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Culminating the Influence of Heat Stress in Broilers by Supplementing Zinc and Vitamin C

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Abstract: Efficacy of supplementing zinc and vitamin-C in reducing heat stress of broiler chicks was assessed. One hundred and sixty, day-old broiler birds were randomly allotted to four replicated (n=4; 10 birds/replicate) dietary treatments (groups A, B, C and D). Treatment A was control and to other were added Zn (60 mg kg^{-1}, B), vitamin-C (300 mg kg^{-1}, C) and Zn + vitamin-C (D). Birds on deep litter had ad libitum access to feed and water. Heat stress was induced during the finisher phase (22-42 days). Birds in treatment-D had better ($p<0.05$) body weight gain and Feed conversion ratio (FCR) comparatively. Feed intake was reduced ($p<0.05$; 2940.44g $\pm$ 21.46) in control group. No significant difference was seen in the antibody titer against Newcastle disease (ND), Infectious Bursal disease (IBD) and Infectious Bronchitis (IB) of treated groups and was lowest ($p<0.05$) in group-A. Total leukocyte count (TLC) was significantly reduced however, neutrophil, eosinophil, lymphocyte and monocyte numbers were increased in control group. Difference in the count of these cells among supplemented groups was non significant. Significantly high mortality was observed in control group. It can be concluded from these findings that dietary supplementation of zinc and vitamin-C can effectively alleviate the adverse effects of heat stress in meat type chickens.

Key words: Antibody Titer · Broilers · Thermal Stress · Vitamin C · Zinc

INTRODUCTION

Heat stress, a potential threat to the fast growing broiler industry of tropical countries got worse when summer temperature reaches to its extremity since most of the farming is still practiced in conventional manner. High summer temperature and stress can cause under-performance, suppressed immunity and high mortality in meat type chicken [1]. Today's modern broiler with its advance genetic improvement and higher body weight gain has become more prone to different environmental stresses [2]. Prolong exposure to high ambient temperature can adversely affect the normal physiological functions of the body [3]. Thermal stress can suppress feed intake, body weight gain and coupled with high humidity can result in increased mortality [4]. Absorption and retention of essential and trace minerals are severely affected [5]. Production of glucocorticoids, immune-suppressing agent increases [6] and paraoxonase, an antioxidant enzyme decreases in heat stressed birds [7].

Bird's mechanism of dissipating heat severely disturbed when ambient temperature exceeds beyond thermo-neutral range. Exposure to high ambient temperature with reduced ability of dissipating heat can result in higher rectal temperature, reduced antioxidant enzyme activity, generation of malondialdehyde (MDA) and impaired mitochondrial function [8]. The inability to dissipate extra heat result in reduced metabolizable energy intake by decreasing feed intake [9]. Body electrolyte balance is disturbed due to high water intake in heat stressed birds and can be repaired by supplementing minerals and vitamins in diet [7]. Vitamin C and selenium (Se) possess antioxidative properties and help in improving immune response of broilers. Similarly, vitamin

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E and selenium (Se) have synergistic effects in reducing heat stress [10]. Zinc (Zn) plays a crucial role in enhancing immune function and maintaining cell integrity by avoiding oxidative damage [5] and its requirements increases in high ambient temperature [11]. Synthesis of vitamin C is inadequate in young chicks and in stressed birds. It enhances both humoral and cell mediated immunity [6].

Certain special types of enzymes are produced in the body of chicken when exposed to thermal stress as defense mechanism to decrease oxidative damage caused by increased lipid peroxidation and free radical generation. Lipid peroxidation can enhance the formation of reactive oxygen species (ROS) that stimulate oxidative stress. As protective mechanism antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) are produced to protect cellular damage from harmful effects of ROS [12]. Vitamin C supplementation leads to strengthening of the antioxidative defense mechanism and is a consequent minimize oxidative stress [13]. Zinc is used in poultry diets because of its anti-stress effects and plays a crucial role in enhancing immune function and maintaining cell integrity by avoiding oxidative damage [5]. Moreover, it is evident from different research work that the requirement of Zn is increased and its retention decreases during stress that indicates the importance of supplementation of Zn during stress [14].

