Influence of dietary *Chlorella vulgaris* and carbohydrate-active enzymes on growth performance, meat quality and lipid composition of broiler chickens

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ABSTRACT Herein, we investigated the effect of *Chlorella vulgaris* as ingredient (10% of incorporation) in broiler diets, supplemented or not with 2 formulations of Carbohydrate-Active enZymes (**CAZymes**; Rovabio Excel AP and a mixture of recombinant CAZymes, composed by an exo-β-glucosaminidase, an alginate lyase, a peptidoglycan N-acetylmuramic acid deacetylase and a lysozyme), on growth performance, meat quality, fatty acid composition, oxidative stability, and sensory traits. One hundred twenty 1-day-old Ross 308 male birds were randomly assigned to one of the 4 experimental diets ($n = 30$): corn-soybean meal–basal diet (control), basal diet with 10% *C. vulgaris* (**CV**), CV supplemented with 0.005% of a commercial CAZyme cocktail (Rovabio Excel AP), (**CV + R**), and CV supplemented with 0.01% of a 4-CAZyme mixture previously selected (**CV + M**) during the experimental period lasted from day 21 to day 35. Body weight gain and feed conversion rate of broilers were not affected by *C. vulgaris* but digesta viscosity increased more than 2-fold ($P < 0.001$) relative to the control. In addition, neither cooking loss, shear force, juiciness, flavor nor off-flavor was impaired by dietary treatments ($P > 0.05$). By contrast, the dietary *C. vulgaris* increased tenderness, yellowness (b*) and total carotenoids in breast and thigh meats. However, no additional protective effect against lipid oxidation was observed in meat with the inclusion of microalga. *Chlorella vulgaris*, independently of CAZymes, had a minor impact on meat fatty acid composition but improved the proportion of some beneficial fatty acids. In summary, our data indicate a slight improvement of broiler meat quality and lipid nutritional value, without impairment of broilers' growth performance, thus supporting the usefulness of this microalga in poultry diets, up to this high level of incorporation. By contrast, the selected CAZyme mixtures used do not significantly improve the release of microalga nutrients in poultry diets, through the disruption of microalga cell wall, which warrants further research.

Key words: microalgae, CAZymes, animal performance, nutritional value, broilers

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INTRODUCTION

The utilization of alternative high-quality protein supplements in animal feeds, replacing conventional protein sources, is currently encouraged because of sustainability issues. Microalgae supplementation of poultry and livestock diets could represent a useful protein candidate among future protein sources to enhance growth performance and meat quality (Madeira et al., 2017).

The unicellular green microalga *Chlorella vulgaris*, known by the relative ease of cultivation and high productivity (Buono et al., 2014), has been incorporated in feeds as a source of protein and other valuable components, such as essential amino acids, polyunsaturated fatty acids, vitamins, and natural pigments (Becker, 2004; Jeon et al., 2012; Swiątkiewicz et al., 2015; Andrade et al., 2018). Dietary *Chlorella* could partially replace soybean in poultry feed because of high protein content (approximately 50%) and balanced amino acid profiles (Lamminen et al., 2019). Besides chemical

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composition, digestibility is also a crucial parameter to take into consideration when assessing the nutritional quality of novel feed ingredients. In this respect, *Chlorella* has a rigid and recalcitrant cell wall, characterized by a complex matrix of polysaccharides and glycoproteins, which is a major barrier for digestibility and extraction of nutritional compounds (Safi et al., 2014; Abdelnour et al., 2019). To overcome this limitation, dietary supplementation with Carbohydrate-Active enZymes (**CAZymes**) may constitute an excellent strategy to disrupt *C. vulgaris* cell wall integrity and, consequently, to increase the bioavailability of target nutrients for poultry diets (Ravindran and Son, 2011; Alagawany et al., 2018). There are several CAZyme mixtures commercially available for cereal-based diets, including Rovabio Excel AP that contains predominantly xylanases and β-glucanases. Although these enzyme mixtures have not yet been tested for microalgae-containing diets, it is not expected a high efficacy because of major differences between terrestrial and marine plant cell walls.

Recently, Coelho et al. (2019) developed a 4-CAZyme mixture that displays the ability to partially degrade *C. vulgaris* cell wall in vitro, with the consequent release of proteins and pigments with antioxidant capacity. Therefore, the objective of the present study was to assess how dietary incorporation of *C. vulgaris* microalga at a high level of 10% influences broilers' performance and meat quality. In addition, the efficacy of 2 exogenous mixtures of CAZymes (Rovabio Excel AP and the 4-CAZyme mixture) at increasing the bioavailability of microalga nutrients was also determined. We hypothesized that the incorporation of high levels of *C. vulgaris* is an effective strategy to partially replace conventional, although unsustainable, protein sources, particularly soybean meal, in poultry nutrition. In addition, we expected that the use of exogenous CAZymes would degrade the recalcitrant microalga cell wall and would improve the bioavailability of its nutrients in poultry diets.

MATERIALS AND METHODS

Animal Care and Experimental Diets

The trial was conducted at the facilities of Instituto Superior de Agronomia, Universidade de Lisboa. All the procedures were reviewed by the Ethics Commission of CIISA/FMV and approved by the Animal Care Committee of the National Veterinary Authority (Direção Geral de Alimentação e Veterinária, Portugal), following the guidelines of the European Union legislation (2010/63/EU Directive). Birds were raised in wired-floor cages in an environmentally controlled room under standard brooding practices, with constant light. Room temperature was maintained at 31°C at day 0, 30°C at day 1, 28°C from day 2 to 4, and then gradually decreased to 21°C by day 22, after which it remained constant. Room temperature and ventilation were monitored continuously from day 1 to day 35. Each cage measured 66 × 66 cm and was equipped with 2 drinking nipples and one feeder. The experimental design was performed with 10 replicate pens per treatment with 3 birds per pen. After 21 d with a corn-based diet, birds received during the experimental period, from day 21 until standard slaughter age of 35 d, one of the 4 dietary treatments: 1) a corn-soybean meal basal diet (as control); 2) the basal diet with 10% of *C. vulgaris* supplied by Allmicroalgae (Natural Products, Portugal) (**CV**); 3) the basal diet with 10% of *C. vulgaris* supplemented with 0.005% of the commercial CAZyme cocktail Rovabio Excel AP from Adisseo (Antony, France), containing predominantly β-xylanase and β-glucanase (**CV + R**); and 4) the based diet with 10% of *C. vulgaris* supplemented with 0.01% of a mixture of 4 CAZymes, as described above (**CV + M**).

