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Organic Chromium and Vitamin E Enhance Physiological Performances, Humoral and Cellular Immune Responses in Heat-stressed Broiler Chickens

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ABSTRACT

Background: The use of chromium (Cr) and vitamin E (Vit-E) to overcome the detrimental effects of heat stress (HS) in broiler chickens has been studied by a few researchers. The study was designed to evaluate the effects of organic chromium picolinate (CrPic) and vit-E on physiological performances and immune responses in broiler chicken exposed to HS.

Methods: A total of 120, day old chicks were reared and divided into four equal groups: A, B, C and D at day 13. Group A considered as non-treated control, Group B, C and D were treated with CrPic, Vit-E and both respectively. The birds were exposed heat to 35 ± 3°C for 6 hours for 22 days.

Result: CrPic, Vit-E and combined groups had significantly higher live body weight and lower FCR ($p<0.05$). Hematological values were not varied among the groups. Liver enzymes AST but not ALT were increased significantly in the Vit-E group. Creatinine level was significantly ($p<0.05$) higher in CrPic and combined groups. Total protein, albumin and glucose were not differed between the treated and non-treated groups. Total cholesterol (TC), triglyceride (TG), HDL-c and LDL-c were significantly ($p<0.05$) higher in treated groups. The highest antibody titers against NDV were detected in birds of CrPic group followed by combined and Vit-E group. The cellular immune response assessed by CBH test revealed significant skin increased in CrPic and Vit-E supplemented birds. It concluded that the dietary CrPic and Vit-E are effective to enhance growth performance and immune responses in broiler chicken under heat-stress condition.

Key words: Antibody titers, Broiler, Cellular immunity, Chromium picolinate, Vitamin E.

INTRODUCTION

Heat stress (HS) is one of the most important environmental stressors challenging poultry production in tropical and sub-tropical countries. It has detrimental effects on poultry feed intake, body weight gain, mortality, hatchability and other important traits (Rao *et al*., 2012). The negative effects of heat stress will increase in the coming years due to temperature rises associated with global warming. Broiler chickens are more susceptible to high temperatures because of their greater metabolic heat production. To attenuate the negative effects of heat stress in broiler production systems, different approaches are adopted including vitamin and mineral supplementation (Lin *et al*., 2006). The inclusion of vit-E in feed for broiler has resulted in positive effects on growth performance when supplemented at higher levels during HS (Souza *et al*., 2011). Vit-E is a component and natural antioxidant of cell membranes. It involved in activation of the immune system (Hashizawa *et al*., 2013). Cr is an essential mineral element, present in small amounts in raw ingredients in broiler diets. Organic Cr plays a crucial role in poultry nutrition, production and health in broiler chicken under HS condtion. It is required for improving productive performance in poultry due to its important functions in metabolism, growth and reduction of lipid and protein peroxidation. Supplementation of Cr may increase body weight gain, improve feed efficiency in broilers (Farag *et al*., 2017). The one of the most common Cr forms used as supplements to poultry and animal diets is Cr picolinate (CrPic). CrPic is an organic source of chromium that has high bioavailability. The precise role of Cr on immune functions has not been fully elucidated. Several studies showed that immunological function has been enhanced by Cr (III) and its effects seem more pronounced during stress (Kafilzadeh *et al*., 2012). On the other hand, HS was shown to adversely affect immune potency (Ghazi *et al*., 2012). Therefore, supplementation of Cr may beneficially influence the immune response in heat-stressed broiler chicken (Lee *et al*., 2003). Another study revealed that the supplementation of dietary

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Cr increases the production of antibodies against IBR (Faldyna *et al*., 2003) and tetanus toxins. The study investigated the effects of CrPic and vit-E on growth performance, hemato-biochemical parameters and immune responses in broiler chicken under HS condition.

MATERIALS AND METHODS

The research was conducted at the Department of Physiology, Bangladesh Agricultural University, Mymensingh during the period from March 1, 2019 to April 5, 2019. All experimental protocols were approved and performed according to the approved guidelines of animal welfare and experimentation ethics committee, Bangladesh Agricultural University, Mymensingh, Bangladesh.

