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Improving Meat Quality and Reducing Breast of Myopathies in Broiler Chickens Subjected to Cyclic Heat Stress by Supplementing of Chromium-Methionine

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ABSTRACT

Chromium-methionine (CrMet) provides essential nutrients and bioactive compounds that may enhance meat quality and reduce stress-related issues in broiler chicken. This study aimed to evaluate the effects of chromium-methionine on carcass yield, relative weights of the liver and fat pad, incidence and severity of myopathies, muscle color quantification, water-holding capacity, cooking loss, shear force, lipid peroxidation in breast muscle, and composition of breast meat in broiler chickens subjected to cyclic heat stress. A total of 1,000 one-day-old male Cobb 500 broiler chickens were divided into 10 replicate pens with 20 birds each, following a completely randomized design with five doses of dietary treatments (0; 0.25; 0.50; 1.0; and 2.0 mg CrMet kg^{-1} diet). Experimental broiler chickens were kept in thermoneutral conditions for 21 days, then subjected to cyclic heat stress (31.1 °C and 60.2% humidity) from 09:00 am to 03:00 pm until 42 days of age. Statistical analysis included Tukey's test and regression analysis. Myopathy scores were assessed using the Kruskal-Wallis test and Dunn's test, all at a significant level of 5%. Inclusion of 0.50 and 1.0 mg CrMet reduced woody breast severity scores. At 15 minutes postmortem, birds fed 0.50 mg CrMet had less redness (a*) than controls and birds fed 2.0 mg CrMet; birds fed 2.0 mg CrMet had lower lightness (L*) than those fed 0.25 and 1.0 mg CrMet. Lipid peroxidation was lower in birds fed 0.50 mg CrMet at 60 days compared to control. Crude fat was lower in birds fed 1.0 mg CrMet. Chromium-methionine supplementation at 0.50 and 1.0 mg/kg diet improved meat quality and reduced woody breast in broiler chickens.

Keywords: *carcass yield; muscle conditions; lipid peroxidation; thermal stress*

INTRODUCTION

Thermal stress poses a substantial challenge in poultry production, impairing zootechnical performance, health, and bird welfare (An *et al.*, 2023; Han *et al.*, 2021). To reduce these adverse effects, researchers have investigated the potential of dietary additives such as chromium-methionine in modulating birds' adaptive response to thermal stress (Das *et al.*, 2016; Van Hoeck *et al.*, 2020; Piray & Foroutanifar, 2022). Chromium-methionine, an organic form of chromium, exhibits antioxidant properties and potential benefits in regulating energy metabolism and the immune response of poultry (Arif *et al.*, 2019; Safwat *et al.*, 2020; Dalólio *et al.*, 2024).

Understanding the effects of this supplementation on performance, meat quality, and physiological parameters is crucial for developing nutritional strategies that promote the health and growth of birds under challenging environmental conditions, thus ensuring the sustainability and efficiency of poultry production (White & Vincent, 2019; Hayat *et al.*, 2020; Safwat *et al.*, 2020). Recent studies have shown that supplementing broiler diets with chromium-methionine under cyclic heat stress can improve carcass cut yields, such as the breast (Huang *et al.*, 2020; Aslam *et al.*, 2021; Amini *et al.*, 2023; Dalólio *et al.*, 2024). It can also minimize the incidence of white striping and the severity of myopathies, conditions that negatively affect meat quality and consumer acceptability (Ebrahimzadeh *et al.*, 2011; Toghyani *et al.*, 2012; El-Tarabany *et al.*, 2021).

Other studies suggest that carcass quality, including parameters such as pH, water-holding capacity, and tenderness, can be optimized, leading to more tender and better-textured meat (Safwat *et al.*, 2020; Dalólio *et al.*, 2021; Saracila *et al.*, 2021; Youssef *et al.*, 2022; Kazakova & Marshinskaia, 2023).

However, despite the promising benefits of chromium-methionine supplementation in broiler diets, the variability in bird responses due to the lack of consensus on appropriate dosage and the influence of interactions with the other dietary nutrients and chromium absorption, limits the effectiveness of the feed additive (Mir *et al.*, 2018; Shakeri *et al.*, 2019; Van Hoeck *et al.*, 2020, El-Tarabany *et al.*, 2021; Kazakova & Marshinskaia, 2023). This underscores the need for further studies to clarify these points and establish clear and safe guidelines for the use of chromium-methionine in broiler diets (Lu *et al.*, 2017; Safwat *et al.*, 2020; Khan *et al.*, 2021; Kuttappan *et al.*, 2021). Furthermore, the environmental impact of using chromium on a large scale in poultry production is another aspect that needs to be considered (Barzegar-Yarmohammadi *et al.*, 2020; Saracila *et al.*, 2021; Dalólio *et al.*, 2024).

The hypothesis of this study is that chromium-methionine supplementation in broiler chickens' diets improves meat quality and reduces the incidence of myopathies, such as woody breast and white striping, especially under cyclic heat stress conditions. To test this hypothesis, the study aimed to evaluate the impact of including different levels of chromium-methionine in the diets of broiler chickens from 1 to 42 days of age on various variables. These variables include carcass yield, relative weights of the liver and fat pad, incidence and severity of myopathies, muscle color quantification, water-holding capacity, and cooking loss. Moreover, shear force, lipid peroxidation in breast muscle, composition of breast meat, and estimation of chromium in feed samples were assessed.

