

Evaluating the impact of organic chromium with flax seed in broiler diets: effects on production performance, breast muscle pathology, and meat quality aspects

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ABSTRACT The study investigated the impact of organic chromium (Cr) and flax seed supplementation on live performance, carcass yield, muscle lipid profile, histopathological aspects, and meat quality parameters in broilers. Ninety (n = 90), day-old Cobb chicks were fed a corn-soybean meal-based diet containing 0% flax seed (**Control**), 10% flax seed (**Diet 1**) and Diet 1 + 0.05% organic Cr (**Diet 2**). The experiment was a completely randomized design and chicks were placed in 6 pens with 5 chicks per each pen. Pen was the experimental unit for production performance and bird collected from each pen was considered as experimental unit for all other analysis. On d 43, 45, and 48, one bird per pen were euthanized. A one-way ANOVA was performed with diet as the main factor and significance was set at $P < 0.05$. Significant differences between each treatment were analyzed by GLM Least Square Method and Tukey's Honestly Significant Difference test. Weight gain and feed:gain was determined at d (1–11), (12–21), and (22–42). For all response variables, the effects among dietary treatments were compared using ANOVA separately using SAS 9.4. P -values were considered significant at ≤ 0.05 . At d 22, Diet 1 and Diet 2 birds had lower BW and feed:gain than Control ($P < 0.05$). At d 42, Diet 2 birds were higher in BW with improved feed:gain when compared to Diet 1 ($P < 0.05$) but were not different from Control ($P > 0.05$). The

overall weight gain was higher in Diet 2 and Control compared to Diet 1 ($P < 0.05$) and overall feed:gain was the highest in Control than the experimental diets ($P < 0.05$). Histopathological changes in breast muscle including floccular/vacuolar degeneration, fibrosis, lipodosis, interstitial inflammation, and muscle lysis were less pronounced in Diet 1 compared to Diet 2 ($P < 0.05$). Breast muscle total fat and cholesterol was lower in Diet 1 compared to Control ($P < 0.05$). Diet 1 and Diet 2 increased (>2–5 fold) total and long chain ($\geq 20C$) n-3 fatty acids (FA) in the breast muscle ($P < 0.05$) compared to Control. Lipid peroxidation products measured as thiobarbituric acid reactive substances were lower in the breast muscle of Diet 1 and Diet 2 compared to Control ($P < 0.05$). Phospholipid n-3 FA molecular species in phosphatidylcholine (PC) 36:5, 38:6, and phosphatidylethanolamine (PE) 36:5 were higher in breast muscle of Diet 1 than Control ($P < 0.05$) and was not different from Diet 2 ($P > 0.05$). A decrease in n-6 FA species (36:4 and 38:4) was observed in PC and PE of Diet 1 and Diet 2 compared to Control ($P < 0.05$). Drip loss values were reduced in Diet 1 and Diet 2 versus Control ($P < 0.05$). As consumer demand for n-3 FA-rich poultry products are on the rise, Cr may serve as a feed supplement that could be used in broilers fed flax seed-containing diets for enriching edible tissues with n-3 FA, while enhancing production performance.

Key words: broiler, flax seed, chromium, n-3 fatty acid, meat quality

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INTRODUCTION

Fatty acids (FA) especially, n-3 polyunsaturated fatty acids (PUFA) play an important role in the normal growth, cognitive functions, improvement of immune functions, and management of cardiovascular diseases in humans (Kris-Etherton et al., 2019; Fard et al., 2019; Shibabaw, 2021). The typical western diet is deficient in n-3 FA while n-6 FA and saturated

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FA predominate the main lipid constituents, consequently leading to various chronic diseases (Stark et al., 2016; Richter et al., 2017). Humans do not have the ability to synthesize α -linolenic acid (18:3 n-3, **ALA**) and linoleic acid (18:2 n-6) the parent n-3 and n-6 FA, hence these are considered as essential FA. To enhance the human intake of n-3 FA, nutritionists have used different strategies to enrich animal food products with n-3 FA. Both plant-based (e.g., flax seed, chia) and marine based ingredients (e.g., microalgae, fish oil, fish meal) in animal diets has been successfully attempted for this purpose (Cherian, 2011; Lee et al., 2019). Among the different feed ingredients, flax seed has the great potential to enrich poultry meat with n-3 FA due to its high metabolizable energy (3,757 kcal/kg) and ALA content (40–50%). However, flax seed feeding may affect broiler production performance by decreasing feed intake, weight gain and feed efficiency due to various antinutritional factors associated with it (Beheshti Moghadam and Cherian, 2017). Moreover, it may compromise organoleptic qualities since n-3 PUFA are highly susceptible to oxidation (Domínguez et al., 2019) affecting meat sensory aspects.

A potential application for meat enrichment with n-3 FA seems to be related with breast muscle myopathies, white striping (**WS**) and wooden breast (**WB**). It has been shown that WB myopathy seems to be associated with imbalance of FA profile and its severity can be ameliorated by modulation of short chain fatty acids and PUFA content in meat (Cauble et al., 2020). Although etiologies of WS and WB are not completely understood, various studies have shown oxidative stress (Mutryn et al., 2015; Abasht et al., 2016) and inflammatory conditions (Sihvo et al., 2014; Mudalal et al., 2015) in WB muscles because of genetic selection for fast growth. These myopathies represent birds' welfare, health, and economic concern and is anticipated to cost over \$700 million economic loss in the United States (Xing et al., 2020). Recent research (Khan et al., 2021) reported that supplementation of methionine (100% more than NRC requirement) can attenuate the incidence of WS in broilers birds fed an n-3 FA-rich diet. As methionine acts as an antioxidant, it may alleviate the oxidative stress and decrease the muscle pathological score (Khan et al., 2021) suggesting that antioxidants can be used as feed additives in broilers fed high n-3 FA diet to combat meat quality aspects.

Chromium (**Cr**) is a trace element that is naturally present in a trivalent form in very low concentrations in feed ingredients. There are no requirements for Cr in poultry diets (NRC, 1997). However, in recent years, organic Cr has achieved greater attention due to its positive impact on the physiological and immunological functions, growth, and performance of animals (Rajalekshmi et al., 2014; Huang et al., 2020; Sahin et al., 2017). Due to its antioxidant properties, Cr is considered as a potential feed additive to attenuate the oxidative stress in birds fed flax seed-based diet by ameliorating lipid peroxidation (Khan et al., 2014). In this context, the objectives of the current study are to assess the effect of organic Cr on production performance, incidence of WS

and breast muscle pathology, and lipid profile (total lipids, FA, cholesterol, phospholipid molecular species, lipid peroxidation products), and meat quality attributes in broilers fed flax seed-containing diets. The hypothesis tested is that supplementing organic Cr will enhance live performance, feed:gain, yield of cut-up-parts; alter muscle lipid composition and lipid peroxidation products; enhance breast muscle quality (chemical and cooking attributes) and reduce incidence of WS in the breast muscle and histopathological lesions of myopathy in broilers fed n-3 FA-rich flax seed-containing diets.

