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Effect of different levels of alpha tocopherol on performance traits, serum antioxidant enzymes, and trace elements in Japanese quail (*Coturnix coturnix japonica*) under low ambient temperature

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ABSTRACT - This study was designed to find the effect of vitamin E supplementation on growth, serum antioxidant enzymes, and some trace elements in Japanese quail (*Coturnix coturnix japonica*) under low ambient temperature. A total of 180 day-old Japanese quails were randomly divided into four groups and provided with 0 (group A), 50 (group B), 100 (group C), and 150 IU/kg (group D) vitamin E (dl-α-tocopherol acetate) under an average temperature of 9±0.5 °C for an experimental period of 42 days. The result showed that feed intake per day, body weight, weight gain per day, and feed conversion ratio did not differ significantly between the groups. Serum concentrations of superoxide and glutathione peroxidase were significantly high in birds supplemented with 150 mg/kg of vitamin E. The concentration of aspartate aminotransferase was not significantly affected between the control and treated groups; however, alanine transaminase concentration significantly reduced in group D. Zinc concentration in the blood increased significantly in group D, with no significant effect on copper and manganese between the control and treated groups. Vitamin E at the level of 150 IU/kg of feed improves the blood antioxidant status and zinc concentration, with no effect on the performance traits of quail reared under low ambient temperature.

Key Words: oxidative stress, trace minerals, vitamin E

Introduction

Ambient temperatures below 16 °C leave significantly detrimental effects on poultry performance. Exposure to cold temperature increases the excretion of vitamins and minerals in poultry, thus increasing the requirements of vitamins C, E, and A (Sahin et al., 2003a; Ipek and Dikmen, 2014). For birds, the thermoneutral (TN) temperature is 18 to 22 ºC and, within this zone, birds do not feel any problem. In many countries of the world, the environmental temperature falls below the TN temperature during winter, which may adversely affect the production performance of birds (Khan et al., 2011). Such ambient temperature can also result in increased mineral excretion (Khan et al., 2014a). To augment production under low ambient temperature, enhanced energy intake is usually suggested; however, it has been previously reported that the negative effects of the environment could be prevented by the use of vitamin and mineral supplements such as vitamin C and chromium (Chand et al., 2014; Ipek and Dikmen, 2014; Khan et al., 2014a).

Vitamin E acts as a biological chain-breaking antioxidant, which is included in the feed of the bird to improve performance (Jena et al., 2013). The results of experiments are variable chiefly depending upon the dose and duration of feeding, genetics, and age (Khan et al., 2011). Unfortunately, birds are unable to synthesize vitamin E in their body and, therefore, they largely depend upon external sources of this vitamin in their diet (Khan, 2011; Ipek and Dikmen, 2014). The minimum requirement of vitamin E in the feed is 10 mg/kg of feed, and a deficiency of this vitamin may result in retarded performance (Khan et al., 2011).

Antioxidants are the substances that can scavenge, dispose of, or reduce the formation of reactive oxygen species (ROS) or suppress their action (Khan, 2011; Majid et al., 2015). As a consequence of extensive lipid peroxidation, ROS are continuously produced, which is detrimental to the productive performance of the birds. Under physiological conditions, the animal body has an appropriate amount of antioxidants to neutralize the production of ROS; however, when the number of these free radicals reaches beyond the antioxidant potential of the body, oxidative stress is developed, deteriorating the performance of the bird (Rahman et al., 2014).

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One of the important components of the antioxidant system is superoxide dismutase (SOD), which plays a key role in protecting the cell against the potentially deteriorating effect of ROS such as superoxide and hydroxyl radicals (Rahman et al., 2014). Other enzymes such as glutathione peroxidase (GPx) detoxify hydrogen peroxide, a potent ROS, and convert it into a stable product. It has been well documented that, together with vitamins and trace elements such as copper, zinc and iron are also essential for biological activities (Naz et al., 2016). These metals are required for the catalytic properties, which have widespread distribution and functions in animal biology (Rahman et al., 2014; Majid et al., 2015). Reports on animal models have shown that antioxidant enzyme level varies with the level of antioxidants in the food (Khan, 2011; Majid et al., 2015).

