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Effects of Vitamin E (α-Tocopherol acetate) on Serum Lipid Profile, Ca and P Levels of Broilers Exposed to Heat Stress

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Abstract: The present study presents effect the diet of broilers exposed to heat stress, which the diet was added vitamin E on serum glucose, total protein, triglyseride, total cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL), hydrocarbon (HC), triacylglycerol (TAG), diacylglycerol (DAG), non-ester fatty acid (NEFA), polar lipids (PL) with calcium (Ca) and phosphorus (P) minerals. Three groups were established, including Control group applied basal ration at +24 °C environmental temperature, Stress group applied basal ration, + 34 °C environmental temperature, and Stress+Vitamin E group applied basal ration by adding vitamin E (150 mg α-tocopherol acetate/kg) at + 34 °C environmental temperature. Blood samples were taken from the animals forty second day and analysed. It was observed that heat stress statistically resulted in an increase in NEFA to ratio, cholesterol, LDL and total protein. Glucose, Ca and P levels also statistically decreased with increase in heat stress, while they increased with applied vitamin E. However, vitamin E did not affect on the value of P. Consequently, the vitamin E added to the ration statistically regulated negative effects of stress on the metabolic profile.
Keywords: broiler; heat stress; lipid profile; vitamin E

INTRODUCTION

High environmental temperature is a major stress factor for poultry and induces adverse effects on performance as well as on anatomical, physiological and behavioural parameters. Under normal conditions, nutrients ingested into the body are used for reproduction (30%), growth (30%), the maintenance of health (10%) and survival (30%). However, when exposed to stress, the organism uses nutrients only for the maintenance of health (80%) and survival (20%)[1]. Research has demonstrated that, in poultry exposed to heat stress, not only does egg quality decrease, but also several physiological changes occur a result of increased water consumption[2]. Furthermore, heat stress increases mortality rates in broiler chickens[3].

Heat stress is one of the most significant stress factors and poses a constant threat to the maintenance of homeostasis in the body. Literature reports indicate that high environmental temperature increases the excretion of minerals from the body[4], and decreases iron, zinc and chromium concentrations in the liver and serum [5]. Similarly, El Husseiny and Creger[6] determined that exposure to high environmental temperature (32 °C) led to decreased calcium, potassium, sodium, magnesium, iron, and zinc levels in broiler chickens. Furthermore, stress leads to the excessive increase of reactive oxygen species (ROS), which disrupts the balance between the antioxidant defense system and oxidation in favour of the latter (oxidative stress) [7] and in result, causes oxidative damage to lipids, carbohydrates, proteins and DNA, a phenomenon referred to as lipid peroxidation [8]. It has been reported that the supplementation of the ration with vitamins, including vitamin E, could reduce such adversities caused by environmental stress[9].

Several methods are used to reduce the adverse effects of high environmental temperature on the metabolism of poultry species. One of these methods is based on the maintenance of the temperature of poultry houses within the comfort zone. However, as this method is rather costly, alternative solutions are sought. An applicable alternative is the supplementation of the ration with vitamins and minerals that have antioxidant property. Vitamin E is an antioxidant, which has found common use in livestock breeding. Also referred to as α-tocopherol, vitamin E is a natural antioxidant, which is found in food and inactivates destructive free radicals that are generated as a result of either normal cell activity or several stress factors, and thereby, contributes to the maintenance of animal health[9].

Several stress factors cause the generation of free oxygen radicals, which by means of lipid peroxidation may result in damage to multiple

unsaturated fatty acids in cells. Normally, organisms have the capacity to eliminate reactive oxygen radicals, but when exposed to environmental stress, this capacity may be inhibited and thus, the organism may not be able to eliminate free radicals [10]. It is known that, in order to eliminate free radicals generated as a result of increased environmental temperature, poultry require an increased level of vitamin E intake [8].Owing to the antioxidant effect it shows in the cell membrane, vitamin E protects tissues against lipid peroxidation caused by free radicals, and thereby, alleviates the adverse effects of environmental stress in poultry [11].

In the present study, broiler chickens were exposed to high temperature (34 °C), and thus, to heat stress. The ration provided to these animals was supplemented with vitamin E, known to have an antioxidant property, and the effects of heat stress on the lipid profile, and protein and mineral metabolism of poultry were investigated.

