control treatment. Present study concluded that inclusion of a mixture of 10 g *C. vulgaris* + 9 mg Cu/kg diet in the diet of lactating Boer goats enhanced nutrient digestibility, ruminal fermentation, milk production, feed efficiency as well as milk nutritive value. Increasing the dose of the mixture to 15 g *C. vulgaris* + 13.5 mg Cu/kg diet is not recommended in the diet of lactating Boer goats.

Key words: *Chlorella vulgaris*, copper, microalgae, lactational performance, nutrient utilisation

The use of microalgae as a feed or feed supplement for ruminants gained increasing interests (Marrez et al., 2017; Gomaa et al., 2018; Kholif et al., 2020). Supplementing diets of animals with microalgae, at small amounts, showed positive effects on animal physiology, health status and productivity (Póti et al., 2015; Kholif et al., 2017 d, 2020). *Chlorella vulgaris* microalgae supplementation to dairy animals enhanced daily milk production and altered milk nutritive value by reducing saturated fatty acids (SFA) and increasing unsaturated fatty acids (UFA) concentrations

(Kholif et al., 2017 d), due to its content of crude protein (CP), amino acids, UFA, minerals and vitamins (Kholif et al., 2017 b). Additionally, *C. vulgaris* microalgae contain a detoxicant with an ability to inhibit oxidation which is known as Chlorella Growth Factor (CGF) (Han et al., 2002; Kholif et al., 2017 b). Supplementing diets of lactating goats with *C. vulgaris* enhanced nutrient utilisation, milk yield and composition in lactating goats (Kholif et al., 2017 d). Moreover, Lamminen et al. (2019) substituted soybean meal with *C. vulgaris* in the diet of lactating Finnish Ayrshire cows and observed a positive effect on milk fat concentration.

The inclusion of copper (Cu) in diets of animals is essential for many enzymes and synthesis of connective tissues, transport of iron and formation of haemoglobin (NRC, 2007). Increasing the concentration of Cu in diets of animals showed some positive effects on the animal performance (Kholif et al., 2020). Moreover, Roshanzamir et al. (2020) observed that supplementing lactating cows with trace minerals such as Cu had positive effects on dry matter (DM) intake and digestibility, lactational performance, somatic cell counts and milk total antioxidant capacity. Recently, Kholif et al. (2020) observed that supplementing diets of lactating Boer goats with *C. vulgaris* and Cu mixture showed positive effects on feed utilization and lactational performance.

Therefore, the present study aimed to compare three doses of a mixture of *C. vulgaris* microalgae and Cu on feed intake and digestibility, ruminal fermentation, blood parameters and lactational performance of Boer goats. We hypothesized that combining *C. vulgaris* and Cu will alter digestion process and improve ruminal fermentation resulting in enhanced production performance of goats.

Material and methods

The experiment was carried out at Dakhla oasis in the New Valley, located in the western Egyptian desert, which is an arid tropical area. The ambient temperature and relative humidity during the experiment ranged from 15 to 19°C and 35 to 41%, respectively. Chemical analyses were performed at the Laboratory of Dairy Animal Production, National Research Centre (Egypt). Goats were managed in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Federation of Animal Science Societies, Champaign, IL, USA).

Chlorella vulgaris microalgae

C. vulgaris biomass was obtained from the algal biotechnology unit, National Research Centre (Egypt). Inoculum was prepared using BG-II growth medium (Stanier et al., 1971) as described in details in Kholif et al. (2017 d). Chemical analysis of the *C. vulgaris* microalgae has been summarised by Kholif et al. (2017 d) as (/kg DM): 942 g DM, 905 g organic matter (OM), 515 g CP, 122 ether extract (EE), 119 g non-structural carbohydrate (NSC) and 92 g fibres. Additionally, the fatty acid profile was (/kg DM): 19 g C14:0, 526 g C16:0, 159 g C17:1, 88 g C18:0, 54 g C18:1, 66 g C18:2, 89 g C18:3, 632 g SFA and 367 g total UFA. The amino acids

content was (/kg DM): 105 g aspartic acid, 52 g threonine, 51 g serine, 107 g glutamic acid, 51 g glycine, 84 g alanine, 64 g valine, 15 g methionine, 50 g isoleucine, 68 g leucine, 52 g tyrosine, 42 g phenylalanine, 50 g histidine, 56 g lysine, 82 g arginine and 64 g proline.

Goats, feeding and experimental design

One week after parturition, 32 multiparous lactating Boer goats (35.2 ± 1.4 kg BW) were assigned randomly to four experimental groups (8 does per treatment) as per a completely randomized design. Goats were housed individually in soil-surfaced pens (1.5 m^2/goat), with unrestricted access to water and experimental diets to meet their energy and nutrient requirements according to NRC (2007) recommendations. Adjustments were made to the diets to ensure collection of orts. Duration of experimental feeding was 12 weeks.