MATERIALS AND METHODS

Ethics Committee

The use of animals and approval for all experimental protocols were granted by the Ethics Committee for Animal Use of Western Paraná State University (Protocol number: 18/2021). The study was conducted at the Poultry Research Center of Western Paraná State University, Marechal Cândido Rondon, Paraná, Brazil.

Broiler Chickens and Diets

A total of 1,000 one-day-old male Cobb 500® broiler chickens with initial weights of 47.8 to 49.2 g purchased from a commercial hatchery (Globoaves, Cascavel, PR, BR) were assigned in a completely randomized design composed of 5 dietary treatments (0; 0.25; 0.5; 1.0; and 2.0 mg CrMet kg^{-1} diet), and 10 replicate pens with 20 animals each as experimental unit. Animals received diet and water *ad libitum* throughout the experimental period.

The experimental diets were mash, isonitrogenous, and isocaloric, formulated to meet the nutritional requirements for broilers (except for selenium) according to the recommendation by Rostagno *et al.* (2024) in four different periods: pre-starter, day 1 to 7; starter, day 8 to 21; grower, day 22 to 33; and finisher, day 34 to 42 (Table 1).

The CrMet used for this study contains 1,000 ppm (0.1%) of chromium, along with a significant amount of crude protein (1.0%) and ash (95.0%). The product has physical characteristics such as a gray-tan color, granular texture, and a density ranging from 1,340 to 1,410 kg/m^3. Its water solubility allows for easy incorporation into feed formulations. The product inclusions were performed in replacement ($g\ g^{-1}$) of inert material (kaolin).

Experimental Period

The study took place at the Poultry Research Center of Western Paraná State University (Unioeste, Marechal Cândido Rondon, PR, Brazil) from September 18th to October 29th, 2020. Throughout the entire experiment, climatic conditions were closely monitored by the environmental control station located at the university's experimental farm. The recorded climate data included minimum, maximum, and average temperatures of 20.3, 36.3, and 26.6 °C, respectively. Additionally, relative humidity values were documented at 29.2%, 73.4%, and 48.5%, respectively.

The facility was divided into concrete floor pens (1.96 m^2/pen). Each pen was covered with wood shavings as bedding material and was provided with a tubular feeder and a nipple drink line. The experimental aviary was equipped with exhaust fans, inlets, an evaporative cooling system, and a commercial pellet stove as a heating source. The light program used in this study followed the guidelines outlined in the Cobb 500 breeder's manual.

Cyclic Heat Stress and Environmental Conditions Management

The birds were reared at comfortable temperatures, as recommended in the breeder's manual throughout the experiment, except for the period between 22 and 42 days of age, from 9:00 am to 3:00 pm, when the animals were exposed to controlled heat stress.

To carry out stress conditions, the temperature and relative humidity were manually changed on the control panel, which activated the pellet combustion stove, which is responsible for heating the house and the evaporative cool cells, to increase the relative humidity, if necessary. During this period of stress, the temperature was maintained at 31 °C with a variation of ±1.5 °C, and the relative humidity at 65% with a variation between 55% to 75%. The mean temperature and relative humidity during the period of heat stress were 31.1 and 60.2, respectively. The temperature at placement was 32 °C, which gradually reduced to 21 °C to ensure a comfortable environment for the birds. However, in the last week of the experiment, the minimum temperature reached was 24 °C.

Considering the lighting conditions, the birds were initially exposed to a continuous 24-hour light period on the first day of age. Subsequently, the light/dark periods adopted were 23 hours of light and 1 hour of darkness until the third day, 21 hours of light and 3 hours of darkness until the eighth day, and 18 hours of light and 6 hours of darkness and until they reached 42 days. The light intensity throughout the entire experimental period remained constant at 20 lux. The environmental conditions were controlled by a control panel (SMAAI 4, InoBram automations, Pato Branco, PR, Brazil), which recorded environmental parameters every 5 minutes.

Carcass Yield and Relative Weights of Liver and Fat Pad

At 42 days of age, after being subjected to 6 h of fasting, four broilers per pen were randomly selected and individually weighed, euthanized, and processed. Broilers were stunned by electronarcosis, followed by bleeding for ventral neck cutting. Then, the carcasses were scalded at 60 °C for 30 seconds, and the feathers

Table 1. Proximate composition calculated and analyzed values of experimental diets for broiler chickens

