Impact of dietary incorporation of Spirulina (*Arthrospira platensis*) and exogenous enzymes on broiler performance, carcass traits, and meat quality

J. M. Pestana,[*] B. Puerta,[*] H. Santos,[*] M. S. Madeira,[*] C. M. Alfaia,[*] P. A. Lopes,[*] R. M. A. Pinto,[†,‡] J. P. C. Lemos,[*] C. M. G. A. Fontes,[*] M. M. Lordelo,[§] and J. A. M. Prates[*,1]

**CIISA - Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Av. da Universidade Técnica, 1300-477 Lisboa, Portugal; †iMed UL, Faculdade de Farmácia, Universidade de Lisboa, Av. Professor Gama Pinto, 1649-003 Lisboa, Portugal; ‡JCS, Laboratório de Análises Clínicas Dr. Joaquim Chaves, Av. General Norton de Matos, 1495-148 Miraflores, Algés, Portugal; and §LEAF - Linking Landscape, Environment, Agriculture And Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal*

ABSTRACT This study assessed the effect of Spirulina (*Arthrospira platensis*), individually and in combination with exogenous enzymes, on growth performance, carcass traits, and meat quality of broiler chickens. One hundred and twenty Ross 308 male chickens were allocated into 40 battery brooders, with 3 birds per cage, and fed ad libitum a corn-based diet during the first 21 D of the trial. The experimental period lasted from day 21 to 35, during which birds were fed 4 different diets: a corn-soybean basal diet, taken as the control group, a basal diet containing 15% Spirulina (**MA**), a basal diet containing 15% Spirulina plus 0.005% Rovabio Excel AP (**MAR**), and a basal diet containing 15% Spirulina plus 0.01% lysozyme (**MAL**). Body weight gain ($P < 0.001$) and feed conversion rate ($P < 0.001$) were improved in control chickens, when compared with those fed with Spirulina. In addition, Spirulina increased the length of duodenum plus jejunum in relation to the other treatment ($P < 0.01$). Chickens on the MAL diet showed a considerable increase in digesta viscosity ($P < 0.05$) compared with the control group. Breast and thigh meats from chickens fed with Spirulina, with or without the addition of exogenous enzymes, had higher values of yellowness (b*) ($P < 0.001$), total carotenoids ($P < 0.001$), and saturated fatty acids ($P < 0.001$), whereas n-3 polyunsaturated fatty acid ($P < 0.01$) and α-tocopherol ($P < 0.001$) decreased, when compared with the control. In conclusion, the incorporation of 15% Spirulina in broiler diets, individually or combined with exogenous enzymes, reduced birds' performance through a higher digesta viscosity, which is likely associated with the gelation of microalga indigestible proteins. In addition, cell wall of Spirulina was successfully broken by the addition of lysozyme, but not by Rovabio Excel AP. Therefore, we anticipate that the combination of lysozyme with an exogenous specific peptidase could improve the digestibility of proteins from this microalga and avoid their detrimental gelation.

Key words: Spirulina, exogenous enzyme, growth performance, meat quality, broiler chicken

2020 Poultry Science 99:2519–2532
https://doi.org/10.1016/j.psj.2019.11.069

INTRODUCTION

The use of microalgae as a feed ingredient contributes for the protection of environment and natural resources, namely land degradation and water deprivation (Madeira et al., 2017). The current trend in poultry nutrition is to use natural ingredients as an alternative to antibiotics, growth factors, or other chemicals. Microalgae are natural feed with high nutritional value and, therefore, might stand as a promising ingredient in poultry diets (El-Hady and El Ghalid, 2018).

Arthrospira platensis species, henceforth denominated as Spirulina, is very rich in protein (50–70%) (Soni et al., 2017) and may be used as partial replacement of conventional protein sources, such as soybean meal (Spolaore et al., 2006; Austic et al., 2013; Swiatkiewicz et al., 2015). Spirulina is an edible microalga classified as a blue-green alga (*Cyanophyceae*, also known as

Received June 20, 2019.
Accepted November 26, 2019.

[1]Corresponding author: japrates@fmv.ulisboa.pt

cyanobacteria), with a filamentous multicellular morphology and ability to thrive in harsh environments (Becker, 2007; Madkour et al., 2012; Seyidoglu et al., 2017). Cyanobacteria have a cell wall that is similar in structure to those of gram-negative bacteria (Drews, 1973). *A. platensis* has a multilayered cell composed, mostly of peptidoglycan and lipopolysaccharides (**LPS**) (Cohen, 1997). Besides being an excellent source of protein, this microalga presents a high content of carbohydrates, vitamins, namely pro-vitamin A, vitamin C, vitamin E, and minerals, such as iron, calcium, chromium, copper, magnesium, manganese, phosphorus, potassium, sodium, and zinc. Spirulina is also a good source of essential fatty acids and pigments, such as chlorophyll a, phycocyanin, carotenes, and xantophylls (Babadzhanov et al., 2004; Howe et al., 2006; Wang et al., 2007; Soni et al., 2017; Hynstova et al., 2018).

It is well recognized that the hard cell wall of the majority of microalgae remains largely indigestible by monogastric animals, preventing the access to their valuable nutritional compounds (Austic et al., 2013; Lum et al., 2013; Shirazi et al., 2017). Spirulina is no exception, displaying a resistant cell wall that acts as an effective barrier for the exposure of intracellular nutritional compounds to the digestive process (Shirazi et al., 2017). Thus, the partial or complete degradation of bacterial cell walls is an important challenge for microalgae utilization, and enzymatic degradation could be the most promising approach to achieve this goal.

Exogenous enzymes have been largely used in animal nutrition to improve the nutritive value of poultry diets (Slominski, 2011). Feed enzymes, mainly phytase (Cowieson and Bedford, 2009) and carbohydrate-degrading enzymes, boost feed conversion by promoting a better access of the endogenous enzymes to their target substrates, thus promoting feed digestibility (Choct, 2006). In addition, exogenous enzymes may generate prebiotic oligosaccharides that alter resident microbiota, thus promoting animal health (Bedford and Morgan, 1996; Apajalahti and Bedford, 1999; Bedford and Cowieson, 2012). In addition, exogenous enzymes allow minimizing environmental concerns due to reduced output of excreta (Choct, 2006). In particular, lysozyme is an antimicrobial enzyme that can hydrolyse the peptidoglycan of bacterial cell walls (Cowieson and Kluenter, 2018) and disrupt the cell wall of different microalgae strains (e.g., *Chlorella* sp., *Scenedesmus* sp., and *Nannochloropsis* sp.), leading to a better exposure of proteins and pigments to the endogenous repertoire of digestive enzymes (Al-Zuhair et al., 2016). Moreover, lysozyme was shown to improve the intestinal barrier function and growth performance of chickens (Liu et al., 2010).

Dietary *A. platensis* has been associated with increased growth rate (Evans et al., 2015; Park et al., 2018), as well as improved carcass quality (Venkataraman et al., 1994; Toyomizu et al., 2001; Altmann et al., 2018). Herein, we investigated the effect of Spirulina as feed ingredient (15% of incorporation) in broiler diets, individually or combined with exogenous enzymes (lysozyme and a mixture of carbohydrate-degrading enzymes that are able to disrupt microalgae cell wall) on animal performance, carcass traits, and meat quality.

MATERIALS AND METHODS

All the procedures used in animal experiments were reviewed by the Ethics Commission of Centro de Investigação Interdisciplinar em Sanidade Animal/Faculty of Veterinary Medicine and approved by the Animal Care Committee of the National Veterinary Authority (Direção Geral de Alimentação e Veterinária, Portugal), following the principles and specific guidelines of the European Union legislation (2010/63/EU Directive).

Animals, Diets, and Management

One hundred and twenty 1-day-old male Ross 308 birds were housed in 40-battery brooders. Birds were raised in wired-floor cages in an environmentally controlled room under standard brooding practices, with constant light. Room temperature and ventilation were monitored continuously from day 1 to 35. Birds were fed ad libitum a corn-based diet during the first 21 D. From day 21 to 35, the 4 dietary treatments were provided to birds. All diets were formulated to contain adequate nutrient levels, as defined by the NRC (1994) and summarized in Table 1: 1) a corn-based control diet; 2) a diet containing 15% Spirulina powder (**MA**); 3) a diet containing 15% Spirulina powder supplemented with 0.005% of the commercial carbohydrate-degrading enzyme cocktail Rovabio Excel AP (**MAR**); 4) a diet containing 15% Spirulina powder supplemented with 0.01% lysozyme powder (**MAL**). There were 10 replicate pens per treatment with 3 birds per pen. Mash feed and water were given to birds ad libitum. Birds and feeders were weighed weekly, and feed was provided daily to determine body weight gain (**BWG**), feed intake, and feed conversion ratio. Commercial Spirulina containing 66% of protein and 8% of ash was supplied by Sopropéche (Boulogne-sur-Mer Cedex, France). Rovabio Excel AP, containing predominantly β-xylanase and β-glucanase, was obtained from Adisseo (Antony, France). Commercial lysozyme from chicken egg white was provided by Merck KGaA (Darmstadt, Germany). Diets were formulated to be isocaloric and isonitrogenous. Diet samples were analyzed for dry matter (**DM**) by drying a sample at 100°C to a constant weight. The nitrogen (N) content of all diets was determined by the Kjeldahl method, as described in AOAC method 954.01 (AOAC, 2000), and crude protein was calculated as 6.25 × N. Ash content was determined according to AOAC method 942.05 (AOAC, 2000). Crude fat was determined by extracting feed samples with petroleum ether using an automatic Soxhlet extractor (Gerhardt Analytical Systems; C. Gerhardt GmbH & Co. KG, Königswinter, Germany). Feed gross energy was determined by adiabatic bomb calorimetry (Parr 1261, Parr Instrument Company, Moline, IL).