Table 1. Ingredient and chemical composition of the control diet (g/kg DM)

Item	Value
Ingredient	
berseem hay	300
wheat straw	200
corn	275
wheat bran	125
soybean meal	85
limestone	10
sodium chloride	2.5
minerals and vitamins mixture[1]	2.5
Analysed composition	
dry matter (g/kg wet material)	908
organic matter	903
crude protein	151
ether extract	20
non-structural carbohydrate	307
neutral detergent fibre	426
acid detergent fibre	271
Cu (mg/kg DM)	15.3

[1]Provided per kilogram of the diet: 30 mg of Zn as Zn $SO_4 \cdot 7H_2O$; 20 mg of Mn as $MnSO_4 \cdot H_2O$; 0.5 mg of I as KI; 0.1 mg of Co as $CoCl_2$; 0.1 mg Se as Na_2SeO_3; 1500 IU of vitamin A; 250 IU of vitamin D and 16 IU of vitamin E.

The basal diet of does contained (/kg DM) 300 g berseem hay (*Trifolium alexandrinum*), 200 g wheat straw (*Triticum aestivum*) and 500 g of concentrates feed mixture (containing per kg DM: 550 g corn, 250 g wheat bran, 170 g soybean meal, 20 g limestone, 5 g sodium chloride, and 5 g minerals and vitamins mixture). Ingredients and chemical composition (g/kg DM) of the control diet are presented in Table 1.

Goats were fed the control diet without supplements or with addition of a mixture of 5 g *C. vulgaris* + 4.5 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg5 treatment), 10 g *C. vulgaris* + 9 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg10 treatment), or a mixture of 15 g *C. vulgaris* + 13.5 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg15 treatment). Diets were offered to each goat individually at 08:00 and 16:00 h in two equal portions. Copper sulphate was used as a source of Cu. Copper requirements were calculated according to NRC (2007). Daily allocation of *C. vulgaris* and Cu mixture for each goat was administered in-feed (in 100 g of the concentrate feed mixture) before morning feeding at 08:00 h to ensure the full dose was received. Samples of the berseem hay, wheat straw and concentrate mixture were taken daily, composited weekly and dried at 60°C in a forced-air oven for 48 h. Samples were ground to pass a 1-mm screen using a Wiley mill and then stored for chemical analysis.

Digestibility and chemical analysis

In normal feeding area, two digestion trials were conducted at the 8th and 12th week (7 d for each collection period) of the experiment. During these periods, DM intake was recorded daily by weighing feeds offered and orts. Faecal grab samples were collected twice daily at 07:00 and 15:00 h, dried at 60°C in a forced-air oven for 72 h (AOAC, 1997) and pooled by goat within period. An acid-insoluble ash was used as the internal indigestibility marker (Sales and Janssens, 2003). Acid-insoluble ash contents of feeds and faeces were determined gravimetrically after drying, ashing, boiling of ash in hydrochloric acid (HCl), filtering and washing of the hot hydrolysate, and re-ashing.

Feed, orts and faecal samples were analysed for DM, ash, nitrogen, and EE, according to AOAC (1997) official methods. Neutral detergent fibre (NDF) was determined by the procedure of Van Soest et al. (1991) with the use of alpha amylase and sodium sulphite. Acid detergent fibre (ADF) was analysed according to AOAC (1997). Acid detergent lignin (ADL) was analysed by solubilisation of cellulose with sulphuric acid in the ADF residue according to Van Soest et al. (1991). Non-structural carbohydrates (1000 – (NDF + crude protein (CP) + EE + ash) and OM (1000 – ash) were calculated.

Moreover, the control diet was analysed for Cu concentration in the Regional Centre for Food and Feed (RCFF) using atomic absorption spectrometry (atomic absorption spectrometry, Thermo Elemental, UK).

Sampling and analysis of rumen fluid

On day 7 of the 8th and 12th week of the experiment, ruminal contents were sampled at 3 h post-morning feeding to determine pH and concentration of fermentation end-products as describes in details in Kholif et al. (2018 a). Rumen contents (~ 100 mL) were collected once from the ventral sac by using a stomach tube, and then composite samples taken from each goat were strained through four layers of cheesecloth. The pH of ruminal fluid was measured immediately using a pH meter (HI98127 pHep®4 pH/Temperature Tester, Hanna® instrument, Italy).

A sub-sample of 5 mL was preserved in 5 mL of 0.2 *M* HCl for ammonia-N analysis and 0.8 mL of rumen liquor was mixed with 0.2 mL of a solution containing

250 g of metaphosphoric acid/L for total volatile fatty acid (VFA) analysis as describes in details in Kholif et al. (2017 a). Samples were stored at –20°C until laboratory analysis. The concentration of ruminal ammonia-N was determined according to AOAC (1997). Total VFA concentration in samples was determined by titration, after steam distillation of a 4 mL sample. The molar proportions of the individual VFA were measured by gas-liquid chromatography (Varian 3700; Varian Specialties Ltd, Brockville, Ontario, Canada). Separation was carried out with a capillary column (30 m × 0·25 mm internal diameter, 1 m film thickness, Supelco Nukol; Sigma–Aldrich, Mississauga, ON, Canada) and flame ionization detection. The column temperature was adjusted to 100°C for 1 min, then increased by 20°C/min to 140°C, then by 8°C/min to 200 °C, and held at this temperature for 5 min. The injector temperature was 200°C while the detector temperature was 250°C More details can be found in Matloup et al. (2017). Helium was used as the carrier gas.

