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RESEARCH ARTICLE

Responses of Broilers under Cold Conditioning (15ºC) to Dietary Triiodothyronine and Iodine Combined to Antioxidants (Selenium and Vitamin C)

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Summary

This experiment was conducted to determine the influence of dietary antioxidants (selenium (Se) and vitamin C), and triiodothyronine (T_3) to iodine on biochemical parameters, malondialdehyde (MDA), catalase (CAT) in broilers exposed cold. In the study, 150, one-day-old, broiler chicks (Ross 308) were used. Chicks were randomized into 1 control and 4 treated groups each containing 30 birds and each experimental group comprised 3 replicates of 10 birds. The experimental groups were as follow: control was fed with basal diet; group I was fed with basal diet supplemented with 1 mg/kg selenium as sodium selenite plus 2 mg/kg iodine as calcium iodate; group II was fed with basal diet supplemented 1 mg/kg selenium plus 1 mg/kg T_3; group III was fed with basal diet supplemented with 250 mg/kg vitamin C as ascorbic acid plus 1 mg/kg iodine; group IV was fed with basal diet supplemented with 250 mg/kg vitamin C plus 1 mg/kg T_3. Plasma T_3, triglyceride and SGPT were significantly different among the groups. MDA level in heart tissue of control group was found significantly higher than those of other groups ($P<0.01$). MDA levels in liver ($P<0.01$) and abdominal fat ($P<0.001$) tissues of control and group I were determined significantly higher than those of other groups, whereas MDA level in lung tissue was similar found in all groups. The CAT activity of liver of control and group I was found significantly the highest ($P<0.001$). Results showed that cold exposure in broilers induced oxidative damage in tissues, but this damage decreased partly in supplement groups, except group I.

Keywords: *Cold conditioning, Antioxidant enzymes, Iodine, Ascorbic acid, Selenium, Triiodothyronine, Broiler*

Soğuk Koşullar Altındaki (15ºC) Broylerlerin Diyetsel Antioksidanlarla (Selenyum ve Vitamin C) Kombine Edilen Triiodotiroidinin ve İyodine Tepkileri

Özet

Bu araştırma soğuğa maruz kalan broylerlerde diyetsel antioksidanların (selenyum (Se) ve vitamin C) ve triiodotiroidinin (T_3) biyokimyasal parametreler, malondialdehit (MDA) ve katalaz (CAT) üzerine etkilerini belirlemek için yapıldı. Çalışmada bir günlük yaşta 150 adet broyler civcivler (Ross 308) kullanıldı. Civcivler her biri 30 civcivden oluşan 1 kontrol ve 4 deneme grubuna tesadüfi olarak ayrıldı ve her deneme grubu 10 civcivden oluşan 3 tekerrür grubu ihtiva etti. Deneme grupları şöyledir; kontrol grubuna bazal diyet yedirildi, grup 1'e sodyum selenit olarak 1 mg/kg selenyum + kalsiyum iyodat olarak 2 mg/kg iyot katkılı basal diyet yedirildi; grup II'ye 1 mg/kg selenyum + 1 mg/kg T_3 katkılı bazal diyet yedirildi; grup III'e askorbik asit olarak 250 mg/kg vitamin C + 1 mg/kg iyot katkılı bazal diyet yedirildi; grup IV'e askorbik asit olarak 250 mg/kg vitamin C + 1 mg/kg T_3 katkılı bazal diyet yedirildi. Plazma T_3, trigliserit ve SGPT gruplar arasında önemli oranda farklıydı. Kontrol grubunun kalp dokusundaki MDA düzeyi ($P<0.01$) diğer gruplarınkinden önemli oranda yüksek bulundu ($P<0.01$). Akciğer dokusunda MDA düzeyi tüm gruplarda benzer bulunurken, kontrol ve grup I'in karaciğer ($P<0.01$) ve kalp ($P<0.001$) dokularında MDA düzeyi diğer gruplarınkinden önemli oranda yüksek tespit edildi. Kontrol ve grup I'in karaciğer CAT aktivitesi önemli oranda en yüksek bulundu ($P<0.001$). Sonuçlar gösterdi ki, broylerlerde soğuk maruziyeti dokularda oksidatif hasarı indükledi, ancak grup I dışındaki katkı gruplarında bu hasar kısmen azaldı.