Production of Recombinant Enzyme Mixture

Plasmids containing the genes encoding the 4 recombinant CAZymes that compose the mixture (exo-β-glucosaminidase, alginate lyase, peptidoglycan N-acetylmuramic acid deacetylase and lysozyme) were obtained according to Coelho et al. (2019). Briefly, BL21 *Escherichia coli* cells were transformed with the generated recombinant plasmids and were grown on Luria-Bertani media, at 37°C under agitation (190 rpm) to mid exponential phase (absorbance was measured at λ = 595 nm as being 0.4–0.6). Isopropyl β-d-thiogalactoside was added to a final concentration of 1 mM to induce recombinant gene expression. Cells were incubated overnight at 19°C with agitation (140 rpm). After induction, the culture media was centrifuged and the protein extracts were prepared by ultrasonication followed by centrifugation. The 4-CAZymes protein extracts were mixed and added, in equal weight proportions, at a final level of 0.01% to the experimental diet containing 10% of *C. vulgaris* (CV + M).

Microalga and Experimental Diets Analysis

The proximal composition of *C. vulgaris* microalga and experimental diets was determined by AOAC (2000) methods. Dry matter (**DM**) was analyzed by drying samples at 103°C to constant weight. The nitrogen (**N**) content of microalga and diets was determined by the Kjeldahl method and crude protein was calculated as 6.25 × N. The determination of ash content in samples was performed in accordance with the AOAC method 942.05 (AOAC, 2000). Crude fat of microalga and experimental diets was determined after automatic Soxhlet extraction with petroleum ether (Gerhardt Analytical Systems, Königswinter, Germany). Gross energy was calculated by complete combustion of samples in an adiabatic bomb calorimetry (Parr 1261, Parr Instrument Company, Moline, IL).

The amino acid composition of *C. vulgaris* and experimental diets was determined in accordance with the AOAC method 994.12 (AOAC, 2005) and quantified

by HPLC (Agilent 1,100, Agilent Technologies, Avondale, PA), as described by Henderson et al. (2000). Fatty acid methyl esters (**FAME**) of *C. vulgaris* and experimental diets were analyzed by one-step extraction and acid transesterification, followed by gas chromatography using heneicosanoic acid (21:0) methyl ester as the internal standard. The analysis of the diterpene profile of *C. vulgaris* and experimental diets was carried out by direct saponification, using a single *n*-hexane extraction followed by HPLC (Prates et al., 2006).

The determination of pigments in *C. vulgaris* and experimental diets was performed according to Teimouri et al. (2013), with slight modifications. Samples were extracted overnight with acetone under agitation in the dark. Then, the solutions were centrifuged at 4,000 rpm for 5 min and analyzed by UV-Vis spectrophotometry measuring the absorbance at different wavelengths (Ultrospec 3100 pro, Amersham Biosciences, Little Chalfont, UK). The contents of pigments in *C. vulgaris* and feed samples were determined according to Hynstova et al. (2018), applying the following equations: Ca: chlorophyll a = 11.24 × A662 nm–2.04 × A645 nm; Cb: chlorophyll b = 20.13 × A645 nm–4.19 × A662 nm; Ca + b: total chlorophylls = 7.05 × A662 nm + 18.09 × A645 nm; Cx + c: total carotenoids = (1,000 × A470 nm–1.90 × Ca–63.14 × Cb)/214 and Ccc: total chlorophylls and carotenoids = (Ca + b) + (Cx + c). Table 1 shows the ingredients and feed additives of the experimental diets. Table 2 describes the chemical composition of *C. vulgaris* microalga and experimental diets.

Animal Slaughtering and Sampling

Birds were weighed weekly and feed was provided daily. Feed intake, weight gain, and feed conversion ratio were determined for performance evaluation. One bird per experimental unit was sacrificed at day 35, using electrical stunning followed by exsanguination. The gastrointestinal (**GI**) organs (crop, gizzard, liver, pancreas, duodenum, jejunum, ileum, and cecum) were removed, emptied, and weighed. The length of the duodenum, jejunum, ileum, and cecum was registered. The viscosity of the duodenum plus jejunum and ileum was measured using a viscometer (model LVDVCP-II, Brookfield Engineering Laboratories, Middleboro, MA). Carcasses were maintained during 12 h in the air-chilled circuit until the carcass temperature reached 4°C, which was monitored with a probe thermometer.

Table 1. Ingredients and feed additives of the experimental diets (% as fed basis).

Item	Dietary treatments			
	Control	CV	CV + R	CV + M
Corn	56.0	55.5	55.5	55.5
Soybean meal	37.0	26.5	26.5	26.5
Soybean oil	3.60	4.14	4.14	4.14
Sodium chloride	0.33	0.33	0.33	0.33
Calcium carbonate	1.06	1.00	1.00	1.00
Dicalcium phosphate	1.44	1.50	1.50	1.50
DL-Methionine	0.28	0.36	0.36	0.36
L-Lysine	0.00	0.37	0.37	0.37
Vitamin-mineral premix[1]	0.30	0.30	0.30	0.30
Chlorella vulgaris powder	-	10.0	10.0	10.0
Rovabio® Excel AP	-	-	0.005	-
Mix of 4 CAZymes	-	-	-	0.01

Dietary treatments: Control, corn-soybean basal diet; CV, basal diet plus 10% *C. vulgaris*; CV + R, basal diet plus 10% *C. vulgaris* + 0.005% Rovabio Excel AP; CV + M, basal diet plus 10% *C. vulgaris* + 0.01% mix of 4 CAZymes.

Abbreviation: CAZymes, Carbohydrate-Active enZymes.

[1]Premix provided the following nutrients per kg of diet: pantothenic acid 10 mg, vitamin D_3 2,400 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.

Determination of Meat Quality Traits

The pH was measured on breast and thigh muscles, located on the right side of the bird, without skin and deboned, with a glass penetration pH electrode (HI9025, Hanna instruments, Woonsocket, RI) at 24 h postmortem. The pH value was calculated as an average of 3 replicate measurements on the same muscle. The standard color parameters, lightness (L*), redness (a*), and yellowness (b*) were determined on breast and thigh meats, after cooling the carcass during 24 h, with a Minolta CR-300 Chroma Meter (Minolta camera Co. Ltd., Osaka, Japan) standardized with a white calibration plate (Y = 93.1; x = 0.3136; y = 0.3192). The final CIELAB color parameters were the average of 3 readings, taken from 3 spots at the surface of breast and thigh meats, approximately 60 min after exposing to air.

Shear force in meat samples was measured with a texture analyzer TA.XTplus from Stable Microsystems (Surrey, UK) using a Warner-Bratzler blade. Data were collected with specific software (Texture Expert Exceed, Stable Micro Systems, Surrey, UK) and meat shear force was expressed as the mean of the peak value of a minimum of 4 replicates. Briefly, the samples were thawed at 4°C during 24 h and individually cooked in a water bath at 80°C in plastic bags, until the internal temperature reached 72°C in the geometric center, which was monitored using an internal thermocouple (Lufft C120, Munchen, Germany). Meat samples were chilled during 2 h at room temperature to measure shear force. Breast and thigh muscles were weighed before and after cooking to determine cooking loss. Samples were prepared by excising strips (1 cm × 1 cm × 5 cm) from cooked breast and thigh muscles along the fibers and, then cut perpendicular to the muscle fibers.

