1 **TITLE PAGE**

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Chromium propionate as a dietary strategy for heat stress mitigation in broilers
Running Title (within 10 words)	Dietary chromium propionate for heat stress mitigation in broilers
Author	Hyuck Kim[1, #], Dongcheol Song[1], Kyeongho Jeon[1], Jinmo Yang[1], Jihwan Lee[2], Seyeon Chang[3], Hans Lee[4], Dexter Lee[4], Jinho Cho[1, *]
Affiliation	[1] Department of Animal Science, Chungbuk National University, Cheongju 28644, Korea [2] Department of Animal Science, Jeonbuk National University, Jeonju 54896, Korea [3] Animal Welfare Division, National Institute of Animal Science, Rural Development Administration, Wanju 55365, Korea [4] Kemin Industries, Singapore 758200
ORCID (for more information, please visit https://orcid.org)	Hyuck Kim / harrck85@naver.com (https://orcid.org/0000-0002-5280-0734) Dongcheol Song / paul741@daum.net (https://orcid.org/0000-0002-5704-603X) Kyeongho Jeon / jeonkh1222@gmail.com (https://orcid.org/0000-0003-2321-3319) Jinmo Yang / mike000315@gmail.com (https://orcid.org/0009-0007-4272-3441) Jihwan Lee / jl26112@jbnu.ac.kr (https://orcid.org/0000-0001-8161-4853)

	Seyeon Chang / angella2425@naver.com (https://orcid.org/0000-0002-5238-2982) Hans Lee / hans.lee@kemin.com (https://orcid.org/0000-0002-8603-947X) Dexter Lee / Dexter.lee@kemin.com / (https://orcid.org/0009-0001-7887-4088) Jinho Cho / jinhcho@cbnu.ac.kr (http://orcid.org/0000-0001-7151-0778)
Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	This manuscript has not received any funding.
Acknowledgements	
Availability of data and material	All data generated or analyzed during this study are included in this published article.
Authors' contributions Please specify the authors' role using this form.	[#] *These authors contributed equally to this work* Conceptualization: Cho JH, Lee JH, Kim H Data curation: Song DC, Jeon KH Formal analysis: Chang SY, Yang JM, Lee H Methodology: Kim H, Song DC, Chang SY Software: Yang JM, Jeon KH Validation: Kim H, Lee D Investigation: Cho JH, Lee H Writing - original draft: Kim H, Lee JH, Lee D Writing - review & editing: Kim H, Lee JH, Chang SY, Song DC, Jeon KH, Yang JM, Lee H, Lee D, Cho JH

Ethics approval and consent to participate	The experimental protocol was approved (CBNUA-24-0071-01) by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea.

2

3 **CORRESPONDING AUTHOR CONTACT INFORMATION**

For the corresponding author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Jinho Cho
Email address – this is where your proofs will be sent	jinhcho@chungbuk.ac.kr
Secondary Email address	
Address	Department of Animal Science, Chungbuk National University, Cheongju, Republic of Korea, 28644
Cell phone number	+82-10-8014-8580
Office phone number	+82-43-261-2544
Fax number	+82-43-273-2240

4

Abstract

This study was conducted to evaluate the effects of chromium (Cr) levels and dilution rates in broiler diets under heat stress (HS) on performance, digestibility, immunity, carcass characteristics, and mineral retention. One-hundred and twenty-eight 1-day-old Arbor Acres broiler chickens (initial body weight of 37.98 ± 0.22 g) were used in this experiment for d 35. They were assigned to four treatment groups (eight replications, four birds each per cage): PC, birds fed a basal diet under thermoneutral temperature; NC, birds fed a basal diet and were exposed to cyclic HS at d 14 of age; CP2, PC with supplementing Cr 200 μg/kg as chromium propionate (Cr-Pro); CP4, PC with supplementing Cr 400 μg/kg as Cr-Pro. In this study, the NC exhibited decreased ($p < 0.05$) performance and nutrient digestibility compared to the PC, while showing increased ($p < 0.05$) mineral excretion and abdominal fat. Additionally, HS increased ($p < 0.05$) cortisol, norepinephrine, and immunoglobulin A and G levels, while decreasing ($p < 0.05$) glucose levels in serum. However, the CP4 showed higher ($p < 0.05$) body weight gain, crude protein digestibility, and retention of copper and chromium than the NC. Also, Cr-supplemented groups showed lower ($p < 0.05$) abdominal fat than the NC. In the serum profiles, Cr supplementation decreased serum norepinephrine levels under HS. Serum glucose levels were significantly higher ($p < 0.05$) in the CP4 compared to the NC. Serum IgG levels were significantly lower ($p < 0.05$) in the CP2 and CP4 compared to the NC. The CP4 showed a significant decrease ($p < 0.05$) in serum cortisol and H:L ratio compared to the NC. In conclusion, Cr-Pro is an additive that can mitigate the effects of HS, supplementation with 400 μg/kg Cr is suggested as a more effective alternative than 200 μg/kg.

Keyword: Heat stress, Chromium propionate, Broiler, Meat quality, Mineral retention

INTRODUCTION

Broilers are covered in feathers and they lack sweat glands, they are highly susceptible to heat stress (HS) due to their limited capacity for heat dissipation [1]. When exposed to high temperatures, broilers will reduce feed intake (FI) to regulate body temperature, which can impair their immunity and performance [2, 3]. HS also induces increased cortisol levels and insulin resistance, leading to altered body protein and fat composition and deteriorating meat quality [4]. Such physical and physiological responses impair broiler growth performance and meat quality, resulting in significant economic losses of the poultry industry.

Chromium plays an essential role in potentiating insulin action by forming the chromodulin complex, which enhances insulin receptor kinase activity and facilitates nutrient uptake [5]. Under HS, impaired insulin sensitivity accelerates protein catabolism, lipid accumulation, and oxidative damage; thus, supplemental Cr may help restore insulin function and alleviate metabolic disturbances [6]. Its bioavailability depends on the chemical form, organic Cr is generally preferred over inorganic Cr because of its lower toxicity and greater absorption efficiency [7, 8]. Among organic Cr sources, chromium propionate (Cr-Pro) has demonstrated superior absorption efficiency compared with other organic forms [9]. Supplementation with 400 μg/kg Cr-Pro has been reported to reduce mortality and increase carcass yield in broilers [10]. Furthermore, dietary inclusion at 0.4 or 2.0 mg Cr/kg under HS conditions decreased breast muscle fat accumulation and improved meat quality compared with Cr-picolinate or $CrCl_3$ [11]. Nevertheless, the overall findings have been inconsistent, and further research is required to clarify its effects. In addition, most studies evaluating Cr-Pro have been conducted under thermoneutral conditions, while information regarding its effects on immunity, growth performance, and carcass traits under HS conditions is limited [12-14]. Therefore, this study aimed to investigate effects of Cr-Pro supplementation at various levels on growth performance, nutrient digestibility, carcass traits, mineral retention, and blood profiles in broilers exposed to HS conditions.

MATERIALS AND METHODS

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Chungbuk National University (approval no. CBNUA-24-0071-01).