Methane (CH_4) production was calculated according to Moss et al. (2000) as: CH_4 production (mmol/L) = 0.45 × acetate − 0.275 × propionate + 0.4 × butyrate.

Sampling and analysis of blood serum

On day 7 of the 8th and 12th week, blood samples (10 mL) were taken 4 h after feeding from the jugular vein of each goat into a clean dry tube, without anti-coagulants. Blood samples were centrifuged at 4000 ×*g* at 4°C for 20 min. Serum was separated into 2-mL clean dried Eppendorf tubes and frozen at –20°C until analysis. More details can be found in Kholif et al. (2018 b).

Blood serum samples were analysed for concentrations of total protein, albumin, urea-N, glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT), glucose, total lipids and Cu using specific kits (Stanbio Laboratory, Boerne, Texas, USA) following manufacturer's instructions. Globulin concentration was calculated by subtracting albumin values from their corresponding total protein values.

Milk sampling, milk composition and fatty acid analysis

Goats were milked by hand twice daily at 09:00 and 21:00 h biweekly (7 days per week), and samples (100 g/kg of recorded milk yield) were collected at each milking. A mixed sample of milk (proportional to amounts produced in the morning and evening) was taken daily as describes in Kholif et al. (2018 c). Milk samples were analysed for total solids, fat, protein, lactose and ash using infrared spectrophotometry (Milkotester LM2, Belovo, Bulgaria). Fatty acids in milk were determined using methyl esters prepared by base-catalysed methanolysis of the glycerides (KOH in methanol) according to International Standards (International Standard ISO 15884-IDF 182. 2002. Brussels, Belgium: International Dairy Federation) on a Perkin-Elmer chromatograph (model 8420, Beaconsfield, Perkin Elmer, Beaconsfield, UK) equipped with a flame ionization detector. More details can be found in Kholif et al. (2017 c). Atherogenic index (AI) was calculated according to Ulbricht and Southgate (1991) as: AI = (C12:0 + 4 × C14:0 + C16:0)/∑ of UFA.

Average yield (g/d) of each milk component was calculated by multiplying milk yield by the component content (g/kg). Gross energy content in milk was calculated according to Tyrrell and Reid (1965) as: milk energy content (MJ/kg) =

4.184 × 2.204 × [(41.63 × fat (g/100 g) + 24.13 × protein (g/100 g) + 21.60 × lactose (g/100 g) – 11.72)/1000]. Milk energy output (MJ/d) was calculated as milk energy (MJ/kg) × milk yield (kg/d).

Energy corrected milk (ECM) was calculated according to Sjaunja et al. (1991) as: ECM (kg/d) = milk (kg/d) × [38.3 × fat (g/kg) + 24.2 × protein (g/kg) + 16.54 × lactose (g/kg) + 20.7]/3140.

Statistical analysis

Data on intake, apparent diet digestibility, ruminal fermentation, blood chemistry parameters, milk yield and composition (including fatty acid profile) were analysed using a completely randomized design with repeated measurements in time, where each goat was an experimental unit using PROC MIXED of SAS (Version 9.4, SAS Inst., Inc., Cary, NC) with the following model:

$$Y_{ijkl} = m + T_i + A_j(T_i) + W_k + (T \times W)_{ik} + E_{ijkl}$$

where:

Y_{ijkl} expressed each observation of the *j*th goat in the *k*th sampling time given *i*th treatment,

T_i expressed the treatments effect,

$A(T)_{ji}$ expressed the goat within treatments,

W_k expressed the sampling week effect,

$(T \times W)_{ik}$ expressed the interaction between the treatments and sampling week,

E_{ijkl} expressed the experimental error.

Two covariance structures were considered in the REPEATED statement in PROC MIXED: compound symmetry (cs) and auto-regressive (AR(1)). The error structure with the lowest Akaike information criteria that fits statistics was selected for the model. When the treatment *F*-test was significant at $P<0.05$, means were then compared by applying the probability of difference option of the least squares means statement and PDIFF option. Additionally, polynomial (linear and quadratic) contrasts (adjusted for the equal spacing of treatments) were used to examine dose responses to increasing doses of the additives mixture and for comparison between control *vs*. the average of additives mixture treatments. Significance was declared at a level of $P<0.05$. The week effect and treatment × week interaction were non-significant (i.e., $P>0.05$) for most of the measurements; thus, only the main effects of treatments were reported.

Results

Feed intake and digestibility

Treatments did not affect feed intake (Table 2). The Alg10 treatment quadratically ($P<0.05$) increased nutrient digestibility compared with the control. The Alg15 treatment quadratically ($P<0.05$) decreased DM digestibility, without affecting the digestibility of other nutrients compared with the control treatment. Both of Alg5 and Alg10 treatments had improved NSC, NDF and ADF digestibilities compared with the control.

