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Meat quality of chickens subjected to cyclic heat stress while supplemented with ZINC-L-SELENOMETHIONINE

Qualidade da carne de frangos submetidos ao estresse térmico cíclico suplementados com ZINCO-L-SELENOMETIONINA

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Highlights

CZinc-L-Selenomethionine (Zn-L-SeMet) is a new organic source of selenium.
The inclusion of Zn-L-SeMet did not affect carcass, liver, or fat characteristics.
The inclusion of 1.30 mg of Zn-L-SeMet leads to better scores of 0 for wooden breast.
Zn-L-SeMet supplementation reduces the incidence of white striping score 3.

Abstract

A significant additional benefit of incorporating organic forms of selenium into poultry diets is the substantial improvement in the nutritional quality of the meat. This study aimed to investigate gradual inclusions of Zinc-L-Selenomethionine in the diet of broiler chickens from one to 42 days of age, reared under cyclic heat stress conditions, and its effects on carcass traits, breast myopathies, quality, and composition. A total of 1,000 one-day-old male Cobb 500® broiler chicks were randomly assigned to five

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treatments: 0, 0.15, 0.23, 0.47, and 1.30 mg of Zn-L-SeMet per kg of feed, with each group consisting of ten replicates of 20 birds. The carcass traits were unaffected ($p \geq 0.102$). All scores of breast myopathy incidence (from 0 to 3) were affected by the treatments ($p < 0.05$). For wooden breast, the inclusion of 1.30 mg provided breasts with a lower incidence. Breasts from the 0.15 mg treatment had a lower incidence within the severe and extreme White striping scores. The treatments had no significant effects on the breast meat quality ($p \geq 0.180$), lipid peroxidation ($p \geq 0.172$), or composition ($p \geq 0.383$). Including 1.30 mg of Zn-L-SeMet for broiler chickens results in better scores of 0 for wooden breast. For white striping, supplementation from 0.15 mg of Zn-L-SeMet reduces the incidence of score 3.
Key words: Antioxidant. Carcass. Growth performance. Nutrition. Organic selenium. Poultry.

Resumo

Um benefício adicional significativo da incorporação de formas orgânicas de selênio nas dietas de aves é a considerável melhoria na qualidade nutricional da carne. O objetivo deste estudo foi investigar as inclusões graduais de Zinco-L-Selenometionina (Zn-L-SeMet) nas dietas de frangos de corte de 1 a 42 dias de idade, criados sob condições de estresse térmico cíclico (ETC), e seus efeitos nas características da carcaça, miopatias do peito, qualidade e composição. Um total de 1.000 pintos machos Cobb 500® de um dia foram distribuídos aleatoriamente em cinco tratamentos: 0, 0,15, 0,23, 0,47 e 1,30 mg de Zn-L-SeMet por kg de ração; cada grupo consistiu em 10 repetições de 20 aves. As características da carcaça não foram afetadas ($p \geq 0{,}102$). Todos os escores de incidência de miopatia do peito (de 0 a 3) foram afetados pelos tratamentos ($p < 0{,}05$). A inclusão de 1,30 proporcionou menor incidência de peitos de madeira (PM). Peitos do tratamento 0,15 tiveram menor incidência nos escores graves e extremos de Listras Brancas. Não houve efeitos significativos dos tratamentos na qualidade da carne do peito ($p \geq 0{,}180$), peroxidação lipídica ($p \geq 0{,}172$) e composição ($p \geq 0{,}383$). A inclusão de 1,30 mg de Zn-L-SeMet para frangos de corte proporciona melhores escores de 0 para Peito de Madeira. Para Listras Brancas, a suplementação a partir de 0,15 mg de Zn-L-SeMet reduz a incidência do escore 3.
Palavras-chave: Antioxidante. Carcaça. Desempenho de crescimento. Nutrição. Selênio orgânico. Aves.

Introduction

Animals are sensitive organisms that face environmental stress due to global warming. High environmental temperatures have serious effects on the production performance of animals raised for protein. Poultry farming is one of the largest industries that are negatively affected by increasing environmental temperatures (Goel et al., 2021). Birds are the most susceptible to heat stress among farmed animals due to their limited ability to dissipate heat, arising from having few sweat glands and bodies covered in feathers (Goo et al., 2019).