MATERIALS AND METHODS

Ethics Approval

An institutional animal care and use committee approved all experimental protocols to ensure adherence to animal care guidelines (ACUP# 5165).

Birds, Experimental Design, and Dietary Treatments

Ninety birds ($n = 90$) 1-day old Cornish cross straight run broiler chicks were obtained from a local hatchery and randomly placed in 18 floor pens bedded with wood shavings. Chicks were weighed and randomly assigned one of the 3 corn-soybean meal-based diets containing 0% flax seed (**Control**), 10% full fat whole flax seed (**Diet 1**), Diet 1 + 0.05 % organic Cr (**Diet 2**) (KemTRACE Chromium (Kemin, Des Moines, IA). The ingredient content and nutrient composition of the experimental diets are shown in Tables 1 and 2. Each treatment was replicated in 6 pens with 5 chicks in each pen. The chicks were fed experimental diets in 3 phases: starter (1–11 d), grower (12–21 d), finisher (22–50 d). All diets made were isocaloric and isonitrogenous. On d 11, 21, and 42, birds and feed were weighed, and BW and feed consumption were recorded for each pen. Average chicken weight and feed:gain was calculated. The chickens were not vaccinated and housed in an environmentally controlled facility with lighting program of 23L:1D. Gross energy (Bomb calorimetry) and crude protein (AOAC Official Method 968.06, AOAC, 2006) of the experimental diets were analyzed at the Centre of Excellence for Poultry Science at University of Arkansas (Fayetteville, AR) (Table 1).

Sample Collection

On d 43, 45, and 50, eighteen birds were randomly selected from each dietary treatment ($n = 6$, birds per dietary treatment per day, 3 per replicate pen). Birds were euthanized with carbon dioxide gas. The carcass was cut open and breast muscles were exposed. Chicken breast muscles were visually scored for WS on scale of 1–3 with 1 being very low striping, 2 mild striping, and 3 very high striping as described earlier (Khan et al., 2021). All scorings were done by the same person to minimize the error. For muscle pathology assessment one

Table 1. Ingredient content calculated and analyzed nutrient content of starter, grower and finisher diets¹.

Ingredients (lb/100 lb)	Starter			Grower			Finisher		
	Control	Diet 1	Diet 2	Control	Diet 1	Diet 2	Control	Diet 1	Diet 2
Corn	55.28	48.73	48.73	58.4	51.78	51.78	62.38	55.68	55.68
Soybean meal	38.0	35.0	35.0	35.0	32.0	32.0	31.30	28.63	28.63
Vegetable oil	2.90	2.45	2.45	2.78	2.35	2.35	2.50	2.00	2.00
Limestone	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
L-Lysine HCL	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
D-L Methionine	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Dicalcium phosphate	1.63	1.63	1.63	1.63	1.63	1.63	1.63	1.63	1.63
Premix ²	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Flax seed	-	10.0	10.0	-	10.0	10.0	-	10.0	10.0
Chromium	-	-	0.05	-	-	0.05	-	-	0.05
Nutrient specifications									
M, kcal/kg	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200
CP (%)	21.5	21.5	21.5	20.5	20.5	20.5	19.3	19.3	19.3
Analyzed values									
Gross energy, kcal/kg	3,967	3,987	3,992	4,011	4,089	4,056	4,017	4,029	4,076
CP (%)	21.7	22.1	22.1	21.0	21.0	20.5	19.2	19.8	19.2

¹Control, Diet 1, and Diet 2 represent corn-soybean meal-based diet containing 0% flax seed, (Control), 10% flax seed (Diet 1), Diet 1 + 0.05% Chromium (Diet 2).

²Supplied 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.

bird/replicate pen (n = 6/treatment) was selected on d 43 and samples were collected from one half of the breast muscle (pectoralis major) and processed as described earlier (Khan et al., 2021). The other half of the breast muscle was taken for lipid profile (total lipids, FA, phospholipid molecular species, lipid oxidation products, cholesterol), nitrogen, and mineral content. Tissue samples (n = 6/treatment) collected on d 43 for lipid profile, nitrogen and mineral analysis were cleaned in saline and were stored at -20°C until analysis. Breast muscle samples collected on d 45 and d 50 were used for assessing pH and other meat quality assays, respectively.

Breast Muscle White Striping Scoring, and Histopathological Assessment

Briefly, tissue samples (n = 6/treatment) collected on d 43 were trimmed, embedded in paraffin, sectioned at 4 micrometer, and stained with hematoxylin and eosin stain. The degree of microscopic changes (lesions) in skeletal muscle were assessed using a Nikon Eclipse

E400 bright-field microscope and were as follows. Chronic myopathic change (enlarged fibers with internal nuclei), fibrosis, floccular/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 taken at 20× to 400× (Nikon Digital Sight DS FiL) 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 about the dietary treatments.

Organ and Cut-Up-Parts Yield

For accessing organ and yield of cut-up-parts (6 birds per treatment, one bird per each replicate pen) selected on d 43 was used. The birds were weighed and were euthanized as described earlier. The carcass was cut

Table 2. Fatty acid composition of starter, grower, and finisher diets.¹

Fatty acids (%)	Starter			Grower			Finisher		
	Control	Diet 1	Diet 2	Control	Diet 1	Diet 2	Control	Diet 1	Diet 2
C14:0	0.11	0.11	0.11	0.09	0.11	0.11	0.12	0.13	0.00
C16:0	15.76	9.22	8.93	15.42	9.28	9.28	15.7	9.78	9.86
C16:1	0.33	0.37	0.17	0.19	0.26	0.26	0.17	0.20	0.28
C18:0	2.00	2.70	2.52	1.97	2.74	2.74	2.00	2.62	2.50
C18:1	26.69	30.2	29.51	26.69	30.5	30.5	26.17	29.64	29.80
C18:2 n-6	52.05	24.76	25.72	52.99	25.6	25.6	53.31	27.17	28.90
C18:3 n-3	2.64	32.38	32.52	2.22	31.4	31.4	2.07	30.14	28.50
Total SFA	17.87	12.03	11.56	17.48	12.13	12.13	17.82	12.53	12.36
Total MUFA	27.02	30.57	29.68	26.88	30.76	30.76	26.34	29.84	30.08
Total n-3 FA	2.64	32.38	32.52	2.22	31.4	31.4	2.07	30.14	28.50
Total n-6 FA	52.05	24.76	25.72	52.99	25.6	25.6	53.31	27.17	28.90

¹Control, Diet 1, and Diet 2 represent corn-soybean meal-based diet containing 0% flax seed, (Control), 10% flax seed (Diet 1), Diet 1 + 0.05% Chromium (Diet 2). n = 6. FA = Fatty acids. FA values reported as percentages of FA methyl esters. SFA = Saturated fatty acids (14:0+16:0+18:0); MUFA = Monounsaturated fatty acids (16:1+18:1); Total n-3 = 18:3 n-3+20:5 n-3+ 22:5 n-3+22:6 n-3.; Total n-6 FA = 18:2 n-6+ 20:4 n-6+ 22:4 n-6.