In the literature, most of the studies are focused on high ambient temperature, especially in broilers or layers. Little attention has been given to study the effect of low ambient temperature in Japanese quail and its ameliorative management. The present study was conducted to demonstrate the influence of graded levels of vitamin E on the growth performance, blood antioxidants, and trace mineral concentration in Japanese quails reared under low ambient temperature (9 ºC).

Material and Methods

This study was conducted in compliance with the Animal Care and Use Committee of the University of Agriculture, Peshawar, Pakistan, under protocol no. FAVS-127.

A total of 180 day-old Japanese quail (*Coturnix coturnix japonica*) were purchased from the local market. After an adaptation period of one week, the chicks were randomly divided into four groups. Each group was further divided into five replicates with 9 chicks per replicate. Up to the slaughter age (42 days), the birds were provided with a commercial feed to meet or exceed NRC (1994) recommendation (Table 1), and water *ad libitum*. The protein content of the feed was determined according to proximate analysis method of AOAC (2000) and metabolizable energy was determined with an adiabatic bomb calorimeter (IKA® WERKE, USA) standardized with benzoic acid. Briefly, 1 g of feces (five birds per replicate) was processed to a tablet form in a pellet press (Parr Instrument Co, USA), and then transferred to a volatile matter (VM) crucible for combustion in the adiabatic bomb calorimeter. The metabolizable energy was calculated as follows:

Energy intake – Energy lost/Feed intake

Throughout the experiment, light was provided continuously for 24 h. The room temperature was maintained at 9±0.5 °C. The chicks were kept on a deep litter housing system using a cemented floor (24 × 24 inches) with wood shavings litter. One group having no vitamin E supplementation (basal diet only) served as a control. Other groups were supplemented with 50 (group B), 100 (group C), and 150 (group D) mg/kg of vitamin E as dl-α-tocopherol acetate (Super's Diana, S.L.) in the feed. Feed intake (FI) was measured on a daily basis, while body weight (BW) was determined at the end of each week. From the data of FI and WG, average daily gain (ADG) and feed conversion ratio (FCR) were calculated for each group. The experiment was carried out for a period of 42 days.

At the end of the experiment, three milliliters of blood sample were randomly collected from three birds per replicate for slaughter. Blood was centrifuged at 2000 rpm for 15 min. Serum samples were stored at –20 °C until further analyses.

Serum glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity was evaluated using a spectrophotometer (IRMECO Model U2020) and a commercial kit (Mountain View, CA, USA). The assay of SOD is based on the principle of utilization of WST-1, a tetrazolium salt, which produces a water soluble formazan after reduced with superoxide anion. The reduction rate of superoxide anion correlates linearly with the concentration of xanthine oxidase activity and is inhibited by SOD. The GPx converts reduced glutathione to oxidized glutathione

Table 1 - Ingredient composition (%) of experimental diet (dry matter basis)

Ingredient (%)	
Corn	41.00
Rice	14.00
Soybean meal (46%)	17.00
Fish meal	3.00
Rice polish	5.00
Canola meal	7.00
Gluten meal (27%)	3.00
Guar meal	3.00
Molasses	4.00
Dicalcium phosphate	1.70
Marble chips	0.50
Salt	0.16
DL-methionine	0.1
L-lysine HCl	0.3
Vitamin + mineral remix[1]	0.24
Analyzed composition	
DL-methionine (%)	0.51
Lysine (%)	1.32
Crude protein (%)	24.00
Metabolizable energy (kcal kg^{-1})	2900

[1] Provided per kg of diet: Mn - 80 mg; Zn - 60 mg; Fe - 60 mg; Cu - 5 mg; Co - 0.2 mg; I - 1 mg; Se - 0.15 mg; choline chloride - 200 mg; vitamin A (retinol) - 12,000 IU; vitamin D3 (cholecalciferol) - 2,400 IU; vitamin E (DL-α-tocopherol) - 50 IU; vitamin K (menadione) - 4 mg; vitamin B1 (thiamine) - 3 mg; vitamin B2 (riboflavin) - 6 mg; vitamin B5 (pantothenic acid) - 25 mg; vitamin B6 (pyridoxine) - 5 mg; vitamin B12 (cyanocobalamin) - 0.03 mg; folic acid - 1 mg.

and lipid hydroperoxides to their corresponding alcohols or free hydrogen peroxide to water which is measured.

