Partial replacement of soybean meal with *Chlorella vulgaris* in broiler diets influences performance and improves breast meat quality and fatty acid composition

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ABSTRACT Alternative feed ingredients, such as microalgae, may be more sustainable in comparison to conventional feedstuffs that need large amounts of arable land and are often imported. This study evaluates the effects of *Chlorella vulgaris* various inclusion levels in the diet of broiler chickens on performance, carcass yield, organ measurements, breast meat quality, fatty acids profile, and antioxidant capacity. A total of two hundred forty 5 d old male Ross 308 broilers were randomly allotted to 4 groups (6 replicates of 10 birds each). Each group received either a control diet or a diet where soybean meal was replaced with 10% (CV10%), 15% (CV15%), or 20% *C. vulgaris* for 40 d. Performance parameters, carcass and meat traits were evaluated. Compared to the control group, birds supplemented with *C. vulgaris* (15% and 20%) had lower body weight, weight gain, and feed intake ($P < 0.0001$), whereas no differences were observed between the control and CV10% groups ($P > 0.05$). Feed conversion ratio did not differ between control and CV groups. Diets containing *C. vulgaris* significantly increased ileal digesta viscosity, weight and size of several gastrointestinal compartments, as well as breast muscle yield ($P < 0.0001$). Incorporation of *C. vulgaris* resulted in yellower breast muscle ($P < 0.0001$), with significantly increased chlorophyll a ($P < 0.05$), chlorophyll b, and total carotenoids contents ($P < 0.0001$). Inclusion of *C. vulgaris* decreased bacterial count in meat samples in comparison to controls ($P < 0.0001$). A 20% *C. vulgaris* inclusion resulted in higher water holding capacity ($P < 0.05$) and lower cooking loss ($P < 0.05$). As dietary *C. vulgaris* increased, concentrations of DHA + EPA ($P < 0.05$) and n-3 PUFA ($P < 0.0001$) increased in breast meat, while the n-6/n-3 PUFA ratio decreased ($P < 0.0001$). Sensory analysis showed that breast meat from the CV10% group had the highest acceptance score. Overall, dietary concentrations of *C. vulgaris* of up to 20% improve breast meat quality, whereas 10% of *C. vulgaris* inclusion is recommended.

Key words: microalgae, growth performances, *Pectoralis major*, n-3 fatty acids, pigments

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INTRODUCTION

Due to the high protein and vitamin B contents, poultry meat is among the most nutritive and most common animal-origin food consumed worldwide (Marangoni et al, 2015). Indeed, poultry meats are affordable and consumers perceive chicken meat as a healthier food, particularly in comparison to red meat (Leinonen et al., 2014). In addition, there are no religious or ethical restrictions to its consumption (Maurer, 2003). All these factors, along with increased convenience and ease of preparation (Wideman et al., 2016), result in chicken meat being the preferred choice for most consumers. In addition, broilers have a very efficient feed conversion ratio and a relatively fast production cycle that can easily be implemented in almost every inhabited region of the globe (Maurer, 2003; Siegel, 2014). Poultry production is one of the fastest-growing agricultural subsectors, especially in developing countries, where it significantly

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contributes to food security and plays a major role in food security (Mottet and Tempio, 2017). Poultry is thus expected to be the fastest-growing meat produced worldwide, with an estimated production increase of 16% by 2029 (OECD/FAO, 2020). It is expected for poultry consumption to increase to 144 874 kt ready to cook equivalent by 2029 (OECD/FAO, 2020).

However, the expansion of the sector increases the pressure on agricultural yield for the production of feedstuffs. It is estimated that 33% of the world's arable land is used for grain and soybean production for animal feeding (Martinelli et al., 2020). Poultry production is the sector that requires most land for grain production (Mottet and Tempio, 2017). To overcome such challenges, significant efforts have been conducted toward the development of alternative sustainable high-quality protein feed resources. These include for instance microalgae (Chaves et al., 2021). The unicellular green microalga, *Chlorella vulgaris,* has a balanced essential amino acid profile (Lamminen et al., 2019), being suitable as a protein source in feeds. In addition, it is rich in bioactive compounds, including carotenoids and other pigments, beta-glucans, polyunsaturated fatty acid (PUFA), vitamins, macro, and micro-minerals that are important in animal feeding (Alfaia et al., 2021). Furthermore, microalgae have up to 50 times higher photoautotrophic efficiency than terrestrial plants thus contributing to decrease CO_2 levels in the atmosphere (Zhou et al., 2017). In addition, Microalgae production does not use arable land (Trentacoste et al., 2015). Large quantities of microalgal biomass can easily be produced in open photobioreactors in the presence of sunlight or closed units under controlled CO_2 and light conditions (Xu et al., 2009).

The concept of using microalgae in boiler feeds has been proposed in previous studies. It was found that *C. vulgaris* supplementation improves broiler performance and meat quality traits (Kang et al., 2013; An et al., 2016; Abdelnour et al., 2019; El-Bahr et al., 2020; Alfaia et al., 2021). However, the majority of these studies used *C. vulgaris* as a feed supplement in low incorporation levels (below 3%). To the best of our knowledge, only a small number of studies, focused on the effect of a higher (but still below 10%) inclusion levels of this microalga on broiler performances and meat quality (Combs, 1952; Lipstein and Hurwitz, 1981; Alfaia et al., 2021), while only one study (Alfaia et al., 2021) provided data on broiler meat quality.

This work aimed to use higher levels of *C. vulgaris* (up to 20%) as a replacement for soybean meal in broilers feeds and study the effects on performance, carcass yield, meat quality, fatty acid profile, antioxidant capacity, texture, and sensory attributes of broiler breast meat.

MATERIAL AND METHODS

Animal Housing and Experimental Diets

The study was conducted at the research facilities of Instituto Superior de Agronomia (**ISA**), University of Lisbon (Portugal). Procedures were approved by the Animal Welfare Research and Ethics Commission (**ORBEA**) of ISA. All appropriate guidelines on animal experimentation of both Portugal and the European Union were followed.

Two hundred and forty, one-day-old male Ross 308 chicks were individually identified with wing bands. Birds were housed in floor pens (2.25 × 0.9 m; 0.2025 m^2 per animal) in an environmentally controlled room. Room temperature and ventilation were constantly monitored for 40 days according to the broiler strain management guidelines. Each pen was equipped with 4 drinking nipples and 1 feeder. Throughout the experiment, clean water and feed were provided *ad libitum*. Birds were submitted to an adaptation period of 4 d, and a corn/soybean meal standard diet was provided during this time. At 5 d of age, chicks were randomly divided into 4 groups of 60 birds each, with 6 replicates. Birds in the control group were fed a corn and soybean meal basal diet. Birds in the other 3 groups were fed a basal diet with a 10 (CV10%), 15 (CV15%) or 20% (CV20%) inclusion rate of *C. vulgaris* as the primary protein source (replacing soybean meal). Birds were fed the starter diet from day 5 to 19 (phase I) and the grower diet from day 19 to 40 (phase II). All diets were formulated to contain adequate nutrient levels as summarized

Table 1. Ingredient composition and nutrient levels of experimental diets (%, as fed basis).

	Dietary treatments							
	Starter				Grower			
Item	C	CV10%	CV15%	CV20%	C	CV10%	CV15%	CV20%
Ingredients, %								
Premix	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Corn	43.58	45.36	46.13	46.51	50.18	52.00	52.71	53.42
Soybean Meal	48.89	39.00	34.00	29.2	41.5	31.56	26.61	21.7
C. vulgaris powder	0	10	15	20	0	10	15	20
Sunflower Oil	3.55	1.64	0.75	0	4.80	2.89	1.99	1.10
Methionine Synthetic	0.13	0.18	0.21	0.24	0.11	0.16	0.19	0.21
Synthetic Lysine	0	0.13	0.30	0.47	0	0.15	0.31	0.47
Calcium Carbonate	1.2	1.5	1.65	1.8	1.06	1.37	1.53	1.67
Dicalcium Phosphate	1.9	1.44	1.22	1.03	1.6	1.13	0.91	0.68
Crude protein	22.46	23.07	23.76	23.95	21.02	21.39	22.14	22.65

Dietary treatments: C: control, corn-soybean basal diet; CV10%, basal diet plus 10% *C. vulgaris*; CV15%, basal diet plus 15% *C. vulgaris*; CV20%, basal diet plus 20% *C. vulgaris* Premix provided the following per kilogram of diet: pantothenic acid 10 mg, vitamin D_3 2400 IU, cyanocobalamin 0.02 mg, folic acid 1 mg, vitamin K_3 2 mg, nicotinic acid 25 mg, vitamin B_6 2 mg, vitamin A 10,000 UI, vitamin B_1 2 mg, vitamin E 30 mg, vitamin B_2 4 mg, Cu 8 mg, Fe 50 mg, I 0.7 mg, Mn 60 mg, Se 0.18 mg, Zn 40 mg.

Table 2. Fatty acid (FA) content (mg/g) and profile, (g/100 of total fatty acids) for grower diets.

