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Communication

[*Comunicação*]

Effect of chromium-methionine supplementation on meat quality of broilers reared under heat stress

[*Efeito da suplementação de cromo-metionina na qualidade da carne de frangos de corte criados em estresse por calor*]

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Heat stress decreases the quality of chicken meat due to the increase in lactate in the muscle, consequently contributing to the decline in the pH levels of the breast meat (Petracci and Baéza, 2011). One of the main deleterious effects occurs in the color of the meat with the possibility of the appearance of PSE meat (pale, soft, exudative). PSE meat in broilers can be detected by combining the pH (below 5.8) and color (L * values above 52.0) values measured 24 hours after slaughter (Olivo *et al*., 2001). In addition, the loss of water (cooking weight loss or water holding capacity) in the breast is another determining factor for measuring meat quality and can directly influence industrial performance in the production of meat products such as pre-made, frozen and processed products. Thus, the objective was to evaluate the meat quality parameters of broilers, from 22 to 43 days, reared under heat stress and fed diets supplemented with chromium-methionine levels.

All experimental procedures were approved by the Animal Ethics Committee of Federal University of Viçosa (Protocol Number 15/2016), Minas Gerais, Brazil. The experiment was conducted at the Animal Bioclimatology Laboratory of the Animal Science Department of Federal University of Viçosa, Minas Gerais State, Brazil. Three hundred and thirty-six 22-day-old male Cobb 500 broilers were used in the study. Animals were kept at thermal comfort temperature from day 1 to 21, as recommended in the Cobb 500 breed line manual. Birds were fed the same basal diet according to nutritional recommendations by Rostagno *et al*. (2011) for 1-to-21 days of age, with 21% of crude protein, 2900 $kcalkg^{-1}$ of metabolizable energy, 0.89% of total calcium and 0.42% non-phytate phosphorus.

During the experimental period from 22 to 43 days of age, broilers were kept in climatic chambers under HS at 33.0 ± 0.8°C for 12 h (7:00 a.m. to 6:59 p.m.). After this period, they were kept at 23.0 ± 0.8°C (7:00 p.m. to 06:59 a.m.). Relative humidity was kept at 65.0 ± 3.5% throughout the experimental period. The objective was to simulate daily thermal amplitude conditions that occur in open side houses in tropical and subtropical regions, such as Brazil. The experiment used a completely randomized block design with four blocks (each block comprised a climatic chamber), 6 treatments, 8 repetitions (2 repetitions per block) and 7 animals per experimental unit.

Treatments consisted of the basal diet with one of 6 chromium-methionine (CrMet) (Availa Cr 1000, Zinpro Corporation, Eden Prairie, MN, USA), levels formulated for 22-to-43-day-old broilers, according to nutritional recommendations by Rostagno *et al*., (2011), with

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19% of crude protein, 3100kcalkg^{-1} of metabolizable energy, 0.68% of total calcium and 0.32% non-phytate phosphorus, supplemented with 500 FTU/kg of phytase. The 6 CrMet inclusion concentrations were 0.00, 0.10, 0.20, 0.40, 0.80 and 1.20mgkg^{-1}. Two 43-day-old birds per experimental unit (total of 96 birds) were selected based on the average body weight of experimental unit (± 5%) to evaluate breast meat quality. After fasting for eight hours, the birds were placed in appropriate plastic boxes and transported to a processing room lit by blue artificial light. Chilled breast meat, without skin and bone, was used to evaluate meat quality. The pH was measured at room temperature (25°C), 15 minutes and 24 hours after harvest, by directly introducing a pH meter probe (Tecnopon, model mPA210) coupled to a penetration electrode (Hanna model Hl 8314) into the *Pectoralis major* muscle of the birds.

Color analysis was based on the CIELAB system (L*, a*, b*); it was conducted in a Minolta colorimeter, model CR 400, which provided three variables, according to the Hunter Diagram. The L* value on the vertical axis of the diagram measures the brightness or reflectance rate, which ranged from 0 (white) to 100 (black). Thus, characteristics such as pale, normal or dark can be objectively determined. The a* value on the horizontal axis measures the variation between red and green, whereas the b* value measures the variation between yellow and blue. From the values of a* e b*, the values of chrome (C*) and hue angle (H°) according to the equations $C = (a^{*2} + b^{*2})^{1/2}$ and $H° = \tan^{-1} b^*/a^*$, respectively. To determine the water holding capacity (WHC), meat samples (mean weight 5g) were weighed and placed between two filter papers (12.5cm diameter) and between two glass plates (12 x 12 x 1cm).