Anahtar sözcükler: *Soğuk koşullar, Antioksidan enzimler, İyot, Askorbik asit, Selenyum, Triiodotiroidinin, Broyler*

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INTRODUCTION

Ambient temperature is an important factor in poultry breeding. The suitable temperature for poultry is between 16-25ºC [1,2]. Environmental stress declines the production and moreover it may increase the death rate in poultry [1,3].

Cold condition, a physical environmental stressor, has been shown to have variable modulator effects on cells of the immune system in animals. Animal exposure to cold environment is associated with increased serum triiodothyronine (T_3) levels, which is felt to be main factor responsible for the sustained phase of cold adaptation characterized by increased heat production. During such a phase, the heat is produced by nonshivering thermogenesis, a process involving an array of changes in metabolic activity of the whole organism [4]. High concentrations of thyroid hormones stimulate free radical formation in mitochondria by affecting oxygen metabolism [5].

Thyroid hormones act on mitochondria by regulating energy metabolism, and mitochondria are a major source of intracellular free radials. Data from in vivo and in vitro studies indicate that thyroid hormones have a considerable impact on oxidative stress. Thyroid hormones are unique in that they require the trace element iodine (I) for biological activity [6]. Selenium (Se) is an essential trace element that regulates a major component of the antioxidant defense mechanism in all living tissues [7]. Types I, II, and III iodothyronine monodeiodinases are now known to be selenoenzymes. Vitamin C supplementation leads to strengthening of the antioxidative defence and consequent lowering of the degree of oxidative stress [8]. In thyroid disorders, it should been taken supplements containing molecules with antioxidant properties, such as vitamin C, Vitamin E, and coenzyme Q10. Especially vitamin C is very important. The play an important role in bringing about an improvement in the general functioning of the individual's immune system along with the functioning of the thyroid gland itself [9]. In addition, several works revealed a beneficial effect of Vitamin C supplementation in stressed-laying hens and broilers [6,10,11].

The present study was conducted by using triiodothyronine (T_3) hormone, Se, I and vitamin C combinations on biochemical parameters and antioxidant enzyme activities of broilers exposed to cold at 15ºC.

MATERIAL and METHODS

The experiment was in accordance with animal welfare, and was conducted under protocols by the Veterinary Faculty in Elazig-Turkey. In this study, 150 one-day-old, broiler chicks (Ross 308) were used. Chicks were randomized into 1 control and 4 experimental groups, each containing 30 birds and each treatment group comprised of 3 replicates of 10 birds. Corn and soybean meal-based feeds were formulated according to the requirements suggested by National Research Council [12]. Diets were formulated as starter, grower and finisher diets *(Table I)*. The experimental groups were as follows; Group I (control) was fed with basal diet, Group II (Se+I) was fed with basal diet supplemented with 1 mg/kg Se as sodium selenite plus 2 mg/kg I as calcium iodate, Group III (Se + T_3) was supplemented with 1 mg/kg Se plus 1 mg/kg T_3 (Sigma, 2877), Group IV (Vitamin C + I) was supplemented with 250 mg/kg Vitamin C as ascorbic acid plus 1 mg/kg I, Group V (Vitamin C+T3) was diet supplemented with 250 mg/kg Vitamin C plus 1 mg/kg T_3. Small amounts of the basal diet were first mixed with the respective amounts of Se, I, Vitamin C and T_3 as a small batch, then with a larger amount of the basal diet so that the total amount of the respective diets was homogeneously mixed. This process was