	Dietary treatments			
Item	C	CV10%	CV15%	CV20%
Total FA	58.77	47.23	39.42	37.62
C12:0	0.042	0.100	0.150	0.179
C14:0	0.076	0.104	0.139	0.175
C15:0	0.013	0.030	0.040	0.026
C16:0	9.319	11.597	12.954	15.400
C16:1c7	0.000	0.298	0.570	1.234
C16:1c9	0.000	0.889	1.499	2.470
C17:0	0.053	0.069	0.089	0.092
C17:1c9	0.018	0.032	0.050	0.046
C18:0	3.086	2.819	2.781	2.636
C18:1c9	27.556	27.729	27.111	25.942
C18:1c11	0.679	0.932	1.205	1.493
C18:2n−6	56.904	51.326	46.113	41.363
C18:3n−3	0.895	1.697	3.214	3.647
C20:0	0.326	0.359	0.351	0.365
C20:1	0.162	0.171	0.178	0.168
C22:0	0.526	0.429	0.397	0.265
C24:0	0.240	0.312	0.261	0.249

Dietary treatments: C: control, corn-soybean basal diet; CV10%, basal diet plus 10% *C. vulgaris*; CV15%, basal diet plus 15% *C. vulgaris*; CV20%, basal diet plus 20% *C. vulgaris*.

in Table 1. The fatty acid composition of the diets are presented in Table 2.

Animal Slaughtering and Sampling

Broiler performance was assessed by body weight (**BW**), weight gain (**WG**), feed intake (**FI**), and feed conversion ratio (**FCR**) during the experiment. Body weight and FI was recorded weekly (days 5, 12, 19, 26, 33, and 40), and WG and FCR was calculated. A known amount of feed was administered daily and feed leftovers were weighed weekly. On day 40, 2 birds per pen (12 per group) were slaughtered, for 48 birds. Birds were electrically stunned and manually exsanguinated. The heart, spleen, and gastrointestinal (**GI**) organs (crop, gizzard, liver, pancreas, duodenum, jejunum, ileum, and cecum), were removed, emptied, and weighed. The length of the intestinal compartments was measured. The viscosity of the *digesta* contents in the duodenum plus jejunum and ileum were determined using a viscometer (LVDVCP-II, Brookfield Engineering Laboratories, Middleboro, MA) with a cup maintained at 24°C. Also, on day 40, 3 broilers per pen, for a total of 72 birds (18 broilers per group) were transported to a commercial poultry abattoir and then electrically stunned and immediately slaughtered by severance of the jugular veins following standard commercial practices in the European Union. Carcass weight was recorded to calculate carcass yield. During the first 24 h *post mortem*, carcasses were stored in a ventilated cold room at 4°C. Carcasses were cut in major retail parts (breast, wings and drumsticks with thighs). Fresh *Pectoralis major* muscles (**PMM**) were vacuum packed and stored at −20°C until further use.

Determination of Quality Traits

Fresh PMM was used for determining pH, drip loss, color, and total viable counts (**TVC**), while the frozen samples were used for evaluation of thawing and cooking loss, texture, sensory characteristics, pigment concentrations, fatty acids profiles, and antioxidant capacity as detailed in Figure 1. At 24 h *postmortem*, the final PMM pH was measured with a pH meter (model 506, Crison Instruments SA, Alella, Barcelona, Spain) calibrated at room temperature with pH 4 and 7 and 9 buffers by inserting a glass electrode directly into the muscle at 3 locations (middle, medial, and lateral). Measurements at each point were done twice.

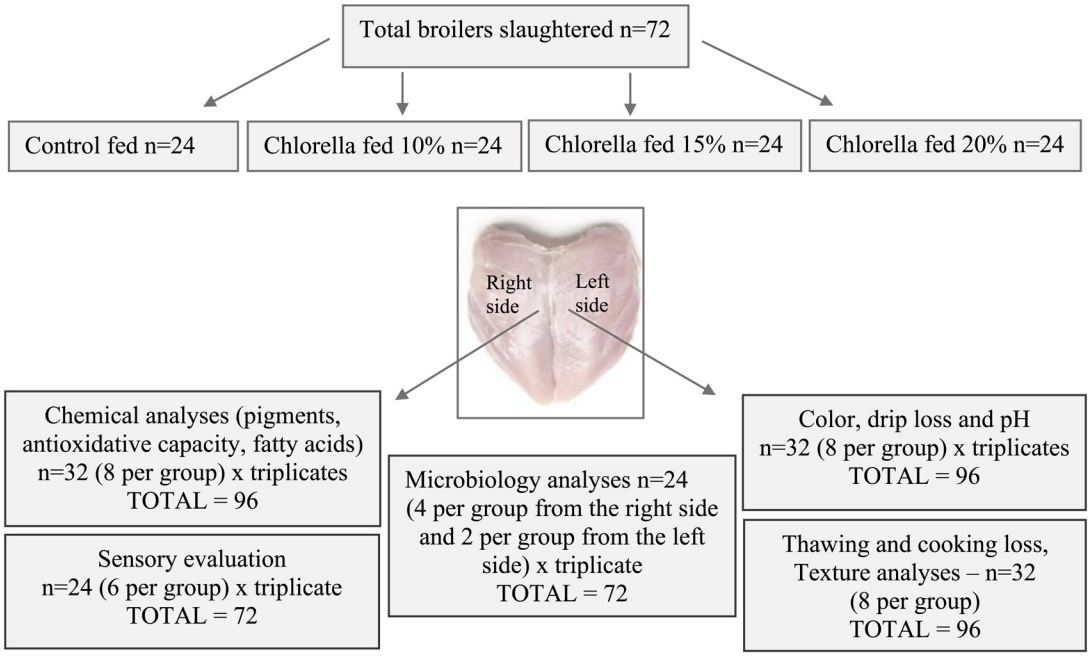

Figure 1. Broiler breast meats sampling plan.

Breast muscle color was estimated with a colorimeter (CR−400, Konica Minolta Sensing, Inc., Marunouchi, Japan), based on the CIE L*a*b* system (L* for lightness, a* for redness, and b* for yellowness) 30 min after air exposure in order to allow blooming. Measurements were evaluated at 6 different points and the average value calculated.

Drip loss was determined using the bag method by Honikel (1987). Briefly, after cutting a 90 ± 5 g block from the PMM, each piece was suspended inside an inflated bag and stored at a cooling chamber at 4°C. After 24 h, samples were wiped gently with absorbent paper and weighed again. Drip loss was expressed as a percentage of the initial weight. Water holding capacity was determined as described by El-Bahr et al. (2020). In this method, approximately 10 g of breast meat was placed in 50 mL centrifuge tubes with glass beads. The tubes were centrifuged at 10,000 rpm for 20 min at 5°C. Then, meat samples were removed, gently dried with filter paper, and weighed.

To determine thawing loss, the frozen breast fillets were thawed for 24 h at 4°C and the final weight recorded. The percentage of the difference between initial and thawed weights was the value of thawing loss. To determine cooking loss, once thawing loss was calculated, the breast muscle was placed in PA/PE bags (FoodSaver, Boca Raton, FL), sealed and cooked sous vide at 80°C for 55 min in a hot water bath to reach a core temperature of 77°C. After room temperature cooling, samples were stored at 4°C for 24 h, weighed, and cooking loss was expressed as the percentage of the initial weight. Once cooking loss was determined, breast samples were used for texture analysis.

Texture Profile Analysis

The instrumental texture profile analysis (**TPA**) of the cooked PMM was conducted using a TA-XT plus texture analyzer (Stable Micro Systems, Godalming, UK) with a P/50 probe (50 mm diameter cylinder aluminum) to compress the samples at room temperature (20 ± 1°C), with a load cell of 5 kg, in compression mode. The breast cylinder was placed on the texture analyzer, and a double compression test was performed perpendicularly to the longitudinal orientation of muscle fiber. The samples were compressed to 45% of the original height and a hold time of 5 s allowed between compressions using a 0.01 N as trigger force and a test speed of 5 mm/s. The hardness, springiness, chewiness, and cohesiveness (Bourne, 1978) were calculated based on the force/distance data collected during the 2-cycle compression test.

Microbiological Analysis

Chicken PMM were analyzed for TVC on days 0 and day 7 after storage. For bacterial counting, 25 g of the breast muscle was weighed after aseptically opening the package and transferred into sterile Stomacher bags, after which 225 mL of Buffered Peptone Water (**BPW**) (Merck, Darmstadt, Germany) was added to each sample. Minced samples were homogenized in a Stomacher blender (Stomacher 400 Circulator, Seward, UK) for 2 min. Serial decimal dilutions were prepared and 1 ml of appropriately diluted suspension were pour-plated in duplicate using Plate Count Agar (**PCA**) (Merck, Darmstadt, Germany) and incubated at 30°C for 72 h according to ISO 4833 (2003). Plates were examined visually for TVC, and then the number of colonies were counted and results were recorded as colony-forming units per g (CFU/g).