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

Diets (day 21–35)				
Item	CON[1]	MA[1]	MAR[1]	MAL[1]
Ingredients, %				
Corn	64.2	69.0	69.0	69.0
Soybean meal 47% CP	30.0	11.3	11.3	11.3
Soybean oil	2.10	1.25	1.25	1.25
Sodium chloride	0.33	0.33	0.33	0.33
Calcium carbonate	1.06	1.22	1.22	1.22
Dicalcium phosphate	1.43	1.00	1.00	1.00
DL-Methionine	0.31	0.23	0.23	0.23
L-Lysine	0.22	0.37	0.37	0.37
Vitamin-mineral premix[2]	0.30	0.30	0.30	0.30
Spirulina powder	–	15.0	15.0	15.0
Rovabio Excel AP	–	–	0.005	–
Lysozyme	–	–	–	0.01
Nutrient content				
Energy, MJ ME/kg DM	13.1	13.1	13.1	13.1
Crude protein, %	20.5	21.1	21.1	21.0
Crude fat, %	6.10	6.10	6.00	6.10
Ash, %	5.60	5.50	5.40	5.40
Calcium, %	0.77	0.78	0.78	0.78
Available phosphorus, %	0.38	0.42	0.42	0.42
Sodium, %	0.15	0.15	0.15	0.15
Available lysine, %	1.11	1.14	1.14	1.14
Available methionine, %	0.60	0.62	0.62	0.62
Fatty acid profile, % total fatty acids				
14:0	0.07	0.21	0.18	0.20
16:0	12.9	19.5	19.4	20.5
16:1c9	0.09	1.32	1.29	1.41
17:0	0.10	0.11	0.13	0.12
18:0	2.56	2.23	2.18	2.37
18:1c9	23.7	19.5	20.6	20.7
18:c11	1.10	0.88	0.93	0.94
18:2n-6	52.4	43.1	45.3	43.2
18:3n-3	3.68	2.35	2.32	2.39
20:0	0.39	0.36	0.36	0.37
20:1c11	0.25	0.41	0.13	0.40
22:0	0.34	0.27	0.22	0.28
22:1n-9	0.11	0.19	0.24	0.24
24:0	0.23	0.13	0.17	0.14
Diterpene profile, μg/g				
α-Tocopherol	18.3	24.5	24.2	23.9
α-Tocotrienol	3.50	3.23	2.71	2.52
γ-Tocopherol	9.24	6.65	6.43	5.35
γ-Tocotrienol	6.89	5.32	4.80	5.07
δ-Tocopherol	1.75	0.80	0.82	0.83
Pigments, μg/g				
β-Carotene	0.48	26.5	28.1	31.5
Chlorophyll a[3]	1.52	419	478	512
Chlorophyll b[4]	2.42	21.9	24.9	28.7
Total chlorophylls[5]	3.95	441	504	541
Total carotenoids[6]	2.03	47.6	54.5	63.9
Total chlorophylls and carotenoids[7]	5.98	489	558	605

[1]CON: corn/soybean, in the control group; MA: 15% Spirulina; MAR: 15% Spirulina + 0.005% Robavio Excel AP; MAL: 15% Spirulina + 0.01% lysozyme.

[2]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.

[3]Ca: chlorophyll a = 11.24 × A662 nm − 2.04 × A645 nm.

[4]Cb: chlorophyll b = 20.13 × A645 nm − 4.19 × A662 nm.

[5]Ca + b: total chlorophylls = 7.05 × A662 nm + 18.09 × A645 nm.

[6]Cx + c: total carotenoids = (1,000 × A470 nm − 1.90 × Ca - 63.14 × Cb)/214.

[7]Ccc: total chlorophylls and carotenoids = (Ca + b) + (Cx + c).

At day 35, one bird per pen was slaughtered. Birds were electrically stunned and manually exsanguinated. Blood samples were collected in a Sarstedt tube (Numbrecht, Germany) and then centrifuged to obtain serum. Gastrointestinal (**GI**) organs were removed and emptied with running water. The weight of the crop, gizzard, liver, and pancreas and the length and weight of the duodenum, jejunum, ileum, and caecum were registered. To measure the viscosity of small intestine contents, samples were collected from the duodenum plus jejunum and ileum and centrifuged for 10 min at 9,000 rpm, and the viscosity of sample's supernatant was measured at 6 rpm using a viscometer (Model LVDVCP-II; Brookfield Engineering Laboratories, Middleboro, MA) with a cup maintained at 24°C. Qualitative analyses for xylanase, β-glucanase, and lysozyme activities in the GI tract were determined as described in the following section. Carcasses were maintained in the air-chilled circuit, until the carcass temperature reached 4°C.

Enzymatic Activity Analysis

Qualitative analysis for xylanase and β-glucanase activity in the digesta contents recovered from the various GI compartments was performed in agar plates, using wheat arabinoxylan (Megazyme, Wicklow, Ireland) and β-glucan (Megazyme, Wicklow, Ireland) at 0.1% final concentration (w/v) in 10-mM Tris HCl pH 8 (Merck KGaA, Darmstadt, Germany). Xylanase and β-glucanase activities were detected after 24 h incubation at 37°C using Congo red assay, as described by Ponte et al. (2004). The qualitative analysis of lysozyme in digesta samples was performed in agarose plate, using 0.1% (w/v) NaCl in phosphate buffer (pH 6.5), followed by the addition of *Micrococcus lysodeikticus* (0.02% w/v). The catalytic activity was detected after 24 h incubation at 47°C with Amido Black 10B and 7% of acetic acid, according to Gosnell et al. (1975) with slight modifications.

Serum Metabolites

Total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triacylglycerols, creatinine, total protein, urea, glucose, aspartate aminotransferase, alanine aminotransferase, gammaglutamyltransferase, and alkaline phosphatase were analyzed through diagnostic kits (Roche Diagnostics, Mannheim, Germany), using a Modular Hitachi Analytical System (Roche Diagnostics). Very low-density lipoprotein cholesterol and total lipids were calculated by using formulas described in the studies by Friedewald et al. (1972) and Covaci et al. (2006), respectively. Total antioxidant capacity was analyzed by using a Quanti-Chrom Antioxidant Assay kit (BioAssaySystems, Hayward, CA).

Determination of Carcass Traits

Breast (pectoralis major) and thigh (biceps femoris) muscles without skin and deboned, located on the right side of the bird, were measured on the same 3 spots by using a glass penetration pH electrode (HI9025; Hanna instruments, Woonsocket, RI), 24 h postmortem. The pH value was an average of 3 replicate measurements

on the same muscle samples. The CIELAB color measurements, lightness (L*), redness (a*), and yellowness (b*), resulted from the average value of 3 readings taken from 3 spots of breast and thigh muscles using a tristimulus analyzer (Chroma Meter CR-300 attached with data processor; Konica Minolta, Tokyo, Japan). Data were obtained after 1-h air exposure to allow blooming. A portion of the right-side breast and thigh was stored at −20°C in a vacuum-sealed bag until analysis. For shear force determination, these portions were thawed at 4°C during 24 h and were then individually cooked in a water bath at 80°C in plastic bags to an internal temperature of 72°C, which was monitored by an internal thermocouple (Lufft C120; Lufft, Munchen, Germany). The samples were then removed and chilled for 2 h at room temperature to measure shear force. Before and after cooking, breast and thigh muscles were weighed to determine cooking loss. Strips (1.0 cm [width] × 1.0 cm [thickness] × 5.0 cm [length]) of both muscles were analyzed using a Warner-Bratzler blade, coupled to a texture analyzer TA.XT.plus from Stable Microsystems (Surrey, UK). The measurements of slices were replicated extensively depending on the size of meat samples (a minimum of 4 replications). Data were collected using a specific software program (Texture Expert Exceed; Stable Micro Systems, Surrey, UK), and meat shear force was expressed as the mean of the peak value of replicates.

Trained Sensory Panel Analysis

Meat sensory analysis was performed only on breast because of the difficulty on separating muscles from thighs. Right skinless breast muscles were stored at −20°C until analysis. Breast meat samples were thawed at 4°C during 24 h and were individually cooked in a water bath at 85°C in plastic bags, until an internal temperature of 78°C was reached. While cooking, meat temperature was monitored using a thermocouple (Lufft C120; Lufft, Munchen, Germany). For sensory analysis, muscle samples were trimmed of external connective tissue, cut into cubes of approximately 1 cm^3, and maintained at 60°C, in heated plaques previously identified. The 11 panelists were selected and trained at the Faculty of Veterinary Medicine (University of Lisbon, Lisbon, Portugal), according to the study by Cross et al. (1978). Samples were randomly distributed across 5 panel sessions, with 8 random samples per session, and the attributes classified were tenderness, juiciness, flavor, off-flavors, and overall acceptability. The scale applied in the sensory analysis was structured into 8 points, with 1 being extremely tough, dry, weak, and negative and 8 being extremely tender, juicy, strong, and positive for tenderness, juiciness, flavor, and overall acceptability, respectively.

Determination of Meat Oxidative Stability

Approximately 15 g of meat from the left breast and thigh of each animal were minced, divided into 4 portions, and kept exposed to air in plastic bags for 0, 2, 4, and 6 D at 4°C. Lipid oxidation in both muscles was determined at day 0, 2, 4, and 6 by measuring the thiobarbituric acid reactive substances (**TBARS**), based on the procedure of Grau et al. (2000), following the spectrophotometric method described by Mercier et al. (2004). This technique is based on the ability of malonaldehyde to form a pink-colored chromogen that absorbs 532-nm light in a UV/visible spectrophotometer (Ultrospec III; Pharmacia LKB Biochrom Ltd. Cambridge, UK). To quantify TBARS, a standard calibration curve was constructed using 1,1,3,3-tetraethoxypropane (Fluka, Neu Ulm, Germany) as a precursor of malonaldehyde. Results are presented as milligrams of malonaldehyde per kilogram of meat.

Determination of Total Cholesterol, β-Carotene, and Diterpenes in Meat and Experimental Diets

The simultaneous analysis of total cholesterol, β-carotene, and tocopherols in fresh meat (0.75 g) and feed (0.10 g) was performed according to Prates et al. (2006). After the direct saponification of samples, an aliquot of the n-hexane layer was filtered and injected into an HPLC system (Agilent 1100 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), with fluorescence detection of tocopherols and tocotrienols (excitation λ = 295 nm and emission λ = 325 nm) and UV-visible photodiode array detection of cholesterol (λ = 202 nm) and β-carotene (λ = 450 nm) in series. Total cholesterol, β-carotene, tocopherols, and tocotrienols contents were calculated, in duplicate, based on the external standard technique from a standard curve of peak area vs. concentration.

Determination of Pigments in Meat and Experimental Diets

The contents of chlorophyll a, chlorophyll b, and total carotenoids were measured in meat and experimental diets, according to Teimouri et al. (2013), with slight modifications. For the pigment determination, 10 mL of acetone (Merck KGaA, Darmstadt, Germany) was added to 1 g of fresh meat or 0.5 g of feed, then incubated at room temperature and shaken in the dark overnight. After extraction, the samples were centrifuged at 4,000 rpm for 5 min and measured by using a UV-Vis spectrophotometer (Ultrospec 3100 pro; Amersham Biosciences, Little Chalfont, UK). All procedures associated with pigments extraction and analyses were carried out in dim light because pigments are very photosensitive. The pigment content was calculated according to the equations described by Hynstova et al. (2018).