Items	Dietary phases for broiler chickens[1]			
	Pre-starter	Starter	Grower	Finisher
Ingredients (g kg^{-1})				
Corn (78.8 g kg^{-1} CP)	509.1	528.0	585.9	640.0
Soybean meal (460 g kg^{-1} CP)	429.5	405.0	343.4	292.7
Soybean oil	24.49	32.55	35.01	36.79
Dicalcium phosphate	19.01	16.79	14.42	10.60
Salt	5.32	5.15	3.70	3.46
DL-Methionine (990)	3.28	3.12	2.66	2.27
Limestone	3.09	2.99	7.24	6.87
Inert	2.00	2.00	2.00	2.00
Byo-Lysine (540)	1.84	2.02	2.19	2.40
Sodium bicarbonate	-	-	1.50	1.50
Vitamin premix[2]	0.50	0.50	0.50	0.50
Mineral premix[3]	0.50	0.50	0.50	0.50
Choline Chloride	0.50	0.50	0.40	-
L-Threonine	0.45	0.44	0.33	0.24
Salinomycin (120)	0.20	0.20	0.15	-
BHT	0.10	0.10	-	-
Avilamicyn (100)	0.05	0.05	0.05	-
Calculated chemical composition (g kg^{-1})				
Metabolizable energy (MJ)	12.46	12.77	13.08	13.40
Crude protein	242	232	209	189
Total calcium	9.71	8.78	7.58	6.34
STTD phosphorus	4.63	4.19	3.74	2.96
Sodium	2.25	2.18	2.00	1.90
Potassium	9.49	9.10	8.16	7.46
SID Lysine	13.07	12.56	11.24	10.14
Digestible methionine	6.63	6.37	5.47	4.87
Digestible Met + Cis	9.67	9.29	8.32	7.50
Digestible threonine	8.63	8.29	7.42	6.69
Digestible tryptophan	2.84	2.71	2.38	2.11
Digestible arginine	12.90	14.67	12.91	11.49
Digestible valine	10.13	9.70	8.65	7.81
Digestible isoleucine	9.51	9.08	8.03	7.19
Analyzed composition (g kg^{-1})				
Dry matter	886.2	880	888.5	896.3
Crude protein	246	221	194	174
Total ash	59.4	56.5	58.1	51.3
Crude fat	42.7	45.1	53.8	54.0

Note: [1]Pre-starter= day 1 to 7; starter= day 8 to 21; grower= day 22 to 33; finisher= day 34 to 42. [2]Content per kilogram of diet: Retinol acetate= 4.8 mg, Cholecalciferol= 200 mg, D-Alpha tocopherol= 44.7 mg, Menadione nicotinamide bisulfite= 3 mg, Thiamine= 3.6 mg, Riboflavin= 10 mg, Pyridoxine= 4.8 mg, Cyanocobalamin= 0.02 mg, Nicotinamide= 54 g, Calcium pantothenate= 18 mg, Folic acid= 1.65 mg, Biotin= 80.0 mg. [3]Content per kilogram of diet: Manganese sulfate= 70 g, Zinc sulfate= 60 g, Iron sulfate= 50 g, Copper sulfate= 8 g, Calcium iodate= 0.8 g.

were mechanically removed using a commercial plucking machine. After removing feathers, viscera, feet, and neck, the weights of the eviscerated hot carcass and organs were obtained. Afterward, the carcass was cooled in a static mixture of ice and water for 1 h and drained for 10 min. Subsequently, the carcasses were weighed again to obtain the cold carcass weight.

The hot and cold carcass yield was determined by dividing the absolute weight of the eviscerated carcass (without head, feet, and neck) by the live broiler weight. The yields of breast (*Pectoralis major*), legs, wings, and tenders (*Pectoralis minor*) were obtained by dividing the absolute weight of each variable by the cold eviscerated carcass weight. The relative weights of the liver and fat pads were calculated in relation to the live broiler weight.

Incidence and Severity of Breast Muscle (*Pectoralis major*) Myopathies

To evaluate the breast muscle (*Pectoralis major*) myopathies, analyses of the incidence and severity of Wooden Breast (WB) and White striping (WS) were performed. The WB incidence analysis was an adaptation of the methodology described by Tijare *et al.* (2016). For this purpose, the incidences were classified in four scores: normal (score 0, no areas of hardness or pallor); mild moderate (score 1, slightly affected in the cranial and/or caudal areas); moderate severe (score 2, moderately affected throughout the muscle); and severe (score 3, with superficial hemorrhage and the presence of a sterile exudate on the muscle surface).

For the incidences of WS, a methodology proposed by de Souza *et al.* (2021) was followed. The striations were classified as normal (score 0, breasts that do not present distinct white lines); moderate (score 1, small white lines, generally < 1 mm thick, but visible on the fillet surface); severe (score 2, large white lines, 1-2 mm thick, very visible on the fillet surface, covering less than 50% of the fillet); extreme (score 3, whitish streaks in parallel to the muscle fiber, > 2 mm thick, covering almost the entire fillet surface). For both WB and WS severities, mean scores were calculated for subsequent statistical analysis.

Instrumental Color (IC) Analyses

A colorimeter (CR-400, Konica Minolta Sensing, São Paulo, SP, BR) was used to measure the expression of IC in the CIELAB (L*, a*, b*) color system at 15-min and 24-h *postmortem*. The L* values indicated the degree of lightness, with higher values indicating a lighter color. The a* values represented the level of redness, with higher values indicating a redder color, while the b* values indicated the degree of yellowness, with higher values indicating a more yellow color. Before its use, the colorimeter was calibrated to black and white references. The assessments were performed at the center of each muscle section and the value was expressed as the average of three measurements. Color measurements were determined at room temperature (20-25 °C) on the surface of each muscle sample at three randomly selected locations using an illuminant and a 0° angle observer.

