Pakistan J. Zool., pp 1-13, 2022. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20220605060642

Effect of Chromium Picolinate, Alone or in Combination with Vitamin C or Formic Acid on Growth, Carcass Traits, Immune and Blood Parameters of Broilers under Heat Stress

Faramin Javandel Soum Sarai[1], Mir Daryoush Shakouri[2]* and Alireza Seidavi[3]*

[1]PhD Candidate, Department of Animal Science, University of Mohaghegh Ardabili, Ardabil, Iran
[2]Department of Animal Science, University of Mohaghegh Ardabili, Ardabil, Iran
[3]Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran

Article Information
Received 05 June 2022
Revised 11 August 2022
Accepted 26 September 2022
Available online 28 December 2022 (early access)

Authors' Contribution
FJS, MDS and AS presented the concept, wrote and edited the mauscript. FJS performed investigation and data collection. FJS, MDS and AS provided resources, and reviewed and edited the mauscript.

Key words
Heat stress, Formic acid, Vitamin C, Chromium picolinate, Broiler, Blood parameters

ABSTRACT

The experiment was performed to evaluate the effect of chromium picolinate, alone or in combinationwith vitamin C or formic acid on performance, carcass traits and some blood biochemical and hematological parameters of broilers under heat stress (34 ˚C for 8 h). A total of 160 28-d-old Ross 308 broiler chickens were divided into 4 treatment groups with 4 replicates and 10 birds per each by employing a completely randomized design. Broilers were fed on corn-soybean meal basal diets with no additive (control) or added chromium picolinate (400 mg/kg), chromium picolinate (400 mg/kg)+ vitamin C (240 mg/kg) or chromium picolinate (400 mg/kg) +formic acid (0.5%) form 29 to 42 days of age. All supplements significantly improved daily feed intake, daily weight gain and feed conversion ratio of birds compared with the control (P<0.05). The treatments reduced the abdominal fat (P<0.05) and had no effect on the other carcass traits. Relative weight of immune organs was significantly increased by the dietary treatments (P<0.05). The supplements caused a significant increase in total protein and a decrease in glucose, total cholesterol, and LDL cholesterol concentrations (P<0.05). All supplements significantly decrease mean corpuscular volume (MCV), percentage of heterophils and the ratio of heterophil to lymphocyte (H/L), and increased mean corpuscular hemoglobin concentration (MCHC) and white blood cells (WBC) (P<0.05). Addition of formic acid lowered the effect of chromium picolinate on performance parameters, abdominal fat and spleen weights (P<0.05). The results suggest that supplemental chromium picolinate alleviate the adverse effects of heat stress. No synergistic effect was observed between vitamin C or formic acid with chromium picolinate, and in some cases formic acid exacerbated the effect of chromium picolinate.

INTRODUCTION

Heat stress is one of the most important environmental stress inducing factors that challenge commercial broilersaround the world, especially in tropical and subtropical regions, due to the economic losses related to reduced production performance and increased mortality.

There are scientific evidences for the destructive effects of heat stress on growth performance (Chougule *et al*., 2018), carcass characteristics (Huang *et al*., 2016), blood biochemical (Ding *et al*., 2020) and hematological parameters (Ribeiro *et al*., 2018).

It is well known that activation of the hypothalamus-pituitary-adrenal (HPA) axis during stress increases the level of corticosterone in blood plasma of the birds (Lu *et al*., 2019), which facilitates the increase of infections by suppressing the immune system (Hirakawa *et al*., 2020). Chromium plays an important role in increasing the metabolism of nutrients such as carbohydrates, lipids, proteins, and nucleic acids by activating the enzymes associated with their metabolic pathways (Haldar *et al*., 2009). In fact, the major physiological role of chromium is to improve glucose tolerance (Hamidi *et al*., 2016), which increases the susceptibility of tissue receptors to insulin (Ezzat *et al*., 2017). Corticosterone secretion reduces

* Corresponding author: mdshakouri@uma.ac.ir, alirezaseidavi@iaurasht.ac.ir
0030-9923/2022/0001-0001 $ 9.00/0

the sensitivity of body cells to insulin during the stress (Sirirat *et al*., 2012), and in this case chromium improves bird performance by lowering blood corticosterone concentrations (Toghyani *et al*., 2006; Dalólio *et al*., 2018). It is illustrated that heat stress increases the mobilization of chromium from tissues, increases urinary excretion, and reduces the retention of chromium, which results in a deficiency of chromium in the body (Sahin *et al*., 2002), thus, the need for chromium increases in such cases. Chromium picolinate is a combination of a low-toxicity trivalent form of chromium combined with picolonic acid (Hamidi *et al*., 2016). The use of chromium picolinate supplement as a nutritional strategy in the diet of broilers under heat stress can improve growth performance (Toghyani *et al*., 2006), and carcass yield (Sahin *et al*., 2003), reduce depot fat (Kulkarni *et al*., 2018), improve immune organ weights (Lu *et al*., 2019) and blood parameters (Toghyani *et al*., 2006).

Vitamin C (ascorbic acid) is an unnecessary vitamin in poultry diets; as birds have the enzyme gluconolactone oxidase in their kidneys, which synthesize vitamin C from glucose (Khan and Sardar, 2005). However, during heat stress the endogenous vitamin C is not sufficient to meet the needs of birds (Abidian and Khatoon, 2013). On the other hand, vitamin C existsin high concentration in immune cells, which undergoes a rapid depletion under stress (Sorice *et al*., 2014). Vitamin C is not a part of the metabolic pathways; however, it is an essential factor for many enzymatic reactions involved in collagen carnitine, catecholamine, and tyrosine biosynthesis (Shakeri *et al*., 2020). Supplementation of vitamin C in the diet of broilers under heat stress reduces the synthesis and secretion of corticosteroids and decreases the plasma corticosterone concentrations by inhibiting the key enzymes involved in the corticosterone biosynthesis pathway (21-hydroxylase and 11-β-hydroxylase). It has been reported that under stress conditions, vitamin C supplementation can increase productivity, immune response, resistancetodiseaseand viability of broiler chickens (Whitehead and Keller, 2003).

Methanoic acid, known as formic acid, is the simplest carboxylic acid. One of the main applications of formic acid is to add it to feeds due to antibacterial activity against pathogens (Milillo *et al*., 2011) to prevent their subsequent colony formation in the digestive system of birds (Ricke *et al*., 2020). Moreover, the positive effects of formic acid have been previously reported on broiler body weight gain (Panda *et al*., 2009), feed intake, and feed efficiency (Kim *et al*., 2015), depot fat (Panda *et al*., 2009), cellular and humoral immunity (Ragaa and Korany, 2016), relative weight of immune organs (Ghazalah *et al*., 2011), and mortality (Brzoska *et al*., 2013) under normal condition. However, based on our knowledge, there is limited information about the effect of supplemental formic acidon broiler performance under heat stress. Therefore, there is room to study whether dietary inclusion of chromium picolinate with an acifieror vitamin C can show any additive effect to eliminate the adverse effects of heat stress. Hence, the aim of the current study was to compare the influence of above mentioned additives on growth, immunity and blood parameters of broiler chickens under heat stress.

MATERIALS AND METHODS

For this experiment, a total of 160 one-day-old male broiler chicks (Ross 308) were obtained from a local hatchery and raised on deep litter pens until 28 days of age, then weighed and randomly assigned to 4 dietary treatments with 4 replicates and 10 birds each with similar group weights. The experimental treatments were (1) basal diet without any supplement (control), (2) basal diet + 400 mg/kg chromium picolinate, (3) basal diet + 400 mg/kg chromium picolinate + 240 mg/kg vitamin C, and (4) basal diet +400 mg/kg chromium picolinate + 0.5% formic acid. The basal diet based on corn and soybean meal was formulated to meet or exceed the nutrients requirements recommended by Ross 308 production manual for starter (1 to 14 days of age), grower (15 to 28 days of age), and finisher (29 to 42 days of age) periods (Table I). The birds in all experimental groups had free access to water and feed for 23 h per day. The room temperature on the first day was set at 32˚C and gradually decreased to approximately 22 ˚C and then remained constant until 28 days of age. For application of heat stress, during 29 to 42 days of age the room temperature was raised to 34˚C for 8 h every day. Feed intake and body weight gain of birds per each replicate were recorded through the expemerimental period and then feed conversion ratio was calculated. The study was approved by the Animal Ethics Committee of the University of Mohaghegh Ardabili.