Determination of Dry Matter and Total Lipids in Meat

The dry matter content of breast and thigh meats was determined as described by Rosenkranz (1993). Samples were lyophilized to constant weight using a lyophilisator Edwards Modulyo (Edwards High Vacuum International, Crawley, UK) at −60°C and 2.0 hPa. After lyophilization, samples were kept in desiccators at room temperature until analysis. Total lipids were extracted from lyophilized breast and thigh, as well as from feed, according to Folch et al. (1957) using a dichloromethane:methanol mixture (2:1, v/v). Total lipids were measured gravimetrically, in duplicate, by weighing the fatty residue obtained after solvent evaporation.

Determination of Fatty Acid Composition in Meat and Experimental Diets

The fatty acids residue of breast, thigh, and feed were converted to fatty acid methyl esters (**FAME**) by a combined transesterification procedure with NaOH in anhydrous methanol (0.5 M), followed by HCl/methanol (1/1 v/v) at 50°C for 30 and 10 min, respectively (Raes et al., 2001). FAME were analyzed using a gas chromatograph (HP6890 A; Hewlett-Packard, Avondale, PA), equipped with a flame ionization detector and a CP-Sil 88 capillary column (100 m × 0.25 mm i.d., 0.20-μm film thickness, Chrompack; Varian Inc., Walnut Creek, CA). The chromatographic conditions were as follows: injector and detector temperatures were maintained at 250°C and 280°C, respectively; helium was used as the carrier gas at a flow rate of 1.0 mL/min; and the split ratio was 1:20. The gas chromatograph oven temperature was programmed to start at 50°C (maintained for 4 min), followed by a 13°C/min ramp to 175°C (maintained for 20 min), and by a 4°C/min ramp to 275°C (maintained for 44 min). The quantification of FAME was performed using heneicosanoic acid (21:0) methyl ester as the internal standard and the conversion of relative peak areas into weight percentages. Fatty acids were expressed as percentage of total fatty acids.

Statistical Analysis

All data were analyzed using the generalized linear mixed (GLM) model of SAS program (SAS Institute Inc., Cary, NC). The cage was used as the experimental unit for feed intake and feed conversion ratio. The individual bird was used as the experimental unit for body weight (**BW**), BWG, serum parameters, enzymes activity, and meat quality measurements. Significant multiple comparisons test was carried out using the PDIFF option adjusted with Tukey-Kramer to determine statistical differences among dietary treatments. A P value lower than 0.05 was considered statistically significant.

RESULTS

Growth Performance and Gastrointestinal Tract Parameters

The performance of birds is summarized in Table 2. On day 35, birds fed Spirulina diets supplemented with lysozyme (MAL) had a lower BW ($P < 0.05$) in comparison to birds fed the control diet. During the entire treatment period, BWG was lower in birds fed diets with Spirulina in comparison to birds fed the control diet ($P < 0.001$). In addition, groups fed on Spirulina, with or without enzyme supplementation, had higher feed conversion ratios relative to the control group ($P < 0.001$). No treatment effects were observed on feed intake ($P > 0.05$). Relative weights of the GI tract organs were not significantly influenced by dietary treatment ($P > 0.05$), except for the ileum, which was heavier in birds fed MAL than in those fed MAR ($P < 0.05$). The relative length of the duodenum was lower in the control group ($P < 0.01$) than in birds fed with Spirulina. Birds fed with Spirulina diet supplemented with lysozyme contributed to a greater increase (190%) in the viscosity of the duodenum plus jejunum contents ($P < 0.05$) than birds fed the control diet.

Serological Measures

The effect of dietary treatments with Spirulina on serological parameters are presented in Table 3 and characterizes birds' health at the end of the experimental assay. Total lipids and total cholesterol were reduced in the control group ($P < 0.001$) relative to the other treatments. MA had increased concentrations of triacylglycerols ($P < 0.001$), very low-density lipoprotein cholesterol ($P < 0.001$), and gammaglutamyltransferase ($P < 0.001$) in comparison to birds fed the control diet and also to birds fed with Spirulina supplemented with exogenous enzymes. MAL increased total protein ($P < 0.001$) compared to birds of the remaining groups. The total antioxidant capacity was not affected by dietary treatments ($P > 0.05$).

Enzymatic Activity in Digesta Samples

Xylanase activity was detected in the duodenum of birds fed MAR ($P < 0.05$) in comparison to the other dietary treatments (Table 4). In addition, β-glucanase activity was also observed in the caecum of all birds. Lysozyme activity was observed in jejunum and ileum of birds fed MA, MAR, and MAL ($P < 0.001$), compared with broilers fed the control diet.

Carcass Traits, Meat Quality, and Sensory Evaluation

No significant differences were found for pH 24 h, lightness (L*), cooking loss, and shear force of breast and thigh meats across dietary treatments ($P > 0.05$) (Table 5). The yellowness (L*) score presented higher

Table 2. Performance, relative weight, and length of gastrointestinal tract of broilers.

Item	CON[1]	MA[1]	MAR[1]	MAL[1]	SEM	*P* value
Body weight, g						
day 21	723	742	787	728	24.8	0.262
day 28	1,244	1,198	1,225	1,137	32.3	0.117
day 35	1,802[b]	1,697[a,b]	1,717[a,b]	1,613[a]	39.2	0.016
Body weight gain, g						
day 21–28	74.5[b]	65.2[a]	62.6[a]	58.5[a]	1.83	<0.001
day 28–35	93.0[b]	83.2[a]	82.0[a]	79.3[a]	2.25	<0.001
day 21–35	83.8[b]	74.2[a]	72.3[a]	68.9[a]	1.83	<0.001
Feed intake, g						
day 21–28	107	105	107	97.9	3.09	0.126
day 28–35	141	138	138	132	3.3	0.342
day 21–35	124	121	122	115	3.0	0.191
Feed conversion ratio						
day 21–28	1.44[a]	1.60[b]	1.71[c]	1.68[b,c]	0.022	<0.001
day 28–35	1.51[a]	1.66[b]	1.68[b]	1.67[b]	0.021	<0.001
day 21–35	1.48[a]	1.63[b]	1.70[b]	1.67[b]	0.017	<0.001
Relative weight of GI tract, g/kg BW						
Crop	2.62	2.91	2.98	2.53	0.221	0.418
Gizzard	12.2	14.3	13.1	14.2	0.61	0.061
Liver	21.8	23.7	22.4	23.5	0.90	0.422
Pancreas	2.30	2.38	2.25	2.37	0.126	0.870
Duodenum	4.77	5.06	5.33	4.82	0.223	0.283
Jejunum	9.45	9.57	9.26	9.03	0.354	0.722
Ileum	8.29[a,b]	8.19[a,b]	7.39[a]	8.52[b]	0.281	0.042
Caecum[3]	3.74	3.79	4.68	3.63	0.361	0.164
Relative length of GI tract, cm/kg BW						
Duodenum	13.1[a]	15.7[b]	15.5[b]	14.8[b]	0.52	0.006
Jejunum	33.6[a]	37.8[b]	37.0[a,b]	37.8[a,b]	1.10	0.034
Ileum	37.2	38.7	38.8	40.7	1.15	0.224
Caecum	8.08	8.44	8.12	8.02	0.329	0.812
Content viscosity, cP						
Duodenum + jejunum	4.84[a]	7.08[a,b]	7.32[a,b]	9.20[b]	1.071	0.039
Ileum	9.19	10.8	11.8	10.8	0.962	0.306

[a-c]Different superscript lowercase letters within a row indicate a significant difference ($P < 0.05$).
Abbreviation: GI, gastrointestinal tract.
[1]CON: corn/soybean, in the control group; MA: 15% Spirulina; MAR: 15% Spirulina + 0.005% Robavio Excel AP; MAL: 15% Spirulina + 0.01% lysozyme.
[3]Caecum: weight of 2 caecum.

values in breast and thigh from birds fed MA, MAR, and MAL ($P < 0.001$) than birds fed the control diet. The trained sensory panel scores in breast muscle are presented in Table 6. Meat tenderness, flavor, off-flavor, and overall acceptability were not affected by dietary treatments ($P > 0.05$). Meat juiciness from birds fed MA and MAL diets was less intense than juiciness from control birds ($P < 0.05$).

Oxidative Stability, Diterpenes, and Total Pigments

Table 7 presents the levels of oxidative stability, vitamin E profile, and total pigments in breast and thigh meats. The oxidative stability was not affected by dietary treatments in both muscles ($P > 0.05$). Birds fed Spirulina diets had lower values of α- and γ-tocopherol in breast and thigh meats ($P < 0.001$) than the control. Conversely, in breast and thigh, the sums of carotenoids and total chlorophylls plus carotenoids in both muscles were 2 times higher in Spirulina treatments than in the control treatment ($P < 0.001$).

Meat Lipids and Fatty Acid Composition

The total lipids, cholesterol, and fatty acid composition of breast and thigh meats is shown in Table 8. In breast, birds fed with MAR presented higher contents of total lipids ($P < 0.05$) than birds fed with MAL. Still for breast, birds fed with MA, MAR, and MAL had increased contents of 17:0 ($P < 0.001$), 17:1c9 ($P < 0.001$), 18:3n-6 ($P < 0.001$), 20:3n-6 ($P < 0.001$), saturated fatty acids (**SFA**) ($P < 0.001$), and n-6/n-3

Table 3. Serum parameters of broilers.

Item	CON[1]	MA[1]	MAR[1]	MAL[1]	SEM	*P* value
Total lipids, mg/dL[2]	490[a]	588[d]	535[b]	556[c]	4.5	<0.001
Triacylglycerols, mg/dL	84.0[a]	142[c]	86.0[a,b]	90.9[b]	1.42	<0.001
Total cholesterol, mg/dL	128[a]	148[b]	157[b,c]	150[c]	2.2	<0.001
HDL-C, mg/dL	107	105	104	110	1.8	0.130
LDL-C, mg/dL	4.50[a]	14.1[b]	28.6[c]	29.3[c]	0.710	<0.001
VLDL-C, mg/dL	16.8[a]	28.4[c]	17.2[a,b]	18.2[b]	0.28	<0.001
Glucose, mg/dL	287[b]	265[a]	263[a]	273[a,b]	5.4	0.017
Urea, mg/dL	1.45[a,b]	1.10[a]	2.74[c]	1.68[b]	0.106	<0.001
Total protein, g/dL	2.73[a]	2.65[a]	3.11[b]	3.33[c]	0.032	<0.001
Creatinine, mg/dL	0.018[b]	0.006[a]	0.019[b]	0.005[a]	0.0010	<0.001
ALT, U/L	12.5	13.5	12.6	12.1	0.91	0.737
AST, U/L	255[b]	152[a]	585[c]	238[b]	15.9	<0.001
ALP, U/L	2,821[c]	1,760[b]	1,063[a]	2,956[c]	65.8	<0.001
γ-GT, U/L	15.5[a,b]	20.7[c]	13.7[a]	17.2[b]	0.84	<0.001
TAC, μM (Trolox equiv.)	460	399	465	432	26.7	0.288

[a-d]Different superscript lowercase letters within a row indicate a significant difference ($P < 0.05$).

