

## Research Article

# Effects of Chromium (III) Picolinate and Chromium (III) Picolinate Nanoparticles Supplementation on Growth Performance, Organs Weight and Immune Function in Cyclic Heat Stressed Broiler Chickens

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### Abstract

This experiment conducted to investigate the effects of dietary chromium (III) picolinate (CrPic) and chromium (III) picolinate nanoparticles (Nano CrPic) supplementation on growth performance, organs weight and immune function of broilers exposed to heat stress. Heat stress (36°C) was applied for 10 h per day from the 21<sup>th</sup> to the 42<sup>nd</sup> days. Among 8 experimental treatments; only group T1 represented the non-heat stressed control group fed with a basal diet in comfort zone whereas group T2 represented the heat stressed control group fed with a basal diet. Heat stressed T3, T4, T5 groups were fed with basal diet supplemented with 500, 1000, 1500 ppb of CrPic/kg while T6, T7, T8 groups were fed with basal diet supplemented with 500, 1000, 1500 ppb of Nano CrPic/kg respectively. Results of the current experiment showed that the non-heat stressed group had a higher final BW, daily weight gain and daily feed intake compared with heat stressed groups during the experiment period (d 21-42), among heat stressed groups, FCR values improved by supplementation of Cr into the diet. NanoCrPic1500 treatment had the lowest ( $P < 0.05$ ) FCR (2.14) of the total experimental period among heat stressed groups. The liver weight values of the day 35 of experiment differed significantly ( $P < 0.05$ ). Serum complement component C3 of experimental broilers was severely affected by the Cr supplementation. The results indicated that the nanoparticle supplementation might be an influential method for reduction of heat stress induced disorders which may attribute to the lowering of FCR and provoking the hepatic related alteration including the liver weight.

**Keywords:** Chromium Picolinate Nanoparticles, Cyclic Heat Stress, Immune Function, Organ Weights, Performance.

### Introduction

Heat Stress (HS) is one of the most important commercial challenges that cause poor growth and has a negative influence on feed efficiency of broiler chickens. Broilers exposed to high environmental temperature display various behavioral and physiological disorders include feather pecking, tendency to inactivity,

increase of body temperature, panting and respiratory alkalosis [1,2]. Briefly, HS is considered by decreased feed consumption, reduced metabolic rate, high mortality, reduced body weight gain, high feed conversion ratio, and per oxidation of lipid, endocrine disorders, immunosuppression and intestinal microbial dysbiosis in poultry [3-6]. Also HS has been shown to decrease the total white blood cell count and antibody production [4], reduction of the peripheral blood lymphocytes number, induction of an electrolyte imbalance [7], decline spleen weight [8] and diminution CD4<sup>+</sup>

T cells (T-helper lymphocytes) and CD8<sup>+</sup> T cells (T-cytotoxic lymphocytes) [9].

Some strategies for resolving this problem have been proposed to manage the negative effects of heat stress, including environmental management, nutritional manipulation as well as the addition of feed additives in the diet. Chromium-supplemented diets have shown to be effective in diminishing the negative effects of stress and improving immunity in broilers [10,11]. Chromium is an ingredient of glucose tolerance factor (GTF) and is essential for carbohydrate, fat, and protein metabolism likely by potentiating the action of insulin (12). Stress and diseases lead to more urinary excretion of Cr and can cause exacerbating of marginal Cr deficiency [12]. Most poultry feedstuffs are mainly composed of plant source components, which are typically a low in contented of Cr [13]. It has been reported that the inclusion of CrPic in diet enhances daily gain, feed utilization and improve growth performance of broilers consumed low protein diets [14,15]. Chromium has been reported to have immunomodulatory properties [16-18], which is assumed to be an indirect effect of chromium on the secretion of glucocorticoids, because corticosteroids have a depressing effect on the immune system [19,20].