Trained Sensory Panel Analysis

The sensory analysis was performed only in the breast (pectoralis major) muscle because it is very hard to separate individual muscles from the entire thigh. After thawed at 4°C during 24 h, meat samples were individually cooked in a water bath set at 85°C in plastic bags until reached an internal temperature of 78°C, which was monitored by a thermocouple (Luft C120, Munchen, Germany). Then, samples were trimmed of external

Table 2. Chemical composition of *Chlorella vulgaris* and experimental diets.

Item	Microalga	Dietary treatments			
	C. vulgaris	Control	CV	CV + R	CV + M
Energy, kcal ME/kg as fed basis	4,586	4,614	4,627	4,650	4,615
Proximate composition, % as fed basis					
Dry matter	93.1	89.0	89.6	89.3	86.4
Crude protein	42.8	19.9	20.4	19.8	19.1
Crude fat	8.73	6.59	7.56	7.63	7.41
Ash	11.8	5.60	6.08	6.21	6.13
Amino acid composition, % as fed basis					
Alanine	2.77	0.95	0.95	1.15	1.00
Arginine	3.89	1.34	1.16	1.63	1.14
Asparagine	0.06	0.02	0.02	0.03	0.02
Aspartate	3.04	1.72	1.36	1.95	1.48
Cysteine	0.66	0.33	0.24	0.13	0.00
Glutamate	4.07	2.94	2.32	3.12	2.29
Glutamine	0.02	n.d.	n.d.	n.d.	n.d.
Glycine	1.72	0.68	0.61	0.82	0.67
Histidine	0.65	0.51	0.36	0.51	0.39
Isoleucine	1.26	0.67	0.55	0.76	0.72
Leucine	2.45	1.39	1.21	1.52	1.42
Lysine	2.63	0.77	0.92	1.14	1.69
Methionine	0.45	0.10	0.21	0.17	0.63
Phenylalanine	1.49	0.85	0.71	0.95	0.84
Proline	1.87	1.23	1.03	1.11	1.04
Serine	1.56	1.03	0.81	1.11	0.85
Threonine	2.32	1.25	1.18	1.42	1.01
Tryptophan	0.47	0.19	0.25	0.21	0.33
Tyrosine	1.18	0.67	0.56	0.74	0.58
Valine	3.52	1.46	1.30	1.71	1.50
Fatty acid profile, % total fatty acids					
14:0	1.10	0.10	0.14	0.14	0.19
16:0	17.2	12.4	12.6	12.6	13.2
16:1c9	3.90	0.09	0.95	0.98	1.15
18:0	3.00	2.77	2.83	2.81	2.99
18:1c9	11.7	21.6	22.1	22.6	23.2
18:1c11	0.00	1.35	1.63	1.58	1.81
18:2n-6	11.2	50.5	47.3	48.0	46.5
18:3n-3	10.1	5.24	5.47	5.58	5.62
20:0	0.20	0.33	0.32	0.33	0.33
20:1c11	0.10	0.22	0.25	0.23	0.27
Diterpene profile, μg/g					
α-Tocopherol	19.2	10.5	42.2	12.4	20.2
α-Tocotrienol	n.d.[+]	1.29	5.94	3.00	2.75
β-Tocopherol	0.34	0.44	0.98	0.52	0.66
γ−Tocopherol+β-tocotrienol	0.52	16.2	26.8	14.7	19.3
γ-Tocotrienol	0.56	2.50	7.60	3.92	3.36
δ-Tocopherol	0.36	2.00	4.44	2.79	2.90
Pigments, μg/g					
β-Carotene	198	n.d.	83.6	37.3	45.1
Chlorophyll a[1]	906	0.67	307	339	200
Chlorophyll b[2]	171	0.90	96.3	104	40.0
Total chlorophylls[3]	1,077	1.57	404	444	240
Total carotenoids[4]	228	3.61	102	108	47.7
Total chlorophylls + carotenoids[5]	1,305	5.17	505	552	288

Dietary treatments: Control, corn-soybean basal diet; CV, basal diet plus 10% *C. vulgaris*; CV + R, basal diet plus 10% *C. vulgaris* + 0.005% Rovabio Excel AP; CV + M, basal diet plus 10% *C. vulgaris* + 0.01% mix of 4 CAZymes.

[+] Co-eluted with α-tocopherol.

Abbreviations: CAZymes, Carbohydrate-Active enZymes; DM, dry matter; ME, metabolized energy; n.d., not detected.

[1]Chlorophyll a = 11.24 × A662 nm–2.04 × A645 nm.

[2]Chlorophyll b = 20.13 × A645 nm–4.19 × A662 nm.

[3]Total chlorophylls (Ca + b) = 7.05 × A662 nm + 18.09 × A645 nm.

[4]Total carotenoids (Cx + c) = (1,000 × A470 nm–1.90 × Ca–63.14 × Cb)/214.

[5]Total chlorophylls and carotenoids = (Ca + b) + (Cx + c).

connective tissue, cut into cubes of approximately 1 cm^3, and maintained at 60°C in heated plaques. Eleven selected and trained panelists from the Faculty of Veterinary Medicine (University of Lisbon, Lisbon, Portugal) composed the sensory panel, according to Cross et al. (1978). Eight breast samples were randomly distributed across 5 panel sessions and the attributes evaluated were tenderness, juiciness, flavor, off-flavors, and overall acceptability. These attributes were classified on a grading scale from 1 (the low score being extremely

tough, dry, weak and negative) to 8 (the high score being extremely tender, juicy, strong, and positive), with the exception of flavor and off-flavor quantified from 0 (absence) to 8 (very intense).

Determination of Total Cholesterol, β-Carotene, Vitamin E, and Pigments in Meat

Total cholesterol, β-carotene, and vitamin E homologs (tocopherols and tocotrienols) were simultaneous analyzed in fresh breast and thigh (750 mg), in duplicate, as described by Prates et al. (2006). After the addition of ascorbic acid to prevent vitamin E degradation, samples were incubated with a saponification solution composed by potassium hydroxide, ethanol, and deionized distilled water in a shaking water bath at 80°C for 15 min under agitation. After centrifugation at 2,500 rpm for 10 min, the *n*-hexane layer was filtered and injected into an HPLC system (Agilent 1,100 Series, Agilent Technologies Inc., Palo Alto, CA), using a normal-phase silica column (Zorbax RX-Sil, 250 mm × 4.6 mm i.d., 5 μm particle size, Agilent Technologies Inc., Palo Alto, CA). The HPLC analysis was performed using 2 detectors set on series, which are the UV-visible photodiode array detector for the determination of cholesterol (λ = 202 nm) and β-carotene (λ = 450 nm), and the fluorescence detector for quantification of vitamin E (excitation λ = 295 nm and emission λ = 325 nm). Total cholesterol, β-carotene, and vitamin E homologs contents were determined based on the external standard method from a standard curve of peak area vs. concentration.

