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The Metabolic Effect of Vitamin E Supplementation to the Diets of Laying Hens under Heat Stress (35°C)

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Abstract: The aim of the study, was to investigate the effects of vitamin E and heat stress on the metabolism of laying hens. In the study, 120 Leghorn line laying hens were used. The study was carried out in 9 weeks and divided into 3 periods. The hens were divided into 2 groups. The control and experimental groups were subdivided into 3 groups of 20 hens each. The hens in the subgroups were fed the standard full feed layer ration supplemented with extra 50 and 75 mg kg^{-1} vitamin E, respectively. The temperature of the room of the experimental groups was raised to 35°C during the second period. It was verified that heat stress lead to significant increase in plasma total protein, albumin, total lipid and cholesterol concentrations during the second period of the experiment. As a result, it might be concluded that supplementing vitamin E to the diet during heat stress does not have any positive effect on the metabolism.

Key words: Heat, stress, laying hens, vitamin E, metabolism, biochemical parameters

INTRODUCTION

For poultry the changes of environmental temperature are among the most important factors that effect energy need and metabolic rate. Under heat stress behavioral, physiological, hormonal and molecular changes take place in poultry. Poultry adapt to the environmental temperature by changing their physiologic activities (Yahav and McMurty, 2001; Yahav *et al*., 1997). Developing such thermotolerance in poultry might be a very important advantage for the poultry industry located on tropical and subtropical regions, especially in summer season (Moraes *et al*., 2003). In parallel with the increase in environmental temperature the poultry will increase their respiratory rate and evaporation in order to reduce their rising body heat consequently their metabolic rates increase. Moreover, high environmental temperatures decrease the egg productivity and quality of laying hens and also negatively affect feed consumption and efficiency, which might lead to economical losses (Donkoh, 1989; Sahin and Kucuk, 2001; Smith and Oliver, 1972). Several methods can be applied in order to reduce the negative effects of heat stress. One of these methods is cooling the buildings. However, as this method exceedingly increases operating costs manipulations concerning the diets are recommended (Bollengier *et al*., 1998). Addition of vitamin E to the diets is recommended as one of the methods that might be used for eliminating the negative effects of heat stress (Ertas and Sahin, 2002). Moreover, the vitamin E supplemented to the diet not only raises the quality of the animal products for human consumption but increases the vitamin E content of the foods as well (Flachowsky, 2000; Sunder *et al*., 1997). The poultry cannot synthesize vitamin E, thus they use the diet as a vitamin E source (Chen *et al*., 1999). As it exists in the lipid components of biological membranes, vitamin E protects the cells and tissues against the negative effects of the free radicals that emerge as a result of oxidative stress with its chain-breaking antioxidant effects. It was formerly reported that the lipid peroxidation in cell membranes increased during heat stress (Sahin and Kucuk, 2001; Edens and Segel, 1975). Many researchers reported that heat stress had negative effects on laying hens in terms of egg productivity and quality and vitamin E addition to the diets was useful in minimizing these effects (Sahin and Kucuk, 2001; Sahin *et al*., 2002). In this study, it was aimed to determine the effects of vitamin E supplementation during heat stress on poultry metabolism by monitoring changes in blood levels of some biochemical parameters.

MATERIALS AND METHODS

Animals: In this study, 120 of 27 week-old Leghorn line laying hens were used. The study has been carried out during 9 weeks of the highest egg productivity. Two

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rooms with identical environmental specifications were used in the study. The hens were grouped in 2 sets of 60, groups not under heat stress (Group 1) and groups under heat stress (Group 2) and placed in separate rooms. Each groups were subdivided into three groups of 20 hens each; control groups (Group 1a and 2a), 50 mg vitamin E resumed groups (Group 1b and 2b) and 75 mg vitamin E resumed groups (Group 1c and 2c).