Table 3. Effect of flax seed with chromium supplementation on production performance of broiler birds.

Production parameter	Dietary treatment			SEM	P-value
	Control	Diet 1	Diet 2		
Starter					
Initial BW at day 10 (g)	46.12	45.51	45.45	0.56	0.654
Final BW at day 10 (g)	200.45	187.45	187.45	8.51	0.478
Feed consumption (g)	185.74	175.86	168.45	6.56	0.208
Feed:gain	1.21	1.26	1.19	0.03	0.368
Grower					
Initial BW at d 11(g)	200.45	187.45	187.45	8.51	0.478
Final BW at d 22(g)	923.33	790.00	838.33	24.82	0.006
Weight gain (g)	880.04 ^a	749.62 ^b	799.29 ^b	23.68	0.005
Feed consumption (g)	1006.01	959.80	980.74	31.43	0.593
Feed:gain	1.15 ^b	1.28 ^a	1.23 ^a	0.02	0.005
Finisher					
Final BW at d 42 (g)	2,830.70 ^a	2,484.58 ^b	2,719.17 ^a	70.63	0.011
Weight gain (g)	1,858.06	1,694.58	1,880.83	62.96	0.107
Feed consumption (g)	3,181.0	3,238.1	3,380.8	84.10	0.255
Feed:gain		1.92 ^a	1.80 ^b	0.03	0.009
Overall					
Initial BW at d 1 (g)	46.12	45.51	45.45	0.56	0.654
Final BW at d 42(g)	2,830.70 ^a	2,484.58 ^b	2,719.17 ^a	70.63	0.011
Weight gain (g)	2,784.58 ^a	2,439.07 ^b	2,673.72 ^a	70.41	0.01
Feed consumption (g)	4,372.7	4,373.8	4,530.0	98.02	0.446
Feed:gain	1.57 ^c	1.80 ^a	1.70 ^b	0.03	0.009

Control, Diet 1, and Diet 2 represent corn-soybean meal-based diet containing 0% flax seed (Control), 10% flax seed (Diet 1), Diet 1 + 0.05% Chromium (Diet 2).

^{a-b}Means within a row with no common superscript differ when $P < 0.05$. n = 6.

open and organ/tissue samples from each chicken including heart, liver, breast, and thigh muscles (pectoralis major and biceps femoris, without skin), and abdominal fat pad (including fat surrounding the gizzard, bursa of Fabricius, and cloaca) were collected, cleaned with saline and weighed, and relative weight (as percent of body weight) is calculated (Table 4).

Total Lipids, Fatty Acids, Lipid Oxidation Products, and Cholesterol

Lipids were extracted from muscle tissue and feed samples as per 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 × 0.25 mm i.d). Conditions of the gas chromatograph are as reported earlier (Apperson and Cherian, 2017). FA methyl esters were identified and quantified by comparison authentic external (PUFA-1, PUFA-2, Matreya, Inc., PA) and internal (methyl docosanoate, 22:0, Matreya, Inc.).

Lipid peroxidation in breast muscle tissue (n = 6/treatment) was evaluated by estimating malondialdehyde (MDA) concentration, a thiobarbituric acid reactive substance (TBARS). Lipid peroxidation products were measured using a colorimetric assay previously described in Salih et al. (1987), with modifications built on those described in Yonke and Cherian (2019). Two duplicates of each sample were averaged for data analysis. Tetraethoxypropane was used as standard. Values represent milligrams of MDA equivalents per gram of tissue. Breast muscle cholesterol assay was done as per AOAC Official Method 994.10 at University of Missouri, Experimental Station, Chemical Laboratory (Columbia, MO).

Phospholipid Molecular Species

Polar lipid molecular species in the breast muscle (n = 6/treatment) were determined by direct infusion electrospray ionization (ESI) and tandem mass spectrometry (MS/MS) at the Kansas Research Center analytical laboratory. An automated ESIMS/MS approach was used, and data acquisition, analysis and acyl group identification were carried out, with some modifications as reported earlier (Cherian, 2009; Head et al., 2019) and values are reported as mol %.

Meat Mineral Composition

Breast muscle mineral composition (n = 6/treatment) was conducted at the Central Analytical Laboratory,

Table 4. Effect of dietary flax seed and chromium supplementation on body weight, relative organ weight (g), and yield of carcass in broiler birds.

Variables	Dietary treatment			SEM	P-value
	Control	Diet 1	Diet 2		
Body weight (g)	2,875.00 ^{ab}	2,558.33 ^b	3,158.33 ^a	110.2	0.006
Liver (%)	2.00	2.10	2.01	0.08	0.678
Gizzard (%)	1.48	1.70	1.45	0.11	0.260
Heart (%)	0.46	0.43	0.42	0.01	0.172
Fat pad (%)	1.19	0.82	0.87	0.24	0.503
Breast (%)	26.64	27.23	28.22	1.02	0.558
Thigh (%)	20.94 ^a	19.20 ^b	20.14 ^{ab}	0.34	0.090

Control, Diet 1, and Diet 2 represent corn-soybean meal-based diet containing 0% flax seed (Control), 10% flax seed (Diet 1), Diet 1 + 0.05% Chromium (Diet 2).

^{a-b}Means within a row with no common superscript differ when $P < 0.05$. n = 6.

Department of Crop and Soil Sciences, Oregon State University.

Meat Quality Parameters

Six samples of whole chicken breast (one per pen) from each treatment ($n = 6/\text{treatment}$) on d 45 and 50 were isolated. The samples were deboned and divided into 2 equal halves. One half was taken for the calculation of color, drip loss, and pH and the second half was used for assessing cooking loss and meat tenderness according to the procedure reported earlier (Khan et al., 2021). Meat quality parameters were conducted at the Clark Meat Science Center, Oregon State University. For assessing cook loss and meat tenderness, samples were vacuum-packed and stored in a commercial freezer at -20°C .