Recently, nanotechnology has rapidly been developing different scientific areas and nanoscale of materials has interested extension because nano-formulation particulates exhibit novel distinguishing quality such as a size, shape, large surface area, high surface activity, high catalytic efficiency and strong adsorb-

ing ability [21]. Limited published data in various experimental conditions implicated higher absorption and bioavailability of chromium nanoparticles [22-24]. Previous researches have shown that chromium nanoparticles had beneficial properties on growth performance, body composition, as well as augmented tissue concentrations of Cr in selected muscles [25] and serum [24]. Also and chromium nanoparticles can improve utilization of Zn, Fe and Ca of broiler chickens [23]. Therefore, the purpose of this study was to investigate the effects of the supplementation of CrPic and Nano CrPic on performance, organs weight and immune function of broiler chickens exposed to cyclic heat stress (10 h/d).

## Materials and Methods

All experiments were carried out under the ethical guidelines of the Islamic Azad University of Tehran Science and Research Branch (93/987, in 2014).

**Birds and Grouping:** A total of 480 broiler chickens (Ross 308), from 21 to 42 days old (equal in both sex), were used in a completely randomized design. Chickens were purchased from a commercial hatchery and were housed in floor pens covered with sterilized and contaminant-free wood shavings with 10 birds/m<sup>2</sup> and with water and food (hanging feeders) provided as *ad libitum*. The broiler chickens were observed for health status and behavior constantly. All chickens consumed a diet based on corn-soybean meal, which provided as mash form was formulated based on NRC 1996 by UFFDA software (Table 1).

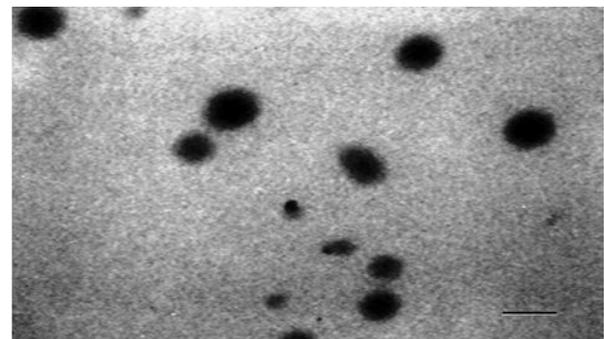
Ingredients (%)	Starter (1-21d)	Finisher (21 - 42 d)							
		Cont - (T1)	Cont + (T2)	Cr 500 (T3)	Cr 1000 (T4)	Cr 1500 (T5)	NCr 500 (T6)	NCr 1000 (T7)	NCr 1500 (T8)
Corn	60.7	66.0	66.0	66.0	66.0	66.0	66.0	66.0	66.0
Soybean Meal	30.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Corn Gluten Meal	2.5	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Soybean Oil	2.3	2.55	2.55	2.55	2.55	2.55	2.55	2.55	2.55
Dicalcium phosphate	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Limestone	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
L-Lysin	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
DL-Methionine	0.14	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral and vitamin mix l	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

CrPic (ppb/kg)	-	-	-	500	1000	1500	-	-	-
NanoCrPic (ppb/kg)	-	-	-	-	-	-	500	1000	1500
Calculated nutrient composition	Starter (1– 21d)					Finisher (21 – 42 d)			
ME (kcal/kg)	3120					3190			
CP (%)	21.1					19.0			
Lysine (%)	1.1					0.95			
Methionine (%)	0.5					0.4			
Calcium (%)	0.9					0.9			
Total phosphorus (%)	0.4					0.4			

**Table 1:** Ingredients and chemical composition of the experimental diets.

<sup>1</sup>Supplied per kilogram of diet: trans-retinyl acetate, 25 mg; cholecalciferol, 6 mg; menadione, 1.2 mg; thiamine, 2.3 mg; riboflavin, 8 mg; nicotinamide, 42 mg; choline chloride, 400 mg; calcium pantothenate, 10 mg; pyridoxine HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; cobalamin, 0.012 mg; Fe (from ferrous sulfate), 82 mg; Cu (from copper sulfate), 7.5 mg; Mn (from manganese sulfate), 110 mg; Zn (from zinc oxide), 64 mg; I (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.28 mg.

