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Effects of Supplemental Chromium Nanoparticles on IFN-γ expression of Heat Stress Broilers

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Abstract

The aim of present study was to investigate the beneficial effect of chromium (III) picolinate (CrPic) and chromium (III) picolinate nanoparticles (NCrPic) addition on growth performance, stress-related hormonal changes, and serum levels of various immunity biomarkers, as well as the gene expression of IFN-γ in broilers exposed to heat stress conditions. Treatments included T1 which received the basal diet with no feed additive; T2 exposed to heat stress; T3, T4, and T5 containing 500, 1000, and 1500 ppb CrPic; as well as T6, T7, and T8 containing 500, 1000, and 1500 ppb NCrPic, respectively. After 2 weeks from CrPic and NCrPic supplementation, IFN-γ mRNA expression was assayed using the RT-PCR technique. The results showed that the lower body weight, daily weight gain, daily feed intake by heat stress, and the feed conversion ratio were recovered remarkably by CrPic and NCrPic supplements. The stress-elevated levels of cortisol and immunoglobulin were reduced significantly using CrPic and NCrPic supplementation ($P \leq 0.05$). The gene expression profile showed that the upregulated expression of IFN-γ was regulated by the addition of CrPic and NCrPic, in particular, to the diet; however, a full downregulation of IFN-γ expression was observed after week 2 of NCrPic supplementation. In conclusion, the results indicated that nanoparticle supplementation could be effective in reducing heat stress–induced detrimental alterations, thereby attributing to substantial changes to the immune system, including IFN-γ expression.

Keywords Anti-stress status · Broilers · Heat stress · IFN-γ expression · Nanoparticle chromium

Introduction

Heat stress (HS) is one of the major environmental concerns, which negatively affects the farm animals' performance. HS is characterized by endocrine disorders, decreased metabolic rates, increased lipid peroxidation, reduced feed intake (FI), body weight (BW) gain, a higher feed conversion ratio (FCR), immunosuppression, and intestinal microbial dysbiosis [1]. Many studies have confirmed the correlation between chromium (Cr) and metabolism under increased physiological, pathological, and nutritional stress conditions [2–4]. Heat stress may increase the urinary excretion of Cr, exacerbate a marginal Cr deficiency in broilers, and increase the Cr demand in humans and animals [5, 6].

Trivalent Cr enhances the immunological function, with its effects seemed to be more pronounced under HS conditions [7]. The anti-stress attributes of Cr are linked to its impact on pro-inflammatory cytokines, such as interleukin 6 [8]. In addition, it modulates the immune response through releasing cytokines [2, 7]. Bhagat et al. [9] observed the significant impact of Cr addition on interferon gamma (IFN-γ) mRNA expression in broilers' splenocytes and attributed an immune-modulatory role to Cr.

Chromium has been contributed to increase cell-mediated immune (CMI) responses in chickens [2]. CMI responses are mediated by type 1 T helper cells (TH1) that commonly generate IFN-γ and are related with inflammatory cytolytic responses, being routinely indispensable for destroying cells infected with viruses and other intracellular pathogens [9].

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IFN-γ induces a wide range of responses in epithelial cells. In addition, evidence verifies the effectiveness of IFN-γ in inducing anti-inflammatory responses [10, 11]. In nanotechnology, being a relatively new science, the properties of materials smaller than 100 nanometers are examined; in this respect, studies on chromium nanoparticles have shown that chromium nanoparticles are more absorbed in intestinal conditions, being more effective than regular chromium particles [12]. According to some studies, the NCrPic increases CrPic digestibility and serum chromium levels in broilers [12] and rats [13] significantly.

During the past decades, many studies have been done on the beneficial effects of Cr and its derivatives, including their immunomodulatory properties; however, their effects on the regulation of IFN-γ at the mRNA level in heat stress–exposed broilers have not been fully elucidated. Hence, the effects of Cr and Cr nanoparticle supplementation on growth performance, stress-related hormones, serum levels of various immunity biomarkers, and IFN-γ gene expression were examined at an mRNA level.

Materials and Methods

Ethical protocol (no. 93/987 - 2014) was approved by the experimental animal ethics committee of Islamic Azad University of Tehran Science and Research Branch, Tehran, Iran.

Birds and Experimental Design

A total of 480 broilers of both sexes, from day 21 to day 42, were randomly assigned in a CRD. The treatments included the negative control group (normal temperature; NT) and the positive control group (heat stress), which were fed with no additive, as well as 6 heat stress (HS) groups that were fed with a diet supplemented with 500, 1000, or 1500 ppb of CrPic or 500, 1000, or 1500 ppb of NCrPic, respectively. As Table 1 shows, the diets were balanced according to Ross 308 Broiler Nutrition Specifications [14]. On day 21, the chicks were weighed and classified into 8 different groups, with 4 replicates of 15 birds in each group. The chicks were monitored for their health and behavior constantly with water and feed provided to them ad libitum in the experimental period.

Chromium picolinate was purchased from Sigma-Aldrich ((C18H12CrN3O6), catalogue no. C4124, CAS no. 14639-25-9, USA), and the nanoparticles of chromium picolinate were provided by the wet polish technique using a ball grinding machine (DECO-PBM-V-4 L-A). The structure of nanoparticles was characterized with a scanning electron microscope (Philips Bio Twin 100, the Netherlands) according to Lin et al. [12], with the average diameter of the particles determined to be 100 nm (Fig. 1).