Experimental animals and treatment

One-hundred and twenty-eight 1-day-old Arbor Acres broiler chickens (initial body weight of 37.98 ± 0.22 g) were obtained from a local hatchery (Dowon Co., Cheongju, Korea) and used in this experiment for d 35. All broilers were assigned to four completely randomized treatment groups based on initial body weight (BW). There were four broilers in a cage and eight replication cages per treatment. The treatments were as follows: PC, birds fed a basal diet under thermoneutral temperature; NC, birds fed a basal diet and were exposed to cyclic HS at d 14 of age; CP2, PC with supplementing Cr 200 μg/kg of elemental Cr; CP4, PC with supplementing Cr 400 μg/kg as elemental Cr. The Cr source was a commercial Cr-Pro (KemTRACE Cr propionate; Kemin Industries, Seongnam, Korea) containing 0.4% elemental Cr (4,000 mg/kg). To achieve target elemental Cr concentrations, 50 mg (for CP2) and 100 mg (for CP4) of the commercial product were imcorporated per kg of the basal diet. This product is a stabilized Cr-pro supplement (molecular formula $(C_6H_5O_2)_3Cr$) with a guaranteed Cr content of 0.4% (w/w) and typical purity > 99%. To ensure homogeneity, Cr-Pro was first mixed with a small portion of the basal diet to create a pre-mixture before being thoroughly blended into the final mash-form diets. Each treatment included four birds per cage, sized 100 cm in width, 40 cm in depth, and 45 cm in height. The experiment initiation temperature was 31 ± 1℃, and after that, the temperature was gradually lowered to maintain 25 ± 1℃ according to the Arbor Acres Broiler Management Guideline from d 1 to 14 of age. Then the PC continued from d 15 to 35 of age [15]. Thus, all treatments were managed identically during this period. In contrast, the other experimental groups were exposed to HS conditions. The HS conditions exposed the birds to cyclic periods of elevated temperature (32 ± 2℃) for 8 h daily, from 10:00 am to 6:00 pm, with the temperature gradually increasing and decreasing over 30 min, to mimic the summer season in Korea. The HS treatment was applied from d 15 to 35. Relative humidity inside the poultry house was kept between 60 and 70% (Table 1). All diets were formulated to meet or exceed the NRC [16] (Table 2) for the pre-starter (d 0 to 7), starter (d 8 to 14), grower (d 15 to 21), and finisher (d 22 to 35) periods. A basal diet was fed to all treatments during the experimental periods, with Cr-Pro supplemented in the CP2 and CP4 groups. All broilers were given *ad libitum* access to diet and water throughout the experiments.

Analysis items and measurements

Growth performance

All broilers and diet were weighed at d 0, 7, 14, 21, and 35 to estimate BWG, FI, FCR, uniformity, and production index (PI). Data collected on d 7 and 14 were used to assess the effect of Cr before HS, while d 21 and 35 were collected during HS conditions. The BWG was calculated as the current week's BW and subtracted from the BW of the previous week for each period (d 0 to 7, d 8 to 14, d 15 to 21, d 22 to 35, d 0 to 35). The ADG was calculated by dividing BWG by the number of days in each period (d 0 to 7, d 8 to 14, d 15 to 21, d 22 to 35, d 0 to 35). The FI was calculated as the residual amount subtracted from the diet amount. The FCR was calculated by dividing FI by BWG. The uniformity % was calculated depending on the following England et al. (2022) method:

"Uniformity % = (1 - (SD / x)) × 100"

SD = standard deviation of BW of the birds for each treatment group, x = the BW mean across the treatment groups. The PI was calculated depending on the following formula:

"PI = {(Average BW, kg × livability) / (Duration of the period, d × FCR)} × 100"

Nutrient digestibility

All treatment groups were fed 0.2% Cr_2O_3 in the diet as an indigestible marker for analyzing nutrient digestibility starting 3 d before the end of the experiment. The ileum of each broiler was dissected from the Meckel's diverticulum to 5 cm above the ileocecal junction. Diet and ileal digesta samples were collected and frozen at – 20℃ until analysis. Ileal digesta samples were dried at 70℃ for 48 h and then ground through a 1 mm screen. The dry matter (DM; method 930.15) and crude protein (CP; method 984.13) contents of both ileal digesta and diet samples were analyzed according to AOAC [17]. Gross energy (GE) content was determined using an adiabatic oxygen bomb calorimeter (Parr 6400 Bomb Calorimeter, Parr Instrument Co., Moline, IL, USA). Cr levels were measured by ultraviolet (UV) absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) following the method of Williams et al. [18]. Apparent ileal digestibility (AID) was calculated using the following formula:

"AID = 1 − [(concentration of nutrient in digesta × concentration of Cr_2O_3 in the diet) / (concentration of nutrient in diet × concentration of Cr_2O_3 in the digesta)] × 100"

106

107 **Carcass traits**

108 At d 35, one broiler with similar BWs from each replicate was selected to measure carcass yield following the
109 method of Zhang et al., [19]. Each bird was euthanized, and feathers were removed using a commercial
110 defeather (Yanglim, Yangpyeong, Korea). The neck, head, and feet were removed, and the birds were
111 eviscerated. Carcass weight, breast muscle weight, and abdominal fat weight were recorded. Carcass yield was
112 calculated by dividing carcass weight by live BW. Additionally, the breast muscle and abdominal fat were
113 weighed to determine their yields as a percentage of carcass weight (part weight / carcass weight × 100).

114

115 **Blood profile**

116 At d 35, blood samples were collected from the brachial wing vein of one broiler per replication into a
117 sterile syringe for blood profile. The blood samples were collected into vacuum tubes containing tri
118 potassium ethylene diamine tetra-acetic acid (K_3EDTA; Becton, Dickinson and Co., Franklin Lakes, NJ,
119 USA) for complete blood count analysis and nonheparinized tubes for serum analysis, respectively. After
120 collection, serum samples were centrifuged at 3,000 rpm for 20 min at 4℃. Subsequently, the blood sample
121 tubes were stored in a −20℃ refrigerator until analysis. The heterophils and lymphocytes were measured
122 using the complete blood cell count method. 10 μL blood samples in K_3EDTA tubes were collected to make
123 blood smears on glass slides. The smears were stained with Wright's stain. Heterophils and lymphocytes
124 were identified using a compound microscope (Axio Scope.A1, Carl Zeiss, Oberkochen, Germany). The
125 heterophil to lymphocyte (H:L) ratio was calculated by dividing the number of heterophils by lymphocytes.
126 Immunoglobulin A (IgA) and immunoglobulin G (IgG) levels were measured in serum using an automatic
127 biochemistry blood analyzer (Hitachi 747; Hitachi, Tokyo, Japan). The cortisol was analyzed using
128 enzyme-linked immunosorbent assay kits (LDN GmbH & Co., Nordhorn, Germany) following the
129 manufacturer's protocol. The glucose was analyzed using an automatic Konelab analyzer (Thermo Clinical
130 Labsystems Oy, Vantaa, Finland) according to the manufacturer's instructions. The norepinephrine was
131 quantified using an ion-exchange purification procedure followed by high-performance liquid
132 chromatography with electrochemical detection as described by Hay and Mormède (1997).

133 For elemental analysis, serum samples were first diluted to 1:15 (w/w) with an acidified (pH = 2.0)
134 diluent consisting (w/w) of 1% 1-butanol (Merck KGaA, Darmstadt, Germany), 0.1% Triton X-100

(Sigma-Aldrich, Co., St. Louis, USA), and 0.07% HNO_3 (Sigma-Aldrich, Co.) in distilled deionized water (18 MΩ/cm) (Merck Millipore, Billerica, Massachusetts, USA). Next, the macronutrient (i.e., Ca and P) and vital microelement (i.e., Zn, Cu, and Cr) of all samples were determined using a NexION 300D spectrometer (Perkin Elmer, USA).