Determination of Fatty Acid Profiles

The fatty acid composition of freeze dried (−60°C and 2.0 hPa) breast meat and feed samples (0.25 g) was determined by gas chromatography of fatty acid methyl ester (**FAME**) derivatives prepared by direct transesterification of samples, using a basic followed by acid catalysis as described (Parente et al., 2020). Briefly, FAME were analyzed by gas chromatography with flame ionization detection (**GC-FID**) using a Shimadzu GC 2010-Plus (Shimadzu, Kyoto, Japan) equipped with a SP-2560 capillary column (100 m × 0.25 mm, 0.20 μm film thickness, Supelco, Bellefonte, PA). The chromatographic conditions were as follows: 1) injector and detector temperatures were set at 220 °C and 280 °C, respectively; 2) helium was used as the carrier gas at 1 mL min^{-1} constant flow; 3) the initial oven temperature of 50 °C was held for 1 min, increased at 50 °C min^{-1} to 150 °C and held for 20 min, increased at 1°C min^{-1} to 190 °C and then increased at 2°C min^{-1} to 220 °C and held for 30 min. Identification was accomplished by comparing the retention times of peaks from samples with those of commercial standard FAME mix C4:0 to C24:0 (37 Component FAME Mix) and a PUFA N°1 Marine Source mix, both from Supelco Inc. (Bellefonte, PA). Quantification of total FAME was done using the chromatographic peak area according to the internal standard method. Nonadecanoic acid (19:0) at 1 mg/mL was used as an internal standard for both meat and feed samples. Fatty acids were expressed as FAME in percentage of total FAME (g/100 g FAME). The identification of FAME on the GC-FID were confirmed by gas, using a Shimadzu GC coupled with a mass spectrometer (MS) 2010-Plus (Shimadzu, Kyoto, Japan) equipped with a SP-2560 capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness). The GC conditions were similar to those used for GC-FID analysis and the MS conditions were as follows: ion source temperature, 200°C; interface temperature, 220°C; ionization energy, 70 eV; scan, 50 to 500 atomic mass units.

Determination of Antioxidant Capacity and Lipid Oxidation

The antioxidant activity of the breast meat was assessed by measuring its scavenging abilities to 2,2-

diphenyl-1-picrylhydrazy (**DPPH**) stable radicals, according to the method adapted from Brand-Williams et al. (1995) and by the ferric reducing ability of plasma (**FRAP**) assay, according with the procedure described by Benzie and Strain (1996). Total phenolic compounds (**TPC**) were also evaluated using the method adapted from Mohankumar et al., 2018, based on the reaction with Folin−Ciocalteu reagent. All tests were estimated with the extract obtained from PMM. Briefly, meat extract was prepared by dissolving 1 g of freeze-dried sample in 10 mL of solvent (ethanol). Meat extracts were placed in an ultrasound bath (35 KHz) for 10 min, and then left stirring at 150 rpm for 4 h at 25°C. After that period, the sample was centrifuged at 6,000 rpm for 10 min and filtered. The supernatant was removed and ethanol extraction of the same sample was repeated again. After evaporation in a rotary evaporator at 40°C, ethanol was added to redissolve the concentrated extract to obtain a final concentration of 1 mg/mL.

DPPH was dissolved in ethanol, and the experiments were performed on freshly prepared solutions. In this assay, a reaction mixture containing 0.1 mL of dry meat extract was added to 3.9 mL of a 0.06 mM DPPH solution and then shaken and left to stand for 1 h in the dark. Decoloring of DPPH-donated protons was determined by measuring the absorbance at 517 nm with a 100 UV-Visible spectrophotometer (Agilent, Santa Clara, CA).

Inhibition of free radicals scavenging activity (**RSA**) by DPPH in percentage was calculated as follows:

$$\mathrm{RSA}(\%) = (\mathrm{Acontrol} - \mathrm{Asample}/\mathrm{Acontrol}) \times 100$$

To determine TPC, 150 μL of meat extract was mixed with 2.4 mL of distilled water, 150 μL of 12.5% (v/v) Folin-Ciocalteu reagent and, 3 min later, 300 μL of 10% (w/v) Na_2CO_3. Double distilled water was used as blank. After a 2 h incubation period, absorbances were recorded at 725 nm (100 UV-Visible spectrophotometer, Agilent, Santa Clara, CA). TPC values were calculated based on the calibration curve for gallic acid (0−250 μg/mL) and expressed as mg of gallic acid equivalents per 100 gram of dry weight meat extract (mg GAE/g DW).

FRAP assay was performed as described by Benzie and Strain (1996) with some modifications. Meat extract (90 μL) was added to 2.7 mL FRAP reagent containing 10 mM TPTZ (Sigma−Aldrich, St. Louis, MO) in 40 mM HCl and 20 mM Fe_2Cl_3 (Sigma−Aldrich, St. Louis, MO) added to 300 mM (pH 3.6) acetate buffer in a proportion of 10:1:1 (v/v/v), respectively. Water was used as blank. Absorbances were recorded after 30 min of incubation, at 37°C, in the dark, at 595 nm. FRAP values were calculated from the calibration curve for ascorbic acid (0−100 μg/mL) and expressed as mg of ascorbic acid equivalents per100 g of dry weight of meat extract (mg AAE/100g DE).

Lipid peroxidation levels in breast meat were measured by the concentration of thiobarbituric acid reactive substances (**TBARS**), as described by Mercier et al. (2004), with slight modifications. Briefly, 1 g of minced breast meat was homogenized in 10 mL sodium phosphate buffer (100 mM, pH 7.0) for one min. After adding a mixture of 0.5 mM ferrous sulfate (100 μL) and 1 mM hydrogen peroxide (100 μL) samples were incubated at 37°C in a water bath under agitation for 30 min and butylated hydroxytoluene was added to stop the reaction and 500 μL of homogenates were frozen at −20°C, until analysis. To determine TBARS, homogenates were incubated with 1% of 2-thiobarbituric acid (250 μL) and 2.8% of trichloroacetic acid (250 μL) for 10 min at 100 °C in a water bath. The pink chromogen was extracted with 2 mL of *n*-butanol and absorbances measured using a UV/visible spectrophotometer (Ultrospec III, Pharmacia LKB Biochrom Ltd., Cambridge, UK) at 535 nm. The malonaldehyde (**MDA**) concentration was expressed as mg MDA/kg of meat.

Pigment Determinations

For the determination of pigments, the ethanol meat extract obtained as described above, was used. The absorbance was measured at 470, 648, and 664 nm using a UV-Vis spectrophotometer (Ultrospec 3100 pro, Amersham Biosciences, Little Chalfont, UK). Ethanol was used as blank. The chlorophyll a (**Chla**), chlorophyll b (**Chlb**) and total carotenoids were calculated using the following equations (Maadane et al., 2015):

$$\mathrm{Chla} = 13.36 \times \mathrm{A664} - 5.19\ \mathrm{A648}$$

$$\mathrm{Chlb} = 27.43 \times \mathrm{A648} - 8.12\ \mathrm{A664}$$

$$\mathrm{Carotenoids} = (1000 \times \mathrm{A470} - 1.63 \times \mathrm{Chla} - 104.96 \times \mathrm{Chlb})/221$$

For determination of myoglobin (**Mb**) and metmyoglobin (**MetMb**) content, 5 g of minced meat was homogenized in 25 mL ice-cold phosphate buffer (40 mM, pH 6.8). The homogenate was placed at 1°C for 1 hour, after which samples were centrifuged at 4,500 g for a half-hour at 4°C. The supernatant was filtered through a Whatman No 1. filter paper and absorbance measured at 525, 572, and 700 nm. Mb and MetMb were calculated as follows:

$$\mathrm{Mb(mg/g)} = (\mathrm{A572} - \mathrm{A700}) \times 2.303 \times 6(\text{dilution factor})$$

$$\%\ \mathrm{MetMb} = 1.395 - [(\mathrm{A572} - \mathrm{A700})/(\mathrm{A525} - \mathrm{A700})] \times 100$$

Sensory Analysis

Breast meat samples were cooked as previously described for the texture analysis and kept at 60°C until serving. All samples were labeled with random 3-digit

codes. The 35 untrained panelists (Gender: 18 females and 17 males) were chosen based on the frequency of chicken meat consumption (at least once a week). The age of consumers ranged as follows: 18 to 25 yr old (11 panelists); 26 to 35 yr old (10 panelists); 36 to 50 yr old (13 panelist); 51 to 65 years old (no panelist); ≥66 yr old (one panelist). Breast meat was evaluated in terms of overall appearance, color, flavor, taste, tenderness, juiciness, and sample overall acceptability using a 5-point hedonic scale where 1 = dislike very much and 5 = like very much. Results from the Just About Right (**JAR**) scale were amalgamated into 3 groups. Categories 1 and 2, corresponded to "too less," category 3 corresponded to "JAR," and category 4 and 5 corresponded to "too much." Water and unsalted crackers were used for palate cleansing. Analysis were conducted in a standardized sensory analysis room according to the standard EN ISO 8589 norm.

Statistical Analysis

Statistical analyses were conducted using the software GraphPad Prism version 9.00 for Windows (GraphPad Software, San Diego, CA). The effects of different dietary treatments on performances and meat quality traits were appraised by one-way Analysis of Variance (**ANOVA**) followed by Duncan's multiple comparison tests (difference considered significant if $P < 0.05$). Data were presented as means and standard error of mean (SEM) calculated. A 3-way analysis of variance (ANOVA) (dietary treatment, gender, age) with 3 first-order interactions (dietary treatment × gender interaction, dietary treatment × age interaction, gender × age interaction) and one second-order interaction (dietary treatment × gender × age) interaction was conducted for sensory analysis. In addition, a one-way analysis (dietary treatment) on sensory scores were performed to examine sample discrimination independently of the previously studied (gender, age) factors.

