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ORIGINAL ARTICLE

Influence of dietary vitamin E and zinc on performance, oxidative stability and some blood measures of broiler chickens reared under heat stress (35 °C)

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Abstract
This study was conducted to determine the effects of vitamin E (αTA; dL-α-Tocopheryl acetate) and zinc (Zn; $ZnCl_2$) on performance, oxidative stability and some blood measures of broiler chickens under normal and hot temperatures. One hundred sixty 10-day-old male chicks were assigned to four groups in four replicates. The birds received two diets, control and enriched with two levels of vitamin E and Zn (100 and 50 mg/kg of diet, respectively) under two ambient temperatures (22 °C and 35 °C). Although the diets enriched with the antioxidant vitamin and element compared with un-enriched diets resulted in the better performance of the birds, significant differences were related to temperature conditions, as the lowest feed conversion ratio (FCR) and the highest body weight (BW) were observed in the group fed the enriched diet under normal temperature. The serum malondialdehyde (MDA), cholesterol (C), triglycerides (TG), glucose (G), total protein (TP), and hematocrit and heterophil : lymphocyte ratio levels were significantly lower in birds reared under 22 °C compared with the group under 35 °C but the hematocrit was not significantly lower in groups fed enriched diets and exposed to normal conditions (P>0.05). It was concluded that a combination of 100 mg of vitamin E and 50 mg of Zn provides the better performance and the least blood C and TG levels accompanying the better oxidative stability in male broilers under normal temperature (22 °C) and that this combination can be considered as a protective strategy in broiler diets for reducing the negative effects of heat stress.

Key words: antioxidant; blood parameters; heat stress; vitamin E; broiler

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INTRODUCTION

The detrimental effects of heat stress on poultry production and well-being have been extensively reviewed. High ambient temperatures compromise performance and productivity through reducing feed intake and decreasing nutrient utilization, growth rate, egg production, egg quality, and feed efficiency, all of which lead to economic loss. (Teeter et al. 1985, Siegel 1995). Heat stress destroys the cellular wall, also causes denaturizing of protein, reduces hematocrit (Yahav et al. 1997) and increases glucose, protein, cholesterol, triglyceride and the heterophil: lymphocyte ratio (Puvadolpirod and Thaxton 2000).

Vitamin E is mainly found in the hydrocarbon part of the membrane lipid bilayer towards the membrane interface and in close proximity to oxidized enzymes which initiate the production of free radicals. Vitamin E therefore protects cells and tissues from oxidative damage induced by free radicals by purifying the blood from malondialdehyde, especially if boosted with an element helper, for example zinc (O'Neill et al. 1998, Grau et al. 2000, Bou et al. 2004). In general, given the potential health effects described for α-tocopherol (Christen et al. 2000, Food and Nutrition Board 2000, 2001, Pryor 2000, Bron and Asmis 2001), enriched poultry meat could be considered a useful source of this vitamin in the human diet.

Zinc (Zn) is also known as an effective antioxidant and essential trace mineral in organisms. One of the main reason why zinc has a good antioxidant effect accompanying vitamin E against heat stresses, is due to its effect on fat metabolism, in part by the fat absorption in the intestine that plays an important role in numerous biological processes in avian and mammalian species (Hunt 2003). In addition, zinc and selenium are commonly referred to as antioxidant nutrients; however these chemical elements have no antioxidant action themselves but are instead required for the activity of some antioxidant enzymes, such as superoxide dismutase (SOD) which are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide (Zelko et al. 2002, Kataria et al. 2008) and are present in almost all aerobic cells and extracellular fluids. SOD and catalase protects cells against damage caused by reactive oxygen species (ROS) and lipoperoxides. Johnson and Giulivi (2005) have reported that Zn as an antioxidant agent can boost activation of antioxidant enzymes in combination with vitamin E, a well known antioxidant.

Oxidative stress is known to be an important contributing factor in many chronic diseases. The combination of vitamin E and Zn is more effective in ensuring adequate stability of the diet and in protecting the bird's immune system and thereby improving performance (Bou et al. 2004). Hence, this approach can be used to aid the development of strategies for growing poultry during hot weather conditions, while maintaining economically feasible stocking densities, because commercial broiler health and performance is the product of many interacting factors, including stocking density, bird environment, and high temperatures (Ryder et al. 2004, Lin et al. 2006, Ahadi et al. 2010).