Management: Two hens were housed in one cage (24×41×45) and received 17 h of light and 7 h of darkness day^{-1}. Feed and water was provided *ad-libitum* prior to the beginning of the experiment. The 9 week long experimental period consisted of 3 periods of 3 weeks. The initial 3 weeks represent the 1st period and the hens were kept under 45% relative humidity and 21°C room temperature for adaptation purposes. The 2nd period, which comprises the period from 4-6th weeks, the temperature for the experimental groups were increased to 35°C and relative humidity, was increased to 65%. During the last period the temperature was reduced to 21°C. The control groups were kept under 21°C room temperature and 45% humidity conditions during the all periods.

Feeding: The hens in the control subgroups were fed with the standard full feed layer ration. The hens in all groups received 125 g feed per day. The content of the standard full feed layer ration and concerning energy calculations are given on Table 1 and 2.

Sample collection: Blood samples were obtained from the brachial vein (V. Subcutanea ulnaris) of the all groups in the first (before heat stress), second (during heat stress) and third periods (after heat stress) into plasma tubes. The samples were centrifuged at 3000 rpm for 10 min for obtaining plasma. The plasma samples were stored at -20°C until the analyses were made.

Blood metabolite analysis: Plasma total protein, albumin, glucose, uric acid, total lipid and cholesterol concentrations were determined using commercial test kits (Spinreact, SA-ctra sahre coloma, 7-E-17176 sant esteve de bas-Spain) and photometer SEAC ch 100.

Statistical analyses: Effects of heat stress and vitamin E supplementation on plasma total protein, globulin, albumin, glucose, uric acid, total lipid, cholesterol concentrations of all groups were determined using General Linear Model (GLM). The SPSS statistical software package (Version 11.5, SPSS Program) was used for all statistical analysis. All results were expressed as mean±SE.

Table 1: The content of the standart full feed layer ration

Ingredients (%)	Values
Corn	30.00
Wheat	25.00
Full fat soybean	11.00
Sunflower meal	15.00
Fat	0.80
Dicalcium phosphate	2.00
DL-Methionine	0.10
Limestone	9.50
Vitamin+mineral premix*	1.00
Salt	0.30
Meat and bone meal	3.00
Gluten	2.00
L-Lysine	0.20
Vitamin E (mg kg^{-1})**	30.00
Antioxdant (mg kg^{-1})	10.00

*Composition of vitamin premix per kilogram of premix: Vitamin A. 10.000.000 IU; vitamin D3. 2.000.000 IU; vitamin K3. 3 mg kg^{-1}; vitamin B1. 3 mg; vitamin B2. 6 mg: vitamin B6. 4 mg; vitamin B1. 2; 15 mg; Ca pantothenate. 10 mg; Niacin 25 mg; Folic acid. 1mg; D Biotin; 25 mg.Composition of trace elements premix per kilogram of premix: Mn. 80 mg; Fe .60 mg; Zn. 60 mg; Cu. 5 mg: Co 500 mg; Se. 150 mg. **Group 1a; 30 mg VitaminE resumed group, Group 1b; 50 mg vitamin E resumed group, Group 1c; 75 mg Vitamin resumed group, Group 2a; 30 mg VitaminE resumed group, Group 2b; 50 mg vitamin E resumed group, Group 2c; 75 mg Vitamin E resumed group

Table 2: Nutrient content and energy levels of the standard full feed layer ration

Parameters	Values
Dry matter (%)	89.00
Crude protein (%)	17.50
Crude fibre (%)	5.00
Ash (%)	12.00
Crude fat (%)	5.20
Ca (%)	3.90
P (%)	0.85
Metabolisable energy (ME Kcal kg^{-1})	2750.00

RESULTS

The least square means for plasma total protein and albumin concentrations during the 3 periods are given on Table 3 and 5. It was observed that, during heat stress (35°C), the total protein and albumin concentrations in laying hens increased by $p<0.05$ compared to laying hens under optimal environmental temperature (21°C). It was also, determined that extra vitamin E supplementation during the all periods had no interactions the plasma levels of total protein and albumin concentrations. The least square means for plasma globulin, glucose, uric acid concentrations are given on Table 4, 6 and 7. It was observed that heat stress and vitamin E supplementation did not cause significant differences among the groups for these 3 parameters during the all periods. Least square means for plasma total lipid and cholesterol concentrations are given on Table 8 and 9. It was determined that the plasma total lipid and cholesterol concentrations for the groups under the heat stress during the 2nd period were $p<0.001$ higher than those not under heat stress during the 2nd period.