At the end of the experiment, two birds per replicate, each with a body weight close to the average body weight of each replicate were slaughtered after 4 h starvation. The feather removal and cutting into different parts were then performed manually. The different parts included carcass, breast, thigh, wing, neck,internal organs (gizzard, liver, heart, pancreas, and abdominal fat), and lymphoid organs (the bursa of Fabricius, thymus, and spleen) were weighed with a 0.001 g precision digital scale and expressed as the percent of live body weight.

To measure the biochemical and hematological parameters of blood, the samples of about 5 ml were taken from wing vein of two birds of each replicate on day 42 of the experiment. The samples were then poured into two test tubes. One tube containing EDTA anticoagulant was used to measure haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), and packed cell volume (PCV). RBC and WBC were counted using NAT solution by a hemocytometer. Hb and PCV levels were measured using cyanmethemoglobin and microhematocrit methods, respectively. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were estimated using the following common methods:

MCV (fL) = PCV × ×1000 / RBC

MCH (pg) = Hb×10 / RBC

MCHC (mmol/l or %) = Hb / PCV

Table I. Ingredients and chemical composition of the basal diets (%).

Ingredient	Starter	Grower	Finisher
Corn	54.32	60	64
Soybean meal	39.43	31.87	27
Corn oil	2.16	4.5	5
Oyster shell	0.90	0.97	1
Dicalcium phosphate	2.05	1.68	1.85
Common salt	0.37	0.37	0.35
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
DL-methionine	0.20	0.22	0.18
L-lysine HCl	0.07	0.05	0.12
Chemical composition (calculated)			
Metabolic energy (kcal/kg)	2900	3200	3220
Crude protein (%)	22.16	21.30	19.5
Calcium (%)	1	0.85	1.03
Available phosphorus (%)	0.50	0.42	0.58
Lysine (%)	1.15	0.96	1.12
Methionine (%)	0.50	0.48	0.49
Methionine + cysteine (%)	0.83	078	0.73
Threonine (%)	0.79	0.71	0.65

[1]Supplied per kg of diet: 3600000 IU vitamin A, 800000 IUvitamin D_3, 7200 IU vitamin E, 710 mg vitamin B_1, 2640 mg vitamin B_2, 1176 mg vitamin B_6, 400 mg vitamin B_9, 6 mg vitamin B_{12}, 800 mg vitamin K_3, 3920 mg pantothenic acid, 12,000 mg niacin, 40 mg biotin and 200,000 mg choline chloride. [2]Supplied per kg of diet: 40,000 mg manganese,20,000 mg iron, 33,900 mg zinc, 4,000 mg copper, 400 mg iodine, and 80 mg selenium.

The blood serum was used to measure blood biochemical parameters including total protein, albumin, glucose, cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, and uric acid. Blood biochemical parameters were measured using the enzymatic method with Randox-Ransod commercial kits and auto-analyzer spectrophotometer. The levels of triiodothyronine (T3) and thyroxine (T4) were measured using ELISA kits, according to the recommended instructions, with the help of an ELISA reader device.

The collected data were statistically analyzed using the General Linear Model procedure of SAS software. Duncan's multiple range test was used to compare the significant difference (P<0.05) between the means. The statistical model of the design was as follows:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where Y_{ij} represents each observation in the experiment, μ indicates the total mean, T_i represents the effect of the i-th experimental treatment, and E_{ij} represents the experimental error.

RESULTS

Performance

The effect of chromium picolinate with or without formic acid and vitamin C on birds growth performance during heat stress (29 to 42 days of age), and the whole experimental (0 to 42 days of age) periods are presented in Table II. The chickens consuming the chromium picolinate supplement alone or in combination with either vitamin C or formic acid had significantly (P<0.05) higher daily weight gain and feed intake compared with the control group. The birds on chromium picolinate plus vitamin C had similar daily weight gain and feed intake with those on chromium picolinate alone, whereas the chickens fed with formic acid and chromium picolinate had significantly (P<0.05) lower daily weight gain and feed intake than those receiving chromium picolinate alone. Moreover, the chickens fed with chromium picolinate alone or in combination with vitamin C showed the best feed conversion ratio and was followed by the group on chromium picolinate and formic acid (P<0.05).

Carcass characteristics

As shown in Table III, supplementation of different dietary treatments had no significant effect on relative weights of carcass, breast, thigh, wing, neck, gizzard, liver, heart, and pancreas compared with the control. However, all treatments significantly (P<0.05) decreased relative weight of abdominalfat of the birds. The effect was more pronounced by chromium picolinate alone or in combination with vitamin C.

Immune organs

Table IV shows the effect of the supplements on immune organ weights (the bursa of Fabricius, thymus, and spleen) of birds at 42 days of age. All supplements significantly (P<0.05) increased the relative weights of bursa of Fabricius and thymus in comparison with the control.The relative weight of spleen was also significantly (P<0.05) increased by the treatments and the birds on chromium picolinate alone or in combination with vitamin C had the heaviest spleen.

Table II. The effects of dietary treatments on boriler chickens growth performance under heat stress during the experimental periods.

Parameters	Treatments				SEM	P-value
	Control	Chromium	Chromium+ Vitamin C	Chromium + Formic acid		
0-28 d						
Daily weight gain (g)	40.26	40.21	40.33	40.18	0.146	0.989
Daily feed intake(g)	62.50	62.26	62.61a	62.31	1.143	0.846
FCR	1.553	1.548	1.552	1.552	0.007	0.997
29-42 d						
Daily weight gain (g)	72.64[c]	83.21[a]	83.62[a]	77.73[b]	0.590	<0.001
Daily feed intake (g)	147.78[c]	157.78[a]	157.95[a]	153.07[b]	1.267	<0.001
Feed conversion ratio	2.09[a]	1.896[c]	1.890[c]	1.970[b]	0.249	<0.001
0-42 d						
Daily weight gain (g)	50.39[c]	54.54[a]	54.76[a]	52.70[b]	0.543	<0.001
Daily feed intake(g)	90.93[c]	94.10[a]	94.40[a]	92.56[b]	0.426	<0.001
Feed conversion ratio	1.804[a]	1.725[c]	1.723[c]	1.756[b]	0.010	<0.001

SEM, Standard error of means. [a,b]Means in the same row with different letters are significantly different (P<0.05).

Table III. The effects of dietary treatments on carcass traits (%, as the percent of live body weight) of boriler chickens under heat stress on day 42.

Parameters	Control	Chromium	Chromium + Vitamin C	Chromium + Formic acid	SEM	P value
Carcass	70.7	72.95	72.98	72.24	1.343	0.940
Breast	25.48	28.36	28.36	27.73	0.583	0.261
Thigh	19.28	19.26	20.68	19.87	0.426	0.651
Wing	7.68	7.97	8.10	7.84	0.186	0.898
Neck	4.57	4.62	4.61	4.58	0.087	0.998
Abdominal fat	1.43[a]	0.81[c]	0.77[c]	1.12[b]	0.775	<0.001
Gizzard	1.45	1.55	1.58	1.46	0.071	0.913
Liver	1.84	2.13	2.21	2.15	0.627	0.146
Heart	0.54	0.56	0.58	0.57	0.021	0.941
Pancreas	0.21	0.25	0.26	0.26	0.006	0.338

SEM, Standard error of means. [a,b]Means in the same row with different letters are significantly different (P<0.05).

