Original Article

Effects of microalgae supplementation on broiler meat quality, antioxidant status, and fatty acid profile

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Abstract

This study aimed to evaluate the dose-dependent effects of dietary supplementation with *Schizochytrium limacinum*, a microalga rich in omega-3 fatty acids and bioactive compounds, on the fatty acid composition and antioxidant status of broiler breast meat, as well as its potential effects on growth performance, carcass characteristics, and meat quality. In a 42-day feeding trial, 192 day-old Ross-308 broiler chicks were randomly allocated to four dietary treatment groups (48 chicks per group): a control group fed the basal diet without *S. limacinum* supplementation (0 g/kg), and three treatment groups fed the basal diet supplemented with 0.25, 0.5, and 1 g/kg *S. limacinum*, respectively. The chicks were fed a starter diet (24% CP, 3000 kcal ME/kg) from days 0 to 21 and a finisher diet (20% CP, 3200 kcal ME/kg) from days 22 to 42. At the end of the study, although notable variations in both body weight and body weight gain were found in birds fed diets containing 1 g/kg of microalgae during the 1st and 2nd weeks, no differences were detected in the subsequent weeks. Liver weight increased in birds given 1 g/kg of microalgae in their diet. Water-holding capacity declined with increasing microalgae levels. Increasing microalgae doses reduced Paraoxonase and arylesterase, whereas no differences were observed in malondialdehyde and glutathione levels. Microalgae supplementation reduced the proportion of saturated fatty acids. In contrast, the proportion of unsaturated fatty acids increased. A notable rise in omega-3 fatty acid levels was observed in birds fed diets containing 0.25 g/kg of microalgae, and the omega-3/omega-6 ratio reached optimal levels.

Keywords: antioxidant status, broiler, meat fatty acid profile, microalgae, performance

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Introduction

Over the past two decades, microalgae have received growing interest as functional feed ingredients in poultry nutrition. Among them, *Schizochytrium limacinum* has attracted particular attention because of its high content of polyunsaturated fatty acids (PUFA), especially long-chain n-3 fatty acids such as docosahexaenoic acid (DHA), which may beneficially modify the fatty acid profile of poultry meat. This marine microalga is also considered an alternative feed additive due to its suitability for large-scale production through heterotrophic fermentation, which enables rapid biomass yield and a relatively stable chemical composition (Wang *et al*., 2025). In broiler diets, its inclusion may enhance the nutritional value of meat by promoting the deposition of long-chain n-3 fatty acids in muscle tissues. Furthermore, *Schizochytrium limacinum* biomass contains bioactive compounds, including carotenoids and sterol-like substances, that may contribute to the protection of highly unsaturated fatty acids against oxidative deterioration (Jeon *et al*., 2022; Wang *et al*., 2025). For these reasons, *Schizochytrium limacinum* is considered a promising functional feed ingredient with the potential to improve the lipid profile and nutritional quality of poultry meat (Altmann *et al*., 2020; Jeon *et al*., 2022).

Previous studies have shown that *Schizochytrium limacinum* supplementation can improve tissue fatty acid composition in poultry and may exert beneficial effects on performance traits, meat quality, and antioxidant-related parameters (Long *et al*., 2018; Khan *et al*., 2021; Jeon *et al*., 2022). In broilers, dietary inclusion of *Schizochytrium limacinum* has been associated with increased DHA deposition in meat and an improved ω-6/ω-3 ratio (Long *et al*., 2018; Jeon *et al*., 2022). Nevertheless, findings across studies are not entirely consistent, and these discrepancies may be related to differences in microalgal strain, inclusion level, broiler genotype, basal diet composition, and feeding duration (Long *et al*., 2020; Saadaoui *et al*., 2021). Despite these promising outcomes, several factors may limit the use of *Schizochytrium limacinum* in poultry diets. One of the most important of these is the structural complexity of its cell wall. Because poultry have a limited capacity to degrade complex cell-wall polysaccharides and associated extracellular polymeric substances, the release of intracellular nutrients may be restricted, which can limit the access of digestive enzymes to proteins and lipids and ultimately reduce nutrient bioavailability and digestibility (Saadaoui *et al*., 2021).

Recent studies have shown increasing interest in the use of *Schizochytrium limacinum* in poultry nutrition. However, limited information is available from individual broiler studies that comprehensively assess the dose-dependent effects of this microalga on growth performance, carcass traits, breast meat quality, antioxidant status, and fatty acid profile. Therefore, it is important to clarify whether *Schizochytrium limacinum* can enhance the nutritional quality of broiler meat without compromising production performance or basic meat quality characteristics. Accordingly, the present study was conducted to evaluate the effects of dietary *Schizochytrium limacinum* supplementation on growth performance, carcass traits, breast meat quality, antioxidant parameters, and fatty acid composition in broilers. It was hypothesized that *Schizochytrium limacinum* supplementation would increase the n-3 fatty acid content of the meat, modulate antioxidant-related parameters in a dose-dependent manner, and preserve performance and carcass traits at acceptable levels.

Materials and Methods

This research was carried out with the approval of the Kafkas University Local Ethics Committee for Experimental Animals (Approval No: KAÜ-HADYEK/2023-126). A total of 192 one-day-old straight-run Ross-308 broiler chicks were used in this study, as the chicks were obtained without sex separation at hatch. The birds were randomly allocated to the treatment groups to minimize potential bias associated with sex distribution among groups. Sex was not determined at placement and was not included as a factor in the statistical analysis. However, chicks were randomly allocated to replicate pens in order to minimize potential bias due to unequal sex distribution among treatments. Therefore, sex-related variation was assumed to be randomly distributed across the experimental groups. Four experimental groups were formed in total, and each group consisted of four replicates containing 12 birds each.