Table 2. Feed intake and digestibility of nutrients in different groups of lactating Boer goat

Item	Group[1]				SEM	P-value			
	control	Alg5	Alg10	Alg15		treatment	control vs. others	linear	quadratic
Dry matter intake (g/d)	799	796	799	804	7.8	0.917	0.960	0.634	0.599
Digestibility (g absorbed/kg ingested DM)									
dry matter	625 b	643 b	682 a	598 c	9.0	<0.001	0.114	0.282	<0.001
organic matter	577 c	614 b	645 a	553 c	9.6	<0.001	0.021	0.347	<0.001
crude protein	566	598	600	577	12.6	0.171	0.083	0.545	0.033
ether extract	578 c	615 b	647 a	569 c	9.0	<0.001	0.004	0.885	<0.001
non-structural carbohydrate	572 b	614 a	638 a	550 b	10.1	<0.001	0.019	0.358	<0.001
neutral detergent fibre	590 b	624 a	646 a	573 b	9.1	<0.001	0.027	0.459	<0.001
acid detergent fibre	541 b	571 a	580 a	534 b	9.8	0.005	0.079	0.777	0.001

Means in the same row with different letters differ, P<0.05. P-value is the observed significance level of the *F*-test for treatment; SEM = standard error of the mean.

[1]The control diet based on (per kg DM): 500 g of concentrates feed mixture, 300 g berseem hay and 200 g wheat straw without addition of supplements (Control treatment) or with addition of a mixture of 5 g *C. vulgaris* + 4.5 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg5 treatment), 10 g *C. vulgaris* + 9 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg10 treatment), or a mixture of 15 g *C. vulgaris* + 13.5 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg15 treatment).

Table 3. Ruminal fermentation and serum metabolites in different groups of lactating Boer goat

	Group[1]				SEM	P-value			
	control	Alg5	Alg10	Alg15		treatment	control vs. others	linear	quadratic
Rumen fermentation (3 h after feeding)									
pH	6.09	6.11	6.12	6.11	0.059	0.987	0.732	0.791	0.799
ammonia-N (g/L)	25.5	26.2	27.0	25.9	0.64	0.240	0.241	0.139	0.113
volatile fatty acids (VFA, mmol/L)	106 b	107 b	117 a	101 c	1.3	<0.001	0.212	0.433	<0.001
acetate (mmol/L)	64.5 b	64.2 b	69.4 a	61.3 c	0.79	<0.001	0.604	0.241	<0.001
propionate (mmol/l)	27.9 b	27.5 b	31.5 a	26.8 b	0.54	<0.001	0.298	0.785	0.001
butyrate (mmol/l)	13.8	14.9	15.7	13.3	0.72	0.106	0.361	0.785	0.023
acetate/propionate ratio	2.32	2.35	2.21	2.29	0.049	0.271	0.573	0.361	0.611
methane (CH_4, mmol/L)[2]	25.3	25.6	24.7	25.2	0.30	0.249	0.722	0.369	0.820
CH_4/VFA ratio	0.24 a	0.24 a	0.21 b	0.25 a	0.004	<0.001	0.365	0.876	0.002
Blood serum chemistry (mg/dL, unless stated otherwise)									
total proteins	6.37	6.34	6.47	6.43	0.149	0.915	0.801	0.633	0.976
albumin	3.73	3.77	3.76	3.77	0.125	0.995	0.787	0.834	0.880
globulin	2.64	2.57	2.71	2.66	0.185	0.960	0.984	0.808	0.938
albumin/globulin ratio	1.57	1.57	1.45	1.49	0.171	0.944	0.729	0.635	0.901
urea-N	28.0	27.2	28.3	27.7	0.64	0.683	0.680	0.978	0.892
glucose	58.8 b	64.6 a	66.7 a	60.9 b	1.10	<0.001	0.003	0.100	<0.001
glutamate-pyruvic transaminase (Units/L)	18.2	17.7	17.6	18.0	0.33	0.526	0.203	0.534	0.181
glutamate-oxaloacetate transaminase (Units/L)	33.5	33.3	33.5	32.7	0.50	0.640	0.575	0.337	0.549
total lipids	225 a	203 b	199 b	201 b	4.0	0.002	<0.001	0.002	0.006
Cu (μg/dL)	84.1 b	86.3 b	89.5 a	90.8 a	1.83	0.033	0.032	0.008	<0.001

Means in the same row with different letters differ, P<0.05. P-value is the observed significance level of the *F*-test for treatment; SEM = standard error of the mean.

[1]The control diet based on (per kg DM): 500 g of concentrates feed mixture, 300 g berseem hay and 200 g wheat straw without addition of supplements (Control treatment) or with addition of a mixture of 5 g *C. vulgaris* + 4.5 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg5 treatment), 10 g *C. vulgaris* + 9 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg10 treatment), or a mixture of 15 g *C. vulgaris* + 13.5 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg15 treatment).

[2]Methane production (mmol/L) = 0.45 (acetate) − 0.275 (propionate) + 0.4 (butyrate); (Moss et al., 2000).