Table IV. The effects of dietary treatments on relative weight of immune organs ((%, as the percent of live body weight) of boriler chickens under heat stress on day 42.

Parameters	Control	Chromium	Chromium + Vitamin C	Chromium + Formic acid	SEM	P value
Bursa of Fabricius	0.110[b]	0.110[a]	0.184[a]	0.176[a]	0.009	<0.001
Thymus	0.130[b]	0.194[a]	0.214[a]	0.206[a]	0.012	0.028
Spleen	0.105[c]	0.161[a]	0.162[a]	0.130[b]	0.006	<0.001

SEM, Standard error of means. [a,b]Means in the same row with different letters are significantly different (P<0.05).

Table V. The effects of dietary treatments on blood biochemical parameters of boriler chickens under heat stress on day 42.

Parameters	Control	Chromium	Chromium + Vitamin C	Chromium + Formic acid	SEM	P value
Total protein (mg/dL)	2.57[b]	3.77[a]	4.03[a]	3.67[a]	0.200	0.016
Albumin (mg/dL)	1.03[b]	1.27[a]	1.25[a]	1.16[ab]	0.365	0.047
Glucose (mg/dL)	238.33[a]	191.67[b]	189[b]	201[b]	6.320	<0.001
Total cholesterol (mg/dL)	173[a]	130.67[b]	120.67[b]	133[b]	7.078	0.013
Triglycerides (mg/dL)	56.33	45.67	37.33	46.00	3.231	0.236
LDL-C (mg/dL)	70.66[a]	45.33[b]	40.33[b]	38.33[b]	6.412	0.009
HDL-C (mg/dL)	79.67	76	47.33	85.67	4.193	0.832
Uric acid (mg/dL)	5.40	3.68	5.53	4.22	0.461	0.463

SEM, Standard error of means. [a,b]Means in the same row with different letters are significantly different (P<0.05).

Table VI. The effects of dietary treatments on hematological parameters of boriler chickens under heat stress on day 42.

Parameters	Control	Chromium	Chromium + Vitamin C	Chromium + Formic acid	SEM	P value
RBC (× 10^6/µl)	2.26	2.52	2.70	2.58	0.068	0.124
Hb	10.23	11.60	12.53	11.80	0.326	0.057
PCV= Hct (%)	30.50	31.90	33.90	31.83	0.655	0.368
MCV(fl)	134.80[a]	126.56[b]	125.50[b]	123.20[b]	1.434	0.001
MCH (pg)	45.16	46.03	46.43	45.66	0.290	0.520
MCHC (%)	33.47[b]	36.37[a]	37.00[a]	37.06[a]	0.473	<0.001
WBC (×10^3/µL)	22.86[b]	23.85[a]	23.97[a]	23.65[a]	0.139	0.001
Heterophil (%)	35.67[a]	25.33[b]	23.67[b]	26.00[b]	1.601	0.005
Lymphocyte (%)	60.33[b]	67.67[a]	66.33[a]	63.0[ab]	0.089	0.039
Heterophil /lymphocyte	0.59[a]	0.37[b]	0.35[b]	0.41[b]	0.293	<0.001
T3 (nmol/l)	0.97	1.82	1.73	1.62	0.173	0.319
T4 (nmol/l)	1.07	2.40	2.30	1.73	0.210	0.064

SEM, Standard error of means. [a,b]Means in the same row with different letters are significantly different (P<0.05).

Biochemical parameters of blood

The concentration of serum metabolites of broilers as affected by dietary treatments are presented in Table V. The supplements caused a significant increase in the concentration of total protein (P<0.05). The level of albumin was increased by chromium picolinate alone or in combination with vitamin C (P<0.05). A significant decrease was observed in the concentration of glucose, total cholesterol, and LDL cholesterolas the influence of the dietary treatments (P<0.05). There were no significant effect of the treatments on triglyceride, HDL cholesterol, and uric acid levels of the birds serum.

Hematological parameters

According to the results shown in Table VI, all dietary supplements significantly (P<0.05) decreased blood MCV, heterophils percent and heterophil to lymphocyte ratio and decreased MCHC percent and WBC counts compared with the control. The percentage of blood lymphocytes was significantly (P<0.05) increased by chromium picolinate and chromium picolinate plus vitamin C. The other hematological parameters including RBC, Hb, PCV, MCH, T3 and T4 were not significantely affected by the treatments (P>0.05).

DISCUSSION

It is demonstrated that heat stress leads to reduced feed intake, growth performance and feed efficiencyin broiler chickens (Hu *et al*., 2019). Weight loss is not only due to less feed intake, but also due to the direct effect of ambient temperature on the physiology and metabolism of broilers (Geraert *et al*., 1996).

The improvement observed in growth response of broilers under heat stress in this study (Table II) is consistent with previous studies which report the similar results by adding chromium picolinate supplement alone (Hamidi *et al*., 2016; Chougule *et al*., 2018; Hridoy *et al*., 2021) or in combination with vitamin C (Sahin *et al*., 2003) to the diet. The increased daily feed intake during heat stress (29 to 42 days of age) and the whole experimental periodis in line with earlier reports indicating increased appetite and feed intake by chromium picolinate supplement alone (Toghyani *et al*., 2006; Hamidi *et al*., 2016; Sahin *et al*., 2017; Chougule *et al*., 2018) or in combination with vitamin C (Sahin *et al*., 2003).

There is contradictory results on chromium picolinate effect on birds feed conversion ratio under heat stress. In keeping with our finding, some studies have been reported improved feed conversion ratio by adding chromium picolinate alone (Samanta *et al*., 2008; Ezzat *et al*., 2017; Chougule *et al*., 2018; Hamidi *et al*., 2019) or in combination with vitamin C (Sahin *et al*., 2003) or with vitamin E (Hridoy *et al*., 2021), whereas the others have not shown any improvement by adding chromium picolinate alone (Toghyani *et al*., 2006; Tawfeek *et al*., 2014; Huang *et al*., 2016) under heat stress or with vitamin C (Haq *et al*., 2018) under normal temperature conditions. Inconsistency in the results obtained by different studies might be explained by the supplemental level of chromium picolinate and other variables such as bird age, health status, and most importantly, the type (acute or chronic) and duration of stress and *etc*.

Chronic heat stress suppresses the activity of the appetite center in the hypothalamus and reduces feed intake, by affecting the ambient temperature receptors and the transmission of nerve impulses to the hypothalamus (Marai *et al*., 2007). Reducing feed intake leads to a reduction in consumption of nutrients used for growth, which is part of the bird's physiological adaptation to heat stress (Lara and Rostagno, 2013), so that fewer nutrients are available to the bird for activity and enzymatic synthesis, hormone production, and heat regulation (Attia *et al*., 2017). In addition, stress increases the mobilization of chromium from tissues and increases urinary excretion, and decreases its retention in the body (Sahin *et al*., 2002). Thus, the need for chromium increases in such cases. In current study, observation of increased feed intake by supplemental chromium picolinatecompared with the control may be because of providing high demand of birds for chromium during heat stress (Sahin *et al*., 2010). On the other hand, heat stress increases the concentration of corticosterone in the blood (Zhao *et al*., 2009) and decreases the sensitivity of body cells to the insulin (Sirirat *et al*., 2012). Chromium supplement reduces blood glucose and increases appetite (Chougule *et al*., 2018) by increasing the sensitivity of tissue receptors to insulin and increasing glucose uptake by cells (Ezzat *et al*., 2017). As a result, increasing feed intake by providing more glucose, amino acids and other nutrients to muscle tissues and cells and increased protein retention can lead to improved weight gain (Hamidi *et al*., 2016), as seen in this study (Table II).