Abbreviations: γ-GT, γ-glutamyltransferase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TAC, total antioxidant capacity; VLDL-C, very low-density lipoprotein cholesterol = 1/5 triacylglycerols.

[1]CON: corn/soybean, in the control group; MA: 15% Spirulina; MAR: 15% Spirulina + 0.005% Robavio Excel AP; MAL: 15% Spirulina + 0.01% lysozyme.

[2]Total lipids = (total cholesterol) × 2 + (triacylglycerols) + 150.

polyunsaturated fatty acids (**PUFA**) ($P < 0.001$) and decreased contents of 18:2n-6 ($P < 0.001$), 18:3n-3 ($P < 0.001$), n-3 PUFA ($P < 0.01$), and PUFA/SFA ($P < 0.001$) when compared with birds fed the basal diet. In the thigh, birds fed with MA, MAR, and MAL had increased contents of 17:0 ($P < 0.001$), 17:1c9 ($P < 0.001$), 18:3n-6 ($P < 0.001$), SFA ($P < 0.001$), and n-6/n-3 PUFA ($P < 0.001$) but decreased 18:3n-3 ($P < 0.001$), n-3 PUFA ($P < 0.001$), and PUFA/SFA ($P < 0.01$) in relation to control birds.

DISCUSSION

The cell wall composition and structure of *A. platensis* is not completely understood (Madeira et al., 2017), and therefore, the process of selecting enzymes for its degradation is difficult. The commercial exogenous enzymes, Rovabio Excel AP and lysozyme, were herein applied to attempt achieving a better digestibility of Spirulina by broiler chickens. In fact, Coelho et al. (2020) have recently reported that lysozyme in combination with α-amylase is able to partially degrade *A. platensis* cell wall in vitro. A wide range of Spirulina incorporation levels (between 0.25% and 20% in the diet) has been previously reported, with largely conflicting results in what concerns broiler performance. However, Becker (2013) reported that up to 10% of dietary inclusion of microalgae can be safely used as a partial replacement for conventional protein sources in poultry diets. According to the same author, higher levels of microalgae incorporation might, in the long term, cause adverse effects, leading to higher feed, protein, and energy conversion ratios. In the present study, birds fed with Spirulina and supplemented with lysozyme in particular had decreased BW and BWG and increased feed conversion ratio. For the results found in the MAL treatment specifically, data suggest that a lower bird performance might be associated with a higher digesta viscosity that limits the access of the endogenous enzymes to their target substrates and, ultimately, affects negatively the feed digestibility (Jonhson and Gee, 1986). According to Evans et al. (2015), when Spirulina is incorporated at higher levels (>10%), gelation of the proteins is observed, which contributes to a lower amino acid digestibility and an increase in viscosity. The high digesta viscosity observed in birds receiving Spirulina and lysozyme relative to the control led to enlargement of the digestive organs, as observed previously in animals on cereal-based diets (Cardoso et al., 2018). However, current data suggest that the increase in digesta viscosity does not result from the presence of soluble polysaccharides, such as arabinoxylans and β-glucans commonly observed in wheat or barley-based diets, because the presence of xylanases and β-glucanases in the MAR group had no effect on viscosity. Thus, the almost 2-fold increase in digesta viscosity in birds supplemented with lysozyme indicates that the observed increase in digesta viscosity may be a direct result of gelation of the poorly digestible Spirulina proteins, as suggested by Evans et al. (2015). Hence, the degradation of Spirulina cell wall by lysozyme, in birds of the MAL group, may have led to a higher release of microalga protein that increased digesta viscosity. Overall, data suggest that Spirulina proteins may be resistant to the proteolytic attack of birds' endogenous peptidases.

Glucose is an important source of energy, while total proteins, including albumin, reflect the synthesis of proteins in the liver, which may be associated with broilers' growth and physiological status (Limdi and Hyde, 2003). In this study, broiler chickens had serum glucose levels slightly above the standards described by Lumeij et al. (1997). Birds from the MAL group, fed a combination of Spirulina and lysozyme, showed increased levels of total protein, in accordance to the study by Fathi

Table 4. Number of birds, out of 10 animals analyzed, fed with diets presenting xylanase, β-glucanase, and lysozyme activities in digesta samples collected from various gastrointestinal compartments.

Item	CON[1]	MA[1]	MAR[1]	MAL[1]	Chi-square value	*P* value
Xylanase activity						
Crop	1	0	1	3	4.34	0.227
Gizzard	0	0	0	0	–	–
Duodenum	0[a]	0[a]	3[b]	0[a]	9.73	0.021
Jejunum	0	2	0	3	6.17	0.104
Ileum	2	2	2	4	1.60	0.659
Caecum	5	8	7	7	2.16	0.538
β-Glucanase activity						
Crop	4	4	8	3	5.91	0.116
Gizzard	1	2	0	1	2.22	0.528
Duodenum	0	0	2	0	6.32	0.097
Jejunum	0	0	0	0	–	–
Ileum	2	1	1	1	0.63	0.890
Caecum	10	10	10	10	–	–
Lysozyme activity						
Crop	0	0	0	1	3.08	0.380
Gizzard	0	0	0	1	3.08	0.380
Duodenum	0	0	0	1	3.08	0.380
Jejunum	0[a]	9[b]	8[b]	10[b]	28.6	<0.001
Ileum	0[a]	9[b]	10[b]	9[b]	31.4	<0.001
Caecum	2[a]	5[a]	8[b]	9[b]	12.5	0.006

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

[1]CON: corn/soybean, in the control group; MA: 15% Spirulina; MAR: 15% Spirulina + 0.005% Robavio Excel AP; MAL: 15% Spirulina + 0.01% lysozyme.

et al. (2018). Birds fed with Spirulina had a reduction in serum urea, suggesting that microalgae alone promoted a more efficient nitrogen utilization (Brown and Cline, 1974), thus contributing to a better balance between body protein synthesis and body protein degradation (Métayer et al., 2008). Signs of oxidative stress were not found in this study. However, it was expected that birds fed Spirulina displayed increased antioxidant action because this microalgae is a rich source of C-phycocyanin, an antioxidant pigment with hypolipidemic activity (Khan et al., 2005; Wu et al., 2005). As opposed to cholesterol, total lipids in serum were higher than the values found by El-Hady and El Ghalid (2018) in broiler chickens fed with 3 and 6% of Spirulina. The hypolipidemic action of Spirulina has been attributed to C-phycocyanin, a blue color pigment able to inhibit the pancreatic lipase activity in a dose-dependent manner (Deng and Chow, 2010). Data presented here suggest that this antioxidant mechanism has not been potentiated by the presence of Spirulina.

Regarding the liver function, results for aspartate aminotransferase are in accordance with those obtained by others (Lumeij, 1997; Fathi et al., 2018). In relation to γ-glutamyl transferase, an indicator of hepatocellular and renal damage (Hochleithner, 1994; Hoffman and Solter, 2008), lower levels were observed here when compared with data obtained by Kuttappan et al. (2013). Overall, our data on hepatic markers suggest that none of the diets compromised the normal function of the liver or kidney.

All birds, whether supplemented or not with exogenous enzymes, displayed high levels of polysaccharidase activity in the caecum. The caecum plays an important role in preventing colonization of pathogens, detoxifying harmful substances, recycling nitrogen, promoting the degradation of some carbohydrates, and absorbing additional nutrients (Clench and Mathias, 1995; Jorgensen et al., 1996; Jeong and Kim, 2014). A high lysozyme activity was detected in jejunum plus ileum of birds fed Spirulina, regardless of the supplementation with

Table 5. The effect of dietary treatment on meat quality and carcass traits of broilers.

	Breast muscle						Thigh muscle					
Item	CON[1]	MA[1]	MAR[1]	MAL[1]	SEM	*P* value	CON[1]	MA[1]	MAR[1]	MAL[1]	SEM	*P* value
pH24 h	5.79	5.77	5.82	5.70	0.060	0.559	5.83	5.84	5.87	5.77	0.060	0.739
Lightness (L*)	49.2	46.6	47.6	46.9	0.84	0.139	49.2	49.0	48.0	47.4	0.47	0.051
Redness (a*)	4.53	5.31	5.50	5.37	0.288	0.089	8.25[a]	9.49[a,b]	9.91[b]	9.67[a,b]	0.430	0.046
Yellowness (b*)	4.38[a]	10.7[b]	11.7[b]	12.3[b]	0.546	<0.001	5.38[a]	12.9[b]	12.4[b]	13.5[b]	0.459	<0.001
Cooking loss, %	14.7	12.6	13.7	12.8	1.05	0.501	17.8	18.3	17.3	19.0	1.02	0.657
Shear force, kg	1.63	1.53	1.61	1.66	0.135	0.909	2.22	2.26	2.26	2.03	0.113	0.444

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

[1]CON: corn/soybean, in the control group; MA: 15% Spirulina; MAR: 15% Spirulina + 0.005% Robavio Excel AP; MAL: 15% Spirulina + 0.01% lysozyme.

Table 6. Sensorial panel traits of broiler breast meat.

Item	CON[1]	MA[1]	MAR[1]	MAL[1]	SEM	*P* value
Tenderness	5.38	5.24	5.69	5.24	0.155	0.130
Juiciness	4.50[b]	4.00[a]	4.27[a,b]	3.96[a]	0.134	0.012
Flavor	4.52	4.40	4.56	4.15	0.124	0.088
Off-flavor	0.650	0.480	0.291	0.348	0.1050	0.077
Overall	5.00	4.84	5.15	4.73	0.126	0.087

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

The different attributes evaluated in sensorial traits were quantified on a rating scale from 1 (low score) to 8 (high score), with the exception of flavor and off-flavor that were quantified from 0 (absence) to 8 (very intense).