Table 1. *Composition of the basal diet, %*
Tablo 1. *Temel diyetin bileşimi, %*

Ingredients	Starter	Grower	Finisher
Corn	56.00	57.03	60.81
Soybean meal	31.70	29.00	30.65
Fish meal	6.50	6.00	-
Soybean oil	3.50	5.00	5.00
Limestone	1.00	1.50	1.60
Dicalcium phosphate	0.20	0.55	0.95
L-Lysine hydrochloride	0.20	0.10	0.04
Vitamin-mineral premix [1]	0.35	0.35	0.50
DL- Methionin	0.30	0.22	0.20
Sodium chloride	0.25	0.25	0.25
Calculated nutrient content [2]			
ME, kcal/kg	3096	3188	3194
CP, %	23.20	21.70	18.8
Calcium, %	1.00	1.02	0.95
Total phosphorus,%	0.56	0.59	0.54
Selenium ppm (analysed)	0.20	0.20	0.19
Iodine, ppm (analysed)	0.45	0.45	0.41

[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 B_1, 3 mg; Vitamin B_2, 6 mg; Vitamin B_6, 5 mg; Vitamin B_{12}, 30 mg; 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

applied to each prepared diet (starter, grower and finisher diets). The birds were initially fed a starter diet until 21 day of age, then a grower diet until 35 day and a finishing diet from 35 day to 40 day. The diets and fresh water were provided ad libitum. Chicks were reared in a warm-room, at 32±1.65ºC in the first week and at 25±1.96ºC in the second week. The birds were exposed to cold from 14 day to the end of 6th week at an average room temperature of 15±2.10ºC. On 40 day, 10 birds in each group were killed by cervical dislocation, taken blood and liver, abdominal fat, lung and heart organ samples. The broiler in control and experimental groups were reared under the same environmental conditions. The basal diet was analysed for Se [13] and I [14]. Triiodothyronine 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 MDA concentration, the end product of lipid peroxidation were measured according to the method of Satoh [15]. MDA contents of tissue homogenates were assayed spectrophotometrically according to the method of Ohkawa et al.[16]. CAT activity was estimated by measuring the breakdown of H_2O_2 at 240 nm according to the method of Aebi [17].

Statistical methods

All values were presented as means±SEM Differences between group means were calculated by a one-way analysis of variance (ANOVA) [18] and post-hoc Duncan test using used the SPSS/PC computer program (version 12.0). Results were considered statistically significant when P<0.05 [19].

RESULTS

In this study, plasma T_3 hormone, glucose, total protein, albumin, total cholesterol, triglyceride and SGPT levels were presented in *Table 2*. MDA levels of plasma and some tissues were showed in *Table* 3. MDA level in heart tissue of control group was found significantly higher than those of other groups (P<0.01).

Table 2. *Plasma T_3 hormone levels and some biochemical parameters of the study groups (n=10)*
Tablo 2. *Araştırma gruplarının plazma T_3 hormon düzeyleri ve bazı biyokimyasal parametreler (n=10)*

Biochemical parameters	Control	Se + I	Se + T_3	Vitamin C + I	Vitamin C + T_3	P
T_3 hormone (nmol/l)	2.71±0.12 ^{a}	2.78±0.27 ^{a}	2.08±014 ^{ab}	1.84±0.18 ^{b}	2.30±0.32 ^{ab}	**
Glucose (mg/dl)	232.66±8.95	203.05±44.34	235.25±9.32	247.00±18.28	260.03±13.11	NS
Total Protein (g/dl)	2.87±0.12	3.25±1.29	2.87±0.06	2.55±0.21	2.72±0.17	NS
Albumin (g/dl)	1.56±0.06	1.52±0.12	1.57±0.06	1.40±0.10	1.47±0.09	NS
Total Cholesterol (mg/dl)	118.66±6.96	110.00±5.33	127.75±7.77	148.50±16.17	115.75±2.02	NS
Triglyceride (mg/dl)	43.33±4.63 ^{b}	67.75±4.21 ^{a}	35.50±2.72 ^{b}	36.75±5.08 ^{b}	35.50±2.28 ^{b}	***
SGPT (IU/l)	4.66±0.66 ^{a}	2.25±0.25 ^{b}	2.00±0.57 ^{b}	2.25±0.94 ^{b}	1.50±0.29 ^{b}	*