Heat stress has an adverse effect on serum concentrations of vitamins such as vitamin C (Kucuk *et al*., 2003). It has been reported that vitamin C supplement improves the absorption and retention of chromiumin the body (Ahmed *et al*., 2005; Dalólio *et al*., 2018), thereby improving physiological functions of broilers under heat stress (Sahin *et al*., 2003; Haq *et al*., 2016). However, in the present study there was no synergy between vitamin C and chromium picolinate. Accordingly, the synergy of chromium picolinate and vitamin C leads to improved feed intake, better nutrient digestibility, and feed efficiency in this group, as previously reported by Sahin *et al*. (2003).

In keeping with our results (Table III), no significant effect of chromium picolinate supplementation on relative weight of broiler carcass (Tawfeek *et al*., 2014; Zheng *et al*., 2016), breast (Xiao *et al*., 2017; Kulkarni *et al*., 2018; Ding *et al*., 2020), and thigh (Xiao *et al*., 2017; Kulkarni *et al*., 2018) has been reportedunder heat stress. However, anincrease in relative carcass and breast weights by adding chromium picolinatehas also been illustrated under heat stress conditions (Samanta *et al*., 2008; Ding *et al*., 2020). Unlike our findings, the increase of hot and cold carcass weights by combination of chromium picolinateand vitamin C under heat stress (Sahin *et al*., 2003) and carcass and breast weights by chromium yeast with vitamin C in normal conditions (Haq *et al*., 2018) has been reported. No significant effect of chromium picolinate supplement has been previously recorded on relative weights of thigh, wing, gizzard, liver, heart, and pancreasunder heat stress (Toghyani *et al*., 2006; Samanta *et al*., 2008; Kulkarni *et al*., 2018), which is consistent with our results. However, Sahin *et al*. (2003) has been reported increased liver, heart, and gizzard weights as the result of chromium picolinate supplementation to the diet of heat stressed broilers. There are contrary reports on the effect of chromium picolinate on the liver weight of broilers under heat stress. Lien *et al*. (1999) reported that supplemental chromium picolinate at the levels of 1600 and 3200 µg/kg had no effect on liver weight, while at 800 µg/kg level increased the organ weight. An increase in the liver weigh has been also observed by Sahin *et al*. (2002) in broilers fed with chromium picolinate. Furthermore, no effect of chromium picolinate supplementation has been shown on liver weight of broilers under heat stress (Toghyani *et al*., 2006;

Tawfeek *et al*., 2014).

Consistent with our results, the reduction of abdominalfathas been previously reported by adding chromium picolinate supplement to the diets of broilers under heat stress (Samanta *et al*., 2008; Huang *et al*., 2016; Kulkarni *et al*., 2018). A similar observation has also been seen by supplemental chromium yeast with vitamin C under normal temperature conditions (Ahmed *et al*., 2005; Haq *et al*., 2018). One probable reason for this observation may be due to the effect of chromium oninhibition of fat synthesis or fat mobilization, or both (Suksombat and Kanchanatawee, 2005). Furthermore, it has been revealed that chromium enhances cellular utilization of glucose by stimulating insulin action (Vincent, 2000). According to Mertz (1993), glucose is converted to lipid and stored in adipose tissue at low levels of insulin. Chen *et al*. (2018) reported that the activity of fatty acid synthase, acetyl coenzyme A carboxylase, hormone-sensitive lipase, and lipoprotein lipase, which are essential enzymes in the metabolism, transport and storage of fatty acids, arereduced by chromium picolinate supplement. Thus, induced lower deposition of fat by chromium supplementation might be occurred by increased utilization of glucose and fatty acids and inhibition of fat biosynthesis.

On the other hand, heat stress increases the level of blood corticosterone by activating the hypothalamic-pituitary-adrenal axis (Zhao *et al*., 2009). Chromium picolinate, however, reduces the level of plasma corticosteroids (Dalólio *et al*., 2018) by inhibiting key enzymes involved in the corticosteroid biosynthesis pathway (21-hydroxylase and 11-β-hydroxylase) (Alhassani and Alshukri, 2016), thereby causing are duction in the synthesis and secretion of corticosterone in broilers under heat stress, which in turn resulted in decreased catabolism and increased protein synthesis in muscle tissue and reduced abdominal fat. This can improve meat quality of under heat stressed broilers.

The bursa of Fabricius, thymus, and spleen are the important organs of the immune systems in broilers. It is shown that heat stress reduces the weight of these organs (Hirakawa *et al*., 2020), may be due to the lowered feed intake and subsequent fewer nutrients providing for their proper growth (Bartlett and Smith, 2003). All supplements increased the birds feed intake (Table II) and led to high relative weight of the bursa of Fabricius, thymus and spleen (Table IV). However, the effect of chromium picolinate with formic acid on both feed intake and spleen relative weight was less than chromium picolinate alone or in combination with vitamin C. Lu *et al*. (2019) reported that different levels of chromium picolinate supplement (0.4, 0.8, 1.6, or 3.2 mg/kg) increased immune organ weights of the broilers under heat stress. Hamidi *et al*. (2016) also reported an increase in thymus and spleen weights by adding chromium picolinate supplement to the diet of heat stressed broilers. However, inconsistent with our results, Toghyani *et al*. (2007) did not report any significant effect of chromium picolinate on immune organs weights. In addition to the effect of feed intake on immune organs, the influence of corticosterone also should be noted, as it is shown that corticosterone causes the weight loss of lymphoid organs (Haq *et al*., 2018). Therefore, the supplementation of chromium picolinatein the current study may has been caused high immune organ weights by affecting on this hormone as mentioned above.

Biochemical parameters provide valuable information about the health and wellbeing status of animals. Heat stress in broilers leads to some changes in serum biochemical parameters such as reducing total protein, albumin, and HDL- cholesterol, and increasing glucose, total cholesteol, triglyceride, and LDL-cholesterollevels (Ding *et al*., 2020). Supplementaion of chromium picolinate, alone or in combination with vitamin C, icreased the levels of total protein and albumin in the blood (Table V). A similar observation has been previously reported by adding chromium picolinatealone (Ezzat *et al*., 2017; Chougule *et al*., 2018; Hamidi *et al*., 2019) or in combination with vitamin C to the diet under heat stress (Sahin *et al*., 2003), or by adding chromium yeast plus vitamin C in the diet of broilers under normal temperature conditions (Haq *et al*., 2018). However, there is a report indicating no significant effect of chromium picolinate on the concentration of blood total protein and albuminunder heat stress (Hridoy *et al*., 2021). It is shown that high ambient temperatures reduces protein synthesis and increases protein catabolism in the body (Gous and Morris, 2005). The chromium supplement enhances glucose uptake by the cells due to stimulating insulin action (Vincent, 2000). Indeed, increasing serum insulin concentration regulates protein metabolism by increasing cellular amino acid uptake and protein synthesis (Colgan, 1993). Therefore, the rate of protein anabolism is higher than the rate of protein catabolism (Sahin *et al*., 2002). On the other hand, vitamin C also limits the catabolism of lipids and proteins by lowering corticosterone levels (Kucuk *et al*., 2003).

The reduction of serum glucoselevelin this trial (Table V) was in keeping with perivious studies by adding chromium picolinatealonealone (Sahin *et al*., 2017; Chougule *et al*., 2018; Hamidi *et al*., 2019) or in combination with vitamin C (Sahin *et al*., 2003; Saracila *et al*., 2021) to the diet of broilers under heat stress. It has been reported that chromium, as an active glucose tolerance factor (GTF), increases the susceptibility of tissue receptors to insulin and thus increases glucose uptake by the cells (Ezzat *et al*., 2017) and decreses its level in the blood.It is possible that chromium and vitamin C supplement also reduced blood glucose levels by activating insulin, thereby increasing insulin uptake into tissues (Khukhodziinai *et al*., 2021).