Mineral retention

At d 31, the excreta output and FI were determined for mineral retention. The excreta samples were collected for 24 h using plastic plates inserted beneath the cages. The diet and excreta samples were dried out in an oven at 105℃ for 24 h and 65℃ for 48 h, respectively. After drying out, samples were pooled by pen and ground in a sample mill through a 1 mm screen to achieve a homogeneous sample for compositional analysis. Dried samples were analyzed for Cu and Zn using an atomic absorption spectrophotometer mercury analyzer (Z2300, Hitachi, Tokyo, Japan), as described by Li et al. (2004). Then dried samples were ashed at 550℃ for 6 h for Ca and P analysis. The amount of Ca (method 968.08) was analyzed by AOAC methods. The amount of P was determined colorimetrically using a wet ash procedure, as described by Sands et al. [20]. Cr analysis was based on the method of Anderson et al. [21]. The retention of Ca, P, Cu, Zn, and Cr was calculated according to the following equation [22]:

"Retention = [intake – excretion / intake] × 100"

Statistical analysis

Data on growth performance, nutrient digestibility, and carcass traits were statistically analyzed by one-way ANOVA using JMP (JMP Pro version 16.0.0, SAS Institute Inc., Cary, NC, USA). Differences between treatment means were determined using Tukey's multiple-range test. A probability level of $p \leq 0.05$ was indicated to be statistically significant, and a level of $0.05 < p \leq 0.10$ was considered to have such a tendency.

RESULTS

Growth performance

The effect of supplementing Cr on growth performance under HS in broilers is presented in Table 2. At d 15 to 21, the NC significantly decreased ($p < 0.05$) in BWG and FI compared to the PC. At d 15 to 21, Cr-supplemented groups significantly increased ($p < 0.05$) in FI compared to the NC. At d 15 to 21, BWG was significantly higher ($p < 0.05$) in the CP4 than in the NC. At d 21 and 35, the CP4 significantly increased ($p < 0.05$) in BW compared to the NC. Over the entire experimental period, the NC significantly decreased ($p < 0.05$) in BWG and FI while FCR significantly increased ($p < 0.05$) compared to the PC. Also, the CP4 significantly increased ($p < 0.05$) in BWG compared to the NC over the entire experimental period. In the NC, the production index was significantly lower ($p < 0.05$) than in the PC. There were no mortalities during the entire experimental period.

The effect of supplementing Cr on BW uniformity under the HS in broilers is presented in Table 3. During the entire experimental period, all treatment groups showed a uniformity rate exceeding 90%, indicating consistent growth performance across treatments.

Nutrient digestibility

The effect of supplementing Cr on nutrient digestibility under the HS in broilers is presented in Fig 2. The PC and CP4 showed higher ($p < 0.05$) CP digestibility than the NC. The NC and CP2 groups showed lower ($p < 0.05$) GE digestibility than the PC.

Carcass traits

The effect of supplementing Cr on carcass traits under the HS in broilers is presented in Fig 3. The NC significantly increased ($p < 0.05$) in abdominal fat compared to other groups.

Blood profiles

The effect of supplementing Cr on blood profile under the HS in broilers is presented in Fig 4. The NC significantly increased ($p < 0.05$) in cortisol, norepinephrine, H:L ratio, IgG, and IgA. The CP4 significantly reduced ($p < 0.05$) norepinephrine levels compared to the NC. IgG levels were significantly lower ($p < 0.05$) in the CP2 and CP4 than in the NC. The CP4 significantly decreased ($p < 0.05$) in cortisol and H:L ratio while increasing the serum glucose levels compared to the NC.

The effect of supplementing Cr on serum minerals under the HS in broilers is presented in Table 4. All treatments had no significant differences ($p > 0.05$) in serum minerals.

Mineral retention

The effect of supplementing Cr on mineral retention under the HS in broilers is presented in Fig 5. The NC significantly decreased ($p < 0.05$) in Ca, P, Cu, and Cr retention compared to the PC. The CP4 group significantly increased ($p < 0.05$) in Cu and Cr retention compared to the NC.

Discussion

This study evaluated the effects of Cr-Pro supplementation on broilers under HS conditions. HS impaired growth performance, as indicated by reduced FI, BWG, and PI, along with increased FCR. These results are consistent with previous studies showing that elevated temperature reduces FI and BWG in broilers [23]. However, supplementation with 400 μg/kg of Cr-Pro alleviated these adverse effects, recovering FI and BWG to levels comparable with the PC. Similar results have been reported in previous studies, with Cr supplementation reducing serum cortisol levels and improving FI and BW [24-26]. Sahin et al. [27] further demonstrated that increasing dietary Cr-Pic from 200 to 1,200 μg/kg under HS conditions significantly improved FI, BWG, and FCR, supporting the dose-dependent efficacy observed in our study. Conversely, Xiao et al. [28] reported that 200 μg/kg of Cr-Pro supplementation did not significantly affect BW under HS conditions, which is consistent with our observation that 200 μg/kg was insufficient to elicit consistent improvements in performance. These findings indicate that higher levels of Cr-Pro are required to counteract the negative impact of HS on performance, and that the efficacy of Cr supplementation depends on the dosage of Cr.

In our study, the reduction in performance was attributed to decreased digestibility. HS can restrict blood flow to the gastrointestinal tract, thereby impairing nutrient absorption and leading to an insufficient nutrient supply for body composition synthesis, resulting in reduced performance [29, 30]. Accordingly, HS reduced CP and GE digestibility in broilers, whereas supplementation with 400 μg/kg of Cr-Pro improved CP digestibility. Consistent with these findings, previous studies reported that the inclusion of Cr-Pic or Cr-Histidine in laying hen diets enhanced CP digestibility [31], and An et al. [32] showed that 400 μg/kg of Cr-Met in broilers under HS numerically increased the AID of CP and GE, resulting in improved BWG, although differences from thermoneutral conditions were not significant. These results suggest that dietary supplementation with 400 μg/kg of Cr-Pro can alleviate the negative effects of reduced FI and nutrient digestibility induced by HS, thereby supporting improved broiler performance.

Cr-Pro effectively reduced abdominal fat levels under HS conditions, comparable to results observed under thermoneutral conditions. Huang et al. [11] have demonstrated that supplementation with 400 μg/kg of Cr-Pro and Cr-Pic can effectively improve carcass yield and reduce abdominal fat under HS conditions. These findings suggest that Cr may help broilers withstand HS and mitigate meat quality impairment. The activation effect of Cr on insulin receptors can regulate nutrient metabolism and mitigate fat accumulation and protein degradation

caused by HS [8, 33]. Through these mechanisms, Cr supplementation has been reported to improve carcass yield under various stress conditions [12, 34]. Also, Cr can inhibit fat synthesis in adipose tissues and reduce serum triglycerides and abdominal fat in broilers [35, 36]. These results suggest that Cr-Pro is a promising additive that can enhance meat quality and contribute to economic value under HS.

