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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

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ABSTRACT: This study was aimed to investigate the effects of different levels of dietary chromium picolinate (CrPic) on performance, some blood biochemical and intestinal morphology and microflora in Ross 308 broiler chicks. A total of 240 broiler chickens Ross 308 strain, from 21 to 42 days old were used in a completely randomized design. All chickens consumed a diet based on the corn-soybean meal, and the treatments were control- (thermo-neutral) and control + (heat stress) with no add-on and the others with CrPic supplementation at levels 500, 1000 and 1500 ppb respectively. The blood serum samples were subjected to biochemical analysis for blood parameters. The histomorphometric examination was performed by light microscopy. The result showed that all the growth and feed intake and feed conversion ratio parameters were declined when the broilers fed the CrPic supplementation ($P \leq 0.05$). The serum glucose concentration decreased CrPic supplemented groups. As a result, revealed that the villus height and crypt depth in the ileum and jejunum. Ileal villus height of birds on CrPic groups was significantly taller than that of chicks on the control. The result of intestinal microbiota investigations suggested that feeding management regimens with CrPic could have a more profound impact on the gut microbiota of experimental chicks than to that of control birds. In conclusion, we could demonstrate that using of different levels of CrPic could have some beneficial effects on performance, some blood biochemical and intestinal morphology and microflora in Ross 308 broiler chicks exposed to the heat stress condition.

INTRODUCTION: Increase environmental temperatures, especially with high humidity, can be devastating to commercial broilers. Nutritional modifications appear to be the much logical approach to overcome the environmental stress, especially heat stress in poultry industry [1].

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Heat stress increases mineral and vitamin mobilization from tissues and their excretion, and it may exacerbate a marginal vitamin and mineral deficiency or an increased mineral and vitamin requirement [2].

Effect of some minerals and vitamin supplements such as chromium to lower the negative effects of heat stress is well documented [3, 4]. It is well known that heat stress is one of the major stressors on poultry production, which produces a wide range of physiological changes. It increases circulating concentrations of corticosterone in broilers, and it is well reported that corticosterone reduces insulin sensitivity in broiler chicks [5, 6]. Chromium (Cr) is a chemical element and atomic number 24 and is the first element in group 6. It is a steely grey, lustrous, hard and brittle metal [7]. It has antioxidant effects, and its deficiency leads to disrupting carbohydrate and protein metabolism [8]. Chromium was identified as an essential nutrient and accepted as a trace element for its roles in the action of insulin, a hormone critical to the metabolism and storage of carbohydrate, fat, and protein [9]. Trivalent Cr is associated with the metabolism of nutrients in animals termed as "glucose tolerance factor" since Cr regulates the metabolic action of insulin [10].

Some researchers suggested that supplementation of Cr at different levels in poultry improved feed intake and its efficiency. Samanta *et al.* reported that the 0.5 mg per kg of Cr to improve the feed intake and feed conversion ratio of broiler chickens [11]. Many studies supported that the Cr supplementation at a dose of 1500 ppb improved bird's performance during heat stress condition significantly [12]. Chromium has been utilized for weight gain, to improve feed conversion ratio, increase relative organ weight, muscle development, decrease cholesterol, increase high-density lipoprotein cholesterol (HDL), and improve nutrient digestion. Halder and Ghosh observed that serum glucose concentration significantly declined in the broiler supplemented with 0.5 and 1 mg Cr/kg diet compared to the control [11].

Moeini *et al*. concluded that using Cr reduces serum triglycerides and low-density lipoprotein cholesterol (LDL) and elevates serum HDL cholesterol heat-stressed broilers instead of [13]. Ibrahim *et al*., observed that total blood lipid was significantly lowered in dietary Cr supplemented [14]. Al-Sultan *et al.* reported Cr supplementation increased the serum concentration of calcium, phosphorous, and potassium and decreased the level of sodium [15]. Amatya *et al*. found that Cu, Zn, Fe, and Mn retention was better when Cr was supplemented in the feed of broilers [16]. The National Research Council has recommended chromium at 300 μg per kg in diets of lab animals [17]; however, currently, there are no NRC recommendations for chromium in poultry diets. Also few studies have been designed to compare the effectiveness of different levels of Cr and CrPic on the blood biochemical and intestinal microflora of broiler chicks, Thus, the present study was aimed to investigate the effects of using different levels of CrPic on performance, some blood biochemical and intestinal microflora in Ross 308 broiler chicks exposed to the heat stress condition.

MATERIALS AND METHODS: All procedures used in this experiment were approved by the Department of Animal Sciences, Faculty of Agriculture and Food industry, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran.