According to the results, all supplements reduced total and LDL cholesterol levels ($P<0.05$) with no effectonthe concentration of triglyceride or HDL cholesterol. Consistent with our results, several studies have shown that the use of chromium picolinate supplements in the diet of broilers under heat stress reduces serum cholesterol (Sahin *et al*., 2017; Chougule *et al*., 2018; Hridoy *et al*., 2021). Also, a reduction in total cholesterol concentrations in broilers blood have been reported by the addition of chromium picolinate plus vitamin C (Sahin *et al*., 2003) or chromium picolinate plus vitamin E under heat stress (Hridoy *et al*., 2021). Inconsistent with our results, Saracila *et al*. (2021) reported no decrease of serum total cholesterol by adding chromium picolinate (200g / kg) with vitamin C (0.25 g / kg) to the diet of broilers under heat stress. Similar to our results, no changes in triglyceride or HDL cholesterol levels was observed by supplementing chromium picolinate (200 or 400 µg/kg) (Sands and Smith, 2002) or in triglyceride level by adding chromium picolinate (200 µg/kg) plus vitamin C (0.25 g/kg) to the diet of heat stressed broilers (Saracila *et al*., 2021). Haq *et al*. (2018) also reported that supplementation of chromium yeast with vitamin C did not affect the concentration of HDL cholesterol of broilers under normal temperature conditions. Moreover, a decrease in triglyceride and an increase in HDL cholesterol concentration of broilers blood have been reported by chromium picolinate under heat stress (Sahin *et al*., 2002; Samanta *et al*., 2008; Tawfeek *et al*., 2014), which is in opposite to our findings.

The increase oftotal cholesterol and triglyceride levelsin the blood of broilers under heat stress might be due to the higher levels of stress hormones that stimulate lipolysis (Hajati *et al*., 2018), or due to the reduced feed intake, which causes the bird to increase lipid metabolism or lipolysis to meet its energy needs (Rashidi *et al*., 2010). The reduction in serum total cholesterol and triglyceride concentrations can be explained by the stimulatory effect of organic chromium on insulin secretion, there by increasing glucose uptake to oxidize or convert to fatty acids for storing as triglyceride in adipose tissue. It has been reported that chromium increases the activity of lipoprotein lipase and lecithin-cholesterol acyltransferase, which in turn accelerates esterification and excretion of cholesterol, stimulates HDL synthesis, and increases LDL receptors in the liver, thereby reducing blood LDL content, and at the same time, increasing HDL levels (Brindley and Salter, 1991). On the other hand, vitamin C reduces the concentration of cholesterol in the liver and serum by accelerating the conversion of cholesterol to bile acids. Because cholesterol is transported in the blood by lipoprotein complexes (VLDL, LDL, and HDL), the concentrations of cholesterol and lipoprotein are positively correlated (Linne and Ringsrud, 1999). As asimultaneous reduction in total cholesterol and LDL cholesterol levels was observed in this study (Table V). However, differences in the results of different studies may be related to chromium sources and levels, stress conditions, and disease incidence (Huang *et al*., 2016).

Consistent with our results, Samanta *et al*. (2008) and Sathyabama *et al*. (2017) reported that chromium picolinate supplement had no significant effect on serum uric acid concentration in broilers under heat stress compared with the control.

It has been shown already that heat stress in broilers reduces RBC (Hassan and Asim, 2020), Hb level (Ding *et al*., 2020), PCV percentage (Ribeiro *et al*., 2018), increases MCV, and decreases MCHC (Jassim and Hassan, 2011), which are part of the bird's response to temperature regulation (Yahav, 1999). Howevere, RBC and Hb did not change in the current study due to the dietary treatments (Table VI). The findings are in line with Toghyani *et al*. (2006) and Hridoy *et al*. (2021), who also reported no effect of chromium picolinate on these parameters. However, Ezzat *et al*. (2017) found that the addition of chromium picolinate at the level of 1200 µg/kg to the diet of broilers under heat stress caused anincrease in RBC and Hb. It is noted that that chromium, as an antioxidant, can protect vitamin C against oxidative damage. Insulin that is activating by chromium,isalso involved in the transport of vitamin C to red blood cells (Sahin *et al*., 2001). Accordingly, chromium picolinate plus vitamin C can more effectively protect RBCs and Hb against oxidative damage and provide more nutrients by increasing the ability of oxygen transferring under stress conditions (Oleforuh-Okoleh *et al*., 2015). In the current study, the positive effect of adding vitamin C to chromium picolinate containing diet is reflected by a non-significant increase in Hb levels ($P=0.057$).

The percentage of PCV was not affected by the supplements (Table VI), which was consistent with the findings of Toghyani *et al*. (2006) and Hridoy *et al*. (2021). MCV, MCH, and MCHC are indicators of red blood cells, and since MCH and MCHC are calculated using Hb, PCV, or RBC values, any changes in Hb, PCV, and RBC are directly reflected in MCH and MCHC (Ding *et al*., 2020). Consistent with our results, Abdelhady *et al*. (2017) reported that chromium supplement (1400 µg/kg) in the diet of Japanese quails under heat stress reduced MCV. However, Toghyani *et al*. (2006) and Hridoy *et al*. (2021) reported that chromium picolinate supplement in the diet of broilers under heat stress did not affect MCV. It is possible that the supplements caused the reduction of MCV due to their antioxidant capacity to maintain the integrity of RBS membranes (Adekola *et al*., 2010). The supplements had no significant effect on MCH (P>0.05), which was in line with the finding of Toghyani *et al*. (2006) and Hridoy *et al*. (2021) and increased MCHC, that was consistent with the result of Toghyani *et al*. (2006). In contrast, Hridoy *et al*. (2021), did not find any changes in MCHC by chromium picolinate supplement. Since MCHC reflects the haemoglobin content of red blood cells, an increase in its amount may indicates increased haemoglobin production and subsequent oxygen-carrying capacity of this cells (Isroli *et al*., 2020). In keeping with our result, Ezzat *et al*. (2017) also reported that the addition of chromium picolinate supplement to the diet of broilers under heat stress caused a significant increase in WBC. It is revealed that broilers exposed to various stresses show an increase in heterophils and a decrease in lymphocytes, which leads to an increase in the H/L ratio (Aengwanich and Suttajit, 2010). Hence, the H/L ratio is a reliable indicator for avian stress (Felver-Gant *et al*., 2012). Gross and Siegel (1983) found that the number of heterophils in the blood of chickens increased by feeding corticosterone. According to the results, the supplements caused a significant decrease inheterophils and H/L ratio (P<0.05). Such reductions might be mediated by decreased corticosterone secretion, which induced by chromium supplement (Dalólio *et al*., 2018). The decrease in the H/L ratio by adding chromium (Norain *et al*., 2013) or vitamin C (Hassan and Asim, 2020) to the diet of broilers under heat stress has already been reported.

It has been shown that under heat stress, dietary supplementation of chromium increases the concentrations of T3 and T4 in the blood of broilers (Dalólio *et al*., 2018). The same observation has been reported by supplemental chromium picolinate alone (Sahin *et al*., 2003; Ezzat *et al*., 2017) or in combination with vitamin C (Sahin *et al*., 2003) in heat stressed broilers. Although in this study the treatments had no significant effect on T3 and T4 concentrations, the influence of chromium picolinate, alone or in combination with vitamin C on T4 tended to be increased (P= 0.064).

CONCLUSION

In conclusion, the use of dietary chromium picolinate (400 mg/kg) alone or in combination with vitamin C (240 mg/kg) ameliorates the adverse effects of heat stress by improving performance and immune status, and reducing abdominal fat of the birds. Supplementation of vitamin C (240 mg/kg) to the diet failed to show any synergistic effect with chromium picolinate, however, addition of formic acid (0.5%) worsens the influence of supplemental chromium picolinate on growth response, abdominal fat andsome blood parameters.

Funding

Not any funds were provided for this study.

IRB approval

The study was approved by the University of Mohaghegh Ardabili, Ardabil, Iran.

