EFFECT OF VITAMIN E AND SELENIUM ON PERFORMANCE, PLASMA AND TISSUE GSH-PX ACTIVITY IN BROILERS

Broylerlerde Vitamin E ve selenyumun performans, plazma ve doku GSH-Px aktivitesi üzerine etkisi

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ABSTRACT

The aim of this study was to determine the effects of Vitamin E and organic or inorganic selenium sources combined with Vitamin E on growth performance and plasma and tissue glutathione peroxidase activity in broilers. A total of 168, one-day old female commercial broiler chicks (Hubbard strain) were randomly allotted to one control and three treatment groups, each containing 42 birds. All experimental groups were replicated three times with 14 chicks per replicate. Feed and water were supplied *ad libitum* during the experimental period. Four experimental diets were provided as follows: a. Basal diet without Vitamin E and any selenium sources, for the control group (control). b. Basal diet supplemented with 250 mg/kg Vitamin E for the treatment group 1 (Vit E). c. Basal diet supplemented with 250 mg/kg Vitamin E plus 0.2 mg/kg organic selenium for the treatment group 2 (Vit E+Or Se) d. Basal diet supplemented with 250 mg/kg Vitamin E, plus 0.2 mg/kg inorganic selenium for the treatment group 3 (Vit E+Inor Se). The experiment lasted 42 days.

At the end of the study, there was a difference ($P<0.05$) regarding body weight gain in the first (Vit E) and the second (Vit E+Or Se) treatment groups compared to the control group.

Feed conversion ratio was more efficient (P<0.05) in the treatment group 2 (Vit E+Or Se) compared to the rest. Glutathione peroxidase activity in plasma (P<0.01), kidney (P<0.05), leg muscle (p<0.05), heart (P<0.01) and liver (P<0.01) elevated in all of the treatment groups compared to the control group. Liver glutathione peroxidase activity in the treatment group 2 (Vit E+Or Se) was higher than the rest.

The results of this study indicated that, utilisation of organic selenium plus vitamin E in broiler diets was effective for improving the body weight gain, the feed conversion ratio, and plasma and tissue glutathione peroxidase activity.

Key words: Broiler, glutathione peroxidase, performance, selenium, Vitamin E.

ÖZET

Bu çalışmanın amacı, broyler rasyonlarına katılan E Vitamini ile organik ve inorganik selenyum kombinasyonlarının büyüme performansı, plazma ve doku glutatyon peroksidaz aktivitesi üzerine etkilerini belirlemektir. Araştırmada toplam 168 adet, günlük dişi broyler civciv, her biri 42 hayvandan oluşan bir kontrol ve üç deneme grubuna rastgele olarak ayrılmış ve her bir grup üç alt gruba bölünmüştür. Deneme süresince yem ve su *ad libitum* olarak sağlanmıştır. Kontrol grubuna E Vitamini ve selenyum kapsamayan yem verilmiştir. Deneme grupları 1, 2 ve 3'e sırasıyla 250 mg/kg E, Vitamini; 250 mg/kg Vitamin E + 0,2 mg/kg organik selenyum; 250 mg/kg Vitamin E + 0,2 mg/kg inorganik selenyum ilavesi yapılmıştır. Araştırma 42 gün sürdürülmüştür.

Araştırma sonunda, Deneme grubu 1 (Vit E) ve 2 (Vit E+Or Se)'de canlı ağırlık artışları kontrol grubundan daha yüksek (P>0.05) bulunmuştur. Yemden yararlanma oranının 2. deneme grubunda (Vit E+Or Se) daha yüksek (P<0.05) olduğu gözlenmiştir. Tüm deneme gruplarının kontrol grubu ile karşılaştırılmasında, plazma (P<0.01), böbrek (P<0.05), kas (p<0.05), kalp (P<0.01) ve karaciğer (P<0.01) glutatyon peroksidaz aktivitesinde önemli bir artış görülmüştür. Karaciğer glutatyon peroksidaz aktivitesi, 2. deneme grubunda (Vit E+Or Se) kontrol ve diğer deneme gruplarından daha yüksek bulunmuştur.

Sonuç olarak; E Vitamini ile organik selenyum kombinasyonunun, canlı ağırlık kazancı, yemden yararlanma oranı, plazma ve dokularda, glutatyon peroksidaz aktivitesini olumlu yönde etkilediği belirlenmiştir.