RESULTS

Effect of Dietary C. vulgaris on Production Performances and Carcass Yields

Animal production performances are shown in Table 3. Dietary inclusion levels of 15% and 20% of *C. vulgaris* resulted in a significant ($P < 0.0001$) decrease in final BW, BW gain, and FI, in comparison to the control animals. Such decrease was recorded in phases I and II and the whole trial (d 5 −40). The 10% dietary incorporation of *C. vulgaris* did not affect growth performance in comparison to control birds ($P > 0.05$). In phase I of the

Table 3. Growth performances of broilers fed control and 10, 15, and 20% *C. vulgaris* diet.

	Dietary Treatment					
Parameter	C	CV10%	CV15%	CV20%	SEM	*P* value
Body weight, g						
Day 5	109	107	109	106	0.637	0.4907
Day 12	306[a]	306[a]	285[b]	253[c]	2.429	<0.0001
Day 19	710[a]	714[a]	649[b]	545[c]	6.537	<0.0001
Day 26	1244[a]	1263[a]	1160[b]	1007[c]	11.10	<0.0001
Day 33	1984[a]	1999[a]	1826[b]	1625[c]	16.98	<0.0001
Day 40	2801[a]	2819[a]	2587[b]	2342[c]	22.28	<0.0001
Weight gain (g/d)						
Day 5−12	28.18[a]	28.49[a]	25.19[b]	20.89[c]	0.318	<0.0001
Day 12−19	57.49[a]	58.23[a]	51.96[b]	41.73[c]	0.661	<0.0001
Day 19−26	76.30[a,b]	78.37[a]	73.05[b]	66.08[c]	0.806	<0.0001
Day 26−33	105.68[a]	105.06[a]	95.12[b]	88.18[c]	1.101	<0.0001
Day 33−40	116.79[a]	117.18[a]	108.68[b]	102.47[b]	1.231	<0.0001
Phase I	42.92[a]	43.36[a]	38.58[b]	31.30[c]	0.456	<0.0001
Phase II	99.59[a]	100.21[a]	92.29[b]	85.58[c]	0.824	<0.0001
Overall	76.92[a]	77.49[a]	70.80[b]	63.87[c]	0.634	<0.0001
Feed intake (g/pen)						
Day 5−12	2375[a]	2435[a]	219[1b]	2094[b]	38.15	0.0007
Day 12−19	5081[a]	5078[a]	4774[a]	4308[b]	81.54	<0.0001
Day 19−26	8110.[a]	8188[a]	7589[b]	7248[b]	99.42	<0.0001
Day 26−33	11255[a]	11145[a]	10330[b]	9950[b]	153.8	0.0009
Day 33−40	13604[a]	13229[a,b]	12498[bc]	12321[c]	169.4	0.0116
Phase I	7456[a]	7513[a]	6965[b]	6402[c]	114.5	<0.0001
Phase II	7456[a]	7513[a]	6965[b]	6402[c]	114.5	<0.0001
Overall	40425[a]	40075[a]	37382[b]	35922[b]	484.0	<0.0001
FCR						
Day 5−12	1.24[a]	1.24[a]	1.26[a]	1.43[b]	0.018	<0.0001
Day 12−19	1.31[a]	1.27[a]	1.34[a]	1.48[b]	0.021	0.0005
Day 19−26	1.57	1.52	1.51	1.57	0.012	0.1442
Day 26−33	1.59	1.54	1.58	1.61	0.020	0.7259
Day 33−40	1.72	1.64	1.67	1.72	0.015	0.1966
Phase I	1.23[a,b]	1.24[a]	1.31[b]	1.46c	0.021	<0.0001
Phase II	1.63	1.57	1.60	1.64	0.012	0.1168
Overall	1.56[a,b]	1.50[a]	1.53[a]	1.61[b]	0.012	0.0064

[ac]Different superscripts within a row indicate a significant difference ($P < 0.05$).

experiment, the FCR of birds fed the control diet did not differ from the FCR of those fed CV10% and CV15% diets. Moreover, as C. vulgaris incorporation increased, the higher was the FCR in phase I ($P < 0.0001$). However, in phase II, no differences ($P > 0.05$) were found between dietary treatments. Significant higher overall FCR was found in birds from the CV20% group in comparison to the CV10% and CV15% groups ($P < 0.05$). Overall, FCR did not differ between broilers fed the control diet and those fed diets with *C. vulgaris* incorporation.

Furthermore, broiler carcass yields between control, CV10% and CV15% groups did not differ. However, it was found that birds fed CV20% had lower carcass yields in comparison to those of the control and CV10% groups ($P < 0.05$) as presented in Table 4. Regarding carcass portions, broilers from groups CV10%, CV15%, and CV20% had higher breast yields when compared to the controls, whereas the controls had higher leg yields in comparison to the other dietary treatments ($P < 0.0001$). Wing yields were not influenced by *C. vulgaris* feed inclusion ($P > 0.05$).

Relative organ measurements are shwon in Table 4. Crop relative weight increased in birds fed CV20% in comparison to control birds ($P < 0.05$). Heart weight, however, was lower in CV15% in comparison to the control group ($P < 0.05$). Incorporation of 10% and 15% *C. vulgaris* decreased proventriculus weights in relation to the controls ($P < 0.05$). Sections of the intestine, such as duodenum, jejunum, ileum, and cecum were heavier in birds fed 20% *C. vulgaris* than those fed the control diet ($P < 0.05$). In addition, birds from the CV15% group had significantly heavier ileum, and cecum when compared to control birds. Moreover, the incorporation of 15 and 20% of *C. vulgaris* resulted in birds with significantly increased ($P < 0.0001$) length of the intestinal tract compartments, with the exception of the cecum length, that did not differ from birds in the control group. The viscosity of duodenum plus jejunum contents was not influenced by dietary treatment ($P > 0.05$). The incorporation of *C. vulgaris*, regardless of the inclusion level, resulted in significantly ($P < 0.0001$) higher ileal content viscosity when compared to animals fed the control diet (Table 4).

Effect of Dietary C. vulgaris on Meat Quality, Microbiology and Texture Parameters

Data regarding the effect of dietary *C. vulgaris* on meat quality parameters, texture and microbiological status of breast muscles are shown in Table 5. No significant differences ($P > 0.05$) were found between dietary treatments for pH, drip and thawing losses. The inclusion of 20% *C. vulgaris* in the broiler diet significantly ($P < 0.05$) reduced cooking loss, compared to the breast muscle from the control and CV10% groups. In addition, our results showed a significant increase ($P < 0.05$) in water holding capacity (**WHC**) of the breast muscles from CV20% group in comparison to the control group.

In terms of color, the inclusion of 15% and 20% *C. vulgaris* resulted in less red (lower a*) meat when compared to the control group. Yellowness (b*) in the muscle was significantly higher ($P < 0.0001$) in groups fed *C. vulgaris*, when compared to control birds. Between meat

Table 4. Carcass traits, relative organs weight and length of gastrointestinal tract, and intestinal content viscosity of broilers fed control and 10, 15 and 20% *C. vulgaris* diet.

		Dietary treatment				
Parameter	C	CV10%	CV15%	CV20%	SEM	*P* value
Carcass traits						
Carcass yield (%)	73.93[ab]	74.46[a]	73.11[bc]	72.58[c]	0.223	0.0112
Breast muscle yield (%)	21.39[a]	25.11[b]	24.67[b]	24.39[b]	0.286	<0.0001
Leg muscle yield (%)	29.59[a]	26.82[b]	26.12[b]	25.81[b]	0.263	<0.0001
Wing muscles yield (%)	7.36	7.24	7.38	7.38	0.049	0.7013
Relative organ weight, g/kg body weight						
Crop	2.76[a]	3.31[ab]	2.89[a]	4.12[b]	0.168	0.0137
Heart	5.21[a]	5.00[ab]	4.55[b]	5.24[a]	0.094	0.0308
Proventriculus	3.59[a]	2.99[b]	3.16[b]	3.29[ab]	0.068	0.0122
Gizzard	13.97	13.19	14.58	14.16	0.255	0.2803
Pancreas	2.03	2.12	2.34	2.17	0.052	0.1810
Spleen	1.08	1.04	1.21	1.16	0.038	0.4008
Liver	19.36[ab]	18.49[a]	17.80[a]	20.42[b]	0.298	0.0087
Duodenum	4.77[a]	4.81[a]	5.13[ab]	5.61[b]	0.118	0.0401
Jejunum	9.43[a]	8.88[a]	9.62[ab]	10.40[b]	0.169	0.0111
Ileum	8.50[a]	8.91[ab]	9.551[b]	9.67[b]	0.165	0.0337
Cecum	3.70[a]	4.13[ab]	4.52[b]	5.47[c]	0.150	<0.0001
Relative length of GI tract, cm/kg body weight						
Duodenum	11.40[a]	11.73[ab]	12.68[b]	14.33[c]	0.246	<0.0001
Jejunum	28.94[a]	29.60[ab]	32.07[b]	37.95[c]	0.689	<0.0001
Ileum	31.48[a]	31.58[a]	35.26[b]	40.28[c]	0.716	<0.0001
Cecum	7.65[a]	7.53[a]	8.26[a]	9.81[b]	0.187	<0.0001
Content viscosity, cP						
Duodenum + jejunum	8.34	13.90	11.25	14.02	0.941	0.1031
Ileum	7.01[a]	16.40[b]	26.96[c]	40.95[d]	2.215	<0.0001

[ad]Different superscripts within a row indicate a significant difference ($P < 0.05$).