MATERIALS AND METHODS

Animals and Experimental Design

The trial was conducted at the Research and Practice Farm of Atatürk University, Faculty of Veterinary Medicine in 3 groups of animals (broilers of the commercial breed Ross 308). This study was approved by the ethics committee of Faculty of Veterinary Medicine in Ataturk University (Decision No: 2007/5f). The animals were housed in 15 three tier cages measuring 100 x 55 x 35 cm. The 9 animals in each cage (total 45 Ross 308 broilers in each group) were placed. Three groups were established with 5 replications for each. The Control group was fed on a basal ration, exposed to an environmental temperature of 24 °C; Stress group was fed on a basal ration, exposed to an environmental temperature of 34 °C; Stress+Vitamin E group was fed on a basal ration, exposed to an environmental temperature of 34 °C, and given vitamin E (150 mg α-tocopherol acetate/kg ration) (Table 1).

Until d 14 of the trial, all animals were raised at comfort temperatures (the temperature was gradually decreased from 36 °C to 24 °C). From d 15 onwards, until the end of the trial, the temperature to which the animals were exposed was 24 °C for the Control group, and 34°C for the Stress and Stress+Vitamin E groups, between 08:00-16:00 h. All groups were reared on a lighting cycle of 17 h light/d. The trial ended when the animals were 42 d old. At the end of the trial, the 2 male animals at random from each sub-group were selected and the blood samples were taken from a total of 30 broilers.

Table 1: Composition and nutrient content of the diets used in the study, %

	Starter (1-15 days)	**Grower (16-42 days)**
Ingredients (%)		
Corn	56.99	58.74
Cornglutein	20.00	20.00
Wheat short*	7.00	7.00
Soybean oil	0.78	3.72
Soybean meal	11.53	7.14
Calcium carbonate	1.36	1.23
Dicalcium phosphate	1.06	0.91
L-lysine	0.40	0.42
Salt	0.26	0.27
Vitamin-mineral premix**	0.20	0.20
Toxinbinder	0.10	0.10
Anticoccidian	0.10	0.10
Sodium bicarbonate	0.10	0.09
Growth factor	0.05	0.05
Phyzyme XP TPT	0.03	0.03
DL methionine	0.04	-
Composition		
Metabolisable energy (kcal/kg)	3000	3200
Crude protein (%)	23	21
Calcium (%)	1.00	0.90
Phosphorus (available) (%)	0.44	0.40
Sodium (%)	0.15	0.15

*The additives (α-tocopherol acetate) were added in place of wheat shorts.

**The vitamin-mineral premix provides the following (per kg): all-trans-retynyl acetate - 1.8 mg; all-rac-α-tocopheryl acetate - 1.25 mg; menadione sodium bisulphate - 1.1 mg; riboflavin - 4.4 mg; thiamine (thiamine mononitrate) - 1.1 mg; vitamin B-6 - 2.2 mg; niacin - 35 mg; Ca-pantothenate - 10 mg; vitamin B-12 - 0.02 mg; folic acid - 0.55 mg; d-biotin - 0.1 mg, manganese (from manganese oxide) - 40 mg; iron (from iron sulfate) - 12.5 mg; zinc (from zinc oxide) - 25 mg; copper (from copper sulfate) - 3.5 mg; iodine (from potassium iodide) - 0.3 mg; selenium (from sodium selenite) - 0.15 mg; choline chloride - 175 mg.

Feed Analysis

The rations fed to the animals were formulated in accordance with the recommendations of the National Research Council [12] (Table 1). The crude protein content of the rations was analysed according to the method described by the Association of Official Analytical Chemists[13] and the crude fibre content as described by Van Soest and Robertson[14]. The content of ME, methionine, lysine, Ca and phosphorus was calculated.

Biochemical Analyses

On the 42nd day of trial, in the morning and before the animals were fed, blood samples were collected by wingve in puncture into sterile tubes, which were not coated with an anticoagulant. The samples, after being kept at room temperature for 2 hours were centrifuged (1500g, 15 min, room temperature). Then sera were carefully harvested and stored at -80°C until analysis. Lipid profile analysis at the biochemistry laboraty of Ataturk University, Faculty of Veterinary Medicine, and other analyzes at the biochemistry laboratory of Faculty of Medicine were performed.

Serum concentrations of glucose, total protein, triglyceride, total cholesterol, HDL, LDL, Ca and P were measured with an automatic analyzer using commercial test kits (Cobas 6000 analyzer, Roche).Additionally, VLDL was calculated by dividing triglyceride by five.