The Birds and the Diets: A total of 240 broiler chickens Ross 308 strain, from 21 to 42 days old were used in a completely randomized design. All chickens consumed a diet based on the corn-soybean meal, which provided as mash form was formulated based on NRC [17] **Table 1**. On day 21, the broiler chicks were weighed and, the birds with same weight allocated into 5 different groups with 4 replications and 12 birds each. The treatments were control- (thermo-neutral) and control + (heat stress) were fed with no additive, and other groups were fed 500; 1.000; or 1.500 ppb of chromium picolinate. The CrPic used in the current study was purchased from Sigma-Aldrich ($C_{18}H_{12}CrN_3O_6$), Cat. no. C4124, CAS Number = 14639-25-9, USA.

Diets and fresh water were provided *ad libitum* during the experimental period. The birds in the control group were kept in comfort zone temperature (23 ± 1°C from 21 to 28 day and 21±1 °C from 28-42 days), and the heat stressed groups were kept under 36 ± 1 °C ambient temperature from 08:00 to 18:00 h. Additionally, the environmental temperature of the heat stressed birds was reduced to the equal to that of the control group.

Performance: During the experimental period, broilers performance was assessed for body weight gain, feed intake, feed conversion ratio, and feed efficiency ratio. The feed conversion ratio was calculated based on feed intake/gain for each replicate. At the end of the experimental period (42 days old), the birds were slaughtered by cervical dislocation method.

Blood Sampling and Biochemical Analysis: The blood samples were taken from the brachial vein from two birds per each replicate and stored at refrigerator at 4 °C. Also, the blood serum samples were subjected to biochemical analysis for blood parameters. Individual serum samples were analyzed for albumin and globulin by subtracting the obtained albumin level from the total protein and glucose, cholesterol by an automatic biochemical analyzer following the instructions of the corresponding reagent kit (Pars Azmoon Co., Teheran, Iran). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by Reitman and Frankel method [18].

Histomorphometric Examination of Intestine: The histomorphometric examination was performed by light microscopy, and the measurement was done using public domain image analysis software (Image J, National Institute of Mental Health, Bethesda, MD, USA) and described by Rezaian [19].

Evaluation Intestinal Microflora: The internal organs were removed after slaughter. About 8 cm from the length of the ileum was sampled to determine the microbial population. Als, one g of ileum content was used to make 10-fold dilution using buffered peptone water, and then 0.1 ml of the appropriate ileum dilution was spread on Lactobacillus MRS Agar-Hi Media Laboratories to detect lactic acid bacteria (*Lactobacillus*) and VRBA (Violet Red Bile Agar) to detect *Escherichia coli* (*E. coli*) form. The cultures of *Lactobacillus* and *E. coli* bacteria were made anaerobically form. The plates were incubated at 37.5 °C for 48 h. After counting the number of colonies in each plate, the number so obtained was multiplied by the inverse of the dilution and the result was stated as the number of colony forming unit (CFU) in 1 g of sample [20].

TABLE 1: INGREDIENTS AND CHEMICAL COMPOSITIONS OF THE EXPERIMENTAL DIETS

Ingredients %	Starter (0-21 days old)	Finisher (21-42 days old)
Corn grain	60.07	66.00
Soybean meal	30.0	24.00
Corn gluten meal	2.50	3.00
Vegetable oils	2.30	2.55
DCP	1.70	1.70
Limestone	1.20	1.20
NaCl	0.30	0.30
L- Lysine	0.16	0.15
DL-Methionine	0.14	0.10
Mineral and vitamin premix*	1.00	1.00
CrPic (ppb per each Kg)	0	500-1000-1500
Calculated nutrient content		
ME($_{Kcal/Kgr}$)	3120	3190
CP (%)	21.10	19.00
Ca (%)	0.90	0.95
Available phosphorus (%)	0.40	0.40
Lysine (%)	1.10	0.90
Methionine + Cystine (%)	0.50	0.40

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.

Statistical Analysis: Data were collected and analyzed using the General Linear Model Procedure (GLM), and the comparison of means was made through Duncan multiple range tests by using SAS 9.1 software [21]. The statistical model was:

$$Yij = \mu + Ti + \beta j + \varepsilon ij$$

Whereas Y_{ij} is the individual observation, μ is the overall mean, T_i is the effect of treatment, T_i is the effect of blocks, and e_{ij} is the remainder effect.

RESULTS: The results of CrPic supplementation on performance of heat-stressed broilers are shown in **Table 2**.

All the growth and feed intake and feed conversion ratio parameters declined when the broilers fed the chromium picolinate supplementation, which indicated that high CrPic supplementation was intolerable to birds leading to the reduced growth rate in (21- 42 days old).

