


# Docosahexaenoic acid (22:6 n-3)-rich microalgae along with methionine supplementation in broiler chickens: effects on production performance, breast muscle quality attributes, lipid profile, and incidence of white striping and myopathy

Ibrahim A. Khan <sup>\*</sup>, Nathan B. Parker,<sup>\*</sup> Christiane V. Löhner,<sup>†</sup> and Gita Cherian<sup>\*,1</sup>

<sup>\*</sup>Department of Animal and Rangeland Sciences, College of Agricultural Sciences, Oregon State University, Corvallis, OR 97331, USA; and <sup>†</sup>Department of Biomedical Sciences, Carlson College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331, USA

**ABSTRACT** The effect of docosahexaenoic acid (DHA, 22:6 n-3)-rich microalgae and methionine (Met) supplementation on production performance, incidence of breast muscle white striping (WS), and pathology, lipid profile, and meat quality aspects in broiler chickens was investigated. The hypothesis tested was that feeding Met and n-3 fatty acid (FA)-rich diet enhances muscle n-3 FA content and meat quality while attenuating breast muscle WS and myopathy in broiler chickens. One hundred and forty four (n = 144) 10-day-old Cornish cross chicks were fed a corn-soybean meal-based diet containing 0% microalgae (control), 2% microalgae (diet 1), and diet 1 + 100% more National Research Council requirement of Met (diet 2) up to day 42 of growth. All diets were isocaloric and isonitrogenous. The chicks were kept in 6 pens with 8 chicks per replicate pen. Feed consumption and feed efficiency were calculated on day 21 and 42. On day 43, 3 chicks per pen (n = 18/treatment) were euthanized. The breast muscle (pectoralis major) was visually scored for muscle WS (1 = no striping, 2 = mild, 3 = severe) and was subjected to histopathology. Breast muscle lipid profile (total lipids, FA composition, cholesterol, lipid oxidation products), quality (moisture, color, drip loss, shear force, cook loss, pH), and chemical characterization (protein,

minerals) were recorded. A one-way analysis of variance was carried out with diet as the main factor and significance was set at  $P < 0.05$ . The incidence of muscle WS was lower ( $P < 0.02$ ) for control vs. diet 2 and a trend for reduction in WS was observed in birds fed diet 1 vs. control ( $P = 0.09$ ). Histopathological changes consisted of floccular or vacuolar degeneration, fibrosis, lipidosis, interstitial inflammation, and lysis of fibers, and were minimal in diet 2 when compared to control ( $P < 0.05$ ). The total lipid content was lowest in birds fed diet 1 ( $P < 0.05$ ). Total n-3 and total long chain ( $\geq 20C$ ) n-3 FA were highest in the breast muscle of diet 2 birds ( $P < 0.05$ ). Muscle drip loss and shear force were highest in diet 2 ( $P < 0.05$ ). Meat color ( $a^*$ , redness) was reduced ( $P < 0.05$ ) and a trend for reduction in  $b^*$  (yellowness) was observed in diet 2 ( $P = 0.07$ ). No effect of diet on body weight gain, feed efficiency, breast muscle yield, pH, moisture, lipid oxidation products, cook loss, minerals (Ca, P, Mg, Na), cholesterol, or protein content was observed ( $P > 0.05$ ). The results demonstrated a significant effect of DHA-rich microalgae along with Met supplementation in reducing the incidence of breast muscle striping and myopathy, while enriching meat with n-3 FA. However, inclusion of Met in microalgae-based diets could influence meat tenderness and color.

**Key words:** broiler, n-3 fatty acids, methionine, white striping, meat quality

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## INTRODUCTION

Advances in genetic selection, coupled with consumer demand for lean animal protein, make broiler chicken

the most common animal food consumed in the U.S. Improved growth rate and high breast muscle (pectoralis major) yield are also associated with disorders negatively affecting breast meat quality and consumer acceptance. The 2 most severe anomalies in chicken breast muscle reported recently are woody breast and white striping (WS) (Kuttappan et al., 2016; Melochie et al., 2018; Petracci et al., 2019), leading to unappealing appearance, carcass downgrading, condemnation, and reduced protein functionality in processed products (Mudalal et al., 2014; Petracci et al., 2014, 2019; Tijare et al.,

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<sup>1</sup>Corresponding author: [gita.cherian@oregonstate.edu](mailto:gita.cherian@oregonstate.edu)

2016). Despite the increased incidence of wooden breast and WS in broilers, the exact reason behind the occurrence of the muscle myopathies is not clearly understood. Assessing the lipid profile of breast fillets from chickens affected with WS, Kuttappan et al. (2013) reported a significant reduction in n-3 fatty acids (FA).

Methionine (Met) is an essential amino acid and is the first limiting amino acid in broilers fed corn-soy-based diets. Met serves as a lipotropic agent and plays many roles in avian nutrition including protein synthesis, oxidative stress prevention, transmethylation, and transsulfuration pathways (Fagundes et al., 2020). In broilers, dietary Met level affects antioxidant status, essential amino acid digestibility, and carcass quality (Pillai et al., 2006; Zhai et al., 2016; Fagundes et al., 2020). In a recent study by Beheshti Moghadam et al. (2017), supplementing Met improved n-3 polyunsaturated fatty acid (PUFA) status and oxidative stability in the breast muscle of broiler chickens fed flaxseed rich in  $\alpha$ -linolenic acid (18:3 n-3).

Western diets generally are high in saturated fats, which lead to a host of chronic metabolic diseases (Doll et al., 2009; Lands, 2014). Consumption of n-3 PUFA can improve lipid metabolism and decrease the incidence of cardiovascular and metabolic disease (Jump et al., 2012). To meet the human need of n-3 FA, strategies such as enriching poultry food products with n-3 FA have been attempted successfully through supplementing flaxseed or marine oils in broiler diets (Gonzalez-Esquerra and Leeson, 2001; Beheshti Moghadam and Cherian, 2017). Recently microalgae-based products as n-3 FA-rich feed supplements are gaining increasing interest and popularity as an alternative to marine oils or flaxseed in poultry feeds due to their low anti-nutritional factors, increased sustainability, and high content of docosahexaenoic acid (DHA, 22:6 n-3), the most biologically active form of n-3 FA (Neijat et al., 2017; Yonke and Cherian, 2019).