Water Holding Capacity (WHC), Cooking Loss (CL), and Shear Force (SF)

The left portion of the pectoralis muscle (*Pectoralis major*) was used to determine the WHC, according to the methodology proposed by Nakamura & Katoh (1981). For this purpose, two samples were cut from the portion and weighed on an analytical balance, thus determining the initial weight of the sample. Then, samples were wrapped in filter paper and placed in a centrifuge for 4 min at 2000 g and were taken to a forced-air oven prior to determine the final weight. Through the difference between the values, the water loss in the sample was determined.

To determine CL, samples from the right portion of the breast fillets were weighed, wrapped in laminated paper, and cooked in a commercial model electric plate up to 180 °C until reaching an internal temperature of 80 °C. Afterward, the samples were kept at rest until stabilized at room temperature. Then, the samples were weighed to obtain the weight after cooking (Honikel, 1998).

The cooked breast portion from the CL analysis was used to assess the shear force (SF). The breast fillet was cut to obtain four rectangular samples in the form of parallelepipeds measuring approximately 1.0 × 1.0 × 4.0 cm (length × thickness × width). These samples were evaluated in a texture analyzer (CT3 Texture Analyzer, Brookfield Engineering Laboratories, Inc., Middleboro, MA, US) using a probe (TA 3/100 and fixture TA-SBA, Brookfield Engineering Laboratories, Inc., Middleboro, MA, US). The equipment was calibrated at 0.01 kg force, 20 mm deformation, 2.5 mm/s test speed. Shear force was expressed as kilogram strength (kgf cm^{-1}), which measured the force required to cut the samples individually.

Thiobarbituric acid Reactive Substances

The evaluation of lipid peroxidation in the breast muscle was performed through the thiobarbituric acid reactive substances (TBARS) analysis at 10, 30, and 60 d of storage at -20 °C. The procedures were performed according to the methodology adapted from Sorensen & Jorgensen (1996) and Vyncke (1975). The aldehydes were extracted by homogenizing 10 mL of trichloroacetic acid (7.5%) and BHT (0.2%) solution with 2.5 g of the meat sample. After homogenization, the solution was filtered using qualitative filter paper and centrifuged at 4,000 *g* for 10 min (Centrifuge Kasvi K14-4000, Kasvi, São Paulo, BR). Posteriorly, 3 mL aliquots were added to 3 mL of thiobarbituric acid (TBA) solution, and this mixture was kept for 40 min at 80 °C in a water bath. The absorbance was measured at 538 nm in a spectrophotometer (600S, FEMTO, São Paulo, SP, BR). To determine the concentration of TBARS in the sample, a standard curve with 1,1,3,3-Tetraethoxypropane was used. The result was expressed in milligrams of malondialdehyde (MDA) per kilogram of meat.

Breast Meat (*Pectoralis major*) Composition

To determine the dry matter (DM) content (Method 934.01; AOAC, 1990), samples were weighed and then dried in an oven at 102 ± 2 °C for 16-18 hours, posteriorly were weighed again to determine the loss of moisture and the DM content.

To determine the total ash (TA) content (Method 942.05; AOAC, 2005a), samples were weighed and then incinerated in a furnace at 600 °C to remove all organic matter; afterward, samples were cooled and weighed again, obtaining the value of TA.

To determine the crude fat (CF) content (Method 954.02; AOAC, 2005b), samples were ground, weighed, rolled in paper cartridges, and then placed into a Soxhlet extractor. The samples were repeatedly washed with an organic solvent (petroleum ether), which extracts the lipids. The cartridges were removed from the Soxhlet apparatus and left to rest for one hour at room temperature. They were then transferred to an oven and heated for one hour before being weighed.

In order to determine the crude protein (CP) content (Method 981.10; AOAC, 2005b), the Kjeldahl method was followed. Samples were homogenized and weighed followed by a digestion with sulfuric acid, distillation to collect the released ammonia gas, and titration with hydrochloric acid to determine the amount of nitrogen present.

Estimation of Chromium in Feed Samples

The oven-dried samples were ignited in porcelain crucibles in a muffle furnace at 600 °C for 4 h. After cooling, the ash was treated with hydrochloric acid and heated on a hot plate until only the mineral concentrate remained, which was washed with distilled water and filtered through filter paper in a volumetric flask and to a final volume of 10 mL. Chromium was analyzed in an atomic absorption spectrophotometer (Perkin Elmer A Analyst 100, Massachusetts, Wellesley, USA) following the methodology of Silva & Queiroz (2009).

Statistical Procedures

The statistical analysis was performed using the SAS® (SAS, 2014). Data were subjected to the test of homogeneity (Levene) and normality (Shapiro-Wilk), using the UNIVARIATE procedure. The outliers were removed, and one-way analysis of variance was performed using the GLM procedure. If a statistical difference was observed, data were submitted to Tukey's test. Furthermore, polynomial and broken line regression analyses were used in isolation or combination to find the optimal inclusion point. The selection of the equation was based on the adjustment between quadratic and broken line regressions, and when they did not align, the equation with the highest R^2 value was selected as the criterion for determining the most accurate predictive equation.

Data of myopathies were grouped, and the percentage of scores within each replicate was calculated. Posteriorly, a nonparametric test (Kruskal-Wallis) was performed, using the procedure NPAR1WAY. Mean scores were compared to the Dunn test. All procedures were performed at 5% probability.