Stress factors such as HS have been reported to increase mineral excretion, while Cr supplementation has been shown to mitigate this effect [37, 38]. Previous studies have reported that NanoCrPic supplementation at 500 μg/kg can increase Cr retention in the body [39]. Additionally, Amatya et al. [40] have observed that Cr supplementation under HS conditions can improve the retention of Cr and other minerals, including Cu. In another study, supplementation with Cr-Met at 600 and 1,200 μg/kg increased serum concentrations of Cr and Zn [41]. Although our study did not find significant differences in serum mineral concentrations, supplementation with 400 μg/kg of Cr-Pro improved the retention of Cr and Cu. These results support previous findings and suggest that Cr supplementation can mitigate the increase of mineral excretion caused by HS.

In this study, HS increased serum IgA, IgG, and the H:L ratio in broilers. These findings might be attributed to the activation of immune responses due to oxidative stress induced by HS, which can increase intestinal permeability [42-44]. However, antioxidant properties of Cr can alleviate intestinal damage caused by HS, potentially leading to immunomodulatory effects [45]. Previous studies have demonstrated that supplementation with 800 μg/kg of Cr-Met can suppress HS-induced heterophil production, resulting in a decreased H:L ratio [46, 47]. Similarly, our study confirmed that supplementation with 400 μg/kg of Cr-Pro under HS conditions reduced serum IgG levels and the H:L ratio. Huang et al. [48] have also reported similar effects of Cr-Pro at levels of 400 μg/kg or higher, including reductions in IgG levels. These results suggest that supplementing broilers with 400 μg/kg of Cr-Pro can stabilize excessive immune responses induced by HS. However, other studies have reported varying results. For instance, Ghazi et al. [41] observed that supplementation with 600 μg/kg of Cr-Met reduced the H:L ratio and increased IgG levels, while Toghyani et al. [49] reported that Cr supplementation under HS conditions had no effect on the H:L ratio but increased immunoglobulin levels. Currently, studies investigating the mechanisms underlying effects of Cr on immunity are still limited. These discrepancies might be due to differences in experimental conditions, such as the intensity of HS, timing of blood sampling, and/or individual variability in immune responses. Immune indicators did not differ significantly between broilers supplemented with 400 μg/kg of Cr-Pro under HS and those in the PC group maintained at thermoneutral conditions. This finding indicates that Cr-Pro supplementation restored immune responses impaired by HS to levels comparable with

thermoneutral controls, supporting its potential as a practical strategy to mitigate heat stress in broilers.

Conclusion

HS negatively affected growth performance (e.g., BWG, FI, and FCR) and immune response. Additionally, HS reduced digestibility (e.g., CP and GE), mineral retention, and serum levels of cortisol and norepinephrine, while increasing abdominal fat and serum glucose compared to the thermoneutral group. The supplementation of Cr in broilers under HS reduced the increase in serum cortisol, norepinephrine, and abdominal fat, while improving FI, indicating its potential to mitigate the adverse effects of HS. Specifically, the inclusion of 400 ppb Cr-Pro improved BW, BWG, and CP digestibility in broilers exposed to HS. Furthermore, the supplementation of 400 ppb Cr-Pro modulated immune response, and reduced serum cortisol and excretion of Cr and Cu in HS broilers. In conclusion, Cr-Pro was effective in alleviating heat stress, with 400 µg/kg identified as the optimal supplementation level based on its combined effects on growth performance, CP digestibility, immune response, mineral retention, and serum cortisol levels.

REFERENCES

1. Varasteh S, Braber S, Akbari P, Garssen J, Fink-Gremmels J. Differences in susceptibility to heat stress along the chicken intestine and the protective effects of galacto-oligosaccharides. PloS one. 2015;10:e0138975. https://doi.org/10.1371/journal.pone.0138975
2. Mcfarlane JM, Curtis SE, Shanks RD, Carmer SG. Multiple concurrent stressors in chicks.: 1. Effect on weight gain, feed intake, and behavior. Poult. Sci. 1989;68:501-9. https://doi.org/10.3382/ps.0680501
3. Mahmoud UT, Abdel-Rahman MAM, Darwish MHA, Applegate TJ, Cheng H-w. Behavioral changes and feathering score in heat stressed broiler chickens fed diets containing different levels of propolis. Appl. Anim. Behav. Sci. 2015;166:98-105. https://doi.org/10.1016/j.applanim.2015.03.003
4. Teyssier JR, Preynat A, Cozannet P, Briens M, Mauromoustakos A, Greene ES, Owens CM, Dridi S, Rochell SJ. Constant and cyclic chronic heat stress models differentially influence growth performance, carcass traits and meat quality of broilers. Poult. Sci. 2022;101:101963. https://doi.org/10.1016/j.psj.2022.101963
5. Vincent JB. The biochemistry of chromium. J. nutr. 2000;130:715-8. https://doi.org/10.1093/jn/130.4.715
6. Lebedev S, Gavrish I, Gubajdullina I, Shabunin S. Effects caused by different doses of dietary chromium nanoparticles fed to broiler chickens. Agric. Biol. 2019;54:820-31. https:// doi: 10.15389/agrobiology.2019.4.820eng
7. Król B, Słupczyńska M, Kinal S, Bodarski R, Tronina W, Mońka M. Bioavailability of organic and inorganic sources of chromium in broiler chicken feeds. J. Elem. 2017;22. https://doi: 10.5601/jelem.2016.21.1.1119
8. Sahin N, Hayirli A, Orhan C, Tuzcu M, Akdemir F, Komorowski J, Sahin K. Effects of the supplemental chromium form on performance and oxidative stress in broilers exposed to heat stress. Poult. Sci. 2017;96:4317-24. https://doi.org/10.3382/ps/pex249
9. Clodfelder BJ, Chang C, Vincent JB. Absorption of the biomimetic chromium cation triaqua-μ 3-oxo-μ-hexapropionatotrichromium (III) in rats. Biol. Trace Elem. Res. 2004;98:159-69. https://doi.org/10.1385/BTER:98:2:159
10. Jackson A, Powell S, Johnston S, Shelton J, Bidner T, Valdez F, Southern L. The effect of chromium propionate on growth performance and carcass traits in broilers. J. Appl. Poult. Res. 2008;17:476-81. https://doi.org/10.3382/japr.2008-00031
11. Huang Y, Yang J, Xiao F, Lloyd K, Lin X. Effects of supplemental chromium source and concentration on growth performance, carcass traits, and meat quality of broilers under heat stress conditions. Biol. Trace