From day 21 to day 28, the control group (NT) was kept under the environmental temperature of 23 ± 1 °C, and from day 28 to day 42, it was kept under the environmental temperature of 21 ± 1 °C for the entire day. The chicks of the heat stress groups, separated by oilskin and tarpaulin fabric covers, were maintained under the environmental temperature of 36 ± 1 °C (heat stress) for 21 days (day 21 to day 42), with the temperature applied daily (from 08:00 a.m. to 06:00 p.m., 10 h a day), using some heaters at the end of the saloon with automatic thermal sensors. The experiment was terminated on day 42, with the chicks euthanized by cervical dislocation.

Performance Parameters

The performance parameters such as FI, BW and FCR were measured according to Abudabos et al. [15]. The data were collected during the experimental period, with the mortality rates noted.

Sample Collection and Organ-Related Weights

On day 42, 8 chicks per each group (2 chicks per each pen) were randomly selected and slaughtered using the cervical dislocation method [16]. At necropsy, the chicks' hearts, livers, spleens, and the bursa of Fabricius were taken to determine the relative weights of the organs.

Plasma Immunoglobulin Measurement (IgA, IgM, and IgG)

Eight broilers were selected randomly from each treatment group, with their blood samples taken and collected from their wing veins on day 42. Next, they were centrifuged at 3,000×*g* for 10 min at 4 °C and then were kept at − 20 °C. The IgA, IgM, and IgG concentrations were determined using the ELISA method (with the category numbers of H108, H109, and H106, respectively).

Stress-Related Hormones and Biochemical Alterations

The concentration of cortisol was measured by ELISA kit (MyBioSource, USA, Category Number of MBS265227). An ELISA reader system (IDEXX Inc., Westbrook, ME 04092, USA) was used to measure serum cortisol. Plasma cholesterol and triglycerides were analyzed spectrophotometrically, making use of commercial kits (Pars Azmoon Kits, product codes: 1 500 010 and 1 500 032).

Table 1 Ingredients and chemical composition of the experimental diets (based on Ross strain rearing catalogue)

Ingredients (%)	Starter (1–21 days)	Finisher (21–42 days)							
		Cont (T1)	HS (T2)	Cr500 (T3)	Cr1000 (T4)	Cr1500 (T5)	NCr500 (T6)	NCr1000 (T7)	NCr1500 (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-lysine. HCl	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[1]	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
Analyzed nutrient composition	Starter (1–21 days)	Finisher (21–42 days)							
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							
Available phosphorus (%)	0.4	0.4							

[1] 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

Interferon Gamma mRNA Changes

RNA Isolation and RT-PCR

To examine the effects of normal and nano-chromium on the IFN-γ expression, the total RNA was extracted from spleen using TRIZOL method [17, 18]. The amount of RNA was determined spectrophotometrically (260 nm and A260/280 = 1.8–2.0), with the samples stored at − 70 °C. For RT-PCR, cDNA was synthesized in a 20 μL reaction mixture containing 1 μg of RNA, the oligo (dT) primer (1 μL), a 5 × reaction buffer (4 μL), an RNAse inhibitor (1 μL), a 10 mM of dNTP mix (2 μL), and M-MuLV Reverse Transcriptase (1 ml), according to the manufacturer's protocol (Fermentas, GmbH, Germany). The cycling protocol for 20 μL reaction mixes included 5 min at 65 °C, followed by 60 min at 42 °C, and 5 min at 70 °C to terminate the reaction.

PCR Reaction

The RT-PCR reaction was triggered in the total volume of 25 μL, containing the PCR master mix (12.5 μL), FWD- and REV-specific primers (each 0.75 μL), cDNA as the template (1 μL), as well as the nuclease-free water (10 μL). PCR conditions were provided by general denaturation at 95 °C for 3 min, one cycle, followed by 40 cycles of 95 °C for 20 s, the annealing temperature of 63 °C for β-actin and 58 °C for IFN-γ for 30 s, as well as elongation at 72 °C for a minute

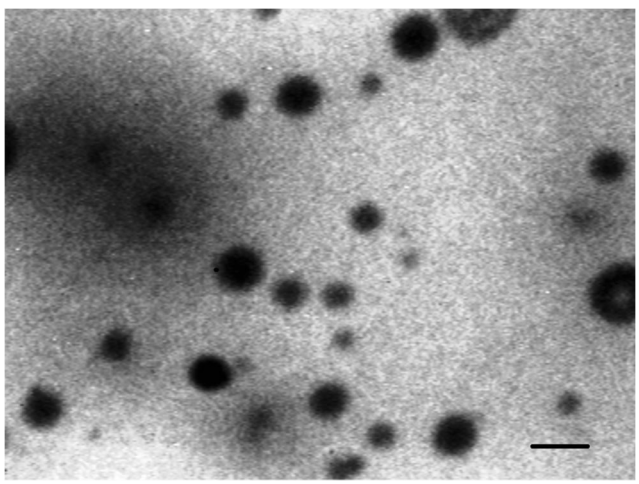

Fig. 1 TEM image of nano-chromium (bar line = 100 nm)

and at 72 °C for 5 min. The products of RT-PCR were separated on the 1.5% agarose gel containing ethidium bromide and were visualized using the Gel Doc 2000 system (Bio-Rad). The specific primers for Gallus IFN-gamma and GAPDH were designed and manufactured by CinnaGen (CinnaGen Co., Tehran, Iran). The primer pairs for RT-PCR are depicted in Table 2.

Statistical Analysis

Obtained data were analyzed using SPSS (19.0), with Duncan's multiple range test used for the comparison of means. The probability value of less than 0.05 was determined to be statistically significant. Following mathematical model was used for statistical analysis:

$$Y_{ijk} = \mu + T_i + \varepsilon_{ij}$$

where Y_{ijk}= a dependent variable, μ = overall mean, A_i = the effect of treatments, and ε_{ij} = the residual deviation of the observation from the effects in the model.