Based on Yan and Kim (2013), the birds were assigned to four dietary treatment groups: a control group fed the basal diet without microalgae supplementation (0 g/kg), and three treatment groups fed the basal diet supplemented with 0.25, 0.5, and 1 g/kg microalgae, respectively. The microalgae (*Schizochytrium limacinum*) additive used in the study was produced by Marin Biotechnology and Food Industry Trade Limited Company, Aydın, Türkiye. The microalgae culture was produced in closed systems using fermenters and harvested at an early stage. According to the information obtained from the manufacturer, it contains 45% crude protein, 5% crude fat, 4% crude ash, and 0.211% trans-linoleic acid + trans-linolenic acid on a dry matter basis. Additionally, it contains 0.662 g total omega-6 fatty acids, 0.369 g total trans fatty acids, and 2.466 g total omega-3 fatty acids per 100 g.

The diets used for feeding the animals in this experiment were prepared to satisfy the nutritional requirements of broilers for the starter (0–21 days) and finisher (22–42 days) periods according to according to NRC (1994), which remains a widely accepted reference in experimental poultry nutrition, and is consistent with current commercial guidelines (Aviagen, 2022) (Table 1). Throughout the experiment, the birds were fed a broiler starter diet containing 23% crude protein and 3000 kcal/kg ME during days 0–21, and a broiler finisher diet containing 21% crude protein and 3200 kcal/kg ME during days 22–42. Diets were formulated with equivalent energy and protein contents. Feed and water were made available *ad libitum* throughout the experimental period. The nutrient composition and chemical composition of the

diets used for animal feeding in the study are presented in Table 2.

The care and feeding of the animals were carried out in floor pens measuring 130 × 54 × 108 cm, bedded with pine shavings approximately 8–10 cm thick. During the first seven days of the experiment, a lighting program providing 23 hours of light followed by 1 hour of darkness was implemented. For the remaining period of the experiment, a 20-hour light and 4-hour dark program was used. The house temperature was maintained at 34 °C at chick level during the first seven days, and the ambient temperature was gradually decreased by an average of 0.5 °C per day until stabilizing at 24 °C, which was then maintained constant throughout the study. The experiment was conducted over a period of 42 days.

Table 1 Basal diet composition.

Ingredients (%)	Starter (d 0-21)	Finisher (d 22-42)
Corn, yellow	46.50	49.40
Soybean meal (48% crude protein)	24.35	20.35
Corn gluten meal (62% crude protein)	7.30	7.00
Barley	4.00	4.00
Sunflower meal	3.00	3.00
Rice bran	2.90	2.90
Wheat bran	1.90	2.10
Crushed grain	2.00	2.10
Coarsely ground grain	1.00	1.00
Vegetable oil	2.57	4.67
Dicalcium phosphate	1.94	1.40
DL-Methionine	0.35	0.20
L-Lysine hydrochloride	0.50	0.40
L-Threonine	0.20	0.10
Marble powder	1.04	0.93
Salt	0.20	0.20
Vitamin–mineral premix[1]	0.25	0.25

[1]Vitamin A, 13000000 IU; Vitamin D_3, 5000000 IU; Vitamin E, 80000 mg; Vitamin K_3, 3200 mg; Vitamin B_1, 3200 mg; Vitamin B_2, 8600 mg; Vitamin B_8, 4500 mg; Vitamin B_{12}, 17 mg; Niacin, 65000 mg; Calcium pantothenate, 20000 mg; Folic acid, 2200 mg; D-biotin, 250 mg; Manganese (Mn), 120000 mg; Iron (Fe), 20000 mg; Zinc (Zn), 110000 mg; Copper (Cu), 16000 mg; Iodine (I), 1250 mg; Selenium (Se), 300 mg; Antioxidant, 100000 mg.

Table 2 Nutrient composition of the basal diet.

Nutrient composition, %	Starter (d 0–21)	Finisher (d 22–42)
Dry matter	89.90	90.10
Crude protein	23.00	21.00
Metabolizable energy, kcal/kg	3000	3200
Crude oil	4.78	7.11
Crude ash	6.37	5.49
Crude fiber	4.17	3.95
Starch	37.16	38.77
Calcium	0.96	0.78
Phosphorus	0.49	0.40

All nutrient values were determined by laboratory analysis and expressed on an as-fed basis.

Growth performance: All birds included in the study were individually weighed weekly on days 7, 14, 21, 28, 35, and 42, and live weight (LW) and live weight gain (LWG) were recorded. LWG was calculated as the difference between the final and initial live weights for each measurement period. To determine feed intake, the amount of feed added weekly was recorded, and the residual feed remaining in the feeders at the end of each week was subtracted from the total amount of feed offered. Feed consumption was then calculated for each replicate, and average feed intake (AFI) per bird was determined by dividing feed consumption by the number of birds in each replicate. Total body weight gain (TBWG) was calculated as the difference between final and initial body weight, and average daily gain (ADG) was calculated by dividing TBWG by the number of days in the relevant period. FCR was

calculated as feed intake divided by body weight gain for each replicate during each measurement period.