The objectives of the current study are to test the effect of DHA-rich microalgae and Met supplementation on the incidence of breast muscle WS, histopathology, lipid profile, chemical composition, and meat quality aspects in broiler chickens. The hypothesis tested was that supplementation of DHA-rich microalgae and Met will enhance muscle n-3 FA content and meat quality while reducing breast muscle myopathies and WS in broiler chickens.

## MATERIALS AND METHODS

### *Animals, Experimental Design, and Diets*

An institutional animal care and use committee approved all experimental protocols to ensure adherence to animal care guidelines (ACUP # 4931).

**Microalgae** The microalgae product DHA Natur used in the current study was provided by Archer Daniels Midland Company (Clinton, IA).

### *Birds and Dietary Treatments*

One hundred and forty four ( $n = 144$ ) 10-day-old Cornish cross broiler chicks were obtained from Oregon State University Poultry Center and randomly placed in 18 floor pens bedded with wood shavings. Chicks were weighed and assigned to 1 of 3 corn-soybean meal-based diets containing 0% microalgae (control), 2% microalgae (diet 1), diet 1 + 100% more Cobb 500 Met requirement (diet 2). Ingredient content and FA composition of the experimental diet are shown in Table 1 and Table 2, respectively. Each treatment was replicated in 6 pens with 8 birds per pen. The chicks were fed grower (days 10–21) and finisher (days 21–42) diets, respectively. All diets were isocaloric and isonitrogenous. Diets within each phase were formulated with similar dietary fat, Ca, P, and lysine levels to ensure that any observed performance differences were due to dietary microalgae and Met. The analyzed Met concentration for the grower and finisher diet was 0.37, 0.36, 0.77% and 0.39, 0.39, 0.63% for control, diet 1, and diet 2, respectively.

On days 21 and 42, chickens and feed were weighed, and body weight gain and feed consumption were recorded for each pen. Average chicken weight and feed conversion ratio (feed:gain) were calculated. During the experiment, birds were provided free access to water and feed. The chicks were not vaccinated and were housed in an environmentally controlled facility with a lighting program of 23L:1D. Gross energy and CP of the experimental diets were analyzed at the Center of Excellence for Poultry Science, Central Analytical Laboratory of the University of Arkansas (Fayetteville, AR) and feed amino acid composition was determined at the University of Missouri, Experiment Station Chemical Laboratories (Columbia, MO).

### *Breast Muscle WS Scoring and Histopathological Assessment*

On days 43 to 45, a total of 54 birds were selected ( $n = 6$ , birds per dietary treatment per day, total 18 birds per treatment, 3 per replicate pen). Birds were weighed individually, and euthanized with carbon dioxide gas. They were cut open and breast muscles were exposed manually. Chicken breast muscle (pectoralis major) was visually scored for WS on a scale of 1 to 3 with 1 being none, 2 referring to mild striping, and 3 very high striping (Kuttappan et al., 2013). All the scoring was done by the same person to minimize variability. Breast muscle samples collected on day 43 were used for histopathological analyses, those collected on day 44 were used for lipid profile and chemical composition, and those collected on day 45 were used for assessing yield, cook loss, and other meat quality assays. For histopathological analyses, 1 bird/replicate pen ( $n = 6$ /treatment) was taken randomly and about 5 cm of breast muscle (pectoralis major and pectoralis minor) tissue were cut along the direction of the muscle fiber, blotted, and

**Table 1.** Ingredient content, and calculated and analyzed composition of experimental diets.

Ingredients (kg/100 kg)	Grower			Finisher		
	Control	Diet 1	Diet 2	Control	Diet 1	Diet 2
Corn	63.40	62.00	61.56	65.47	63.47	63.54
Soybean meal	30.00	29.85	29.78	28.00	28.00	27.5
DHA Natur	0.00	2.00	2.00	0.00	2.00	2.00
Corn oil	2.80	0.00	0.00	3.10	0	0
Canola oil	0	2.33	2.43	0	3.10	3.10
Salt	4.00	4.00	4.00	3.70	3.70	3.70
Vitamin-mineral premix <sup>1</sup>	5.00	5.00	5.00	5.00	5.00	5.00
Dicalcium phosphate	1.63	1.63	1.63	1.42	1.42	1.42
Limestone, ground	1.01	1.010	1.01	0.94	0.94	0.94
L-lysine HCL	0.15	0.15	0.15	0.09	0.09	0.09
DL-methionine	0.13	0.13	0.54	0.11	0.11	0.54
Nutrient specifications						
ME (kcal/kg)	3,280	3,280	3,280	3,330	3,330	3,330
CP (%)	20	20	20	18.0	18.0	18.0
Analyzed values						
CP (%)	19.6	19.0	20.2	19.1	19.4	19.0
Gross energy (kcal/kg)	4,097	4,059	4,046	3,906	4,053	4,011
Crude fat (%)	5.01	5.23	5.01	5.74	6.45	6.44
Methionine (%)	0.37	0.36	0.77	0.39	0.39	0.63

Control, diet 1, and diet 2 represent corn-soybean meal-based diet containing 0% microalgae (control), control + 2% microalgae (diet 1), and diet 1 + 100% more methionine (diet 2).

Microalgae was supplied as DHA Natur (Archer Daniels Midland Company, Clinton, IA). The analyzed CP and crude fat content of DHA Natur was 9.8 and 16.57%, respectively.

Abbreviation: DHA, docosahexaenoic acid.