297 Elem. Res. 2016;170:216-23. https://doi.org/10.1007/s12011-015-0443-z
298 12. Van Hoeck V, Sonawane M, Sanchez ALG, Van Dosselaer I, Buyens C, Morisset D. Chromium propionate
299 improves performance and carcass traits in broilers. Anim. nutr. 2020;6:480-7.
300 https://doi.org/10.1016/j.aninu.2020.03.005
301 13. Hayat K, Bodinga BM, Han D, Yang X, Sun Q, Aleya L, Abdel-Daim M, Yang X. Effects of dietary
302 inclusion of chromium propionate on growth performance, intestinal health, immune response and nutrient
303 transporter gene expression in broilers. Sci. Total Environ. 2020;705:135869.
304 https://doi.org/10.1016/j.scitotenv.2019.135869
305 14. Segun O, Bosede AF, Adesanya AO, Adinma CC, Taiwo AO. Broiler Chickens Fed Chromium Propionate
306 Supplemented Diets in a Tropical Environment: Serum Biochemical and Intestinal Morphology. International
307 Journal of Environment, Agric. Biotechnol. J. 2022;7:1. https://doi: 10.22161/ijeab
308 15. Aviagen Inc. (2023) 'PARENT STOCK Management Handbook 2023.' (Newbridge: Huntsville, AL, USA),
309 https://aviagen.com/assets/Tech Center/AA Breeder ParentStock/Aviagen ArborAcres PS Handbook 2023
310 Interactive EN.pdf.
311 16. NRC. Nutrient requirements of poultry. 9th ed. Washington, DC: National Academy of Press; 1994.
312 17. AOAC. (2005). [Association of Official Analytical Chemists]. Official method of analysis of AOAC
313 International. 18th ed. Gaithersburg, MD; AOAC International.
314 18. Williams C, David DJ, Iismaa O. The determination of chromic oxide in faeces samples by atomic
315 absorption spectrophotometry. J. Agric. Sci.1962;59:381-5. https://doi.org/10.1017/S002185960001546X
316 19. Zhang Q, Zhang H, Jiang Y, Wang J, Wu D, Wu C, Che L, Lin Y, Zhuo Y, Luo Z, Nie K, Li J. Chromium
317 propionate supplementation to energy-and protein-reduced diets reduces feed consumption but improves feed
318 conversion ratio of yellow-feathered male broilers in the early period and improves meat quality. Poult. Sci.
319 2024;103:103260. https://doi.org/10.1016/j.psj.2023.103260
320 20. Sands J, Ragland D, Baxter C, Joern B, Sauber T, Adeola O. Phosphorus bioavailability, growth
321 performance, and nutrient balance in pigs fed high available phosphorus corn and phytase. J. Anim. Sci.
322 2001;79:2134-42. https://doi.org/10.2527/2001.7982134x
323 21. Anderson R. Chromium. Trace elements in human and Anim. nutr. Academic Press New York. 1987;9:225-
324 44.
325 22. Almeida F, Stein H. Performance and phosphorus balance of pigs fed diets formulated on the basis of values

for standardized total tract digestibility of phosphorus. J. Anim. Sci. 2010;88:2968-77. https://doi.org/10.2527/jas.2009-2285

23. Piray A, Foroutanifar S. Chromium supplementation on the growth performance, carcass traits, blood constituents, and immune competence of broiler chickens under heat stress: A systematic review and dose–response meta-analysis. Biol. Trace Elem. Res. 2022;200:2876-88. https://doi.org/10.1007/s12011-021-02885-x

24. Zha LY, Zeng JW, Chu XW, Mao LM, Luo HJ. Efficacy of trivalent chromium on growth performance, carcass characteristics and tissue chromium in heat-stressed broiler chicks. J. Sci. Food Agric. 2009;89:1782-6. https://doi.org/10.1002/jsfa.3656

25. Norain TM, Ismail IB, Abdoun KA, Al-Haidary AA. Dietary inclusion of chromium to improve growth performance and immune-competence of broilers under heat stress. Italian J. Anim. Sci. 2013;12:e92.

26. Jahanian R and Rasouli E. Dietary chromium methionine supplementation could alleviate immunosuppressive effects of heat stress in broiler chicks. J. Anim. Sci. 2015;93:3355-63. https://doi.org/10.2527/jas.2014-8807

27. Sahin K, Sahin N, Onderci M, Gursu F, Cikim G. Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities, and some serum metabolites of broiler chickens. Biol. Trace Elem. Res. 2002;89:53-64. https://doi.org/10.1385/BTER:89:1:53

28. Xiao F, Ao D, Zhou B, Spears JW, Lin X, Huang Y. Effects of supplemental chromium propionate on serum lipids, carcass traits, and meat quality of heat-stressed broilers. Biol. Trace Elem. Res. 2017;176:401-6. https://doi.org/10.1007/s12011-016-0852-7

29. Song J, Xiao K, Ke Y, Jiao L, Hu C, Diao Q, Shi B, Zou X. Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poult. Sci. 2014;93:581-8. https://doi.org/10.3382/ps.2013-03455

30. de Souza LFA, Espinha LP, de Almeida EA, Lunedo R, Furlan RL, Macari M. How heat stress (continuous or cyclical) interferes with nutrient digestibility, energy and nitrogen balances and performance in broilers. Livest. Sci. 2016;192:39-43. https://doi.org/10.1016/j.livsci.2016.08.014

31. Orhan C, Tuzcu M, Deeh PBD, Sahin N, Komorowski JR, Sahin K. Organic chromium form alleviates the detrimental effects of heat stress on nutrient digestibility and nutrient transporters in laying hens. Biol. Trace Elem. Res. 2019;189:529-37. https://doi.org/10.1007/s12011-018-1485-9

32. An J, Lee J, Song M, Oh H, Kim Y, Chang S, Go Y, Song D, Cho H, Park H, Kim H, Cho J. Effects of supplemental different clay minerals in broiler chickens under cyclic heat stress. J. Anim. Sci. Technol. 2023;65:113. https://doi: 10.5187/jast.2022.e94

33. Samanta S, Haldar S, Bahadur V, Ghosh TK. Chromium picolinate can ameliorate the negative effects of heat stress and enhance performance, carcass and meat traits in broiler chickens by reducing the circulatory cortisol level. J. Sci. Food Agric. 2008;88:787-96. https://doi.org/10.1002/jsfa.3146

34. Youssef IM, Abdo IM, Elsukkary HF, El-Kady MF, Elsayed M. Effects of dietary supplementation of chromium methionine chelate on growth performance, oxidative stress, hematological indices, and carcass traits of broiler chickens. Trop. Anim. Health Prod. 2022;54:267. https://doi.org/10.1007/s11250-022-03260-1

35. Kim Y, Han IK, Choi Y, Shin I, Chae B, Kang T. Effects of dietary levels of chromium picolinate on growth performance, carcass quality and serum traits in broiler chicks. Asian-Australasian J. Anim. Scis. 1996;9:341-7.

36. Toghyani M, Toghyani M, Shivazad M, Gheisari A, Bahadoran R. Chromium supplementation can alleviate the negative effects of heat stress on growth performance, carcass traits, and meat lipid oxidation of broiler chicks without any adverse impacts on blood constituents. Biol. Trace Elem. Res. 2012;146:171-80. https://doi.org/10.1007/s12011-011-9234-3

37. Schrauzer GN, Shrestha KP, Molenaar TB, Mead S. Effects of chromium supplementation on food energy utilization and the trace-element composition in the liver and heart of glucose-exposed young mice. Biol. Trace Elem. Res. 1986;9:79-87. https://doi.org/10.1007/BF02916517

38. Mir SH, Pal RP, Mani V, Malik TA, Ojha L, Yadav S. Role of dietary minerals in heat-stressed poultry: A review. J. Entomol. Zool. Stud. 2018;6:820-6.

39. Sirirat N, Lu J, Hung AT, Chen S, Lien T. Effects different levels of nanoparticles chromium picolinate supplementation on growth performance, mineral retention, and immune responses in broiler chickens. J. Agric. Sci. 2012;4:48. http://dx.doi.org/10.5539/jas.v4n12p48

40. Amatya J, Haldar S, Ghosh T. Effects of chromium supplementation from inorganic and organic sources on nutrient utilization, mineral metabolism and meat quality in broiler chickens exposed to natural heat stress. Anim Sci. 2004;79:241-53. https://doi.org/10.1017/S135772980009010X

41. Ghazi S, Habibian M, Moeini M, Abdolmohammadi A. Effects of different levels of organic and inorganic chromium on growth performance and immunocompetence of broilers under heat stress. Biol. Trace Elem.

