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The Effects of Vitamin E on Some Blood Parameters in Broilers

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Abstract: The purpose of this study was to determine the effects of different doses of vitamin E supplemented into the diet on erythrocyte osmotic fragility and some biochemical parameters. Sixty broilers were randomly divided into four groups of 15 each (one control and three experimental groups). Experimental groups (D1, D2, D3) were fed with a diet supplemented with 100, 200 and 300 ppm of vitamin E respectively. The plasma vitamin E level, and other blood parameters, of all the test subjects were measured in the fifth and seventh week of the study. The plasma vitamin E level of D2 and D3 experimental groups in the fifth week, and the plasma vitamin E level of all three experimental groups in the seventh week of the study increased statistically when compared to the control group. Erythrocyte osmotic fragility of all three experimental groups decreased significantly by the fifth and seventh weeks of the study in comparison with the control group. No significant difference in plasma cholesterol, triglyceride, total protein, ALP (alkaline phosphatase), Ca (calcium), P (phosphorus), AST (aspartate aminotransferase) or ALT (alanin aminotransferase) was found between control and experimental groups by the fifth and seventh weeks. Only the ALP level of all groups decreased statistically by the seventh week of the study in comparison to the fifth week.

Key Words: Vitamin E, Broiler, Erythrocyte, Osmotic Fragility, Biochemical Parameters

Broylerlerde E Vitamininin Bazı Kan Parametreleri Üzerine Etkileri

Özet: Bu araştırma yeme katılan farklı dozlardaki vitamin E'nin alyuvar ozmotik frajilite ve bazı biyokimyasal parametrelere etkisini belirlemek amacıyla yapıldı. Altmış broylerden her biri 15 hayvandan oluşan biri kontrol, üçü deneme olmak üzere dört grup oluşturuldu. Deneme gruplarına (D1,D2,D3) sırasıyla 100 ppm, 200 ppm, 300 ppm vitamin E verildi. Tüm hayvanlarda 5. ve 7. haftalarda plazma vitamin E düzeyi ve bazı kan parametreleri ölçüldü.

Plazma Vitamin E düzeyi uygulamanın 5. haftasında D2 ve D3 gruplarında, 7. haftada ise her üç deneme grubunda kontrol grubuna göre istatistiksel önemde farklı olacak şekilde arttı. Alyuvar ozmotik frajilitesi 5 ve 7. haftalarda tüm deneme gruplarında kontrol gruplarına göre önemli oranda azaldı. Plazma kolesterol, trigliserit, total protein, ALP (alkalin fosfotaz), Ca (kalsiyum), P (fosfor), AST (aspartat aminotransferaz) ve ALT (alanin aminotransferaz) açısından her iki haftalıklarda deneme ve kontrol grupları arasında önemli bir fark gözlenmezken, haftalar arası değerlendirmede sadece ALP düzeyi 7. haftada 5. haftaya göre tüm gruplarda istatistiksel önemde azaldı.

Anahtar Sözcükler: E vitamini, Broyler, Alyuvar, Ozmotik Frajilite, Biyokimyasal Değerler

Introduction

Vitamin E (α-tocopherol) is a biological antioxidant, soluble in fat (1), which inhibits the oxidation of long chained unsaturated fatty acids of the cell membrane (2,3). Unsaturated fatty acids react with oxygen, and form superoxide, peroxide and hydroperoxides (4).These free radicals cause cell damage by disturbing the metabolism and structure of the biological membranes of those organs that contain excessive amount of unsaturated fatty acids (5). Vitamin E inhibits the effects of hydrogen protons and free radicals by saturating them, and so inhibits autooxidation (2,4). It has been reported that lipid peroxidation is stopped in chicks fed with a vitamin E supplemented diet (6). It has also been proposed that vitamin E inhibits the oxidation of unsaturated fatty acids such as linoleic acid on the erythrocyte membrane (4), and the deficiency of this vitamin increases the hemolysis of red cells (7,8).

Plasma alkaline phosphatase (ALP) level is accepted as an indicator of cell activity. The increase in plasma ALP, dependent on vitamin E, helps cell multiplication be come more active and assists the metabolism work in good order (9).

However, it has also been declared that the excess supplementation of vitamin E decreases plasma

cholesterol and triglyceride levels and increases the ALP level in hens (9).

Changes in plasma cholesterol, triglyceride and lipoprotein levels have been reported in those studies in which high doses of vitamin E were given to humans (10) and rats (11).