<sup>1</sup>Supplied per kg feed: vitamin A, 8,000 UI; vitamin D3, 2,000 UI; vitamin E, 30 UI; vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg; pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg.

placed in a 250 mL jar containing 10% buffered neutral formalin. From each tissue sample, a longitudinal and a transverse section were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin at the Oregon Veterinary Diagnostic Laboratory, yielding 2 sections per slide for each of the pectoralis major and pectoralis minor. The degree of myopathic lesions and changes was assessed using a Nikon Eclipse E400 bright-field microscope (Nikon, Tokyo, Japan) and included: chronic myopathic change (enlarged fibers with internal nuclei), fibrosis, floccular or vacuolar degeneration, necrosis (hypereosinophilic fibers with

loss of striation with or without fragmentation of sarcoplasm), interstitial inflammation (leukocytes within endomysium and/or perimysium), mineralization (basophilia of sarcoplasm), regeneration (basophilic fibers with rows of satellite cell nuclei), and lipidosis (clearly defined empty intrasarcoplasmic vacuoles). Images of representative areas of muscle were observed at 20× to 400× (Nikon Digital Sight DS FiL, Nikon) to depict the range of changes present. The slides were scored according to [Kuttappan et al., 2012](#) by a board-certified veterinary pathologist, who was blinded to the dietary treatments.

**Table 2.** FA composition of the experimental diets.

Fatty acids (%)	Grower			Finisher		
	Control	Diet 1	Diet 2	Control	Diet 1	Diet 2
16:0	16.05	11.79	11.36	15.73	10.66	11.07
16:1	0.39	0.49	0.49	0.42	0.39	0.50
18:0	1.91	2.09	1.99	1.89	1.90	1.98
18:1	27.53	42.86	43.94	27.67	45.94	44.55
18:2 n-6	51.99	31.78	30.75	52.03	30.58	29.51
18:3 n-3	0.23	0.28	0.28	0.22	0.28	0.22
20:1	1.65	5.05	5.32	1.62	5.17	5.24
22:6 n-3	0.00	1.87	1.80	0.00	1.84	1.88
Total SFA	18.20	16.87	16.61	17.87	15.21	17.22
Total MUFA	29.57	48.41	49.75	29.70	51.49	50.29
Total n-6 FA	51.99	31.78	30.75	52.03	30.58	29.51
Total n-3 FA	0.23	2.95	2.90	0.41	2.91	2.98

Control, diet 1, and diet 2 represent corn-soybean meal-based diets containing 0% microalgae (control), 2% microalgae (diet 1), and diet 1 + 100% more methionine.

Microalgae was supplied as DHA Natur (Archer Daniels Midland Company, Clinton, IA) which contained 9.8% CP and 16.57% DHA (as per product label).

SFA, saturated fatty acids (14:0 + 16:0 + 18:0); MUFA, monounsaturated fatty acids (16:1 + 18:1 + 20:1); total n-3 = 18:3 n-3 + 20:2 n-3 + 22:5 n-3 + 22:6 n-3.

Abbreviations: DHA, docosahexaenoic acid; FA, fatty acids.

## Total Lipids, FA, Lipid Oxidation Products, and Cholesterol

For breast muscle total lipids, FA composition, lipid oxidation products, and cholesterol analyses 1 bird/replicate pen ( $n = 6$ /treatment) was used on day 44. Lipids were extracted by using the method of Folch et al. (1957) using chloroform:methanol (2:1, vol. vol.). Total lipid content of samples was measured gravimetrically. FA methyl esters were prepared from extracted lipids using boron trifluoride methanol as the derivatizing agent and were analyzed using an HP 6890 gas chromatograph (Hewlett-Packard Co., Wilmington, DE) equipped with an autosampler, flame ionization detector, and SP-2330 fused silica capillary column (30 mm  $\times$  0.25 mm  $\times$  0.2  $\mu$ m film thickness). Conditions of the gas chromatograph were as reported earlier (Apperson and Cherian, 2017). FA methyl esters were identified and quantified by comparison of authentic external (PUFA-1, PUFA-2, Matreya, Inc., State College, PA) and internal (methyl docosanoate, 22:0, Matreya, Inc.) standards. FA values were reported as percentages of FA methyl esters or as mg/g tissue.

Lipid peroxidation in breast muscle tissue was evaluated by estimating the malondialdehyde concentration, a thiobarbituric acid reactive substance (TBARS). Lipid peroxidation products were measured by using a colorimetric assay previously described in Salih et al. (1987), with modifications built on those described in Cherian et al. (2002). Two duplicates of each sample were averaged for data analysis. Tetraethoxypropane was used as the standard. Values represent milligrams of malondialdehyde equivalents per gram of tissue. Breast muscle cholesterol assay was carried out at the University of Missouri, Experiment Station Chemical Laboratories.

## Muscle Quality Attributes

Six samples of whole chicken breast meat ( $n = 6$ ) (1 bird/replicate pen) from each treatment were selected on day 45. Meat quality parameters such as instrumental color, drip loss (water holding capacity), cook loss, and tenderness (texture analysis) were recorded at the Clark Meat Science Center, Oregon State University. The samples were divided into 2 equal halves. One half (left) was used for color assessment and drip loss and the other half (right) was used for cook loss and meat tenderness assessments. Cook loss and meat tenderness samples were vacuum packed and stored at  $-20^{\circ}\text{C}$  until further analysis.

**Meat Color** Breast samples were deboned and residual fat removed; water was removed by blotting them with paper towel and they were weighed. Instrumental color readings were noted using a HunterLab MiniScan EZ

portable spectrophotometer (model 45/0 LAV, Hunter Associates Laboratory, Inc., Reston, VA) with a 1.54 cm aperture, calibrated with black and white standards. CIE  $L^*$ ,  $a^*$ ,  $b^*$  color space values were calculated (CIE, 1978). Three readings were recorded across the entire breast and they were averaged. All color readings were noted by the same person to minimize variability and were observed in a commercial retail cooler at  $3^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . Breast fillets were packaged in  $21 \times 14.6 \times 1.9$  cm black polystyrene trays (CKF Inc., Langley, BC, Canada) with a soaker pad (Dri-Loc AC-25, Sealed Air Cryovac, Duncan, SC) in each tray and overwrapped using a polyvinyl chloride overwrap machine (model 625, Heat Seal Co., Cleveland, OH) with clear stretch  $\text{O}_2$  permeable film (RMF-61-HY, AEP Industries Inc, South Hackensack, NJ). After the readings were taken, the samples were vacuum sealed and placed in a commercial freezer at  $-20^{\circ}\text{C}$ .