Determination of carcass traits and internal organ weights: Upon completion of the experiment, two birds were randomly selected from each replicate pen (eight birds per treatment group; 32 birds in total) for slaughter analysis (Ölmez *et al*., 2021). Birds were selected without prior consideration of body weight or sex in order to avoid selection bias. Immediately after the slaughtering process, hot carcass weights were measured and recorded. The hot carcass yield was calculated by multiplying the hot carcass weight (g) by 100 and dividing it by the slaughter weight (g). During slaughter, the internal organs (liver, heart, spleen, and gizzard) of a total of 32 animals were individually weighed using a precision balance (0.001 g), and the obtained data were recorded. After the carcasses were kept in the cold storage, they were maintained at 4 °C for 24 hours and reweighed, and the cold carcass yield was calculated using the same method.

Determination of breast meat glutathione, malondialdehyde, paraoxonase, and arylesterase parameters: Paraoxonase (PON) and arylesterase measurements were performed according to the kit procedure (RelAssay Diagnostics®, Mega Tıp, Türkiye). Glutathione (GSH) and malondialdehyde (MDA) analyses were carried out using tissue homogenates according to the described methods (Beutler *et al*., 1963).

Breast meat quality and nutrient composition: For the determination of breast meat quality and some nutrient compositions, pH, water-holding capacity (WHC), cooking loss (CL), dry matter (DM), and crude ash (CA) parameters were examined. For the analysis of WHC and CL, meat samples were taken from the breast fillet, stored at 4 °C for 24 hours, and then analyzed.

The breast meat pH value was measured at the 15th minute after slaughter (pH15) and 24 hours after slaughter (pH24). The samples were stored at 4 °C until the post-slaughter measurements. pH measurements were performed using a pH meter probe (Hanna Instruments, Inc., Woonsocket, USA) at three different locations (cranial, cranio-axial, and cranio-abaxial) of the pectoralis major, at a depth of approximately 2–2.5 cm. The average of the three values obtained was taken as the meat pH value (ISO, 1999; Kahraman *et al*., 2011).

After the collected samples had been stored at 4 °C for a period of 24 hours, a 5 g breast meat sample taken from the cranial part was placed between pre-weighed No. 1 Whatman filter papers, and a pressure of 2250 g was applied for five minutes. At the end of the pressing period, the applied pressure was removed, and the meat samples were collected and weighed. The initial and final weights of the samples were measured using a precision balance (0.001 g), and the difference between them was calculated to determine the WHC percentage (Barton-Gade *et al*., 1993).

The determination of cooking loss was carried out 24 hours after slaughter following the procedure described by Honikel (1998). Breast meat samples (10 g) taken after slaughter were kept at 4 °C, then placed in numbered nylon bags sealed airtight. The meat samples were processed in a water bath (Şimşek Laboteknik Ltd. Şti., Ankara, Türkiye) at 80 °C for one hour. After cooking, the cooled meat samples were gently dried with paper towels and subsequently weighed. The cooking loss percentage was determined by calculating the difference between the initial and post-cooking weights (g), multiplying by 100, and dividing by the post-cooking weight (g).

For breast meat nutrient composition analysis, breast meat samples (eight samples from each replicate) collected after slaughter were stored at -20 °C until nutrient analyses were performed. Before conducting the analysis, the samples were brought to room temperature, and DM and CA contents of the meat were determined according to the reported methods (AOAC, 1990).

To determine the fatty acid composition, breast meat samples collected post-slaughter were stored at -20 °C until analysis. Prior to analysis, the samples were thawed and homogenized at room temperature using a tissue homogenizer (Homogenizer HS-30E, witeg Labortechnik GmbH, Wertheim, Germany) equipped with a polytetrafluoroethylene-headed probe (No. 5553855, witeg Labortechnik GmbH, Wertheim, Germany).

The homogenate (0.7 mL of 10 M potassium hydroxide and 5.3 mL of methanol) was incubated at 55 °C for 45 minutes in an incubator (Nüve FN 120, Ankara, Türkiye). Subsequently, 0.58 mL of 10 M sulfuric acid was added, the mixture was vortexed, and incubation was continued at 55 °C for an additional 45 minutes. After incubation, 3 mL of n-hexane was added, and the mixture was centrifuged at 1600 × g for 5 minutes.

Diet samples were milled to a particle size of 1 mm and subjected to the same methylation procedure for fatty acid composition analysis, as described by Kara (2023) (Table 3).

Statistical analyses: Statistical analyses were performed using SPSS (PASW Statistics 22). Data for growth performance parameters (BW and BWG) measured repeatedly over time were analyzed using a Repeated Measures ANOVA to determine the effects of treatment, time, and the treatment × time interaction. Sphericity was assessed using Mauchly's test, and Greenhouse-Geisser corrections were applied when the sphericity assumption was violated. For other overall growth performance variables (TBWG, ADG, AFI, and FCR), the replicate pen was considered the experimental unit (n = 4 per treatment), and data were analyzed by one-way ANOVA. For carcass traits, organ weights, meat quality, antioxidant parameters, and fatty acid composition, two birds were sampled from each pen; the mean value of birds sampled within the same pen was used for statistical analysis, so that the pen remained the experimental unit (n = 4 per treatment). Data were checked for normality and homogeneity of variance using the Shapiro–Wilk and Levene's tests, respectively, before performing ANOVA. When significant treatment effects were detected, Tukey's post hoc test was applied. Differences were considered significant at $P < 0.05$.

Table 3 Fatty acid composition of microalgae and experimental diets.