On day 21, the broiler chickens were weighed and, selected based on weight (630±50g), re-allocated into 8 different groups: a control- (thermo neutral) group and 7 independent heat-stressed groups and for each group of birds, 4 replications with 15 birds per box were made. Broilers in the control- (TN) and control+ (heat stress) groups were fed with no additive, whereas other groups were fed 500; 1000; or 1500 ppb of CrPic, or 500; 1000 or 1500 ppb of Nano CrPic, respectively. CrPic purchased from Sigma-Aldrich [(C<sub>18</sub>H<sub>12</sub>CrN<sub>3</sub>O<sub>6</sub>), Catalogue no. C4124, CAS Number = 14639-25-9, USA] and Nanoparticles of chromium picolinate were prepared using the method that described by Lin et al 2015, briefly” mixture of dry ingredients contained of 10 g of chromium and 2.5 g of dispersed reagent silica was added to 240 ml of 95% ethanol to make a semi-liquid mixture. The mixture was premixed for 1.5 h and then placed in a grind chamber with 500 g of 0.2 mm zirconium particles. The mixture was then ground for 1.5 h at 960 g. After the grinding, the mixture was passed through a 0.074 mm (200 mesh) sieve to remove large particles. The mixture was then oven-dried at 50 °C overnight. The chromium nanoparticle powder was passed through a 0.074 mm sieve again” (24), finally the nanoparticles size was determined by a transmission electronic microscope (Philips Bio Twin 100/The Netherland) according to *linet al* (24) and the average diameter of particles was 100 nm (Figure-1).



**Figure 1:** TEM image of Nano-chromium (the average diameter was 100 nm)

The birds of control group (C) were kept comfort zone temperature (23 ± 1°C from 21 to 28 day and 21 ± 1°C from 28-42 day). The birds of heat-stressed groups were kept under 36 ± 1°C ambient temperature from 08:00 to 18:00 h = 10 h/d (from 21-42 day). From 18:00 to 08:00 h, the environmental temperature of the heat-stressed groups was reduced to the equal to that of the control- group. The birds in 42 days old were euthanized by cervical dislocation.

**Vaccination:** All chickens were immunized intramuscularly with a killed vaccine of Newcastle virus at 8 d of age (Nobilis ND La-Sota, Intervet/Schering-Plough Animal Health, Boxmeer, and the Netherlands). Live Newcastle disease vaccine was administered orally (drinking water) at 22 d of age.

**Performance Parameters:** Broilers performance was assessed for mortality rate, BW gain, feed consumption per bird, and feed conversion. The feed conversion ratio was calculated on the basis of feed intake/gain for each replicate. Data were collected during the experimental period (ED21 to ED42).

**Organ weights:** On ED28 and ED35 immediately after weighing, 8 birds per group (2 birds per pen) were randomly selected and euthanized by cervical dislocation. At necropsy, heart, liver and lymphoid organs (spleen, and bursa of Fabricius) were then picked up for relative weight determinations,  $\pm 0.01$  g (connective tissue was removed before weighing).

**Plasma C3 and C4:** At the end of experiment period 8 birds per pen were randomly selected, and blood samples were collected from the wing vein. Blood samples were kept to at 4°C to coagulate, then samples centrifuged at  $3,000 \times g$  for 10 min (at 4°C) to separation of serum. Blood serum samples were stored at -20°C until they were analyzed. Serum complement component 3 (C3), and complement component 4 (C4) concentrations were determined by commercial kits (Jiancheng Biological Engineering Research Institute, Nanjing, China, Cat. Nos. E032 and E033). The

procedure was carried out according to the advised protocol of the company.

**Statistical Analysis:** All data were subjected to a one-way ANOVA procedure of SPSS 19.0 for Windows [26], and the differences among means were separated by Duncan's multiple range test. A probability value of less than 0.05 was described to be statistically significant. Because there was no mortality during the experimental period so it has not been analyzed statistically.

## Results

### Performance parameters

The effects of different treatments on BW gain, feed intake and feed conversion ratio (FCR) throughout the experiment are presented in Table 2.