Serum AST and ALT were measured by using a commercial kit (Randox Laboratories Ltd. United Kingdom, BT29 4 QY) using a UV-Vis spectrophotometer (IRMECO Model U2020).

Serum samples were analyzed for the determination of manganese (Mn), zinc (Zn), and copper (Cu) as suggested by Khan et al. (2012). Two serum samples of known quantity (1 mL) were digested in the presence of nitric acid followed by perchloric acid. The samples were filtered and stored at –20 °C until analyses. An atomic absorption spectrophotometer (PRKIN-ELMER, Connecticut, USA) was used to measure the absorbance which was converted into concentration with the help of a standard curve for each element.

Data were statistically analyzed with analysis of variance (ANOVA) using SPSS (version 12.0). Means were separated by least significant difference (LSD). P-value less than 0.05 was considered statistically significant.

Results

The results for performance traits indicated that FI, FCR, WG, and BW had no significant (P>0.05) differences among the groups (Table 2). However, FI and WG were numerically higher in the treated groups, especially in group D.

Mean serum concentrations of GPx and SOD were significantly (P<0.05) higher in group D as compared with the other groups (Table 3).

The concentration of AST was not significantly influenced between the control and treated groups. Concentration of ALT significantly decreased in all treated groups as compared with control. The lowest ALT was found in the D group.

No significant difference (P>0.05) was observed in serum concentration of Cu and Mn between the control and treated groups (Table 4). Serum concentration of Zn was significantly (P<0.05) higher in group D than in the other groups.

Discussion

It is neither feasible nor desirable to heat animal buildings to alleviate the negative impact of low ambient temperature; therefore, the situation can be minimized by manipulation of diet. During environmental stress, the serum and tissue level of vitamin E decreases; thus, supplementation of vitamin E is beneficial (Ipek and Dikmen,

Table 2 - Effect of vitamin E on the performance traits of Japanese quail

Parameter	Group A	Group B	Group C	Group D	Pooled SEM	P-value
Feed intake (g/day)	19.72	21.03	20.53	22.41	0.21	0.12
Feed conversion ratio (g/g)	2.13	2.42	2.15	2.26	0.73	0.32
Weight gain (g/day)	9.33	8.61	9.61	9.84	0.82	0.19
Final body weight (g)	145.71	145.27	145.10	147.32	0.18	0.09

SEM - standard error of the mean.
Group A - control; Group B - vitamin E 50 mg/kg; Group C - vitamin E 100 mg/kg; Group D - vitamin E 150 mg/kg of feed.

Table 3 - Effect of vitamin E on serum antioxidant and liver enzymes of Japanese quail

Parameter	Group A	Group B	Group C	Group D	Pooled SEM	P-value
GPx (mU/mL)	1.33b	1.61b	3.32b	3.61a	0.01	0.04
SOD (% inhibition)	23.32c	24.45c	27.74b	41.12a	0.04	0.03
AST (U/I)	43.78	45.35	43.73	43.94	0.12	0.07
ALT (U/I)	38.57a	37.27b	36.86b	34.93c	0.11	0.04

a, b, c - values bearing different letters in a row differ significantly (P<0.05).
SEM - standard error of the mean; GPx - glutathione peroxidase; SOD - superoxide dismutase; AST - aspartate aminotransferase; ALT - alanine transaminase.
Group A - control; Group B - vitamin E 50 mg/kg; Group C - vitamin E 100 mg/kg; Group D - vitamin E 150 mg/kg of feed.

Table 4 - Effects of vitamin E supplementation on serum trace elements of quail

Parameter	Group A	Group B	Group C	Group D	Pooled SEM	P-value
Cu (ppm)	1.12	1.71	1.34	0.88	0.03	0.55
Mn (ppm)	7.01	6.15	5.41	4.75	0.04	0.74
Zn (ppm)	8.64c	12.75b	12.34b	14.73a	0.12	0.03

a, b, c - values bearing different letters in a row differ significantly (P<0.05).
SEM - standard error of the mean.
Group A - control; Group B - vitamin E 50 mg/kg; Group C - vitamin E 100 mg/kg; Group D - vitamin E 150 mg/kg of feed.