**Drip Loss (Water Holding Capacity)** Drip loss of the breast muscle was determined by weighing the samples before and after storage at  $3^{\circ}\text{C} \pm 1^{\circ}\text{C}$  in commercial retail coolers. Sample were stored in Ziploc plastic bags and were weighed at 4, 8, and 16 h of storage. Weights were recorded in grams using a digital scale (model SP6001, OHAUS Corporation, Parsippany, NJ). After each interval before weighing samples were blotted with paper towels to remove excess moisture. After weighing, the samples were placed in a new Ziploc plastic bag to prevent water contamination to avoid affecting results. All the samples were placed on stainless steel trays under even lighting.

$$\text{Drip loss (\%)} = \frac{\text{Initial weight (g)} - \text{final weight (g)}}{\text{Initial weight (g)}} \times 100$$

**Cook Loss** After storage, samples were thawed in a commercial retail cooler at  $3^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for 24 h and weighed using a digital scale before placing them in the oven. Samples were placed in a conventional cooking oven on aluminum foil-lined pans. The temperature of the oven was set at  $176^{\circ}\text{C}$  and samples were cooked until an internal temperature of  $77^{\circ}\text{C} \pm 1^{\circ}\text{C}$  was reached. In order to ensure an internal temperature of  $77^{\circ}\text{C}$ , cooking was carried out in batches of 3 with 2 samples from each dietary treatment. A probe (copper constant type T thermocouple) was inserted into the breast samples, which measured the temperature every second. After about 1 h, the samples were removed and allowed to cool until a temperature of  $32^{\circ}\text{C} \pm 1^{\circ}\text{C}$  was reached. After cooling the samples were blotted with paper towels to remove any excess moisture and weighed. Cook loss percentage was calculated using the formula given below:

$$\text{Cook loss (\%)} = \frac{\text{Initial weight before cooking (g)} - \text{final weight after cooking (g)}}{\text{Initial weight before cooking (g)}} \times 100$$

After cooking, the samples were wrapped in aluminum foil and placed in a commercial cooler at  $3^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for further meat tenderness analysis.

### **Meat Tenderness (Warner Bratzler Shear Force)**

Samples were cored and 1.27-cm core samples were taken in triplicates from the rostral and middle section of each cooked breast fillet, in the longitudinal orientation of the muscle fiber. The core samples were analyzed using a Shimadzu EZ-X Series Table-top Testing Machine (Shimadzu Corporation, Japan) with a load cell size of 500 N. The gravel distance was set at 39 mm and test travel speed was set at 4 mm/s. The values were reported in Newtons (N) and stored in Trapezium X version 1.4.0 software (Shimadzu Scientific Instruments, Columbia, MD) for data analysis.

### **Muscle Chemical Composition (CP, Minerals, and Moisture) and pH Analyses**

Breast muscle (pectoralis major) (1 bird/replicate pen, 6 birds/treatment) was used for measuring CP, minerals (Ca, P, Mg, Na), pH, and moisture content on day 44. CP and mineral observations were recorded at the Central Analytical Laboratory, Department of Crop and Soil Science, Oregon State University testing laboratory. To assess the moisture content, about 5 g of muscle tissue was incised, minced, and the weighed samples were placed in a hot air oven for 6 h at  $85^{\circ}\text{C}$ . The dried samples were reweighed, weight loss was calculated, and moisture content was reported as dry weight/total weight before drying  $\times 100$ . To determine meat pH, about 7 g of tissue sample was minced and mixed with 43 mL of distilled water and shaken vigorously to allow even mixing. The pH of the solution was measured by inserting a probe attached to a pH meter (model pH 3210, WTW, Weilheim, Germany); 2 readings were noted for each tissue and were averaged.

### **Carcass Yield**

For assessing carcass yield, 6 birds per treatment (1 bird per replicate pen) were selected, breast muscle (with bones intact and skin removed) was collected and weighed, and the relative weight (as percentage of body weight) was determined.

### **Statistical Analysis**

The number of pens was considered as the experimental unit for assessing the production performance and the number of birds collected from each pen was considered as the experimental unit for all other tests. To assess the effect of dietary treatment on all response variables, a one-way ANOVA was used. To analyze the effect of diet and storage time on drip loss, a 2-way ANOVA was performed with diet and time as the main factors. Visual and pathological scores were

compared using Fisher's exact test. *P* values were considered significant at  $\leq 0.05$ . All data analyses were carried out using SAS (version 9.4) (SAS Institute Inc., Cary, NC). Significant differences between each treatment means were analyzed by Tukey's honestly significant difference test.

## **RESULTS**

### **Feed Analysis**

Analysis of the microalgae showed that it contains 16.49% crude fat, 5.60% CP, and 17% DHA. Ingredient content composition, and calculated and analyzed nutrient contents of the experimental diets are shown in [Table 1](#). Microalgae increased the DHA content of feed from undetectable to 1.87 and 1.80% and 1.84 and 1.88% in the grower and finisher diet 1 and diet 2 groups, respectively ([Table 2](#)).

### **Bird Production Performance and Breast Muscle Yield**

Experimental diet had no effect ( $P > 0.05$ ) on overall weight gain and feed consumption in the grower or finisher phase ([Table 3](#)). However, during the grower phase, diet 1 had a lower feed conversion ratio compared to control ( $P < 0.05$ ) and was not different from diet 2 ( $P > 0.05$ ). No effect of diet on breast muscle weight or yield of breast muscle when expressed as a percentage of body weight was observed ( $P > 0.05$ ) ([Table 3](#)).