Items	Treatments								
	Control (-)	Control (+)	Cr 500	Cr 1000	Cr 1500	NCr 500	NCr 1000	NCr 1500	SEM
<b>21-28d (1th week)</b>									
Initial weight (day 21) (kg bird <sup>-1</sup> )	0.593	0.661	0.591	0.621	0.619	0.625	0.638	0.615	0.0087
Final weight (day 28) (kg bird <sup>-1</sup> )	0.874 <sup>a</sup>	0.872 <sup>a</sup>	0.837 <sup>ab</sup>	0.855 <sup>ab</sup>	0.832 <sup>ab</sup>	0.805 <sup>b</sup>	0.822 <sup>ab</sup>	0.870 <sup>a</sup>	0.0063 <sup>*</sup>
Daily feed intake (kg bird <sup>-1</sup> )	0.068 <sup>c</sup>	0.083 <sup>a</sup>	0.082 <sup>a</sup>	0.082 <sup>a</sup>	0.077 <sup>ab</sup>	0.072 <sup>bc</sup>	0.081 <sup>a</sup>	0.080 <sup>a</sup>	0.0010 <sup>**</sup>
Daily weight gain (kg bird <sup>-1</sup> )	0.040	0.032	0.031	0.036	0.033	0.035	0.035	0.035	0.0010
FCR	1.72 <sup>b</sup>	2.62 <sup>a</sup>	2.66 <sup>a</sup>	2.28 <sup>ab</sup>	2.36 <sup>ab</sup>	2.07 <sup>ab</sup>	2.32 <sup>ab</sup>	2.29 <sup>ab</sup>	0.0951 <sup>*</sup>
<b>29-35d (2th week)</b>									
Final weight (kg bird <sup>-1</sup> )	1.595 <sup>a</sup>	1.367 <sup>b</sup>	1.437 <sup>b</sup>	1.350 <sup>b</sup>	1.354 <sup>b</sup>	1.325 <sup>b</sup>	1.392 <sup>b</sup>	1.374 <sup>b</sup>	0.0196 <sup>*</sup>
Daily feed intake (kg bird <sup>-1</sup> )	0.154 <sup>a</sup>	0.154 <sup>a</sup>	0.155 <sup>a</sup>	0.149 <sup>ab</sup>	0.148 <sup>ab</sup>	0.140 <sup>b</sup>	0.150 <sup>ab</sup>	0.148 <sup>ab</sup>	0.0011 <sup>**</sup>
Daily weight gain (kg bird <sup>-1</sup> )	0.102 <sup>a</sup>	0.063 <sup>c</sup>	0.092 <sup>ab</sup>	0.069 <sup>bc</sup>	0.075 <sup>bc</sup>	0.066 <sup>bc</sup>	0.081 <sup>bc</sup>	0.068 <sup>bc</sup>	0.0033 <sup>**</sup>
FCR	1.51 <sup>c</sup>	2.44 <sup>a</sup>	1.68 <sup>bc</sup>	2.16 <sup>ab</sup>	1.97 <sup>abc</sup>	2.12 <sup>abc</sup>	1.85 <sup>bc</sup>	2.18 <sup>ab</sup>	0.0756 <sup>*</sup>
<b>36-42d (3th week)</b>									
Daily feed intake (kg bird <sup>-1</sup> )	0.221 <sup>a</sup>	0.167 <sup>bc</sup>	0.179 <sup>b</sup>	0.162 <sup>c</sup>	0.164 <sup>c</sup>	0.165 <sup>bc</sup>	0.165 <sup>bc</sup>	0.170 <sup>bc</sup>	0.0038 <sup>**</sup>
Daily weight gain (kg bird <sup>-1</sup> )	0.082 <sup>ab</sup>	0.064 <sup>ab</sup>	0.071 <sup>ab</sup>	0.070 <sup>ab</sup>	0.070 <sup>ab</sup>	0.067 <sup>ab</sup>	0.059 <sup>b</sup>	0.089 <sup>a</sup>	0.0025 <sup>**</sup>
FCR	2.70 <sup>a</sup>	2.61 <sup>a</sup>	2.52 <sup>ab</sup>	2.31 <sup>ab</sup>	2.34 <sup>ab</sup>	2.46 <sup>ab</sup>	2.80 <sup>a</sup>	1.91 <sup>b</sup>	0.0735 <sup>*</sup>
<b>21-42d (total)</b>									
Final weight (kg bird <sup>-1</sup> )	2.176 <sup>a</sup>	1.763 <sup>c</sup>	1.986 <sup>b</sup>	1.769 <sup>c</sup>	1.840 <sup>bc</sup>	1.730 <sup>c</sup>	1.811 <sup>c</sup>	1.849 <sup>bc</sup>	0.0313 <sup>*</sup>
Daily feed intake (kg bird <sup>-1</sup> )	0.148 <sup>a</sup>	0.135 <sup>bc</sup>	0.139 <sup>b</sup>	0.131 <sup>cd</sup>	0.130 <sup>cd</sup>	0.126 <sup>d</sup>	0.132 <sup>cd</sup>	0.132 <sup>c</sup>	0.0013 <sup>**</sup>
Daily weight gain (kg bird <sup>-1</sup> )	0.075 <sup>a</sup>	0.053 <sup>c</sup>	0.065 <sup>b</sup>	0.058 <sup>bc</sup>	0.059 <sup>bc</sup>	0.056 <sup>bc</sup>	0.058 <sup>bc</sup>	0.064 <sup>b</sup>	0.0015 <sup>**</sup>
FCR	1.97 <sup>b</sup>	2.55 <sup>a</sup>	2.14 <sup>ab</sup>	2.26 <sup>ab</sup>	2.20 <sup>ab</sup>	2.25 <sup>ab</sup>	2.28 <sup>ab</sup>	2.06 <sup>b</sup>	0.0403 <sup>**</sup>