Anahtar kelimeler: Broyler, glutatyon peroksidaz, performans, selenyum, E Vitamini.

INTRODUCTION

Vitamin E is essential for growth performance, health and maintenance of tissue integrity in broilers. Vitamin E, a fat-soluble vitamin, is an intracellular antioxidant for all cells. It prevents oxidation of unsaturated lipids within cells and protects cell membrane from oxidative damages (4).

Selenium is an essential trace mineral in poultry for body development, maintenance of glutathione peroxidase activity (14) and avoidance of exudative diathesis (6). Rotruck et al. (8) demonstrated the selenium necessity for proper glutathione peroxidase enzyme function, which is an antioxidant enzyme destroying free radicals produced during normal metabolic activity in the organism. It is stated that there are two selenium sources, as inorganic selenium and organic selenium which is part of the organic molecules. On the other hand, utility of these selenium sources are different. Inorganic selenium has toxic effects and a little accumulation into meat, in addition to interacting with other minerals. However, its retention and availability are lower than that of organic selenium. Organic selenium remains at higher concentrations in muscle tissue compared to inorganic selenium. Therefore, not only does organic selenium improve animal health and productivity, but it also contributes to production of selenium–enriched meat, hence, it can be considered as an advantage for improvement of meat quality. Because of these reasons, inorganic selenium is used limitedly in poultry diets.

Metabolic functions of selenium are similar to Vitamin E. A well known synergy exits between selenium and Vitamin E. Selenium and Vitamin E, both act as the primer antioxidant by suppressing oxidative damages. Marsh et al (5) reported that body weights decreased in chicks fed with Vitamin E and selenium deficient diets. Selenium deficiency, especially together with the low level of Vitamin E in chickens, is responsible for arising of diseases such as exudative diathesis (6) and pancreatic atrophy (12). Devore et al (2), demonstrated that dietary supplementation of 0.25 ppm selenium substantially increased GSH-Px activity in breast and leg muscles.

The aim of this study is to determine the effects of Vitamin E and organic or inorganic selenium sources combined with Vitamin E on growth performance, and plasma and tissue glutathione peroxidase activity in broilers.

MATERIALS AND METHODS

Diets and Experimental Design

One hundred and sixty eight, one-day-old female commercial broiler chicks (Hubbard strain) were used in this study. The chicks were randomly separated to one control and three treatment groups, and each group consisted of three replicates of 14 chicks in each. All groups were fed broiler starter diets from day 1 to 21 and, grower diets from day 22 to 42. Feed and water were provided *ad libitum* throughout the experiment. The experimental period lasted 42 days.

Four experimental diets (iso-nitrogenous, 22.83 % crude protein and iso-caloric, 3102 Kcal ME/kg during the starter period; 21.02 % crude protein with 3209 kcal ME/kg during the grower period) were provided as follows: a. Basal diet without Vitamin E or selenium sources for the control group (control). b. Basal diet supplemented with 250 mg/kg Vitamin E (Kartal Chemistry[2]) for the treatment group 1 (Vit E) c. Basal diet supplemented with 250 mg/kg Vitamin E plus 0.2 mg/kg organic selenium (Sell-Plex; Alltech Inc.[1]) for the treatment group 2 (Vit E+Or Se) d. Basal diet supplemented with 250 mg/ kg Vitamin E plus 0.2 mg/kg inorganic selenium (Kartal Chemistry[2]) for the treatment group 3 (Vit E+Inor Se). The study was carried out in AYPI Incorporation[3]. The formulation of feeds and nutrients in the basal diet are presented in Table 1.