Although much research invesgating the effects of high levels of vitamin E on the metabolism of humans (10,12) and various animals (8,13,14) has been done, little work has been attempted on animals undergoing rapid growth, such as broilers (9). Therefore, in this study, we aimed to investigate the effects of different doses of vitamin E supplemented into the diet for different terms on a number of blood parameters.

Material and Methods

A total of 60 Shewver male chicks were used in this study. The chicks were divided into four groups of three experimental groups (D1, D2, D3) and a control group, with 15 chicks in each. Vitamin E in amounts of 100, 200 and 300 ppm was added to the diets of the experimental groups respectively. All groups were fed with commercial starter diet from JET YEM A.Ş. for the first three weeks and they were fed with a commercial growing diet until the end of the seventh week. Food and water were supplied *ad libitum*.

All animals were placed in a temperature controlled room at 32°C. The temperature of the room was reduced 2°C every week up to five-weeks of age. After the fifth week, the temperature of the room was fixed at 20°C, and 23 hours of light/day was provided in the room where the animals were placed.

At the age of five and seven weeks, 5ml blood samples were taken from the v. cutanea ulnaris of all the animals by using heparinized syringes. The first part of the blood sample 1.5 ml was used to determine osmotic fragility. The remaining part (3.5ml) was centrifuged at 3500xg for 5 minutes to remove the plasma. Samples were stored at -20°C in propylene tubes until they were analyzed.

Plasma vitamin E levels (15) and the osmotic fragility of erythrocytes (7) were determined by the spectrophotometric method. Plasma cholesterol, triglyceride, total protein, ALP, calcium (Ca), phosphorus (P), aspartate aminotransferase (AST) and alanin aminotransferase (ALT) levels were measured on a Ciba Corning Express Plus autoanalyzer by using Bio Clinica commercial kits.

The significance of differences among the groups was determined by using variance analysis, and the significant differences between the weeks were identified by t-test (16).

Results

The means of vitamin E, erythrocyte osmotic fragility, cholesterol, triglyceride, total protein, ALP, Ca, P, AST and ALT levels, and the standard deviations of the means, and the significant differences among the groups, and between the weeks (five and seven) are presented in the Table.

The plasma vitamin E levels of the D2 and D3 experimental groups at five-weeks of age, and plasma vitamin E levels of all three experimental groups at seven weeks of age increased statistically in comparison to the control group.

The erythrocyte osmotic fragility of all experimental groups decreased significantly both at five and seven-weeks of age in comparison to the control group. However, no significant difference was observed in plasma cholesterol, triglyceride, total protein, ALP, Ca, P, AST and ALT levels of the experimental groups compared to the control group.

When we compared the means of the control and experimental groups over the weeks, in respect of all properties, we determined a significant decrease only in ALP level, which was statistically lower at seven weeks of age than that at five-weeks of age.

Discussion

In comparison to the control group, at five weeks of age, the plasma vitamin E level of D2 and D3 experimental groups increased, but not group D1 statistically, while at seven-weeks of age, the plasma vitamin E levels of all experimental groups increased statistically. In contrast, the erythrocyte osmotic fragility of all experimental groups decreased significantly both at five and seven-weeks of age. It has been found that the supplementation of vitamin E into the diet increases the plasma vitamin E level (17,18), though the changes between weeks were found to be insignificant (19). These findigns are similar to our results.

Table The Effects of Vitamin E on Erythrocyte Osmotic Fragility and Some Biochemical Parameters in Broilers.