TABLE 2: EFFECTS OF CRPIC SUPPLEMENTATION ON PERFORMANCE OF HEAT STRESSED BROILERS

Treatments	FI (kg bird^{-1})	BW (kg bird^{-1})	FCR	FER
Control (-)	0.148^{a*}	0.075^{a}	1.97^{b}	0.50^{a}
Control (+)	0.135^{bc}	0.053^{c}	2.55^{a}	0.39^{c}
CrPic (500)	0.139^{b}	0.065^{b}	2.14^{ab}	0.46^{ab}
CrPic (1000)	0.131^{cd}	0.058^{bc}	2.26^{ab}	0.44^{b}
CrPic (1500)	0.130^{cd}	0.059^{bc}	2.20^{ab}	0.45^{b}
SEM	0.001	0.015	0.040	0.012

*Means within a row with no common on the letter are significantly different ($p<0.05$).

The blood parameters (glucose, cholesterol, albumin, globulin, total protein, AST, and ALT) of bids serum values and contents are shown in **Table 3**. The serum glucose concentration decreased in broilers received CrPic supplements compared with other treatments. Slight and significant decreases were observed in cholesterol serum concentration of treated groups than controls while the mean serum albumin and globulin concentration was higher in the CrPic group compared with the controls group. Also, serum ASR and ALT level increased in broiler received CrPic supplements ($p<0.05$).

TABLE 3: EFFECTS OF CRPIC SUPPLEMENTATION ON SOME BLOOD BIOCHEMICAL OF HEAT STRESSED BROILERS

Treatments	Glucose (mg dl^{-1})	Cholesterol (mg dl^{-1})	Albumin (g dl^{-1})	Globulin (g dl^{-1})	Total protein (g dl^{-1})	AST (IU dl^{-1})	ALT (IU dl^{-1})
Control (-)	221.5^{a*}	139.1^{a}	1.82^{b}	2.90^{b}	4.65^{c}	45.24^{b}	9.00^{b}
Control (+)	220.2^{a}	138.2^{a}	1.89^{b}	3.00^{b}	4.76^{b}	45.39^{a}	9.04^{a}
CrPic (500)	208.5^{ab}	135.4^{ab}	2.04^{ab}	3.07^{ab}	4.94^{ab}	45.40^{a}	9.02^{a}
CrPic (1000)	205.7^{b}	133.2^{b}	2.12^{a}	3.24^{a}	5.00^{a}	45.61^{a}	9.06^{a}
CrPic (1500)	204.1^{b}	131.6^{b}	2.35^{a}	3.38^{a}	5.21^{a}	46.10^{a}	9.08^{a}
SEM	0.067	0.078	0.041	0.053	0.034	0.044	0.001

*Means within a row with no common on the letter are significantly different ($p<0.05$).

The morphological indices evaluated were the villus height and crypt depth in the ileum and jejunum. Ileal villus height of birds on CrPic groups was significantly taller than that of chicks on the control. The crypt depth in the jejunum was significantly affected by the dietary treatments. Ileal and jejunal morphology was affected more profoundly in CrPic groups than control groups.

TABLE 4: EFFECTS OF CRPIC SUPPLEMENTATION ON INTESTINAL MORPHOLOGY OF HEAT STRESSED BROILERS (µm)

Treatments	Villus height (Ileum)	Villus height (Jejunum)	Crypt depth (Ileum)	Crypt depth (Jejunum)
Control (-)	362.10^{c*}	510.23^{a}	86.30^{a}	80.34^{c}
Control (+)	365.14^{c}	509.14^{a}	82.21^{a}	82.14^{c}
CrPic (500)	400.10^{b}	468.32^{b}	76.14^{b}	88.23^{b}
CrPic (1000)	436.16^{a}	460.25^{b}	70.32^{bc}	91.02^{a}
CrPic (1500)	495.20^{a}	429.12^{c}	65.19^{c}	94.26^{a}
SEM	16.20	18.46	5.21	6.44

*Means within a row with no common on the letter are significantly different ($p<0.05$).

TABLE 5: EFFECTS OF CRPIC SUPPLEMENTATION ON INTESTINAL MICROFLORA OF HEAT STRESSED BROILERS

Treatments	*Escherichia coli* (log $_{10}$CFU g^{-1})	*Lactobacillus* (log ^{10}CFU g^{-1})
Control (-)	7.21^{a}	7.86^{b}
Control (+)	7.06^{a}	7.92^{b}
CrPic (500)	6.65^{b}	8.11^{ab}
CrPic (1000)	6.20^{bc}	8.36^{a}
CrPic (1500)	6.00^{c}	8.55^{a}
SEM	0.45	0.57

*Means within a row with no common on the letter are significantly different ($p<0.05$).