Determination of the Lipid Profile (Thin Layer Chromatography)

Thin layer chromatography was performed using a 20 x 10 cm Silica Gel 60 F254 High Performance Thin Layer Chromatography (HPTLC) Plate. 1 ml of serum homogenate or serum was added 1 ml of n-hexane/iso-propanol (2:1 (v/v)) mixture in a tube. After being mixed thoroughly, the tube content was maintained for 10 minutes and mixed once again. This procedure was repeated for a further two times[15].Subsequently, the tubes were centrifuged at 8000 rpm for 10 minutes and the upper phases were loaded onto the HPTLC plate. The plates were developed in a hexane: diethyl ether: formic acid (80:20:2 (v/v/v) mixture for 15 cm and then dried. The spots on the dry plates were made visible by means of treatment with 3% $CuSO_4$ in 8% phosphoric acid followed by burning on hot plates[16].The standard lipid mixture consisting of L-α-phosphatidylcholine, cholesterol, palmiticacid, triolein, squalene, and serum lipids were separeted as follows: hydrocarbons, diacylglycerol, triacylglycerol, esterified fatty acids, cholesterol and polar lipids.

Statistical Analysis

All values measured were tested with One-way ANOVA (total). Any differences between the groups were determined by the Duncan Multiple Comparison Range Test requiring a $p < 0.05$ for significance. All statistical analyses have been made with the SPSS[17] package software.

RESULTS AND DISCUSSION

While heats tress increased serum cholesterol, LDL and total protein levels significantly in Stress group ($p<0.05$), these parameter were determined similar in the Control and Stress+Vitamin E groups (Table 2). Furthermore, serum glucose, Ca and P levels were significantly decreased in the Stress group, whilst serum glikoz and Ca levels were also found to be similar in the Control and Vitamin E+Stress groups, but, P levels was significantly decreased in the Stress+Vitamin E group ($p<0.05$) (Table 2). Hydrocarbons, TAG, DAG and polar lipids rates were similar in all three groups (Table 3). While heat stress mathematically increases trigliyceride, HDL and VLDL levels, Vitamin E were reduced these values($p>0.05$) (Table 2).

Heat stress is one of the most significant environmental stress factors. The optimum environmental temperature range for poultry species is 18-24 °C, and when these temperatures are exceeded, animals become exposed to heat stress [18]. When exposed to heat stress, the antioxidant defence system is inhibited and oxidative stress induces adverse effects on several tissues [11]. The supplementation of the ration with feed additives is one of the methods used to prevent the adverse effects of environmental stress, and many studies have demonstrated that antioxidant feed additives prevent the adverse effects triggered by heat stress[8, 19]. Although many researches have been conducted on the effects of feed additives on the antioxidant defence system, the number of studies investigating the effects of feed additives on the metabolic profile of animalsis scarce. The present study was aimed at the investigation of the effects of vitamin E on the metabolic profile of broiler chickens, and to contribute to scientific literature in this field.

In their study, in which they supplemented the ration of quails exposed to heat stress with vitamin E and selenium, Gursu*et al.*[20]determined that serum glucose, urea, triglyceride and cholesterol levels had decreased and ascertained that, when the concentration of vitamin E and selenium in the ration was increased, plasma albumin and protein levels also increased. In a similar study, the supplementation of the ration with vitamin E was shown to decrease serum glucose and cholesterol levels and to increase plasma protein levels [4]. Furthermore, Imik *et al.*[21] reported that, the administration of vitamin E to quails exposed to heat stress significantly reduced serum glucose levels, but did not affect the lipid profile or total protein, Ca and P levels. Sujatha*et al.*[22] reported that the supplementation of the ration with vitamin C decreased plasma cortisol, cholesterol, glucose, total protein and albumin levels, which had increased with heat. On the