Table 4. Milk yield and composition in different groups of lactating Boer goat

	Group[1]				SEM	P-value			
	control	Alg5	Alg10	Alg15		treatment	control vs. others	linear	quadratic
Production (g/d)									
milk	745 c	782 b	820 a	720 d	19.0	0.002	0.190	0.665	0.004
energy corrected milk (ECM)	729 c	786 b	827 a	703 c	13.2	<0.001	0.005	0.527	<0.001
total solids	93.5 b	100.6 a	104.7 a	90.7 b	1.87	<0.001	0.020	0.582	<0.001
solids not fat	65.8 b	70.4 a	71.4 a	63.9 b	1.42	0.004	0.092	0.467	<0.001
fat	27.7 b	30.2 a	33.2 a	26.7 b	0.51	<0.001	0.001	0.990	<0.001
protein	28.3 b	30.3 a	29.9 a	26.6 b	0.45	<0.001	0.221	0.007	<0.001
lactose	31.7 b	34.1 a	35.2 a	31.6 b	0.92	0.013	0.072	0.830	0.002
ash	5.75	5.99	6.29	5.64	0.20	0.106	0.334	0.972	0.027
milk energy output (MJ/d)	2.25 c	2.43 b	2.55 a	2.17 c	0.041	<0.001	0.005	0.585	<0.001
Milk component (g/kg)									
total solids	127	129	129	127	1.1	0.229	0.200	0.959	0.039
solids not fat	88.9	90.4	87.8	89.3	0.59	0.170	0.686	0.605	0.976
fat	37.9 b	38.8 b	41.2 a	37.7 b	0.56	<0.001	0.049	0.524	0.001
protein	38.8	39.3	37.3	37.8	0.67	0.131	0.432	0.110	0.997
lactose	42.5	43.5	42.9	43.7	0.97	0.600	0.601	0.110	0.833
ash	7.63	7.61	7.61	7.74	0.088	0.685	0.792	0.385	0.413
milk energy content (MJ/kg)	3.06	3.12	3.16	3.05	0.033	0.066	0.170	0.925	0.010
Milk efficiency									
milk yield/DM intake	0.94 b	0.98 ab	1.03 a	0.90 b	0.025	0.002	0.238	0.566	0.004
ECM yield/DM intake	0.92 b	0.99 a	1.00 a	0.88 b	0.019	<0.001	0.014	0.479	<0.001

Means in the same row with different letters differ, P<0.05. P-value is the observed significance level of the *F*-test for treatment; SEM = standard error of the mean.

[1]The control diet based on (per kg DM): 500 g of concentrates feed mixture, 300 g berseem hay and 200 g wheat straw without addition of supplements (Control treatment) or with addition of a mixture of 5 g *C. vulgaris* + 4.5 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg5 treatment), 10 g *C. vulgaris* + 9 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg10 treatment), or a mixture of 15 g *C. vulgaris* + 13.5 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg15 treatment).

Table 5. Fatty acids profile (g/100 g total fatty acids) in milk of different groups of lactating Boer goat

Fatty acid	Group[1]				SEM	P-value			
	control	Alg5	Alg10	Alg15		treatment	control vs. others	linear	quadratic
C4:0	4.02	4.38	4.38	4.27	0.146	0.294	0.074	0.287	0.132
C6:0	1.46	1.49	1.47	1.52	0.045	0.780	0.521	0.428	0.790
C8:0	2.58	2.60	2.51	2.43	0.099	0.984	0.977	0.981	0.985
C10:0	5.77	6.01	5.70	5.70	0.123	0.193	0.812	0.315	0.301
C11:0	0.95	0.99	0.91	0.91	0.032	0.301	0.650	0.189	0.638
C12:0	2.51	2.60	2.89	2.95	0.164	0.209	0.123	0.045	0.923
C14:0	9.80 a	9.23 a	8.88 b	9.36 a	0.187	0.017	0.007	0.066	0.011
C14:1	0.29	0.32	0.37	0.32	0.029	0.262	0.175	0.314	0.166
C15:0	1.17 a	0.84 b	0.94 b	0.85 b	0.030	0.044	0.011	0.042	0.211
C16:0	26.8	26.1	25.6	24.9	1.40	0.220	0.010	0.102	0.945
C16:1	1.23	1.31	1.36	1.11	0.094	0.309	0.827	0.461	0.101
C17:0	0.99 a	0.88 b	0.79 b	0.84 b	0.026	0.018	0.031	0.047	0.017
C18:0	12.8	12.7	11.8	13.1	0.42	0.135	0.510	0.915	0.090
C18:1n9c	2.95	2.74	3.22	2.99	0.188	0.356	0.868	0.492	0.938
C18:1n9t	24.7 b	25.9 b	27.2 a	26.9 a	0.39	0.006	0.002	0.002	0.064
cis-9, trans-11C18:2	0.15	0.15	0.14	0.15	0.006	0.727	0.673	0.899	0.389
trans-10, cis-12 C18:2	0.47	0.48	0.55	0.50	0.081	0.890	0.660	0.688	0.672
C18:3n-3	0.14	0.14	0.15	0.15	0.009	0.703	0.348	0.299	0.734
C18:3n-6	0.32	0.32	0.35	0.35	0.010	0.071	0.086	0.020	0.835
C20:0	0.79	0.78	0.73	0.74	0.024	0.210	0.177	0.083	0.687