Fatty acids	*Schizochytrium limacinum* (fatty acid profile)	Starter (d 0–21)	Finisher (d 22–42)
C16:0	18.15	12.73	13.17
C18:0	3.82	2.90	2.66
C18:1n-9c	16.20	26.27	25.88
C18:2n-6c	23.26	57.56	57.78
C20:1	0.22	0.18	0.19
C20:4n-6	0.73	0.25	0.22
C24:0	0.25	0.02	0.03
C20:5n-3 (EPA)	10.06	0.02	0.02
C24:1	1.31	0.02	0.01
C22:6n-3 (DHA)	7.67	0.03	0.01
∑SFA	26.85	15.67	15.89
∑UFA	73.19	84.33	84.11
∑MUFA	26.08	26.47	26.10
∑PUFA	47.11	57.86	58.03
ω-3	20.38	0.05	0.03
ω-6	26.73	57.81	58.00
ω-9	18.72	26.45	26.07
∑PUFA/SFA	1.75	3.69	3.65
∑UFA/SFA	2.73	5.38	5.29

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; EPA, eicosapentaenoic acid (C20:5n-3); DHA, docosahexaenoic acid (C22:6n-3); ω-3, omega-3 fatty acids; ω-6, omega-6 fatty acids; ω-9, omega-9 fatty acids. Values are expressed as percentages of total fatty acids. Microalgae biomass represents the fatty acid profile of the raw microalgae material used in the experimental diets. Grower diet was fed from day 7 to 21, and finisher diet was fed from day 22 to 42.

Results

The repeated measures ANOVA showed a significant main effect of time on body weight and body weight gain ($P < 0.001$), while no significant effect was observed for treatment (BW: $P = 0.319$; BWG: $P = 0.159$). Importantly, the treatment × time interaction was not significant for either parameter (BW: $P = 0.880$; BWG: $P = 0.188$), indicating that the growth patterns were similar across all experimental groups throughout the study period. As presented in Table 4, dietary microalgae supplementation had no significant effect on the overall performance parameters of broilers during days 0–42, including TBWG, ADG, AFI, and FCR ($P > 0.05$). Although the highest TBWG and ADG values were observed in the 1.00 g/kg group, no statistically significant differences were detected among the treatments ($P > 0.05$). AFI also showed slight numerical variation among the groups, with the highest value detected in the 1.00 g/kg group, while FCR was comparable across all treatments.

No statistical difference was observed in slaughter weight, hot and cold carcass weight, or carcass yield ($P > 0.05$) (Table 5). When internal organ weights were examined, no differences were detected in heart, gizzard, or spleen weights ($P > 0.05$). However, liver weight was found to increase due to microalgae-enriched diets ($P < 0.05$) (Table 5).

The highest liver weight (53.12 g) was recorded in the group whose diet was enriched with 1 g/kg of microalgae, while the lowest liver weight (44.26 g) was found in the group without supplementation.

No notable changes were detected among the experimental groups in terms of cooking loss, meat DM, CA, or pH15 and pH24 values after slaughter ($P > 0.05$). Analysis showed that the WHC decreased with increasing levels of microalgae supplementation ($P = 0.024$) (Table 6).

No significant differences were observed in MDA and GSH values among the experimental groups ($P \geq 0.05$). In contrast, PON and arylesterase activities significantly decreased with increasing levels of microalgae supplementation ($P = 0.0006$ and $P = 0.0027$, respectively) (Table 7) (Fig. 1).

When the effects related to the meat fatty acid profile were examined, it was determined that microalgae supplementation exhibited both increasing and decreasing effects on some saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) (Table 8). The level of C16:1 was significantly increased, with the highest value observed in broilers receiving 0.50 g/kg microalgae ($P = 0.028$). In addition, C17:0 concentrations were significantly higher in the 0.50 and 1 g/kg supplementation groups compared with the control group ($P = 0.001$). In contrast, the levels of C14:1, C15:0, and C18:0 were significantly reduced in specific microalgae-supplemented groups ($P < 0.05$).

Notably, arachidonic acid (C20: 4n-6) and lignoceric acid (C24:0) concentrations were significantly increased in broilers receiving 0.25 g/kg microalgae compared with the other groups ($P = 0.004$

and P = 0.021, respectively). With respect to PUFA, alpha-linolenic acid (ALA; C18:3n-3) levels were significantly higher in broilers fed diets containing 0.50 and 1 g/kg microalgae (P = 0.001). Moreover, a significant decrease was observed in C20:1 levels in response to increasing microalgae supplementation (P = 0.001).

∑SFA values showed a numerical decrease in microalgae-supplemented groups compared with the control; however, the difference did not reach statistical significance (P = 0.06). In contrast, total unsaturated fatty acids (∑USFA) increased significantly following microalgae supplementation (P = 0.022), whereas no significant differences were detected in total MUFA or PUFA contents (P > 0.05).

Omega-3 fatty acid levels were significantly affected by microalgae supplementation, reaching the highest value at the 0.25 g/kg inclusion level, while a decline was observed at the 1 g/kg dose (P = 0.005). A marked increase in eicosapentaenoic acid (EPA; C20:5n-3) was recorded in the 0.25 g/kg group (P = 0.001), whereas the increase in docosahexaenoic acid (DHA; C22:6n-3) was not statistically significant (P = 0.071). Furthermore, the omega-3/omega-6 ratio reached its highest value in broilers supplemented with 0.25 g/kg microalgae (P = 0.001).