RESULTS

Chromium Analysis in the Diets

The experimental diets were analyzed for the presence of Cr. The results showed that the basal diets contained 0.02 mg kg^{-1} of Cr in the pre-starter, 0.03 mg kg^{-1} in the starter and grower, and 0.02 mg kg^{-1} in the finisher (Table 2). The total concentrations of analyzed Cr in the experimental diets were slightly higher than the calculated concentrations for the experimental treatments.

Carcass Traits and Liver and Fat Pad Relative Weights

The carcass and parts yield, as well as liver and fat pad relative weights determined in this study were not affected ($p \geq 0.168$) by the treatments (Table 3).

Incidence and Severity of Wooden Breast (WB) and White Striping (WS) Myopathies

Considering the scores of the incidence of WB and WS (Table 4), there was no effect on the WB ($p \geq 0.340$). For WS, animals that were fed 0.5 and 1.0 mg had a higher proportion of unaffected breast fillets (score 0) than treatment 0.25 mg ($p \leq 0.034$). Additionally, the breasts of animals that received 1.0 mg CrMet had the lowest incidence of score 3 ($p \leq 0.023$). There was no significant effect of CrMet ($p \geq 0.557$) on the severity of WB and WS myopathies (Table 5).

Instrumental Color (IC), Water Holding Capacity (WHC), Cooking Loss, and Shear Force (SF)

The meat quality was affected by the treatments (Table 6). Broilers that were fed 0.5 mg of CrMet kg^{-1} ration had reduced a* ($p \leq 0.004$) compared to those fed the control diet and 2.0 mg of CrMet kg^{-1} ration. According to the quadratic equation, the inclusion of 0.99 mg was predicted to optimize breast a* values. In addition, the breasts of animals that consumed 2.0 mg of CrMet kg^{-1} ration were darker ($p \leq 0.006$) than those consuming treatments 0.25 and 1.0 mg of CrMet kg^{-1} ration. The inclusion of 0.79 was estimated to optimize meat L*. No difference was observed for b* ($p \geq 0.418$), nor IC at 24-h ($p \geq 0.301$), WHC ($p \geq 0.439$), CL ($p \geq 0.253$), or SF ($p \geq 0.184$).

Thiobarbituric Acid Reactive Substances (TBARS)

Considering analysis of lipid peroxidation in the breast meat at 10, 30, and 60 days of storage (Table 7), animals receiving 0.5 mg CrMet kg^{-1} ration had lower breast MDA levels than those receiving the control and 2.0 of CrMet kg^{-1} diets ($p \leq 0.036$). Furthermore, the predicted inclusion of 0.68 mg of CrMet kg^{-1} ration was determined to reduce lipid peroxidation. No difference was observed at d 10 and 30 of storage ($p \geq 0.476$).

Proximal Composition of the Breast Muscle

The proximal composition of the breast meat of heat-stressed broilers that were fed CrMet is presented in Table 8. The CF content was lower in animals that received 1.0 mg of CrMet kg^{-1} ration than those that consumed 0.25 mg of CrMet kg^{-1} ration ($p \leq 0.202$). The data of CF did not fit the polynomial or broken line regression equations. No difference was observed for DM, TA, and CP ($p \geq 0.419$).

Table 2. Analyzed chromium concentrations in the experimental diets for broiler chickens

Treatments	Inclusions of CrMet (mg kg^{-1})	Analyzed Cr (mg kg^{-1}) - (as-fed basis)			
		Pre-starter	Starter	Grower	Finisher
Control	0.00	0.02	0.03	0.03	0.02
Cr 0.25	0.25	0.36	0.33	0.33	0.36
Cr 0.5	0.50	0.60	0.63	0.64	0.59
Cr 1.0	1.00	1.09	1.16	1.13	1.03
Cr 2.0	2.00	2.02	2.09	2.11	2.04

Note: Pre-starter: day 1 to 7; starter: day 8 to 21; grower: day 22 to 33; finisher: day 34 to 42. CrMet = Chromium-methionine.

Table 3. Chromium-methionine (CrMet) in diets of broiler chickens subjected to cyclic heat stress and its effects on carcass and parts yield and liver and fat pads relative weights at 42 days of age

Supplementation of CrMet (mg kg^{-1})	Variables							
	HCY[2]	CCY[2]	BFY[3]	LGY[3]	WNY[3]	TRY[3]	LIY[2]	FPY[2]
0	68.06	68.50	26.47	31.88	10.11	5.48	1.89	1.42
0.25	68.91	69.91	26.54	31.45	9.77	5.22	1.93	1.42
0.5	68.38	69.51	25.76	31.38	9.84	5.28	1.91	1.40
1	68.00	69.19	25.84	31.68	10.04	5.37	1.85	1.49
2	67.13	68.79	26.43	31.54	10.06	5.38	1.90	1.47
SEM	1.53	1.31	1.15	0.92	0.44	0.25	0.23	0.16
CV	2.25	1.90	4.40	2.91	4.45	4.69	16.05	8.67
p-value[1]	0.168	0.169	0.376	0.782	0.335	0.217	0.918	0.884

Note: [1] = ANOVA p-values, [2] = % in relation to the live broiler weight (without head, feet, and neck), [3] = % in relation to cold eviscerated carcass weight, HCY= hot carcass yield, CCY= cold carcass yield, BF= breast fillet yield, LGY= legs yield, WNY= wings yield, TRY= tender yield, LIY= liver relative weight, FPY= fat pads relative weight, SEM= pooled standard error of the mean, CV= coefficient of variation.