Moreover, recent intensive farming methods, while enhancing bird performance, also increase heat production, thus decreasing the capacity of these animals for thermoregulation (He et al., 2018). Birds exposed to heat stress (HS) exhibit several physiological and behavioral problems, significantly reducing their productive performance, health, and welfare (Kang et al., 2020). It has been reported that HS can also lead to undesirable changes in carcass traits (Emami et al., 2021), meat composition (Lu et al., 2007), and meat quality characteristics, such as lower pH, reduced water-holding capacity, and impaired meat color (Ma et al., 2015).

Additionally, high temperature conditions can increase lipid oxidation, a critical factor impacting the quality of poultry meat products (Habibian et al., 2016). Heat stress may also lead to an increase in the production of reactive oxygen species (ROS) within cells (Miao et al., 2020), causing an imbalance between ROS production and the antioxidant defense system. This results in cellular oxidative stress (Ngoula et al., 2020), damaging cell components including proteins, lipids, and DNA (Wasti et al., 2021). Therefore, developing effective strategies to reduce HS in birds is essential for the current poultry industry.

Dietary adjustments, such as modified energy and nutrient concentrations, may be a potential solution to problems associated with HS in birds. A practical and appropriate approach is functional nutrient supplementation, which plays an important role in mitigating the physiological and metabolic disturbances caused by HS (D. Y. Kim et al., 2021).

Selenium (Se) is an essential element in the human and animal diet, as it is part of at least 25 proteins that play a crucial role in regulating homeostasis (Hariharan & Dharmaraj, 2020). Zinc-L-Selenomethionine (Zn-L-SeMet), a new organic source of Se formed by a complex of essential metals and amino acids (Chaosap et al., 2020), provides significant benefits. Incorporating organic forms of Se into poultry diets considerably improves the nutritional quality of the meat (Khan et al., 2018), enhancing chicken meat production and increasing carcass quality, thereby reducing the incidence of wood breast (Zheng et al., 2022).

The purpose of this study was to investigate the effects of gradual inclusions of Zinc-L-Selenomethionine (Zn-L-SeMet) in broiler chicken diets from one to 42 days of age, reared under cyclic heat stress conditions, on carcass traits, breast myopathies, quality, and composition.

Material and Methods

Animal welfare and health

This experiment was conducted at the Poultry Research Center of the Universidade Estadual do Oeste do Paraná (Unioeste) in Marechal Cândido Rondon, PR, Brazil. Careful handling of the experimental animals was ensured to minimize any unnecessary discomfort. All procedures followed the guidelines set by the Animal Use Ethics Committee of Unioeste and complied with Normative Act No. 37, dated February 15, 2018, from the National Committee for the Control of Experimental Animals (CONCEA), which outlines the Euthanasia Practice Guidelines.

Experimental design, diets, and facilities

One thousand one-day-old male Cobb 500® broiler chicks, with an initial average weight of 49.23 ± 0.60 g, were obtained from a commercial hatchery. These chicks were randomly assigned to five different diets, each replicated in 10 replicates of 20 chicks per replicate. The control group was fed a mash basal diet formulated to meet the nutritional requirements of broiler chickens, except for Se, based on the recommendations by Rostagno et al. (2017). This diet (Table 1) was administered in four phases: pre-starter (days one to seven), starter (days eight to 21), grower (days 22 to 33), and finisher (days 34 to 42). The experimental groups received the basal diet supplemented with Zinc-L-Selenomethionine (Zn-L-SeMet) at concentrations of 0.00, 0.15, 0.23, 0.47, and 1.30 mg Zn-L-SeMet/kg, replacing an equivalent amount (g g^{-1}) of inert material (kaolin). Throughout the experimental period, the chicks had *ad libitum* access to feed and water.

Table 1
Proximate composition, calculated and analyzed values of basal diets: Pre-starter (days 1 to 7), starter (days 8 to 21), grower (days 22 to 33), and finisher (days 34 to 42)

Ingredient g/kg	Pre-starter	Starter	Grower	Finisher
Corn (78.8 g/kg/CP)	509.1	528.0	585.9	640.0
Soybean meal (460 g/kg/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 premix1	0.50	0.50	0.50	0.50
Mineral premix2	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				
Met. En. (MJ)	12.46	12.77	13.08	13.40
Crude protein	242	232	209	189
Calcium	9.71	8.78	7.58	6.34
Total phosphorus	7.18	6.68	6.10	5.22
Available phosphorus	4.63	4.19	3.74	2.96
Sodium	2.25	2.18	2.00	1.90

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Potassium	9.49	9.10	8.16	7.46
Digestible 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				
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

[1]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)
[2]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).