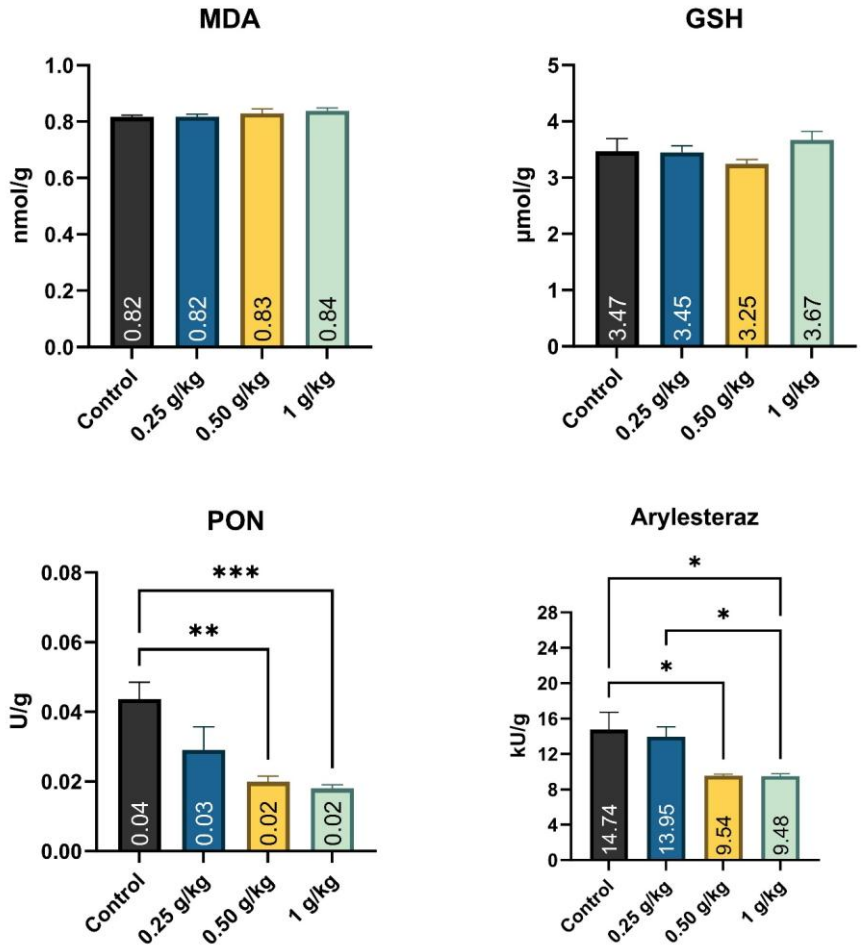


Figure 1 The effect of microalgae supplementation in broiler diets on breast meat MDA, GSH, PON, and arylesterase.

Table 4 The effect of microalgae supplementation in broiler diets on performance values during days 0–42.

Parameters	Control	0.25 g/kg	0.50 g/kg	1.00 g/kg	*P*-value
TBWG, g	2589.57 ± 20.29	2603.90 ± 5.90	2630.79 ± 18.06	2638.74 ± 26.00	0.277
ADG, g/day	61.66 ± 0.48	62.01 ± 0.14	62.64 ± 0.43	62.83 ± 0.62	0.275
AFI, g/day	107.62 ± 0.99	107.13 ± 0.59	108.81 ± 2.04	109.74 ± 1.04	0.496
FCR	1.69 ± 0.02	1.69 ± 0.01	1.69 ± 0.03	1.70 ± 0.01	0.981

Differences between means are significant at the P < 0.05 level.
Values are presented as mean ± Standard Error of the Mean (SEM). The replicate pen was considered the experimental unit for all performance parameters (n = 4 pens per treatment, with 12 birds per pen).
TBWG, total body weight gain; ADG, average daily gain; AFI, average feed intake; FCR, feed conversion ratio.
Repeated Measures ANOVA for weekly Body Weight (BW): Treatment P = 0.319; Time P < 0.001; Treatment × Time P = 0.880.
Repeated Measures ANOVA for weekly Body Weight Gain (BWG): Treatment P = 0.159; Time P < 0.001; Treatment × Time P = 0.188.

Table 5 Effects of microalgae supplementation at different levels in broiler diets on carcass yield characteristics.

Parameters	Control	0.25 g/kg	0.50 g/kg	1.00 g/kg	*P*-value
Slaughter weight, g	2615.89 ± 24.31	2605.90 ± 41.63	2663.75 ± 48.63	2621.44 ± 53.72	0.794
Hot carcass yield, %	75.13 ± 0.52	75.41 ± 0.58	74.73 ± 0.55	75.77 ± 0.47	0.569
Cold carcass yield, %	69.06 ± 0.50	69.71 ± 0.54	69.06 ± 0.55	70.25 ± 0.48	0.324
Heart, g	12.56 ± 0.49	11.26 ± 0.79	12.68 ± 0.81	12.37 ± 0.61	0.456
Liver, g	44.26 ± 0.96^{b}	45.11 ± 0.99^{b}	50.13 ± 1.75^{ab}	53.12 ± 2.38^{a}	0.002
Gizzard, g	25.93 ± 1.15	23.71 ± 0.96	24.44 ± 1.62	23.62 ± 0.87	0.500
Spleen, g	3.05 ± 0.21	3.13 ± 0.18	3.47 ± 0.26	3.77 ± 0.30	0.147

Differences between means are significant at the $P < 0.05$ level.
Values are presented as mean ± SEM. The replicate pen was considered the experimental unit (n = 4 pens per treatment).
a,b,c: Each superscript indicates differences among the means within the same row.

Table 6 The effect of microalgae supplementation in broiler diets on breast meat quality after slaughter.