Results

Performance

Table 3 demonstrates the performance data. The final weight in the heat stress animals that received CrPic and NCrPic for 3 weeks increased significantly in comparison to the heat stress control; however, chicks treated with CrPic at 500 ppb and NCrPic at 1500 ppb doses showed a remarkable increase in their daily WG (Table 3). The FCR was significantly lower ($P < 0.01$) in CrPic- and NCrPic-treated broilers.

Organs' Relative Weight

Table 4 shows a summary of the effects of various treatments on organs' weight in broilers. There was a significant difference between the relative weights of the spleens and the bursa of Fabricius among experimental treatments ($P < 0.05$). In addition, the weights of the heart and the liver were not affected significantly.

Hormones and Serum Biochemical Parameters

In the anti-stress effects of CrPic and NCrPic under stress conditions, the cortisol concentration was measured as a stress-related factor (Table 5). The results demonstrated that the cortisol content of serum increased significantly ($P < 0.05$) on day 42 in the heat stress birds, while CrPic at 500 and NCrPic at 1500 ppb doses reduced the cortisol content of serum significantly in heat stress broilers (Table 5). Furthermore, the cholesterol content of the heat stress broilers increased significantly ($P < 0.05$) after CrPic and NCrPic treatments. In addition, the level of serum triglycerides was affected significantly by the addition of CrPic and NCrPic in different treatments.

The Immune Function

As Table 6 demonstrated, serum immunoglobulin analyses showed that in the CrPic- and NCrPic-treated birds, the important blood immunoglobulins, i.e., IgA, IgG, and IgM, increased significantly ($P < 0.01$). The dose of 1500 ppb CrPic has had the highest value among all immunoglobulins. In addition, immunoglobulins increased significantly upon the addition of NCrPic to the treatments.

Interferon Gamma Gene Expression

The mRNA level of IFN-γ-expressing splenocytes was determined using the PCR technique after 1 and 2 weeks of exposure to heat stress, with the results having been normalized against the mRNA level of IFN-γ-actin, a house-keeping gene (Fig. 2). The expression profile of IFN-γ after days 7 and 14 from treatment with CrPic and NCrPic showed a different feature, for a significant upregulation of IFN-γ expression was observed on day 7 in the group of birds that received 1000 ppb CrPic, while all three groups that received NCrPic showed the remarkable downregulation of IFN-γ expression. In the meantime, the 14-day treatment with CrPic at 1500 ppb resulted in the significant downregulation of IFN-γ

Table 2 Sequences of real time PCR primers for β-actin and IFN-γ (Bhagat et al. 2008)

RNA target	Primer sequence (5′–3′)	Accession number	Product size (bp)
β-actin			
F	CATCACCATTGGCAATGAGAGG	L08165	353
R	GCAAGCAGGAGTACGATGAATC		
IFN-γ			
F	TGAAGAACTGGACAGAGAGAAATGA	AJ634956	227
R	GGCTTTGCGCTGGATTCTCA		

Table 3 Effects of CrPic and NanoCrPic supplementation on production performance of heat stress broilers on experimental period (days 21–42)

Items	Treatments[1]								
	Cont	HS	Cr500	Cr1000	Cr1500	NCr500	NCr1000	NCr1500	SEM
Final weight (kg)	2.176^{a}	1.763^{c}	1.986^{b}	1.769^{c}	1.840^{bc}	1.730^{c}	1.811^{c}	1.849^{bc}	0.03^{*}
Daily feed intake (g bird^{-1})	148^{a}	135^{bc}	139^{b}	131^{cd}	130^{cd}	126^{d}	132^{cd}	132^{c}	1.34^{**}
Daily weight gain (g bird^{-1})	75^{a}	53^{c}	65^{b}	58^{bc}	59^{bc}	56^{bc}	58^{bc}	64^{b}	1.57^{**}
FCR (feed /gain)	1.97^{b}	2.55^{a}	2.14^{ab}	2.26^{ab}	2.20^{ab}	2.25^{ab}	2.28^{ab}	2.06^{b}	0.04^{**}

[a–d] Different superscript letters indicate a significant difference between data presented in the same row, $^{*}P < 0.05$, $^{**}P < 0.01$

[1] Cont = no stress no additive; HS = heat stress no additive; Cr500 = heat stress and CrPic at 500 ppb of diet; Cr1000 = heat stress and CrPic at 1000 ppb of diet; Cr1500 = heat stress and CrPic at 1500 ppb of diet; NCr500 = heat stress and NanoCrPic at 500 ppb of diet; NCr1000 = heat stress and NanoCrPic at 1000 ppb of diet; NCr1500 = heat stress and NanoCrPic at 1500 ppb of diet

expression, and all three given concentrations of NCrPic led to the substantial downregulation of IFN-γ expression.