The contents of chlorophyll a, chlorophyll b, and total carotenoids in breast and thigh meats were measured in accordance with the modified procedure of Teimouri et al. (2013). Samples (1 g) were incubated overnight with 10 mL of acetone (Merck KGaA, Darmstadt, Germany) under dark agitation at room temperature. After centrifugation at 4,000 rpm for 5 min, the absorbance was measured using a UV-Vis spectrophotometer (Ultrospec 3100 pro, Amersham Biosciences, Little Chalfont, UK). The results were calculated according to Hynstova et al. (2018), as previously described for *C. vulgaris* microalga and experimental diets.

Evaluation of Meat Lipid Oxidative Stability by Thiobarbituric Acid Reactive Substances Assay

Lipid peroxidation levels in breast meat were measured by the concentration of thiobarbituric acid reactive substances (**TBARS**), after chemical oxidation through a ferrous/hydrogen peroxide system, as described by Mercier et al. (2004). Meat samples were ground in sodium phosphate buffer 100 mM, pH 7.0, with a Polytron homogenizer and incubated at 37°C in water bath under agitation during 30 min with a mixture of 0.5 mM ferrous sulfate and 1 mM hydrogen peroxide. After incubation, the oxidation was stopped with addition of butylated hydroxytoluene and aliquots of homogenates were frozen at −80°C, until analysis. Then, the homogenates were incubated with 1% of 2-thiobarbituric acid in 50 mM of sodium hydroxide and 2.8% of trichloroacetic acid in a boiling water bath during 10 min. The pink chromogen was extracted with *n*-butanol and the absorbance was measured at λ = 532 nm using a UV/visible spectrophotometer (Ultrospec III, Pharmacia LKB Biochrom Ltd., Cambridge, UK). Thiobarbituric acid reactive substances levels were quantified in breast meat after 0, 2, 4, and 6 d storage at 4°C. Thiobarbituric acid reactive substance concentration was calculated using 1,1,3,3-tetraethoxypropane (Fluka, Neu Ulm, Germany), as the standard and precursor of malonaldehyde. The results were expressed as mg of malonaldehyde/kg of meat.

Determination of Total Lipids and Fatty Acid Composition in Meat

Total lipids were extracted from lyophilized breast and thigh muscles (−60°C and 2.0 hPa) using a lyophilizator Edwards Modulyo (Edwards High Vacuum International, Crawley, UK) in accordance with the modified method of Folch et al. (1957), by using dichloromethane:methanol (2:1, v/v) instead of chloroform:methanol (2:1, v/v). Total lipids were determined gravimetrically, in duplicate, by weighing the fatty residue obtained after solvent evaporation.

Afterward, the fat residue of breast and thigh were suspended in dry toluene and fatty acids converted to FAME by sequential alkaline and acid transesterification procedure at 50°C for 30 and 10 min, respectively (Raes et al., 2001). The chromatographic separation of FAME was performed by gas chromatography (HP7890A Hewlett-Packard, Avondale, PA) comprising a Supelcowax 10 capillary column (30 m × 0.20 mm internal diameter, 0.20 μm film thickness; Supelco, Bellefonte, PA) and flame ionization detector. Helium was used as the carrier gas and the injector and detector temperatures were maintained at 250°C and 280°C, respectively. The oven temperature was programmed to start at 150°C, held for 11 min and followed by an increase of 3°C/min to 210°C. The final oven temperature was maintained for 30 min. The identification of FAME was based on a reference standard (FAME mix 37 components, Supelco Inc. Bellefonte, PA) confirmed by gas chromatography coupled to mass spectrometry using a GC-MS QP2010-Plus (Shimadzu, Kyoto, Japan). The quantification of FAME was performed by the internal standard method, using heneicosanoic acid (21:0) methyl ester, to compensate for sample losses during extraction and chromatographic analysis. The fatty acids identified were expressed as percentage of total fatty acids.

Statistical Analysis

Data were analyzed by ANOVA using the Generalized Linear Mixed model of Statistical Analysis System

program (SAS Institute Inc., Cary, NC), considering the cage as the experimental unit for feed intake and feed conversion ratio, and the individual bird for body weight, body weight gain, and meat quality variables. Dietary treatment was a fixed effect in the statistical model. Least squares means for multiple comparisons were generated using the PDIFF option adjusted with the Tukey-Kramer method. P values lower than 0.05 were considered statistically significant.

RESULTS

Growth Performance of Broilers and GI Tract Parameters

Data on growth performance, relative weight, and length of GI tract of broilers are shown in Table 3. Diets had no effect on body weight gain, feed intake, and feed conversion ratio of broilers ($P > 0.05$). In addition, no changes in the relative weight of GI organs were found in broilers fed experimental diets ($P > 0.05$). However, the viscosity content of the duodenum plus jejunum was higher in birds fed *C. vulgaris*, especially in those supplemented with Rovabio Excel AP ($P < 0.001$) compared with the control. The ileum content viscosity was found to be increased more than two-fold in broilers fed *C. vulgaris* relative to the control ($P < 0.001$).

Meat Quality Traits and Sensory Scores

The effect of dietary treatments on meat quality traits of broilers are shown in Table 4. Breast from birds fed on CV + M had higher values of pH 24 h postmortem ($P = 0.022$) than birds from the control group. By contrast, breast lightness (L*) was significantly higher in control and CV + R groups ($P < 0.001$). Dietary incorporation of *C. vulgaris*, including those supplemented with CAZymes, promoted higher values of yellowness (b*) in breast and thigh meats compared with the control group ($P < 0.001$). However, neither cooking loss nor shear force was affected by dietary treatments ($P > 0.05$) in both meats. The trained sensory panel scores in breast are summarized in Table 5. Dietary incorporation of *C. vulgaris*, alone or supplemented with CAZymes, had no influence on juiciness, flavor, off-flavor, and overall acceptability ($P > 0.05$), but meat tenderness was higher in *C. vulgaris* treatments than in control ($P < 0.001$).

Vitamin E Profile, Pigments, and Oxidative Stability

Table 6 presents the diterpene profile and total pigments in breast and thigh meats. Dietary treatments did not affect vitamin E homologs in breast ($P > 0.05$). In thigh, the dietary incorporation of *C. vulgaris*, with and without exogenous CAZymes, decreased the levels of α-tocopherol (CV + R and CV + M, $P = 0.013$) and γ-tocopherol (CV, CV + R, and CV + M, $P < 0.001$) relative to the control. Conversely, breast from birds fed CV and CV + R had higher values of carotenoids than control ($P = 0.002$). In addition, the sum of total chlorophylls and carotenoids were almost two-fold higher in all *C. vulgaris* treatments compared

Table 3. Growth performance (Day 21–Day 35), relative weight and length of gastrointestinal (GI) tract, and intestinal content viscosity of broilers ($n = 10$).