A total of 120 healthy day old Lohman broiler chicks were purchased from local hatchery. On the 13th day of age, chicks were randomly divided into the four equal groups (n=30): A, B, C and D. Group A was considered as non-treated control, Group B, C and D were treated with CrPic (250 mg/kg feed), Vit-E (1mL/2L) drinking water and both respectively. All groups were reared at room temperature of 28 ± 3°C followed by HS condition at a temperature range of 35 ± 3°C for 6 hours daily throughout the experimental period. Broiler starter, grower and broiler finisher feed was provided according the standard broiler farming system (Table 1). CrPic and vit-E were chosen from market available poultry products. Birds received their freshly prepared daily medication in the morning hour of each day.

Measurements of body weight and feed conversion ratio (FCR)

The body weight of each bird was measured in a weekly basis using digital balance and total body weight gain was calculated (body weight gain= final body weight - initial body weight). FCR was determined by the formula: total feed consumed by birds divided total body weight gain. Feed consumption is the amount of feed consumed by the birds in a period of time.

Blood collection and hemato-biochemical analysis

Blood collection, serum separation and selected hematological parameters (Hb, TEC, PCV and erythrocyte indices) were performed according to standard method (Khalil *et al*., 2020). The biochemical tests were performed using a UV spectrophotometer T 80, PG instruments, Great Britain.

Measurement of antibody titers against ND vaccine by hemagglutination inhibition (HI) test

HI tests on serum samples were carried out according to the method described (Yadav *et al.,* 2018). The HI titer of the Newcastle disease virus (NDV) antigen (LaSota stock virus) was adjusted by dilution to contain 4 units of hemagglutination activity. HI titer was determined as the highest dilution of serum samples that inhibited NDV agglutination of chicken RBCs.

Determination of cell-mediated immune (CMI) response

The CMI response was assessed using the cutaneous basophil hypersensitivity (CBH) response to phyto hemagglutinin (PHA-P) (Sikandar *et al.,* 2017). Briefly, on day 32, six healthy broilers per treatment were intradermal injected between the third and fourth digits of the right foot with 100 µg of PHA-P in 0.10 ml of sterile PBS solution. The left foot of each bird was similarly injected with 0.10 ml PBS solution to serve as control. The thickness of the skin was measured with a veriner caliper just before and 12h, 24 h and 72 h following the injection.

Statistical analysis

The hematological and biochemical parameters of broilers corresponding to Cr and vit-E supplementation are compared by performing analysis of variance using Graph Pad Prism 8.

RESULTS AND DISCUSSION

Effects of CrPic and vit-E on growth performances and FCR in broilers

The effects of organic CrPic and vit-E on live body weight, body weight gain and FCR are presented in Fig 1 and Table 2. After one week of the CrPic and vit-E supplementation, the highest body weight was recorded in group B (CrPic) followed by groups D, C and A. The values were differed significantly ($p<0.05$). On the 28th day of age, the highest body weight was recorded in group B (CrPic) followed by group C and D. On the 35th day, there was a sharp rise in body weight in the birds of treatment groups. The highest body weight was recorded in group B (CrPic) and the lowest body weight was in group A (control). All the data were

Table 1: Composition of broiler feed.

Nutrients	Broiler starter (g /100 g)	Broiler grower (g /100 g)	Broiler finisher (g /100 g)
Moisture (Max)	11	11	11
Protein (Min)	25	24	24
Fat (Min)	5	5	5
Fiber (Max)	4	4	4
Calcium (Min)	1.20	1.20	1.20
Phosphorus (Min)	0.75	0.75	0.75
Methionine (Min)	0.65	0.60	0.60
Lysine (Min)	1.40	1.40	1.30
ME (Min) Kcal/kg	3150	3175	3175

statistically significant (p<0.05) among the groups. The result showed the body weights and body weight gain were increased slowly in the control group but a rise in body weight was noticed during the last three weeks in the treated groups (B, C and D). Organic CrPic had a more pronounced effect on body weight gain in HS conditions. In parallel, FCR values were significantly reduced in all treated groups (Fig 1 and Table 2). Increased body weight gain in the treated group might be due to an increased feed utilization, digestion, absorption and metabolism of supplied feed nutrient. The present observation was in line with the previous findings of Haq *et al*., 2016. Both Cr and vit-E improved feed efficiency in the later phases of growth in the broiler. Under HS conditions, CrPic might play a crucial role in poultry nutrition, production and health. It enhances growth performance. Rao *et al.,* 2012 also stated that Cr supplementation alone increases weight gains of poultry. Vit-E with drinking water was also found to be useful to improve feed intake, body weight gain and feed efficiency broilers. CrPic and vit-E singly or in combination had a significant (p<0.05) lowering effect on FCR in broiler and especially CrPic treated birds achieved minimum FCR than other groups. A similar decrease was observed by previous findings of Moeini *et al*., 2011; Niu *et al*., 2009.