Res. 2012;146:309-17. https://doi.org/10.1007/s12011-011-9260-1

42. Selhorst P, Van Ierssel SH, Michiels J, Mariën J, Bartholomeeusen K, Dirinck E, Vandamme S, Jansens H, Ariën K. Symptomatic severe acute respiratory syndrome coronavirus 2 reinfection of a healthcare worker in a Belgian nosocomial outbreak despite primary neutralizing antibody response. Clin. Infect. Dis. 2021;73:e2985-e91. https://doi.org/10.1093/cid/ciaa1850

43. Ruytinx P, Vandormael P, Fraussen J, Pieters Z, Thonissen S, Hellings N, Stinissen P, Callebaut I, Penders J, Vanhove K, Kieffer D, Rummens J, Valkenborgh T, Messiaen P, Stessel B, Mesotten D, Somers V. Comprehensive antibody and cytokine profiling in hospitalized COVID-19 patients in relation to clinical outcomes in a large Belgian cohort. Sci. Rep. 2023;13:19322. https://doi.org/10.1038/s41598-023-46421-4

44. Kusakabe T, Lin W-Y, Cheong J-G, Singh G, Ravishankar A, Yeung ST, Mesko M, DeCelie MB, Carriche G, Zhao Z, Rand S, Doron I, Putzel G, Worgall S, Cushing M, Westblade L, Inghirami G, Parkhurst C, Guo C, Schotsaert M, García-Sastre A, Josefowicz S, Salvatore M, Iliev I. Fungal microbiota sustains lasting immune activation of neutrophils and their progenitors in severe COVID-19. Nat. immunol. 2023;24:1879-89. https://doi.org/10.1038/s41590-023-01637-4

45. Sahin K, Sahin N, Kucuk O. Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature (32 °C). Nutr. Res. 2003;23:225-38. https://doi.org/10.1016/S0271-5317(02)00513-4

46. Bahrami A, Moeini M, Ghazi S, Targhibi M. The effect of different levels of organic and inorganic chromium supplementation on immune function of broiler chicken under heat-stress conditions. J. Appl. Poult. Res. 2012;21:209-15. https://doi.org/10.3382/japr.2010-00275

47. Ebrahimzadeh S, Farhoomand P, Noori K. Immune response of broiler chickens fed diets supplemented with different level of chromium methionine under heat stress conditions. Asian-Australasian J. Anim. Sci. 2012;25:256.https://doi: 10.5713/ajas.2011.11217

48. Huang C, Feng W, Xiong J, Wang T, Wang W, Wang C, Yang F. Impact of drying method on the nutritional value of the edible insect protein from black soldier fly (Hermetia illucens L.) larvae: amino acid composition, nutritional value evaluation, *in vitro* digestibility, and thermal properties. Eur. Food Res. Technol. 2019;245:11-21. https://doi.org/10.1007/s00217-018-3136-y

49. Toghyani M, Zarkesh S, Shivazad M, Gheisari A. Immune responses of broiler chicks fed chromium picolinate in heat stress condition. J. Poult. Sci. 2007;44:330-4. https://doi.org/10.2141/jpsa.44.330

Table 1. Ingredient composition of basal diets

Items	Pre-starter, d 0 to 7	Starter, d 8 to 14	Grower, d 15 to 21	Finisher, d 22 to 35
Ingredient, %				
Corn fine	31.66	35.15	39.68	43.00
Wheat fine	19.46	19.96	20.46	19.96
Rice bran	2.79	2.79	3.49	2.79
Soybean meal 45%	26.18	19.14	17.03	14.97
DDGS 28%	3.49	6.99	3.49	3.49
Greaves	6.05	5.37	5.55	5.42
Poultry meal	2.56	2.69	2.69	3.12
Animal fats	2.11	2.00	2.00	2.15
L-lysine SO_4 55%	0.87	1.06	0.98	0.88
DL-methionine 99%	0.53	0.53	0.57	0.57
L-threonine 98%	0.26	0.32	0.30	0.26
L-tryptophan 10%	0.18	0.41	0.38	0.32
Arginine 98%	0.01	0.01	0.01	0.01
Salt	0.15	0.15	0.15	0.15
Limestone	1.23	1.12	1.01	0.90
Mono-dicalcium phosphate	1.68	1.53	1.38	1.24
Sodium bicarbonate	0.10	0.10	0.10	0.10
Mineral premix[1)]	0.20	0.20	0.20	0.20
Vitamin premix[2)]	0.14	0.14	0.14	0.14
Liquid-Choline 50%	0.09	0.09	0.08	0.07
Zeolite	0.10	0.10	0.10	0.10
Mold-Zero-Liquid	0.05	0.05	0.05	0.05
Phytase 1000[3)]	0.11	0.11	0.11	0.11
Total	100.00	100.00	100.00	100.00
Calculated value				
Metabolic energy, kcal/kg	3000	3020	3070	3100
Moisture, %	12.057	12.186	12.293	12.444
Crude protein, %	23.000	21.170	20.040	19.000
Crude fat, %	5.699	5.700	5.781	5.743
Crude fiber, %	3.591	3.767	3.489	3.104
Crude ash, %	5.883	5.581	5.290	5.032
Calcium, %	0.850	0.770	0.710	0.650
Phosphorus, %	0.675	0.618	0.616	0.579
Lysine, %	1.627	1.554	1.445	1.341
Methionine, %	0.860	0.841	0.849	0.849
Sulfur amino acid, %	1.236	1.207	1.202	1.191
Threonine, %	1.086	1.056	0.989	0.924
Tryptophan, %	0.270	0.257	0.239	0.221

[1)]Provided per kg of diet: 35 mg of Fe, 0.2 mg of Co, 93 mg of Cu, 65 mg of Mn, 65 mg of Zn, 0.65 mg of I, 0.20 mg of Se, 0.20 mg of Molybdenum.
[2)]Provided per kg of diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D_3, 37.5 mg of vitamin E, 2.55 mg of vitamin K_3, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B_6, 24 μg of vitamin B_{12}, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin and 13.5 mg of pantothenic acid.
[3)]Phytase was supplemented at 1,000 FTU/kg using Ronozyme® HiPhos (DSM Nutritional Products, Basel, Switzerland

413

414

Table 2. Effects of different levels of supplemental chromium on growth performance in broilers under heat stress

	PC	NC	CP2	CP4	SE	*p*-value
BW						
d 0	37.99	38.01	37.94	37.99	0.056	0.812
d 14	370.76	378.52	378.95	381.07	3.930	0.287
d 21	955.78a	878.75b	914.06ab	932.92a	11.987	<0.001
d 35	2222.81a	2011.88c	2088.13bc	2120.63ab	27.745	<0.001
d 0 to 14						
BWG	332.77	340.51	341.02	343.07	3.938	0.288
FI	447.69	451.53	455.49	455.33	6.694	0.821
FCR	1.36	1.33	1.34	1.33	0.023	0.886
Mortality, %	0.00	0.00	0.00	0.00	-	-
d 15 to 21						
BWG	585.02a	500.23b	535.11ab	551.85a	12.917	<0.001
FI	797.85a	692.10c	745.52b	761.01b	9.009	<0.001
FCR	1.38	1.42	1.40	1.39	0.045	0.917
Mortality, %	0.00	0.00	0.00	0.00	-	-
d 22 to 35						
BWG	1267.03a	1133.13b	1174.06ab	1187.71ab	24.767	0.006
FI	2225.22a	2189.65ab	2170.34b	2195.45ab	12.029	0.026
FCR	1.77b	1.96a	1.87ab	1.87ab	0.037	0.016
Mortality, %	0.00	0.00	0.00	0.00	-	-
d 0 to 35						
BWG	2184.82a	1973.87c	2050.19bc	2082.63ab	27.749	<0.001
FI	3470.75a	3333.27c	3371.35bc	3411.80ab	19.618	<0.001
FCR	1.60b	1.70a	1.66ab	1.65ab	0.022	0.025
Mortality, %	0.000	0.000	0.000	0.000	-	-
Production index	397.97a	338.67b	361.41ab	367.78ab	9.593	0.002