### **Meat Muscle Visual Scoring and Histopathology**

Overall, visual scores and histopathological findings showed significant differences when comparing the control group to treatment groups. A significant effect of diet on visual mean scores for WS was observed ( $P = 0.01$ ). The breast muscle of diet 2 birds had the lowest WS score compared to control ( $P < 0.05$ ), and a trend for reduction in visual WS score was observed between control and diet 1 groups ( $P = 0.09$ ) ([Table 4](#)). Histopathological changes consisted of floccular or vacuolar degeneration, fibrosis, lipidosis, interstitial inflammation, and necrosis or lysis of fibers, and were minimal in diet 2 when compared to control ( $P < 0.05$ ). Least severe changes in myofiber damage or minimal inflammation were observed in diet 2 birds followed by diet 1 birds ([Figure 1](#), [Table 4](#)).

### **Breast Muscle Total Lipid, FA Composition, and Lipid Oxidation Products**

Breast muscle FA composition, total lipids, cholesterol, and TBARS content are shown in [Table 5](#). Palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2 n-6) acids were the 3 major FA in breast muscle. Palmitic acid and stearic acid were highest in diet 1 ( $P < 0.05$ ). Supplementing Met led to an increase in oleic

**Table 3.** Effect of microalgae and methionine supplementation on broiler production performance and relative yield of breast muscle.

Production parameter	Dietary treatment <sup>1</sup>			Pooled SEM	P-value
	Control	Diet 1	Diet 2		
BW at day 10 (g)	190.83	198.40	190.03	5.35	0.492
BW at day 22 (g)	811.32	846.78	820.07	11.51	0.109
Weight gain (g)	620.48	648.38	630.04	8.62	0.099
Feed consumption (g)	725.0	725.0	725.0	0	0.000
FCR	1.16 <sup>a</sup>	1.12 <sup>b</sup>	1.16 <sup>a,b</sup>	0.01	0.048
Finisher					
BW at day 22 (g)	811.32	846.78	820.07	11.51	0.109
BW at day 42 (g)	2,689.58	2,713.13	2,709.38	46.50	0.954
Weight gain (g)	1,878.27	1,856.34	1,889.30	49.92	0.894
Feed consumption (g)	3,210.42	3,328.13	3,155.21	68.74	0.225
FCR	1.72	1.80	1.65	0.051	0.144
Overall					
BW at day 0 (g)	39.55	39.50	39.45	0.333	0.978
BW at day 42 (g)	2,689.58	2,713.13	2,709.38	46.50	0.954
Weight gain (g)	2,650.03	2,663.62	2,669.92	46.62	0.954
Feed consumption (g)	4,097.40	4,204.56	4,032.17	69.18	0.238
FCR	1.55	1.58	1.51	0.02	0.134
Breast muscle weight (g) and relative weight (%)					
BW at day 45 (g)	3,048.67	3,081.10	3,058.67	20.55	0.937
Breast muscle weight <sup>2</sup> (g)	788.83	740.17	743.20	23.46	0.291
Breast muscle relative weight (%)	25.87	24.04	24.33	0.63	0.128

<sup>a,b</sup>Means within a row with no common superscript differ when  $P < 0.05$ .  $n = 6$ .

Abbreviation: FCR, feed conversion ratio.

<sup>1</sup>Control, diet 1, and diet 2 represent corn-soybean meal-based diets containing 0% microalgae (control), 2% microalgae (diet 1), and diet 1 + 100% more methionine (diet 2).

<sup>2</sup>Breast muscle weight (g) is reported with bones and without skin in samples collected at day 45 of feeding.

acid in diet 2 when compared to diet 1 ( $P < 0.05$ ). Addition of microalgae and Met led to a decrease in linoleic acid (18:2 n-6) in diet 1 and diet 2 ( $P < 0.05$ ). The control group had the highest linoleic acid (22.33%) while diet 1 had the lowest (9.28%). Arachidonic acid (20:4 n-6) was lower in diet 1 and diet 2 than control ( $P < 0.05$ ). Microalgae supplementation led to an increase in the deposition of DHA in diet 1 and diet 2. No DHA could be detected in the breast muscle of control birds. Total saturated FA were lower in diet 1 and diet 2 than control ( $P < 0.0001$ ). Total lipid concentration in the muscle of diet 1 birds was lower compared to control ( $P < 0.05$ ) and was not different from the diet 2 group (Table 5). Dietary microalgae and Met supplementation had no effect on breast muscle cholesterol or TBARS content ( $P > 0.05$ ). When breast muscle FA concentration was expressed as mg/g basis, a significant effect of microalgae and Met supplementation in DHA incorporation was observed. Supplementing Met led to an over 2-fold increase in DHA in diet 2 when compared to diet 1 ( $P < 0.05$ ) (Figure 2).

**Table 4.** Visual score comparison of white striping in the breast muscle of broiler birds fed the experimental diets.

Visual score comparison	P-value
Control vs. diet 1	0.09
Control vs. diet 2	0.02
Control vs. diet 1 and diet 2	0.01

Control, diet 1, and diet 2 represent corn-soybean meal-based diets containing 0% microalgae (control), 2% microalgae (diet 1), and diet 1 + 100% more methionine.