Discussion

Results of current study demonstrated that the administration of CrPic and NCrPic supplements in cyclic heat stress chickens resulted in the side effects of typical anti-stress agents, including an increase in the daily WG and the ultimate body weight, having been consistent with other reports [19]. Besides, Cr was observed to modulate the FI of heat stress chickens, with our findings having been in line with some other studies [20–22]. Chromium is usually recognized as an active component in glucose metabolism, which enhances the sensibility of tissue receptors to insulin and glucose absorption by cells, thereby increasing the glucose oxidation. It is supposed that increased glucose absorption, diminished blood glucose, and increased appetite enhance FI. Increased FI may lead to an increase in the BW gain in the absence of malnutrition, mal-adsorption factors, and especially degenerative diseases. In the present study, administration of NCrPic resulted in significant effects on FCR. The addition of 1500 ppb nanoparticles of chromium resulted in 2.06 FCR, having been better than other treatments and too close to the control group. It is assumed that when large particles are converted into small particles using nanotechnology, they will easily absorb through the intestinal mucosa. Moreover, the particles' surface area will increase, thereby enhancing digestion. Thus, nanoparticle feed may increase intestinal absorption [12]. Some studies have reported that nanoparticle drugs and minerals could increase absorption [23–25]. Lien et al. [13] demonstrated that as against normal CrPic, the NCrPic significantly increased CrPic digestibility in rats. Pursuant to observations, chromium nanoparticles are absorbed more easily and exert a strong impact. Interestingly, the weight of lymphatic organs (bursa of Fabricius and spleen) in heat stress chickens were significantly ($P < 0.05$) affected by general size Cr and nano-sized Cr. Improvements in lymphoid organs are consistent with the studies done by Lu et al. [21] and Valera et al [26]; however, others reported no significant effect by Cr addition on the weight of lymphatic organs [27, 28]. In addition, some findings have showed that physiological stress is usually linked with the degeneration of lymphoid organs [29–31], yet the effects of Cr on the regeneration of these organs are not determined. Serum immunoglobulins have

Table 4 Effects of chromium and nanoparticles chromium supplementation on organs weight at 42 days of age

Items (% of live weight)	Treatments[1]								
	Cont	HS	Cr500	Cr1000	Cr1500	NCr500	NCr1000	NCr1500	SEM
Heart	0.56	0.59	0.46	0.50	0.54	0.47	0.43	0.54	0.01
Liver	2.74	2.66	2.82	2.80	2.49	3.02	2.56	2.85	0.06
Spleen	0.11^{a}	0.10^{ab}	0.09^{ab}	0.10^{ab}	0.09^{ab}	0.09^{ab}	0.07^{b}	0.07^{b}	0.00^{*}
Bursa of Fabricius	0.21^{a}	0.17^{ab}	0.24^{a}	0.23^{a}	0.18^{ab}	0.11^{b}	0.22^{a}	0.15^{ab}	0.01^{*}

[a–b] Means in the same row with different superscript letters differ significantly, $^{*}P < 0.05$

[1] Cont = no stress no additive; HS = heat stress no additive; Cr500 = heat stress and CrPic at 500 ppb of diet; Cr1000 = heat stress and CrPic at 1000 ppb of diet; Cr1500 = heat stress and CrPic at 1500 ppb of diet; NCr500 = heat stress and NanoCrPic at 500 ppb of diet; NCr1000 = heat stress and NanoCrPic at 1000 ppb of diet; NCr1500 = heat stress and NanoCrPic at 1500 ppb of diet

Table 5 Effect of chromium and nanoparticles chromium supplementation on serum cortisol, cholesterol, and triglycerides in heat stress broilers at 42 days of age

Items	Treatments[1]								SEM
	Cont	HS	Cr500	Cr1000	Cr1500	NCr500	NCr1000	NCr1500	
Cortisol (ng/ml)	5.403^{ab}	5.673^{a}	5.116^{b}	5.143^{b}	5.260^{b}	5.233^{b}	5.283^{b}	5.176^{b}	0.04*
Cholesterol (mg/dl)	125.33^{a}	90.33^{b}	122.00^{ab}	116.33^{ab}	120.33^{ab}	104.00^{ab}	124.33^{ab}	114.66^{ab}	3.80*
Triglycerides (mg/dl)	115.66^{ab}	102.66^{b}	131.00^{ab}	153.33^{ab}	142.00^{ab}	174.00^{ab}	179.33^{ab}	210.66^{a}	12.13*

$^{a–d}$ Means in the same row with different superscript letters differ significantly, $^{*}P < 0.05$

[1] Cont = no stress no additive; HS = heat stress no additive; Cr500 = heat stress and CrPic at 500 ppb of diet; Cr1000 = heat stress and CrPic at 1000 ppb of diet; Cr1500 = heat stress and CrPic at 1500 ppb of diet; NCr500 = heat stress and NanoCrPic at 500 ppb of diet; NCr1000 = heat stress and NanoCrPic at 1000 ppb of diet; NCr1500 = heat stress and NanoCrPic at 1500 ppb of diet

been reported to increase significantly ($P < 0.01$) upon the addition of CrPic and NCrPic to the diet of heat stress broilers, with numerous reports indicating the triggering of the immune function upon Cr inclusion [7, 19, 27, 32], with our findings being consistent with these studies. Bahrami et al. [7] indicated that the concentration of serum IgG increased in heat stress broilers supplemented with Cr. In addition, Huang et al. [33] noted that serum IgG and IgM increased in dietary supplementation of Cr. To illustrate the improvement in the immune function in the heat stress cases, it should be noted that heat stress induces a secretion of hormonal events. These events begin with hypothalamic excitation and production of the corticotropin-releasing factor, which induce the anterior pituitary to release ACTH, thereby leading to the instigation of the adrenal cortical tissue by ACTH to enhance the production and release of corticosteroids [26, 34]. Corticosterone prevents the production of antibodies [35]. Hirakawa et al. [36] concluded that heat stress conditions cause immune abnormalities in broiler chickens. Antibodies reduction might have been obliquely due to the increase in inflammatory cytokines under stress, which stimulates the hypothalamic production of the corticotropin-releasing factor [37, 38]. Chromium supplementation enhances the immune response or via a direct effect on cytokines [39], either the indirect efficacy of reducing the level of glucocorticoids [40, 41]. The accurate mechanism by which chromium improves the immune system is not yet fully known. Nonetheless, a trustworthy result showed that Cr reduced the levels of serum cortisol, as observed in the current study (Table 4). It may not be surprising that the depletion of the serum cortisol content is one of the main mechanisms by which Cr alleviates heat stress-related depression in immunocompetent broilers. In addition, according to Spears et al. [42] and Valera et al. [26], Cr addition increases the serum insulin concentration, yet it decreases the corticosterone concentration. There is a negative correlation between insulin (anabolic) and corticosterone (catabolic), in which they have opposite effects on metabolism. In addition, IFN-γ exerts its impact using various methods and is the most effective inducer of the reactive oxygen and nitrogen species (ROS-RNS) in target cells, such as macrophages. The immunomodulation that stimulates tryptophan degradation through the enzyme indoleamine 2, 3-dioxygenase is triggered during the cellular immune response, following the production of ROS-RNS by immunocompetent cells [43]. In this study, it was assumed that the upregulation of IFN-γ expression by the addition of CrPic and NCrPic could be a key factor in overcoming heat stress and the resulting immunosuppression. However, according to the results of the present study, the addition of Cr