Meat Color

Breast samples were deboned and residual fat removed, water was removed by blotting them with paper towel and weighed. Instrumental color readings were taken at 0 and 24 h using HunterLab MiniScan EZ portable spectrophotometer (Model 45/0 LAV, Hunter Associates Laboratory, INC, Reston, VA) with a

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

1.54 cm aperture, calibrated with black and white standards. Meat color was measured as a^* (redness), b^* (yellowness), and L^* (lightness) values CIE L^* , a^* , b^* color space values were calculated (CIE., 1978). Three readings were taken across the entire breast and were averaged as described in detail earlier (Khan et al., 2021).

Drip Loss (Water Holding Capacity)

Drip loss of breast muscle was determined by weighing the samples before and after storage at $3^{\circ}\text{C} \pm 1$ 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 towel to remove excess moisture. After weighing, samples were placed in a new Ziploc plastic bag to prevent water contamination, which may affect the result. All the samples were placed on stainless steel trays under even lighting.

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

pH Analysis

To determine muscle pH, about 7 g of tissue sample was minced and mixed with 43 mL of distilled water 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 GmbH, Weilheim, Germany) and 2 readings were taken for each tissue and were averaged.

Cook Loss

After storage, samples were thawed in a commercial retailer cooler at $3 \pm 1^{\circ}\text{C}$ for 24 h and were weighed using a digital scale. Samples were placed in a conventional cooking oven on aluminum foil lined pans. The temperature of the oven was set at 176°C and samples were cooked until an internal temperature of $77 \pm 1^{\circ}\text{C}$ was reached (Khan et al., 2021). Cook loss percentage was calculated using the formula shown below: After cooking, samples were wrapped in aluminum foil and placed in a commercial cooler at $3^{\circ}\text{C} \pm 1$ for further meat tenderness analysis.

Meat Tenderness (Warner-Bratzler Shear Force)

Samples were cored and the core samples were analyzed using Shimadzu EZ-X Series Tabletop Testing Machine with a load cell size of 500N as described earlier (Khan et al., 2021). The travel distance was set at 39 mm and a 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 for analysis.

Statistical Analysis

The data were analyzed using the statistical software SAS (version 9.4) (SAS Institute). The data from bird production performance, lipid profile (total lipids, cholesterol, FA, TBARS, phospholipid species), and minerals were analyzed using a one-way ANOVA. Pen was considered as the experimental unit for production performance and bird collected from each pen was considered as the experimental unit for all other tests. To analyze the effect of diet on meat quality aspects a two-way ANOVA was done with diet, day or time as main factors. Significant differences between each treatment means were analyzed by GLM Least Square Method and Tukey's Honestly Significant Difference test (Steel and Torrie, 1980). Visual and pathological scores were

compared using Fisher Exact test. P values were set at ($P < 0.05$) for all test scorings and $P < 0.1$ indicated a trend.

RESULTS

Bird Production Performance and Organ Yield

Experimental diets had no effect on the BW, weight gain, feed consumption, and feed:gain in the starter phase (1–11 d) ($P > 0.05$; Table 3). However, during grower phase, lower weight gain and high feed:gain was observed in Diet 1 and Diet 2 compared to Control ($P < 0.05$). No effect of experimental diets on feed consumption was observed in grower phase (12–21 d) ($P > 0.05$). In the finisher phase (22–42 d), feed:gain was higher in Diet 1 compared to Control and Diet 2 ($P < 0.05$), while no effect observed in weight gain and feed consumption ($P > 0.05$). Overall (1–42 d), feed:gain was the highest in Control than Diet 1 and Diet 2 ($P < 0.05$). Diet 1 birds had the lowest final BW and weight gain compared to Control and Diet 2 ($P < 0.05$). Diet 2 caused an improvement in final BW of birds by 234.6 g than Diet 1 ($P < 0.05$). Total feed consumption was not affected by experimental diets ($P > 0.05$). The relative organ weight (liver, fat pad, heart, gizzard) and yield of breast muscle was not different ($P > 0.05$) among the diets, though a trend for increase ($P = 0.09$) in yield of thigh muscle was observed for Diet 2 vs. Diet 1 (Table 4).

Breast Muscle Visual Scoring and Pathology

No significant dietary effect was observed on incidence of WS in breast muscle when scored visually (Table 5). However, a decreasing trend for Diet 1 and Diet 2 vs. Control was observed in the reduction of WS ($P = 0.08$). A trend for reduction in muscle histopathological score was observed in Diet 1 compared to Control ($P = 0.08$). Diet 2 had lesions similar to those of Control diet (Figure 1, Table 6). Overall, histopathological changes including floccular/vacuolar degeneration, fibrosis, lipodosis, interstitial inflammation, and muscle lysis were less pronounced in Diet 1 compared to Diet 2 ($P < 0.05$).

Table 5. Effect of flax seed and chromium supplementation on the visual score representing white striping on breast muscle in broilers.

Dietary treatment	P -value	Score ¹
Control vs. Diet 1	0.25	Control = 1.67
Control vs. Diet 2	0.18	Diet 1 = 1.56
Control vs. Diet 1 and Diet 2	0.08	Diet 2 = 1.67

Control, Diet 1, and Diet 2 represent corn-soybean meal-based diet containing 0% flaxseed (Control), 10% flax seed (Diet 1), Diet 1 + 0.05% Chromium (Diet 2).

¹ $n = 18$.

¹Chicken breast muscles were visually scored for white striping on scale of 1-3 with 1 being very low striping, 2 mild striping, and 3 very high striping.

Table 6. Effect of flax seed and chromium supplementation on the histopathological score representing pathological lesions in the breast muscle of broilers.

Dietary treatment	P -value	Score ¹
Control vs. Diet 1	0.08	Control = 2.00
Control vs. Diet 2	1.00	Diet 1 = 1.67
Diet 1 vs. Diet 2	0.02	Diet 2 = 2.17
Control vs. Diet 1 and Diet 2	0.01	

Control, Diet 1, and Diet 2 represent corn-soybean meal-based diet containing 0% flax seed (Control), 10% flax seed (Diet 1), Diet 1 + 0.05% Chromium (Diet 2).

¹ $n = 6$.

¹Chicken breast muscles were scored for histopathological lesions on scale of 1-3 with 1 being minimal lesions, 2 mild to moderate lesions, and 3 moderate to severe lesions. Following pathological lesion were considered while for scoring: follicular degeneration, lysis, mineralization, regeneration, interstitial inflammation, fibrosis, lipodosis, myopathic changes (enlarged hyper eosinophilic fibers with internal nuclei), and internal nuclei.