Table 4. Chromium-methionine (CrMet) in diets of broiler chickens subjected to cyclic heat stress and its effects on the scores of incidences of wooden breast and white striping myopathies in the breast muscle (*Pectoralis major*) at 42 days of age

Supplementation of CrMet (mg kg^{-1})	Wooden breast				White striping			
	Scores (%)				Scores (%)			
	0	1	2	3	0	1	2	3
0	30.0	40.0	27.5	2.5	5.0[ab]	40.0	32.5	22.5[a]
0.25	25.0	47.5	20.0	7.5	2.5[b]	27.5	47.5	22.5[a]
0.5	30.0	37.5	27.5	5.0	10.0[a]	30.0	37.5	22.5[a]
1	37.5	37.5	17.5	7.5	12.5[a]	37.5	42.5	7.5[b]
2	27.5	45.0	22.5	5.0	5.0[ab]	35.0	42.5	17.5[a]
p-value[1]	0.383	0.493	0.340	0.498	0.034	0.309	0.249	0.023

Note: [1] = Kruskal-Wallis p-values, [a-b] = means with no common superscript differ for each treatment.

Table 5. Chromium-methionine (CrMet) in diets of broiler chickens subjected to cyclic heat stress and its effects on the scores of severities of wooden breast and white striping myopathies in the breast meat (*Pectoralis major*) at 42 days of age

Supplementation of CrMet (mg kg^{-1})	Average scores	
	Wooden breast	White striping
0	1.03	1.73
0.25	1.10	1.90
0.5	1.08	1.73
1	0.95	1.56
2	1.05	1.73
SEM	0.40	0.43
CV	38.36	24.91
p-value[1]	0.932	0.557

Note: [1] = chi-squared p-values, SEM= pooled standard error of the mean, CV= coefficient of variation.

DISCUSSION

The analyses of the experimental diets showed that the chromium content in the diets was slightly higher than the calculated concentrations. This increase in chromium concentrations can be attributed to the inclusion of CrMet, which is an organic form of chromium that enhances the absorption and bioavailability of this mineral (Barzegar-Yarmohammadi *et al.*, 2020; Dalólio *et al.*, 2021). The adequate presence of chromium in the diets may contribute to the regulation of glucose and lipid metabolism, resulting in better nutrient utilization (Han *et al.*, 2021; Dalólio *et al.*, 2024). During heat stress, the efficiency of food utilization becomes critical, and chromium can help minimize the negative effects (El-Tarabany *et al.*, 2021; Aslan *et al.*, 2021; Amini *et al.*, 2023). Previous studies indicate that chromium may act as an antioxidant, reducing oxidative stress in birds (Arif *et al.*, 2019; Amini *et al.*, 2023; An *et al.*, 2023).

The absence of a significant effect of the treatments on carcass yield and the relative weights of the liver and fat suggests that the inclusion of CrMet in the diets did not impact these characteristics. This result may be attributed to the ability of chromium to improve nutrient metabolism efficiency without causing adverse changes in the birds' body composition (Lu *et al.*, 2017; Huang *et al.*, 2020; Van Hoeck *et al.*, 2020). Furthermore, the lack of variation in the relative weights of the liver and fat suggests that the birds did not experience nutritional or metabolic stress associated with the treatments (Mir *et al.*, 2018; Safwat *et al.*, 2020; Piray & Foroutanifar, 2022).

The inclusion of CrMet in the diets of broiler chickens showed significant results for the incidence of white striping myopathies. The higher proportion of unaffected breast fillets in birds receiving 0.5 and 1.0 mg of CrMet indicates that chromium may have a protective effect against the development of this condition (Amini *et al.*, 2023; Dalólio *et al.*, 2024). These results reinforce the hypothesis that chromium plays an important role in improving meat quality, possibly through the modulation of oxidative stress and the improvement of metabolic health (Sahin *et al.*, 2017; Huang *et al.*, 2020; Amini *et al.*, 2023). These results may also be attributed to the role of chromium in enhancing insulin sensitivity, which can positively influence the metabolism of birds and

Table 6. Chromium-methionine (CrMet) in diets of broiler chickens subjected to cyclic heat stress and its effects on meat quality (*Pectoralis major*) at 42 days of age

Supplementation of CrMet (mg kg^{-1})	Variables after postmortem								
	15 minutes			24 hours			24 hours		
	a*	b*	L*	a*	b*	L*	WHC	CL	SF
0	1.97[a]	1.4	47.63[ab]	4.57	3.88	47.12	72.56	31.50	3.15
0.25	1.74[ab]	1.97	49.35[a]	3.08	4.56	46.60	72.13	30.39	3.03
0.5	0.61[b]	0.73	48.96[ab]	4.01	4.34	48.17	72.46	28.89	2.68
1	1.03[ab]	1.17	50.77[a]	3.48	4.28	48.13	73.95	28.72	3.08
2	1.92[a]	0.77	43.58[b]	4.15	4.30	46.94	72.39	31.71	2.49
SEM	0.90	1.71	4.19	1.55	1.41	3.72	2.22	3.71	0.71
CV (%)	62.88	49.14	8.73	40.22	30.25	7.84	3.06	12.27	24.68
p-value	0.004[(Q)]	0.418	0.006[(Q)]	0.301	0.886	0.826	0.439	0.253	0.184
Polynomial regression equations							R^2	CrMet	Response
[(Q)]a*15min = 1.964768 – 0.00234 * CrMet + 0.00000117 * CrMet^2							0.75	0.99	0.79
[(Q)]L*15min = 47.47531 + 0.00719 * CrMet – 0.00000455 * CrMet^2							0.95	0.79	50.31