Item	Control	CV	CV + R	CV + M	SEM	P-value
Initial body weight, g	787	788	780	783	12.7	0.969
Final body weight, g	1,867	1,928	1,923	1,929	52.9	0.811
Body weight gain, g/d	77.2	81.4	81.6	81.8	2.401	0.991
Feed intake, g/pen	385.3	371.9	393.5	373.4	10.93	0.463
Feed conversion ratio	1.59	1.54	1.53	1.60	0.037	0.395
Relative weight of GI tract, g/kg body weight						
Crop	2.62	2.39	2.30	2.72	0.116	0.547
Gizzard	13.9	13.7	13.7	13.0	0.29	0.680
Pancreas	2.86	3.09	3.09	3.18	0.074	0.485
Liver	23.4	24.3	26.6	26.7	0.68	0.357
Duodenum	6.99	7.76	7.96	7.56	0.219	0.444
Jejunum	13.7	13.6	13.7	14.3	0.38	0.929
Ileum	11.1	10.9	11.0	11.8	0.27	0.611
Cecum[1]	4.58	4.72	5.41	5.21	0.173	0.285
Relative length of GI tract, cm/kg body weight						
Duodenum	18.8	18.3	18.9	19.3	0.27	0.596
Jejunum	45.3	46.4	45.4	49.4	0.62	0.057
Ileum	45.6	44.8	47.0	48.6	0.78	0.312
Cecum[1]	10.8	10.2	10.8	9.79	0.175	0.108
Content viscosity, cP						
Duodenum + jejunum	3.63[a]	5.38[b]	6.79[c]	6.52[b,c]	0.362	<0.001
Ileum	5.21[a]	10.5[b]	12.7[b]	13.6[b]	1.06	<0.001

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

Dietary treatments: Control, corn-soybean basal diet; CV, basal diet plus 10% *C. vulgaris*; CV + R, basal diet plus 10% *C. vulgaris* + 0.005% Rovabio Excel AP; CV + M, basal diet plus 10% *C. vulgaris* + 0.01% mix of 4 CAZymes.

Abbreviation: CAZymes, Carbohydrate-Active enZymes.

[1]Cecum: weight of 2 ceca.

Table 4. Meat quality and carcass traits of broilers ($n = 10$).

	Control	CV	CV + R	CV + M	SEM	*P*-value	Control	CV	CV + R	CV + M	SEM	*P*-value
Item	Breast						Thigh					
pH 24 h	5.69[a]	5.77[a,b]	5.79[a,b]	5.82[b]	0.029	0.022	5.86	5.87	5.87	5.85	0.041	0.978
Color parameters												
Lightness (L*)	47.9[a]	44.1[b]	45.6[a,b]	42.8[b]	0.75	<0.001	47.9	48.7	50.1	47.3	0.83	0.109
Redness (a*)	4.40	4.45	3.89	4.89	0.325	0.213	10.6	8.23	8.66	9.73	0.853	0.202
Yellowness (b*)	5.73[a]	9.96[b]	10.3[b]	9.40[b]	0.584	<0.001	6.67[a]	12.0[b]	11.8[b]	12.3[b]	0.45	<0.001
Cooking loss, %	24.4	23.0	23.9	22.9	0.70	0.395	27.8	27.2	27.6	26.4	0.51	0.211
Shear force, kg	2.85	2.64	2.60	2.50	0.172	0.523	3.02	2.87	2.68	2.67	0.237	0.690

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

Dietary treatments: Control, corn-soybean basal diet; CV, basal diet plus 10% *C. vulgaris*; CV + R, basal diet plus 10% *C. vulgaris* + 0.005% Rovabio Excel AP; CV + M, basal diet plus 10% *C. vulgaris* + 0.01% mix of 4 CAZymes.

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with the control ($P = 0.001$). In thigh, the control group had lower levels of carotenoids ($P < 0.001$) and sum of total chlorophylls plus carotenoids ($P = 0.004$) compared with *C. vulgaris* dietary treatments, including those supplemented with feed CAZymes. Moreover, the effect of dietary incorporation of *C. vulgaris*, alone or combined with CAZymes, in the oxidative stability of breast meat is shown in Figure 1. The TBARS values after chemical induction were similar among dietary treatments ($P > 0.05$). Still, TBARS formation decreased during storage at 4°C from day 2 to day 4 ($P < 0.001$).

Total Lipids and Fatty Acid Composition

The effect of 10% *C. vulgaris* incorporation, alone or combined with exogenous CAZymes, on total lipids, cholesterol, and fatty acid composition of breast and thigh meats is presented in Table 7. Total lipids were not affected by dietary treatments ($P > 0.05$) in both meats. In thigh, broilers fed CV + M diet showed lower cholesterol content in relation to the control ($P < 0.05$). Dietary treatments had a minor impact on fatty acid profile of both muscles. In breast, the percentages of 16:0 ($P < 0.001$) and saturated fatty acids (**SFA**) ($P = 0.022$) were lower in broilers fed CV and CV + M diets, whereas the proportion of 22:0 ($P = 0.016$) decreased in CV + R diet when compared with the control diet. By contrast, the percentage of 16:1c7 was higher in CV diet ($P = 0.022$), and the percentage of 18:3n-3 ($P = 0.027$) was increased in both CV and CV + R diets, in comparison with the control. In thigh, the proportion of 16:1c7 was higher in broilers fed *C. vulgaris*, individually or combined with CAZymes ($P < 0.001$). In addition, CV + R and CV + M diets increased 17:1c9 ($P = 0.014$) relative to the control diet. In addition, the percentage of 18:4n-3 ($P = 0.009$) in thigh was higher in CV + M diet than in control. By contrast, broilers fed CV + R diet had lower DMA18:0 ($P = 0.017$) and DMA18:1 ($P = 0.018$) compared with broilers fed control diet. Concerning the fatty acid ratios, CV + M diet decreased the n-6/n-3 ratio ($P = 0.049$) in thigh muscle when compared with the control.

DISCUSSION

The dietary incorporation of 10% *C. vulgaris* microalga, whether supplemented or not with exogenous enzymes, in broiler diets was not detrimental to growth performance of broilers. In fact, body weight gain, feed intake, and feed conversion ratio of broilers were unchanged among the experimental diets. Lipstein and Hurwitz (1983) reported that *Chlorella* was a suitable protein supplement in broiler diets at 5 or 10% dietary level, without impacting negatively the body weight gain and feed conversion ratio. By contrast, An et al. (2016) and Abdelnour et al. (2019) have shown that growth performance of broilers was positively influenced by *C. vulgaris* supplementation at very low amounts (0.15–1.0% of the diet). Thus, it seems that responses on growth performance parameters depend to a large extent on the level of microalga incorporation in the diet. However, a high viscosity content of the duodenum plus jejunum and ileum was observed in birds fed *C.*

Table 5. Sensorial attributes of broiler breast meat ($n = 10$).

