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The Effects of Selenium and Vitamin C Supplementation on Lipid Peroxidation in Broilers Reared Cold Environment (15°C) and Diets of High Energy

Pınar TATLI SEVEN [1]
İsmail SEVEN [2]
Seval YILMAZ [3]
Bestami DALKILIÇ [1]

[1] Fırat Üniversitesi, Veteriner Fakültesi, Hayvan Besleme ve Beslenme Hastalıkları Anabilim Dalı, Elazığ, TÜRKİYE

[2] Fırat Üniversitesi, Sivrice Meslek Yüksekokulu, Elazığ, TÜRKİYE

[3] Fırat Üniversitesi, Veteriner Fakültesi, Biyokimya Anabilim Dalı, Elazığ, TÜRKİYE

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Yazışma Adresi
Correspondence

Pınar TATLI SEVEN
Fırat Üniversitesi,
Veteriner Fakültesi,
Hayvan Besleme ve
Beslenme Hastalıkları,
23119
Elazığ - TÜRKİYE

ptatli@firat.edu.tr

One hundred and twenty broilers (one-day old) were divided into 1 control and 3 experimental groups consisting 30 animals each. The experimental groups were as follow; Group I (control) was fed with basal diet, Group II was fed with high energy diet (3250 kcal/ kg as starter diet; 3300 kcal/kg as grower diet), Group III was fed with high energy diet supplemented with 1mg/kg Se as sodium selenite, Group IV was fed with high energy diet supplemented with 250 mg /kg Vit C as ascorbic acid. Plasma triiodothyronine and triglyceride levels were found significantly increased in group II compared to those of groups supplemented selenium and vitamin C. Malondialdehyde level was higher in liver ($P<0.01$) and abdominal fat ($P<0.05$) in control and group II in compared to those in other groups. The catalase (CAT) activity of liver was observed the highest in control and group II in compared to those in other groups. The CAT activity of abdominal fat was seen significantly higher in control than group III. Reduced glutathione activities for liver, abdomial fat and heart were not significantly different among all the groups. Results showed that cold exposure and diets of high energy induced oxidative damage in tissues, but this damage decreased partly with selenium and vitamin C supplementing to diet.

Key Words: *High energy, selenium, vitamin C, antioxidant enzymes, blood parameters, cold conditioning, broilers.*

Yüksek Enerjili Diyetler ve Soğuk Çevre Şartlarında (15 °C) Yetiştirilen Broylerlerde Selenyum ve Vitamin C Katkısının Lipit Peroksidasyon Üzerine Etkileri

120 broyler (1 günlük), her biri 30 hayvan içeren 1 kontrol ve 3 deneme grubuna ayrıldı. Deneme grupları şöyledir; temel diyet yedirilen Grup I (kontrol), yüksek enerji diyeti yedirilen (başlangıç diyeti olarak 3250 kcal/ kg; büyütme diyeti olarak 3300 kcal/kg) Grup II, yüksek enerjili diyete sodyum selenit olarak 1 mg/kg Se katılan Grup III, yüksek enerjili diyete askorbik asit olarak 250 mg /kg Vitamin C katılan Grup IV olarak belirlendi. Plazma triiodotiroidin ve trigliserit düzeyleri selenyum ve vitamin C katılan gruplarla karşılaştırıldığında grup II'de önemli oranda arttı. Karaciğer ve abdominal yağ malandialdehit aktiviteleri kontrol ve grup II'de daha yüksek ($P<0.01$) bulundu. En yüksek karaciğer katalaz (CAT) düzeyi kontrol ve grup II de bulundu. Abdominal yağın CAT düzeyi kontrol grubunda grup III' den önemli oranda daha yüksekti. Karaciğer, abdominal yağ ve kalp redükte glutatyon düzeyleri istatistiksel olarak önemli bulunmadı ($P>0.05$). Sonuçlar gösterdi ki soğuğa maruz kalma ve yüksek enerjili diyetler dokularda oksidatif hasarı indükledi, fakat bu hasar diyete selenyum ve vitamin C katkısıyla kısmen azaldı.