Effects of CrPic and vit-E on hemato-biochemical parameters

The mean values of Hb, TEC, PCV and erythrocyte indices (MCV, MCH and MCHC) were slightly increased in the treated groups but remained within the normal range and statistically non-significant (p>0.05) (Table 3). The mean serum ALT values were varied among the groups but didn't differ significantly. The highest AST level was recorded in group C and the lowest was in group A . The mean values of creatinine levels also varied among the groups (Table 4). The data indicated that birds fed supplemented CrPic had increased creatinine levels. The mean values of serum total protein, albumin and glucose of different treatment groups were found differed slightly among the treatment groups. Still, the values were within the normal ranges. Broilers fed dietary chromium and vit-E had increased serum cholesterol, triglyceride, LDL-c and HDL-c (Table 4). The data were statistically significant at p<0.05. It is reported that Cr supplemented birds had significantly increased hemoglobin,

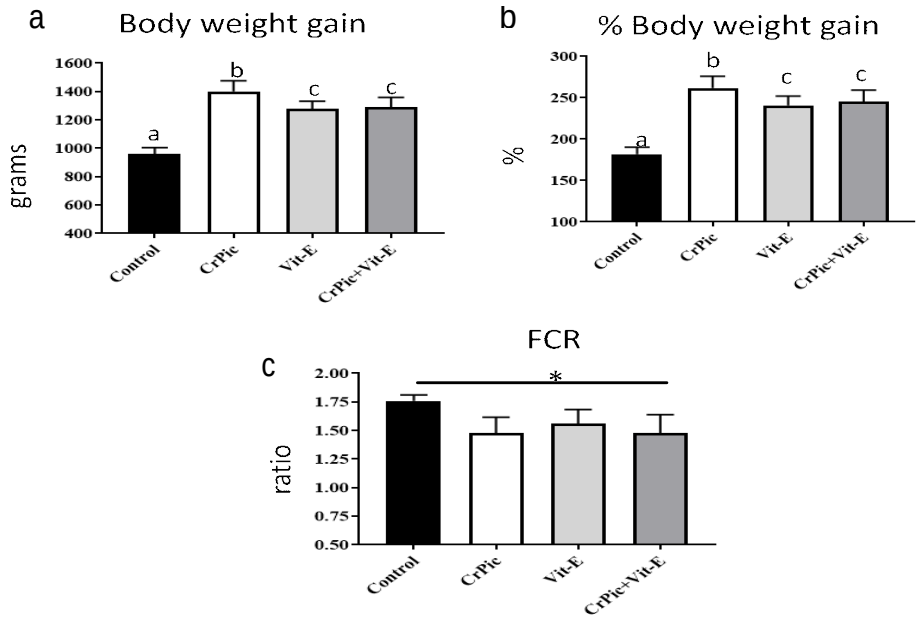

Fig 1: Effects chromium and vit-E on (a) body weight gain (g), (b) % of body weight gain and (c) feed conversion ratio (FCR) (Mean ± SEM), in broiler chickens. #Values with dissimilar letters over graph differs significantly (p<0.05). *p <0.05 (control vs. treated group).

Table 2: Effects of chromium and Vit-E on live body weight in different age groups of broiler chicken.

Groups	Body weight (g) (Mean ± SEM)			
	14th day	21st day	28th day	35th day
Control	526.0±4.40[a]	980±6.95[a]	1330±8.85[a]	1680±45.62[a]
CrPic	533.0±3.78[a]	1170±11.76[b]	1690±10.64[b]	2260±59.77[b]
Vit-E	494.5±8.93[a]	1100±11.76[b]	1595.8±14.51[c]	2057.5±51.61[c]
CrPic+Vit-E	500.8±8.98[a]	1120.8±12.54[b]	1580.8±15.99[c]	1995.8±72.18[c]

*Values with dissimilar letters in a column differs significantly (p<0.05).