Item	Control	CV	CV + R	CV + M	SEM	*P*-value
Tenderness	4.90[a]	5.76[b]	5.71[b]	5.73[b]	0.142	<0.001
Juiciness	4.20	4.45	4.38	4.33	0.131	0.584
Flavor	4.71	4.44	4.43	4.62	0.114	0.201
Off-flavor	0.164	0.203	0.309	0.078	0.065	0.088
Overall acceptability	4.96	5.27	5.18	5.32	0.127	0.200

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

Dietary treatments: Control, corn-soybean basal diet; CV, basal diet plus 10% *C. vulgaris*; CV + R, basal diet plus 10% *C. vulgaris* + 0.005% Rovabio Excel AP; CV + M, basal diet plus 10% *C. vulgaris* + 0.01% mix of 4 CAZymes.

Abbreviation: CAZymes, Carbohydrate-Active enZymes.

Table 6. Diterpene profile and pigments in breast and thigh meats of broilers ($n = 10$).

Item	Control	CV	CV + R	CV + M	SEM	*P*-value	Control	CV	CV + R	CV + M	SEM	*P*-value
	Breast						Thigh					
Diterpene profile, μg/g												
α-Tocopherol	3.92	3.26	3.15	3.41	0.267	0.200	5.09^{b}	4.14^{a,b}	3.40^{a}	3.15^{a}	0.428	0.013
γ-Tocopherol	0.638	0.537	0.376	0.405	0.0743	0.062	0.969^{a}	0.608^{b}	0.588^{b}	0.564^{b}	0.0696	<0.001
Pigments, μg/100 g												
Chlorophyll a	9.75	19.3	12.1	15.5	3.91	0.347	9.73	6.20	7.42	8.38	3.13	0.876
Chlorophyll b	19.9	35.1	26.1	28.7	9.23	0.709	17.6	10.3	12.5	15.1	5.41	0.795
Total chlorophylls	29.2	54.4	38.2	44.2	13.0	0.582	27.4	16.5	19.9	24.7	8.48	0.806
Total carotenoids	84.6^{a}	202^{b}	197^{b}	164^{a,b}	21.6	0.002	107^{a}	208^{b}	196^{b}	201^{b}	14.3	<0.001
Total chlorophylls and carotenoids	114^{a}	256^{b}	235^{b}	208^{b}	24.1	0.001	134^{a}	225^{b}	216^{b}	226^{b}	19.2	0.004

^{a,b}Different superscripts within a row indicate a significant difference ($P < 0.05$).

Dietary treatments: Control, corn-soybean basal diet; CV, basal diet plus 10% *C. vulgaris*; CV + R, basal diet plus 10% *C. vulgaris* + 0.005% Rovabio Excel AP; CV + M, basal diet plus 10% *C. vulgaris* + 0.01% mix of 4 CAZymes.

Abbreviation: CAZymes, Carbohydrate-Active enZymes.

vulgaris relative to the control group. The addition of high levels of *C. vulgaris* in broiler diets, regardless the presence of feed enzymes, releases a large amount of proteins from microalga that increases digesta viscosity, which may be a direct result of proteins gelation when microalgae is incorporated at higher levels (>10%) (Evans et al., 2015). The increase in digesta viscosity as a consequence of the presence of soluble polysaccharides, for example, arabinoxylans and β-glucans, is excluded here, as the presence of xylanases and β-glucanases in CV + R group had no effect on viscosity. Hence, the positive effects from adding low levels of *Chlorella* to broiler diets, widely described in other reports, may have been counterbalanced by the increase of digesta viscosity, when high levels are added, as the case of the present study. Such a contrast has also been reported in humans, with high *Chlorella* levels leading to GI problems (Barkia et al., 2019).

Concerning meat quality parameters and sensory properties, the effect of 10% *C. vulgaris* in broiler diets, alone or combined with exogenous CAZymes, promoted a yellowness color to breast and thigh meats due to the high amounts of carotenoids in *C. vulgaris*. Similar results were reported by Oh et al. (2015) who shown increased yellowness in breast meats of male ducks fed very low levels (0.1–0.2%) of *C. vulgaris* during 42 d, indicating an efficient transfer of active carotenoids to meat. By contrary, An et al. (2016) found that low levels (0.05, 0.15, and 0.5%) of incorporation of Chlorella in broiler diets had no significant effect on color of breast meat. In the present study, the trained panelists were unable to identify color changes in cooked breast from broilers fed the experimental diets, probably due to the difficult in detecting color differences on meat cuts with only 1 cm^2 surface area. Furthermore, juiciness, flavor, off-flavor, and overall acceptability of breast meat were not discriminated by the panelists, with the exception of tenderness. This absence of off-flavors in meat from broilers fed with high levels of *C. vulgaris* could be an advantage in comparison with meats supplemented with fish (Wood et al., 2008) and algae oils (Ribeiro et al., 2013). Surprisingly, breast from broilers fed with *C. vulgaris* was positively perceived by the trained panelists as being tenderer. Although tenderness

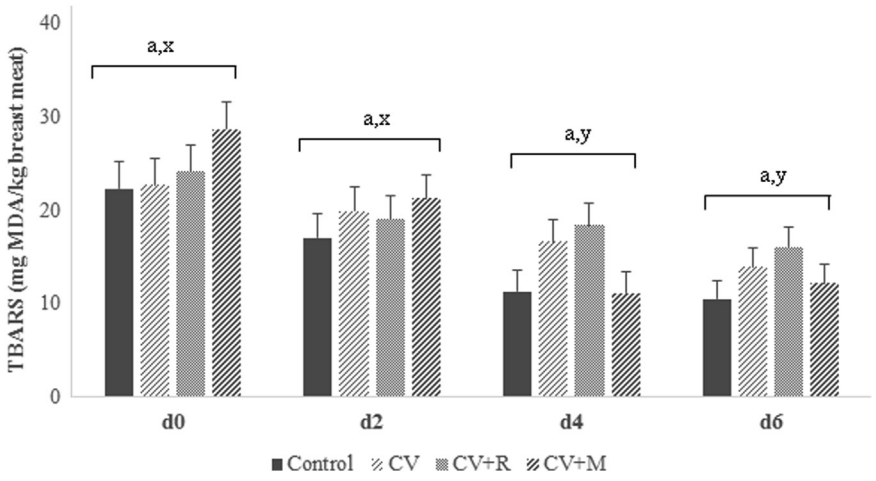

Figure 1. Thiobarbituric acid reactive substances (TBARS) after lipid oxidation with chemical induction in breast meat determined at 0, 2, 4, and 6 d under refrigeration. Means with different letters within diet (a,b,…) and time (x,y,…) are significantly different ($P < 0.05$). Dietary treatments: Control, corn-soybean basal diet; CV, basal diet plus 10% *Chlorella vulgaris*; CV + R, basal diet plus 10% *C. vulgaris* + 0.005% Rovabio Excel AP; CV + M, basal diet plus 10% *C. vulgaris* + 0.01% mix of 4 CAZymes. Abbreviation: CAZymes, Carbohydrate-Active enZymes.