Note: [1] = ANOVA p-values, a* = redness, b* = yellowness, L* = lightness, WHC = water holding capacity %, CL = cooking loss %, SF = shear force, kgf cm^{-1}. [a-b] = means with no common superscript differ for each treatment, [Q] = quadratic, SEM = pooled standard error of the mean, CV= coefficient of variation.

Table 7. Thiobarbituric acid reactive substances (TBARS, MDA kg^{-1} of meat) in the breast muscle of broiler chickens subjected to diets with chromium-methionine (CrMet) and cyclic heat stress during different days of storage

Supplementation of CrMet (mg kg^{-1})	Days of storage		
	10	30	60
0	0.17	0.26	0.60[a]
0.25	0.15	0.23	0.54[ab]
0.5	0.12	0.20	0.34[b]
1	0.11	0.17	0.56[ab]
2	0.13	0.29	0.71[a]
SEM	0.01	0.01	0.02
CV (%)	76.82	52.4	41.77
p-value[1]	0.842	0.476	0.036[(Q)]
Polynomial regression equation	R^2	CrMet	Response
[(Q)]TBd60 = 0.000007145 * CrMet + 0.0000000521317 * CrMet^2	0.58	0.68	0.038

Note: [1] = ANOVA p-values, [a-b] = means with no common superscript differ for each treatment, [Q] = quadratic, SEM = pooled standard error of the mean, CV = coefficient of variation.

Table 8. Proximate composition of breast meat (*Pectoralis major*) in broiler chickens at 42 days of age fed chromium-methionine (CrMet) and subjected to cyclic heat stress

Supplementation of CrMet (mg kg^{-1})	Breast composition (%)			
	DM	TA[2]	CF[2]	CP[2]
0	25.73	1.56	1.37[ab]	23.80
0.25	25.92	1.70	2.12[a]	23.20
0.5	26.03	1.77	1.71[ab]	23.60
1	25.13	1.93	1.08[b]	23.80
2	25.29	1.98	1.65[ab]	23.50
SEM	0.94	0.48	0.44	1.10
CV (%)	3.66	25.38	29.03	3.65
p-value[1]	0.492	0.644	0.022	0.891

Note: [1] = ANOVA p-values, [2] = expressed as fed-basis, DM = dry matter, TA = total ash, CF = crude fat, CP = crude protein, [a-b] = means with no common superscript differ for each treatment, SEM = pooled standard error of the mean, CV = coefficient of variation.

reduce the negative impacts associated with stress (Lu *et al.*, 2017; Sahin *et al.*, 2018; Saftwar *et al.*, 2020; Saracila *et al.*, 2022). This is relevant in production systems where environmental stress can increase the occurrence of myopathies (Huang *et al.*, 2016; Fraz *et al.*, 2023).

The inclusion of CrMet in the diets of broiler chickens demonstrated a significant effect on meat quality, especially regarding coloration. The reduction in a* values in birds fed with 0.5 mg of CrMet per kilogram diet indicates a decrease in the intensity of red coloration, suggesting that this dose may optimize the desired appearance of the meat (Dalólio *et al.*, 2021). The prediction that 0.99 mg is the ideal inclusion to maximize a* value highlights the importance of adjusting chromium concentrations to achieve specific visual characteristics (Untea *et al.*, 2019; Untea *et al.*, 2021; Dalólio *et al.*, 2021).

The darker coloration observed in the breasts of birds consuming 2.0 mg of CrMet, compared to the treatments of 0.25 and 1.0 mg, may be related to physiological changes in the pigment deposition processes in the meat (Xiao *et al.*, 2017; Haq *et al.*, 2018). High concentrations of chromium may influence the activity of enzymes involved in melanin synthesis or in the oxidation of compounds affecting meat color (Untea *et al.*, 2019; Hoseini *et al.*, 2020; Dalólio *et al.*, 2021).

Moreover, the elevated presence of chromium may affect iron metabolism, which is an essential component in the formation of myoglobin, the protein responsible for meat color (Untea *et al.*, 2019; Dalólio *et al.*, 2021; Zhang *et al.*, 2024). An increase in chromium concentration could potentially alter the bioavailability of iron or modify the expression of proteins involved in iron transport and storage, resulting in greater deposition of dark pigments in the meat (Safwat *et al.*, 2020; Kazakova & Marshinskaia, 2023). These color changes may also be attributed to oxidative stress induced by high doses of chromium, which could affect cellular integrity and muscle characteristics, influencing meat appearance (Safwat *et al.*, 2020; Dalólio *et al.*, 2021).