	Week	Control x Sx	100 ppm x Sx	200 ppm x Sx	300 ppm x Sx
Plasma Vit-E	5	$0.79^{a}\pm0.07$	$2.42^{a}\pm0.10$	$3.53^{b}\pm0.14$	$4.52^{c}\pm0.12$
(mg/100 ml)	7	$0.80^{a}\pm0.06$	$2.41^{b}\pm0.08$	$3.34^{c}\pm0.14$	$4.59^{d}\pm0.11$
		-	-	-	-
Osmotic	5	$0.46^{a}\pm0.05$	$0.28^{bc}\pm0.02$	$0.26^{c}\pm0.04$	$0.22^{cd}\pm0.02$
Fragility (%)	7	$0.41^{a}\pm0.04$	$0.26^{bc}\pm0.04$	$0.22^{c}\pm0.02$	$0.21^{cd}\pm0.02$
		-	-	-	-
Cholesterol	5	$149.70^{a}\pm8.72$	$145.70^{a}\pm6.84$	$144.80^{a}\pm5.08$	$141.54^{a}\pm4.19$
(mg/dl)	7	$143.40^{a}\pm6.11$	$141.88^{a}\pm4.52$	$136.80^{a}\pm7.06$	$134.58^{a}\pm3.81$
		-	-	-	-
Triglyceride	5	$121.82^{a}\pm9.04$	$104.80^{a}\pm9.75$	$114.10^{a}\pm9.90$	$83.60^{a}\pm7.82$
(mg/dl)	7	$108.22^{a}\pm18.70$	$79.55^{a}\pm10.97$	$70.20^{a}\pm10.47$	$88.16^{a}\pm12.81$
		-	-	-	-
Total Protein	5	$1.83^{a}\pm0.28$	$2.91^{a}\pm0.67$	$2.74^{a}\pm0.47$	$2.62^{a}\pm0.41$
(g/dl)	7	$2.81^{a}\pm0.38$	$2.34^{a}\pm0.46$	$2.21^{a}\pm0.41$	$2.55^{a}\pm0.36$
		-	-	-	-
ALP (U/L)	5	$1816.40^{a}\pm27.10$	$1947.00^{a}\pm35.67$	$1929.08^{a}\pm33.52$	$1942.50^{a}\pm41.59$
	7	$1218.70^{a}\pm19.34$	$1218.70^{a}\pm26.37$	$1224.50^{a}\pm33.27$	$1247.10^{a}\pm31.51$
		*	*	**	***
Ca (mg/dl)	5	$8.11^{a}\pm0.33$	$9.36^{a}\pm0.45$	$10.19^{a}\pm0.55$	$8.61^{a}\pm0.76$
	7	$8.40^{a}\pm1.32$	$9.91^{a}\pm1.00$	$11.60^{a}\pm1.84$	$11.39^{a}\pm0.67$
		-	-	-	-
P (mg/dl)	5	$6.51^{a}\pm0.41$	$7.17^{a}\pm0.31$	$7.29^{a}\pm0.30$	$6.53^{a}\pm0.33$
	7	$6.98^{a}\pm0.37$	$7.06^{a}\pm0.39$	$7.34^{a}\pm0.12$	$7.22^{a}\pm0.29$
		-	-	-	-
AST (U/L)	5	$176.90^{a}\pm5.65$	$178.80^{a}\pm6.80$	$174.60^{a}\pm5.74$	$180.09^{a}\pm5.27$
	7	$174.77^{a}\pm10.68$	$167.88^{a}\pm6.52$	$164.10^{a}\pm7.54$	$172.58^{a}\pm4.47$
		-	-	-	-
ALT (U/L)	5	$6.22^{a}\pm0.80$	$5.77^{a}\pm0.64$	$4.00^{a}\pm0.67$	$5.00^{a}\pm0.83$
	7	$10.50^{a}\pm0.87$	$10.40^{a}\pm1.35$	$9.40^{a}\pm1.26$	$8.64^{a}\pm0.65$

^{abcd}: In each line, the differences between the means with different letters are significant ($p< 0.05$).
* :$p< 0.05$ ** :$p< 0.01$ *** :$p< 0.001$

Erythrocyte osmotic fragility of the control group broilers, determined at five and seven-weeks of age, were within the ranges declared in the literature (7,19). In comparison to the control group, decreases in the erythrocyte osmotic fragility of all three experimental groups at five and seven-weeks of age, resembled the results of those studies which were carried out on chicks (7) and rats (20,21). This case may depend on the reduction of the harmful effects of O_2 on the cell membrane by vitamin E, and so vitamin E increases the stability of the cell membrane. However, it has been found that vitamin E prevents the oxidation of unsaturated fatty acids especially the linoleic acid of the erythrocyte membrane (4).

Plasma cholesterol levels of the control group, found to be 149.7±8.72 mg/dl and 143.40±6.11 mg/dl at five and seven-weeks of age respectively, are close to those levels in the literature (9). It was determined that increasing the levels of vitamin E decreases the level of cholesterol in the experimental groups, whereas this decrease was found to be insignificant statistically. However, the maximum decrease was found in experimental D3 group. The same also occurred in triglyceride levels. Similar results have been shown in poultry fed with vitamin E supplemented diet (22,23). It has also been found that the supplementation of vitamin E inhibits atherosclerosis in poultry, but has no effect on the plasma cholesterol level (24,25). In this study,

although a decrease in plasma cholesterol and triglyceride levels was determined, related to the age of the birds, this difference was found to be insignificant statistically (Table). Francini et al. (9) reported that the supplementation of vitamin E at a dosage of 325 ppm results in a decrease in cholesterol and triglyceride levels, and the decrease related to age is definite by day 49.