MCH and MCHC (Farag *et al.,* 2017). Birds fed supplemented vit-E also had slightly increased hematological values in this current study, which is supported by a previous report (Biswas *et al.* 2011).

Two important liver enzymes were varied slightly in the present research. AST values but not ALT, were increased significantly in the birds of Vit-E fed group. Dietary addition of Se and bacterial organic Se (vitamin E) significantly affects broiler serum ALT, AST, LDH activities and reduces serum creatinine level (Farag *et al*., 2017). The underlying mechanism of increase activity of AST in treated group need to investigate. Current study didn't find any alternation of serum total protein, albumin and glucose level in blood although glucose levels were reduced in CrPic treated birds. it is reported (Haldar and Ghosh 2008; Tawfeek *et al*., 2014) that serum glucose was decreased. In contrast, total protein, albumin concentrations increased when dietary Cr and Se singly or in combination, were supplemented. TC, LDL-c and HDL-c were found increased in the sera of CrPic and Vit-E fed birds in the present study. The results are not agreed with other findings that supplementation of Cr decreased the blood cholesterol and LDL-c whereas HDL-c concentration was increase during starter and finisher phase of broilers (Aslanian *et al.,* 2011). Under heat stress conditions, the serum concentrations of TC, TG and LDL-cholesterol were increased. Serum HDL-cholesterol decreased in broilers supplemented with selenium and vit-E (Habibian *et al.,* 2014). It was also reported that cholesterol decreased when dietary Cr and Se singly or combined were supplemented (Tawfeek *et al.* 2014).

Effects of CrPic and vit-E on humoral and cellular immune response

The HI titer values were calculated in different groups of broilers (Fig 2). It was clearly found that birds of group B had the highest antibody titers against the ND vaccine compared with the control and other groups ($p<0.01$). These results revealed that the administration of Cr to ND vaccinated birds orally resulted in potentiation of the chicken immune response to ND vaccination under HS. Cellular immunity of broiler chickens fed CrPic and vit-E were assessed by CBH response (Table 5). The CBH response elicited by the PHA-P was evaluated by determining toe web skin thickness. Results revealed that significant skin increases were observed in PHA-P treated right toe web compared to PBS treated left foot. Supplementation of Cr and Vit-E in birds achieved a different degree of CBH responses in a time-dependent manner. The current study confirmed that Cr has immune stimulants activities that can promote recovery from immune suppression states and can enhance the innate and specific immunity. This result is in line with the findings of previous reports (Eze *et al.,* 2014)

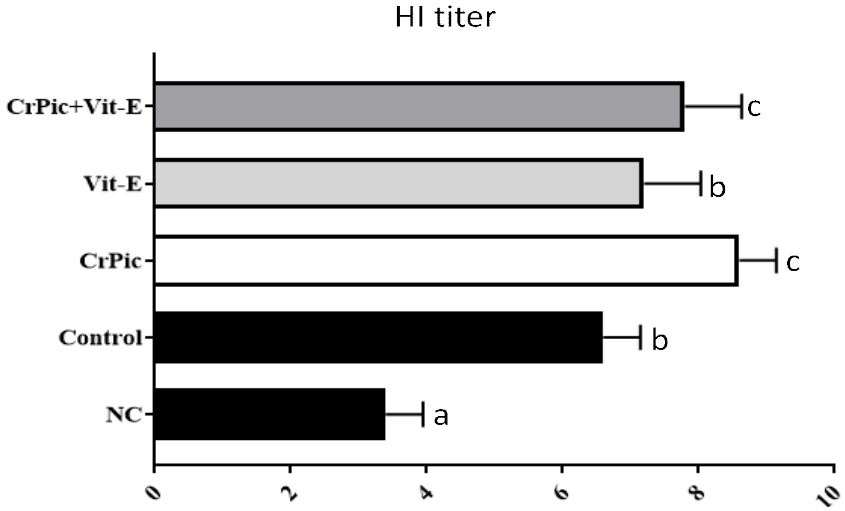

Fig 2: Effects of CrPic and vit-E on antibody titer (HI titer) against ND vaccine in broiler chickens* Values with dissimilar letters beside bargrpah differs significantly ($p<0.05$).