The birds were housed in concrete floor pens of 1.96 m² (experimental units), each lined with fresh wood shavings and equipped with a semiautomatic feeder and a nipple drink line. The experimental barn stove exhaust fans, air inlets, evaporative cooling systems (EVAPs), and a commercial pellet stove for heating. The lighting program adhered to the guidelines provided in the breeder strain recommendations manual.

Heat stress management

The birds were raised under comfortable temperature conditions following the guidelines specified in the breeder's manual throughout the experiment. However, from 22 to 42 days of age, between 9:00 and 15:00, they were subjected to heat stress.

To induce stressful conditions, manual adjustments were made to the control panel to modify the temperature and relative humidity. This process involved activating the pellet combustion stove to heat the house and the evaporative cooling cells to increase humidity when necessary. During this stress period, the temperature was maintained at 31 °C with a variation of ±1.5 °C, and the relative humidity was kept at 65%, ranging from 55% to 75%.

The average temperature and relative humidity recorded during the heat stress period were 31.1 °C and 60.2%, respectively. Initially, the temperature at placement was 32 °C and was gradually reduced to 21 °C to provide a comfortable environment for the birds. Nonetheless, in the final week of the experiment, the lowest recorded temperature reached 24 °C.

Regarding lighting conditions, the birds were exposed to 24 h of light on their first day. Subsequently, the light/dark cycles were adjusted to 23 h of light and 1 h of darkness until the third day; 21 h of light and 3 h of darkness until the eighth day; and 18 h of light and 6 h of darkness until they reached 42 days of age. Throughout the entire experimental period, the light intensity was consistently maintained at 20 lux.

The environmental conditions were monitored by a control panel (SMAAI 4, InoBram Automations, Pato Branco, PR, Brazil), which recorded the environmental parameters at five-minute intervals.

Carcass traits and liver and fat pad relative weights

After 6 h of fasting, four broilers per replicate were randomly selected, weighed, euthanized, and slaughtered at 42 days of age. The animals were stunned by electronarcosis and bled through ventral neck cutting. The carcasses, without heads, were scalded, and feathers were mechanically removed using a commercial plucking machine. The feet were removed, and visceral organs were detached to obtain the eviscerated hot carcass, including the weights of the fat pad and liver.

Subsequently, the carcasses were cooled in a static mixture of ice and water for one hour and drained for 10 min. The carcasses were then weighed again to obtain the cold carcass weight.

Carcass yield was determined by dividing the weight of the eviscerated carcass by the initial weight of the live broiler. To calculate the yields of the breast (*pectoralis major*), legs, wings, and tenders, their weights were divided by the weight of the cold eviscerated carcass. The weights of the liver and fat pad were determined by calculating their relative weights based on the initial weight of the live broiler.

Wooden breast (WB) and white striping (WS) myopathies in the breast muscle (pectoralis major)

The incidence of wooden breast (WB) and white striping (WS) was evaluated in 200 broilers (four broilers per pen, selected for the determination of carcass traits and fat pad and relative weights). To assess the incidence of WB, an analysis adapted from the methodology described by Tijare et al. (2016) was performed. The incidences were categorized using a four-point scoring system: 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 sterile exudate on the muscle surface).

The incidences of WS were assessed according to the methodology proposed by Souza et al. (2021), with the following criteria: normal (score 0, breasts that do not exhibit 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 parallel to the muscle fiber, > 2 mm thick, covering almost the entire fillet surface).

Breast meat quality assessments

At 42 days old, one broiler per pen had its right and left *pectoralis major* muscles analyzed to measure the quality of meat using instrumental color (IC), water holding capacity (WHC), cooking loss (CL), and shear force (SF) parameters.

Instrumental color was assessed using a portable colorimeter (CR-400, Konica Minolta Sensing, São Paulo, SP, BR) directly on the right portion of the pectoralis major at 15 min and 24 h *postmortem.* The color was expressed using the CIE L* a* b* system profile, which includes lightness (L*), redness (a*), and yellowness (b*).