Dietary manipulation can be an effective tool to handle heat stress during high summer temperatures. The aim of this study was to assess the potential benefits of zinc and vitamin C in reducing heat stress in meat type chicken during finisher phase of growth.

MATERIALS AND METHODS

Present research was done at the Poultry Research Unit of The University of Agriculture Peshawar, Pakistan and was pre-approved by the departmental scientific research committee for issues involving live birds handling, welfare and standard lab procedures.

Bird Husbandry and Dietary Treatments: 160, day-old male broiler birds (Cobb), obtained from a commercial hatchery were randomly assigned to four dietary treatments (A, B, C and D) replicated 4 times (10 birds/replicate). Treatment A was control and others were either supplemented Zn (60 mg kg^{-1}, B), vitamin-C (300 mg kg^{-1}, C) and Zn +vitamin-C (D). All birds were reared in an environmentally controlled house and had unlimited access to a commercial feed and water. Heat stress was induced during the finisher phase (22-42 days) by increasing shed temperature to 40°C from 7:00 am to 7:00 pm. Birds were vaccinated against NewCastle, Infectious Bronchitis and Infectious Bursal diseases. Birds were fed commercial diets both at starter and finisher phase. Known amount of vitamin-C and Zn for 50 kg bag was mixed in a small amount of finisher mash feed (protein; 21%, metabolisable energy; 13.33 MJ kg^{-1}) using small micro-ingredient mixer. This feed was then mixed with the rest of feed in the bag.

Data Collection and Sampling

Feed Intake

Body weight gain: Birds were initially weighed on day first and then at the end of every week to determine total body weight gain.

Feed Conversion Ratio (FCR): was weekly measured basis and adjustment for mortality were made. The FCR given are recorded as (FCR = weight of feed consumed per weight gain of survivors) and as adjusted for mortality (AFCR = weight of feed consumed)/ (weight gain of survivors + weight gain of mortalities).

Weight of Lymphoid Organ: Weight of the spleen, bursa of fabricius and thymic lobes were measured. Relative organ weights were calculated as percentages of body weight = [(organ weight/ body weight) × 100]

Antibody Titer and Cell Mediated Immunity (CMI): Antibody titer against ND, IB [15] and IBD [16] was determined using heamagglutination inhibition and ELISA tests, respectively. Blood samples were randomly collected from two birds in all replicates to measure total leucocytes count (TLC) and differential leucocytes count (DLC) as outlined by Banjamin [17]. Weight of bursa, spleen and thymus was also measured.

Data Analysis: Data were analyzed using completely randomized block design (replicate as an experimental unit) and mean were compared using Fisher's LSD test in statistical package SAS [18].

RESULTS AND DISCUSSION

Bird Performance: Average feed intake and body weight gain was greater in birds of group-D followed by group-C and B, respectively. Better FCR was observed in group-D and C, having insignificant difference compared to group-B and A (Table 1). These outcomes indicate that heat stress can negatively affect feed intake, body weight gain and feed efficiency. In high summer temperature birds

Table 1: Mean± SE feed intake (g), weight gain (g) and feed conversion ratio (FCR) in heat stressed broiler chicks fed Zn and vitamin C[1].

Groups	Feed intake	Weight gain	FCR
A	2940.44 ^{d} ± 21.463	1511.76 ^{d} ± 16.371	1.77 ^{a} ± 0.015
B	3038.98 ^{c} ± 1.478	1704.5 ^{c} ± 4.618	1.65 ^{b} ± 0.004
C	3162.03 ^{b} ± 11.860	1826.79 ^{b} ± 4.900	1.6 ^{c} ± 0.00
D	3217.85 ^{a} ± 24.034	1898.1^{a} ± 15.169	1.57 ^{c} ± 0.002

Means in columns with different superscripts are significantly different at á=0.05

[1]Values are means of four replicates per treatment

Table 2: Mean± SE weight of spleen, thymus ad bursa (g) in heat stressed broiler chicks fed Zn and vitamin C[1].