Table 3: Effects of chromium and vitamin E on hematological parameters (Mean ± SEM) in broiler chickens.

Parameters	Control	CrPic	Vit-E	CrPic+vit-E
Hb (g%)	6.88±0.15[a]	7.08±0.20[a]	7.12±0.05[a]	7.34±0.17[a]
TEC (10^6/uL)	2.20±0.11[a]	2.60±0.17[a]	2.73±0.10[a]	2.66±0.09[a]
PCV (%)	25.40±1.03[a]	28.50±1.65[a]	26.20±0.50[a]	25.80±0.86[a]
MCV (fl)	133.37±8.68	131.40±5.53	132.61±8.47	136.91±8.40
MCH (pg)	34.31±1.94	33.33±1.30	33.36±1.14	34.35±3.42
MCHC (%)	26.02±1.37	24.90±0.71	24.99±1.66	25.27±2.19

*Values with dissimilar letters in a row differs significantly ($p<0.05$).

Table 4: Effects of chromium and vitamin E on biochemical markers (Mean ± SEM) in broiler chickens.

Parameters	Control	CrPic	Vit-E	CrPic+vit-E
ALT (U/L)	3.17±0.75[a]	4.07±1.01[a]	4.27±1.34[a]	2.91±0.58[a]
AST (U/L)	9.13±2.99[a]	13.80±4.21[b]	15.16±4.55[b]	12.05±1.78[b]
Creatinine (mg/dL)	0.72±0.21[a]	1.10±0.38[b]	0.74±0.13[a]	1.18±0.22[b]
Total protein (g/dL)	4.99±0.29[a]	5.07±0.53[a]	5.35±1.13[a]	4.61±0.56[a]
Albumin (g/dl)	3.07±0.15[a]	2.85±0.53[a]	2.97±0.35[a]	3.20±0.25[a]
Glucose (mg/dl)	100.80±32.19[a]	101.89±16.87[a]	91.37±24.67[a]	98.83±8.87[a]
TC (mg/dl)	116.27±3.30[a]	151.46±29.42[b]	129.14±4.46[c]	145.03±30.31[b]
TG (mg/dl)	98.62±11.86[a]	108.45±7.04[b]	105.28±23.21[b]	109.20±9.35[b]
HDL-c (mg/dl)	41.66±1.52[a]	52.93±2.40[b]	51.04±0.97[b]	52.30±2.64[b]
LDL-c (mg/dl)	54.88±3.22[a]	76.83±29.18[b]	83.44±22.48[b]	54.31±10.04[a]

*Values with dissimilar letters in a row differs significantly (P<0.05).

Table 5: CBH response (% increase in toe web thickness in mm) in broiler chickens fed organic chromium and Vit-E.

Group	12 h		24 h		72 h	
	PBS	PHA-P	PBS	PHA-P	PBS	PHA-P
Control	2.50±0.07[a]	2.48±0.05[a]	3.62±0.10[a]	4.94±0.14[a]	3.75±0.05[a]	4.94±0.09[a]
CrPic	2.43±0.04[a]	4.43±0.05[b]	3.53±0.09[a]	6.62±0.16[b]	3.70±0.21[a]	6.98±0.38[b]
Vit-E	2.38±0.05[a]	4.31±0.19[b]	3.27±0.21[a]	6.54±0.21[b]	3.32±0.22[a]	6.87±0.25[b]
CrPic+vit-E	2.45±0.05[a]	4.60±0.10[b]	3.58±0.366[a]	6.82±0.22[b]	3. 71±0.20[a]	7.21±0.37[b]

*Values with dissimilar letters in a column differs significantly (p<0.05).

which concluded that dietary supplementation of Cr improved the immune response to ND vaccine under HS conditions in broilers. Assessment of the cellular immunity of broiler chickens fed CrPic and vit-E by CBH response induced the immune responses of birds under HS conditions.

CONCLUSION

Supplementation of CrPic at the dose rate of 250 mg/kg feed improves the physiological performances, antibody titers against NDV and cellular immune response in heat-stressed broiler chickens.

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Conflict of interest

The author declares no conflict of interest.

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