[1]: Alltech Türkiye, Hacılarkırı Cad. No:5 Naldöken, Bornova, İzmir / TÜRKİYE

[2]: GOSB Çıkışı, Mollafeneri yolu üzeri Gebze, Kocaeli / TÜRKİYE

[3]: Bafra-Sinop Karayolu 13. km Alaçam/Samsun/TÜRKİYE

Table 1. Feed ingredients and the chemical composition of basal diets (%)

Ingredient	**Starter Diet**	**Grower Diet**
Corn	54.60	57.25
Full fat soybean	17.00	17.00
Soybean meal	22.10	17.50
Poultry meal	2.00	2.30
Vegetable oil	1.60	3.00
Dicalcium phosphate	0.75	1.00
Limestone	1.20	1.35
Salt	0.25	0.25
Vitamin+Mineral premix*	0.25	0.25
Anticoccidial	0.10	0.10
DL-Methionine	0.15	-
Nutrients and metabolizable energy		
Dry matter	88,76	89,19
Crude protein	22,83	21,02
Calcium	0,77	0,89
Total phosphorus	0,56	0,59
Metabolizable energy for poultry, (kcal/ kg DM)	3 102	3 209

*Composition (per 1 kg of mixture): 6 000 000 IU Vitamin A, 2 000 000 Vitamin D3, 50 000 mg Vitamin E, 2 500 mg Vitamin K3, 1 500 mg Vitamin B1, 3 000 mg Vitamin B2, 25 000 mg Niacin, 7 500 mg Cal. D.Pantothenate, 3 000 mg Vitamin B6, 15 mg Vitamin B12, 750 mg folic acid, 75 mg D. Biotin, 62 500 mg choline chloride, 250 fyt Phytase, 50 000 mg Mn, 30 000 mg Fe, 30 000 mg Zn, 2 500 mg Cu, 100 mg Co, 500 I, 100 mg Selenium, 62 500 mg antioxidant.

Body Weight, Feed Consumption

During the experimental period, chicks were weighed individually every week to determine body weight (BW) and body weight gain (BWG). Feed consumption was recorded weekly and was calculated as, g per day per chick. Feed conversion ratio (FCR) was calculated as, feed consumption per day per chick (g) divided by body weight gain (g).

Chemical Analyses

All diets were analyzed for moisture, crude ash, crude fiber, ether extract, crude protein, calcium and total phosphorus, according to the Association of Official Analytical Chemists methods (2). The metabolizable energy levels of diets were calculated according to the Turkish Standards Institution (12)

Plasma and Tissue Analyses

Whole blood was collected by vena ulnaris, in heparinized tubes and plasma was separated by centrifugation at 4 °C and 3000 rpm for 10 minutes. Tissue samples were homogenized in 5 ml of cold GPx Assay Buffer per wet weight of tissues, and centrifuged mixture for 15 minutes at 4 °C and 8000 rpm according to total GSH-Px assay kit (OXItek, ZMC Catalog #: 0805002). Plasma and tissue specimen were stored at -82 °C for analyses. Glutathione peroxidase activity in plasma and tissues were measured by using a kinetic UV visible spectrophotometer (Shimadzu 1700). The oxidation of NADPH to $NADP^+$ was measured by a decrease in absorbance at 340 nm.

Statistical Analyses

All data were statistically analyzed, using the analysis of variance (ANOVA) by Least Square Method of the SAS GLM procedure (9) and the differences among the means of groups were determined by using Duncan's multiple-range test. All results were summarized as mean ± standard error of means (Stderr).

RESULT AND DISCUSSION

The effects of control, Vitamin E and organic or inorganic selenium source combined with Vitamin E, on body weight gain, feed consumption and feed conversion ratio have been presented in Table 2. Mean of final body weights for control, Vitamin E, organic or inorganic selenium source, combined with Vitamin E were, 1791.17, 1842.50, 1841.33 and 1796.67 g, respectively. There was no significant difference among groups for body weight; body weight was higher ($P>0.05$) in Vitamin E (Vit E) and treatment group 2 (Vit E+Or Se) than that of control and treatment group 3 (Vit E+Inor Se). Total body weight gain was higher ($P<0.05$) in the first and the second treatment groups compared to the other groups There were no differences among groups regarding total feed intake.

There were significant (P<0.05) differences for feed conversion ratio, between the organic or inorganic selenium group combined with Vitamin E. Swain et al. (11) reported broilers fed a diet excluding Vitamin E and selenium had the lowest body weight gain. Chicks fed diet supplemented with Vitamin E 150 and Vitamin E 300 IU/kg, had higher (P<0.05) body weight gain than the control and these results are similar to the present study.