Table 6 Effect of chromium and nanoparticles chromium supplementation on serum immunoglobulins in heat stress broilers

Items (mg/ml)	Treatments[1]								SEM
	Cont	HS	Cr500	Cr1000	Cr1500	NCr500	NCr1000	NCr1500	
IgA	0.397^{ab}	0.316^{cd}	0.320^{cd}	0.357^{bc}	0.439^{a}	0.278^{d}	0.321^{cd}	0.388^{ab}	0.01**
IgG	0.711^{ab}	0.591^{c}	0.616^{c}	0.603^{c}	0.758^{a}	0.601^{c}	0.654^{bc}	0.684^{b}	0.01**
IgM	1.035^{a}	0.931^{c}	0.964^{bc}	0.963^{bc}	1.053^{a}	0.918^{c}	1.016^{ab}	0.998^{ab}	0.01**

$^{a–d}$ Means in the same row with different superscript letters differ significantly, $^{**}P < 0.01$

[1] Cont = no stress no additive; HS = heat stress no additive; Cr500 = heat stress and CrPic at 500 ppb of diet; Cr1000 = heat stress and CrPic at 1000 ppb of diet; Cr1500 = heat stress and CrPic at 1500 ppb of diet; NCr500 = heat stress and NanoCrPic at 500 ppb of diet; NCr1000 = heat stress and NanoCrPic at 1000 ppb of diet; NCr1500 = heat stress and NanoCrPic at 1500 ppb of diet