The inclusion of 0.79 mg of CrMet may optimize meat luminosity, indicating a direct relationship between chromium concentration and the capacity to reflect light in the meat (Nadaf Fahmideh *et al.*, 2023). Physiologically, this may be related to chromium's effect on the structure of muscle fibers and myoglobin formation, which can influence how light is absorbed and reflected (Untea *et al.*, 2019; Mazhari & Ranjbari-nasab, 2022).

The absence of significant differences in b* values suggests that the yellow coloration of the meat was not altered by the different chromium inclusions. This implies that the inclusion of CrMet did not interfere with the biochemical processes responsible for yellow pigment formation, maintaining uniformity in meat quality (Lu *et al.*, 2017; Shakeri *et al.*, 2019). This stability in yellow coloration may be beneficial for the market, as it ensures a consistent appearance of the product (Kuttappan *et al.*, 2021).

These results emphasize the importance of optimizing diet formulations to achieve the desired characteristics in meat, such as luminosity, without compromising overall coloration (Han *et al.*, 2021; Youssef *et al.*, 2022; Dalólio *et al.*, 2024). The ability of chromium to influence luminosity without affecting the other color characteristics demonstrates its potential as an effective dietary supplement for improving the quality of broiler chicken meat (Hoseini *et al.*, 2020; Zhang *et al.*, 2024).

The maintenance of water-holding capacity, cooking loss, and shear force without significant changes indicates that the inclusion of CrMet did not compromise the physical properties of the meat (An *et al.*, 2023; Zhang *et al.*, 2024). Physiologically, this may be attributed to chromium's interaction with muscle proteins and its ability to stabilize the protein matrix of the meat (Dalólio *et al.*, 2021). Water retention is closely related to the structure of muscle proteins, which form a network capable of trapping water (Fathi *et al.*, 2023). Additionally, the stability of shear force suggests that the inclusion of chromium did not affect the integrity of muscle fibers (An *et al.*, 2023). These results are significant, as they indicate that CrMet supplementation can be implemented in broiler chicken diets without compromising the sensory quality of the meat (Untea *et al.*, 2019; Dalólio *et al.*, 2021).

The analysis of thiobarbituric acid reactive substances (TBARS) suggests that the inclusion of CrMet in the diets had a beneficial effect on the lipid oxidation of breast meat (Dalólio *et al.*, 2024). The observation of lower malondialdehyde (MDA) levels in birds receiving 0.5 mg of CrMet per kilogram ration indicates that this dose may promote the antioxidant action of chromium (Dalólio *et al.*, 2021; Fathi *et al.*, 2023).

Physiologically, chromium's effectiveness in reducing lipid peroxidation may be related to its ability to stabilize cell membranes and modulate oxidative processes (Han *et al.*, 2021; Kazakova & Marshinskaia, 2023). Chromium may interact with enzymatic systems that protect cells from oxidative damage, resulting in less degradation of the lipids present in the meat (Untea *et al.*, 2021). This action is essential for preserving meat quality, as lipid oxidation can lead to the formation of undesirable compounds that affect flavor and texture (Khalifah *et al.*, 2021). Furthermore, reducing lipid peroxidation is crucial for extending the shelf life of meat during storage, ensuring that its sensory and nutritional properties are maintained (Zhang *et al.*, 2024; Waleed *et al.*, 2024).

The prediction that 0.68 mg of CrMet per kilogram ration is the ideal inclusion to minimize lipid peroxidation highlights the importance of finding the correct dosage to maximize chromium's antioxidant benefits. This ability to reduce lipid oxidation is particularly relevant, as peroxidation can negatively impact the sensory quality and food safety of meat (Youssef *et al.*, 2022; Dalólio *et al.*, 2024).

The inclusion of CrMet in broiler chicken diets affected the proximal composition of breast meat, particularly with a reduction in crude fat content in animals receiving 1.0 mg of CrMet compared to those consuming 0.25 mg. Physiologically, the reduction in fat content may be related to chromium's effect on regulating lipid metabolism and insulin sensitivity (Barzegar-Yarmohammadi *et al.*, 2020; Dalólio *et al.*, 2024). Chromium helps improve glucose uptake by cells, resulting in greater efficiency in converting nutrients into lean mass instead of fat storage (Lu *et al.*, 2017; Aslam *et al.*, 2021).

The maintenance of dry matter, total ash, and crude protein levels without significant differences indicates that the inclusion of CrMet did not negatively impact meat quality (Kazakova & Marshinskaia, 2023). This result suggests that while chromium supplementation may lead to a reduction in fat content, it does not compromise the other essential components of meat, such as protein and minerals (Safwat *et al.*, 2020; Youssef *et al.*, 2022; Kazakova & Marshinskaia, 2023). Physiologically, this may be attributed to chromium's ability to positively influence nutrient metabolism without causing imbalances in the nutritional composition of the meat (Piray & Foroutanifar, 2022). Preserving these parameters is crucial, ensuring that meat retains its nutritional value and functional properties (Barzegar-Yarmohammadi *et al.*, 2020; Dalólio *et al.*, 2021).

CONCLUSION

Chromium-methionine supplementation at doses of 0.50 and 1.0 mg kg^{-1} ration improved meat quality and reduced woody breast in broiler chickens. These results demonstrate the potential of chromium-methionine as a nutritional strategy to enhance muscle integrity and reduce the occurrence of myopathies in intensive broiler production systems.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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