Table 5. Meat quality and carcass traits of broilers fed control and 10, 15, and 20% *C. vulgaris* diet.

Parameter	Dietary treatment				SEM	*P* value
	C	CV10%	CV15%	CV20%		
Drip loss %	2.26	2.17	1.98	1.92	0.310	0.399
pH 24h	6.00	6.08	6.06	6.15	0.072	0.0641
Color						
L*- lightness	56.03	54.63	54.87	51.02	3.095	0.150
a*- redness	2.15[a]	1.40[a,b]	0.83[b]	0.97[b]	0.450	0.006
b*-yellowness	6.24 [a]	17.46[b]	20.14[b]	19.39[b]	1.916	<0.0001
Thawing loss (%)	4.07	3.43	3.96	4.04	0.805	0.6479
Cooking loss (%)	31.21[a]	29.18[a]	27.06[a,b]	24.13[b]	2.250	0.0018
WHC (%)	76.78[a]	79.21[a,b]	80.94 [a,b]	83.62[b]	2.760	0.0092
Hardness (N)	25.03	28.09	25.99	26.91	4.069	0.7450
Chewiness	11.70	14.16	12.31	13.17	2.183	0.4300
Springiness	0.77	0.77	0.76	0.77	0.027	0.9299
Cohesiveness	0.61	0.62	0.64	0.61	0.022	0.1395
TVC day 0 (log CFU/g)	4.79[a]	4.53[ab]	4.40[b]	4.40[b]	0.147	0.0034
TVC day 7 (log CFU/g)	8.30[a]	7.57[b]	7.20[c]	7.22[bc]	0.186	<0.0001
TVC's growing volumes	3.51	3.03	2.79	2.83		

[ac]Different superscripts within a row indicate a significant difference ($P < 0.05$).

samples from birds fed with higher levels of *C. vulgaris*, however, no differences ($P > 0.05$) were observed in yellowness (b*). The addition of different concentrations of *C. vulgaris* did not affect hardness, springiness, and chewiness ($P > 0.05$).

Initial TVCs in the breast muscle was found to be lower in the CV15% and CV20% than in the control group ($P < 0.05$). After 7 d of refrigerated storage, TVCs remained lower in the meat from the *C. vulgaris* fed birds when compared to control animals ($P < 0.05$).

Effect of Dietary C. vulgaris on Pigments in Breast Meat

Pigments in the breast meat are presented in Table 6. In the breast muscle, the dietary incorporation of *C. vulgaris* increased the levels of total carotenoids ($P < 0.00001$), Chlorophyll a ($P < 0.05$) and Chlorophyll b ($P < 0.00001$), resulting in higher yellowness in the meat (Figure 2). The sum of total carotenoids was 3-fold higher in 10% *C. vulgaris* and 5-fold higher in 15% and 20% *C. vulgaris* treatments, compared to the controls. Meanwhile, the carotenoids concentrations were found in the CV15% and CV20% groups. The amount of Mb and MetMb did not differ between treatments.

Effect of Dietary C. vulgaris on Fatty Acid Composition of Breast Meat

The effect of *C. vulgaris* on fatty acid composition of breast meat is presented in Table 7. *C. vulgaris* inclusion of 15 and 20% led to a significant decrease in

Table 6. Pigments in breast meats of broilers fed control and 10, 15 and 20% *C. vulgaris* diet.

Parameter	Dietary treatment				SEM	*P* value
	C	CV10%	CV15%	CV20%		
Total carotenoids (μg/gDW)	11.77[a]	33.98[b]	57.22[c]	51.73[c]	5.741	<0.00001
Chlorophyll a (μg/g DW)	3.028[a]	4.194[a]	8.113[b]	12[b]	2.572	0.0003
Chlorophyll b (μg/g DW)	n.d.	3.36[a]	10.26[b]	17.88[c]	4.478	<0.00001
Mb (mg/g WW)	1.29	1.24	1.36	1.62	0.392	0.8760
MMb (%)	44.85	44.35	45.42	45.66	0.989	0.0480

[ac]Different superscripts within a row indicate a significant difference ($P < 0.05$).

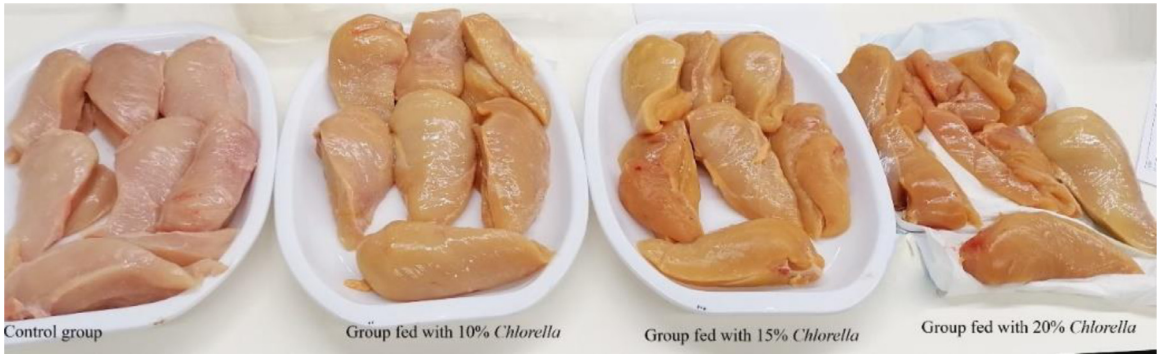

Figure 2. The color of the breasts from broiler fed control and 10, 15, and 20% *C. vulgaris* diet.

Table 7. Fatty acid (FA) content (mg/g DM) and composition (g/100 g total FA) in breast meat of broilers fed control and 10, 15, and 20% *C. vulgaris* diet.

Parameter	Dietary treatment				SEM	*P* value
	C	CV10%	CV15%	CV20%		
Total FA	137.15[a]	90.42[ab]	61.10[b]	59.44[b]	10.52	0.0018
C12:0	0.040	0.040	0.037	0.029	0.008	0.0294
C14:0	0.322	0.378	0.369	0.389	0.036	0.0447
C14:1c9	0.023	0.034	0.032	0.042	0.011	0.1796
C15:0	0.077[a]	0.091[b]	0.108[c]	0.108[c]	0.007	<0.0001
C16:0	15.615[a]	17.752[b]	18.903[bc]	19.844[c]	0.632	<0.0001
C16:1c7	0.351[a]	0.534[b]	0.660[b]	0.887[c]	0.083	<0.0001
C16:1c9	0.884[a]	1.703 [b]	1.611[b]	2.123[b]	0.344	0.0006
C17:0	0.149[a]	0.173[ab]	0.205[b]	0.203[b]	0.022	0.0049
C17:1c9	0.036[a]	0.044[ab]	0.048[ab]	0.054[b]	0.009	0.0451
C18:0	7.794[a]	8.760[ab]	10.103[bc]	10.941[c]	1.011	0.0014
C18:1c9	26.879[a]	24.993[ab]	23.866[ab]	22.913[b]	1.769	0.029
C18:1c11	0.951 [a]	1.421 [b]	1.891[c]	2.462 [d]	0.210	<0.0001
C18:2n−6	39.705[a]	33.422[b]	28.609[c]	23.537[d]	1.513	<0.0001
C18:3n−6	0.306[a]	0.225[a,b]	0.190b[c]	0.155[c]	0.031	<0.0001
C18:3n−3	0.577[a]	1.335[b]	1.563[b]	1.662[b]	0.231	<0.0001
C20:0	0.081	0.081	0.081	0.073	0.010	0.3258
C20:1	0.198	0.204	0.202	0.199	0.010	0.6397
C20:2n−6	0.527	0.610	0.681	0.665	0.145	0.4634
C20:3n−6	0.485[a]	0.615[a,b]	0.645[a,b]	0.834[b]	0.124	0.0062
C20:3n−3	0.013[a]	0.043[ab]	0.090[bc]	0.127[c]	0.029	<0.0001
C20:4n−6	3.152[a]	4.264[ab]	5.203[a,b]	6.585[b]	1.381	0.0152
C20:5n−3	0.041[a]	0.109[ab]	0.170[b]	0.303[c]	0.058	<0.0001
C22:4n−6	1.062	1.219	1.227	1.332	0.306	0.6688
C22:5n−6	0.300	0.281	0.268	0.226	0.076	0.5824
C22:5n−3	0.150[a]	0.481[ab]	0.919[bc]	1.386[c]	0.239	<0.0001
C22:6n−3	0.067[a]	0.270[ab]	0.643[bc]	1.120[c]	0.310	0.0006
Other FA	0.215	0.918	1.676	1.801		
SFA	24.08[a]	27.27[b]	29.80[c]	31.59[c]	1.171	<0.0001
MUFA	29.32	28.93	28.31	28.67	0.340	0.9128
PUFA	46.38[a]	42.87[b]	40.72[bc]	38.14[c]	1.537	<0.0001
PUFA/SFA	1.93[a]	1.58[b]	1.37[c]	1.21[d]	0.079	<0.0001
n3 PUFA	0.85[a]	2.24[b]	3.39[c]	4.60[d]	0.390	<0.0001
n6 PUFA	45.54[a]	40.64[b]	37.33[c]	33.54[d]	1.311	<0.0001
n6/n3 ratio	53.94[a]	18.17[b]	11.11[c]	7.46[d]	1.599	<0.0001
DHA+EPA	0.107[a]	0.379 [ab]	0.813[bc]	1.423[c]	0.344	0.0002