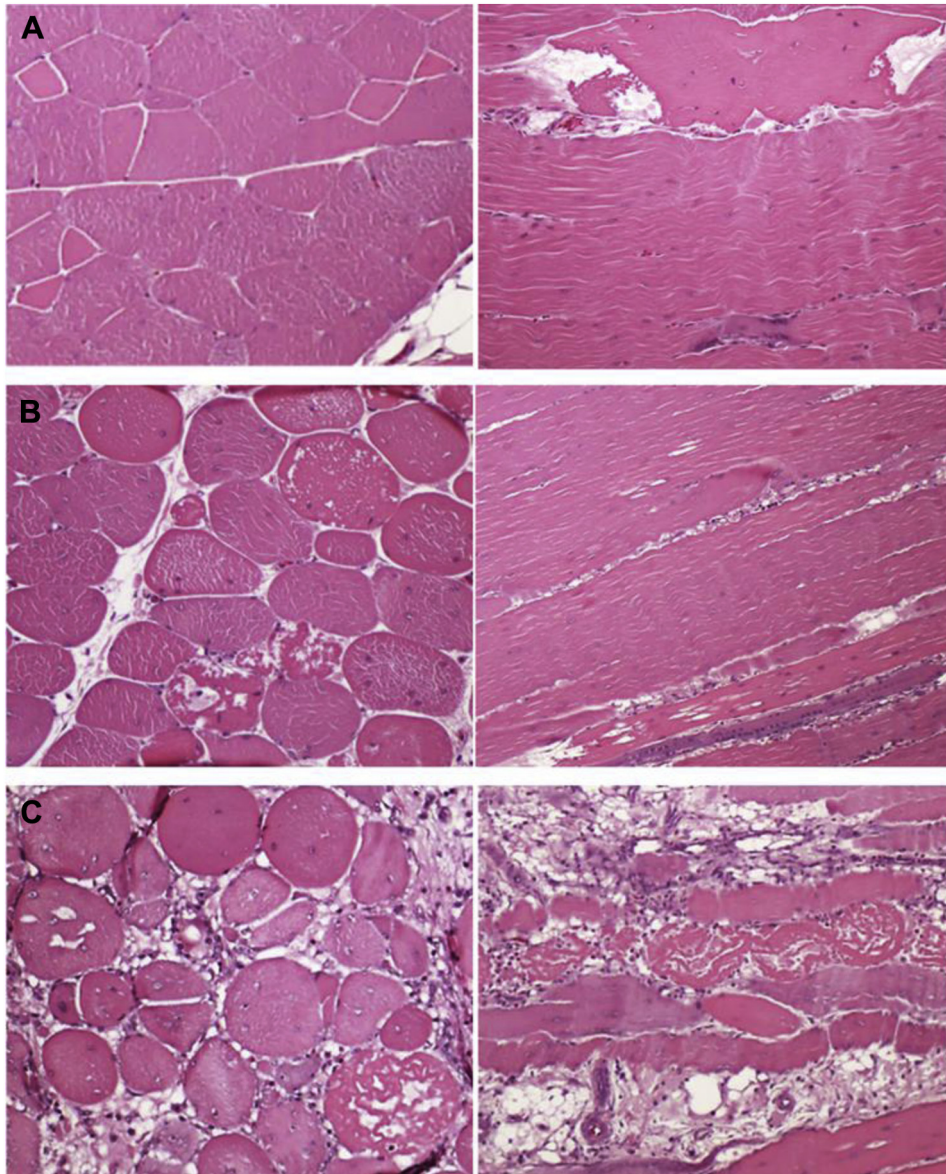
$n = 6$ .

## Muscle Quality Attributes

Meat quality aspects, chemical composition, and pH values are shown in Table 6. Meat color was measured as  $a^*$ ,  $b^*$ , and  $L^*$  values. Breast meat color ( $a^*$ , redness) was reduced ( $P < 0.05$ ) and a trend for reduction in  $b^*$  (yellowness) was observed in the breast muscle of birds fed diet 2 ( $P = 0.07$ ) (Table 6). Meat tenderness was significantly effected ( $P < 0.05$ ) with the control group having the lowest shear force and diet 2 having the highest ( $P < 0.05$ ). Experimental diets had no effect on the cook loss values, pH, mineral composition, nitrogen, or moisture content of the chicken breast muscle ( $P > 0.05$ ) (Table 6). A significant effect of diet on the drip loss values of chicken breast meat with time was observed ( $P < 0.05$ ). Diet 2 lost the most water content while the control group lost the least amount of moisture ( $P < 0.05$ ) (Table 7).

## DISCUSSION

The aim of the current study was to assess the impact of Met supplementation on the incidence of breast muscle WS and histopathology in broiler birds fed microalgae containing diets. In addition, bird performance indices, breast muscle total lipids, FA, lipid oxidation products, cooking quality attributes, and chemical composition were assessed. Results obtained clearly demonstrate a beneficial effect of Met supplementation in microalgae-based diets on WS score, muscle histopathology, and lipid profile. An important highlight from the current study is that Met supplementation



**Figure 1.** Muscle histopathology. (A) Minimal changes. Internal nuclei are infrequent. Rare fibers have floccular sarcoplasm or are swollen with hypereosinophilic sarcoplasm. Fiber lysis or regeneration is infrequent. Neither cellular infiltrates nor fibrosis or edema are present. (B) Mild to moderate lesions. Many fibers have a few internal nuclei. A few fibers have floccular sarcoplasm or swelling with hypereosinophilic sarcoplasm. Scattered fibers show lysis or regeneration. There is mild interstitial edema. Neither cellular infiltrates nor fibrosis are present. (C) Moderate to severe lesions. Moderate fibrosis and edema and small foci of leukocytes expand the endomysium. Most fibers have multiple internal nuclei. Some fibers have floccular sarcoplasm or are swollen with hypereosinophilic, occasionally fragmented sarcoplasm. Occasional fibers show lysis or regeneration. Hematoxylin and eosin. 400 $\times$ .

attenuated the incidence of breast muscle WS while reducing myopathic lesions compared to the control group. In this study, many degenerative and/or necrotic fibers accompanied by an interstitial inflammatory infiltrate were observed in the control group, which were in agreement with previously reported research on WS pathology in broilers (Kuttappan et al., 2013; Ferreira et al., 2014; Sihvo et al., 2014). The major histopathological changes associated with WS reported previously consisted of loss of cross-striations, variability in fiber size, floccular or vacuolar degeneration, lysis of fibers, mild mineralization, occasional regeneration (nuclear rowing and multinucleated cells), mononuclear cell

infiltration, lipidosis, and fibrosis (Kuttappan et al., 2012.; Ferreira et al., 2014). These pathological features leading to myopathy and tissue damage are suggested to be associated with chronic inflammatory processes, fast growth, and oxidative stress (MacRae et al., 2006; Kuttappan et al., 2016). The beneficial effects of Met in diet 2 birds could be attributed to the fact that Met functions as a lipotropic agent with antioxidant properties. Assessing the WS score in broilers collected from a processing plant, Kuttappan et al. (2012) reported that higher degrees of WS were associated with greater amounts of lipid and lower amounts of protein, resulting in a higher net calorie content in the meat. In the current

**Table 5.** Effect of microalgae and methionine supplementation on major fatty acids, total lipids, cholesterol, and lipid oxidation products measured as TBARS in breast muscle.