In the understanding of metabolic changes in heat stressed poultry fed on diet supplemented with vitamin E and Z in compared to normal conditions, the measurement of performance, oxidative stability and blood health indexes have a substantial role. The objective of the current study was to evaluate the effects of vitamin E and Zn supplementation on performance, oxidative stability and some important serum parameters in male broiler chickens reared under normal and hot temperature (22 °C and 35 °C).

MATERIALS AND METHODS

A total of 160 1-day-old broiler chickens (Ross 308 strain) were obtained from a commercial hatchery. The birds were randomly assigned, according to their initial body weights, to four treatment groups (either two groups in normal or hot rooms), four replicates of 10 birds each. The study was started from day 10 after setting the starter rearing period.

The birds received two diets: control and enriched with two levels of vitamin E (DL-a-Tocopheryl acetate) (100 mg/kg of diet) and Zn (50 mg/kg of diet, respectively), at two ambient temperature conditions: room with normal temperature (control, 22 °C) and room with hot temperature (35 °C) in a 2 × 2 factorial design. Treatments were as follows: T1 – diet + heat stress (D+H, 35 °C); T2 – diet + control (D+C, 22 °C); T3 – diet containing antioxidant + heat stress (DA+H, 35 °C); T4 – diet contain antioxidant + control (DA+C, 22 °C). Vitamin E (ROVIMIXÒ E-50 SD; fairly stable source of vitamin E in feed) and Zn were provided by a commercial company (Roche, Tehran, Iran). The birds were fed a starter diet up to 21 days of age, followed by the experimental growing diet to day 42. The ingredients and chemical compositions of the diet are shown in Table 1. The diets were formulated in accordance with the NRC (1994) and contained 222.3 and 200 g/kg crude protein and 12.78 and 12.91 MJ/kg ME (starter and grower, respectively).

Table 1. Ingredients and chemical analyses composition of the starter and grower diets

Ingredients (g/kg)	Starter[1]	Grower
Maize	557	300
Wheat	–	330
Soybean meal	370	300
Soybean oil	30	40
Fish meal	20	–
Limestone	10	–
Oyster shell	–	12
Dicalcium phosphate	5	15
Vitamin-mineral mix[2]	5	5
dl-methionine	1	1
Sodium chloride	2	2
Vitamin E (mg/kg)	–	100
Zn (mg/kg)	–	50
Analyzed chemical composition (g/kg)		
Dry matter	892.2	893.5
Crude protein	222.3	200.7
Fat	62.4	62.9
Fiber	36.1	35.6
Ash	61.7	57.0
Calcium	8.22	8.15
Phosphorus	5.48	5.57
Selenium (mg/kg)	0.53	0.58
ME by calculation (MJ/kg)	12.78	12.91

[1] Starter diet fed to birds from 0 to 21 days. [2] Provides per kilogram of diet: vitamin A, 9,000 IU; vitamin D3, 2,000, IU; vitamin E, 18 IU; vitamin B1, 1.8 mg; vitamin B2, 6.6 mg B2; vitamin B3, 10 mg; vitamin B5, 35 mg; vitamin B6, 3.0 mg; vitamin B9, 1 mg; vitamin B12, 1.5 mg; vitamin K3, 2 mg; vitamin H2, 0.01 mg; folic acid, 0.21 mg; nicotinic acid, 0.65 mg; biotin, 0.14 mg; choline chloride, 500 mg; Fe, 50 mg; Mn, 100 mg; Cu, 10 mg; Zn, 85 mg; I, 1 mg; Se, 0.2 mg.