Glutathione peroxidase activity in plasma and tissues has been presented in Table 3. Mean plasma glutathione peroxidase activity of the control, Vitamin E and the organic or inorganic selenium groups combined with Vitamin E were 27.66, 34.72, 43.47 and 43.28 µkat / L, respectively. Plasma glutathione peroxidase activity was higher (P<0.01) in treatment groups compared to the control. Glutathione peroxidase activity in kidney, muscle (P<0.05) and heart (P<0.01) were statistically important in treatment groups compared to the control.

Hassan et al. (3), reported chicks fed adequate levels of selenium and Vitamin E had higher (P<0.05) enzyme activity, compared to chicks fed diets without selenium or Vitamin E, which agrees with the present study. Glutathione peroxidase activity in kidney (P<0.05), femoral muscle (P<0.05) and heart (P<0.01) tissues were higher in treatment groups than control. Liver glutathione peroxidase activity was statistically important (P<0.01) in treatment group 2 (Vit E+Or Se) compared to the control and treatment group 2 (Vit E+Inor Se).

Surai (10) demonstrated the addition of 100 ppm Vitamin E and 0.4 ppm organic selenium to broiler diets caused a significant increase in the activity of GSH-Px in the chick liver at 10 days of age (40 U/g). Similarly, in another experiment , dietary inclusion of 0,5 ppm Se or its combination with 40-100 ppm Vitamin E augmented glutathione peroxidase activity in tissues, in agreement with the present study.

Diets for chicks were supplemented with 0, 0.05, 0.10 or 0.30 ppm Se from Sel-Plex with or without 100 IU Vitamin E per kg feed. Plasma GSH-Px increased in all of the treatments at 28 days, except for diet supplemented with only Vitamin E. There were no significant differences among treatments with the exception of control for glutathione peroxidase activity at 28 days of age (7), which was in agreement with the results of present study.

CONCLUSION

It is known that both the first and second antioxidant defence lines in the cells depend on the activity of GSH-Px in presence of adequate selenium. Lipid peroxidation induces destructive effects which can be lethal for cells in selenium deficiency. Results of the present study indicated that organic selenium combined with Vitamin E may be beneficial in broiler diets in increasing plasma and tissue glutathione peroxidase activity and improving animal health and productivity.

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Table 2. Means of the body weight gain, feed intake and feed:gain ratio by groups.

Days	Treatment groups				P
	Control	Vit E	Vit E+Or Se	Vit E+ Inor Se	
	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	
Feed intake (g/bird)					
0-21	790.52±13.29	777.31±12.96	784.96±8.68	816.60±14.13	NS
22-42	2298.06±83.68	2510.56±33.27	2307.07±29.10	2522.94±116.01	NS
0-42	3088.58±83.57	3287.87±37.84	3092.03±21.87	3339.54±129.94	NS
Body weight gain (g/bird)					
0-21	511.76±1.01	528.13±11.93	517.92±4.86	521.87±5.63	NS
22-42	1227.24±3.25	1270.77±5.03	1279.73±31.73	1229.67±12.78	NS
0-42	1739.00±3.99b	1798.90±13.53a	1797.65±27.91a	1751.53±9.56ab	*
Feed conversion ratio					
0-21	1.54±0.02	1.47±0.04	1.52±0.01	1.57±0.04	NS
22-42	1.87±0.07ab	1.98±0.03ab	1.80±0.06b	2.05±0.09a	*
0-42	1.78±0.05ab	1.83±0.03ab	1.72±0.03b	1.91±0.08a	*

NS: Non Significant *: P<0.05,; a, b ve c: Means within a row with different letter differ (P<0.05).

Table 3. The levels of glutathione peroxidase in plasma and tissues

		Treatment groups				
		Control	Vit E	Vit E+Or Se	Vit E+ Inor Se	
	n					P
Kidney	21	19.71±2.142b	27.71±2.142a	28.32±2.142a	27.44±2.195a	*
Liver	21	22.30±2.058c	36.23±2.058b	49.35±2.058a	38.56±2.109b	**
Leg muscle	21	12.62±1.603b	15.26±1.442a	18.46±1.478a	16.57±1.409a	*
Heart	21	16.13±2.190b	23.77±2.083a	27.46±2.315a	26.58±2.135a	**
Plasma	21	27.66±1.236c	34.72±1.236b	43.47±1.236a	43.28±1.236a	**

*: P<0.05; **: P<0.01; a, b, c: Means within a row with different letter differ (P<0.05)

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