Table 3: Effects of total protein concentrations in all control and experimental groups (mg dL^{-1})

Concentrations	n	1st period	2nd period	3rd period
Heat stress application		Ns[1]	*	Ns
Groups not under heat stress[2]	60	4.46±0.38	5.53±0.58	5.29±0.53
Groups under heat stress[3]	60	4.37±0.51	4.40±0.59	5.14±0.61
Vitamin E supplementation		ns	ns	ns
Control Groups[4]	40	4.15±0.52	5.28±0.64	5.11±0.58
Total 50 mg vitamin E resumed groups[5]	40	4.62±0.51	4.95±0.62	5.27±0.57
Total 75 mg vitamin E resumed groups[6]	40	4.48±0.54	4.36±0.68	5.27±0.60
Heat stress X Vitamin E interaction		ns	ns	ns
Overall mean	120	4.41±0.49	4.86±0.50	5.22±0.46

[1]Non significant; [2]C1, C2, C3; [3]E1, E2, E3; [4]C1, E1; [5]C2, E2; [6]C3, E3; * $p<0.05$

Table 4: Effects of globulin concentrations in all control and experimental groups (mg dL^{-1})

Concentrations	n	1st period	2nd period	3rd period
Heat stress application		ns[1]	ns	ns
Groups not under heat stress[2]	60	1.99±0.48	1.66±0.55	2.23±0.49
Groups under heat stress[3]	60	1.71±0.49	2.03±0.53	1.71±0.48
Vitamin E supplementation		ns	ns	ns
Control groups[4]	40	1.75±0.52	2.15±0.60	1.97±0.53
Total 50 mg vitamin E resumed groups[5]	40	2.12±0.56	1.89±0.59	2.18±0.57
Total 75 mg vitamin E resumed groups[6]	40	1.68±0.54	1.50±0.63	1.77±0.54
Heat stress X Vitamin E interaction		ns	ns	ns
Overall mean	120	1.85±0.49	1.85±0.48	1.97±0.43

[1]Non significant; [2]C1, C2, C3; [3]E1, E2, E3; [4]C1, E1; [5]C2, E2; [6]C3, E3

Table 5: Effects of albumin concentrations in all control and experimental groups (mg dL^{-1})

Concentrations	n	1st period	2nd period	3rd period
Heat stress application		ns[1]	*	ns
Groups not under heat stress[2]	60	2.47±0.45	3.86±0.55	3.03±0.53
Groups under heat stress[3]	60	2.71±0.41	2.91±0.59	3.55±0.52
Vitamin E supplementation		ns	*	ns
Control groups[4]	40	2.40±0.43	4.04±0.60[a]	3.27±0.53
Total 50 mg vitamin E resumed groups[5]	40	2.59±0.43	3.13±0.59[b]	2.89±0.57
Total 75 mg vitamin E resumed groups[6]	40	2.77±0.44	2.99±0.64[b]	3.65±0.59
Heat stress X Vitamin E interaction		ns	ns	ns
Overall mean	120	2.59±0.40	3.39±0.48	3.29±0.46

[1] Non significant; [2]C1, C2, C3; [3]E1, E2, E3; [4]C1, E1; [5]C2, E2; [6]C3, E3; * $p<0.05$

Table 6: Effects of glucose concentrations in all control and experimental groups (mg dL^{-1})