[1]CON: corn/soybean, in the control group; MA: 15% Spirulina; MAR: 15% Spirulina + 0.005% Robavio Excel AP; MAL: 15% Spirulina + 0.01% lysozyme.

exogenous enzymes. In birds fed Spirulina supplemented with lysozyme, a degradation of peptidoglycan of cell wall was observed, leading to a release of proteins and LPS and promoting an increase in digesta viscosity, as discussed previously. LPS is a component of cell wall of gram-negative bacteria (Tran and Whitfield, 2009) that could cause an intense systemic inflammatory response (Takahashi et al., 2008) and suppress birds' growth (Xie et al., 2000). Thus, the reduced growth performance observed in birds fed Spirulina combined with lysozyme might also be associated with the presence of LPS formation. The observation of exogenous xylanase activity in the GI tract of birds fed Spirulina supplemented with Rovabio Excel AP suggests that carbohydrate-degrading enzymes, in particular xylanases and β-glucanases, are not effective to attack the microalgae cell wall.

Venkataraman et al. (1994) showed that color pigmentation of skin, breast, and thigh muscles was stronger in broilers when groundnut protein was replaced by Spirulina. In line with this, Arbor Acres broilers fed 2.5 and 5% of Spirulina presented yellowness of breast and skin (Raach-Moujahed et al., 2011). In contrast, Park et al. (2018) using Ross 308 male broilers fed with low levels of Spirulina (0.25, 0.5, 0.75, and 1%) reported no changes in the color of breast meat. Herein, redness and yellowness scores were higher in thighs than in breast of birds fed Spirulina. In both muscles, yellowness was affected by the addition of Spirulina in all dietary treatments. These results are similar to those reported by Venkataraman et al. (1994), Toyomizu et al. (2001), and Altmann et al. (2018), which revealed distinct breast colors when Spirulina was incorporated into poultry diets. Dietary *Arthrospira sp.* influences both the yellowness and redness of broiler flesh (Toyomizu et al., 2001), being the increments in yellowness most probably associated with the accumulation of zeaxanthin within the flesh. In accordance to the study by Altmann et al. (2018), the distinctive color was not just the result of an instrumental analytical measurement, but it was also detected by the naked eye. Surprisingly, when the breast muscles were cooked, a color difference between samples was not distinguishable by our trained sensory panel. From the point of view of consumers, the orange color could be advantageous when feeding broiler chickens with Spirulina because meat color is one of the most important quality indicators perceived by consumers in some countries, such as Japan (Komai, 1997), the United States, and Mexico (Castaneda et al., 2005). The majority of meat

Table 7. Oxidative stability of broiler breast and thigh meats measured as TBA reactive substances, diterpene profile, and total pigments.

	Breast muscle						Thigh muscle					
Item	CON[1]	MA[1]	MAR[1]	MAL[1]	SEM	*P* value	CON[1]	MA[1]	MAR[1]	MAL[1]	SEM	*P* value
Malondialdehyde, mg/kg												
day 0	0.266	0.248	0.241	0.320	0.0253	0.140	0.369	0.322	0.317	0.317	0.0374	0.709
day 2	0.390	0.274	0.356	0.346	0.0996	0.866	0.434	0.521	0.491	0.545	0.0673	0.676
day 4	0.279	0.236	0.326	0.328	0.0784	0.814	0.378	0.448	0.421	0.387	0.0255	0.212
day 6	0.398	0.266	0.423	0.370	0.1172	0.790	0.559	0.613	0.429	0.516	0.0661	0.266
Diterpene profile, μg/g												
α-Tocopherol	4.03[a]	2.18[b]	2.18[b]	2.35[b]	0.187	<0.001	7.33[a]	3.65[b]	3.29[b]	4.33[b]	0.294	<0.001
γ-Tocopherol	0.618[a]	0.270[b]	0.279[b]	0.288[b]	0.0329	<0.001	0.658[a]	0.345[b]	0.308[b]	0.379[b]	0.0326	<0.001
α-Tocotrienol	nd	nd	nd	nd	–	–	0.313	0.249	0.260	0.301	0.0274	0.296
γ-Tocotrienol	nd	nd	nd	nd	–	–	0.291	0.247	0.297	0.298	0.0394	0.767
Pigments, μg/100 g												
Chlorophyll a[2]	8.78	10.6	10.7	9.53	1.491	0.774	4.90	10.1	9.75	8.36	1.905	0.219
Chlorophyll b[3]	13.7	16.6	17.7	15.5	2.50	0.703	8.51	13.8	12.8	10.8	2.974	0.608
Total chlorophylls[4]	22.4	27.2	28.4	25.1	3.89	0.716	13.4	23.9	22.6	19.2	4.721	0.414
Total carotenoids[5]	48.6[a]	161[b]	159[b]	183[b]	11.45	<0.001	48.8[a]	153[b]	158[b]	186[b]	9.46	<0.001
Total chlorophylls and carotenoids[6]	71.0[a]	188[b]	187[b]	208[b]	13.00	<0.001	62.2[a]	177[b]	181[b]	208[b]	10.70	<0.001

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

Abbreviation: nd, not detected.

[1]CON: corn/soybean, in the control group; MA: 15% Spirulina; MAR: 15% Spirulina + 0.005% Robavio Excel AP; MAL: 15% Spirulina + 0.01% lysozyme.

[2]Ca: chlorophyll a = 11.24 × A662 nm − 2.04 × A645 nm.

[3]Cb: chlorophyll b = 20.13 × A645 nm − 4.19 × A662 nm.

[4]Ca + b: total chlorophylls = 7.05 × A662 nm + 18.09 × A645 nm.

[5]Cx + c: total carotenoids = (1,000 × A470 nm − 1.90 × Ca − 63.14 × Cb)/214.

[6]Ccc: total chlorophylls and carotenoids = (Ca + b) + (Cx + c).

Table 8. Total lipid content, cholesterol content, and fatty acid profile of breast and thigh.

	Breast muscle						Thigh muscle					
Item	CON[1]	MA[1]	MAR[1]	MAL[1]	SEM	*P* value	CON[1]	MA[1]	MAR[1]	MAL[1]	SEM	*P* value
Total lipids, g/100 g	1.33^{a,b}	1.36^{a,b}	1.57^{b}	1.29^{a}	0.071	0.039	3.45	3.05	3.56	3.60	0.301	0.560
Cholesterol, mg/g	0.620	0.671	0.684	0.618	0.0393	0.525	0.732	0.727	0.766	0.734	0.0276	0.744
Fatty acid composition, g/100 g FA												
10:0	0.10	0.12	0.11	0.12	0.012	0.678	0.03	0.03	0.02	0.02	0.003	0.509
12:0	0.03	0.03	0.03	0.04	0.007	0.939	0.04	0.02	0.02	0.02	0.010	0.360
14:0	0.40	0.36	0.37	0.37	0.010	0.159	0.40^{a}	0.41^{a}	0.45^{b}	0.43^{a}	0.010	0.007
14:1c9	0.08	0.07	0.08	0.08	0.006	0.728	0.12^{a}	0.12^{a,b}	0.15^{b}	0.14^{b}	0.008	0.013
15:0	0.09	0.09	0.08	0.08	0.006	0.570	0.07	0.07	0.08	0.08	0.004	0.345
DMA16:0	2.46	3.08	2.95	3.04	0.229	0.205	0.75	0.88	0.68	0.67	0.122	0.607
16:0	19.9^{a}	20.8^{a,b}	20.6^{b}	21.3^{a,b}	0.25	0.005	20.9^{a}	22.5^{a,b}	22.8^{b}	22.7^{b}	0.32	<0.001
16:1c7	0.52	0.53	0.55	0.49	0.025	0.414	0.49	0.55	0.52	0.53	0.020	0.238
16:1c9	3.16	3.40	3.76	3.75	0.222	0.182	4.74^{a}	5.45^{a,b}	5.98^{b}	6.01^{b}	0.260	0.004
17:0	0.21^{a}	0.38^{b}	0.35^{b}	0.39^{b}	0.013	<0.001	0.14^{a}	0.27^{b}	0.27^{b}	0.27^{b}	0.012	<0.001
17:1c9	0.05^{a}	0.09^{b}	0.10^{b}	0.10^{b}	0.005	<0.001	0.07^{a}	0.11^{b}	0.12^{b}	0.12^{b}	0.006	<0.001
DMA18:0	0.61	0.71	0.73	0.69	0.056	0.440	0.21	0.23	0.20	0.18	0.030	0.667
DMA18:1	0.63	0.64	0.59	0.63	0.048	0.910	0.16	0.17	0.11	0.12	0.028	0.326
18:0	7.70	8.13	7.84	8.01	0.234	0.592	6.12	6.23	6.03	5.94	0.175	0.687
18:1c9	26.4	24.9	24.8	24.9	0.76	0.420	32.9	32.5	33.0	33.0	0.68	0.940
18:1c11	2.42^{a}	2.65^{a,b}	2.70^{a,b}	2.72^{b}	0.073	0.023	2.02	2.15	2.19	2.19	0.054	0.104
18:2n-6	20.6^{a}	18.0^{b}	17.7^{b}	17.9^{b}	0.32	<0.001	22.9^{a}	20.1^{b}	18.9^{b}	20.3^{a,b}	0.69	0.002
18:2t9t12	0.05	0.04	0.04	0.04	0.002	0.072	0.06	0.05	0.04	0.05	0.004	0.198
18:3n-6	0.20^{a}	0.56^{b}	0.56^{b}	0.54^{b}	0.028	<0.001	0.30^{a}	0.71^{b}	0.63^{b}	0.68^{b}	0.045	<0.001
18:3n-3	1.04^{a}	0.65^{b}	0.66^{b}	0.66^{b}	0.034	<0.001	1.43^{a}	1.04^{b}	0.97^{b}	1.07^{b}	0.058	<0.001
18:4n-3	nd	nd	nd	nd	–	–	0.06^{a}	0.04^{a}	0.04^{b}	0.04^{a}	0.004	0.018
20:0	0.06	0.06	0.07	0.06	0.008	0.580	0.14	0.08	0.09	0.11	0.030	0.508
20:1c11	0.39^{a}	0.27^{b}	0.34^{a,b}	0.33^{a,b}	0.027	0.026	0.28	0.27	0.28	0.25	0.012	0.331
20:2n-6	0.66	0.56	0.54	0.57	0.043	0.211	0.25	0.21	0.18	0.19	0.020	0.162
20:3n-6	0.97^{a}	1.37^{b}	1.35^{b}	1.41^{b}	0.072	<0.001	0.40	0.58	0.46	0.47	0.048	0.071
20:4n-6	3.92	4.89	4.66	4.65	0.369	0.284	1.54	1.74	1.42	1.43	0.179	0.571
20:3n-3	0.10	0.08	0.07	0.08	0.007	0.066	0.04	0.04	0.04	0.04	0.004	0.469
20:5n-3	0.20	0.18	0.19	0.19	0.014	0.713	0.07	0.06	0.06	0.06	0.007	0.219
22:0	0.05	0.05	0.05	0.06	0.005	0.460	0.04	0.04	0.03	0.04	0.006	0.334
22:1n-9	0.12^{a}	0.10^{a,b}	0.09^{b}	0.11^{a,b}	0.007	0.007	0.08	0.08	0.09	0.08	0.012	0.928
22:5n-3	0.63	0.61	0.59	0.58	0.048	0.890	0.23	0.20	0.17	0.18	0.024	0.221
22:6n-3	0.39	0.47	0.46	0.47	0.043	0.493	0.15	0.18	0.15	0.15	0.019	0.675
Others	5.82	6.11	6.86	5.60	0.389	0.134	3.00	3.43	3.84	2.40	0.413	0.117
Partial sums of fatty acids												
SFA	28.6^{a}	30.0^{b}	29.6^{b}	30.4^{b}	0.25	<0.001	27.8^{a}	29.2^{b}	29.8^{b}	29.6^{b}	0.33	<0.001
MUFA	33.1	31.9	32.4	32.5	0.91	0.850	40.7	41.3	42.3	42.4	0.83	0.388
PUFA	28.8	27.4	26.8	27.1	0.53	0.060	27.4^{a}	24.9^{a,b}	23.0^{b}	24.6^{a,b}	0.88	0.012
DMA	3.69	4.43	4.28	4.36	0.311	0.333	1.12	1.28	0.99	0.97	0.178	0.591
n-6 PUFA	26.4	25.4	24.8	25.0	0.47	0.107	25.3^{a}	23.3^{a,b}	21.6^{b}	23.0^{a,b}	0.82	0.023
n-3 PUFA	2.35^{a}	1.98^{b}	1.97^{b}	1.97^{b}	0.078	0.002	2.00^{a}	1.56^{b}	1.42^{b}	1.54^{b}	0.068	<0.001
Nutritional ratios												
n-6/n-3	11.3^{a}	13.0^{b}	12.7^{b}	12.8^{b}	0.34	<0.001	12.7^{a}	15.0^{b}	15.6^{b}	15.0^{b}	0.33	<0.001
PUFA/SFA	1.01^{a}	0.91^{b}	0.91^{b}	0.89^{b}	0.078	<0.001	0.98^{a}	0.85^{b}	0.78^{b}	0.83^{b}	0.034	0.001