Total saturated fatty acids (SFA)	69.7 a	68.7 a	66.6 b	67.6 b	0.39	<0.001	<0.001	<0.001	0.015
Total unsaturated fatty acids (UFA)	30.3 b	31.3 b	33.4 a	32.4 a	0.38	<0.001	<0.001	<0.001	0.015
Monounsaturated fatty acids	29.1 b	30.2 b	32.2 a	31.3 ab	0.38	<0.001	0.001	<0.001	0.010
Polyunsaturated fatty acids	1.20	1.10	1.20	1.10	0.082	0.729	0.468	0.423	0.671
Total conjugated linoleic acid	0.62 b	0.63 b	0.70 a	0.65 b	0.011	0.013	0.080	0.694	0.015
ω-6/ω-3 ratio	2.47	2.40	2.43	2.47	0.213	0.994	0.885	0.969	0.798
UFA/SFA ratio	0.43	0.46	0.50	0.48	0.009	<0.001	0.001	0.001	0.012
Atherogenic index[2]	2.28 a	2.11 a	1.94 b	2.02 a	0.044	0.001	<0.001	0.001	0.011

Means in the same row with different letters differ, P<0.05. P-value is the observed significance level of the *F*-test for treatment; SEM = standard error of the mean.

[1]The control diet based on (per kg DM): 500 g of concentrates feed mixture, 300 g berseem hay and 200 g wheat straw without addition of supplements (Control treatment) or with addition of a mixture of 5 g *C. vulgaris* + 4.5 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg5 treatment), 10 g *C. vulgaris* + 9 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg10 treatment), or a mixture of 15 g *C. vulgaris* + 13.5 mg of supplemental Cu/kg diet from $CuSO_4$ (Alg15 treatment).

[2] Calculated according to Ulbricht and Southgate.

Ruminal fermentation kinetics and blood parameters

There were no treatment effects on ruminal pH, ammonia-N, butyrate, acetate/propionate ratio, CH_4, or CH_4/VFA ratio, but Alg10 treatment quadratically increased ($P<0.01$) ruminal total VFA, propionate and acetate concentrations, and decreased CH_4/VFA ratio compared with the control (Table 3). The Alg15 quadratically decreased VFA and acetate concentrations. Treatments did not affect butyrate, acetate/propionate ratio and CH_4 concentration.

Treatments had no effect on the concentration of serum total proteins, albumin, globulin, albumin/globulin ratio, urea-N, GOT or GPT (Table 3). The Alg10 treatment quadratically increased ($P<0.001$) serum glucose and Cu, while Alg5, Alg10 and Alg15 treatments decreased ($P=0.002$) serum total lipids compared with the control treatment.

Milk yield and composition

The Alg5 and Alg10 treatments quadratically increased ($P<0.001$) daily milk production, ECM and yields of milk components compared with the control treatment, but the Alg15 treatment decreased them (Table 4). Additionally, the Alg10 treatment quadratically increased ($P<0.001$) the concentration of fat, and enhanced milk (feed) efficiency.

Milk fatty acid profile

The Alg10 treatment quadratically decreased ($P<0.05$) the concentration of C14:0 and AI, and increased ($P<0.05$) the concentration of total conjugated linoleic acids (CLA) (Table 5). Both of Alg10 and Alg15 treatment quadratically decreased ($P<0.05$) total SFA and increased the concentrations of C18:1n9t and total UFA compared with the control treatment. Alg5, Alg10 and Alg15 treatment decreased the proportion of the linear odd-chain (C15:0 and C17:0 fatty acids) compared with the control.

Discussion

Feed intake and apparent diet digestibility

No effect of feed intake with *C. vulgaris* and Cu supplementation is consistent with previous reports for the mixture (Kholif et al., 2020) or *C. vulgaris* alone (Lamminen et al., 2019). Kholif et al. (2020) reported that feeding the same mixture of additives at 10 g of *C. vulgaris* microalgae and 9 mg Cu/kg diet did not affect feed intake. However, Kholif et al. (2017 d) observed that the inclusion of *C. vulgaris* at 10 g daily in the diet of lactating Damascus goats increased feed intake by 12%. In another study, Roshanzamir et al. (2020) observed that Cu supplementation to Holstein cows increased feed intake due to the enhanced DM and NDF digestibilities and the influence of the trace mineral on appetite (Wu, 2018). The inconsistency from these studies may be related to differences between diets and feeding levels of *C. vulgaris* or Cu.

Increased nutrient digestibility with Alg10 treatment inclusion may be related with the presence of Cu and CGF in *C. vulgaris* microalgae which are known to increase digestibility (Han et al., 2002; Kholif et al., 2017 d, 2020). The CGF is very rich in nucleic acids, amino acids, peptides, vitamins, minerals, polysaccharides, glycoproteins, and β-glucans. In addition, CGF, the most powerful constituent of *C. vulgaris* microalgae, works as a probiotic that enhances the growth and quality of ruminal and intestinal microflora life, resulting in enhanced nutrients digestion (Han et al., 2002). In their experiment, Tsiplakou et al. (2017) observed that *C. vulgaris* increased the number of ruminal *Butyrivibrio fibrisolvens*, *Ruminococcus albus* and *Clostridium sticklandii* in goats. Additionally, Iwamoto (2004) reported that *C. vulgaris* contains β-glucan, which plays a role in scavenging free radicals resulting in improved ruminal fermentation and digestion. The presence of poly UFA, carotenoids, phycobiliproteins, polysaccharides and phycotoxins in *C. vulgaris* microalgae cannot be ignored for greater microbial growth (Kotrbáček et al., 2015). Moreover, the inclusion of Cu in the diet enhanced nutrient digestibility in Holstein cows as reported by Roshanzamir et al. (2020). Copper has beneficial effects on promoting a healthy gut, thereby enhancing enzymatic digestion of nutrients and rumen fermentation (Wu, 2018; Roshanzamir et al., 2020). Moreover, Cu is essential for the growth and activity of ruminal microbes and enzymatic activity responsible for fibre digestion (NRC, 2007).