Ethical statement

The study was approved by the ethics committees of the authors' institutions.

Statement of conflict of interest

The authosr have declared no conflict of interest.

REFERENCES

Abdelhady, D.H., Moshira, A. El-Abasy, M.A., Atta, E., Ghazy, W., Abuzed, T.K. and El-Moslemany, A.M., 2017. Synergistic ameliorative effects of organic chromium and selenium against heat stress in Japanese quails: Performance, immunological, hematological, biochemical and antioxidant studies. *Alex. J. Vet. Sci*., **55**: 113-123. https://doi.org/10.5455/ajvs.282703

Abidin, Z. and Khatoon, A., 2013. Heat stress in poultry and the beneficial effects of ascorbic acid (vitamin C) supplementation during periods of heat stress. *World's Poult. Sci. J*., **69**: 135-152. https://doi.org/10.1017/S0043933913000123

Adekola, A.Y., Kaankuka, F.G., Ikyume, T.T. and Yaakugh, I.D.I., 2010. Ascorbic acid effect on erythrocyte osmotic fragility, haematological parameters and performance of weaned rabbits at the end of rainy season in Makurdi. *Niger. J. Anim. Sci*., **9**: 1077-1085. https://www.researchgate.net/publication/282612580

Aengwanich, W. and Suttajit, M., 2010. Effect of polyphenols extracted from tamarind (*Tamarindus indica* L.) seed coat on physiological changes, heterophil/ lymphocyte ratio, oxidative stress and body weight of broilers (*Gallus domesticus*) under chronic heat stress. *Anim. Sci. J*., **81**: 264-270. https://doi.org/10.1111/j.1740-0929.2009.00736.x

Ahmed, N., Haldar, S., Pakhira. M.C. and Ghosh, T.K., 2005. Growth performances, nutrient utilization and carcass traits in broiler chickens fed with a normal and a low energy diet supplemented with inorganic chromium (as chromium chloride hexahydrate) and a combination of inorganic chromium and ascorbic acid. *J. agric. Sci*., **143**: 427-439. https://doi.org/10.1017/S0021859605005617

Alhassani, D.H. and Alshukri, A.Y., 2016. Comparative efficacy of different supplements with drinking water used to alleviate body temperature of heat-stressed broiler chickens. *Iraqi J. agric. Sci*., **47**(**Special Issue**): 12-18. https://www.researchgate.net/publication/295402371

Attia, Y.A., Al-Harthi, M.A., El-Shafey, A.S., Rehab, Y.A. and Kim, W.K., 2017. Enhancing tolerance of broiler chickens to heat stress by supplementation with vitamin E, vitamin C and/ or probiotics. *Annls Anim. Sci*., **17**: 1155-1169. https://doi.org/10.1515/aoas-2017-0012

Bartlett, J.R. and Smith, M.O., 2003. Effects of different levels of zinc on the performance and immune competence of broilers underheat stress. *Poult. Sci*., **82**: 1580-1588. https://doi.org/10.1093/ps/82.10.1580

Brindley, D.N. and Salter, A.M., 1991. Hormonal regulation of the hepatic low density lipoprotein: Relationship with the secretion of very low density lipoprotein. *J. Lipid Res*., **30**: 349-360. https://doi.org/10.1016/0163-7827(91)90003-N

Brzoska, F., Sliwinski, B. and Michalik-Rutkowska, O., 2013. Effect of dietary acidifier on growth, mortality, post-slaughter parameters and meat composition of broiler chickens. *Annls Anim. Sci*., **13**: 85-96. https://doi.org/10.2478/v10220-012-0061-z

Cai, Y., Song, Z., Zhang, X., Wang, X., Jiao, H. and Lin, H., 2009. Increased de novo lipogenesis in liver contributes to the augmented fat deposition in dexamethasone exposed broiler chickens (*Gallus gallusdomesticus*) comparative biochemistry and physiology part C. *Regul. Toxicol. Pharmacol*., **150**: 164-169. https://doi.org/10.1016/j.cbpc.2009.04.005

Chen, G., Gao, Z., Chu, W., Cao, Z., Li, C. and Zhao, H., 2018. Effects of chromium picolinate on fat deposition , activity and genetic expression of lipid metabolism-related enzymes in 21 day old ross broilers. *Asian Australas. J. Anim. Sci*., **31**: 569-575. https://doi.org/10.5713/ajas.17.0289

Chougule, A.J., Wade, M.R., Manwar, S.J., Kuralkar, S.V., Khose, K.K. and Hedau, M., 2018. Effect of supplementation heat stress alleviating agents on growth performance of broilers subjected to heat stress. *Indian J. Poult. Sci*., **53**: 82-87. https://www.researchgate.net/publication/329189469, https://doi.org/10.5958/0974-8180.2018.00005.3

Colgan, M., 1993. Chromium boosts insulin efficiency. In: *Optimum Sports Nutrition*. Advanced Research Press, New York, pp. 313-320.

Dalólio, F.S., Albino, L.F.T., Silva, J.N., Campos, P.H.R.F., Lima, H.J.D., Moreira, J. and Ribeiro, J.V., 2018. Dietary chromium supplementation for heat-stressed broilers. *World's Poult. Sci. J*., **74**: 101-116. https://doi.org/10.1017/S0043933917001064

Ding, J., He, S., Xiong, Y., Liu, D., DaiI, S. and Hu, H., 2020. Effects of dietary supplementation of fumaric acid on growth performance, blood hematological and biochemical profile of broiler chickens exposed to chronic heat stress. *Braz. J. Poult. Sci*., **22**: 1-8. https://doi.org/10.1590/1806-9061-2019-1147

Ezzat, W., Abdallah, E.A., Rizk, A.M., Ouda, M.M.M. and Raga, E.A., 2017. Impact of chromium picolinate supplementation on productive performance, immune response and heat shock proteins of broiler chickens under heat-stress condition. *Egypt. Poult. Sci*., **37**: 559-583.

Felver-Gant, J.N., Mack, L.A., Dennis, R.L., Eicher, S.D. and Cheng, H.W., 2012. Genetic variations alter physiological responses following heat stress in 2 strains of laying hens. *Poult. Sci*., **91**: 1542-1551. https://doi.org/10.3382/ps.2011-01988

Geraert, P.A., Padilha, J.C.F. and Guillaumin, S., 1996. Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: growth performance, body composition and energy retention. *Br. J. Nutr*., **75**: 195-204. https://doi.org/10.1079/BJN19960124

Ghazalah, A.A., Atta, A.M., Elkloub, K., Mustafa, M.E.L. and Shata, R.F.H., 2011. Effect of dietary supplementation of organic acids on performance, nutrients digestibility and health of broiler chicks. *Int. J. Poult. Sci*., **10**: 176-184. https://doi.org/10.3923/ijps.2011.176.184

Gous, R.M. and Morris, T.R., 2005. Nutritional interventions in alleviating the effects of high temperatures in broiler production. *World's Poult. Sci. J*., **61**: 463-475. https://doi.org/10.1079/WPS200568

Gross, W.B. and Siegel, H.S., 1983. Evaluation of the heterophil/ lymphocyte ratio as a measure of stress in chickens. *Avian Dis*., **27**: 972-979. https://doi.org/10.2307/1590198

Habibian, M., Ghazi, S. and Moeini, M.M., 2013. Lack of effect of dietary chromium supplementation on growth performance and serum insulin, glucose, and lipoprotein levels in broilers reared underheat stress condition. *Biol. Trace Elem. Res*., **153**: 205-

211. https://doi.org/10.1007/s12011-013-9663-2

Hajati, H., Hassanabadi, A., Golian, A., Nassiri-Moghaddam, H. and Nassiri, M.R., 2018. The effect of grape seed extract supplementation on performance, antioxidant enzyme activity, and immune responses in broiler chickens exposed to chronic heat stress. *Iran. J. appl. Anim. Sci*., **8**: 109-117. https://www.researchgate.net/publication/323724222

Haldar, S., Mondal, S., Samanta, S. and Ghosh, T.K., 2009. Effects of dietary chromium supplementation on glucose tolerance and primary antibody response against peste des petits ruminants in dwarf Bengal goats (*Capra hircus*). *Animal*, **3**: 209-217. https://doi.org/10.1017/S1751731108003418

Hamidi, O., Chamani, M., Ghahri, H., Ali, A.S. and Kafkas, H.M., 2016. 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. *Kafkas Univ. Vet. Fak. Derg*., **22**: 373-380. https://doi.org/10.29011/2637-9988/100013

Hamidi, O., Chamani, M., Ghahri, H., Sadeghi, A.A. and Malekinejad, H., 2019. Effects of using different levels of chromium picolinate on performance, some blood biochemical and intestinal morphology and microflora in ross 308 broiler chicks exposed to the heat stress condition. *Int. J. Pharm. Sci. Res*., **10**: 4494-4500.