Table 7. Total lipid content, cholesterol content, and fatty acid (FA) composition in breast and thigh meats of broilers ($n = 10$).

	Control	CV	CV + R	CV + M	SEM	*P*-value	Control	CV	CV + R	CV + M	SEM	*P-value*
Item	Breast						Thigh					
Total lipids, g/100 g	0.96	0.97	0.89	1.00	0.043	0.334	2.61	2.47	2.91	2.92	0.203	0.307
Cholesterol, mg/g	0.65	0.59	0.66	0.64	0.042	0.588	0.817^{b}	0.633^{a,b}	0.701^{a,b}	0.578^{a}	0.056	0.028
FA composition, g/100 g FA												
14:0	0.28	0.28	0.31	0.31	0.015	0.355	0.41	0.40	0.38	0.41	0.022	0.752
14:1c9	0.01	0.02	0.02	0.02	0.008	0.891	0.01	0.09	0.09	0.08	0.007	0.133
15:0	0.10	0.10	0.12	0.09	0.009	0.566	0.12	0.10	0.11	0.11	0.017	0.794
DMA16:0	0.40	0.30	0.30	0.36	0.043	0.276	0.13	0.11	0.14	0.07	0.019	0.063
16:0	20.6^{b}	19.3^{a}	19.8^{a,b}	19.4^{a}	0.22	<0.001	19.9	19.5	20.1	20.8	0.73	0.646
16:1c7	0.37^{a}	0.47^{b}	0.44^{a}	0.40^{a}	0.023	0.022	0.43^{a}	0.587^{b}	0.57^{b}	0.65^{c}	0.016	<0.001
16:1c9	1.22	1.44	1.54	1.40	0.144	0.467	3.23	3.42	3.44	3.45	0.166	0.773
17:0	0.21	0.19	0.18	0.18	0.010	0.145	0.18	0.18	0.16	0.18	0.016	0.861
17:1c9	0.05^{a,b}	0.07^{b}	0.07^{b}	0.03^{a}	0.011	0.027	0.04^{a}	0.06^{a,b}	0.07^{b}	0.07^{b}	0.008	0.014
DMA18:0	0.19	0.32	0.32	0.39	0.060	0.123	0.09^{b}	0.07^{a,b}	0.00^{a}	0.01^{a,b}	0.023	0.017
DMA18:1	0.23	0.35	0.32	0.48	0.067	0.084	0.08^{b}	0.06^{b}	0.00^{a}	0.01^{b}	0.018	0.018
18:0	9.96	9.48	9.26	9.38	0.322	0.445	7.15	7.15	7.22	7.20	0.290	0.997
18:1c9	20.0	21.3	21.6	21.6	0.80	0.436	30.5	30.2	29.2	30.8	0.73	0.438
18:1c11	2.85	2.75	2.70	2.64	0.107	0.556	2.06	2.14	2.18	2.27	0.067	0.180
18:2n-6	24.5	25.8	25.8	25.7	0.58	0.360	27.9	28.4	28.7	26.3	1.31	0.576
18:2t9t12	0.14	0.15	0.16	0.16	0.011	0.451	0.16	0.16	0.15	0.14	0.020	0.874
18:3n-6	0.12	0.12	0.11	0.11	0.006	0.192	0.10	0.11	0.11	0.09	0.01	0.222
18:3n-3	1.15^{a}	1.58^{b}	1.58^{b}	1.45^{a,b}	0.111	0.027	2.02	2.23	2.33	2.17	0.142	0.474
18:4n-3	0.03^{a,b}	0.04^{a,b}	0.07^{b}	0.01^{a}	0.010	0.003	0.01^{a}	0.02^{a,b}	0.03^{a,b}	0.04^{b}	0.007	0.009
20:0	0.13	0.12	0.14	0.10	0.009	0.051	0.13	0.12	0.11	0.11	0.016	0.723
20:1c11	0.23	0.24	0.25	0.26	0.016	0.496	0.28	0.25	0.25	0.28	0.011	0.114
20:2n-6	1.29	1.10	1.08	1.17	0.085	0.322	0.38	0.37	0.38	0.31	0.034	0.387
20:3n-6	0.99	0.84	0.91	1.09	0.091	0.264	0.33	0.33	0.32	0.29	0.030	0.789
20:4n-6	7.46	6.42	5.93	6.06	0.508	0.156	2.09	2.26	1.99	1.74	0.163	0.171
20:3*n*-3	0.21	0.19	0.19	0.19	0.018	0.824	0.06	0.05	0.05	0.04	0.010	0.471
20:5*n*-3	0.28	0.27	0.25	0.25	0.020	0.618	0.08	0.08	0.07	0.07	0.013	0.870
22:0	0.12^{b}	0.09^{a,b}	0.08^{a,b}	0.07^{a}	0.010	0.016	0.05	0.06	0.05	0.06	0.009	0.828
22:1n-9	0.04	0.07	0.04	0.04	0.015	0.456	0.12	0.07	0.06	0.06	0.039	0.674
22:5n-3	0.53	0.41	0.39	0.41	0.042	0.088	0.13	0.10	0.10	0.09	0.018	0.500
22:6n-3	1.41	1.34	1.03	1.10	0.154	0.260	0.37	0.59	0.48	0.54	0.107	0.508
Others	4.86	4.89	4.93	5.18	0.378	0.928	1.06	1.21	1.55	1.20	0.203	0.387
Partial sums of FA, g/100 g FA												
SFA	31.4^{b}	29.5^{a}	29.9^{a,b}	29.5^{a}	0.47	0.022	28.0	27.5	28.2	28.9	0.95	0.785
cis-MUFA	24.8	26.3	26.7	26.4	0.89	0.427	36.7	36.8	35.8	37.7	0.86	0.517
PUFA	38.1	38.2	37.6	37.7	0.48	0.678	33.7	34.7	34.7	31.8	1.52	0.499
n-6 PUFA	34.4	34.2	33.9	34.1	0.42	0.848	30.8	31.4	31.5	28.7	1.42	0.480
n-3 PUFA	3.61	3.86	3.51	3.42	0.137	0.147	2.67	3.08	3.07	2.95	0.139	0.149
Ratios of FA												
PUFA/SFA	1.22	1.30	1.26	1.28	0.024	0.112	0.861	0.807	0.833	0.985	0.085	0.470
n-6/n-3	9.74	8.96	9.74	10.1	0.36	0.174	11.5^{b}	10.3^{a,b}	10.3^{a,b}	9.88^{a}	0.414	0.049

^{a,b,c}Different superscripts within a row indicate a significant difference ($P < 0.05$).