PC, birds fed a basal diet under thermoneutral temperature; NC, birds fed a basal diet and were exposed to cyclic heat stress at d 14 of age; CP2, NC with supplementing chromium 200 μg/kg as chromium propionate; CP4, NC with supplementing chromium 400 μg/kg as chromium propionate; BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; PI, Production index; SE, standard error.

a-c Means in the same row with different superscripts differ ($p < 0.05$).

415

416

Table 3. Effects of different levels of supplemental chromium on uniformity in broilers under heat stress

Items, %	PC	NC	CP2	CP4
D 0	99.44	99.58	99.45	99.47
D 7	93.64	92.90	91.60	93.04
D 14	91.88	92.70	92.91	92.82
D 21	90.83	90.92	92.76	92.35
D 35	91.22	91.65	91.71	91.46

PC, birds fed a basal diet under thermoneutral temperature; NC, birds fed a basal diet and were exposed to cyclic heat stress at d 14 of age; CP2, NC with supplementing chromium 200 μg/kg as chromium propionate; CP4, NC with supplementing chromium 400 μg/kg as chromium propionate.

417

418

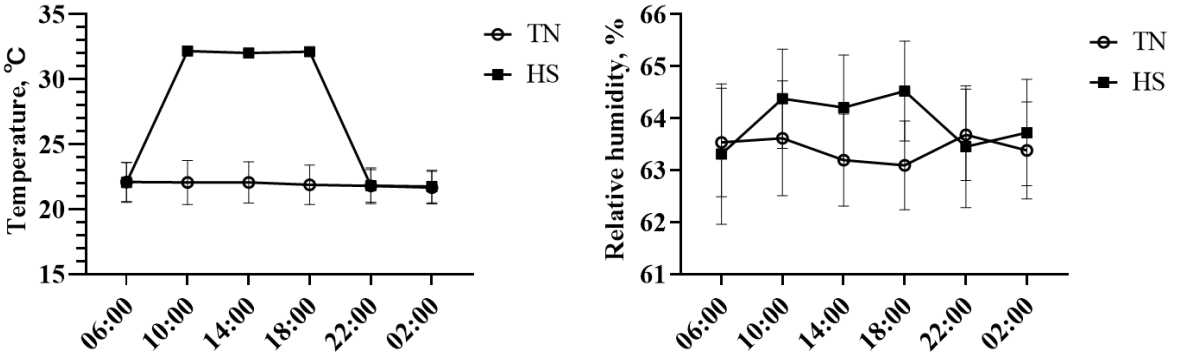
Table 4. Effects of different levels of supplemental chromium on serum minerals in broilers under heat stress

Items, mg/dL	PC	NC	CP2	CP4	SE	*p*-value
Ca	0.38	0.26	0.32	0.31	0.030	0.051
P	0.18	0.12	0.16	0.13	0.021	0.203
Cu	0.19	0.15	0.14	0.16	0.025	0.540
Zn	2.62	2.37	2.33	2.47	0.252	0.849
Cr	0.08	0.06	0.07	0.07	0.008	0.631

PC, birds fed a basal diet under thermoneutral temperature; NC, birds fed a basal diet and were exposed to cyclic heat stress at d 14 of age; CP2, NC with supplementing chromium 200 μg/kg as chromium propionate; CP4, NC with supplementing chromium 400 μg/kg as chromium propionate; SE, standard error.

419

420

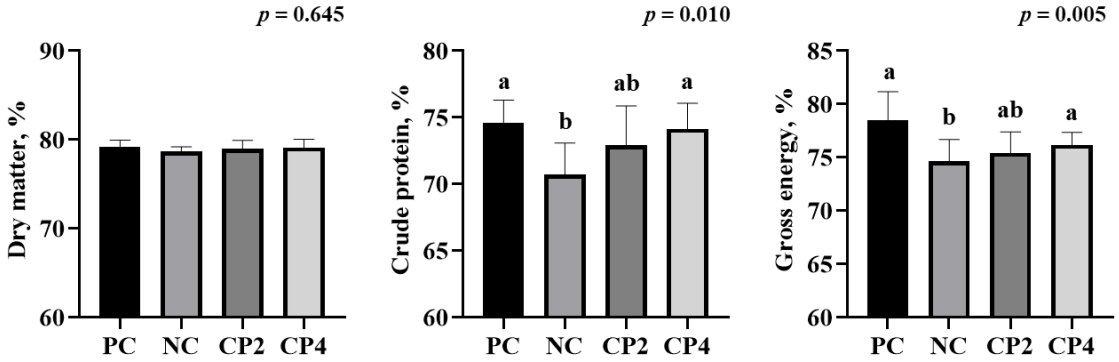


421

422 **Fig 1.** Temperature and relative humidity during the experimental period (d 15 to 35). TN,
423 thermoneutral temperature; HS, heat stress.