A 10kg load was applied to the plates for five minutes to apply pressure. The difference between the initial and final weights of the samples was used to calculate the WHC. To determine the cooking weight loss (CWL) the breast fillets were cut, weighed, wrapped in aluminum foil and placed on a plate heated at 180°C. A thermometer was used to monitor the heating process until the internal breast temperature reached 74°C. After cooking, the sample was left to cool at room temperature. The CWL was calculated by using the weight of the uncooked fillet (WUF) and the post-cooking weight loss (PCFW), as follows: CWL % = (PCFW x 100/WUF). Shear force (SF) was analyzed in a Stable Micro Systems TAXT 2 PLUS texturometer, coupled to a Wanner Bratzler V probe-blade set, measuring the maximum peak force generated during the analysis. The equipment was calibrated at standard weight 5kg, whereas the descent/cut speed of the device was set at 200mm min^{-1}. Parallelepiped-shaped subsamples (1 x 1 x 2cm; height, width and length) were collected from samples remaining from the CWL analysis and arranged in a way that their fibers were perpendicular to the probe for analysis purposes.

Data were subjected to variance and regression analyses based on the following statistical model: $Y_{ij} = \mu + CrMet_i + Block_j + e_{ij}$; were, Y_{ij} = is the value of the response variable at the ith level of dietary CrMet concentration and the jth block; μ = is a constant common to all the observations; $CrMet_i$ = is the fixed effect of the dietary CrMet concentration level, i=6; $Block_j$= is the random effect of the block, j =4;e_{ij}= is the random residual associated to the Y_{ij}. The residuals from the models for all the variables were submitted to assumptions tests. Residual normality was verified based on the visualization of histograms, quantile-quantile plots and the Shapiro-Wilk's test. Residual independence was verified trough graphics of predicted versus residual values. Homogeneity of variances was verified trough box-plotgraphs and the Levene's test. In general, all the assumptions were met for all the variables with some exceptions. When an assumption was not attained, an exclusion of extreme values was performed based on the standardized residuals, where values outside the range of ± 3 were excluded (representing 3 standard deviations from the mean, which is zero).

Such approach solved the problems and resulted in the assumptions attainment. Following, the regression effects (linear and quadratic) were tested using orthogonal contrasts. When linear polynomial regressions or non-linear regressions (LBL and QBL) were significative, the equations, determination coefficient (R^2) and root mean squared error (RMSE) were presented for comparisons. All the analyses were performed in the SAS University Edition (Statistical…, Institute, 2012). Analysis of variance, regression and orthogonal contrasts were carried out using the SAS PROC MIXED. Assumptions of residual normality, homogeneity and independence were made using SAS PROC UNIVARIATE, SAS PROCGLM and SAS PROC REG, respectively. Non-linear regression models (LBL and QBL) were performed using SAS PROC NLIN. Significance was considered at the level of <0.05 (5%) of probability.

Effects of the increasing dietary levels of CrMet on breast meat quality are presented on Table 1. The CrMet supplementation levels affected quadratically (*P*=0.0070) the WHC in the breast meat of broilers slaughtered at 43 days of age. No effect (P>0.05) of CrMet supplementation levels was on the pH_{15min}, pH_{24h}, coloration (L*, a*, b*, C and Hº), CWL and SF in the breast muscle. For higher WHC in the breast meat the best CrMet supplementation level predicted for QR model, was 0.59mgkg^{-1} (Table 2).

Table 1. Mean values of the physical characteristics of the breast meat of broilers, Cobb 500 line, slaughtered at 43 days of age and fed with diets containing different levels of chromium-methionine and raised under heat stress (33ºC), in the phase from 22 to 43 days of age

Variables	Levels of chromium-methionine (mgkg^{-1})						SEM	*p*-value*	
	0	0.10	0.20	0.40	0.80	1.20		Linear	Quadratic
pH_{15min}	6.06	6.11	6.12	6.06	6.04	6.09	0.03	0.7884	0.6228
pH_{24h}	5.90	5.93	5.93	5.94	5.91	5.89	0.03	0.3456	0.2548
L*	44.52	43.83	44.07	43.56	44.40	44.07	1.47	0.9733	0.6782
a*	3.78	3.64	3.80	3.89	4.04	3.93	0.12	0.0502	0.2860
b*	7.07	6.84	6.76	7.00	6.88	6.66	0.15	0.0948	0.5759
C	8.01	7.74	7.75	8.00	7.98	7.73	0.19	0.1132	0.4752
H°	61.86	61.98	60.65	60.93	59.58	59.47	0.71	0.1893	0.5530
WHC[1] (%)	41.73	40.87	43.18	43.54	43.02	41.16	0.99	0.8737	0.0070[1]
CWL (%)	25.55	26.48	26.63	26.24	24.87	26.52	0.74	0.8615	0.5438
SF (kgf^{-1})	3.21	3.27	3.12	3.21	3.21	3.18	0.07	0.7784	0.9775

significance of the regression effects from orthogonal contrasts; L = lightness; a* = redness; b* = yellowness; WHC = water holding capacity; CWL = cooking weight loss; SF = shear force. [1] significative variable (P<0,05) for quadratic effect.