Small amounts of the basal diet were first mixed with the respective amounts of vitamins as a small batch, then with larger amounts of the basal diet until the total amount of the respective diets were homogeneously mixed. The diets and fresh water were offered ad libitum. The experiment rearing period was conducted between 1st July and 15th August. The temperature of the control room was maintained according to established standard operating procedures. Brooders were set at 32.3 °C for the first week, after which room temperatures were maintained at 35±1.5 °C for the heat stress groups and at 22±1.5 °C for the controls using an automated air handling system for 24 h/day. 24 h lighting was maintained during the experiment. Relative humidity inside the room was 45±5% prior to the beginning of the experiment. At weekly intervals, feed intake and body weight were determined on a group basis as replicates of each treatment. The weight gain and feed efficiency of the groups were then calculated. Birds were deprived of feed 4 h before being weighed. At the end of the experiment, 8 birds from each treatment group were randomly selected for slaughter. Prior to slaughtering, blood samples were taken from selected birds from the wing vein by injection into vacuum tubes and were collected in non-heparinized tubes by puncturing the brachial vein for a duration of 4–5 h. All samples were kept at room temperature for 2 hours and then at 4 °C overnight. In the laboratory, blood samples were centrifuged for 10 min at 580 ×g, and serum was isolated and stored at –80 °C.

Chemical analyses

Heparinized blood samples (3 mL) were obtained from broilers for determination of malondialdehyde (MDA), cholesterol (C), triglycerides (TG), glucose (G), total protein (TP), hematocrit and heterophil : lymphocyte ratio (H : ratio) levels, which were determined via enzymatic methods using a commercial kit (Kone commercial kit, Japan) by autoanalyser (ALCYON-300, American). The zinc in plasma samples was measured by electrothermal atomic absorption spectrophotometry, using a Shimadzu AA-680 (*Shimadzu Corporation*, Tokyo, Japan) flame atomic absorption spectrophotometer (AAS).

The α-tocopherol content of diets and meat was determined according to the EVS-EN 12822 European standard (EESTI STANDARD 2000) by HPLC (Shimadzu, VP series) equipped with a diode-array detector. Lipid peroxidation in the serum was measured by the thiobarbituric acid method accordingly to Piette and Raymond (1999) and the results were expressed as thiobarbituric acid-reactive substances (TBARS) in mg of malondialdehyde/dl. All analyses were carried out in the Research Station of Medical Sciences, Tabriz University, Iran.

Statistical analyses

The experiment was carried out on basis of a CRD (completely randomised design) with a factorial arrangement, and crude data from the study were subjected to ANOVA using the general linear model (GLM) procedures of SAS (SAS Institute 1988). Significant differences observed in the means were subjected to Duncan's multiple range test. For significant differences ($P<0.01$), means were compared by the LSD method of the same statistical package.

RESULTS

The results relating to the influence of temperature and diet on weight and feed consumption are shown in Table 2. In this study, the hot weather conditions (35 °C) affected the growth performance ($P<0.01$), significantly reduced feed intake ($P<0.05$) and body weight ($P<0.01$) and increased the feed conversion rate (FCR, $P<0.01$) in comparison to the cool weather conditions (22 °C). However, diet enrichment with vitamin E and Zn resulted in the better performance of the birds ($P<0.05$), in comparison with those fed the control diet. The lowest feed conversion ratio (FCR) and the highest body weight (BW) were observed in those fed the enriched diet and exposed to normal conditions, followed by the control groups exposed to 22 °C. The serum malondialdehyde (MDA), cholesterol (C), triglyceride (TG), glucose (G), total protein (TP) levels; hematocrit percentage and heterophil : lymphocyte ratio (Table 2) were found to be significantly lower in birds reared under 22 °C compared to those subjected to heat stress, and fortifying vitamin E and Zn in the broiler diet caused a decrease in the blood levels measured. Also, the amount of hematocrit was not significantly lower in the groups fed enriched diets and reared under normal weather conditions ($P>0.05$).

Table 2. Effect of diets enrichment with two antioxidants (vitamin E and Zn) on growth performance of broiler chickens reared under normal or heat temperature conditions (means of nine observations per treatment)

Treatment		Variable		
Diet	Condition	Final Body weight (gr)	FCR (g : g)	Feed intake (gr)
D	Normal	3 114^{b}	1.90^{b}	5 921^{b}
D+A	Normal	3 254^{a}	1.87^{b}	6 160^{a}
D	Heat stress	2 261^{d}	1.97^{a}	4 422^{d}
D+A	Heat stress	2 433^{c}	1.95^{a}	4 747^{c}
SE		1.63	0.09	2.12
Significance		**	*	**