[ad]Different superscripts within a row indicate a significant difference ($P < 0.05$).SFA = Sum of C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0. PUFA = Sum of C18:2n−6, C18:3n−6, C18:3n-3, C20:2n−6, C20:3n−6, C20:4n−6, C20:3n−3, C20:5n−3, C22:4n−6, C22:5n−6, C22:5n−3, C22:6n−3. n−6 PUFA = Sum of C18:2n−6, C18:3n−6, C20:2n−6, C20:3n−6, C20:4n−6,C22:4n−6, C22:5n−6.n−3 PUFA = Sum of C18:3n−3, C20:3n−3, C20:5n−3, C22:5n−3, C22:6n−3.

total fatty acid content ($P < 0.05$). Broilers fed with higher levels *C. vulgaris* had significantly increased contents of C15:0, C16:0, C16:1c7, C16:1c9, C18:1c11, C18:3n−3, and had decreased contents of C18:2n−6 in breast meat when compared to the control group ($P < 0.05$). The inclusion of 15 and 20% of *C. vulgaris* in the feed led to significantly higher amounts of C17:0, C18:0, C20:3n−3, C20:5n−3, C22:5n−3, and C22:6n−3. It led also to lower amounts of C18:3n−6 in relation to the breast meat from the control group, while no significant differences between these fatty acids were found between CV10% and the control group. In comparison to the control groups, breast meat from CV20% group had significantly higher amount of C17:1c9, C20:4n−6 and C20:3n−6. Incorporation of *C. vulgaris* in broiler diets caused a significant ($P < 0.0001$) increase in saturated fatty acids (**SFA**), and a decrease in PUFA and PUFA/SFA ratio in the broiler breasts comparing to control. The monounsaturated fatty acids (**MUFA**) level in meat was not influenced by microalgae inclusion ($P > 0.05$). Furthermore, the addition of these microalgae resulted in a significant ($P < 0.0001$) increase of total n−3 PUFA and a decrease in total n−6 PUFA contents (especially linoleic acid) in the muscle. Moreover, the n−6/n−3 ratio of fatty acids decreased with *C. vulgaris* inclusion between 3 and 7 times when compared to control animals.

Effect of Dietary C. vulgaris on Meat Oxidative Stability and Antioxidant Capacity

Antioxidant potential of breast meat was measured by DPPH free RSA, FRAP test and TPC. Free radical inhibition percentage of breast meat ranged from 9.23 (C) to 11.58% (CV15%) as showed in Figure 3a. The results of DPPH free scavenging assay of breast meat showed that there was no significant effect of dietary treatment. The results indicated that the highest TPC ($P < 0.05$) were found in the breast from the CV15% and CV20% groups (Figure 3a). Meat samples from the control group had the lowest amount of TPC, and did not differ from the CV10% group. Values of FRAP means were 206.38 ± 4.43, 287.3 ± 11.02, 414.09 ± 29.43, 405.97 ± 42.94, mg GAE/100 g DW meat for control, CV10%, CV15% and

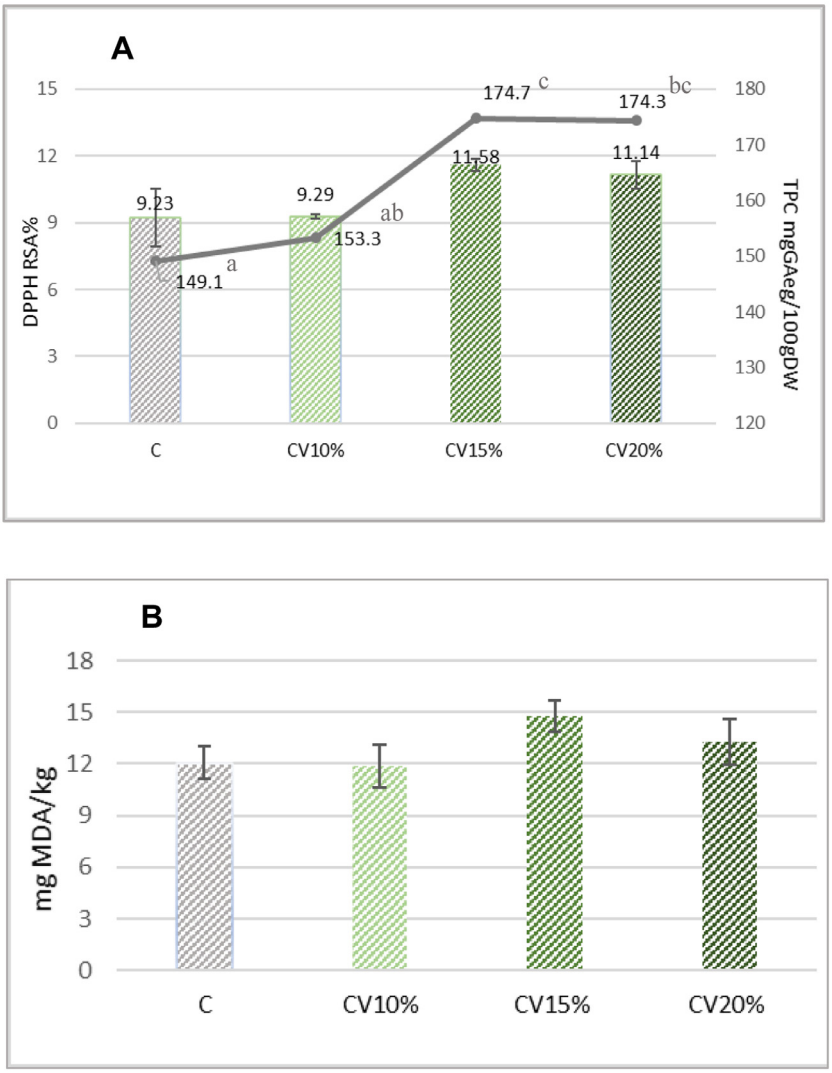

Figure 3. (A) DPPH radical scavenging activities and TPC of breast meats (values ±SD) [a–.c] Different superscripts indicate a significant difference ($P < 0.05$). (B) Thiobarbituric acid reactive substances (TBARS) after lipid oxidation with chemical induction in breast meat (values ± SD). Different superscripts indicate a significant difference ($P < 0.05$).

CV20% treatment groups, respectively. Among the raw meat samples, the meat from the CV15% and CV20% treatment groups was a more effective ($P < 0.05$) reducer of Fe3+ than CV10% and the control samples. Furthermore, the lipid oxidation (Figure 3b) was not affected by dietary treatments ($P < 0.05$).

Effect of Dietary C. vulgaris on Breast Meat Sensory Analysis

The untrained sensory panel scores for breast muscle are presented in Figure 4. No differences were reported for the liking scale between groups. Sample overall scores on hedonic scale were best for the meat from the CV10% group (3.82), followed by CV15% (3.71) and control (3.59), while meat from the CV20% group had the lowest score (3.44). However, there were no differences ($P > 0.05$) between groups for any of the sensory parameters, and all meat samples had scores between 3 and 4 on the hedonic scale. Neither gender nor age effects were detected for meat sensory descriptors in the present study. In addition, no significant dietary treatment × gender × age interaction was observed ($P > 0.05$). Furthermore, 50% of the panelists evaluated the color of the meat samples from the CV10% group to be JAR (Figure 5), while more than 55% found breast meat from the control group to be "too light." Contrarily, the meat from CV15% and CV20% groups were defined as "too dark" by 58% and 64% of the panelists, respectively. Moreover, approximately 50% of panelists evaluated that meat from the CV20% group had JAR juiciness, and 41% of panelists perceived meat from the CV10% and CV20% group as JAR regarding tenderness. Meanwhile, only 29% and 24% found control meat to have JAR tenderness and juiciness, respectively. From the JAR results, CV10% samples showed the highest percentage of respondents for JAR level for all evaluated parameters.