Parameters	Control	0.25 g/kg	0.50 g/kg	1.00 g/kg	*P*-value
Cooking loss (%)	27.07 ± 1.14	27.45 ± 1.01	27.08 ± 1.56	26.95 ± 0.40	0.990
Water-holding capacity (%)	73.53 ± 0.35^{a}	73.11 ± 0.29^{ab}	72.57 ± 0.34^{ab}	72.23 ± 0.18^{b}	0.024
Dry matter (%)	77.20 ± 0.27	76.71 ± 0.32	77.09 ± 0.26	77.10 ± 0.21	0.591
Crude ash (%)	1.63 ± 0.04	1.65 ± 0.06	1.67 ± 0.05	1.66 ± 0.02	0.957
pH15	6.48 ± 0.05	6.58 ± 0.03	6.43 ± 0.06	6.59 ± 0.07	0.128
pH24	5.93 ± 0.11	5.99 ± 0.08	5.92 ± 0.24	5.80 ± 0.08	0.823

Differences between means are significant at the $P < 0.05$ level.
Values are presented as mean ± SEM. Two birds were sampled from each replicate pen, and the pen mean was used for statistical analysis; therefore, the replicate pen was considered the experimental unit (n = 4 pens per treatment).
a,b,c: Each superscript indicates differences among the means within the same row.

Table 7 The effect of microalgae supplementation in broiler diets on MDA, GSH, PON, and arylesterase.

Parameters	Control	0.25 g/kg	0.50 g/kg	1.00 g/kg	*P*-value
MDA	0.82 ± 0.01	0.82 ± 0.01	0.83 ± 0.02	0.84 ± 0.01	0.0500
GSH	3.47 ± 0.23	3.45 ± 0.12	3.25 ± 0.08	3.67 ± 0.16	0.0500
PON	0.04 ± 0.00^{a}	0.03 ± 0.01^{ab}	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}	0.0006
Arylesterase	14.74 ± 1.98^{a}	13.95 ± 1.10^{ab}	9.54 ± 0.17^{bc}	9.48 ± 0.29^{c}	0.0027

Differences between means are significant at the $P < 0.05$ level.
Values are presented as mean ± SEM. Two birds were sampled from each replicate pen, and the pen mean was used for statistical analysis; therefore, the replicate pen was considered the experimental unit (n = 4 pens per treatment).
a,b,c: Each superscript indicates differences among the means within the same row.
MDA, malondialdehyde (nmol/g); GSH, reduced glutathione (µmol/g);
PON, paraoxonase (U/kg); Arylesterase (kU/g).

Discussion

Our study is consistent with other reports in which *Schizochytrium limacinum* supplementation in diets did not affect LW and LWG (Yan and Kim, 2013; Khan *et al*., 2021). In addition, previous research has indicated that adding *Chlorella vulgaris* and *Nannochloropsis oceanica* to broiler diets did not alter LW and LWG values (Tao *et al*., 2018; Alfaia *et al*., 2021; Coelho *et al*., 2021). In contrast, some studies have demonstrated that adding *Spirulina platensis* and *Aurantiochytrium* sp. to diets improved LW and LWG (Kalia *et al*., 2023; Sharmin *et al*., 2025). The differences observed among these studies may be attributed to variations in animal species, the use of different microalgae strains, and differences in dosage and form. We also believe that the lack of change in LW and LWG, except at the end of the first week, may be due to the use of the microalgae species *Schizochytrium limacinum*—rich in fatty acids, especially n-3 fatty acids—at low inclusion levels.

There are studies in which the inclusion of Schizochytrium JB5 in diets, similar to our study, did not affect BWG and FCR (Yan and Kim, 2013). There are also studies in which *Chlorella vulgaris* supplementation to the diet did not affect FCR (Kang *et al*., 2013; Oh *et al*., 2015). In contrast to these findings, some studies indicated that dietary supplementation with *Schizochytrium* sp. enhanced BWG and FCR in broilers (Hassan *et al*., 2022). Moreover, there are also studies indicating that microalgae supplementation worsened BWG and FCR (Pestana *et al*., 2020). The structural complexity of microalgal cell walls may limit nutrient bioavailability and digestibility in broilers, thereby reducing the efficiency with which ingested nutrients are converted into growth (Saadaoui *et al*., 2021). In addition, differences in nutrient composition and bioavailability among microalgal sources may lead

to metabolic adaptation rather than additional muscle deposition (Sun *et al*., 2024). This may help explain why higher feed intake did not result in improved growth performance or feed efficiency. The differences among studies may be explained by the harvesting methods applied during microalgae production and the reduced digestibility caused by their inclusion at high levels in diets. Additionally, variations in the microalgae strains and inclusion levels used in the diets might have contributed to these results.

In accordance with our findings, some studies have reported that slaughter weight as well as hot and cold carcass weights did not change (Tao *et al*., 2018). In contrast, there are also studies in which improvements in these parameters were observed (Megahed *et al*., 2025). Regarding internal organ weights, consistent with our data, many studies have reported that different microalgae strains increased liver weight (Long *et al*., 2020). In many studies in which *Schizochytrium limacinum*, particularly, as well as Enteromorpha intestinalis and *Chlorella vulgaris* were supplemented to broiler diets, liver weight was reported to increase at a statistically significant level (Fathi *et al*., 2018; Akter *et al*., 2024). In contrast, dietary supplementation of *Spirulina platensis* at levels of 2.5, 5, 7.5, and 10 g/kg was reported to have no statistically significant effects on heart, gizzard, or spleen weights (Megahed *et al*., 2025). The variations among research findings are thought to be due to the fact that certain microalgae species contain higher protein levels, whereas others have a greater fat content. The microalga used in the present study is a lipid-rich species, which may have promoted hepatic lipid metabolism and lipid transport, thereby contributing to the increased liver weight. In poultry, the liver is the principal organ involved in fatty acid synthesis and lipid metabolism; therefore, dietary supplementation with DHA-rich microalgae may increase hepatic metabolic activity and lipid processing (Cartoni *et al*., 2022).