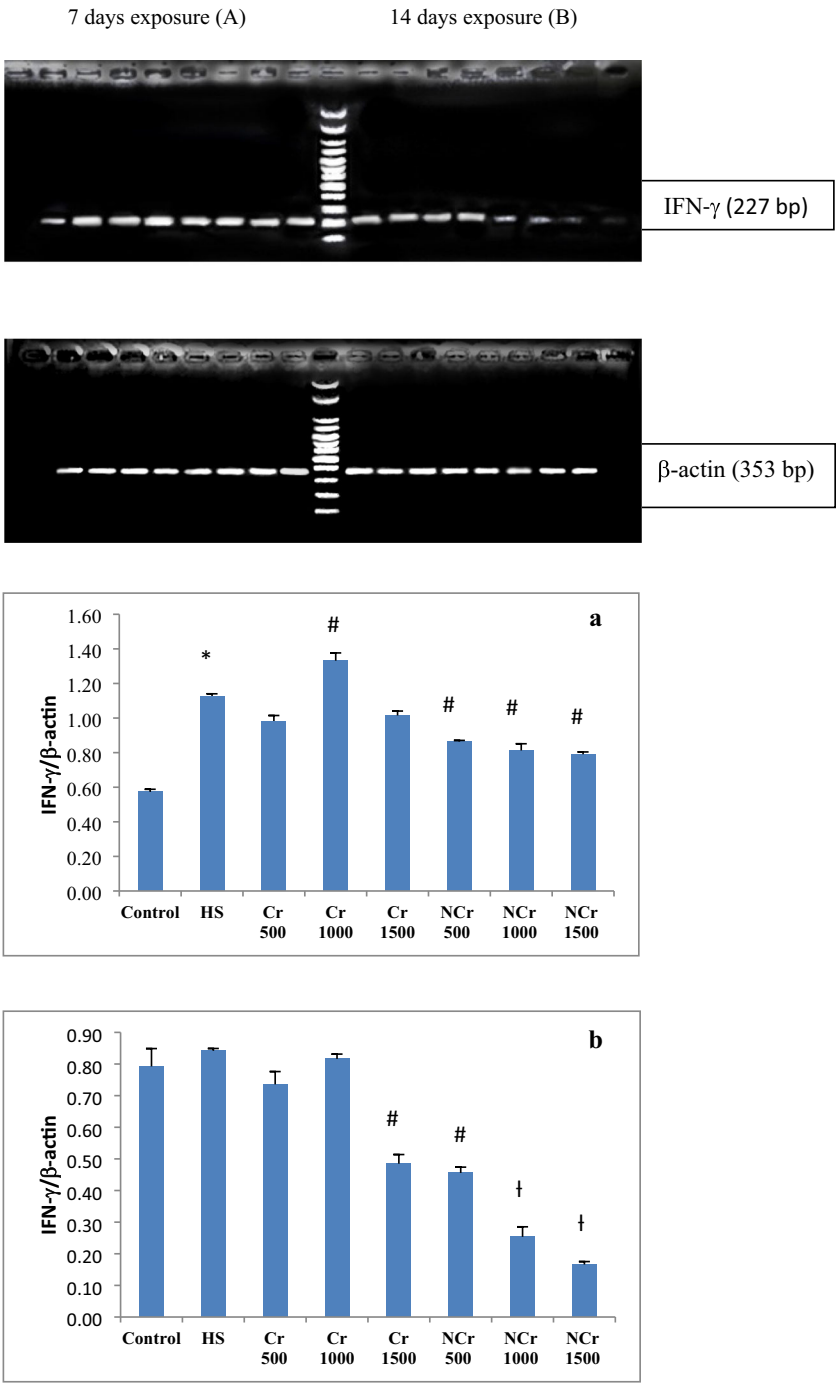

Fig. 2 Effect of different levels of chromium and nanoparticles of chromium on interferon gamma mRNA expression after 7 (**a**) and 14 (**b**) days CrPic and NCrPic inclusion in heat stress broilers. β-actin considered to be a housekeeping gene in our analysis. Star represents a significant different between the control and HS-non-treated groups and # and ǂ indicate significant differences between HS-treated groups

to the diet especially at 1000 ppb dosage upregulated IFN-γ mRNA expression after 1 or 2 weeks, yet the addition of NCrPic resulted in the downregulation of the proposed gene expression in a concentrated manner. These results may have been due to the genotoxic effects of the high levels of chromium on the diet chosen for use [44]. As mentioned in past research [45–47], the use of nano-size nutrients and nutritional materials makes them more prone to absorption in the GI tract. Since in the present study, there was no reliable reference for the suitable dose of NCrPic as against the normal sized Cr, we used the same doses of NCrPic.

Conclusions

The findings of the study demonstrate that both diets supplemented with CrPic and NCrPic could be effective in overcoming heat stress setbacks, including body weight gain and food

intake reduction. Moreover, they were determined to improve stress-related hormonal and immunological markers. In general, the effects of Cr derivatives on IFN-γ expression at the mRNA level were highlighted, and the use of NCrPic was shown to reduce the heat stress–upregulated expression of IFN-γ. In the end, it is recommended that the beneficial effects of both compounds be adjusted properly so as to prevent the toxic effects of the NCrPic supplement.

Authors' Contributions OH and MC contributed to the project idea, design and execution of the study. HG, AS, and HM were in charge of laboratory analyses. VP was responsible for scientific editing and finalizing the manuscript.

Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical protocol was approved by the experimental animal ethics committee of Islamic Azad University of Tehran Science and Research Branch, Tehran, Iran.

Conflict of Interest The authors declare no competing interests.