Haq, Z., Jain, R., Mahajan, A., Ganai, I.A., Khan, N. and Mudasir, S., 2018. Dietary supplementation of chromium yeast alone and in combination with antioxidants for designing broiler meat. *J. Ent. Zool. Stud*., **6**: 766-770.

Haq, Z., Jain,R.K., Khan, N., Dar, M.Y., Ali, S., Gupta, M. and Varun, T.K., 2016. Recent advances in role of chromium and its antioxidant combinations in poultry nutrition: A review. *Vet. World*, **9**: 1392-1399. https://doi.org/10.14202/vetworld.2016.1392-1399

Hassan, A.A. and Asim, R.A., 2020. Effect of vitamin C and acetylsalicylic acid supplementation on some hematological value, heat shock protein 70 concentration and growth hormone level in broiler exposed to heat stress. *Iraqi J. vet. Sci*., **34**: 357-363. https://doi.org/10.33899/ijvs.2019.125950.1195

Hirakawa, R., Nurjanah, S., Furukawa, K., Murai, A., Kikusato, M., Nochi, T. and Toyomizu,T., 2020. Heat stress causes immune abnormalities viavmassive damage to effect proliferation and differentiation of lymphocytes in broiler chickens. *Front. Vet. Sci*., **7**: 1-13. https://doi.org/10.3389/fvets.2020.00046

Hridoy, A.F.M., Hossain, M.S., Haque, M.I., Sujan, K.M., Khalil, K.K.I., Mustari, A. and Miah, M.A., 2021. Organic chromium and vitamin E enhance physiological performances, humoral and cellular immune responses in heat stressed broiler chickens. *J. Dairy. Fd. Home Sci*., **40**: 94-99. https://doi.org/10.18805/ajdfr.DR-206

Hu, R., He, Y., Arowolo, M.A., Wu, S. and He, J., 2019. Polyphenols as potential attenuators of heat stress in poultry production. *Antioxidants*, **8**: 1-11. https://doi.org/10.3390/antiox8030067

Huang, Y., Yang, J. Xiao, F. Lloyd, K. and Lin, X., 2016. Effects of supplemental chromium source and concentration on growth performance, carcass traits and meat quality of broilers under heat stress conditions. *Biol. Trace Elem. Res*., **170**: 216-223. https://doi.org/10.1007/s12011-015-0443-z

Isroli, I., Murwani, R., Yudiarti, T., Widiastuti, E., Wahyuni, H.I., Sartono, T.A. and Sugiharto, S., 2020. Body weight, haematological indices and intestinal morphometric of broilers provided with diets containing formic acid, butyric acid or their blends. *J. Indones. Trop. Anim. Agric*., **45**: 37-46. https://doi.org/10.14710/jitaa.45.1.37-46

Jassim, H.M. and Hassan, A.A., 2011. Changes in some blood parameters in lactating female rats and their pups exposed to lead: Effects of vitamins C and E. *Iraqi J. Vet. Sci*., **25**: 1-7. https://doi.org/10.33899/ijvs.2011.5708

Khan, S.H. and Sardar, R., 2005. Effect of vitamin C supplementation on the performance of Desi, Fayoumi and commercial white leghorn chicken exposed to heat stress. *Pak. Vet. J*., **25**: 163-166.

Khukhodziinai, J.S., Ayub Ali, M., Lallianchhunga, M.C., Goswami, R., Tolenkhomba, T.C. and Mayengbam, P., 2021. Supplementation of chromium and vitamin c to alleviate cold stress in white leghorn chicken. *J. Ent. Zool. Stud*., **9**: 1295-1298. https://www.researchgate.net/publication/349075613

Kim, J.W., Kim, J.H. and Kil, D.Y., 2015. Dietary organic acids for broiler chickens: A review. *Rev. Colomb. Cienc. Pecu*., **28**: 109-123. https://doi.org/10.17533/udea.rccp.v28n2a01

Kucuk, O., Sahin, N. and Sahin, K., 2003. Supplemental zinc and vitamin A can alleviate negative effects of heat stress in broiler chickens. *Biol. Trace Elem. Res*., **94**: 225-235. https://doi.org/10.1385/BTER:94:3:225

Kulkarni, R.M. andal, A., Bhanja, S., Goel, A. and Mehara, M., 2018. Dietary supplementation of chromium picolinateon productive performanc eandcost economics of coloured broiler chicken during hot-humid summer. *Int. J. Livest. Res.*, **8**: 132-139. https://doi.org/10.5455/ijlr.20171230053426

Lara, L.J. and Rostagno, M.H., 2013. Impact of heat stress on poultry production. *Animal*, **3**: 356-369. https://doi.org/10.3390/ani3020356

Lien, T.F., Horng, Y.M. and Yang, K.H., 1999. Performance, serum characteristics, carcass traits and lipid metabolism of broilers as affected by supplement of chromium picolinate. *Br. Poult. Sci.*, **40**: 357-363. https://doi.org/10.1080/00071669987458

Linne, J.J. and Ringsrud, K.M., 1999. *Chemistry in clinical laboratory science* (eds. J.J. Linne and K.M. Ringsrud), Fourth edition, Mosby Inc. USA, pp. 264-266.

Lu, L., Zhao, L.L., Dong, S.Y., Liao, X.D., Dong, X.Y., Zhang, L.Y. and Luo, X.G., 2019. Dietary supplementation of organic or inorganic chromium modulates the immune responses of broilers vaccinated with avian influenza virus vaccine. *Animal*, **13**: 1-9. https://doi.org/10.1017/S1751731118002379

Marai, I.F. M., El-Darawany, A.A., Fadiel, A. and Abdel-Hafez, M.A.M., 2007. Physiological traits as affected by heat stress in sheep. A review. *Small Rumin. Res.*, **71**: 1-12. https://doi.org/10.1016/j.smallrumres.2006.10.003

Mertz, W., 1993. Chromium in human nutrition: A review. *J. Nutr.*, **123**: 626-633. https://doi.org/10.1093/jn/123.4.626

Milillo, S.R., Martin, E., Muthaiyan, A. and Ricke, S.C., 2011. Immediate reduction of *Salmonella enterica* serotype typhimurium following exposure to multiple-hurdle treatments with heated, acidified organic acid salt solutions. *Appl. environ. Microbiol.*, **77**: 3765-3772. https://doi.org/10.1128/AEM.02839-10

Norain, T.M., Ismail, I.B., Abdoun, K.A. and Al-Haidary, A.A., 2013. Dietary inclusion of chromium to improve growth performance and immue-competence of broilers under heat stress. *Ital. J. Anim. Sci.*, **12**: 562-566.