Dietary treatments: Control, corn-soybean basal diet; CV, basal diet plus 10% *C. vulgaris*; CV + R, basal diet plus 10% *C. vulgaris* + 0.005% Rovabio Excel AP; CV + M, basal diet plus 10% *C. vulgaris* + 0.01% mix of 4 CAZymes.

SFA = Sum of (10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0).

cis-MUFA = Sum of (14:1c9, 16:1c7, 16:1c9, 17:1c9, 17:1c10, 18:1c9, 18:1c11, 20:1c11, 22:1n-9).

PUFA = Sum of (18:2n-6, 18:2t9t12, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:5n-3, 22:6n-3).

n-6 PUFA= Sum of (18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6).

n-3 PUFA = Sum of (18:3n-3, 18:4n-3, 20:3n-3, 20:5n-3, 22:5n-3, 22:6n-3).

Abbreviations: CAZymes, Carbohydrate-Active enZymes; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

is strongly dependent on the amount and chemical composition of connective tissue (Sentandreu et al., 2002), the mechanisms underlying the rise in tenderness perception by the panelists, which is not associated with lower shear force values, remain to be explained.

Regarding vitamin E and total pigments, the diterpene profile in breast and thigh agrees with those of *C. vulgaris* and the experimental diets. In thigh, the concentrations of vitamin E homologs increased relative to breast due to the higher lipid content (Ribeiro et al., 2013). Not surprisingly, α-tocopherol was the major diterpene followed by γ-tocopherol, in both meats, and within the range usually found in broilers (Ponte et al., 2008; Ribeiro et al., 2013). Similar to α-tocopherol, the levels of total carotenoids in breast and thigh reflect the values of *C. vulgaris* and the experimental diets. This increase in meat carotenoids from birds fed *C. vulgaris* might explain some of the differences found in yellowness scores of breast and thigh. Because β-carotene was not detected in both meats, it is suggested that the excess β-carotene in the experimental diets is metabolized into retinol (Nogareda et al., 2016). These findings reveal that the inclusion of 10% *C. vulgaris* in broiler diets, alone or with the 2 exogenous CAZymes, enhanced the carotenoid content of chickens, thereby providing additional benefits for consumers. Although *C. vulgaris*

contains relevant amounts of carotenoids and tocopherols with antioxidant activity (Safi et al., 2014), the microalga was unsuccessful to protect chicken meat against lipid oxidation compared to reference diet. In fact, dietary *C. vulgaris*, supplemented or not with exogenous CAZymes, had no influence on TBARS levels in breast, which is probably related to similar contents of polyunsaturated fatty acids (**PUFA**) across the dietary treatments in spite of an increase in total carotenoids. The decrease of TBARS in breast meat during 6 d of storage agrees with the findings reported by Grau et al. (2001). The TBARS reduction as a function of storage time is probably associated with increased concentrations of high polar products formed by polymerization of secondary oxidation products (Cortinas et al., 2005).

As far as fat content and fatty acid composition concern, minor differences between breast and thigh meats were observed. Total lipids ranged from 0.9 to 1.0% in breast, and from 2.5 to 2.9% in thigh. In accordance with the criteria of the Food Advisory Committee (1990), both breast and thigh are considered lean meats (fat content <5%). The thigh, as expected, had higher cholesterol content than breast; however, slightly above the amounts reported by Ponte et al. (2008) and Ribeiro et al. (2014). Albeit the incorporation of 10% *C. vulgaris*, whether supplemented or not with CAZymes, had a small impact on the fatty acid profile of breast and thigh, the results are in line with Gatrell et al. (2015) and Pestana et al. (2020), who studied the effects of *Nannochloropsis oceanica* and *Arthrospira platensis* (Spirulina) inclusion in broiler diets, respectively. The major fatty acids found in breast and thigh muscles were palmitic (16:0) and stearic (18:0) acids as SFA, oleic acid (18:1c9) as monounsaturated fatty acids, and linoleic acid (LA, 18:2n-6) as n-6 PUFA. This fatty acid profile in the breast and thigh is consistent with the fatty acid composition of *C. vulgaris* microalga and the experimental diets. Breast muscle presented LA as predominant fatty acid, whereas the thigh shows oleic acid as the most abundant fatty acid in all dietary treatments. Furthermore, broiler chickens fed diets supplemented with *C. vulgaris*, alone or with Rovabio Excel AP, had lower concentrations of SFA in breast muscle mainly due to the percentages of 16:0.

Current nutritional recommendations advice to decrease the intake of SFA, in particular lauric (12:0), myristic (14:0), and palmitic (16:0) acids, which have been documented to increase low-density lipoproteins cholesterol, a recognized risk factor for cardiovascular diseases (Mensink, 2016). Similar results were observed by Yan and Kim (2013), who also reported lower concentrations of SFA with dietary *Schizochytrium JB5* microalga. Given the healthy roles of n-3 PUFA, individual n-3 fatty acids responses diverge between dietary treatments in breast and thigh meats. A small, but significant, increase in the percentages of 18:3n-3 and 18:4n-3 in breast was observed with *C. vulgaris* incorporation, alone or combined with Rovabio Excel AP. In thigh, the microalgae plus the 4-CAZyme mixture also increased the percentage of 18:4n-3 fatty acid and decreased the n-6/n-3 ratio. Moreover, our data did not point out to an increase in the conversion rate of the alpha-linolenic acid (18:3n-3), an essential fatty acid precursor to the n-3 long-chain PUFA [eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3), and docosahexaenoic acid (22:6n-3)] due to *C. vulgaris* incorporation, with or without the addition of CAZymes. Following on the nutritional guidelines for human diets, the PUFA/SFA and the n-6/n-3 ratios should be above 0.45 and not exceed 4.0, respectively (Burghardt et al., 2010). In view of these recommendations, the n-6/n-3 ratios found here are above the recommended values in all dietary treatments.

CONCLUSION

The inclusion of 10% *C. vulgaris* in broiler chicken diets, individually or combined with CAZymes (commercial Rovabio Excel AP and the 4-CAZyme mixture previously selected) had no impact on growth performance of broilers throughout the experimental period. However, a much higher digesta viscosity was observed in birds fed microalga. In addition, dietary *C. vulgaris*, regardless the presence of CAZymes, slightly improved meat quality, through higher tenderness and yellowness color scores, as well as the nutritional value of meat lipids, with higher proportions of some beneficial fatty acids and carotenoids.

Overall, our data validate the viable use of *C. vulgaris* at a high level of incorporation in broiler diets, without impairing birds' growth performance and with an added value on meat nutritional quality. The inclusion of high percentages of *C. vulgaris* in poultry diets could contribute to reduce the dependency of the poultry industry on soybean meal. In addition, our findings suggest a negligible degradation of *C. vulgaris* cell wall by the selected CAZymes in vivo, with a nonsignificant improvement of nutrients released, being therefore recommended to prospect novel combinations of exogenous enzymes on forthcoming studies.

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DISCLOSURES

The authors declare no conflicts of interest.

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