Anahtar Kelimeler: *Yüksek enerji, selenyum, vitamin C, antioksidan enzimler, kan parametreleri, soğuk şartlar, broyler.*

Introduction

Stress factors reduce the production along with increase the death rate in poultry (1, 2). Cold conditioning, a physical environmental stressor, has been shown to have variable modulator effects on cells of the immune system in animals (3). Despite the general awareness that energy demands are increased by cold and that the magnitude of those demands is moderated by total body insulation, few quantitative data exist relating environment, nutrient need, and productive efficiency (4). Low ambient temperature causes to increased feed intake and decreased performance in poultry (5, 6). A study indicated that animals acclimated to cold could be able to achieve a higher maximum sustained energy intake rate than non acclimated ones (7). Besides, the negative effects of cold conditioning might be lowered by antioxidants such as dietary selenium (Se) and vitamin C (Vit C) (8, 9).

Se is a type of trace mineral which supports healthy activity within your immune system, functions as an important part of the potent antioxidant glutathione. Se supplementation could be of great interest in protecting cells against oxidative stress (10). Vit C has been supplemented to diets of poultry reared under stress. It is known that Vit C are used for reducing the negative effects of environmental stress in the diets of poultry because of the reported benefits of Vit C supplementation on poultry reared under heat or cold stress (3, 11).

This study was planned to determine the effects of Se and Vit C supplemented on high energy diet and low enviromental temperature on blood parameters and antioxidant enzymes in broilers.

Materials and Methods

In this study, 120 broiler chicks (Ross 308) at one-day-old were used. Chicks were randomized into 3 experimental and a control group containing 30 birds in each. Treatment groups were comprised of 3 replicates of 10 birds. Corn and soybean meal-based diets were formulated according to the requirements of the National Research Council (12). Diets were formulated as starter and finisher (Table 1). The experimental groups were as follow; Group I (control) was fed with basal diet, Group II was fed with high energy diet (3250 kcal/kg as starter diet; 3300 kcal/kg as finisher diet), Group III was fed with high energy diet supplemented with 1mg/kg Se as sodium selenite, Group IV was fed with high energy diet supplemented with 250 mg/kg Vit C as ascorbic acid. The birds were fed with a starter diet until 28 d of age, then fed with finisher diet until 40 d. Diets and water were offered ad libitum. Chicks were warm-room reared at 33.20±2.50 ^{o}C during the first week and 26.20±1.85 ^{o}C for the second week. Starting at day 14 and continuing through week 6, birds were cold-stressed at an average room temperature of 15.10 ±1.98 ^{o}C. On days 40, 10 birds from each group were killed by cervical dislocation. Blood samples were collected from brachial vein. Liver, abdominal fat and heart tissue samples were taken immediately.

Table 1. Composition of the experimental diets, %

Ingredients	The Diets of Control Group		The Diets of Groups Fed with High Energy	
	Starter	Finisher	Starter	Finisher
Corn	56.50	60.81	54.93	58.63
Soybean meal	32.10	30.65	26.50	31.80
Fish meal	5.00	-	9.00	-
Soybean oil	3.00	5.00	6.60	6.60
Limestone	1.30	1.50	1.10	1.60
Dicalcium phosphate	1.00	0.95	0.80	0.30
L-Lysine hydrochloride	0.20	0.04	0.10	0.10
Vitamin-mineral premix[1]	0.35	0.50	0.50	0.50
DL- Methionin	0.30	0.30	0.22	0.22
Sodium chloride	0.25	0.25	0.25	0.25
Nutrient contents				
ME, kcal/kg[2]	3036	3190	3250	3300
CP, %[2]	22.40	19.20	22.40	19.20
Calcium[2]	1.00	0.90	1.00	0.80
Total phosphorus[2]	0.48	0.54	0.60	0.42
Selenium ppm (analysed)	42.20	39.65	44.10	39.20

[1]:Vitamin and mineral premix provided per kilogram of diet: vitamin A, 12.000 IU; cholecalciferol, 1.500 IU; vitamin E, 30 mg; vitamin K 3,5 mg; vitamin B1, 3 mg; vitamin B2, 6 mg; vitamin B6, 5 mg; vitamin B12, 30 μg; Ca-D- pantothenate, 10 mg; Folic acid, 0.75 mg; D-biotin, 0.08 mg; Mn, 80 mg; Zn, 60 mg; Fe, 40 mg; Cu, 5 mg; Se, 0.15 mg; Co, 0.1 mg; I, 0.4 mg.
[2]:Based on NRC (1994) feed composition tables.