D = diet, A = antioxidant, vitamin E + Zn (100 + 50 mg/kg), D+A = diet + antioxidant, Heat stress = 35 °C, Normal = 22 °C, D+Normal = Control, FCR = feed conversion ratio, SE = standard error, * = $P<0.05$, ** = $P<0.01$, $^{a–d}$ values in the same row and variable with no common superscript differ significantly

DISCUSSION

Performance

In the present study, the inclusion of a diet enriched with vitamin E (100 mg/kg) and Zn (50 mg/kg) resulted in a better performance in the male broilers exposed to heat stress – indicating the reduced effect of heat stress – and the best performance was detected in those reared under normal conditions. On the other hand, the results did show higher adverse effects of chronic heat stress in comparison to normal for the growth performance of the birds.

These results are consistent with those reported by De Winne and Dirinck (1996), Yahav et al. (1997), and Sahin et al. (2002). Some studies have reported that a high ambient temperature reduces body weight (Siegel 1995), increases cholesterol concentration and enhances the denaturation of protein (Puvadolpirod and Thaxton 2000). Sijben et al. (2002) in an investigation of the interactions of dietary unsaturated fatty acids and vitamin E regarding vitamin E status, fat composition and antibody responsiveness in layer hens, reported that vitamin E and elements such as zinc and selenium have the protective effect of polyunsaturated fatty acids against lipid oxidation that helps to ensure deposits of omega-3 fatty acids such as eicosapentaenoic acid (EPA, C22:5 n-3) and docosahexaenoic acid (DHA, C22:6n-3) in the body (Fritsche et al. 1992), thus achieving a higher immune system response to stress. Diets rich in vitamin E and zinc have been shown to reduce the catabolic response induced by immune stimulation and may be effective in promoting growth (Rymer and Givens 2005). On the other hand, the drinking water provided is of the same temperature as the conditions in the breeding location, and that could affect the broilers' body temperature and performance. Morever, hot weather conditions can decrease the humidity ratio of the breeding place and a subsequent increase in water consumption and consequent wet bed can occur.

Blood parameters

In the presented study, serum MDA, cholesterol, triglyceride and glucose levels increased (P<0.01) and the blood heterophil : lymphocyte ratio decreased (P<0.05) at the higher ambient temperature compared to the normal ambient temperature (Table 3). When the vitamin E and Zn enriched diet was provided, these blood measures significantly (P<0.05) decreased compared with the control diet (see results of D+H and D+N groups vs. results of DA+H and DA+N groups). The results from the current study found higher negative effects in the blood health indices of male broilers exposed to heat stress compared to those under normal weather conditions.

It was demonstrated that increasing the cholesterol, triglycerides and glucose levels in blood may elevate adrenal cortisol and the other glucocorticoid hormons secretion which plays a major role in glucose metabolism (Puvadolpirod and Thaxton 2000). In the current study, hot

Table 3. Effect of diets enrichment with two antioxidants, vitamin E and Zn on some blood measures of broiler chickens reared under normal and heat temperature conditions (means of nine observations per treatment)

Treatment		Variable						
		(mmol/l)				(g/dl)	(%)	
Diet	Condition	MDA	Chol	TG	G	TP	Hem	H : l ration
D	Normal	1.023^{b}	2.56^{c}	0.49^{c}	11.10^{c}	2.10^{b}	26.97^{bc}	0.29^{c}
D+A	Normal	0.800^{c}	2.24^{d}	0.41^{d}	10.78^{c}	2.08^{b}	26.80^{c}	0.27^{c}
D	Heat stress	1.170^{a}	3.32^{a}	0.67^{a}	12.65^{a}	2.90^{a}	28.55^{a}	0.44^{a}
D+A	Heat stress	0.955^{b}	3.04^{b}	0.58^{b}	11.73^{b}	2.85^{a}	27.45^{ab}	0.36^{b}
SE		0.15	0.09	0.03	0.11	0.06	0.42	0.04
Significance		*	**	*	**	*	*	NS