**Table 2:** Effects of CrPic and Nano CrPic supplementation on production performance of heat stressed broilers on experimental period (day 21-42).

<sup>a-d</sup> different superscript letters indicate a significant difference between data presented in the same row, \* = (P < 0.05), \*\* = (P < 0.01).

The control group (TN group) had a higher final BW, daily weight gain and daily feed intake compared with heat stressed groups during the experiment period (d 21-42), whereas, all of these parameters improved significantly with the Cr inclusion to diet with different dosage and particle size. Moreover, the FCR value of control group (1.97) was found lower (P < 0.01) than that in heat-stressed groups and treatment Nano CrPic 1500 (2.06) had the lowest (P < 0.01) FCR value among heat stressed groups. Besides, as there was no mortality during the experimental period it has not been reported.

## Organ weight

The effect of different treatments on organs weight of broilers has been summarized in (Table 3).

Items	Treatments								SEM
	Contr -	Cont <sup>+</sup>	Cr500	Cr1000	Cr1500	NCr500	NCr1000	NCr1500	
Day 28(% live weight)									
Heart	0.59 <sup>ab</sup>	0.58 <sup>abc</sup>	0.46 <sup>c</sup>	0.50 <sup>bc</sup>	0.56 <sup>abc</sup>	0.62 <sup>a</sup>	0.57 <sup>abc</sup>	0.52 <sup>abc</sup>	0.0149*
Liver	2.58	2.59	2.4	2.53	2.5	2.77	2.4	2.66	0.051
Spleen	0.08	0.1	0.1	0.09	0.1	0.07	0.1	0.07	0.0044
Bursa of Fabricius	0.29 <sup>a</sup>	0.22 <sup>ab</sup>	0.23 <sup>ab</sup>	0.23 <sup>ab</sup>	0.22 <sup>ab</sup>	0.23 <sup>ab</sup>	0.29 <sup>a</sup>	0.24 <sup>ab</sup>	0.0110*
<b>Day 35 (% live weight)</b>									
Heart	0.58	0.54	0.46	0.51	0.53	0.54	0.49	0.47	0.013
Liver	2.66 <sup>b</sup>	2.65 <sup>b</sup>	3.10 <sup>ab</sup>	3.06 <sup>ab</sup>	2.64 <sup>b</sup> 3.75 <sup>a</sup>	2.67 <sup>b</sup>	3.25 <sup>ab</sup>		0.0983*
Spleen	0.07	0.09	0.13	0.08	0.1	0.11	0.11	0.1	0.0067
Bursa of Fabricius	0.21	0.18	0.22	0.19	0.21	0.18	0.17	0.19	0.0093

**Table 3:** Effect of CrPic and Nano CrPic supplementation on organs weight (percentage of live body weight) at 28 and 35 days of age.

<sup>a-d</sup> different superscript letters indicate a significant difference between data presented in the same row ( $P < 0.05$ ).