*NS: Non significant, *: P<0.05, **: P<0.01, ***: P<0.001, a,b: Mean values with different superscripts within a row differ significantly*

Table 3. *MDA levels of plasma (nmol/ml) and some tissues (nmol/mg protein) of the study groups (n=10)*
Tablo 3. *Araştırma gruplarının plazma (nmol/ml) ve bazı dokuların (nmol/mg protein) MDA düzeyleri (n=10)*

Plasma and tissues	Control	Se + I	Se + T_3	Vitamin C + I	Vitamin C + T_3	P
Plasma	7.08±0.30 ^{a}	8.08±0.41 ^{a}	6.95±0.40 ^{ab}	6.75±0.35 ^{ab}	5.52±0.74 ^{b}	*
Liver	0.49±0.04 ^{a}	0.66±0.11 ^{a}	0.42±0.04 ^{b}	0.39±0.01 ^{b}	0.43±0.10 ^{b}	**
Abdominal Fat	5.38±0.38 ^{a}	6.20±0.63 ^{a}	2.25±0.15 ^{b}	3.20±0.25 ^{b}	2.48±0.29 ^{b}	***
Lung	0.24±0.02	0.25±0.01	0.22±0.01	0.27±0.02	0.20±0.01	NS
Heart	0.88±0.07 ^{a}	0.58±0.05 ^{b}	0.44±0.02 ^{b}	0.52±0.09 ^{b}	0.57±0.07 ^{b}	**

*NS: Non significant, *: P<0.05, **: P<0.01, ***: P<0.001, a,b: Mean values with different superscripts within a row differ significantly*

Table 4. *CAT activities (k/g protein) in some tissues of the study groups (n=10)*
Tablo 4. *Araştırma gruplarının bazı dokularda CAT aktiviteleri (k/g protein) (n=10)*

Tissues	Control	Se + I	Se + T_3	Vitamin C + I	Vitamin C + T_3	P
Liver	494.90±63.82 ^{a}	474.13±26.10 ^{a}	342.44±19.43 ^{b}	338.83±15.12 ^{b}	317.43±15.54 ^{b}	*
Abdominal fat	83.57±4.30	61.80±8.26	64.40±5.22	70.00±11.35	70.66±8.81	NS
Heart	29.80±5.18	25.44±2.14	24.21±4.16	24.37±3.01	24.45±5.78	NS

*NS: Non significant, *: P<0.001, a,b: Mean values with different superscripts within a row differ significantly*

MDA levels in liver (P<0.01) and abdominal fat (P<0.001) tissues of control and group I were determined significantly higher than those of other groups, whereas MDA level in lung tissue was similar found in all groups. CAT activities of some tissues were presented in *Table 4*. The CAT activity of liver of control and group I was found significantly the highest (P<0.001).

DISCUSSION

Thyroid function is known to be altered by many environmental factors, such as energy intake, dietary composition and ambient temperature. At subthermo-neutral temperatures the thyroid hormone-induced heat production would partly compensate for the need for extra heat production in the cold [18]. In addition to, it is known that the involvement of Se in the regulation of thyroid hormone metabolism underlies interactions between Se and I. Dietary vitamin C alleviated the negative effects of cold stress and decreased lipid peroxidation [19]. In this study, T_3 level in vitamin C+I group was the lowest among groups (P<0.01). T_3 supplementation groups did not affect plasma T_3 level. Tona et al.[20] reported that T_3 hormone levels in standart broiler breeder lines were 3.84, 2.80 and 1.55 nmol/l at 14, 28 and 41 days, respectively. T_3 level (1.84 nmol/l) in group supplemented vitamin C+I at 40 days was in an aggrement with Tona et al.[20]. Significant reduction in T_3 level of this group may be result from antioxidant effects of vitamin C and I [5,21]. In our results, dietary Se and cold conditioning increased plasma T_3 [22]. However, results showed that the nutritional interrelationships of Se and I are complex, and more through research is needed. We found that plasma glucose, total protein, albumin and total cholesterol levels were not different among groups. However, plasma triglyceride in Se+I group and plasma SGPT in control group were significantly higher. Martinello et al.[23] indicated that the acute cold stress caused an increase in plasma triglyceride. Plasma triglyceride level in Se+I group was significantly higher than other groups. Using accompaniment of Se and I may be caused lipid peroxidation. Because, insufficiency or excess of antioxidants like Se may cause lipid peroxidation [24]. Lipid peroxidation increased plasma triglyceride level [23]. Cold exposure induced lipid peroxidation and higher plasma triglyceride level by supplementing Se+I. In the present study, increased SGPT in control group is an agreement with Ruhl and Everhart [25].