To determine the water holding capacity (WHC), two samples were cut from each left portion of the *pectoralis major,* weighed, wrapped in filter paper, and centrifuged for four minutes at 2000 rpm. They were then placed in a forced-air oven. The water loss in the sample was determined by the difference between the initial and final weights (Nakamura & Katoh, 1981).

The determination of cooking loss (CL) was performed using the left breast fillet following the method described by Honikel (1998). The breast fillets were weighed, wrapped in laminated paper, and cooked on a commercial electric plate heated up to 180 °C until reaching an internal temperature of 80 °C. The samples were then allowed to rest until they stabilized at room temperature, and weighed again to determine the weight after cooking.

The cooked portion from the CL analysis was used to assess the shear force (SF). Three rectangular samples, each measuring approximately 1.0 × 1.0 × 4.0 cm (length × thickness × width), were taken from each piece and evaluated in a texture analyzer (CT3 Texture Analyzer, Brookfield Engineering Laboratories, Inc., Middleboro, MA, US). The analysis was conducted using a probe (TA 3/100 and TA-SBA fixture, Brookfield Engineering Laboratories, Inc., Middleboro, MA, US). The equipment was calibrated to apply a force of 0.01 kg, with a deformation of 20 mm and a test speed of 2.5 mm/s. SF was expressed in kilogram-force per centimeter (kgf cm^{-1}), which measured the force required to cut through the samples individually.

Lipid peroxidation in the breast muscle (pectoralis major)

The lipid peroxidation of the breast muscle, stored at -20 °C for 10, 30, and 60 days, was determined based on the analysis of thiobarbituric acid reactive substances (TBARS), which measure the level of malondialdehyde (MDA) per kilogram of sample. The procedures were adapted from the methodologies described by Sorensen and Jorgensen (1996) and Vyncke (1975).

A total of 2.5 g of ground meat samples were homogenized with 10 ml of a trichloroacetic acid (7.5%) and BHT (0.2%) solution. The homogenate was filtered through qualitative filter paper directly into a 100 ml conical flask. Subsequently, 3-ml aliquots of the homogenate were combined with 3 ml of thiobarbituric acid (TBA) solution and heated at 80 °C for 40 min in a water bath. After cooling to room temperature, the absorbance was measured at 538 nm using a spectrophotometer (600S, FEMTO, São Paulo, BR). The concentration of MDA in the samples was assessed using a standard curve prepared with 1,1,3,3-Tetraethoxypropane.

Determination of breast meat (pectoralis major) proximate composition

Breast muscle samples from each treatment were collected for proximate composition analysis. All exterior fat and connective tissue were removed, and the samples were ground before analysis, which was performed in duplicates.

Dry matter (DM) was determined by weighing the samples, drying them in an oven at 102 ± 2 °C for 16-18 h, and re-weighing to calculate moisture loss and DM content through Method 934.01 (Association of Official Analytical Chemists [AOAC], 1990).

Total ash (TA) content was measured by weighing samples, incinerating them in a furnace at 600 °C to remove all organic matter, and then re-weighing to determine TA using Method 942.05 (AOAC, 2005).

Crude fat (CF) content was assessed using Method 954.02 (AOAC, 1990). Samples were ground, weighed, and wrapped in paper cartridges, then placed in a Soxhlet extractor. The samples were washed repeatedly with petroleum ether to extract the lipids, cooled, and reweighed to determine the weight without fat.

To determine the crude protein (CP) content, the Kjeldahl method (AOAC, 2005) was employed. Samples were homogenized, weighed, and digested with sulfuric acid. The released ammonia gas was collected through distillation and titrated with hydrochloric acid to determine the nitrogen present.

Estimation of selenium in feed and breast meat (pectoralis major) samples

The concentration of Se in feed (Table 2) and meat samples was evaluated using atomic absorption spectroscopy. The samples were incinerated in a furnace at 600 °C to remove all organic matter, yielding TA. After cooling, the samples were digested in a solution of hydrochloric acid and distilled water until only the mineral solution remained. This solution was then filtered using paper filters into flasks to obtain a volume of approximately 10 ml, which was stored for further analysis. The sample solution was aspirated into an atomic absorption spectrophotometer (iCE 3000 Series AAS, Thermo Scientific, Waltham, Massachusetts, United States of America). The spectrophotometer measured the amount of light absorbed by Se atoms in the sample, and the concentration of Se was calculated based on the absorption reading and a calibration curve obtained from standard solutions.