Concentrations	n	1st period	2nd period	3rd period
Heat stress application		ns[1]	ns	ns
Groups not under heat stress[2]	60	191.38±13.01	224.71±31.17	212.55±16.78
Groups under heat stress[3]	60	183.18±10.21	196.20±32.36	265.47±14.73
Vitamin E supplementation		ns	ns	ns
Control groups[4]	40	177.35±14.02	200.18±37.44	252.94±17.86
Total 50 mg vitamin E resumed groups[5]	40	207.44±13.51	238.39±35.84	262.34±16.44
Total 75 mg vitamin E resumed groups[6]	40	177.06±14.62	192.79±41.59	246.98±17.86
Heat stress X Vitamin E interaction		ns	ns	ns
Overall mean	120	187.26±9.38	210.45±23.42	246.98±17.8

[1]Non significant; [2]C1, C2, C3; [3]E1, E2, E3; [4]C1, E1; [5]C2, E2; [6]C3, E3

Table 7: Effects of uric acid concentrations in all control and experimental groups (mg dL^{-1})

Concentrations	n	1st period	2nd period	3rd period
Heat stress application		ns[1]	ns	ns
Groups not under heat stress[2]	60	3.37±0.21	4.13±0.29	4.61±0.35
Groups under heat stress[3]	60	3.84±0.15	4.84±0.31	4.44±0.46
Vitamin E supplementation		ns	ns	ns
Control groups[4]	40	3.72±0.23	4.51±0.57	3.76±0.51
Total 50 mg vitamin E resumed groups[5]	40	3.72±0.20	4.49±0.56	5.03±0.50
Total 75 mg vitamin E resumed groups[6]	40	3.28±0.24	4.46±0.58	4.80±0.53
Heat stress X Vitamin E interaction		ns	ns	ns
Overall mean	120	3.61±0.15	4.49±0.21	4.53±0.31

[1]Non significant; [2]C1, C2, C3; [3]E1, E2, E3; [4]C1, E1; [5]C2, E2; [6]C3, E3

However, a significant effect of vitamin E addition to the diet of the laying hens on the plasma levels of these 2 parameters was not determined. A significant interaction between vitamin E supplementation and heat stress did not exist for biochemical blood parameters.

Table 8: Effects of total lipid concentrations in all control and experimental groups (mg dL^{-1})

Concentrations	n	1st period	2nd period	3rd period
Heat stress application		ns[1]	***	ns
Groups not under heat stress[2]	60	238.44±14.27	226.75±17.13	244.93±23.57
Groups under heat stress[3]	60	218.71±17.27	357.99±21.73	285.30±23.93
Vitamin E supplementation		ns	ns	ns
Control groups[4]	40	220.55±17.37	268.87±19.54	270.70±28.31
Total 50 mg vitamin E resumed groups[5]	40	226.54±16.66	289.36±19.16	275.63±26.51
Total 75 mg vitamin E resumed groups[6]	40	237.27±18.11	318.88±23.54	249.03±30.57
Heat stress X Vitamin E interaction		ns	ns	ns
Overall mean	120	228.12±11.30	292.37±13.31	265.12±17.73

[1]Non significant; [2]C1, C2, C3; [3]E1, E2, E3; [4]C1, E1; [5]C2, E2; [6]C3, E3; ***$p < 0.001$

Table 9: Effects of cholesterol concentrations in all control and experimental groups (mg dL^{-1})

Concentrations	n	1st period	2nd period	3rd period
Heat stress application		ns[1]	***	ns
Groups not under heat stress[2]	60	74.39±7.55	82.06±10.94	67.98±8.21
Groups under heat stress[3]	60	64.51±6.87	155.85±11.48	52.13±8.11
Vitamin E supplementation		ns	ns	ns
Control groups[4]	40	65.59±8.80	116.39±12.70	63.46±9.25
Total 50 mg vitamin E resumed groups[5]	40	70.11±8.30	122.65±12.25	60.93±8.92
Total 75 mg vitamin E resumed groups[6]	40	72.65±8.86	117.83±14.26	55.77±9.78
Heat stress X Vitamin E interaction		ns	ns	ns
Overall mean	120	69.45±6.22	118.95±8.84	55.77±9.78