References

1. Sohail MU, Hume ME, Byrd JA, Nisbet DJ, Ijaz A, Sohail A, Shabbir MZ, Rehman H (2012) Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poult Sci 91:2235–2240. https://doi.org/10.3382/ps.2012-02182
2. Shabana N, Kuldeep D, Mani S, Ruchi T, Jeon G, Laudadio V, Tufarelli V (2014) Modes of action and beneficial applications of chromium in poultry nutrition, production and health: a review. Int J Pharmacol 10(7):357–367. https://doi.org/10.3923/ijp.2014.357.367
3. Farag MR, Alagawany M, El-Hack MEA, Muhammad A, Ayasan T, Kuldeep D, Kumaragurubaran K (2017) Role of chromium in poultry nutrition and health: beneficial applications and toxic effects. Int J Pharmacol 13(7):907–915. https://doi.org/10.3923/ijp.2017.907.915
4. Saracila M, Panaite T, Tabuc C, Soica C, Untea A, Ayasan T, Criste RD (2020) Dietary ascorbic acid and chromium supplementation for broilers reared under thermoneutral conditions vs. high heat stress. Sci Pap Anim Sci Ser 73(1):41–47
5. Keshri A, Roy D, Kumar V, Kumar M, Kushwaha R, Vaswani S, Choudhury S (2019) Effect of chromium supplementation on rhythmic alterations in growth performance and nutrient utilization of growing cattle during heat stress. Biol Rhythm Res:1–9. https://doi.org/10.1080/09291016.2019.1616143
6. Kargar S, Habibi Z, Karimi-Dehkordi S (2019) Grain source and chromium supplementation: effects on feed intake, meal and rumination patterns, and growth performance in Holstein dairy calves. Animal 13(6):1173–1179. https://doi.org/10.1017/S1751731118002793
7. Bahrami A, Moeini MM, Ghazi SH, Targhibi MR (2012) 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 21(2):209–215. https://doi.org/10.3382/japr.2010-00275
8. Wang S, Wang J, Liu Y, Li H, Wang Q, Huang Z, Shi P (2019) Trivalent chromium supplementation ameliorates oleic acid-induced hepatic steatosis in mice. Biol Trace Elem Res 187(1):192–201. https://doi.org/10.1007/s12011-018-1368-0
9. Bhagat J, Ahmed KA, Tyagi P, Saxena M, Saxena VK (2008) Effects of supplemental chromium on interferon-gamma (IFN-γ) mRNA expression in response to Newcastle disease vaccine in broiler chicken. Res Vet Sci 85:46–51. https://doi.org/10.1016/j.rvsc.2007.08.003
10. Talukder AK, Rashid MB, Yousef MS, Kusama K, Shimizu T, Shimada M, Miyamoto A (2018) Oviduct epithelium induces interferon-tau in bovine day-4 embryos, which generates an anti-inflammatory response in immune cells. Sci Rep 8(1):1–13. https://doi.org/10.1038/s41598-018-26224-8
11. Barjesteh N, Taha-Abdelaziz K, Kulkarni RR, Sharif S (2019) Innate antiviral responses are induced by TLR3 and TLR4 ligands in chicken tracheal epithelial cells: Communication between epithelial cells and macrophages. Virology 534:132–142. https://doi.org/10.1016/j.virol.2019.06.003
12. Lin YC, Huang JT, Li MZ, Cheng CY, Lien TF (2015) Effects of supplemental nanoparticle trivalent chromium on the nutrient utilization, growth performance and serum traits of broilers. J Anim Physiol Anim Nutr 99(1):59–65. https://doi.org/10.1111/jpn.12215
13. Lien TF, Yeh HS, Lu FY, Fu CM (2009) Nanoparticles of chromium picolinate enhance chromium digestibility and absorption. J Sci Food Agric 89(7):1164–1167. https://doi.org/10.1002/jsfa.3569
14. Aviagen R (2009) Ross broiler management manual, vol 2014, pp 350–364 http://pt.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross_Broiler_Manual_9
15. Abudabos AM, Ali MH, Nassan MA, Saleh AA (2019) Ameliorative effect of Bacillus subtilis on growth performance and intestinal architecture in broiler infected with Salmonella. Animals 9(4):190. https://doi.org/10.3390/ani9040190
16. Martin JE, McKeegan DE, Sparrey J, Sandilands V (2017) Evaluation of the potential killing performance of novel percussive and cervical dislocation tools in chicken cadavers. Br Poult Sci 58(3):216–223. https://doi.org/10.1080/00071668.2017.1280724
17. Huang C, Liang D, Tatomer DC, Wilusz JE (2018) A length-dependent evolutionarily conserved pathway controls nuclear export of circular RNAs. Genes Dev 32(9-10):639–644. https://doi.org/10.1101/gad.314856.118
18. Ingerslev LR, Donkin I, Fabre O, Versteyhe S, Mechta M, Pattamaprapanont P, Barrès R (2018) Endurance training remodels sperm-borne small RNA expression and methylation at neurological gene hotspots. Clin Epigenetics 10(1):12. https://doi.org/10.1186/s13148-018-0446-7
19. Naghieh A, Toghyani M, Gheisari AA, Egbal Saeed S, Mirzazade H (2010) Effect of different sources of chromium on performance and immune responses of broiler chicks. J Anim Vet Adv 9(2):354–358. https://doi.org/10.3923/javaa.2010.354.358
20. Sahin N, Hayirli A, Orhan C, Tuzcu M, Komorowski JR, Sahin K (2018) Effects of the supplemental chromium form on performance and metabolic profile in laying hens exposed to heat stress. Poult Sci 97(4):1298–1305. https://doi.org/10.3382/ps/pex435
21. Lu L, Zhao LL, Dong SY, Liao XD, Dong XY, Zhang LY, Luo XG (2019) Dietary supplementation of organic or inorganic chromium modulates the immune responses of broilers vaccinated with Avian Influenza virus vaccine. Animal 13(5):983–991. https://doi.org/10.1017/S1751731118002379
22. Arif M, Hussain I, Mahmood MA, El-Hack A, Mohamed E, Swelum AA, Komany A (2019) Effect of varying levels of chromium propionate on growth performance and blood biochemistry of broilers. Animals 9(11):935. https://doi.org/10.3390/ani9110935