Table 2
Analyzed selenium (Se) concentrations in experimental diets: Pre-starter (days 1 to 7), starter (days 8 to 21), grower (days 22 to 33), and finisher (days 34 to 42)

Inclusions of Zn-L-SeMet, mg/kg	Analyzed Se, mg/kg (as-fed basis)			
	Pre-starter	Starter	Grower	Finisher
0.00	0.05	0.06	0.05	0.06
0.15	0.17	0.18	0.18	0.19
0.23	0.28	0.28	0.27	0.27
0.47	0.57	0.54	0.57	0.52
1.30	1.33	1.24	1.35	1.35

Statistical analysis

The Univariate procedure was used to test the data for homogeneity (Levene) and normality (Shapiro-Wilk). After the removal of outliers, the General Linear Model (GLM) procedure was applied to perform a one-way ANOVA to test for variance among the data. If a statistically significant difference was found, Tukey's test and polynomial and broken line regression analyses were conducted. The equations polynomial, broken line, or a combination of both were used individually or together to determine the optimal inclusion point. In cases where results did not align, the equation with the highest R^2 value was selected as the criterion for choosing the most accurate predictive equation.

Data on the severity and incidence of myopathies were grouped and then the percentage of scores within each replicate was calculated. Subsequently, a nonparametric test (Kruskal-Wallis) was performed using the procedure NPAR1WAY. Mean scores were compared using the Dunn test. All procedures were performed using the Statistical Analysis System Software – SAS (OnDemand Edition for academics) at a 5% probability level.

Results and Discussion

The yields of the carcass and its parts (table 3), as well as the relative weights of the liver and abdominal fat of chickens fed Zn-L-SeMet and subjected to heat stress, were not affected ($p \geq 0.102$).

Table 3

Zinc-L-Selenomethionine (Zn-L-SeMet) in diets of broiler chickens subjected to cyclic heat stress and its effects on carcass and parts yield and liver and fat pad relative weights at 42 days of age

Zn-L-SeMet mg/kg	HCY[2]	CCY[2]	BFY[3]	LGY[3]	WNY[3]	TRY[3]	LIY[2]	FPY[2]
0.00	67.60	68.50	26.72	31.88	10.11	5.48	1.89	1.50
0.15	68.07	69.08	26.22	31.98	9.97	5.29	1.86	1.54
0.23	67.82	68.89	27.08	31.18	9.89	5.32	1.92	1.45
0.47	68.47	69.27	26.13	31.29	10.20	5.41	1.84	1.37
1.30	68.13	68.95	26.69	31.04	10.20	5.33	1.91	1.38
SEM	1.19	1.44	0.71	1.87	0.51	0.24	0.13	0.19
CV	1.75	2.09	2.69	5.95	5.05	4.49	6.78	13.14
p-value[1]	0.647	0.851	0.102	0.759	0.565	0.420	0.695	0.246

[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.

In this study, birds were subjected to heat stress with temperatures maintained at 31°C. Ideal growth conditions for poultry typically range between 18–21 °C (Kumari & Nath, 2018). At higher temperatures, birds lose the ability to regulate their own body temperature, resulting in poor performance and adversely affecting carcass characteristics and meat quality (Teyssier et al., 2022). Selenium is known to improve food utilization by regulating the metabolism of carbohydrates, lipids, and proteins (Stapleton, 2000). Consequently, it enhances growth performance and mitigates the adverse effects of heat stress on skeletal muscle (Abdel-Moneim et al., 2022). However, although Se reduces the negative impacts of heat stress (Sahin et al., 2008; Krstić et al., 2012), Zinc-L-Selenomethionine supplementation did not affect any of the components of the carcass, liver, or abdominal fat.

The findings of this experiment align with those of Bakhshalinejad et al. (2019), who evaluated several sources of Se and reported no significant effects on carcass characteristics. Furthermore, other studies have suggested that the addition of Se, whether alone or in combination with vitamins E or C, did not lead to significant changes in carcass traits (Habibian et al., 2016; Leskovec et al., 2019).