Data belongs to two consecutive weeks of experiment (4<sup>th</sup> to 5<sup>th</sup> weeks). At the end of the 4th week (one week after CrPic addition), there was a significant difference ( $P < 0.05$ ) between the heart and bursa of Fabricius percentage among experimental treatments. Also, the spleen and liver weights were not affected significantly. As well, in the 5th week of finishing period (d 35), Nano CrPic 500 (T6) had higher liver weight (3.75% of live weight) in comparison with other treatments ( $P < 0.05$ ).

## Immune Function

The effects of CrPic and Nano CrPic supplementation on serum complement components in heat stressed broilers are shown in Table 4.

Items (mg/MI)	Treatments								SEM
	Cont -	Cont +	Cr 500	Cr 1000	Cr 1500	NCr 500	NCr 1000	NCr 1500	
C3	0.197 <sup>ab</sup>	0.173 <sup>b</sup>	0.174 <sup>b</sup>	0.179 <sup>b</sup>	0.181 <sup>b</sup>	0.190 <sup>ab</sup>	0.194 <sup>ab</sup>	0.225 <sup>a</sup>	0.0042**
C4	0.122	0.119	0.117	0.120	0.115	0.117	0.116	0.129	0.0029

**Table 4:** Effect of CrPic and Nano CrPic supplementation on serum complement components in heat stressed broilers.

<sup>a-d</sup> different superscript letters indicate a significant difference between data presented in the same row ( $P < 0.01$ ).

The results indicated that, serum complement component C3 of experimental broilers was considerably affected by Cr addition. The concentrations of serum complement component C3 in the birds fed 1500 ppb Nano CrPic diet (0.225 mg/mL) were greater than birds fed chromium free or other levels of Cr and Nano Cr diet from d 21 to 42 ( $P < 0.01$ ). No significant differences were observed in serum complement component C4 concentrations among the control and test groups, although C4 values increased in some CrPic and Nano CrPic treatments but not significantly ( $P > 0.05$ ).

## Discussion

Heat stress (36°C), applied for 10<sup>th</sup> per day from the 21<sup>th</sup> to the 42<sup>nd</sup> days of life decreased performance parameters in broil-

ers in the present study. These data are in agreement with those reported elsewhere in similar contexts [5,6, 27,28]. Chromium was reported to modulate feed intake of heat stressed chickens [27, 29,30], and our findings are corresponded with those documents. Also, the data showed that body weight ( $P < 0.05$ ) and daily weight gain ( $P < 0.01$ ) improved with Cr addition significantly, and these are in agreement with (30). Concerning to performance observations, it is well established that stress and disease exacerbate urinary excretion of Cr and this occurrence can worth a marginal Cr deficiency (12). Cr is usually recognized as the active component in the Glucose Tolerance Factor (GTF), which increases the sensitivity of tissue receptors to insulin and subsequent increase in glucose uptake by cells and finally increases oxidation of glucose.

It was assumed that increased glucose uptake, reduced blood glucose and increased appetite should increase feed intake. Increased feed intake will tend to increase BW gain, because of improving amino acid and other nutrients consumption by tissues and muscle cells and increase protein retention which tends to increased body weight.

In the current study, the Nano CrPic administration resulted in influential effects on feed conversion ratio in the experimental period. It is theorized that when nutrients are digested, large particle size is degraded into small particle size, so they can be absorbed easily through the intestinal mucosa. In addition, the surface area of particles will increase and then enhance the digestion. Therefore, feed at nanoparticle scale may improve intestinal absorption. Some reports have indicated that nanoparticle drugs and minerals could increase absorption [31,32]. Reported that as compared with regular CrPic, the Nano CrPic significantly increased the CrPic digestibility in rats [33]. According to the description, nanoparticles of chromium absorbed more and easier and made a strong impact.

Significant differences ( $P < 0.05$ ) were found in the heart, liver and lymphoid organs (Bursa of Fabric us and spleen) weight of heat stressed chickens treated with general sized Cr and Nano sized Cr. The improvements in lymphoid organs are in agreement with [28,30], although, others reported no significant effects of Cr supplementation on lymphoid organs weight [11,34]. In regards to, Bartlett and Smith [35] suggest that the decrease in lymphoid organ weights might have been as a result of the reduction in feed consumption, so providing fewer nutrients for suitable growth of these organs under heat stress condition. Also, some findings showed that physiological stress is frequently associated with degeneration of lymphoid organs [36] but the effects of Cr on these organs regeneration are not clear yet.