other hand, in another study, it was determined that vitamin C and α-lipoic acid did not have any effect on glucose, total protein, albumin and globulin levels in broiler chickens exposed to heat stress[23].Moeini*et al.*[24] observed positive effects on serum lipid (cholesterol, HDL, LDL and triglyceride) concentrations in broiler chickens exposed to heat stress upon the supplementation of the ration with chromium. It was ascertained that the supplementation of quail rations with vitamin C significantly reduced serum cholesterol, VLDL and triglyceride levels, but did not affect glucose levels[25]. While Turrens*et al.*[26] reported that resveratrol, known to have antioxidant property, did not induce any effect on the lipid profile of rats, Imik *et al.*[23]indicated that vitamin C and α-lipoic acid had no effect on triglyceride, cholesterol, HDL, LDL, and VLDL levels in broiler chickens. In the present study, it was determined that, vitamin E significantly reduced serum cholesterol levels increased by heat stress ($p<0.05$), and mathematically reduced triglyceride and VLDL levels (Table 2). The increase of the level of HDL, also known as good cholesterol, is a favourable condition and plays an important role in the prevention of cardiovascular diseases and the alleviation of the impact of these diseases [27]. Increased levels of LDL, also known as bad cholesterol, constitute a risk factor for cardiovascular diseases, and it has been reported that decreased LDL levels reduce this risk [28]. Therefore, the increased LDL levels observed in the broiler chickens included in the stress group, having been observed to have significantly decreased in the broiler chickens included in the stress + vitamin E group, is considered as an indicator of the positive effect of vitamin E ($p<0.05$).

Previous research demonstrated that in quails, exposed to heat stress and provided with a ration supplemented with vitamin E, plasma calcium and potassium levels had increased and plasma sodium levels had decreased[4]. Furthermore, it has been reported that, in broiler chickens[29] and turkeys[30] exposed to high temperature, plasma Ca, Na and P levels decrease. On the other hand, Koelkebeck and Odom[31] indicated no change in the serum inorganic phosphorus, calcium, potassium and sodium levels of laying hens exposed to heat stress.

In their study carried out in quails, Seyrek*et al.*[25]determined that the supplementation of the ration with vitamin C had no effect on glucose and Ca levels. In an investigation on broiler chickens, it was ascertained that the decrease observed in blood Ca and Mg levels as a result of exposure to heat stress was not affected by vitamin C, whereas it was determined that α-lipoic acid significantly increased the reduced Ca and Mg levels, but had no effect on P and Fe levels[23]. In the present study, it was observed that while vitamin E significantly increased the Ca levels, which had decreased as a result of exposure to heat stress, it had no effect on the P levels, which had also decreased with heat stress (Table 2).

Table 2: The average values of some parameters in serum of the groups (n=10)

Parameters, mg/dl		Groups			p-value
		Control	Stress	Stress+Vitamin E	
	Triglyceride	116.86	137.86	125.86	NS
	Cholesterol	142.14^{b}	155.14^{a}	140.00^{b}	*
	HDL	94.71	99.43	94.14	NS
	LDL	15.43^{b}	21.00^{a}	15.29^{b}	*
	VLDL	23.29	27.43	25.14	NS
	HDL/LDL	6.13	4.73	6.15	NS
	Total protein	3.69^{b}	3.92^{a}	3.69^{b}	*
	Glucose	196.20^{a}	194.17^{b}	197.86^{a}	*
	Ca	12.04^{a}	10.69^{b}	11.72^{a}	*
	P	4.90^{a}	4.65^{b}	4.59^{b}	*

HDL: High density lipoproteins; LDL: low density lipoproteins; VLDL: very low density lipoproteins; Ca: calcium; P:phosphorus. a, b, c: a letter in the same line means significantly different (*: $p<0.05$). NS: Non Significant.

Table 3: Serum lipid profiles of groups, (n=10).

Parameters, %	Groups			p-value
	Control	Stress	Stress+Vitamin E	
HC	57.72	57.09	57.74	NS
TAG	14.44	13.86	14.47	NS
NEFA	2.43^{c}	3.70^{a}	3.04^{b}	*
CH	15.87^{a}	15.93^{a}	15.25^{b}	*
DAG	1.28	1.14	1.27	NS
PL	8.26	8.28	8.23	NS

HC: hydrocarbons ; TAG: triacylglycerol ; NEFA: *non-ester fatty acid ;*CH: cholesterol ; DAG: diacylglycerol ; PL: polar lipids. a, b, c: a letter in the same line means significantly different (*: $p<0.05$). NS :Non Significant.

CONCLUSION

In result, in the present study, it was determined that in broiler chickens exposed to heat stress, while the supplementation of the ration with vitamin E decreased the NEFA rate and cholesterol, LDL and total protein levels, and increased glucose and Ca levels, it had no effect on hydrocarbon, TAG, DAG, polar lipid, triglyceride, HDL, VLDL and P levels. Therefore, it was concluded that vitamin E can be used as an antioxidant feed additive for the alleviation of the adverse effects caused by heat stress in broiler chickens.

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