In this study, concerning WB (Table 4), there was a higher frequency of normal breasts (score 0) in birds that received 1.30 mg of Zn-L-SeMet compared to those treated with 0.15, 0.47 mg, and the control (p = 0.025). For breasts with a mild to moderate incidence (score 1), birds supplemented with 1.30 mg/

kg of Zn-L-SeMet exhibited a lower incidence, whereas those supplemented with 0.47 mg/kg showed a higher incidence. For the other treatments, the means were the same (p = 0.037). Regarding breasts with a moderate to severe incidence (score 2), the control group exhibited the highest percentage of scores, while the group supplemented with 0.23 mg/kg had the lowest. In the other treatments, the scores were equal (p = 0.030). For breasts with severe conditions (score 3), birds treated with 0.23 mg/kg exhibited a higher incidence, while the control group and those receiving the highest level of supplementation had a lower and equal incidence. The means were equal for the other treatments (p = 0.035).

For WS incidences (Table 04), the breasts of birds receiving 0.23 and 1.30 mg of Zn-L-SeMet showed a higher proportion of normal breasts (score 0) compared to those receiving 0.15 mg and the control treatment (p = 0.008), as well as a lower proportion of breasts with moderate indices (score 1) compared to the same treatments (p = 0.004). Considering breasts with severe conditions (score 2), those from birds treated with 0.23 and 0.47 mg of Zn-L-SeMet showed a higher incidence than the control (p = 0.013). Regarding breasts with extreme conditions (score 3), birds that received 0.23 and 0.47 mg of Zn-L-SeMet exhibited a lower incidence compared to the control treatment (p = 0.003).

Table 4
Zinc-L-Selenomethionine (Zn-L-SeMet) in diets of broiler chickens subjected to cyclic heat stress and its effects on the scores of incidence of Wooden Breast and White Striping myopathies in the breast muscle (*Pectoralis major*) at 42 days of age

Zn-L-SeMet mg/kg	Wooden Breast Scores (%)				White Striping Scores (%)			
	0	1	2	3	0	1	2	3
0.00	30.0^{b}	40.0^{ab}	27.5^{a}	2.50^{b}	5.00^{b}	40.0^{a}	32.5^{b}	22.5^{a}
0.15	32.50^{b}	40.0^{ab}	20.0^{ab}	7.50^{ab}	5.00^{b}	45.0^{a}	37.5^{ab}	12.5^{ab}
0.23	42.5^{ab}	37.5^{ab}	10.0^{b}	10.0^{a}	15.0^{a}	22.5^{b}	52.5^{a}	10.0^{b}
0.47	30.0^{b}	47.5^{a}	17.5^{ab}	5.00^{ab}	12.5^{ab}	32.5^{ab}	50.0^{a}	5.0^{b}
1.30	47.5^{a}	27.5^{b}	22.5^{ab}	2.50^{b}	17.5^{a}	27.5^{b}	37.5^{ab}	17.5^{ab}
p-value[1]	0.025	0.037	0.030	0.035	0.008	0.004	0.013	0.003

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

Myopathies lead to significant economic losses due to carcass condemnation and consumer rejection (Bailey et al., 2015). Breasts with a "woody" condition have hardened areas on the surface that are pale and protrude, sometimes covered by a slightly cloudy viscous fluid and multifocal petechiae (Mudalal et al., 2015). White striping involves the appearance of visible white lines that run parallel to the breast muscle fibers, with varying proportions and thickness (Kuttappan et al., 2012).

Although the etiology of WS has not been fully elucidated, it is believed to be caused by the insufficiency of the vasculature to meet the needs of the breast muscle (Ahsan & Cengiz, 2020). Subsequently, localized hypoxia occurs, leading to the degradation of muscle fibers. This creates an imbalance between the degradation and regeneration of fibers, which are gradually replaced by fat deposits, manifesting as WS in the *pectoralis major* muscle (Aslam et al., 2021). In this study, all treatments were affected by heat stress, showing that stress influences both wooden breast and white striping. Chronic conditions of hypoxia related to heat stress cause severe oxidative damage to lipids, mitochondrial proteins, and the cell membrane, thus exacerbating the severity of WS in broilers subjected to heat stress (Mujahid et al., 2007; Petracci et al., 2019).