Total Lipids, Fatty Acids, Cholesterol, and Lipid Oxidation Products

Total lipids, FA composition, cholesterol content, and lipid oxidation products are shown in Table 7. The content of palmitic acid (16:0) was lower in Diet 1 and Diet 2 compared to Control with an increase in the proportion of oleic acid (18:1) ($P < 0.05$). No effect of experimental diets on 14:0 and 18:0 was observed ($P < 0.05$). Addition of flax seed led to over 6-fold increase in ALA along with a significant reduction in linoleic acid ($P < 0.05$). However, no effect of Cr supplementation was observed in the content of linoleic acid or ALA in the breast muscle compared to Diet 1 ($P > 0.05$). Arachidonic acid (20:4 n-6) was lower in Diet 2 ($P < 0.05$) than Control and was not different from Diet 1 ($P > 0.05$). Docosatetraenoic acid (22:4 n-6) was lower in Diet 1 and Diet 2 than Control ($P < 0.05$) consequently leading to overall decrease in total n-6 FA ($P < 0.05$). Total long chain ($\geq 20C$) n-6 FA were lower in Diet 2 when compared to Control ($P < 0.05$) and was not different from Diet 1 ($P > 0.05$). Addition of flax seed led to an increase the content of eicosapentaenoic acid (EPA, 20:5 n-3) and docosapentaenoic acid (DPA, 22:5 n-3) ($P < 0.05$). The content of docosahexaenoic acid (DHA, 22:6 n-3) was higher in Diet 1 ($P < 0.05$) than Control and was not different from Diet 2 ($P > 0.05$). Overall total n-3 FA and total long chain n-3 FA were higher in Diet 1 and Diet 2 than Control ($P < 0.05$). No difference was observed in the total saturated FA and total PUFA content of breast muscle ($P > 0.05$). Total monounsaturated FA were higher in Diet 2 than Control ($P < 0.05$) and was not different from Diet 1 ($P > 0.05$). Experimental diets had a significant effect on breast muscle total fat content ($P < 0.05$) with Diet 1 having the lowest (0.88%) and Control having the highest lipid content (1.56%). The cholesterol content was lower in Diet 1 than Control ($P < 0.05$). No effect of Cr supplementation on total lipids or cholesterol content of breast muscle of birds fed flax seed-based diet was observed ($P > 0.05$). Experimental diets had significant effect on the TBARS values for breast muscle ($P < 0.05$; Table 7). Both Diet 1 and Diet 2 caused a decrease (>2-fold) in lipid oxidation products in breast muscle than Control ($P < 0.05$).

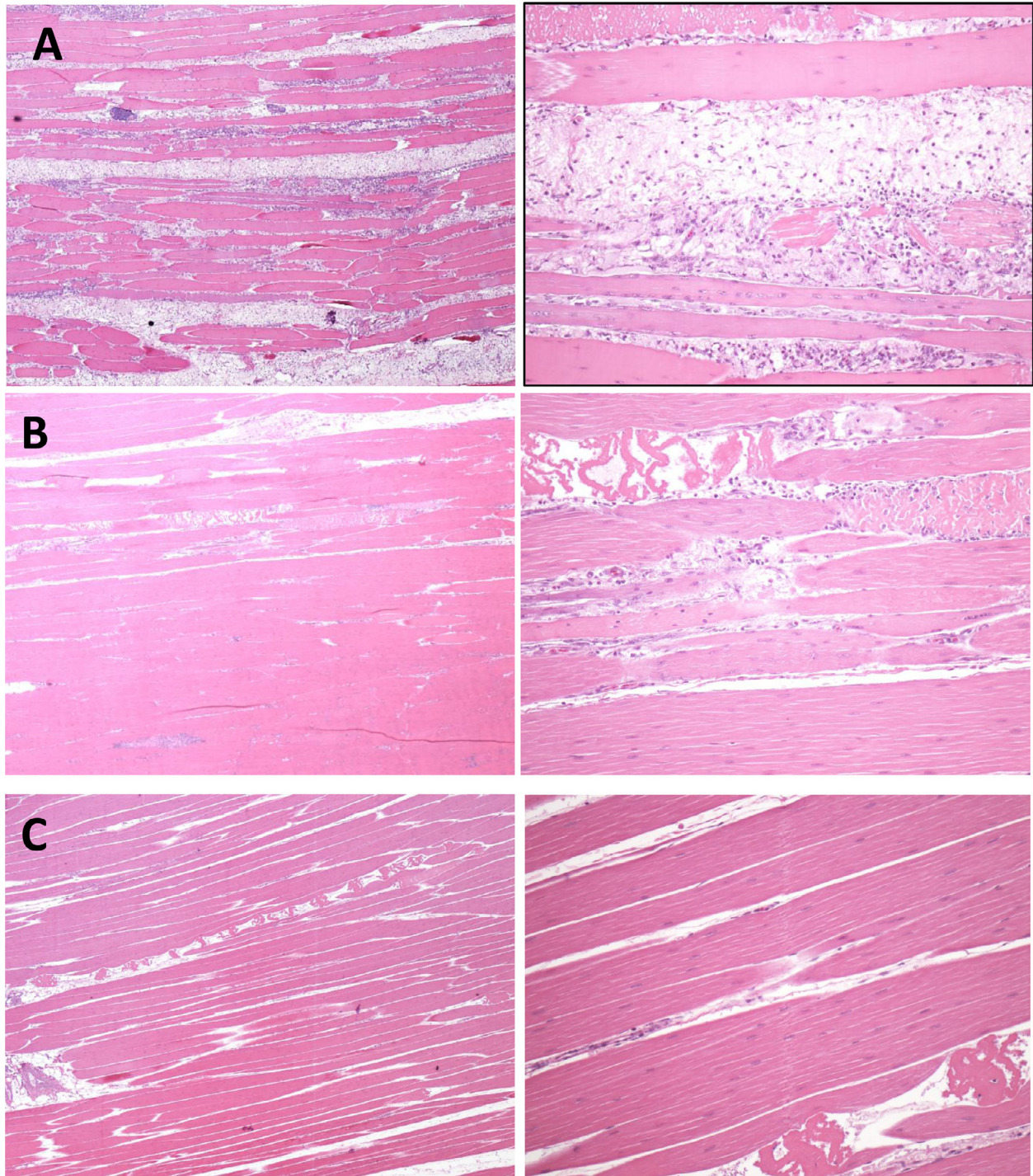


Figure 1. Muscle histopathology. Muscle histopathology. (A) Moderate to severe lesions (histologic score=3), as seen in single samples from Control and Diet 2 groups. Moderate fibrosis and edema and small foci of leukocytes expand the endomysium. Most fibers have multiple internal nuclei. Some fibers have floccular sarcoplasm or are swollen with hyper eosinophilic, occasionally fragmented sarcoplasm. Occasional fibers show lysis or regeneration. (B) Mild to moderate lesions (histologic score=2), as seen in most samples in Control and Diet 2 groups, and a single Diet 1 sample. Many fibers have a few internal nuclei. A few fibers have floccular sarcoplasm or swelling with hyper eosinophilic sarcoplasm. Scattered fibers show lysis or regeneration. There is mild interstitial edema. Neither cellular infiltrates nor fibrosis are present. (C) Minimal changes (histologic score = 1), as seen in most Diet 1 samples and a single Diet 2 sample. Internal nuclei are infrequent. Rare fibers have floccular sarcoplasm or are swollen with hyper eosinophilic sarcoplasm. Fiber lysis or regeneration is infrequent. Neither cellular infiltrates nor fibrosis or edema are present. Hematoxylin and eosin.