Group	Spleen	Thymus	Bursa
A	1.1^{d} ± 0.04	6.12^{d} ± 0.26	6.12^{d} ± 0.26
B	1.44^{c}± 0.02	7.45^{c} ± 0.22	7.45^{c} ± 0.22
C	2.08^{b} ± 0.06	9.57^{b} ± 0.27	9.57^{b} ± 0.27
D	2.57^{a} ± 0.04	11.03^{a} ± 0.06	11.03^{a} ± 0.06

Means in columns with different superscripts are significantly different at á=0.05

[1]Values are means of four replicates per treatment

Table 3: Mean± SE weight of spleen, thymus ad bursa (% of body weight) in heat stressed broiler chicks fed Zn and vitamin C

Group	*Spleen*	*Thymus*	*Bursa*
A	0.073^{d}±0.001	0.405^{d}±0.011	0.405^{d}±0.013
B	0.084^{c}±0.001	0.437^{c}±0.012	0.437^{c}±0.010
C	0.114^{b}±0.002	0.524^{b}±0.013	0.524^{b}±0.012
D	0.135^{a}±0.012	0.581^{a}±0.012	0.581^{a}±0.011

Means in columns with different superscripts are significantly different at á=0.05

[1]Values are means of four replicates per treatment

ability to dissipate heat is drastically affected [6] and birds reduce feed intake to avoid generation of metabolic heat to compensate heat stress that ultimately result in poor FCR and body weight gain. Moreover, reduced digestibility of nutrients (not determined in present study) in heat stressed broilers could be a factor for poor performance. Present findings can be related to the very earlier work of Adams *et al.* [19] who reported reduced feed intake and body weight gain in birds exposed to high environmental temperature. Similar findings were also reported by Bartlett and Smith [20]. It has been previously examined that vitamin supplementation and mineral can effectively minimize the negative impacts associated with high summer temperature [21-24]. Present findings depicts that vitamin C supplementation might have lead to the strengthening of the antioxidative defense mechanism of birds to reduce the severity of oxidative stress [13]. Supplementation of zinc in poultry diets have been reported to possess anti-stress effects. The exact mechanism of combating heat stress is however not clearly understood. Moreover, it is evident from different research work that the requirement of Zn is increased and its retention decreases during stress that indicates the importance of supplementation of Zn during stress [14].

Weights of Lymphoid Organs: Mean weight (Table 2) of spleen, thymus and bursa and weight percent to body weigh (Table 3) were similarly affected by different dietary treatments. Simultaneous supplementation of Zn and Vit-C (group-D) increased significantly ($p<0.05$) weight of these organs followed by group-C and B, respectively. Heat stress has immune suppressing influence not only by interfering antibody production but oxidative damage [5] of cells in various lymphoid organs can badly affect the functionality of these organs and their weight [6, 7]. Stress might have caused degeneration of soft tissues of these organs resulting in abnormal growth and weight. Lagana *et al.* [11] assessed that heat stressed broilers had decreased bursa, thymus and spleen weight and was recovered with zinc and vitamins. This reflects that oxidative damage and other problems associated with heat stress can be overcome in broilers if supplemented with vitamin C and Zn as seen in present study. Similar improvements of increased lymphoid organs weight were observed in previous studies [25, 26]. This however did not happen in the study of Bartlett *et al.* [20] who reported no significant effect of Zn on the immune related organs in male broilers probably due to low dose of zinc and outbreak of infectious diseases.