Ruminal fermentation

Treatments did not affect ruminal pH, and values ranged from 6.06 to 6.16 which is greater than values reported by Ørskov and Ryle (1990) for normal ruminal fermentation and fibre digestion. Moreover, ruminal ammonia-N concentration ranged from 25.5 to 27.0 g/L, being approximately five times greater than the recommended base level for efficient microbial production (Satter and Slyter, 1974). Results of ruminal ammonia-N concentrations are paralleled with those of CP digestibility.

C. vulgaris microalgae and Cu at 10 g and 9 mg, respectively had greater ruminal total VFA, which may be a result of enhanced nutrient digestibility. Kholif et al. (2020) observed increased VFA with the mixture of *C. vulgaris* microalgae and Cu (i.e., Alg10 treatment) fed to lactating goats without affecting ruminal pH and ammonia-N concentrations. Increased acetate concentration with Alg10 treatment is consistent with the result of increased fibre digestion, as reported by France and Dijkstra (2009) who stated that increasing fibre digestion or intake favours the growth of acetate producing rumen bacteria. These observations can partially explain the observed increased milk fat concentration with Alg10 treatment.

The increased propionate concentration with Alg10 treatment may be a result of NSC digestibility. Increasing concentration of propionate is considered beneficial in ruminant nutrition and dairy production because propionate provides greater energy for milk production than acetate, as propionate is the primary gluconeogenic VFA that can be used for lactose biosynthesis (Kholif, 2019). Altered ruminal individual VFA might be related to the positive effects of components in the mixture of *C. vulgaris* microalgae and Cu on ruminal bacteria. As direct measurements of the

microbial population were not made in the present study, additional research will be needed to address this possibility.

Decreasing CH_4/VFA ratio in Alg10 treatment indicates a change in the ruminal microbial profile. The reduction in CH_4/VFA ratio may be related to the presence of poly UFA in *C. vulgaris* which have antimicrobial effects on methanogens and protozoa (Gomaa et al., 2018). Kholif et al. (2020) observed a decreased CH_4/VFA ratio with feeding the same additive mixture at 10 g *C. vulgaris* microalgae and 9 mg Cu.

Blood metabolites

The minimal effects of treatments on blood metabolites, which were within reference ranges for healthy goats, indicate minimal effects on the nutritional status, protein catabolism in muscles and kidney function.

However, liver is the main site of Cu storage in animal body, the minimal effects of *C. vulgaris* microalgae and Cu mixture on the concentrations of serum GPT and GOT are important indicators of normal liver activity, function or hepatotoxicity with feeding additives mixture. Kholif et al. (2020) observed unaffected values of serum GPT and GOT with feeding *C. vulgaris* microalgae and Cu mixture to goats, indicating a probable protective role for *C. vulgaris* against liver dysfunction.

C. vulgaris and Cu enhanced serum glucose concentration as a result of the improved OM and NSC digestibilities, and greater total VFA and propionate production. Kholif et al. (2020) observed similar results with the same additives mixture.

The mechanism by which *C. vulgaris* and Cu mixture supplementation reduced plasma total lipids has not been fully explored. By up-regulation of the activities of lipoprotein and triglycerides lipase, microalgae are able to modify lipoprotein metabolism (Karkos et al., 2011), due to its content of α-glucan which may have contributed to reduced plasma lipids (Iwamoto, 2004).

Both of Alg10 and Alg15 treatments increased serum Cu concentration, which may be due to more absorption of Cu from the gastrointestinal tract into the blood (Wallace and Underwood, 1968). Increased serum Cu concentration with feeding *C. vulgaris* microalgae and Cu was observed by Kholif et al. (2020). Moreover, Roshanzamir et al. (2020) reported that supplementing diets of lactating cows with Cu increased serum Cu concentration.

Milk production and composition

Supplementation of *C. vulgaris* microalgae and Cu in the Alg10 treatment enhanced daily milk production and ECM by 10.1 and 13.5%, respectively. Additionally, increased milk production without corresponding increase in feed intake indicates an enhanced feed efficiency by 10.2 and 13.6% for milk/DM intake and ECM/DM intake, respectively. Increasing nutrient digestibility and ruminal fermentation (total VFA and propionate) are the main reasons for the enhanced milk yield. Additionally, *C. vulgaris* contains several essential nutrients which could have contributed to the observed increase in milk yield (Kholif et al., 2017 d). Our results are consistent with those reported by Kholif et al. (2020). Additionally, Kholif et al. (2017 d) and

Lamminen et al. (2019) reported that feeding *C. vulgaris* to lactating cows or goats increased milk and ECM yields.