2014). Secondly, poultry cannot synthesize vitamin E, unlike vitamin C, and therefore it must be added to the diet (Khan et al., 2011; Ipek and Dikmen, 2014). In the present study, the inclusion levels of vitamin E had no significant effect on the performance traits of quails reared under low ambient temperature. Previously, improved performance in poultry has been reported under higher doses ranging from 250 to 500 mg/kg. Improved body weight, digestibility, and carcass characteristics have been documented in Japanese quail under environmental heat stress (Sahin and Kucuk, 2001; Ipek and Dikmen, 2014). Sahin et al. (2003a) reported improved body weight, dry matter intake, FCR, and egg production when vitamin E was supplemented at the level of 250 mg/kg of feed in Japanese quail under cold stress condition (6 °C). Therefore, it is inferred from the present and the previous observation that for enhanced performance of quails reared under cold temperature, higher doses of vitamin E must be supplemented.

In the present study, the concentration of GPx and SOD decreased under cold condition in the control group; however, the concentration of these enzymes increased when vitamin E was supplemented at the levels of 100 and 150 mg/kg in the feed. Antioxidant vitamins have an inverse relationship with lipid peroxidation inside the body, resulting in damage of cells. It is well documented that vitamin E is an antioxidant vitamin and plays an essential role in the defense against lipid peroxidation (Khan, 2011). In the current work, serum level of the antioxidant enzymes, SOD and GPx, was significantly higher in group D. Jena et al. (2013) reported that SOD and catalase increased significantly when broilers were supplemented with vitamin E under heat stress condition. Previously, Özkan et al. (2007) found that at low temperature (14.5 to 16.8 °C), supplementation of vitamin E in combination with selenium enhanced liver glutathione peroxidase and glutathione in broiler. Stress produces free radicals and ROS, which can potentially harm the cell membrane by the process of peroxidation of the polyunsaturated fatty acids of the cell membrane (Khan et al., 2014a). Because of the quenching activities of vitamin E, it breaks the chain of free radicals at an early stage.

In the current study, ALT concentration decreased significantly in the group of birds fed vitamin E at the level of 150 mg/kg. A lower level of liver enzyme indicates the better health of the quails. Its determination is indicative of oxidative stress in the liver tissue, since a high concentration of the enzymes is linked to the liver disease (Perić et al., 2010). In the study of Ajakaiye et al. (2010) and Jiang et al. (2013), the level of AST and ALT did not differ between the control and the vitamin E treated birds up to the levels of 150 mg/kg and 200 mg/kg, respectively, which may be due to the difference in experimental protocol, dose, and duration of vitamin E. Similar results were reported by Perić et al. (2010) when broiler chicks were supplemented with 0.3 ppm selenium. In this study, reduced concentration of liver enzymes may be due to the antioxidative effect of vitamin E.

Trace minerals are considered very useful for the domestic animals and play an essential role in the structural and functional co-factor in metal containing enzyme. In the present study, we found that Zn concentration increased significantly in the quails supplemented with vitamin E at the level of 150 mg/kg. Sahin et al. (2003b) and Khan et al. (2014b) reported that vitamin E supplementation increased serum concentration of Zn in birds subjected to various kinds of stresses. Sahin et al. (2002) concluded that vitamin E supplementation at various levels (125, 250, and 500 mg/kg) increased significantly the Zn concentration in the egg yolk in laying Japanese quail. Environmental stress has been found to reduce the concentration of certain vitamins and minerals (Khan et al., 2014a, 2014b). Majid et al. (2015) suggested that vitamin E increases the capability of the body to retain Zn by decreasing its excretion from the body. The increased concentration of Zn in serum of Japanese quail under cold stress may be due to increased absorption of this element under the increased supplementation of vitamin E.

Conclusions

Supplementation of vitamin E at the rate of 150 mg/kg improves the serum antioxidant status and zinc concentration and reduces the liver enzymes in Japanese quail.

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