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

Abbreviations: DMA, dimethylacetals; MUFA, monounsaturated fatty acid; nd, not detected; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

SFA = 10:0 + 12:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 22:0.

MUFA = 14:1c9 + 16:1c7 + 16:1c9 + 17:1c9 + 18:1c9 + 18:1c11 + 20:1c11 + 22:1n-9.

PUFA = 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.

DMA = 16:0 + 18:0 + 18:1.

n-3 PUFA = 18:3n-3 + 18:4n-3 + 20:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3.

n-6 PUFA = 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 + 20:4n-6.

[1]CON: corn/soybean, in the control group; MA: 15% Spirulina; MAR: 15% Spirulina + 0.005% Robavio Excel AP; MAL: 15% Spirulina + 0.01% lysozyme.

attributes were not discriminated by our trained sensory panel, as in the study by Raach-Moujahed et al. (2011). Overall appreciation was positive for all meats (higher than 4). However, the panelists found metallic flavors. Metallic flavor is usually considered an off-flavor in poultry products (Neethling et al., 2016), resulting from lipid oxidation of meat (Jayasena et al., 2013). Therefore, a low score in off-flavor is highly advantageous in Spirulina-fed breast chicken, particularly when compared with meats resulting from birds fed on fish oil diets (Wood et al., 2008) and even algae products rich in docosahexaenoic acid (Ribeiro et al., 2013). The juiciness of breast from birds fed on Spirulina combined with lysozyme was affected, which was probably due to the presence of lysozyme.

If lipid oxidation increases linearly as the concentration of PUFA increases, the oxidative stability of unsaturated fatty acids decreases as the degree of unsaturation increases (Cortinas et al., 2005). The higher susceptibility of thigh to oxidation, relative to

breast, is the result of higher lipid content (Leskanich and Noble, 1997; Betti et al., 2009). Increased TBARS values reported in thigh muscle are likely due to high haem iron and myoglobin contents (Min et al., 2008). In fact, levels of TBARS above 0.8 mg/kg are indicative of rancidity in poultry meat (O'Neil et al., 1998), providing an oxidized flavor and turning meat unacceptable to consumers (Bonos et al., 2016). This threshold of 0.8-mg TBARS/kg for meat is remarkably higher and far apart the values observed in this trial. *A. platensis* is an excellent source of antioxidants, such as β-carotene, tocopherol, and carotenoids (Soni et al., 2017), which protect endogenous lipids from oxidation (Long et al., 2018). Although β-carotene was not detected in breast and thigh, the level of total carotenoids observed in meat may reflect the antioxidant action of Spirulina and the ability of this feed source to produce high-oxidative stability in chicken meat. In relation to the absence of β-carotene, it is suggested that the excess β-carotene in the diet is metabolized into retinol (Nogareda et al., 2015), as poultry cannot synthesize these compounds (Blanch, 1999). Moreover, total carotenoids were strongly in agreement with the diet profiles. The highest content of total carotenoids found in birds fed the Spirulina diet might explain some of the original differences found in pigments on breast and thigh meats, that is, on yellowness and redness scores. The thigh displayed higher diterpene concentrations than breast, which is likely due to the higher fat content (Ribeiro et al., 2013). Not surprisingly, α-tocopherol was the major diterpene, while the other vitamin E homologues were present at smaller concentrations and within the range usually found in broilers (Ponte et al., 2008; Ribeiro et al., 2013). Thus, our findings suggest that 15% of Spirulina can increase the antioxidant capacity of chicken meat.

According to the criteria of the Food Advisory Committee (1990), breast and thigh are considered lean meats (fat content <5%). Here, total lipids ranged from 1.33 to 1.57% in breasts and from 3.05 to 3.60% in thighs, meeting that criterion. As expected, thigh meat had higher cholesterol content than breast meat, which was slightly above the levels reported in other studies (Ponte et al., 2008; Ribeiro et al., 2014). The predominant fatty acids in chicken meat from all dietary treatments were palmitic (16:0) and stearic (18:0) acids as SFA, palmitoleic (16:1c9) and oleic acid (18:1c9) as monounsaturated fatty acids, and linoleic acid (18:2n-6) and arachidonic acid (20:4n-6) as n-6 PUFA. This fatty acid profile agrees with Spirulina composition in oleic acid (32%), linoleic acid (23%), palmitic acid (16%), stearic acid (3%), and γ-linoleic acid (18:3n-6) (3%) (Tokusoglu and Unal, 2003; Park et al., 2018). For both muscles, Spirulina incorporation resulted in a higher SFA content, where predominant 14:0, 16:0, and 18:0 fatty acids are not as easy to be oxidized and more ready to be deposited in breast and thigh meats (Long et al., 2018). The content of arachidonic acid was higher in all dietary treatments, which may be due to the conversion of linoleic acid (Martins et al., 2014). In both breast and thigh, the incorporation of Spirulina resulted in a 2-fold increase of γ-linolenic acid relative to the control diet. γ-Linoleic acid intake may have an anti-inflammatory effect due to production of prostaglandin E1 and other eicosanoids from 20:3n-6 (Innes and Calder, 2018). Cereal-based feed is a weak source of α-linolenic acid, and the same applies to *A. platensis* (<1%) (Tokusoglu and Unal, 2003), resulting in a negative impact on n-3 PUFA content in breast and thigh, although not physiologically relevant because of the low percentages found. The conversion of essential fatty acids, mainly α-linolenic acid, along the entire n-3 pathway, is a limited process because of the competition for desaturase enzymes, which are the same for both n-6 and n-3 pathways (Emken et al., 1994; Lopez-Ferrer et al., 2001). Thus, it is mandatory to assure an adequate level of dietary linoleic acid/α-linolenic acid ratio to obtain an efficient conversion of α-linolenic acid into the beneficial eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) (Griffin, 2008). Moreover, the increase in monounsaturated fatty acid and reduction of PUFA may suggest an augment in the de novo synthesis of fatty acids, promoted by delta 9 desaturase (Ribeiro et al., 2014). Data reported here demonstrate that the fatty acid profile in breast and thighs is in accordance with diets, as reported by other studies (Ribeiro et al., 2013, 2014). The addition of exogenous enzymes did not alter these fatty acid profiles. Current nutritional recommendations are that the PUFA/SFA ratio in human diets should be above 0.45, and within PUFA, the n-6/n-3 ratio should not exceed 4.0 (Burghardt et al., 2010). In view of these figures, the n-6/n-3 ratio of breast and thighs across dietary treatments is not in accordance with the recommended guidelines, in contrast to PUFA/SFA ratio.

CONCLUSIONS

The incorporation of 15% of Spirulina, individually and in combination with a commercial mixture of carbohydrate-degrading enzymes (Rovabio Excel AP) or lysozyme, decreased birds' performance and increased the weight and size of some GI compartments. Although birds remained healthy along the entire experimental period, a higher digesta viscosity was observed in animals fed diets with Spirulina, in particular those supplemented with lysozyme. Overall, data suggest that Spirulina proteins impair the nutritive value of microalga for broilers through an increase in digesta viscosity and a lower digestibility, as a consequence of their proteolytic resistance to the broiler endogenous peptidases. In addition, we propose that lysozyme, but not Rovabio Excel AP, might have disrupted Spirulina cell wall in birds' intestine. In general, meat quality traits do not seem to be impaired, neither by Spirulina nor by enzymes.

The underlying mechanisms responsible for the poor performance associated with the intake of Spirulina provided by this study point out to future work on this scientific field. We speculate that the addition of an

exogenous peptidase active on the majority of Spirulina proteins, most likely from a marine organism, might improve the digestibility of Spirulina proteins and avoid the gelation aggravated by lysozyme. Therefore, the supplementation of diets receiving Spirulina with lysozyme and a Spirulina-specific peptidase is a promising approach for the effective use of microalgae in poultry nutrition. Future work should exploit this research opportunity.