The basal diet and high energy diet was analysed for Se (13). Triiodothyronine (T3) concentration was determined using commercially available radioimmunoassay kit (Byk-Sangtec Diagnostica, Dietzenbach-Germany; Immulite 2000, DPC, LA). Plasma biochemical parameters were measured using an auto analyzer (Olympus AU 600, Japan).

Plasma (14) and tissue (15) MDA concentration was measured. Catalase (CAT) level was estimated by measuring the breakdown of H_2O_2 at 240 nm (16). Tissue reduced glutation (GSH) concentration was measured by using the dithionitrobenzoic acid recycling (17). Hemoglobin concentration and tissue protein contents were determined according to Drabkin and Austin (18) and Lowry *et al.* (19), respectively.

Data collected were subjected to analysis of variance, and where significant differences were observed, means were further subjected to Duncan's multiple range test (20). The results were considered as significant when p values were less than 0.05.

Results and Discussion

According to our results, T3 level in group supplemented with Se was the lowest among groups (P<0.01) (Table 2). Tona *et al.* (21) reported that T3 hormone levels in standart broiler breeder lines were 3.84, 2.80 and 1.55 nmol/l at 14, 28 and 41 days, respectively. T3 level (1.72 nmol/l) in group supplemented Se at 40 days was in an aggrement with Tona *et al.* (21). Significant reduction in T3 levels of Se and Vit C groups in compared to control and high energy groups may be result from antioxidant effects of Se and Vit C (22, 11).

In our study, we found that plasma glucose, total protein, albumin and total cholesterol levels were not different among groups. However, plasma triglyceride in group supplemented with Se were significantly found higher than control and high energy group. A study indicated that the acute cold stress caused an increase in plasma triglyceride because of lipid peroxidation (23). The control and high energy group without Se and Vit C supplement may be increased lipid peroxidation, and this following, lipid peroxidation may be increased plasma triglyceride level (23). Cold conditioning induced a significant increase in MDA levels in liver and abdominal fat, thus, suggesting cold stress during this treatment (Table 3). Besides, in liver and abdominal fat, MDA level of the high energy group in compared to that of control group was similar, while its MDA level was increased in compared to those of Se and Vit C supplement groups. Alptekin *et al.* (24) showed that stress induces a significant increase in lipid peroxide levels in the liver and small intestine. McArdle and Jackson (25) have demonstrated a significant increase in free radical production together with an increase in the expression of antioxidant enzymes during a period of non-damaging exercise. These increases in antioxidant enzyme activities have been considered as a protective response against oxidative stress (26). Hydrogen peroxide, a precursor of more potent radical species, is scavenged at higher concentrations by CAT. In the present study, the CAT activity was increased significantly in liver and abdominal fat of broilers exposed to cold (Table 3), while it was similar in the heart. Kaushic and Kaur *et al.* (27) reported that the activities of antioxidant enzymes such as CAT and glutation peroxidase may change according to different tissues. They reported that the CAT activity was increased significantly in the kidney of stressed animals, while it was decreased in the heart, liver and small intestine. In the liver and abdominal fat, hydrogen peroxide produced in response to stress is taken care of primarily by CAT, which is shown to be present in higher concentrations in these tissues. The decreased activities of CAT in the livers of groups supplemented with Se and Vit C and in the abdominal fat of group supplemented with Se indicate the highly reduced capacity to hydrogen peroxide produced in these tissues in response to cold conditioning (28). Factors such as housing in low temperature, rapid growth rates, high energy rations are known to influence the occurence of ascites in broilers (29). Previous studies reported that cold temperature is one of the most effective factors ascites. In this study, control and high energy group may have induced to ascites (29). The MDA levels of liver and abdominal fat, and CAT activities of liver in control group and high energy group without supplement significantly increased ($P<0.05$). This may be due to rising tend to ascites in these groups. Likewise, the previous study reported that lipid peroxidation were elevated in birds with ascites (8). Moreover, it was found that the MDA and CAT activities of groups supplemented with Se (group III) and Vit C (group IV) decreased. In this study, especially, Se supplemention on account of decreasing of lipid peroxidation found the more effective than Vit C supplemention as agreement with study of Stanley *et al.* (28). Because, Se levels in diet could be also be critical since Se has long been recognised to have antioxidant proporties due to its importance for glutathione peroxidase activity (9). In this study, GSH activities of liver, abdomial fat, lung and heart were not found statistically significant between all groups (Table 4). In a previous study indicated that GSH activities may be not significant statistically as related to factors caused and exposed period to oxidatif damage (11).