DISCUSSION

In the present research, it was demonstrated that birds fed diets supplemented with higher amounts of *C.*

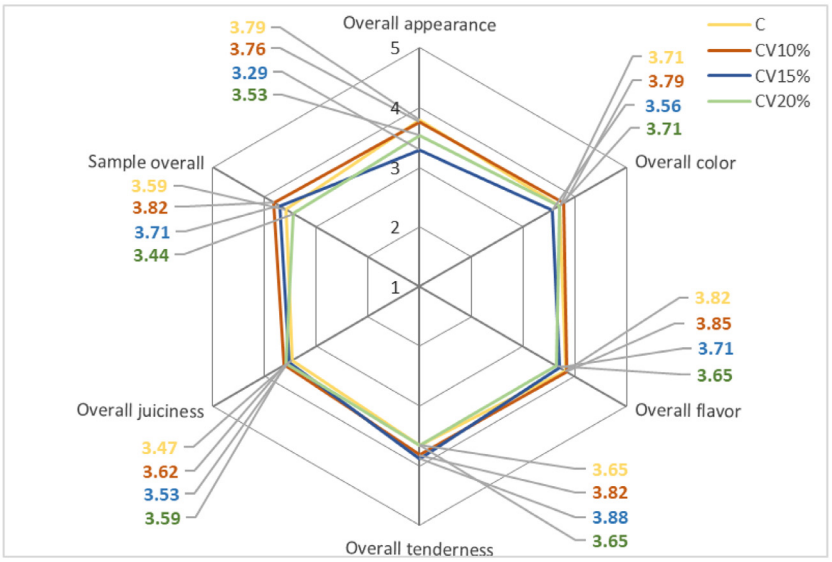

Figure 4. Sensorial attributes on hedonic scale of breast meats from broilers fed control and 10, 15, and 20% *C. vulgaris* diet.

vulgaris showed lower FI, and consequently a decrease in BWG. This result may be the consequence of a reduced palatability of the feed due to microalgae inclusion as suggested by other researchers (Abdelnour et al, 2019). Carcass yield of broilers fed the highest concentration of *C. vulgaris* was lower compared to that of control due to lower body weight and higher relative organ weights. Regarding carcass portions, we found higher breast yields in *C. vulgaris* treatments. It has been hypothesized that an increase in PMM in birds, results in an increase in metabolic costs associated with moving a disproportionately heavy sternal mass during breathing, causing in turn a possible decline in respiratory capacity (Tickle et al., 2018). Since oxygen delivery is of crucial importance for locomotion, a relative reduction in respiratory capacity can cause behavioral changes and an increased tendency to lay down (Tickle et al., 2018). Although bird's behavior was not monitored in the present study, it was apparent that birds fed *C. vulgaris* had decreased mobility in phase II. This, in turn, may be the reason for lower leg muscle yield in *C. vulgaris* fed groups.

There is little information regarding the effect of microalgae feeding inclusion on the GI tract of broilers. However, similarly to the present study, Alfaia et al. (2021) also reported no influence on weight and length of the duodenum, jejunum, and ileum in broilers fed with 10% *C. vulgaris*. The increase of intestinal weight and length in birds from CV15% and CV20% groups may result from slower digesta passage rate caused by increased viscosity, leading to an over development of the ileum and jejunum and a decrease in digestibility (Wu et al., 2013). It has been reported that dietary inclusion of 10% of *C. vulgaris* increases digesta viscosity in broilers, even when exogenous enzymes are added (Alfaia et al., 2021). Increased viscosity may be a consequence of the gelation of proteins, released when microalgae are added in concentrations above 10% (Evans et al., 2015; Pestana et al., 2020; Alfaia et al.,

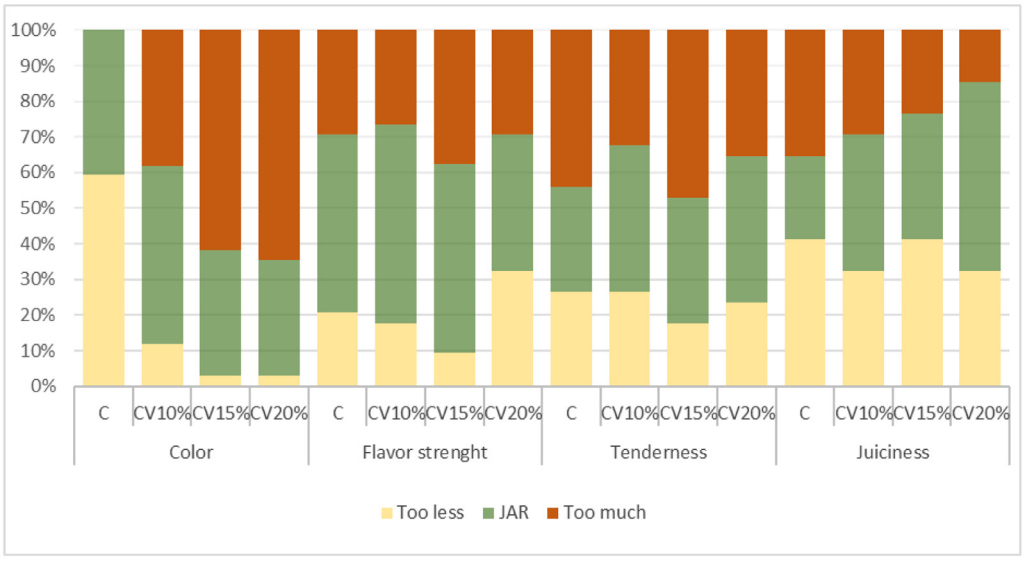

Figure 5. Sensorial attributes of broiler breast meat on just about right (JAR) scale.

2021). In addition, microalgae also increased viscosity due to their high non-starch polysaccharide content (Safi et al., 2014; Abdelnour et al., 2019). Therefore, depressed performances by CV15% and CV20% diet are also likely due to the reduction of feed passage rate and nutrient digestibility caused by the increased digesta viscosity. Although dietary incorporation of 10% of *C. vulgaris* increased digesta viscosity, the broilers' performance parameters were not affected. However, it is worth mentioning that despite having lower productive performances when compared to control and CV10% groups, broilers from the CV15% group achieved the expected performances at day 40 according to the Ross 308 management guidelines (Annex Ross 308, 2014). In fact, the mean BW, BWG, and FCR for CV10% and control broilers were above the expected levels. Similarly, previous studies have reported the positive influence of lower *C. vulgaris* inclusions (0.1% −1.0%) on growth performance of broilers (An et al., 2016; Abdelnour et al., 2019; El-Bahr et al., 2020). Therefore, the effect of *C. vulgaris* on broilers' performances may largely depend on inclusion levels.

The dietary *C. vulgaris* inclusion played a relatively minor role in meat pH and thawing loss. While in the present study only 20% of *C. vulgaris* inclusion had a significant effect on cooking loss, El-Bahr et al. (2020) reported that supplementation with *C. vulgaris, A. platensis*, and *Amphora coffeaformis* in much lower concentrations (0.1%) than used in the present study, significantly decreased cooking loss. However, cooking loss results are difficult to compare between studies as they can differ substantially depending on various factors including the cooking method, temperature, and cooking time. The increase in WHC of breast meat from broilers fed 20% *C. vulgaris*, in the present study, could be due to several reasons. Namely, the lower pH influences the structure of myofibrils due to postmortem myofibrillar and sarcoplasmic protein denaturation, causing the reduction of solubility and decrease in WHC (Warris, 2000; Castellini et al., 2002; Bowker and Zhuang, 2015). Moreover, Kalbe et al. (2019) suggested that the presence of n-3 PUFA enables the muscle cells to build a flexible lipid bilayer membrane and consequently leads to increased WHC. Therefore, a higher amount of omega 3 fatty acids (as depicted in Table 7) in breast muscle is probably responsible for a better WHC in the present study. Even though the WHC, drip and cooking loss were not significantly changed by the 10 and 15% *C. vulgaris* inclusion levels, a numerical reduction in drip and cooking losses and an increase in WHC of breast meat would result in fewer losses at large-scale production.

Color is one of the most important meat quality attributes that indicate meat freshness and influences consumer acceptance (Fletcher, 2002). In the present study, significant effects of *C. vulgaris* inclusion were observed on the color characteristics of the breast. An increased yellowness indicated in turn an efficient transfer of algal pigments into the meat. Furthermore, the deposition of carotenoids and chlorophylls in the meat was found to be dose-dependent. Alfaia et al. (2021) and Pestana et al. (2020) reported similar findings and showed that the incorporation of 10% of *C. vulgaris* and 15% of Spirulina in broiler feed increased chlorophylls and carotenoids in meat 2-fold compared to the controls. Since animals are not able to synthesize chlorophylls and carotenoids *de novo* (Maoka, 2020), these pigments found in breast muscles are likely the result of deposition through dietary components. While the source of carotenoids found in the meat from the control group was mainly from corn (rich in xanthophylls), the majority of the pigments in the *C. vulgaris* groups were derived from the microalgae. Chlorophylls and carotenoids are vitamin precursors, antioxidants, enhancers of immunity, and anti-inflammatory agents, which is why microalgae pigments can be considered as promising functional ingredients in the feed industry (Christaki et al., 2015), providing additional benefits for consumers (Alfaia et al., 2021). Unlike yellowness in breast muscles, redness decreased by the addition of *C. vulgaris* in higher concentrations. In general, lower redness values are associated with higher concentrations of MetMb. However, no differences were observed in MetMb levels between groups, leading to the conclusion that the difference in redness may be due to the other pigments present in the muscle tissue of the birds fed with the *C. vulgaris*. Moreover, the color of the muscle from the experimental groups was not only detected by instrumental analytical measurement, but it was possible to detect visual differences by the panelists among groups, even after cooking. Previously, Altmann et al. (2018), Pestana et al. (2020), and Alfaia et al. (2021) reported that 50% and 15% substitution of soy proteins with Spirulina, and 10% substitution of soy proteins with *C. vulgaris* cause the visual differences in raw breast color. However, in those studies, panelists were not able to detect a color difference after cooking. This could be due to the small surface area of the cuts used (1 cm^2), while in the present study, cuts presented to panelists were 1 cm high and 2.5 cm wide. In addition, color of the cooked meat was found to be most appropriate (JAR) from CV10% group. More than half of the panelists perceived control breast meat as too pale and meat from CV20% as too dark. Broiler chickens with a yellow skin and meat color have been shown to be considered desirable by consumers in different parts of world (Fletcher, 1999; M. T. et al., 2013; Grashorn, 2016).