D = diet, A = Antioxidant, vitamin E + Zn (100 + 50 mg/kg), D+A = diet + antioxidant, Heat stress = 35 °C, Normal = 22 °C, D+Normal = Control; MDA = malondialdehyde, Chol = cholesterol, TG = triglyceride, G = glucose, TP = total protein, Hem = hematocrit, H : L ratio = heterophil : lymphocyte ratio, * = P<0.05, ** = P<0.01, NS = not significant, $^{a–d}$ values in the same row and variable with no common superscript differ significantly weather condition significantly elevated blood MDA and hematocrit contents in broiler chickens. Yahav et al. (1997) reported that the increased amount of MDA could be due to an increase of free radicals and lipid peroxidation in body cells, and that is in agreement with recent reports (Christen et al. 2000, Valçınkaya et al. 2010). To date, it is well known that the protective effect of vitamin E accompanying an antioxidant element for cell membranes can be boosted against free radicals which are vulnerable to molecular oxygen injury, when those react with free radicals, notably peroxyl radicals (H-O-O-H), and with singlet molecular oxygen ($^1O_2$). Grau et al. (2000) reported that the antioxidant function of tocopherols in poultry meat prevents the formation of primary and secondary oxidation products and total volatile compounds. On the other hand, the essential trace mineral, zinc, iron, selenium, etc., are of substantial importance to human health (Food and Nutrition Board 2001). The quality and nutritional value of animal meat can be improved by decreasing oxidative deterioration and especially, the deleterious biological effects that are of importance in human nutrition (Ryder et al. 2004, Ahadi et al. 2010).

The results presented show that cholesterol and triglyceride levels were significantly higher in the groups not provided with antioxidants than those provided with vitamin E and Zn. Blood glucose and MDA were found in significantly higher amounts in the blood of broilers fed the enriched diet, due to its antioxidant action in cells, which prevents the production of MDA in the liver by increasing the amount of the glutathione peroxidase enzyme that protects tissues from oxidative damage by removing the peroxides produced by free radicals, especially in heat stress (Naziroglu et al. 2000). One of the key reasons for the increase of blood malondialdehyde (MDA) levels in the groups fed on the diet without antioxidants, is the presence of unsaturated fatty acids in the diet. The premier reason in this regard is the increased temperature which can increase the lipid peroxidation of the diet and subsequent detriments to the body. But diet management from the view point of lipid stability and transferring antioxidant materials, such as vitamins and trace minerals to body is necessary, especially in hot weather conditions.

There was no significant finding in hematocrit percentage among all the groups and in total protein among groups reared under normal temperature condition. The blood amounts of TP, hematocrit levels and the heterophil : lymphocyte ratio, were significantly higher in the birds exposed to heat stress ($P<0.05$, $P=0.07$ and $P<0.05$, respectively, Table 3). Although the changes in the heterophil : lymphocyte ratio were not significant in the cooler weather condition, the blood H : L ratio significantly decreased ($P<0.05$) in birds fed the enriched diet compared with control diet with the heat stress condition. It has been reported that heat stress increases lipid peroxidation in poultry due to the increase in free radicals and also changes in blood pH (level of acidity) (Teeter et al. 1985, Siegel 1995). Heat stress causes destruction of the cellular wall, and denaturation of protein; reduces the hematocrit and heterophil : lymphocyte ratio (Yahav et al. 1997) and increases glucose, protein, cholesterol, triglycerides and the heterophil : lymphocyte ratio. Adding vitamin E and antioxidant components such as Zn to diets can be useful in controlling these blood levels (Puvadolpirod and Thaxton 2000).

The increased blood measures, particularly MDA, TG and C levels at the normal and hot weather conditions, decreased with the diet supplementation. But the serum amounts of the lipids mentioned were higher in broilers reared under normal ambient temperature. A cold ambient temperature can increase the plasma triglyceride which impedes lipid peroxidation in spite of antioxidant activity (Yahav et al. 1997). But, the only effective way to maintain lipid oxidation, other blood health indices and the immune system of broilers exposed to heat stress, is fortifying the diet with antioxidants that can significantly neutralize the chronic effect of heat stress.

From the presented results in this study, it is concluded that enhancing diet with two types of antioxidant in combination – vitamin E with direct effect and element Zn with second activation – significantly regulated the blood health indices and improved the performance of male broiler chickens grown under heat stress.

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