Table 2. Equation from linear regression model for the significative variable and their respective determination coefficient (R^2) and root mean squared error (RMSE)

Variables	Model[1]	Equation of regression	R^2	RMSE	Min./Max. (mgkg^{-1})	*p*-value*
Breast Meat Quality						
WHC (%)	QR	$y = 41.2903 + 7.6651 \times x - 6.5243 \times x^2$	0.20	3.0	0.59	0.0070

[1]QR = quadratic polynomial regression model: $y = \beta_0 + \beta_1 \times X + \beta_2 \times X^2$, where y is the response variable, X is the dietary chromium-methionine concentration, β_0 is the intercept, β_1 and β_2 are the linear and quadratic coefficients of the regression, respectively [maximum response concentration was obtained by: $-(\beta_1/2 \times \beta_2)$]; *significance of the linear regression effects from orthogonal contrasts when model equal to QR; R^2 = determination coefficient for the regression equation; RMSE = root mean squared error; WHC = water holding capacity.

The Cr increases cell signaling for insulin recognition through low-molecular weight Cr-binding-substance (LMWCr) with anabolic effects by increasing the percentage of protein in the breast muscle by increasing actin and myosin mRNA, reducing production tumor necrosis factor (TNF) that can inhibit lipogenesis and increase GLUT-2 expression (Vincent 2010, Rao *et al*., 2012; Pan *et al*., 2013; Sahin *et al*., 2017). The increase in the protein content in the broiler meat increases the water concentration with reflexes in the reduction of the denaturation of sarcoplasmic proteins, which can benefit the WHC (Bowker and Zhuang, 2015).

In the present study it was observed that CrMet supplementation up to 0.59mgkg^{-1} promoted increased WHC. The increase in WHC with CrMet supplementation may imply less exudate loss and higher yield in the production of frozen prepared processed meat derivatives or even in the appearance and commercialization of fresh chicken meat. Rouhalamini *et al*. (2014) found that supplementation with 1.00mgkg^{-1} of CrPic provided an increase in WHC in the breast of meat-type Japanese quails raised under HS compared to the control diet. Yang *et al*. (2017) observed higher WHC in broiler chickens slaughtered at 28 days of age receiving diets containing $CrCl_3$ associated with probiotic (*Bacillus subtilis*). Mir *et al*. (2017) observed that supplementation with 1.50mgkg^{-1} of CrPicolinate increased WHC in broiler chicks slaughtered at 42 days of age compared to a control diet.

According to these authors the increase in insulin sensitivity caused by Cr increases the protein deposition and glucose uptake by the cell, reducing the proportion of fat. This occurs mainly in the breast which represents 50% of the total protein and about 30% of the edible protein in the broiler carcass of modern breeds. Due to this, there is a greater accumulation of water in the breast muscle, favoring the increase of WHC. Variables such as color (L, a* and b*), WHC and SF are meat quality parameters that can be influenced by pH_{24h} after slaughter (Sterten *et al*., 2009). There was no effect of CrMet supplementation on pH, which might explain the lack of meat-quality parameters response in the current study, except for WHC.

In the present study, no effect of CrMet supplementation on color and cooking weight loss in broiler meat was observed. Vaz *et al*. (2009) also stated that supplementing up to 1.40mgkg^{-1} of CrMet does not alter the quality parameters of broiler breast meat. However, Zhang and Kim (2014) observed an increase in L * when supplementing 0.40mgkg^{-1} of CrMet in the broiler diet. Huang *et al*. (2016) observed a reduction in the values of b * and cooking weight loss when supplementing 2.00mgkg^{-1} of CrPropionate. The supplementation of 0.59 mgkg^{-1} of CrMet increased water holding capacity of breast of broiler chickens reared in heat stress and harvested at 43 days. However, the supplementation of CrMet did not affect pH_{15min}, pH_{24h}, coloration, cooking weight loss and shear force.

Keywords: breast meat quality, chromium, poultry industry, water in breast

RESUMO

Objetivou-se avaliar o efeito da suplementação de cromo-metionina em dietas para frangos de corte criados em estresse por calor, no período de 22 a 43 dias de idade, nos parâmetros de qualidade da carne. Foram utilizados 336 frangos de corte, machos, da linhagem Cobb 500, com 21 dias de idade, distribuídos em delineamento em blocos inteiramente ao acaso, com quatro blocos (cada câmara climática), seis tratamentos (0; 0,10; 0,20; 0,40; 0,80 e 1,20mgkg^{-1} de Cr na forma de Cr-metionina), oito repetições e sete aves por unidade experimental. Aos 43 dias de idade, duas aves por unidade experimental foram selecionadas e abatidas para avaliação da qualidade da carne de peito, por meio dos parâmetros de pH_{15min}, pH_{24h}, luminosidade (L), teor de vermelho (a*), teor de amarelo (b*), croma (C*), ângulo hue (Hº), capacidade de retenção de água, perda de peso por cozimento e força de cisalhamento. Houve efeito quadrático (P=0,0070) na capacidade de retenção de água da carne de peito. A suplementação de CrMet não afetou (P>0,05) os demais parâmetros de qualidade da carne. Assim, recomenda-se a suplementação de 0,59mgkg^{-1} de CrMet para frangos de corte para melhoria da capacidade de retenção de água do peito.*

Palavras-chave: qualidade da carne de peito, cromo, indústria avícola, água no peito

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