Phospholipid Molecular Species

Phospholipid FA in the breast muscle of broilers receiving Diet 1 and Diet 2 were differentially enriched with n-3 FA species than Control and is displayed as mol % (Table 8). In analysis of breast muscle

phospholipid FA molecular species dataset, n-3 FA molecular species in phosphatidylcholine (PC) 36:5 (16:0+20:5 n-3), PC 38:6 (16:0+22:6 n-3) were higher in Diet 1 and Diet 2 compared to Control ($P < 0.05$). Phosphatidylethanolamine (PE) 36:5 showed an increase in Diet 1 compared to Control ($P < 0.05$) but was not

Table 7. Effect of dietary flax seed and chromium supplementation on breast muscle fatty acid composition, total lipids, cholesterol, and lipid oxidation products measured as thiobarbituric acid reactive substances in breast muscle.

Variables	Dietary treatment			SEM	P-value
	Control	Diet 1	Diet 2		
Fatty acids (%)					
14:0	0.63	0.79	0.77	0.09	0.367
16:0	27.22 ^a	25.01 ^b	25.11 ^b	0.48	0.008
16:1	5.69 ^a	3.96 ^c	4.84 ^b	0.29	0.003
18:0	7.53	8.11	7.46	0.45	0.537
18:1	30.86 ^b	34.98 ^a	36.18 ^a	0.80	0.00
18:2 n-6	22.40 ^a	17.02 ^b	16.48 ^b	0.64	<0.0001
18:3 n-3	0.811 ^b	4.59 ^a	4.19 ^a	0.43	<0.0001
20:4 n-6	3.29 ^a	2.60 ^{ab}	2.08 ^b	0.35	0.085
20:5 n-3	0.00 ^c	0.71 ^a	0.39 ^b	0.08	<0.0001
22:4 n-6	0.52 ^a	0.00 ^b	0.07 ^b	0.03	<0.0001
22:5 n-3	0.00 ^b	0.80 ^a	0.61 ^a	0.07	<0.0001
22:6 n-3	0.00 ^b	0.47 ^a	0.25 ^{ab}	0.09	0.01
Total SFA	35.37	34.03	33.70	0.86	0.393
Total MUFA	36.5 ^b	38.95 ^{ab}	41.03 ^a	1.01	0.023
Total n-6 FA	26.57 ^a	20.09 ^b	19.02 ^b	0.73	<0.0001
Total n-3 FA	1.17 ^b	6.92 ^a	6.11 ^a	0.37	<0.0001
Total PUFA	27.74	27.02	25.14	0.84	0.109
Total LC n-6	4.17 ^a	3.07 ^{ab}	2.54 ^b	0.43	0.049
Total LC n-3	0.28 ^b	2.09 ^a	1.60 ^a	0.27	0.001
Total Lipids %	1.56 ^a	0.88 ^b	1.34 ^{ab}	0.40	0.039
Cholesterol (mg/ 100g)	71.92 ^a	61.85 ^b	69.02 ^{ab}	2.28	0.020
TBARS (mg/g MDA)	28.34 ^a	12.31 ^b	13.05 ^b	0.75	<0.0001

Control, Diet 1, and Diet 2 represent corn-soybean meal-based diet containing 0% flax seed (Control), 10% flax seed (Diet 1), Diet 1 + 0.05% Chromium (Diet 2).

Abbreviations: FA, Fatty acid; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substances. SFA = Saturated fatty acids (14:0+16:0+18:0); MUFA = Monounsaturated fatty acids (16:1+18:1); Total n-3 = 18:3 n-3+20:5 n-3+22:5 n-3+22:6 n-3.; Total n-6 FA = 18:2 n-6+20:4 n-6+22:4 n-6. Total long chain (LC) n-3 = 20:5 n-3+22:5 n-3+22:6 n-3.; Total LC FA = 20:4 n-6+ 22:4 n-6.

Fatty acid values reported as percentages of FA methyl esters.

^{a-b}Means within a row with no common superscript differ when $P < 0.05$. n = 6.

different from Diet 2 ($P > 0.05$; Table 8). Similarly, n-6 FA molecular species, PC and PE, 36:4 (16:0+20:4 n-6) and 38:4 (18:0+20:4 n-6) were lower in Diet 1 and Diet 2 when compared to Control ($P < 0.05$; Table 8). No

Table 8. Effect of flax seed with chromium supplementation on breast muscle phospholipid molecular n-3 and n-6 fatty acid species.

Fatty acid species (mol, %)	Dietary treatment			SEM	P-value
	Control	Diet 1	Diet 2		
n-3 Fatty acid species					
PC (36:5)	0.18 ^b	0.52 ^a	0.51 ^a	0.082	0.017
PC (38:6)	0.25 ^b	0.45 ^a	0.48 ^a	0.060	0.029
PE (36:5)	0.09 ^b	0.26 ^a	0.22 ^{ab}	0.042	0.035
PE (38:6)	0.32	0.38	0.41	0.067	0.673
n-6 Fatty acid species					
PC (36:4)	3.16 ^a	1.55 ^b	1.69 ^b	0.216	0.0004
PC (38:4)	2.07 ^a	0.86 ^b	1.04 ^b	0.011	0.0001
PE (36:4)	1.59 ^a	0.89 ^b	0.91 ^b	0.106	0.004
PE (38:4)	7.42 ^a	3.42 ^b	3.97 ^b	0.456	0.0001

Control, Diet 1, and Diet 2 represent corn-soybean meal-based diet containing 0% flaxseed (Control), 10% flaxseed (Diet 1), Diet 1 + 0.05% Chromium (Diet 2).

PC = phosphatidylcholine; PE = phosphatidylethanolamine. 36:5 = (16:0+20:5 n-3); 38:6 = (16:0+22:6 n-3); 38:4 = (18:0+20:4 n-6); 36:4 = (16:0+20:4 n-6).