The presence of Cu in the additives mixture resulted in positive effects on milk production (Wu, 2018; Roshanzamir et al., 2020). Copper plays an important role in structural proteins, enzymes and hormones necessary for biochemical and physiological functions (Wallace and Underwood, 1968). Roshanzamir et al. (2020) reported that supplementing diets of lactating cows increased milk production. The negative effects of increasing the dose of *C. vulgaris* and Cu supplementation (i.e., Alg15 treatment) may be due to their effects on feed digestion and ruminal fermentation.

C. vulgaris and Cu at 10 g and 9 mg, respectively (i.e. Alg10 treatment) enhanced the concentrations of fat by 8.7, which is consistent with the result of Kholif et al. (2020) and inconsistent with the results of Kholif et al. (2017 d) and Lamminen et al. (2019) who observed increased concentrations of milk fat with *C. vulgaris* supplementation. Increased milk fat with *C. vulgaris* microalgae and Cu inclusion is consistent with observed increase in ruminal acetate concentration in Alg10 treatment. Milk fat can originate from more acetate intensive ruminal fermentation leading to increased amounts of milk fat precursors in blood (Kholif et al., 2017 d; Lamminen et al., 2019). Lamminen et al. (2019) reported that increasing ruminal acetate increases mammary uptake of acetic acid about two fold.

Additionally, *C. vulgaris* enriched with Cu in the Alg10 treatment enhanced the concentration of lactose which is not consistent with the result of ruminal propionate in the present study. Ruminal propionate can be efficiently utilised to produce milk lactose and provide goats with energy (El-Zaiat et al., 2019; Kholif, 2019). The observed increase in serum glucose with *C. vulgaris* and Cu (i.e., Alg10 treatment) supports this assumption/speculation (Sallam et al., 2020).

Milk fatty acid profile

The profile of fatty acids in milk is very sensitive to dietary changes or feed additives (Morsy et al., 2015, 2018; Kholif et al., 2019). Treatments decreased the concentrations of the linear odd-chain fatty acids (C15:0 and C17:0) suggesting a decreased *de novo* synthesis from ruminal propionate in mammary gland due to *C. vulgaris* microalgae and Cu mixture, as rumen bacteria are the major source of odd fatty acids in ruminant milk (Vlaeminck et al., 2006). The presence of fatty acids in the *C. vulgaris* microalgae is the main reason of lowering the proportions of C15:0 and C17:0 fatty acids (Vlaeminck et al., 2006).

The Alg10 and Alg15 decreased the concentrations of SFA by 4.5 and 3%, respectively and increased the concentrations of total UFA by 10.5 and 7.1%, respectively. Others (Kholif et al., 2017 d, 2020) observed that feeding *C. vulgaris* to lactating goats reduced the concentration of SFA and concomitantly increased the proportion of DHA and UFA. In human nutrition, reduction of SFA with concomitant increase in UFA are important targets for the prevention of cardiovascular diseases. The present results confirm the assumption that *C. vulgaris* and Cu mixture contains some active compounds that caused some changes in rumen microbiota resulting in changes in the structure of fatty acids from the diet. Increasing milk UFA concentrations

indicates that a considerable amount of algal poly UFA escaped ruminal biohydrogenation (Kholif et al., 2017 d) as *C. vulgaris* are rich in poly UFA (Kholif et al., 2017 d, b). Feeding UFA rich additives to lactating animals dramatically alters milk fatty acid profile (Morsy et al., 2015; Kholif et al., 2016, 2018 d). More than 60% of milk fatty acids originate from plasma uptake, whereas the rest are synthesized in the mammary gland (Chilliard and Ferlay, 2004).

The Alg10 treatment decreased AI by 15.1%. Atherogenic index is widely used to evaluate the nutritional value of milk fat. Decreasing AI is important to relate the profile of fatty acids to the risk of cardiovascular disorders. Lowering the value of AI is beneficial to human health. The consumption of dairy products with lower AI values leads to a decrease of the total cholesterol in human blood plasma (Ulbricht and Southgate, 1991). Additionally, Alg10 treatment increased total CLA concentration by 12.4% making the milk healthier for consumers. The concentration of CLA produced in the rumen and in milk depend mainly on the diets that are fed (Rojo et al., 2015). The origins of milk CLA are the ruminal biohydrogenation of C18:2 and the endogenous synthesis from vaccenic acid through ruminal biohydrogenation via Δ^9-desaturase in the mammary gland (Corl et al., 2001). *B. fibrisolvens* and *B. proteoclasticum* are the main ruminal bacteria responsible for the production of significant amounts of cis-9, trans-11 C18:2 from linoleic acid in rumen, and cis-9, trans-11 C18:2 before their transformation to the mammary glands (Wang and Lee, 2015). As previously noted, *B. fibrisolvens* and *B. proteoclasticum* are strongly sensitive to the presence of *C. vulgaris* microalgae (Tsiplakou et al., 2017).

Conclusions

Daily inclusion of *C. vulgaris* at 10 g mixed with 9 mg Cu/kg feed in lactating Boer goats' diets enhanced nutrient digestibility and enhanced ruminal fermentation, and consequently increased milk yield. Moreover, the additives mixture in the Alg10 modified milk fatty acid profile, and increased total UFA, mono UFA and CLA proportions and decreased SFA proportion. Increasing the dose of *C. vulgaris* and Cu mixture showed negative effects on the performance of goats and is not recommended for practical implementation.

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