Variables	Dietary treatments			Pooled SEM	P-value
	Control	Diet 1	Diet 2		
Fatty acids (%)					
16:0	28.36 <sup>b</sup>	33.02 <sup>a</sup>	26.51 <sup>b</sup>	0.70	<0.0001
16:1	4.41 <sup>a</sup>	1.46 <sup>b</sup>	3.29 <sup>a</sup>	0.39	<0.0001
18:0	8.64 <sup>b</sup>	14.66 <sup>a</sup>	8.92 <sup>b</sup>	0.70	<0.0001
18:1	30.40 <sup>b</sup>	28.26 <sup>b</sup>	37.25 <sup>a</sup>	1.02	<0.0001
18:2 n-6	22.24 <sup>a</sup>	9.28 <sup>c</sup>	15.40 <sup>b</sup>	1.03	<0.0001
20:4 n-6	2.24 <sup>a</sup>	1.62 <sup>b</sup>	1.48 <sup>b</sup>	0.21	0.021
22:6 n-3	0.00 <sup>b</sup>	2.13 <sup>a</sup>	2.80 <sup>a</sup>	0.22	<0.0001
Total lipids (%)	2.98 <sup>a</sup>	1.57 <sup>b</sup>	2.27 <sup>a,b</sup>	0.22	0.0015
Cholesterol (mg/100 g)	72.49	73.20	71.81	0.82	0.505
TBARS (mg/g)	5.28	5.63	3.21	0.94	0.181

Control, diet 1, and diet 2 represent corn-soybean meal-based diets containing 0% microalgae (control), 2% microalgae (diet 1), and diet 1 + 100% more methionine.

<sup>a-c</sup>Means within a row with no common superscript differ when  $P < 0.05$ .

n = 6.

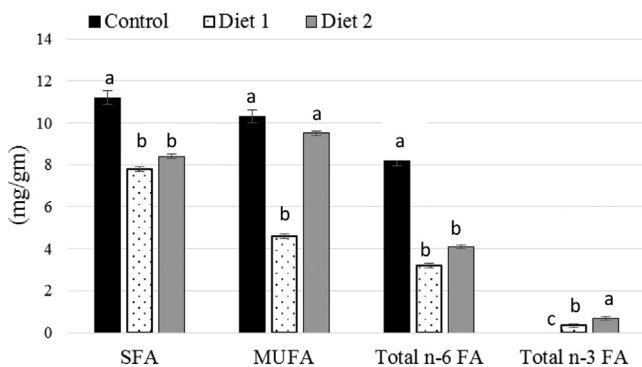
Abbreviation: TBARS, thiobarbituric acid reactive substances.

study, although visual WS scores and pathological lesions were higher in the control birds than diet 2 ( $P < 0.05$ ), no difference in breast muscle total lipids, TBARS, and minerals or protein content was observed. It should be noted that the birds in this study were kept in hygienic conditions and were fed nutritionally balanced diets with minimal external stressors. No difference was observed in the feed consumption or weight gain in the grower or finisher phase, 42-d body weight, or breast muscle relative yield. Thus the hypothesis that incorporating microalgae with Met in broiler diets reduces breast muscle WS score and myopathy without effecting production performance could be accepted. However, the use of Met and microalgae-based diets in reducing WS and breast muscle myopathies in broiler birds raised in commercial field conditions warrants further investigation.

In monogastric animals like chickens in positive energy balance, dietary PUFA are digested, absorbed, and incorporated into the tissues without much modification; so the composition of carcass fat tends to reflect that of dietary fat (Lopez-Ferrer et al., 2001). It is

interesting to note that supplementing Met led to a significant increase in long chain ( $\geq 20C$ ) n-3 FA (mg/g) in the breast muscle with a concomitant decrease in mono-unsaturated FA in chickens fed microalga-based diets. Tissue n-3 FA enriching effects of microalgae in broiler diets have been previously reported (Long et al., 2018). However, the synergistic role of Met in increasing long chain n-3 FA with a concomitant reduction in monounsaturated FA (16:1, 18:1) in the breast muscle of broiler-fed microalgae containing diets is not known to these authors. In this context, a recent study by Beheshti Moghadam et al., 2017 reported muscle tissue n-3 PUFA and  $\alpha$ -tocopherol-enriching effects of Met in broilers fed a diet containing flaxseed rich in  $\alpha$ -linolenic acid. Met is the first limiting amino acid for commercial broilers fed corn-soybean-based diets. Broilers are unable to synthesize the carbon skeleton of Met; thus Met is an essential amino acid and must be supplemented in the diet to optimize poultry growth and feed efficiency. Met has an important role as a sulfur and methyl donor and as a lipotropic agent. Overall, these and previously reported research (Beheshti Moghadam et al., 2017) suggest a unique role of S containing amino acids in lipid and FA metabolism in avians.