^{a-b}Means for each lipid class with no common superscript differ when $P < 0.05$. n = 6.

significant difference was noted between Diet 1 and Diet 2 ($P > 0.05$) in the PC and PE n-3 and n-6 species.

Meat Mineral Content, pH, and Quality Attributes

Meat mineral content, pH, and quality aspects are shown in Tables 9–11. Among the different minerals, a reduction in Mg in Control when compared to Diet 1 was observed ($P < 0.05$). Manganese content was higher in Diet 2 when compared to Diet 1 ($P < 0.05$) and was not different from Control ($P > 0.05$). Other minerals (carbon, nitrogen, phosphorous, potassium, calcium, boron, iron, zinc, and sodium) in breast muscle were not affected by dietary treatments ($P > 0.05$). Experimental diet has no effect on pH of breast meat ($P > 0.05$; Table 10). No effect of day of collection was observed on pH of breast muscle. Meat tenderness and cook loss was significantly affected by day ($P < 0.05$). Shear force and cook loss was higher at d 50 than d 45 for all the diets ($P > 0.05$). However, no effect of diet on shear force or cook loss of breast muscle was observed ($P > 0.05$). Breast meat color (a^* and b^*) was reduced in d 50 compared to d 45 ($P < 0.05$). No dietary effect was observed on meat color ($P > 0.05$). Meat lightness (L^*) at 24 h. was higher than at 0 h. ($P < 0.05$). There was no Diet \times Day and Diet \times Time interactions ($P > 0.05$). Drip loss was affected by both diet and day ($P < 0.05$; Table 11). Both Diet 1 and Diet 2 caused a reduction in drip loss compared to the Control ($P < 0.05$). Higher drip loss was observed at d 50 than d 45 ($P < 0.05$).

DISCUSSION

The current study shows that Cr supplementation has reversed the negative impact of flax seed-based diets on growth of birds by improving BW and feed:gain

Table 9. Effect of dietary flax seed and chromium supplementation on breast muscle chemical composition.

Minerals	Dietary treatment			SEM	P-value
	Control	Diet 1	Diet 2		
Carbon (%)	49.14	48.69	48.00	0.38	0.135
Nitrogen (%)	14.79	15.15	14.89	0.18	0.351
Phosphorous (ppm)	0.86	0.89	0.86	0.01	0.081
Potassium (ppm)	1.61	1.61	1.64	0.04	0.815
Calcium (ppm)	0.03	0.02	0.03	0.00	0.328
Magnesium (ppm)	0.12 ^b	0.13 ^a	0.13 ^{ab}	0.00	0.020
Boron (ppm)	3.00	2.50	3.17	0.52	0.649
Manganese (ppm)	0.50 ^{ab}	0.17 ^b	1.67 ^a	0.39	0.038
Iron (ppm)	79.17	75.67	108.83	11.65	0.120
Zinc (ppm)	36.00	30.67	38.83	2.99	0.180
Sodium (ppm)	1715.8	1371.7	2054.3	295.23	0.292

Control, Diet 1, and Diet 2 represent corn-soybean meal-based diet containing 0% flax seed (Control), 10% flax seed (Diet 1), Diet 1 + 0.05% Chromium (Diet 2).

Nitrogen reported on wet basis.

^{a-b}Means within a row with no common superscript differ when $P < 0.05$. n = 6.

Table 10. Effect of dietary flax seed and chromium supplementation on breast muscle pH, shear force, and cook loss.

Meat quality	Dietary treatment						SEM	P-value		
	Control		Diet 1		Diet 2			Diet	Day	Diet × Day
	D 45	D 50	D 45	D 50	D 45	D 50				
pH	5.68	5.79	5.67	5.79	5.70	5.66	0.05	0.413	0.104	0.159
Shear value (N)	13.97 ^b	26.12 ^a	15.92 ^b	22.96 ^a	11.84 ^b	28.65 ^a	3.14	0.965	<0.0001	0.312
Cook loss (g)	61.24 ^b	103.00 ^a	59.57 ^b	96.55 ^a	55.38 ^b	96.18 ^a	5.91	0.387	<0.0001	0.915

Control, Diet 1, and Diet 2 represent corn-soybean meal-based diet containing 0% flax seed (Control), 10% flax seed (Diet 1), Diet 1 + 0.05% Chromium (Diet 2).

^{a-b}Means with no common superscript differ for each treatment when $P < 0.05$. n = 6.

($P < 0.05$). Beneficial effect of Cr on production performance can be attributed to its role in enhancing insulin action and metabolism of macronutrients via activation of enzymes involved in related pathways (Anderson, 1987; Hayirli 2005; Ognik et al., 2020). Low fat and cholesterol concentration in Diet 1 and Diet 2 breast muscle may be justified by the impact of n-3 FA on lipogenesis. Polyunsaturated FA in muscle go through rapid oxidation reactions compared to saturated FA. Head et al. (2019) demonstrated the role of n-3 FA in repressing the genes involved in de-novo FA synthesis and activating the genes responsible for FA catabolism. In addition, antinutritional factors in flax seed are known to increase the intraluminal viscosity that impedes micelle formation and hence lessen the lipid absorption and re-uptake of bile acids (Beheshti Moghadam et al., 2020; Mir et al., 2017). Consequently, the increased use of cholesterol to synthesize bile acids in liver can reduce the cholesterol level in the body. The fat and cholesterol reduction in broiler muscle due to inclusion of flax seed or flax oil in broiler diet has been reported earlier (Ajuyah et al., 1991; Abdullah et al., 2015). We did not observe an effect of Cr supplementation on breast muscle fat and cholesterol content. However, Mir et al. (2017) reported a decline in the fat and cholesterol content in the breast muscle of birds fed organic Cr in flax seed-fed birds. Total n-3 FA was higher and n-6 FA was lower in breast muscle for Diet 1 and Diet 2 compared to the Control ($P < 0.05$). This could be due to the higher level of ALA and lower level of linoleic acid in the experimental diets as shown in Table 2. In non-ruminant animals like chickens having positive energy balance, dietary PUFA are digested, absorbed, and are incorporated into the tissues without much modification, so the composition of carcass tends to reflect that of dietary fat (Lopez-Ferrer et al., 2001).