Studies have reported that the inclusion of selenium in broiler diets can reduce the incidence and severity of pectoral myopathies (Mudalal et al., 2015; Sihvo et al., 2014). In the present study, all WB and WS scores were influenced by the treatments.

However, the current study did not observe any noticeable impact of Se inclusions on meat quality, suggesting that inclusions of up to 1.30 mg of Se in the diet did not influence meat quality (table 5).

Table 5
Zinc-L-Selenomethionine (Zn-L-SeMet) in diets of broiler chickens subjected to cyclic heat stress and its effects on breast meat (*Pectoralis major*) quality at 42 days of age

	15 min			24 h					
Zn-L-SeMet (mg/kg)	a*	b*	L*	a*	b*	L*	WHC	CL	SF
0.00	1.97	1.40	47.63	4.87	3.88	47.12	72.56	30.53	3.15
0.15	1.83	0.27	43.86	3.72	3.00	45.79	71.97	29.90	2.99
0.23	1.56	0.43	45.79	3.38	3.47	47.58	72.81	28.86	3.13
0.47	1.63	0.47	44.40	3.14	3.71	47.02	72.84	30.44	3.33
1.30	1.97	1.74	46.28	3.78	3.03	45.03	72.36	29.33	2.73
SEM	1.26	1.95	4.98	1.73	1.25	3.67	1.89	3.42	0.84
CV (%)	70.39	226.31	10.93	45.69	36.55	7.90	2.60	11.50	24.68
p-value[1]	0.922	0.345	0.464	0.240	0.427	0.514	0.834	0.815	0.180

[1]: ANOVA P-values
a*: redness; b*: yellowness; L*: lightness
WHC: water holding capacity (%); CL: cooking loss (%); SF: shear force (kgf/cm)
SEM: pooled standard error of the mean
CV: coefficient of variation.

This outcome is consistent with the findings of Silva et al. (2022), who also reported no effects on meat quality when sodium selenite and selenium yeast were added to broiler diets. Conversely, Juniper et al. (2011) noted that while SeMet supplementation increased Se and GPx activity in both blood and breast meat samples, it did not result in any significant improvement in meat quality. Nonetheless, some researchers, including J. L. Li et al. (2018), Rajashree et al. (2014), and Silva et al. (2019), reported positive effects on meat quality when diets were supplemented with SSe, Se-yeast, or SeMet.

In terms of lipid peroxidation in the breast muscle of chickens fed Zn-L-SeMet and subjected to cyclic heat stress, stored at -20 °C for 10, 30, and 60 days (Table 6), no significant differences were observed across the different storage days or inclusion levels ($p \geq 0.172$).

Table 6
Zinc-L-Selenomethionine (Zn-L-SeMet) in diets of broiler chickens subjected to cyclic heat stress and its effects on the thiobarbituric acid reactive substances (TBARS, MDA/kg of meat) in the breast muscle (Pectoralis major) at different days of storage at -20° C

Zn-L-SeMet mg/kg	Days of storage		
	10	30	60
0.00	1.97	1.40	47.63
0.15	1.83	0.27	43.86
0.23	1.56	0.43	45.79
0.47	1.63	0.47	44.40
1.30	1.97	1.74	46.28
SEM	1.26	1.95	4.98
CV (%)	70.39	226.31	10.93
p-value[1]	0.922	0.345	0.464

[1]: ANOVA P-values
SEM: pooled standard error of the mean
CV: coefficient of variation.

Lipid peroxidation is one of the most important factors that can degrade the physicochemical characteristics of meat and meat products (Pereira & Abreu, 2018). Exposure to high temperatures typically leads to excessive production of free radicals, particularly ROS, which can disrupt the body's antioxidant systems and lead to oxidative stress (Belhadj et al., 2014; Lin et al., 2006). Additionally, it is well-documented that the increase in storage time generally leads to an increase in malondialdehyde (MDA) levels in meat products (Y. J. Kim et al., 2010; Mikulski et al., 2009).