424

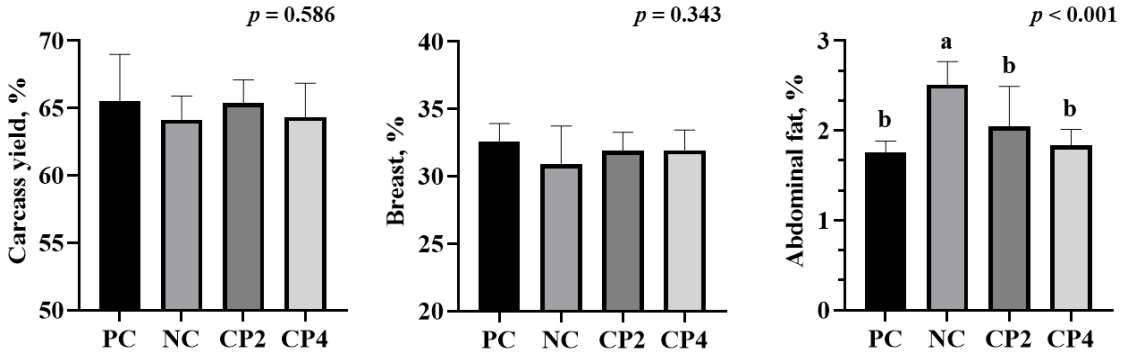
425



426

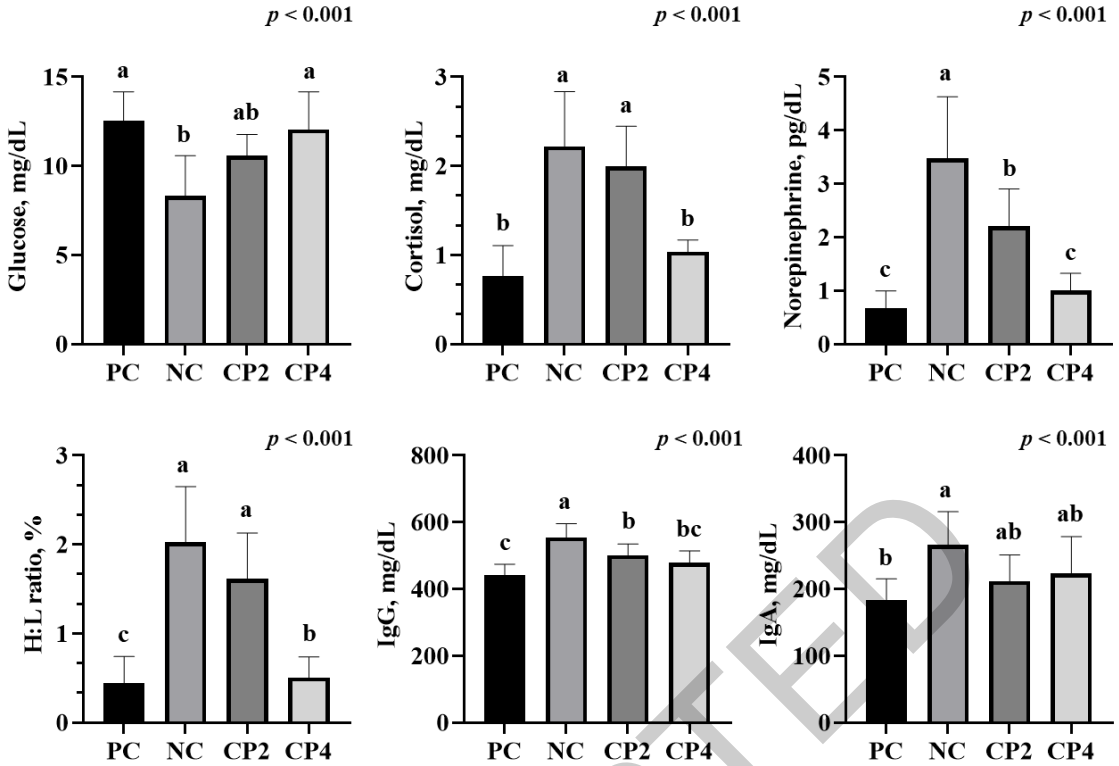
427 **Fig 2.** Effects of different levels of supplemental chromium on nutrient digestibility in
428 broilers under heat stress. PC, birds fed a basal diet under thermoneutral temperature; NC,
429 birds fed a basal diet and were exposed to cyclic heat stress at d 14 of age; CP2, NC with
430 supplementing chromium 200 μg/kg as chromium propionate; CP4, NC with
431 supplementing chromium 400 μg/kg as chromium propionate; SE, standard error; DM, dry
432 matter; CP, crude protein; GE, gross energy; SE, standard error.
433 a, b Means in the same row with different superscripts differ ($p < 0.05$).

434



435

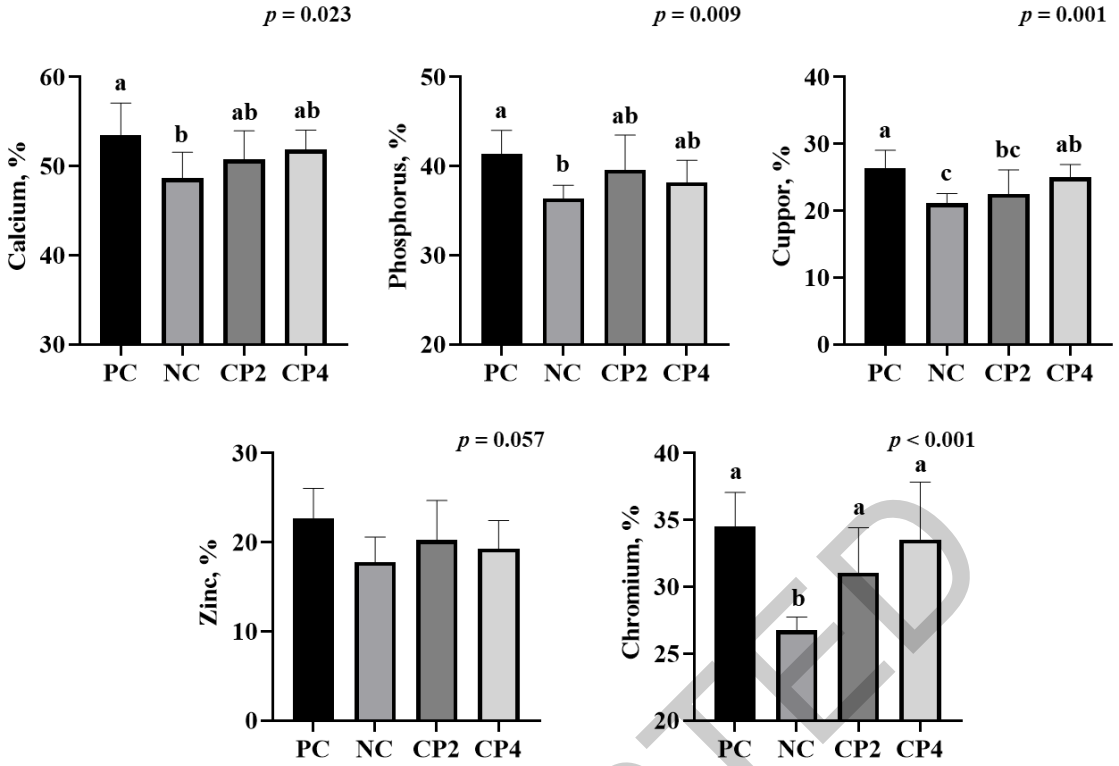
436 **Fig 3.** Effects of different levels of supplemental chromium on carcass traits in broilers under heat
437 stress. PC, birds fed a basal diet under thermoneutral temperature; NC, birds fed a basal diet and
438 were exposed to cyclic heat stress at d 14 of age; CP2, NC with supplementing chromium 200 μg/kg
439 as chromium propionate; CP4, NC with supplementing chromium 400 μg/kg as chromium
440 propionate; SE, standard error.
441 a, b Means in the same row with different superscripts differ ($p < 0.05$).
442



443

444 **Fig 4.** Effects of different levels of supplemental chromium on blood profile in broilers under heat
445 stress. PC, birds fed a basal diet under thermoneutral temperature; NC, birds fed a basal diet and
446 were exposed to cyclic heat stress at d 14 of age; CP2, NC with supplementing chromium 200 μg/kg
447 as chromium propionate; CP4, NC with supplementing chromium 400 μg/kg as chromium
448 propionate; H:L ratio, heterophil to lymphocyte ratio; IgG, immunoglobulin G; IgA, immunoglobulin
449 A; SE, standard error.
450 a-c Means in the same row with different superscripts differ ($p < 0.05$).
451

452



453

454 **Fig 5.** Effects of different levels of supplemental chromium on mineral retention in broilers under
455 heat stress. PC, birds fed a basal diet under thermoneutral temperature; NC, birds fed a basal diet
456 and were exposed to cyclic heat stress at d 14 of age; CP2, NC with supplementing chromium 200
457 μg/kg as chromium propionate; CP4, NC with supplementing chromium 400 μg/kg as chromium
458 propionate; SE, standard error.
459 a, b Means in the same row with different superscripts differ ($p < 0.05$).
460