Meat color is usually used for assessing freshness and meat quality (Uhlirva et al., 2018). In the current study, meat color ( $a^*$ , redness) was reduced ( $P < 0.05$ ) and a trend for reduction in  $b^*$  (yellowness) was observed in diet 2. With respect to meat color,  $a^*$  values are indicative of the degree of muscle pigment oxidation (Suman and Joseph, 2013). Higher redness values are associated with higher concentrations of oxymyoglobin, while lower values typically represent myoglobins' transition to oxidized metmyoglobin (AMSA, 2012). The lower  $a^*$  values for diet 2 may suggest a unique antioxidant or lipotropic effect of microalgae along with Met. Higher muscle concentrations of PUFA in diet 2 may have accelerated the transition of myoglobin to metmyoglobin or the significant increase in drip loss in the muscle of diet 2 compared to other treatments may have



**Figure 2.** Effect of microalgae and methionine supplementation on fatty acid content of breast muscle. Control, diet 1, and diet 2 represent corn-soybean meal-based diets containing 0% microalgae (control), 2% microalgae (diet 1), and diet 1 + 100% more methionine. n = 6. <sup>a-d</sup>Different letters for each bar cluster indicate a significant difference  $P < 0.05$ .



**Table 6.** Effect of microalgae and methionine supplementation on breast muscle color, cook loss, shear force, pH, and chemical composition.

Variables	Dietary treatment			Pooled SEM	P-value
	Control	Diet 1	Diet 2		
Color					
<i>a</i> *	14.58 <sup>a</sup>	13.63 <sup>a,b</sup>	12.27 <sup>b</sup>	0.56	0.033
<i>b</i> *	18.90	17.53	16.23	0.74	0.072
<i>L</i> *	64.55	64.53	65.39	1.25	0.858
Cook loss (%)	65.28	59.97	59.25	0.76	0.529
Shear force (N)	23.28 <sup>b</sup>	32.63 <sup>a,b</sup>	46.63 <sup>a</sup>	0.65	0.008
pH	5.89	6.03	5.93	0.06	0.332
Minerals (ppm)					
Ca	248.99	188.36	220.53	34.10	0.471
Mg	1,200.8	883.6	959.4	126.21	0.212
P	9,915.0	7,291.0	8,005.0	1,040.46	0.216
Na	2,074.3	1,694.3	1,515.2	317.14	0.463
Nitrogen (%)	3.33	3.55	3.43	0.09	0.282
Moisture (%)	72.49	73.20	71.81	0.82	0.505

Control, diet 1, and diet 2 represent corn-soybean meal-based diets containing 0% microalgae (control), 2% microalgae (diet 1), and diet 1 + 100% more methionine.

<sup>a,b</sup>Means within a row with no common superscript differ when  $P < 0.05$ .

n = 6.

Nitrogen reported on wet basis.

contributed to lower *a*\* values. Since more exudate was lost in diet 2, it may have increased water reflectance and myoglobin denaturation due to myoglobins' water solubility (Aberle et al., 2013). No difference was observed in *b*\* and *L*\* values of breast muscle. Given lipid oxidations' impact on accelerating myoglobin oxidation (Faustman et al., 2010), and no differences in TBARS in the breast muscle, samples from all dietary treatments in this study would likely account for the lack of significant differences in *b*\* and *L*\* color values. Tenderness (shear force) is one of the most important indicators that reflect meat quality. Diet 2 breast muscle had the highest shear force followed by drip loss. The major factor which determines the tenderness of poultry meat is mainly the contractile state of myofibrillar protein (Mudalal et al., 2014, 2015). The higher shear force value for diet 2 may be indicative of high intramuscular contractile strength which may have resulted in less denaturation of protein fibers compared to the control group. Elevated intramuscular fat deposits have been shown to disrupt connective tissue cross-linkages in muscle fibers, increasing meat tenderness (Nishimura, 2010). In this study, a significant increase in muscle total fat content was observed between control and diet 1 birds, but not diet 2. It should be noted that drip loss was also reduced significantly in diet 2. Drip loss assesses

the water holding capacity, which is the ability of meat to retain water and is related to various quality attributes like juiciness and tenderness. It should be noted that no difference was observed in the breast muscle weight or yield among the 3 treatments. Overall, these results suggest that supplementing Met in microalgae-based diets could negatively influence meat quality and flavor by increasing liquid flow and flow of soluble nutrients.

In conclusion, the results from this study demonstrate that Met supplementation in DHA-rich microalgae-based diets enriches chicken tissues with n-3 FA, and reduces breast muscle myopathic lesions and WS without affecting productivity. However, inclusion of Met in microalgae-based diets could influence meat tenderness and color. As the demand for health promoting n-3 FA rich foods is on a global rise and poultry meat is a major source of animal food protein consumed globally, further research on improving meat quality attributes in n-3 FA-enriched poultry meat is warranted.

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**Table 7.** Effect of microalgae and methionine supplementation on drip loss in chicken breast muscle.

Drip loss	Dietary treatments			Pooled SEM	P-value		
	Control	Diet 1	Diet 2		Diet	Time	Diet × time
Initial weight (g)	302.63 <sup>a</sup>	282.90 <sup>a,b</sup>	261.80 <sup>b</sup>	4.36	<0.0001	0.977	1.000
Weight after 4 h (g)	301.73 <sup>a</sup>	281.35 <sup>a,b</sup>	260.00 <sup>b</sup>	4.36	<0.0001	0.977	1.000
Weight after 8 h (g)	300.00 <sup>a</sup>	280.63 <sup>a,b</sup>	259.85 <sup>b</sup>	4.36	<0.0001	0.977	1.000
Weight after 16 h (g)	300.00 <sup>a</sup>	280.03 <sup>a,b</sup>	259.22 <sup>b</sup>	4.36	<0.0001	0.977	1.000
Overall mean	301.09 <sup>a</sup>	281.08 <sup>b</sup>	260.21 <sup>c</sup>	4.36	<0.0001	0.977	1.000

Control, diet 1, and diet 2 represent corn-soybean meal-based diets containing 0% microalgae (control), 2% microalgae (diet 1), and diet 1 + 100% more methionine (diet 2).

<sup>a-c</sup>Means within a row with no common superscript differ when  $P < 0.05$ .

n = 6.

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## DISCLOSURES

The authors declare that there are no conflict of interests.

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