While consumers choose their meat based on color, the texture is one of the major quality traits, influencing consumer's final acceptance (Fletcher, 2002). In this regard, the present study confirms that the texture of breast muscles from the birds fed with *C. vulgaris* is not different from the breast meat from the control group. Similarly, other authors reported that the inclusion of different microalgae did not affect shear force of the breast muscle (Altmann et al., 2018, 2020; Alfaia et al., 2021). The results from texture analysis were confirmed by sensory evaluation.

Regarding microbiological status, initial TVCs indicated an acceptable microbial quality of raw breast meat used in the present study (Table 5). After 7 d of

refrigerated storage, a shelf-life threshold (7 log CFU/g) was exceeded in all groups. However, significantly lower TVCs was found in breast from broilers fed *C. vulgaris*. El-Bahr et al. (2020) reported the antibacterial effect of microalgae supplementation on total aerobic bacteria plate count in broiler breasts. These authors furthermore suggested that high levels of bioactive and antioxidant peptides and eicosapentaenoic acids (EPA, C20:5n−3) may affect antimicrobial capacity. This finding is in agreement with the present study, where EPA amounts increased significantly in *C. vulgaris* treatment groups (Table 7). In addition, breast meat from *C. vulgaris* fed groups contained more phenolic compounds (Figure 1a). Phenolic compounds are well-known antibacterial substances that may perhaps explain the lower bacterial count in those groups. Although *C.* vulgaris are considered to have antimicrobial activities (Pradhan et al., 2021), the exact mechanism on how it affects the microbiota of the meat warrants further studies.

As far as total fatty acid composition is concerned, 15 and 20% incorporation of *C. vulgaris* in the feed, significantly decreased fatty acid content in meat. This finding is possibly due to a lower fat deposition reflecting in turn a lower fatty acid content in groups containing microalgae (Table 2). *C. vulgaris* incorporation resulted in a higher SFA and lower total PUFA content, leading to a reduction in PUFA/SFA ratio in the broiler breasts of the *C. vulgaris* groups comparing to controls. However, the PUFA/SFA ratio in all groups was above 0.45 (Table 7), which is considered the limit ratio for food that may increase the incidence of cardiovascular diseases (Burghardt et al., 2010). The higher deposition of myristic and palmitic acids in CV10%, CV15% and CV20% compared to control group, was due to their higher amount in *C. vulgaris* diets. The 10%, 15%, and 20% *C. vulgaris* diets contained 1.70 %, 3.21%, and 3.65% of α-linolenic acid (LNA, C18:3n−3), respectively, while 0.89% of this fatty acid is reported in the control diet. Endogenous desaturases and elongases are responsible for LNA conversion to the EPA and docosahexaenoic acid (DHA). However, EPA and DHA production are not solely influenced by the amount of LNA in the diet but rather by the linoleic acid (LA, C18:2n−6) to LNA ratio of the diet, because enzymes are the same for both omega-3 and omega-6 pathways (Lee et al., 2019). Therefore, dose-dependent LNA diet enrichment and lower amounts of LA in diets where microalgae were incorporated, resulted in a significant increase of EPA and DHA in broiler meat relative to the controls. These results are in agreement with findings previously reported in broilers fed microalgae (Yan and Kim, 2013; Pestana et al., 2020; El-Bahr et al., 2020; Alfaia et al., 2021). To decrease the risk of cardiovascular diseases, n−3 PUFA intake is recommended (Yagi et al., 2017). In the present study, *C. vulgaris* inclusion resulted in a significant increase ($P < 0.0001$) of total n−3 fatty acid, compared to the breast muscle in the control group. Some of previous nutritional strategies to increase omega 3 fatty acids, in particular DHA, in broiler meat with fish oil inclusion resulted in reduced or unacceptable odors (Gonzalez-Esquerra and Leeson, 2000, 2001). Based on the sensory results from the present study, dietary inclusion of *C. vulgaris* does not affect acceptability of broiler breast meat. According to nutritional recommendations, the n−6/n−3 PUFA ratio should not exceed 4:1 (Burghardt et al., 2010), while Sugano (1996) suggested recommended doses between 3:1 and 6:1. Although the n−6/n−3 ratio of meat across treatments is not in accordance with the recommended guidelines, the meat from the group fed 20% *C. vulgaris* had the n6/n3 ratio of 7.5:1, closest to the recommended level. Thus, the inclusion of *C. vulgaris* in broilers diets could represent a novel approach toward human health improvement through a more balanced nutrition, in terms of increased n−3 fatty acid, more beneficial n−6/n−3 PUFA ratio and SFA/PUFA ratio.

Meat from groups fed 15 and 20% *C. vulgaris* had higher TPC and higher FRAP compared to controls. The FRAP assay is the only assay that directly measures antioxidants in a sample, while other assays measure the inhibition of free radicals. The antioxidant activity of carotenoids has been well documented (Pérez-Gálvez et al., 2020). Different studies reported lutein (xanthophyll) to be the most abundant carotenoid (up to 88%) in *Chlorella* spp including *C. vulgaris* (Cordero et al., 2011; Kulkarni and Nikolov, 2018). Thus, higher FRAP in meat from groups fed *C. vulgaris* is probably the result of pigments deposition in muscles through diet. However, despite higher amounts of pigments with antioxidant activity in meat from broilers fed *C. vulgaris*, the microalga did not improve oxidative stability of meat. Consistent with our findings, Alfaia et al., (2021) and Pestana et al., (2020) did not report significant differences between TBARS values in meat from control and groups fed microalgae.

Studies investigating the effect of microalgae inclusion in broiler feed on the sensory quality of meat are limited to a descriptive analysis run by trained panelists (Altmann et al., 2018, 2020; Alfaia et al., 2021). To the best of our knowledge, only articles focused on the impact of dietary Spirulina inclusion in red sea bream (Mustafa et al., 1994*)* and common carp (Nandeesha et al., 1998) have investigated the actual consumer acceptance of farmed fish fed microalgae. In accordance, Altmann et al. (2020) stressed the need for further studies to investigate consumer acceptance of meat resulting from microalgae fed animals. The present study is the first in recent years to conduct such an evaluation, albeit with a limited size panel. However, it should be highlighted that meat from all treatment groups received mean scores between 3 and 4 for all evaluated attributes. No differences in eating quality factors (flavor, taste, tenderness, juiciness) between the chicken breasts from groups fed *C. vulgaris* and the control were reported.

The results were neither influenced by panelist gender or age. The panelist liking, evaluated with scores below 5, can be explained by the lack of salt or seasoning. In addition, based on the amino acid profile of the control, CV10%, CV15%, and CV20% meats, the taste profile

was analyzed (data not shown) as described by Žugčić et al. (2018). Alanine, glycine, proline, serine, and threonine determine a sweet taste, while histidine, isoleucine, leucine, methionine, phenylalanine, tryptophan, and valine contribute to a bitter taste (Pérez-Santaescolastica et al. 2018). Umami taste is in turn determined by the presence of aspartic and glutamic acids (Hwang et al., 2020). The predominant taste was sweet and bitter in all groups. Even though the taste profile was not significantly changed by the dietary treatments ($P \geq 0.05$), a numerical increase in sweet and umami taste was observed in groups from broilers fed *C. vulgaris*. Altman et al. (2020) reported that Spirulina inclusion in the broiler diet resulted in significantly higher umami taste recorded by trained panelists, however, amino acid content and taste profile was not analyzed in that study. In addition, these authors used much higher concentrations of microalgae, replacing 50% of soybean meal in the growing diet. In addition to amino acid composition, fat content and fatty acid composition has been shown to affect palatability and flavor (Calkins and Hodgen, 2007). Thus, it could be hypothesized that CV10% and control groups meat could have obtained numerically higher aroma scores due to higher amount of fat.

Based on the present results, we hypothesize also that the inclusion of *C. vulgaris* in broiler feeds influences the color of the meat due to pigment deposition. It is worth noting that the lower number of panelists could be seen as a limitation of the sensory study leading to neither gender nor age effect observed. Nevertheless, due to the Covid-19 pandemic, it was impossible to conduct a consumer study on a larger scale. Further investigation should thus be focused on consumer acceptance and their purchasing intentions using a larger panel.

CONCLUSIONS

Results from the present study demonstrate that the effect of dietary *C. vulgaris* on broiler growth performances and meat quality is dependent on the inclusion levels. The inclusion of 10% of *C. vulgaris* improves some quality parameters and fatty acid composition of broiler meat without compromising growth performance. Considering that conversion of fatty acids to DHA and EPA is limited in humans, our study showed that the use of *C. vulgaris* as a primary source of omega-3 fatty acids in the broilers feed is an efficient way to improve the concentration of DHA and EPA and n6/n3 ratio in broilers breast meat. Moreover, dietary *C. vulgaris* is a useful strategy to improve broiler meat color, increase pigments, total phenols, and decrease bacterial counts without affecting texture and sensory acceptance of the breast meat. Therefore, from a nutritional point of view, 15 and 20% concentrations of *C. vulgaris* in the feed can successfully be used as a partial replacement of soybean meal while improving breast meat quality, whereas 10% of *C. vulgaris* inclusion is recommended in production performance.

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DISCLOSURES

The authors declare no conflicts of interest.

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