It was found similar effects on lipit peroxidation and antioxidant enzymes of control and diet with high energy. Lipid peroxidation and antioxidant enzyme activities (CAT) were significantly affected negatively to diets of control and group II in broilers exposed to cold conditioning. The supplementation to diet of Se and Vit C decreased significant these negative effects.

Table 2. Plasma triiodothyronine hormone (T3) levels and some biochemical parameters of in the study groups (n=10)

	Group I (Control)	Group II	Group III	Group IV	P
T3 hormone (nmol/l)	2.78^{ab}±0.47	3.68^{a}±0.22	1.72^{c}±0.38	2.19^{bc}±0.07	**
Triglyceride (mg/dl)	42.94±4.10^{ab}	51.40±6.12^{a}	31.66±4.97^{b}	38.25±3.25^{ab}	*
Glucose (mg/dl)	230.20±10.50	220.80±18.81	216.00±10.69	227.25±7.45	NS
Total Protein (g/dl)	2.75±0.10	2.52±0.15	2.56±0.29	2.72±0.17	NS
Albumin(g/dl)	1.55±0.04	1.38±0.08	1.40±0.17	1.45±0.08	NS
Total Cholesterol (mg/dl)	118.50±5.42	106.00±6.72	125.33±8.51	118.75±8.71	NS

NS: Non significant, *: P<0.05, **: P<0.01, a, b, c: Mean values with different superscripts within a row differ significantly

Table 3. MDA (nmol/mg protein) and CAT levels (k/g protein) of liver and abdominal fat in groups (n=10) (mean ± SE)

		Group I (Control)	Group II	Group III	Group IV	P
Plasma	MDA	7.32±0.29	6.03±0.54	7.48±0.48	6.08±0.56	NS
Liver		0.54±0.06^{a}	0.59±0.20^{a}	0.20±0.18^{b}	0.19±0.10^{b}	**
Abdominal Fat		4.54±0.28^{a}	4.20±0.57^{a}	3.95±0.38^{b}	2.97±0.10^{b}	*
Heart		0.92±0.08	0.78±0.05	0.83±0.17	0.82±0.06	NS
Liver	CAT	444.72±48.67^{a}	383.55±3.08^{a}	314.90±22.00^{b}	326.79±11.41^{b}	**
Abdominal Fat		113.60±27.15^{a}	84.00±17.72^{ab}	54.80±9.82^{b}	71.01±7.15^{ab}	*
Heart		48.30±13.35	33.17±6.46	25.28±6.63	36.50±5.75	NS

NS: Non significant, *: P<0.05, **: P<0.01, a, b: Mean values with different superscripts within a row differ significantly

Table 4. Reduced glutation (GSH) levels (k/g protein) of some tissues in the groups (n=10) (mean ± SE)

	Group I (Control)	Group II	Group III	Group IV	P
Liver	9.14±0.35	8.73±0.21	9.00±0.47	9.56±0.13	NS
Abdominal Fat	7.26±0.20	7.70±0.12	7.58±0.04	6.77±0.46	NS
Heart	4.52±0.58	4.20±0.16	3.50±0.11	3.61±0.42	NS

NS: Non significant

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