Measuring MDA concentrations helps determine the degree of lipid peroxidation in various tissues (W. Wang et al., 2022), as MDA is a by-product of the oxidation of lipid substrates that reacts with thiobarbituric acid (TBA), enabling quantification (Kumar et al., 2018). The findings of this study show that although lipid peroxidation in the breast muscle numerically increased with storage time, the treatments did not influence MDA levels, which remained within the tolerable limit of 0.2 to 0.5 mg/kg. This level is considered acceptable to prevent the meat from being categorized as "slightly oxidized," which ranges from 0.5 to 1.5 mg/kg (Coetzee & Hoffman, 2001). Therefore, the stress conditions might not have been sufficient to observe a notable Se effect on lipid peroxidation.

Habibian et al. (2016) investigated the effect of Se inclusion on animals reared under TN and HS conditions. Their results indicated that while animals in the TN group did not show notable changes in MDA levels, those in the HS group experienced elevated concentrations, which decreased with the addition of Se to their diets. The beneficial effects of Se in reducing lipid peroxidation in meat products have been supported by other studies (Gul et al., 2023; Mohamed et al., 2020; C. Wang et al., 2021). However, these findings are not consistent with the current study, aligning instead with the results of Leskovec et al. (2019) and Nemati et al. (2021), who found no significant differences in MDA concentrations in goose and chicken breast muscles, respectively. The level of dietary Se and the presence of other pro- and antioxidant compounds in the diet can influence an animal's oxidative status (National Research Council [NRC], 1994). Furthermore, comparing results across different studies is challenging due to the significant variation in measurement methods (Habibian et al., 2016).

There were no differences ($p \geq 0.383$) in the effect of Zn-L-SeMet on the breast meat composition of heat-stressed broilers (Table 7). It is well-documented that HS can impair meat chemical composition, as animals expend more energy maintaining their body temperature, leading to increased nutritional demands for maintaining homeostasis. If these demands are not met, the nutritional value of the meat can diminish (Ma et al., 2019; Rinaldo & Mourot, 2001). Supplementing animal diets with trace minerals can enhance muscle tissue growth while reducing fat deposition in the meat through nutrient repartitioning, affecting the metabolism of carbohydrates and lipids (Apple et al., 2000). Selenium is known to improve the quality and nutritive value of meat (Surai et al., 2006), making the fortification of poultry meat with Se a viable strategy to increase human consumption of this essential nutrient (Ibrahim et al., 2019).

Table 7

Zinc-l-selenomethionine (Zn-L-SeMet) in diets of broiler chickens subjected to cycliheat stress and its effects on the proximate composition of the breast meat (Pectoralis major)

Zn-L-SeMet mg/kg	Breast composition				
	DM (%)	TA (%)	CF (%)	CP (%)	Se[2]
0.00	25.7	1.56	1.56	23.8	0.132
0.15	25.6	1.75	1.46	24.3	0.132
0.23	25.6	1.49	1.26	24.2	0.145
0.47	25.4	1.39	1.42	23.9	0.150
1.30	25.0	1.40	1.24	23.3	0.150
SEM	0.75	0.51	0.36	0.63	0.28
CV (%)	2.96	33.53	25.56	2.63	19.86
p-value[1]	0.626	0.790	0.571	0.234	0.816

[1]: ANOVA P-values
[2]: mg/kg of breast
DM: dry matter; TA: total ash; CF: crude fat; CP: crude protein
SEM: pooled standard error of the mean
CV: coefficient of variation.

The use of SeMet as a dietary supplement for animals provides a significant advantage over other inorganic or organic Se sources. This is due to its metabolism as part of the methionine pool, which helps to build a Se storage depot in animal body tissues (Hosnedlova et al., 2017; Surai et al., 2018).

Prior studies, such as those conducted by Rossi et al. (2015) and Grossi et al. (2021), did not identify differences in the content of DM, EE, CF, or CP in meat samples supplemented with selenium. However, these researchers observed increased Se retention in the breast meat, contrasting with the findings of Arnaut et al. (2021), who found no increase in Se retention in breast meat. Nonetheless, other researchers have also reported enhanced Se content in chicken breast meat (Payne & Southern, 2005; Pan et al., 2007; Silva et al., 2019).

Conclusion

The treatments did not significantly affect breast meat quality ($p \geq 0.180$), lipid peroxidation ($p \geq 0.172$), or composition ($p \geq 0.383$).

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