[1]Non significant; [2]C1, C2, C3; [3]E1, E2, E3; [4]C1, E1; [5] C2, E2; [6]C3, E3; ***$p<0.001$

DISCUSSION

The poultry cannot synthesize vitamin E, thus they use the diet as a vitamin E source (Chan and Decker, 1994). It is stated that a significant decrease occurs in the feed consumption and plasma vitamin E concentrations of poultry under high environmental temperature (Donkoh, 1989; Sahin and Kucuk, 2001; Simith and Oliver, 1972). In the poultry that try to keep their body temperature at an optimal level breathing and evaporation increases in parallel with the increasing environmental temperature and this leads to an increase in poultry metabolism and energy consumption. In cases where increasing energy need cannot be supplied with feed lipids in reserve fats are mobilized (Gomez *et al*., 2002). Sahin and Kucuk (2001) reported that feed consumption decreased in the Japanese quails under heat stress (34°C). As a result of this study, it might be asserted that the reason for the significant difference between the total lipid and cholesterol levels of hens under heat stress and those not under heat stress during the 2nd period was the mobilization of lipid in reserve fats for supplying the increasing energy need. Our hypothesis concerning the mobilization of the fatty acids in reserve fats was supported by the fact that heat stress in both groups did not have a significant effect on the plasma glucose concentration of laying hens. Another task carried out by albumin, one of the plasma proteins, is the transportation of the lipids to target tissues and organs (El-Sherif and Assad, 2001). It might be asserted that the reason for the minor effect of heat stress on plasma albumin concentrations might be the transportation of the lipids to the target tissue through blood in parallel with the increasing lipid mobilization. The total protein concentration in the plasma is equal to the sum of plasma albumin and globulin concentrations (Chen *et al*., 1999).

In the 2nd period of the study, heat stress effected critically the total protein concentration because we hold the view that while it had no effect on plasma globulin concentration it effected albumin concentrations. Also the reason for the tiny effect of heat stress on plasma uric acid concentrations was the fact that the increasing energy need was primarily supplied from reserve fats Bollengier-Lee *et al*. (1999) and Puthpongsiriporn *et al*. (2001) reported that the plasma vitamin E concentrations were increased by addition of vitamin E to the diet at different rates. Former studies reported that under high environmental temperature the Reactive Oxygen Species (ROS) levels were amplified in parallel with increasing energy consumption and lipid peroxidation increased in parallel with increasing lipid mobilization (Bollengier-Lee *et al*., 1999; Sahin and Kucuk, 2001, 2002; Papa and Skulachev, 1997). Sahin *et al*. (2002) reported that as the result of a study they carried out on broilers they have found out that heat stress increased the MDA levels that are indicators of lipid peroxidation and vitamin E supplementation lead to a decrease in plasma MDA values. As stated in previous researches in such cases it is highly anticipated that the laying hens have a low egg yield and quality (Simith and Oliver, 1972; Bollengier-Lee *et al*., 1998; Sahin *et al*., 1999; Sahin *et al*., 2002; Kirunda *et al*., 2001). Bollenger-Lee *et al*. (1998) asserted that instead of expensive operations for cooling the buildings in tropical and subtropical regions

manipulations about the diet should be made and especially, addition of extra vitamin E to the diet should be effective on reducing operating cost.

CONCLUSION

It might be asserted that addition of extra vitamin E to the diet of laying hens under heat stress has no role in laying hen metabolism short of diminishing the negative effects of ROS and lipid peroxidation. It might also be asserted that only diet manipulations would not suffice to prevent possible economic losses in an enterprise under high environmental temperature risk and this also would not be profitable for the enterprise.

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