Serum complement component C3 increased significantly ( $P < 0.01$ ), with CrPic and Nono CrPic addition to diet of heat stressed broilers and there were lots of reports that indicate improving immune function with Cr inclusion (10-11, 28, 30, 34), and our findings are in agreement with those data. Also Serum complement component C4 increased in some treatments, especially in treatment Nano CrPic 1500 (0.129 mg/mL), but not significantly ( $P > 0.05$ ). Reported that serum IgG concentration increased in broiler chickens supplemented with Cr under heat-stress conditions [10,34]. Moreover, [37] observed that total IgG increased in feeder calves receiving supplemental chromium nicotinate under stress. In addition, [38,39] reported that total IgG and IgM increased after transportation stress in calves supplemented with high-Cr yeast. To explain the improvement of immune function, heat stress induce a cascade of neural and hormonal events, beginning with hypothalamic stimulation and the production of corticotrophin-releasing factor, which stimulates the anterior pituitary to release

ACTH, and ending with stimulation of adrenal cortical tissue by ACTH to increase the production and release of corticosteroids (e.g. corticosterone and cortisol) primarily corticosterone, in birds [40]. Corticosterone prevents antibody production [41]. Reported that antibody production in young broiler chicks decreased in heat-stress conditions [42]. This decline might be indirectly owing to an increase in inflammatory cytokines under stress, which stimulates the hypothalamic production of corticotrophin-releasing factor [43]. Chromium supplementation is detected to enhance the immune response, either through a direct effect on the cytokines [17] or through the indirect effect of decreasing the glucocorticosteroid levels [19-20]. Observed that dietary chromium supplementation has an optimistic effect on the interleukin-6 levels in swine [44]. The exact mechanism by which Cr improves the immune system is not known. However, a reliable result showed that Cr reduces serum cortisol levels. It is possibly not surprising that depletion in serum cortisol content is one of the principal mechanisms by which Cr alleviates heat stress-related depression in immune competent of broilers. Additionally, [45] found out that Cr supplementation improved serum insulin concentration while noticeably decreasing corticosterone concentration in laying hens at a low ambient temperature. This is a typical metabolic relation between insulin (anabolic) and corticosterone (catabolic), in which they have opposite effects on metabolism. A Cr deficiency can disrupt the metabolism of carbohydrates and protein and reduce insulin sensitivity in peripheral tissues [29, 46]. Dietary Cr supplementation increased the plasma insulin concentration, indicating the physiological part of Cr in empowering the insulin to act as an insulin cofactor [45].

Nanoparticles are of great scientific concern as they are effectively a bridge between bulk materials and atomic or molecular structures. With the knowledge of that the particle, when its dimension is reduced to nanometer size, exhibits new electrical, magnetic, mechanical and biological properties. Previous reports have shown that nanoparticle drugs and minerals possibly will increase absorption [31,32]. Described that as compared with regular CrPic, the Nano CrPic significantly increased the CrPic digestibility in rats [33]. Furthermore, [25] and [47] reported that dietary supplementation with nanoparticle chromium increased the lean ratio, longissimus muscle area and tissue chromium content, while decreasing the fat ratio and back fat thickness in pigs. Pointed out that Nano size chromium in the rat diet can significantly increase the average body weight gain [22], feed efficiency and lean mass weight, and reduce the body fat ratio and serum insulin concentration. Besides, numerous studies have shown that nanoparticles are more inclined to be recognized by the immune system and ingested by immune cells, such as macrophages, monocytes and leukocytes [48]. However, reports concerning nanoparticle chromium supplementation in broiler diets are rare, as only three published reports [23,24,49] were found and there was not a considerable outstand marked study about using nanoparticles chromium in heat stressed

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broilers. Totally, although all of our findings were reaffirmation of chromium improve impact in heat stressed broilers, there were remarkable differences between normal and Nano sizes of Cr particles in this experiment that can be considered.

## Conclusions

We tested the details of Chromium and Nano chromium effects on heat stressed broilers in this study. We concluded that chromium and chromium nanoparticles will be effective in heat stress situations, but the results indicated that nanoparticles may be more effective, although more research is needed to firm further.

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