ACKNOWLEDGEMENTS

This research was financially supported by Fundação para a Ciência e a Tecnologia (FCT, Lisbon, Portugal; grant: PTDC/CVT-NUT/5931/2014), Portugal2020 (grant: 08/SI/3399/2015), CIISA (project UIDP/CVT/00276/2020), and Post-Doc fellowships to J.M.P. (SFRH/BPD/116816/2016), M.S.M. (SFRH/BPD/97432/2013), and P.A.L. (08/SI/3399/2015).

REFERENCES

Altmann, B. A., C. Neumann, S. Velten, F. Liebert, and D. Mörlein. 2018. Meat quality derived from high inclusion of a micro-alga or insect meal as an alternative protein source in poultry diets: a pilot study. Foods 34:1–15.

Al-Zuhair, S., S. Ashraf, S. Hisaindee, N. A. Darmaki, S. Battah, D. Svistunenko, B. Reeder, G. Stanway, and A. Chaudhary. 2016. Enzymatic pre-treatment of microalgae cells for enhanced extraction of proteins. Eng. Life Sci. 00:1–11.

AOAC. 2000. Official Method of Analysis, 17th ed. Assoc. Off. Anal. Chem., Washington, DC.

Apajalahti, J., and M. R. Bedford. 1999. Improve bird performance by feeding its microflora. Worlds Poult. Sci. J. 15:20–23.

Austic, R., A. Mustafa, B. Jung, S. Gatrell, and X. Lei. 2013. Potential and limitation of a new defatted diatom microalgal biomass in replacing soybean meal and corn in diets for broiler chickens. J. Agric. Food Chem. 31:7341–7348.

Babadzhanov, A. S., N. Abdusamatova, F. M. Yusupova, N. Faizullaeva, L. G. Mezhlumyan, and M. K. Malikova. 2004. Chemical composition of Spirulina platensis cultivated in Uzbekistan. Chem. Nat. Compd. 40:276–279.

Becker, E. W. 2007. Micro-algae as a source of protein. Biotechnol. Adv. 25:207–210.

Becker, E. W. 2013. Microalgae for human and animal nutrition. Pages 461–503. in Handbook of Microalgal Culture: Applied Phycology and Biotechnology. A. Richmond and Q. Hu Eds. Wiley Blackwell, West Sussex, United Kingdom.

Bedford, M. R., and A. J. Morgan. 1996. The use of enzymes in poultry diets. Worlds Poult. Sci. J. 52:61–68.

Bedford, M. R., and A. J. Cowieson. 2012. Exogenous enzymes and their effects on intestinal microbiology. Anim. Feed Sci. Technol. 173:76–85.

Betti, M., B. L. Schneider, W. V. Wismer, V. L. Carney, M. J. Zuidhof, and R. A. Renema. 2009. Omega-3-enriched broiler meat: 2. Functional properties, oxidative stability, and consumer acceptance. Poult. Sci. 88:1085–1095.

Blanch, A. 1999. Getting the color of yolk and skin right. Worlds Poult. Sci. J. 15:32–33.

Bonos, E., E. Kasapidou, A. Kargopoulos, A. Karampampas, E. Christaki, P. Florou-Paneri, and I. Nikolakakis. 2016. Spirulina as a functional ingredient in broiler chicken diets. S. Afr. J. Anim. Sci. 46:94–102.

Brown, J. A., and T. R. Cline. 1974. Urea excretion in the pig: an indicator of quality and amino acid requirements. J. Nutr. 104:542–545.

Burghardt, P. R., E. S. Kemmerer, B. J. Buck, A. J. Osetek, C. Yan, L. G. Koch, S. L. Britton, and S. J. Evans. 2010. Dietary n-3:n-6 fatty acid ratios differentially influence hormonal signature in a rodent model of metabolic syndrome relative to healthy controls. Nutr. Metab. 7:53–59.

Cardoso, V., E. A. Fernandes, H. M. M. Santos, B. Maças, M. M. Lordelo, L. T. Gama, L. M. A. Ferreira, C. M. G. A. Fontes, and T. Ribeiro. 2018. Variation in levels of non-starch polysaccharides and endogenous endo-1-4-β-xylanases affects the nutritive value of wheat for poultry. Br. Poult. Sci. 59:218–226.

Castaneda, M. P., E. M. Hirschler, and A. R. Sams. 2005. Skin pigmentation evaluation in broilers fed natural and synthetic pigments. Poult. Sci. 84:143–147.

Choct, M. 2006. Enzymes for the feed industry: past, present and future. Worlds Poult. Sci. J. 62:5–15.

Clench, M. H., and J. R. Mathias. 1995. The avian cecum: a review. Wilson Bull 107:93–121.

Coelho, D., P. A. Lopes, V. Cardoso, P. Ponte, J. Brás, M. S. Madeira, C. M. Alfaia, N. Bandarra, C. M. G. A. Fontes, and J. A. Prates. 2020. A two-enzyme constituted mixture to improve the degradation of *Arthrospira platensis* microalga cell wall for monogastric diets. J. Anim. Physiol. Anim. Nutr. 104:310–321.

Cohen, Z. 1997. The chemicals of Spirulina. Pages 198–199. in Spirulina *Platensis* (Arthospira): Physiology, Cell-Biology and Biotechnology. A. Vonshak Ed. Taylor & Francis Ltd., London, United Kingdom.

Cortinas, L., A. Barroeta, C. Villaverde, J. Galobart, F. Guardiola, and M. Baucelis. 2005. Influence of the dietary polyunsaturation level on chicken meat quality: lipid oxidation. Poult. Sci. 84:48–55.

Covaci, A., S. Voorspoels, C. Thomsen, B. van Bavel, and H. Neels. 2006. Evaluation of total lipids using enzymatic methods for the normalization of persistent organic pollutant levels in serum. Sci. Total Environ. 366:361–366.

Cowieson, A. J., and M. R. Bedford. 2009. The effect of phytase and carbohydrate on ileal amino acid digestibility in monogastric diets: complementary mode of action? Worlds Poult. Sci. J. 65:609–624.

Cowieson, A. J., and A. M. Kluenter. 2018. Contribution of exogenous enzymes to potentiate the removal of antibiotic growth promoters in poultry production. Anim. Feed Sci. Technol. 250:91–92.

Cross, H. R., R. Moen, and M. S. Stanfield. 1978. Training and testing of judges for sensory analysis of meat quality. Food Technol. 32:48–54.

Deng, R., and T. J. Chow. 2010. Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae Spirulina. Cardiovasc. Ther. 28:33–45.

Drews, G. 1973. Fine structure and chemical composition of the cell envelopes. Pages 99–116. in The Biology of Blue-Green Algae. N. G. Carr and B. A. Whitton Eds. Blakwell, Oxford, United Kingdom.

El-Hady, A. M. A., and O. A. H. El-Ghalid. 2018. Spirulina platensis Algae (SPA): a novel poultry feed additive. Effect of SPA supplementation in broiler chicken diets on productive performance, lipid profile and calcium-phosphorus metabolism. Worlds Poult. Sci. J. 7498:1–7.

Emken, E. A., R. O. Adlof, and R. M. Gulley. 1994. Dietary linoleic acid influences desaturation and acylation of deuterium- labelled linoleic and linolenic acids in young adult males. Biochim. Biophys. Acta Lipids Lipid Metab. 1213:277–288.

Evans, A. M., D. L. Smith, and J. S. Moritz. 2015. Effects of algae incorporation into broiler starter diet formulations on nutrient digestibility and 3 to 21 d bird performance. J. Appl. Poult. Res. 24:206–214.

Fathi, M. A., M. M. M. Namra, M. S. Ragab, and M. M. M. Aly. 2018. Effect of dietary supplementation of algae meal (Spirulina platensis) as growth promoter on performance of broiler chickens. Egypt. Poult. Sci. 38:375–389.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509.

Food Advisory Committee 1990. Report on Review of Food Labelling and Advertising. Her Majesty's Stationery Office, London.

Friedewald, W. T., R. I. Levy, and D. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 18:499–502.

Gosnell, W. W., M. Kroger, and K. Friedrich. 1975. Improvements on the agar plate method to determine lysozyme. J. Milk Food Technol. 38:457–460.

Grau, A., F. Guardiola, J. Boatella, A. M. D. Baucells, and R. Codony. 2000. Evaluation of lipid ultraviolet absorption as a parameter to measure lipid oxidation in dark chicken meat. J. Agric. Food Chem. 48:4128–4135.

Griffin, B. A. 2008. How relevant is the ratio of dietary n-6 to n-3 polyunsaturated fatty acids to cardiovascular disease risk? Evidence from the OPTILIP study. Curr. Opin. Lipidol. 19:57–62.

Hochleithner, M. 1994. Biochemistries. Pages 223–245. in Avian Medicine: Principles and Application. B. W. Ritchie, G. J. Harrison and L. R. Harrison Eds. Wingers Publishing Inc., Lake Worth, FL.

Hoffman, W. E., and P. F. Solter. 2008. Diagnostic enzymology of domestic animals. Pages 351–378. in Clinical Biochemistry of Domestic Animals. J. J. Kaneko, J. W. Harvey and M. L. Bruss Eds, 6th ed. Academic Press, Burlington, MA.

Howe, P., B. Meyer, S. Record, and K. Baghurst. 2006. Dietary intake of long-chain omega-3 polyunsaturated fatty acids: contribution of meat sources. Nutrition 22:47–53.

Hynstova, V., D. Sterbova, B. Klejdus, J. Hedbavny, D. Huska, and V. Adam. 2018. Separation, identification and quantification of carotenoids and chlorophylls in dietary supplements containing *Chlorella vulgaris* and *Spirulina platensis* using high performance thin layer chromatography. J. Pharm. Biomed. Anal. 148:108–118.

Innes, J. K., and P. C. Calder. 2018. Omega-6 fatty acids and inflammation. Prostaglandins Leukot. Essent. Fatty Acids 132:41–48.

Jayasena, D. D., D. U. Ahn, K. C. Nam, and C. Jo. 2013. Flavour chemistry of chicken meat: a review. Asian-Australas. J. Anim. Sci. 26:732–742.

Jeong, J. S., and I. H. Kim. 2014. Effect of Bacillus subtilis C-3102 spores as a probiotic feed supplement on growth performance, noxious gas emission, and intestinal microflora in broilers. Poult. Sci. 93:3097–3103.

Jonhson, I. T., and J. M. Gee. 1986. Gastrointestinal adaptation in response to soluble non-available polysaccharides in rat. Br. J. Nutr. 55:497–505.

Jorgensen, H., X. Q. Zhao, K. E. Knudsen, and B. O. Eggum. 1996. The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. Br. J. Nutr. 75:379–395.