These results of **Table 5** suggested that feeding management regimens with CrPic could have a more profound impact on the gut microbiota of experimental chicks than to that of control birds. The *E. coli* populations decreased and *Lactobacillus* increased in CrPic supplemented groups significantly ($P\leq0.05$).

DISCUSSION: The result of the current study showed that the CrPic supplementation improved the performance of the broilers in terms of the final weight, weight gain, feed conversion ratio and better feed efficiency ratio and this is an agreement with the reports of Sands and Smith [22]. Toghyani *et al.*, reported an increase in body weight gain and feed intake of broilers under heat stress conditions when supplemented with chromium picolinate [23]. They mentioned that due to insulin-glucose can be utilized by body cells, and adequate amino acids enter the cells; therefore, the muscle can be built. Zhang *et al.* reported that CrPic improved FCR in broilers chicks [24]. As a result, revealed form our study, there were significant differences (P≤0.05) in blood biochemical as affected by dietary supplementations comparing to their control counterparts.

Imik *et al.*, documented free radical generation affects blood serum metabolites of plasma total protein, cholesterol, and glucose, which are manifested in bird's adaptation response through decreased production performance and the birds health [25]. Patil *et al.*, who reported a decrease in serum total cholesterol, triglycerides, and increased serum HDL when broiler chickens were fed diet supplemented with chromium [26]. Our observation in this study is in line with that Ezzat *et al.*, result who reported that concentrations of blood serum glucose, triglyceride, and cholesterol were significantly higher whereas, levels of total protein and albumin were significantly lower in birds exposed to heat stress [27].

Chromium supplementation increases the biological activity of insulin which decreases adipocyte lipolysis by reducing the activities of adenylate cyclase and hormone-sensitive lipase; additionally, it can decrease triglycerides rich lipoprotein by increasing the lipoprotein lipase activity which in turn increases serum triglyceride clearance [28]. It was hypothesized that increased glucose uptake should increase oxidation of glucose, which would be otherwise converted to fatty acids and stored as triglycerides in adipose tissues [29].

Sahin *et al.* observed that the addition of Cr picolinate caused a decrease in serum cortisol concentration [30]. It has an impact on specific insulin binding in the liver of broilers. Cr is also required for normal functioning of the cells in the pancreas, preventing hyperresponsiveness of insulin secretion to glucose stimulation, shown to be a protective factor against heart disease and achieving regression of cholesterol-induced arteriosclerosis [31].

Our results about serum blood glucose and cholesterol agreed with Hanafy [7], found that serum cholesterol levels of birds were decreased chromium supplementation. The difference in total protein among experimental groups was similar with the result of Sahin *et al.*, who found that supplemental chromium had no effects on serum total protein levels in the blood of experimental broiler chicks [6]. About the current study result, the jejunal villus height and ileal crypt depth were not affected by the heat stress as it was observed that birds on the control diet had taller jejunal villus height and deeper ileal crypts than birds on the chromium picolinate supplemented diets. Among the intestinal cells, the maximum reduction of 100% was observed with the upper villus cells and 72% with the middle villus cells while the reduction was the least 4% with the crypt cells since the most common route of entry of chromium is through drinking water and food [32].

Also, resistance to various antibiotics shown by the resident gut bacteria following chromium ingestion indicates that use of chromium as a nutritional supplement and micronutrient may provide significant protection to the gut microflora, particularly *Lactobacillus*, against some of the commonly used antibiotics [33]. The stress has an important role in determining the extent and type of bacteria colonization. Some of the bacteria can modulate the expression of genes in host epithelial cells, thus creating a favorable habitat for themselves, and can prevent the growth of other bacteria introduced later. The result of this study is in line with the report of Sandikci *et al.*, reported that heat stress could significantly modify intestinal histological parameters [34]. The jejunal crypt depth values showed no differences. It could be that the chromium levels were suitable and high enough to alleviate the effect of heat stress on the parameters in question.

CONCLUSION: We could demonstrate that the different levels of CrPic supplementation of broiler chicks' diets exerted some benefits on the performance and some blood biochemical characteristics. Even though higher supplementation of CrPic caused significant lower values of total serum glucose and cholesterol. Additionally, in this study, the optimum inclusion levels of CrPic in broiler chicks for a better influence on the intestinal mucosa and microflora development were at the 1000 and 1500 ppb. Totally result of this study showed that the possibility of the potential use of CrPic for better results achievements compared to the control group.

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CONFLICT OF INTEREST: The authors declare that they have no competing interests.

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