Oleforuh-Okoleh, V.U., Ndofor-Foleng, H.M., Olorunleke, S.O. and Uguru, J.O., 2015. Evaluation of growth performance, haematological and serum biochemical response of broiler chickens to aqueous extract of ginger and garlic. *J. agric. Sci.*, 7: 167-173. https://www.researchgate.net/publication/273383163, https://doi.org/10.5539/jas.v7n4p167

Panda, A.K., Raju, M.V.L.N., RamaRao, S.V., Shyam, S.G. and Reddy, M.R., 2009. Effect of graded levels of formic acid on gut microflora count, serum biochemical parameters, performance, and carcass yield of broiler chickens. *Indian J. Anim. Sci.*, **79**: 1165-1168.

Ragaa, N.M. and Korany, R.M.S., 2016. Studying the effect of formic acid and potassium diformate on performance, immunity and gut health of broiler chickens. *Anim. Nutr.*, **2**: 296-302. https://doi.org/10.1016/j.aninu.2016.08.003

Rashidi, A.A., Ivari, Y.G., Khatibjoo, A. and Vakilia, R., 2010. Effects of dietary fat, vitamin e and zinc on immune response and blood parameters of broiler reared under heat stress. *Res. J. Poult. Sci.*, **3**: 32-38. https://doi.org/10.3923/rjpscience.2010.32.38

Ribeiro, M.N., Ribeiro, N.L., Bozzi, R. and Costa, R.G., 2018. Physiological and biochemical blood variables of goats subjected to heat stress. A review. *J. appl. Anim. Res.*, **46**: 1036- 1041. https://doi.org/10.1080/09712119.2018.1456439

Ricke, S.C., Dittoe, D.K. and Richardson, K.E., 2020. Formic acid as an antimicrobial for poultry production: A review. *Front. Vet. Sci.*, **7**: 1-13. https://doi.org/10.3389/fvets.2020.00563

Sahin, K., Kucuk, O. and Şahin, N., 2001. Effects of dietary chromium picolinate supplementation on performance and plasma concentrations of insulin and corticosterone in laying hens under low ambient temperature. *J. Anim. Physiol. Anim. Nutr.*, **85**: 142-147. https://doi.org/10.1046/j.1439-0396.2001.00314.x

Sahin, K., Sahin, N., Kucuk, O., 2003. Effects of chromium, and ascorbicacid supplementation on growth, carcasstraits, serum metabolites, and antioxidant status of broiler chickens reare data high ambient temperature (32°C). *Nutr. Res.*, **23**: 225-238. https://doi.org/10.1016/S0271-5317(02)00513-4

Sahin, K., Sahin, N., Onderci, M., Gursu, F. and Cikim, G., 2002. 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.*, **89**: 53-64. https://doi.org/10.1385/BTER:89:1:53

Sahin, N., Akdemir, F., Tuzcu, M., Hayirli, A., Smith, M.O. and Sahin, K., 2010. Effects of supplemental chromium sources and levels on performance, lipid peroxidation and proinflammatory markers in heat-stressed quails. *Anim. Feed Sci. Technol.*, **159**: 143-149. https://doi.org/10.1016/j.

anifeedsci.2010.06.004

Sahin, N., Hayrli, A., Orhan, C., Tuzcu, M., Akdemir, F., Komorowski, J.R. and Sahin, K., 2017. Effects of supplemental chromium form on performance and oxidative stress in broilers exposed to heat stress. *Poult. Sci*., **96**: 4317-4324. https://doi.org/10.3382/ps/pex249

Samanta, S., Haldar, S., Bahadur, V. and Ghosh, T.K., 2008. 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. Fd. Agric*., **88**: 787-796. https://doi.org/10.1002/jsfa.3146

Sands, J.S. and Smith, M.O., 2002. Effects of dietary manganese proteinate or chromium picolinate supplementation on plasma insulin, glucagon, glucose and serum lipids in broiler chickens reared under thermoneutral or heat stress conditions. *Int. J. Poult. Sci*., **1**: 145-149. https://doi.org/10.3923/ijps.2002.145.149

Saracila, M., Panaite, T.D., Mironeasa, S. and Untea, A.E., 2021. Dietary supplementation of some antioxidants as attenuators of heat stress on chicken meat characteristics. *Agriculture*, **11**: 1-14. https://doi.org/10.3390/agriculture11070638

Sathyabama, T., Nanjappan, K., Ravi, R., Jagadeeswaran, A. and Visha, P., 2017. Effect of various forms of chromium supplementation on growth performance, blood biochemical constituents of broiler chickens. *Indian Vet. J.,* **94**: 22-24. https://www.researchgate.net/publication/322417247

Shakeri, M., Oskoueian, E., Le, H.H. and Shakeri, M., 2020. Strategies to combat heat stress in broiler chickens: Unveiling the roles of selenium, vitamin E and vitamin C. *Vet. Sci*., **7**: 1-9. https://doi.org/10.3390/vetsci7020071

Sirirat, N., Lu, J., Hung, A.T., Chen, S., Lien, T., 2012. Effects different levels of nanoparticles chromium picolinate supplementation on growth performance, mineral retention, and immune responses in broiler chickens. *J. agric. Sci*., **4**: 48-58. https://doi.org/10.5539/jas.v4n12p48

Sorice, A., Guerriero, E., Capone, F., Colonna, G., Castello, G. and Costantini, S., 2014. Ascorbic acid: Its role in immune system and chronic inflammation diseases. *Mini Rev. Med. Chem*., **14**: 444-452. https://doi.org/10.2174/1389557514666140428112602

Suksombat, W. and Kanchanatawee, S., 2005. Effects of various sources and levels of chromium on performance of broilers. *Asian Australas. J. Anim. Sci*., **18**: 1628-1633. https://doi.org/10.5713/ajas.2005.1628

Tawfeek, S.S., Hassanin, K.M.A. and Youssef, I.M.I., 2014. The effect of dietary supplementation of some antioxidants on performance, oxidative stress, and blood parameters in broilers under natural summer conditions. *J. World's Poult. Res*., **4**: 10-19.

Toghyani, M., Shivazad, M., Gheisari, A.A. and Zarkesh, S.H., 2006. Performance, carcass traits and hematological parameters of heat-stressed broiler chicks in response to dietary levels of chromium picolinate. *Int. J. Poult. Sci*., **5**: 65-69. https://doi.org/10.3923/ijps.2006.65.69

Toghyani, M., Zarkesh, S., Shivazad, M. and Gheisari, A., 2007. Immune responses of broiler chicks fed chromium picolinate in heat stress condition. *Poult. Sci. J*., **44**: 330-334. https://doi.org/10.2141/jpsa.44.330

Vincent, J.B., 2000. The biochemistry of chromium. *Nutr. J*., **130**: 715-718. https://doi.org/10.1093/jn/130.4.715

Whitehead, C.C. and Keller, T., 2003. An update on ascorbic acid in poultry. *World's Poult. Sci. J*., **59**: 161-184. https://doi.org/10.1079/WPS20030010

Xiao, F., Ao, D., Zhou, B., Spears, J.W., Lin, X. and Huang, Y., 2017. Effects of supplemental chromium propionate on serum lipids, carcass traits, and meat quality of heat-stressed broilers. *Biol. Trace Elem. Res*., **176**: 401-406. https://doi.org/10.1007/s12011-016-0852-7

Yahav, S., 1999. The effect of constant and diurnal cyclic temperatures on performance and blood system of young turkeys. *J. Therm. Biol*., **24**: 71-78. https://doi.org/10.1016/S0306-4565(98)00042-4

Zhao, Y., Yang, X., Lu, W., Liao, H. and Liao, F., 2009. Uricase based methods for determination of uric acid in serum. *Microchim. Acta*, **164**: 1-6. https://doi.org/10.1007/s00604-008-0044-z

Zheng, C., Huang, Y., Xiao, F., Lin, X. and Lloyd, K., 2016. Effects of supplemental chromium source and concentration on growth, carcass characteristics, and serum lipid parameters of broilers reared undernormal conditions. *Biol. Trace Elem. Res*., **169**: 352-358. https://doi.org/10.1007/s12011-015-0419-z