Table 4: Mean± SE antibody titer against Newcastle disease (ND), Infectious Bursal disease (IBD) and Infectious Bronchitis (IB) in heat stressed broiler chicks fed Zn and vitamin C[1]

Group	ND	IBD	IB
A	$20^{b} \pm 15.418$	$1373.3^{b} \pm 272.489$	$1292^{b} \pm 523.324$
B	$88^{ab} \pm 24.000$	$2687.5^{a} \pm 244.399$	$4519^{ab} \pm 1116.36$
C	$176^{a} \pm 48.000$	$2976^{a} \pm 133.589$	$6495^{a} \pm 1100.25$
D	$192^{a} \pm 36.950$	$3316^{a} \pm 225.578$	$6625^{a} \pm 1227.10$

Means in columns with different superscripts are significantly different at á=0.05

[1]Values are means of four replicates per treatment

Table 5: Mean± SE neutrophil, lymphocyte, eosinophil, monocytes and total leukocyte count (TLC) in heat stressed broiler chicks fed Zn and vitamin C[1].

Group	Neutrophil	Lymphocyte	Eosinophil	Monocytes	TLC
A	$39.75^{a} \pm 1.652$	$53.25^{b} \pm 1.493$	$4.5^{a} \pm 0.288$	$2.5^{b} \pm 0.288$	$21750^{b} \pm 853.91$
B	$32.75^{b} \pm 0.853$	$59.75^{a} \pm 0.629$	$2.5^{b} \pm 0.288$	$5^{a} \pm 0.40$	$26000^{a} \pm 408$
C	$32.25^{b} \pm 0.478$	$60.00^{a} \pm 0.408$	$3^{b} \pm 0.408$	$4.75^{a} \pm 0.47$	$27500^{a} \pm 1040$
D	$29.75^{b} \pm 0.629$	$62.75^{a} \pm 0.478$	$2.75^{b} \pm 0.25$	$4.75^{a} \pm 0.25$	$28250^{a} \pm 478.71$

Means in columns with different superscripts are significantly different at á=0.05

[1]Values are means of four replicates per treatment

Antibody Titer: Mean antibody titer against different diseases given in Table-4 represent that birds given vitamin-C (group-C) and zinc +vitamin C (group-D) had developed better immunity due to its increased antibody titer again ND, IB and IBD. As mentioned previously oxidative damage to lymphoid organs and reduced weight by heat stress could probably be the reason of lacking a proper antibody titer against these diseases. Moreover, immune suppressing agents and free radicals are released when birds are under heat stressed and their level can be minimized by supplementing antioxidants e.g. vitamin C and zinc in the diet [6]. Zinc suppresses the production of free radicals and prevent lipid per oxidation by binding copper and iron to the cell membrane and also provide protection against immune-mediated free- radicals attack. Previous studies have reported increased immune response with either vitamins or zinc supplementation [21, 22, 27, 28] as seen in the present research study.

Cell Mediated Immunity: Total leucocytes count (TLC) was lower ($p<0.05$) in control group-A. The difference between treated groups was insignificant and had high TLC. Count of neutrophil, eosinophil, lymphocyte and monocyte (differential leucocytes count: DLC) was significantly increased in heat stressed control group-A compared to treated groups (Table 5). Heat stress reduces significantly TLC in heat stressed broilers [29, 30]. Islam *et al.* [31] studied that vitamin supplementation can fix disturbance in TLC and DLC caused by heat stress in broiler birds. This is probably due to depressing effect of vitamins and zinc on glucocarticoid production and stimulates Thymulin hormone production that further enhances lymphocyte development that is normally prevented when birds are exposed to heat stress [32]. However, Borges *et al.* [33] reported negligible physiological response of broiler chickens to dietary electrolytes supplementation and lymphocytes production and activity. Joachim *et al.* [30] that observed significant reduction in and lymphocytes and monocytes production in heat stressed broilers and match with present findings.

It can be deduced from present findings that birds reared under summer temperature in tropical regions if supplemented with a blend of vitamin C and Zn will perform much better indicating a synergistic effect. Further research work is however warranted to assess the in depth mechanism of this synergism.

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