It has been reported that the cholesterol level of turkeys fed with a vitamin E supplemented diet decreased on the 42nd day and it reached its maximum level on the 86th day (26). It has also been reported that (26) the reason for the decrease in the cholesterol level on the 42nd day was the usage of cholesterol for the biosynthesis of steroid hormones, and the reason for the increase on the 86th day was the increased production of endogen for covering the needs of the augmented metabolism. In the same study (26), it was found that increasing the levels of vitamin E decreases the level of triglyceride on the 28th day, and on day 42 it increased the level of triglyceride. A minimal decrease in triglyceride levels were found in the experimental group fed the maximum level of dietary vitamin E by day 140. And also it has been proposed that this case can be explained by weight gain in parallel with the growth of the animal. In another study (27), it was noted that the level of triglyceride may increase due to the increase in fat stores so as to reach the best fat/muscle rate necessary for the beginning of ovulation in females.

No difference was found between the total plasma protein of the control and experimental groups, or between the weeks. These results confirm previous literature (9).

In this study, plasma ALP, Ca and P levels of the control group were close to those declared in the literature (9) for hens, and in the same study it was proposed that the increase in ALP, Ca and P levels of broilers fed with excess dietary vitamin E may be related to osteoblastic activity.

In this study, although the ALP levels of the experimental groups were higher than those of the control group, this difference was found not to be significant statistically (Table). It has been declared that the plasma ALP levels of hens increases as the level of vitamin E increases (9), but no relationship was found between the level of vitamin E and ALP level in turkeys (26). A significant decrease in the plasma ALP level of all three experimental groups and the control group was determined in the seventh week of the study in comparison to the fifth week. The decrease in plasma ALP levels may be related to the increase in age. This has also been confirmed by previous studies (9,26).

In comparison to the control group, although an increase in both Ca and P levels in all experimental groups was determined, the difference was found not to be significant statistically. When comparing between the weeks, an insignificant increase in the plasma Ca level of all groups, including the control group, was observed in the seventh week of the study. However, fluctuations were observed in plasma P levels. The finding regarding Ca levels is also supported by the statement (9) that an increase in Ca levels is related to the age of hens. A decrease in plasma P and Ca levels of 100-day old broilers, fed with 25 and 10,000 IU of vitamin E per kg diet has been reported, but the reason for this was not explained (28). The reason for the contradiction between the results of this study and the results of other studies can be explained by the different age of the animals (28) and the amount of vitamin E supplemented to the diet. A decrease has also been found in plasma Ca and P levels of turkeys fed with 90 ppm of vitamin E per kg in their diet (26). There were also some results showing that oral and intramuscular application of vitamin E does not result in a significant difference, in respect of Ca and P levels, between control and experimental groups of calves (29,30). The reason for the contradiction among the results might be the result of the difference in species used. However, it has been supposed that the tissue and plasma levels of vitamin E in different species were found to be different, eventhough the same amount of dl-α-tocopherol was given (26). This conception is supported by information (31) concerning the absorbtion capacity of vitamin E, with it being lower in turkeys than in hens.

In this study, supplementation of vitamin E had no significant effect on the activity of AST (Table). In comparisons between either groups or weeks, no significant difference was observed among the values statistically. It was noted in a study (9) in which the effects of vitamin E on enzyme activity in broilers was investigated, that no significant change was observed between the control and experimental groups with respect to AST activity on the 49th day of the study. Francini et al. (26) reported that the AST level increases together with vitamin E in young turkeys, although it

decreases in older turkeys (140 days old), and these results show no similarity to those observed in hens.

The ALT level of the control group is similar to that declared in the literature (9). Francini et al. (26) noted that no significant difference was observed in the plasma ALP levels of turkeys fed with 30, 90, 180, 360 ppm of vitamin E per kg of diet, and they observed an increase in plasma ALP levels, related with age, in turkeys. Our findings about the levels of ALT (Table) resemble the findings of Francini et al.

As a result, it can be said that the supplementation of high levels of vitamin E does not change the metabolism of broilers significantly, and as it protects the biologic membranes and affects the cell multiplication, the utilisation of vitamin E can be beneficial for those animals undergoing very rapid growth, such as broilers. However, the limited utilisation of vitamin E would be more suitable until we get confident results concerning the use of high doses of vitamin E for the purpose of treatment over a long period.

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