Additionally, high level of total n-3 FA in breast muscle with concomitant decrease in total n-6 FAs can be explained by the competition that exists between these 2 FA for the same enzymes. Desaturases and elongases are the enzymes used by n-3 and n-6 FA for elongation and desaturation to form respective long chain PUFA like EPA and DHA (Gonzalez-Soto and Much, 2021). Having higher levels of ALA than linoleic acid in flax seed-based diets have resulted in higher concentration of EPA, DPA, and DHA and overall, long chain n-3 FA in breast muscles compared to Control. In the current study, saturated FA was not different but monounsaturated FA concentration was enhanced in breast muscle due to Cr supplementation compared to Control. Contrary to our findings, Beheshti Moghadam et al. (2017) reported the increase in total saturated FA in breast muscle at 15% flax seed supplementation compared to the Control, which can be explained by the inhibitory role of n-3 FA on delta-9 desaturase enzyme that is involved in desaturating saturated FA to monounsaturated FA (Zuidhof et al., 2009).

Phospholipids are the major FA reservoirs that regulate the metabolic activity as well as production of secondary signaling molecules (Sugiura et al., 2009). The FA composition of PC and PE reflects both dietary lipid composition and long chain products of FA synthesis. In this study, n-3 molecular species of PC 36:5, PC 38:6, and PE 36:5 were higher in Diet 1 compared to Control ($P < 0.05$). Conversely, n-6 molecular species 36:4 (16:0 +20:4 n-6) were decreased in the PC and PE of Diet 1 and Diet 2 birds ($P < 0.05$). It can be linked to low content of precursor linoleic acid in the breast muscle of Diet 1 and Diet 2 birds (Table 7).

An important highlight from this study is that Diet 1 decreased breast muscle histopathological score (pectoralis major) compared to other treatments ($P < 0.05$).

Table 11. Effect of dietary flax seed and chromium supplementation on breast muscle color and drip loss.

Meat color	Dietary treatment						SEM	P-value				
	Control		Diet 1		Diet 2			Diet	Day	Time	Diet × Day	Diet × Time
	D 45	D 50	D 45	D 50	D 45	D 50						
a	17.58 ^a	13.45 ^b	18.68 ^a	13.05 ^b	17.08 ^a	13.18 ^b	0.68	0.32	<0.0001	0.05	0.16	0.97
b	22.64 ^a	17.36 ^b	22.06 ^a	16.6	22.53 ^a	17.20 ^b	0.77	0.47	<0.0001	0.10	0.99	0.92
L	62.64	59.51	60.01	60.22	61.30	62.12	1.62	0.38	0.458	0.010	0.19	0.88
Drip loss	331.3 ^b	451.2 ^a	285.1 ^b	410.4 ^a	281.0 ^b	407.1 ^a	24.52	0.00	<0.0001	0.98	0.96	0.00

Control, Diet 1, and Diet 2 represent corn-soybean meal-based diet containing 0% flax seed (Control), 10% flax seed (Diet 1), Diet 1 + 0.05% Chromium (Diet 2).

^{a-b}Means within a row with no common superscript differ for each treatment when $P < 0.05$. n = 6.

The breast muscle myopathies are correlated with increased growth rate of birds and breast muscle (Petracci et al., 2019; Xing et al., 2020). The decrease in final BW and weight gain of birds caused by Diet 1 may have resulted in lesser damage to muscle.

The meat stability was improved by feeding Diet 1 and Diet 2 compared to Control. The reduction of TBARS values in flax seed-based diets may be due to the bioactive molecules present in flax seed such as choline (Aziza et al., 2019). Besides that, another possible explanation for the decrease in TBARS values in breast muscles may be due to the presence of Vitamin E in breast membranes thus raising antioxidant potential, decreasing lipid peroxidation, (Jensen et al., 1998), and maintaining membrane bound lipid stability (Lauridsen et al., 1997). However, it should be mentioned that the premix provided similar amounts of vitamin E (30 IU) to all the birds and the content of vitamin E in muscle was not measured in current study. A similar finding was reported in a recent study where TBARS levels were decreased in breast muscle ($P < 0.05$) and were unaffected in thigh muscle of broilers fed marine rest raw materials rich in n-3 FA when compared to the Control (Cherian et al., 2022). It contradicts other studies that have confirmed the increase in TBARS level in poultry meat from birds fed flax seed-based diets (Betti et al., 2009; Rahimi et al., 2011; Mir et al., 2017). In the current study, we did not observe any effect of Cr supplementation on TBARS of breast muscles compared to Diet 1. Our findings disagree with Mir et al. (2017) who found the improvement in meat stability by decreasing TBARS in the breast muscle of broilers, when Cr was supplemented up to 1.5 mg/kg along flax seed-based diets. This disparity may be explained by the different dosage rates, level of oxidative stress, and source of organic Cr used in these studies.

Drip loss values were decreased in breast muscle Diet 1 and Diet 2 compared to Control ($P < 0.05$) that may be explained because of decreased lipid peroxidation in breast muscle (Table 7). Water is held within the muscle fibers and its loss depends upon the cell integrity, protein denaturation, and protein degradation that are based on rigor mortis and ultimate pH. The improvement in water holding capacity of breast meat measured as drip loss in Diet 1 and Diet 2 may be related to decreased protein oxidation and more availability of soluble proteins, postmortem as shown by lower TBARS values (Table 7).

With respect to meat color, fresh meat, a^* , or redness, values are indicative of the degree of muscle pigment oxidation (Suman and Joseph, 2013). Higher redness values are associated with higher concentrations of oxy or myoglobin, while lower values typically represent myoglobin's transition to oxidized metmyoglobin (AMSA, 2012). Breast meat color (a^* and L^*) was affected ($P < 0.05$) when values were taken at 0 and 24 h after slaughtering birds. We noticed the decline in redness (a^*) and increase in lightness (L^*) at 24 h compared to 0 h. It can be reasoned on basis of increased water loss from muscle fibers with the passage of time after

slaughter affecting a^* and L^* values. In the current study, shear values were high for birds slaughtered at D 50 compared to D 45 that can be due to the increase in cross-linking of collagen and changes in collagen solubility leading to an increase connective tissues maturity as the birds get older (Fang et al., 1999; Morey., 2017). High cook losses for birds slaughtered at D 50 than D 45 can be linked to the high oxidative stress in older birds causing increased lipid peroxidation and degrading proteins responsible for holding water within myofibrils (Viera et al., 2021).

In summary, the results from the current study demonstrate that Cr supplementation may serve as feed supplement that could be used in broilers fed flax seed-based diets for enriching breast meat with n-3 FA, while improving final body weight and feed efficiency. However, Cr inclusion did not influence breast muscle pathological score. As the demand for health-promoting foods is on a global rise with increased interest in n-3 FA and poultry meat being a major source of animal food protein consumed globally, further research on improving meat quality attributes in n-3 FA enriched poultry meat is necessary. Further studies which analyze the cost of production per mg increase in n-3 FA enrichment in meat would provide further information and may pave the way for a consumer shift.

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DISCLOSURES

The authors declare that there is no conflict of interests.

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