In this study, a significant reduction in MDA level of liver, abdominal fat and heart were occurred in Se+T_3, Vitamin C+I and vitamin C+T_3 groups compared to control group *(Table 3)*. Kaushic and Kaur [26] reported that a significant increase in MDA levels of brain, heart, kidney, liver and small intestine was occurred in rats exposed to cold for 21 days. Exposure of growing chickens (21-day old) to temperature (12-14°C) has been reported to increase the plasma MDA. In addition, stress under low-temperature can distrupt the balance in oxidant/antioxidant system and cause oxidative damage to several tissues by altering the enzymatic and non-enzymatic antioxidant status, protein oxidation and lipid peroxidation. In this study, lipid peroxidation and antioxidan status were affected with cold [27]. Dietary supplements decreased to MDA levels in plasma and tissues. But, dietary Se+I supplementation increased MDA level in plasma, liver and abdominal fat but did not affect lung and heart tissues. Jianhua et al.[28] reported that dietary Se supplementation increased plasma T_3 concentration significantly. Rising of MDA could have been caused from increasing of T_3 in plasma in control and Se+I groups. In a previous study [18], different results were declared in respect to MDA activity of some tissues in hyperthyroid. They announced that [18] MDA concentration were unaltered by T_3 treatment in cold exposure in rat. Cold exposure of hyperthyroid rats induced different changes in MDA levels, depending on whether T_4 or T_3 was used for provoking hyperthyoidism. In this study, levels of MDA in plasma and tissues were generally decreased by the groups of T_3 treatment compared to control and Se+I groups. This may be due to thyroid hormone-induced heat production may partly compensate for the need for extra heat production in the cold [18]. Therefore, lipid peroxidation may be reducing in these groups.

CAT activity reduces hydrogen peroxide to water and oxygen, and it is found mainly in peroxisomes, and to a lesser extent in the cytosol and microsomal fraction of the cell [29]. Zamoner et al.[29] reported that hyperthyroidism increased CAT activity. In other words, thyroid secretion rate increased at low temperatures. Agreement with a previous study [29,30] , in this study, thyroid secretion rised due to cold conditioning and T_3 level in plasma was increased *(Table 1)*, and therefore CAT activitiy in the liver of control group higher than those of supplement groups (except of Se+I group) in our study *(Table 4)*. These results demonstrated that increasing of CAT activities in these groups caused by oxidative damage [26]. In the present study, low CAT activity in vitamin C+I groups might result from decreasing lipoperoxidation of

these two antioxidants [7,21]. Once more, low CAT activity of supplementary T_3 groups in liver may be due to lipid peroxidation in these groups [31]. Likewise, dietary T_3 supplementation reduced lipid peroxidation, which is arranging thyroid hormone rising resulted from cold conditioning [32]. We noticed that dietary T_3 decreased plasma T_3 when it was used especially with vitamin C. It can be said that these positive effects might have been resulted from restoring of thyroid hormone rising by T_3 and lowering lipid peroxidation by vitamin C supplementation. These results show that cold conditioning induced oxidative damage in tissues, but in supplement groups, except selenium plus iodine group, decreased tissue damage.

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