In agreement with our results, there are studies showing that cooking loss, meat DM and CP contents, as well as pH15 and pH24 values, did not change (Park *et al*., 2018; Altmann *et al*., 2020; Göçmen, 2022; Sun *et al*., 2024; Mendes *et al*., 2024). In contrast to these parameters, some studies have reported an increase in WHC (Mohamed *et al*., 2025). In addition, there are studies reporting a reduction in cooking loss, while pH and WHC remained unchanged (El-Gogary *et al*., 2023). Microalgae contain high amounts of bioactive compounds such as polyphenols, pigments, insoluble fibers, and some antinutrients (Saadaoui *et al*., 2021). These compounds, particularly phenolic compounds, may interact with muscle proteins and alter protein structure, which could disrupt the protein matrix responsible for water binding and consequently reduce WHC (Huff-Lonergan and Lonergan, 2005; Ozdal *et al*., 2013; Bowker and Zhuang, 2015).

No significant alterations were detected in MDA and GSH values in our study, whereas some studies have reported increased MDA and decreased GSH levels (Moustafa *et al*., 2021; Ranjbarinasab *et al*., 2024). Moreover, there are studies reporting that the supplementation of *Chlorella vulgaris* and *Nannochloropsis oceanica* to broiler diets reduced MDA levels and increased GSH concentrations. (Dlouhá *et al*., 2008; Tao *et al*., 2018; Varzaru *et al*., 2024; Salah *et al*., 2025). To the best of our knowledge, no studies have been reported in the literature that have examined PON and arylesterase activities following microalgae supplementation in broiler diets. MDA is a terminal product of lipid peroxidation and is widely used as an indicator of oxidative damage, whereas GSH is an important non-enzymatic antioxidant involved in cellular redox balance. Therefore, the absence of significant changes in MDA and GSH suggests that dietary *Schizochytrium limacinum* did not induce marked lipid peroxidation or deplete the non-enzymatic antioxidant defense system in breast muscle under the present experimental conditions. However, the significant decrease in PON and arylesterase activities indicates that *Schizochytrium limacinum* supplementation may have modulated enzymatic antioxidant defense mechanisms rather than causing overt oxidative stress. PON1, which exhibits both paraoxonase and arylesterase activities, is associated with the hydrolysis of oxidized lipids and the protection of lipoproteins against oxidative modification (Jaouad *et al*., 2003). Since dietary lipid source and fatty acid composition can influence fatty acid deposition and antioxidant capacity in broilers, the PUFA-rich nature of *Schizochytrium limacinum*, particularly its DHA and EPA content, may have contributed to the observed enzymatic response (Long *et al*., 2018; Long *et al*., 2020). In addition, the biological effects of microalgae in poultry may depend on their nutritional composition, bioactive compounds, and inclusion level (Van Nerom *et al*., 2024). Long-chain PUFA are susceptible to oxidation, and oxidized lipid products may reduce PON and arylesterase activities by affecting enzyme stability or activity (Jaouad *et al*., 2003). Thus, the observed decrease in PON and arylesterase activities, together with unchanged MDA and GSH levels, should be interpreted as a modulation of antioxidant-related enzymatic responses rather than clear evidence of oxidative damage.

Supporting our results, earlier studies have demonstrated that the inclusion of *Schizochytrium limacinum*, *Spirulina platensis*, *Nannochloropsis oceanica*, and *Aurantiochytrium* sp. in broiler diets increased EPA, DHA, total n-3 PUFA, and the n-3/n-6 ratio, while decreasing n-6 PUFA levels (Bonos *et al*., 2016; Tao *et al*., 2018; Long *et al*., 2018; Tolba *et al*., 2019; Petrolli *et al*., 2019; Long *et al*., 2020; Zanaty *et al*., 2024;). However, there are also studies in which no changes were observed in these parameters (Coelho *et al*., 2021). The present findings suggest a non-linear dose-response relationship, as omega-3 enrichment was greatest at the 0.25 g/kg inclusion level rather than at higher doses. This may indicate that supplementation at 0.25 g/kg was sufficient to enhance omega-3 deposition in breast muscle, whereas increasing the dose did not provide proportional additional enrichment, possibly because of limitations in lipid bioavailability and tissue incorporation (Demarco *et al*. 2022). Moreover, the differences among studies may be attributed to variations in microalgal species, inclusion dose, feeding period, basal diet composition, and broiler genotype (Van Nerom *et al*., 2024). Moreover, differences in the bioavailability of algal lipids, which

may be influenced by cell wall characteristics and processing methods, as well as variations in rearing conditions, could also explain the conflicting results (Demarco *et al.*, 2022).

Our study demonstrates that the inclusion of *Schizochytrium limacinum* improves the omega-3 fatty acid content of breast meat. The daily omega-3 fatty acid requirement for humans is known to be 1.1 g (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005). Results from the current study indicated that the inclusion of *K* at a level of 0.25 g/kg in broiler diets could provide approximately 54% of the recommended daily omega-3 fatty acid requirement for humans, assuming a consumption of 100 g of chicken breast meat. The differences observed in certain fatty acid levels may be attributed to factors such as the type and dosage of microalgae used, the genetic structure of the broilers, feeding duration, and environmental conditions. Therefore, more standardized and controlled studies are needed to fully evaluate the effects of microalgae as a feed additive. A limitation of the present study is that sex was not included as a factor in the statistical analysis, and its potential influence cannot be completely excluded.