23. Ognik K, Stępniowska A, Kozłowski K (2017) The effect of administration of silver nanoparticles to broiler chickens on estimated intestinal absorption of iron, calcium, and potassium. Livest Sci 200:40–45. https://doi.org/10.1016/j.livsci.2017.04.002
24. Song Y, Shi Y, Zhang L, Hu H, Zhang C, Yin M, Sun K (2019) Synthesis of CSK-DEX-PLGA nanoparticles for the oral delivery of exenatide to improve its mucus penetration and intestinal absorption. Mol Pharm 16(2):518–532. https://doi.org/10.1021/acs.molpharmaceut.8b00809
25. Talegaonkar S, Bhattacharyya A (2019) Potential of lipid nanoparticles (SLNs and NLCs) in enhancing oral bioavailability of drugs with poor intestinal permeability. AAPS PharmSciTech 20(3):121. https://doi.org/10.1208/s12249-019-1337-8
26. Valera M, Casasola R, Gutiérrez O, Sánchez-Chiprés DR, Mireles S (2021) Effects of supplementation with a novel organic chromium product on metabolic and physiological indicators of broilers. J Anim Health Prod 9(1):13–21. https://doi.org/10.17582/journal.jahp/2021/9.1.13.21
27. Ebrahimzadeh SK, Farhoomand P, Noori K (2012) Immune response of broiler chickens fed diets supplemented with different level of chromium methionine under heat stress conditions. Asian Australas J Anim Sci 25(2):256–260. https://doi.org/10.5713/ajas.2011.11217
28. Tahir SK, Yousaf MS, Rashid MA, Khan AF, Ahmad S, Zaneb H, Rehman H (2019) Supplemental chromium-loaded chitosan nanoparticles affect growth, serum metabolites and intestinal histology in broilers. S Afr J Anim Sci 49(6):1072–1082. https://doi.org/10.4314/sajas.v49i6.11
29. Rifaai RA, Abd El Baky FAZF (2017) Histological and immunohistochemical study on the effect of constant light exposure on T lymphocyte subsets in the thymus and lymph node of male albino rats. Egypt J Histolog 40(1):116–128. https://doi.org/10.21608/EJH.2017.3697
30. Odinokov D, Hamblin MR (2018) Aging of lymphoid organs: Can photobiomodulation reverse age-associated thymic involution via stimulation of extrapineal melatonin synthesis and bone marrow stem cells? J Biophotonics 11(8):e201700282. https://doi.org/10.1002/jbio.201700282
31. ThyagaRajan S, Hima L, Pratap UP, Priyanka HP, Vasantharekha R (2019) Estrogen-induced neuroimmunomodulation as facilitator of and barrier to reproductive aging in brain and lymphoid organs. J Chem Neuroanat 95:6–12. https://doi.org/10.1016/j.jchemneu.2018.02.008
32. Moeini MM, Bahrami A, Ghazi S, Targhibi MR (2011) The effect of different levels of organic and inorganic chromium supplementation on production performance, carcass traits and some blood parameters of broiler chicken under heat stress condition. Biol Trace Elem Res 144(1-3):715–724. https://doi.org/10.1007/s12011-011-9116-8
33. Huang YL, Luo QH, Xiao F, Lin X, Spears JW (2020) Research Note: Responses of growth performance, immune traits, and small intestinal morphology to dietary supplementation of chromium propionate in heat-stressed broilers. Poult Sci 99(10):5070–5073. https://doi.org/10.1016/j.psj.2020.07.005
34. Wasti S, Sah N, Mishra B (2020) Impact of heat stress on poultry health and performances, and potential mitigation strategies. Animals 10(8):1266. https://doi.org/10.3390/ani10081266
35. Sharma P, Pande VV, Moyle TS, McWhorter AR, Chousalkar KK (2017) Correlating bacterial shedding with fecal corticosterone levels and serological responses from layer hens experimentally infected with Salmonella Typhimurium. Vet Res 48(1):5. https://doi.org/10.1186/s13567-017-0414-9
36. Hirakawa R, Nurjanah S, Furukawa K, Murai A, Kikusato M, Nochi T, Toyomizu M (2020) Heat stress causes immune abnormalities via massive damage to effect proliferation and differentiation of lymphocytes in broiler chickens. Front Vet Sci 7:46. https://doi.org/10.3389/fvets.2020.00046
37. Kojima M, Oji S, Tanaka S, Izaki S, Hashimoto B, Fukaura H, Nomura K (2020) Tacrolimus is effective for neuromyelitis optica spectrum disorders with or without anti-AQP4 antibody. Mult Scler Relat Disord 39:101907. https://doi.org/10.1016/j.msard.2019.101907
38. Shen W, Poliquin S, Macdonald RL, Dong M, Kang JQ (2020) Endoplasmic reticulum stress increases inflammatory cytokines in an epilepsy mouse model Gabrg2+/Q390X knockin: a link between genetic and acquired epilepsy? Epilepsia 61(10):2301–2312. https://doi.org/10.1111/epi.16670
39. Podojil JR, Glaser AP, Baker D, Courtois ET, Fantini D, Yu Y, Meeks JJ (2020) Antibody targeting of B7-H4 enhances the immune response in urothelial carcinoma. OncoImmunology 9(1):1744897. https://doi.org/10.1080/2162402X.2020.1744897
40. Mirfendereski E, Jahanian R (2015) Effects of dietary organic chromium and vitamin C supplementation on performance, immune responses, blood metabolites, and stress status of laying hens subjected to high stocking density. Poult Sci 94(2):281–288. https://doi.org/10.3382/ps/peu074
41. Acharya N, Madi A, Zhang H, Klapholz M, Escobar G, Dulberg S, Anderson AC (2020) Endogenous glucocorticoid signaling regulates CD8+ T cell differentiation and development of dysfunction in the tumor microenvironment. Immunity 53(3):658–671. https://doi.org/10.1016/j.immuni.2020.08.005
42. Spears JW, Lloyd KE, Siciliano P, Pratt-Phillips S, Goertzen EW, McLeod SJ, Rounds W (2020) Chromium propionate increases insulin sensitivity in horses following oral and intravenous carbohydrate administration. J Anim Sci 98(4):skaa095. https://doi.org/10.1093/jas/skaa095
43. Song H, Park H, Kim YS, Kim KD, Lee HK, Cho DH, Yang JW, Hur DY (2011) L-kynurenine-induced apoptosis in human NK cells is mediated by reactive oxygen species. Int Immunopharmacol 11:932–938. https://doi.org/10.1016/j.intimp.2011.02.005
44. Landman GW, Bilo HJ, Houweling ST, Kleefstra N (2014) Chromium does not belong in the diabetes treatment arsenal: current evidence and future perspectives. World J Diabetes 5(2):160–164. https://doi.org/10.4239/wjd.v5.i2.160
45. Hassan S, Hassan FU, Rehman MSU (2020) Nano-particles of trace minerals in poultry nutrition: potential applications and future prospects. Biol Trace Elem Res 195(2):591–612. https://doi.org/10.1007/s12011-019-01862-9
46. Lee J, Hosseindoust A, Kim M, Kim K, Choi Y, Lee S, Chae B (2020) Biological evaluation of hot-melt extruded nano-selenium and the role of selenium on the expression profiles of selenium-dependent antioxidant enzymes in chickens. Biol Trace Elem Res 194(2):536–544. https://doi.org/10.1007/s12011-019-01801-8
47. Stępniowska A, Tutaj K, Drażbo A, Kozłowski K, Ognik K, Jankowski J (2020) Estimated intestinal absorption of phosphorus and its deposition in chosen tissues, bones and feathers of chickens receiving chromium picolinate or chromium nanoparticles in diet. PLoS One 15(11):e0242820. https://doi.org/10.1371/journal.pone.0242820