Khan, Z., P. Bhadouria, and P. S. Bisen. 2005. Nutritional and therapeutic potential of Spirulina. Curr. Pharm. Biotechnol. 6:373–379.

Komai, T. 1997. Branded quality chicken booms in Japan. Poult. Int. 36:34–35.

Kuttappan, V. A., G. R. Huff, W. E. Huff, B. M. Hargis, J. K. Apple, C. Coon, and C. M. Owens. 2013. Comparison of hematologic and serologic profiles of broilers birds with normal and severe degrees of white striping in breasts fillets. Poult. Sci. 92:339–345.

Leskanich, C. O., and R. C. Noble. 1997. Manipulation of the n-3 polyunsaturated fatty acid composition of avian eggs and meat. Worlds Poult. Sci. J. 53:155–183.

Limdi, J. K., and G. M. Hyde. 2003. Evaluation of abnormal liver function tests. Postgrad. Med. J. 79:307–312.

Liu, D., Y. Guo, Z. Wang, and J. Yuan. 2010. Exogenous lysozyme influences *Clostridium perfringes* colonization and intestinal barrier function in broiler chickens. Avian Pathol. 39:17–24.

Long, S. F., S. Kang, Q. Q. Wang, Y. T. Xu, L. Pan, J. X. Hu, M. Li, and X. S. Piao. 2018. Dietary supplementation with DHA-rich microalgae improves performance, serum composition, carcass trait, antioxidant status, and fatty acid profile of broilers. Poult. Sci. 97:1881–1890.

Lopez-Ferrer, S., M. D. Baucells, A. C. Barroeta, J. Galobart, and M. A. Grashorn. 2001. n-3 enrichment of chicken meat. 2. Use of precursors of long-chain polyunsaturated fatty acids: linseed oil. Poult. Sci. 80:753–761.

Lum, K., J. Kim, and X. Lei. 2013. Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. J. Anim. Sci. Biotechnol. 4:53.

Lumeij, J. T. 1997. Avian Clinical Biochemistry. Pages 900–901. in Clinical Biochemistry of Domestic Animals. J. J. Kaneko, J. W. Harvey and M. L. Bruss Eds, 5th ed. Academic Press, San Diego.

Madeira, M. S., C. Cardoso, P. A. Lopes, D. Coelho, C. Afonso, N. M. Bandarra, and J. A. M. Prates. 2017. Microalgae as feed ingredients for livestock production and meat quality: a review. Livest. Sci. 205:111–121.

Madkour, F. F., A. E. W. Kamil, and H. S. Nasr. 2012. Production and nutritive value of Spirulina *platensis* in reduced cost media. Egypt. J. Aqua. Res. 38:51–57.

Martins, S. V., C. M. Alfaia, P. A. Lopes, and J. A. M. Prates. 2014. Dietary sources and health implications of arachidonic acid. Pages 31–33. in Arachidonic Acid - Sources, Biosynthesis and Health Effects. J. M. O`Keefe Ed. Nova Science Publishers Inc., New York, USA.

Mercier, Y., P. Gatellier, and M. Renerre. 2004. Lipid and protein oxidation in vitro, and antioxidant potential in meat from Charolais cows finished on pasture or mixed diet. Meat Sci. 66:467–473.

Métayer, S. I., A. Seiliez, S. Collins, Y. Duchene, P. A. Mercier, P. A. Geraert, and S. Tesseraud. 2008. Mechanisms through which sulfur amino acids control protein metabolism and oxidative status. J. Nutr. Biochem. 19:207–2015.

Min, B., K. C. Nam, J. Cordray, and D. U. Ahn. 2008. Endogenous factors affecting oxidative stability of beef loin, pork loin, and chicken breast and thigh meats. J. Food Sci. 73:C439–C446.

National Research Council. 1994. Nutrient Requirements of Poultry, 9th rev. ed. Natl. Acad. Press, Washington, DC.

Neethling, J., L. C. Hoffman, and M. Muller. 2016. Factors influencing the flavour of game meat: a review. Meat Sci. 113:139–153.

Nogareda, C., J. A. Moreno, E. Angulo, G. Sandmann, M. Portero, T. Capell, C. Zhu, and P. Christou. 2016. Carotenoid-enriched transgenic corn delivers bioavailable carotenoids to poultry and protects them against coccidiosis. Plant Biotechnol. J. 14:160–168.

O'Neil, L. M., K. Galvin, P. A. Morrissey, and D. J. Buckley. 1998. Comparison of effects of dietary olive oil, tallow and vitamin E on the quality of broiler meat and meat products. Br. Poult. Sci. 39:365–371.

Park, J. H., and S. I. Lee. 2018. Effect of dietary Spirulina (Arthrospira) platensis on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens. Poult. Sci. 97:2451–2459.

Ponte, P. I. P., L. M. A. Ferreira, M. A. C. Soares, L. T. Gama, and C. M. G. A. Fontes. 2004. Xylanase inhibitors affect the action of exogenous enzymes used to supplement Triticum durum-based diets for broiler chicks. J. Appl. Poult. Res. 13:660–666.

Ponte, P. I. P., S. P. Alves, R. J. B. Bessa, L. M. A. Ferreira, L. T. Gama, J. L. A. Brás, C. M. G. A. Fontes, and J. A. M. Prates. 2008. Influence of pasture intake on the fatty acid composition, and cholesterol, tocopherols, and tocotrienols content in meat from free-range broilers. Poult. Sci. 87:80–88.

Prates, J. A. M., M. A. G. Quaresma, R. J. B. Bessa, C. M. G. A. Fontes, and C. M. P. M. Alfaia. 2006. Simultaneous HPLC quantification of total cholesterol, tocopherols and beta-carotene in Barrosã-PDO veal. Food Chem. 94:469–477.

Raach-Moujahed, A., S. Hassani, S. Zairi, M. Bouallegue, C. Darej, B. Haddad, and C. Damergi. 2011. Effect of dehydrated Spirulina platensis on performances and meat quality of broilers. Res. Opin. Anim. Vet. Sci. 1:505–509.

Raes, K., S. De Smet, and D. Demeyer. 2001. Effect of double-muscling in Belgian Blue young bulls on the intramuscular fatty acid composition with emphasis on conjugated linoleic acid and polyunsaturated fatty acids. Anim. Sci. 73:253–260.

Ribeiro, T., M. M. Lordelo, S. P. Alves, R. J. B. Bessa, P. Costa, J. P. C. Lemos, L. M. A. Ferreira, C. M. G. A. Fontes, and J. A. M. Prates. 2013. Direct supplementation of diet is the most efficient way of enriching broiler meat with n-3 long-chain polyunsaturated fatty acids. Br. Poult. Sci. 54:753–765.

Ribeiro, T., M. M. Lordelo, P. Costa, S. P. Alves, W. S. Benevides, R. J. B. Bessa, J. P. C. Lemos, R. M. A. Pinto, L. M. A Ferreira, C. M. G. A. Fontes, and J. A. M. Prates. 2014. Effect of reduced dietary protein and supplementation with docosahexaenoic acid product on broiler performance and meat quality. Br. Poult. Sci. 55:752–765.

Rosenkranz, F. D. 1993. Freeze drying. Pages 201–215. in Vacuum Products. Edwards High Vacuum International, West Sussex, UK.
Seyidoglu, N., S. Inan, and C. Aydin. 2017. A prominent superfood: Spirulina platensis. Pages 1–28. in Superfood and Functional Food - the Development of Superfoods and Their Roles as Medicine. N. Shiomi and V. Waisundara Eds. IntechOpen, United Kingdom.
Shirazi, H. M., J. Karimi-Sabet, and C. Ghotbi. 2017. Biodiesel production from Spirulina microalgae feedstock using direct transesterification near supercritical methanol condition. Bioresour. Technol. 239:378–386.
Slominski, B. A. 2011. Recent advances in research on enzymes for poultry diets. Poult. Sci. 90:2013–2023.
Soni, R. A., K. Sudhakar, and R. S. Rana. 2017. Spirulina - from growth to nutritional product: a review. Trends Food Sci. Technol. 69:157–171.
Spolaore, P., C. Joannis-Cassan, E. Duran, and A. Isambert. 2006. Commercial applications of microalgae. J. Biosci. Bioeng. 101:87–96.
Swiatkiewicz, S., A. Arczewska-Wlosek, and D. Józefiak. 2015. Application of microalgae biomass in poultry nutrition. Worlds Poult. Sci. J. 71:663–672.
Takahashi, K., A. Aoki, T. Takimoto, and Y. Akiba. 2008. Dietary supplementation of glycine modulates inflammatory response indicators in broiler chickens. Br. J. Nutr. 100:1019–1028.
Teimouri, M., A. K. Amirkolaie, and S. Yeganeh. 2013. The effects of Spirulina *platensis* meal as feed supplement on growth performance and pigmentation of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 396-399:14–19.
Tokusoglu, O., and M. K. Unal. 2003. Biomass nutrient profiles of three microalgae: Spirulina platensis, Chlorella vulgaris, and Isochrisis galbana. J. Food Sci. 68:1144–1148.
Toyomizu, M., K. Sato, H. Taroda, T. Kato, and Y. Akiba. 2001. Effects of dietary Spirulina on meat color in muscle of broiler chickens. Br. Poult. Sci. 42:197–202.
Tran, A. X., and C. Whitfield. 2009. Lipopolysaccharides (endotoxins). Encycl. Microbiol. 71:513–528.
Venkataraman, L. V., T. Somasekaran, and E. W. Becker. 1994. Replacement value of blue-green alga (Spirulina platensis) for fishmeal and a vitamin-mineral premix for broiler chicks. Br. Poult. Sci. 35:373–381.
Wang, L., B. Pan, J. Sheng, J. Xu, and Q. Hu. 2007. Antioxidant activity of Spirulina platensis extracts by supercritical carbon dioxide extraction. Food Chem. 105:36–41.
Wood, J. D., M. Enser, A. V. Fisher, G. R. Nute, P. R. Sheard, R. I. Richardson, S. I. Hugher, and F. M. Whittington. 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat Sci. 78:343–358.
Wu, L. C., J. A. A. Ho, M. C. Shieh, and I. W. Lu. 2005. Antioxidant and antiproliferative activities of Spirulina and Chlorella water extracts. J. Agric. Food Chem. 53:4207–4212.
Xie, H., N. C. Rath, G. R. Huff, W. E. Huff, and J. M. Balog. 2000. Effects of Salmonella typhimurium lipopolysaccharide on broiler chickens. Poult. Sci. 79:33–40.