Table 8 The effect of microalgae supplementation in broiler diets on meat fatty acid parameters.

Fatty acid	Control	0.25 g/kg	0.50 g/kg	1.00 g/kg	*P*-value
C14:0	0.34 ± 0.17	0.07 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.061
C14:1	0.07 ± 0.00^{a}	0.06 ± 0.01^{a}	0.01 ± 0.01^{c}	0.03 ± 0.01^{b}	0.001
C15:0	0.05 ± 0.00^{a}	0.06 ± 0.00^{a}	0.01 ± 0.01^{b}	0.02 ± 0.01^{b}	0.001
C16:0	23.30 ± 0.44	21.79 ± 0.32	22.50 ± 0.38	22.13 ± 0.43	0.064
C16:1	0.01 ± 0.01^{b}	0.09 ± 0.39^{a}	0.40 ± 0.02^{ab}	0.30 ± 0.07^{ab}	0.028
C17:0	0.16 ± 0.03^{b}	0.20 ± 0.01^{b}	1.56 ± 0.12^{a}	1.06 ± 0.24^{a}	0.001
C18:0	10.62 ± 0.50^{a}	9.28 ± 0.62^{a}	8.24 ± 0.61^{b}	8.59 ± 0.44^{a}	0.025
C18:1n-9c	28.21 ± 0.28	27.06 ± 0.87	29.61 ± 0.71	29.33 ± 0.89	0.075
C18:2n-6c	20.07 ± 0.44	20.15 ± 0.50	21.26 ± 0.76	22.25 ± 0.64	0.051
C18:3n-6	0.33 ± 0.05	0.43 ± 0.02	0.32 ± 0.02	0.35 ± 0.02	0.067
C20:0	0.10 ± 0.02	0.13 ± 0.01	0.13 ± 0.02	0.12 ± 0.02	0.311
C20:1	0.66 ± 0.10^{a}	0.85 ± 0.03^{a}	0.01 ± 0.01^{b}	0.02 ± 0.01^{b}	0.001
C18:3n-3 (ALA)	0.09 ± 0.08^{b}	0.01 ± 0.01^{b}	1.01 ± 0.06^{a}	0.89 ± 0.14^{a}	0.001
C22:1n-9	0.33 ± 0.02	0.37 ± 0.02	0.18 ± 0.02	0.32 ± 0.10	0.061
C20:4n-6	6.31 ± 0.35^{ab}	7.35 ± 0.50^{a}	5.36 ± 0.16^{b}	5.57 ± 0.41^{b}	0.004
C24:0	0.32 ± 0.05^{ab}	0.35 ± 0.02^{a}	0.23 ± 0.02^{b}	0.24 ± 0.02^{b}	0.021
C20:5n-3 (EPA)	0.84 ± 0.06^{ab}	0.97 ± 0.05^{a}	0.70 ± 0.03^{b}	0.72 ± 0.05^{b}	0.001
C22:6n-3 (DHA)	1.91 ± 0.23	3.00 ± 0.31	2.20 ± 0.27	2.01 ± 0.38	0.071
∑SFA	36.48 ± 0.90	33.82 ± 0.86	33.89 ± 0.74	33.59 ± 0.77	0.060
∑UFA	63.53 ± 0.90^{b}	66.54 ± 0.78^{a}	66.32 ± 0.71^{ab}	66.63 ± 0.66^{a}	0.022
∑MUFA	30.78 ± 0.27	31.15 ± 0.42	32.07 ± 0.58	31.99 ± 0.65	0.211
∑PUFA	32.76 ± 0.79	35.39 ± 1.02	34.25 ± 0.94	34.64 ± 0.95	0.259
ω-3	4.52 ± 0.29^{ab}	5.91 ± 0.37^{a}	4.90 ± 0.35^{ab}	3.88 ± 0.46^{b}	0.005
ω-6	28.24 ± 0.64	29.48 ± 0.79	29.35 ± 0.85	30.76 ± 0.61	0.137
ω-9	29.45 ± 0.30	28.47 ± 0.88	29.82 ± 0.71	29.70 ± 0.90	0.566
ω-3/ω-6	0.16 ± 0.01^{ab}	0.20 ± 0.01^{a}	0.17 ± 0.01^{ab}	0.13 ± 0.01^{b}	0.001
∑PUFA/SFA	0.90 ± 0.04	1.06 ± 0.06	1.02 ± 0.05	1.04 ± 0.05	0.140
∑UFA/SFA	1.76 ± 0.06	1.98 ± 0.07	1.97 ± 0.06	1.99 ± 0.07	0.054

Differences between means are significant at the $P < 0.05$ level.
Values are presented as mean ± SEM. Two birds were sampled from each replicate pen, and the pen mean was used for statistical analysis; therefore, the replicate pen was considered the experimental unit (n = 4 pens per treatment).
a,b,c: Each superscript indicates differences among the means within the same row.
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; EPA, eicosapentaenoic acid (C20:5n-3); DHA, docosahexaenoic acid (C22:6n-3); ω-3, omega-3 fatty acids; ω-6, omega-6 fatty acids; ω-9, omega-9 fatty acids.

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