Kafkas Univ Vet Fak Derg
22 (6): 903-908, 2016
DOI: 10.9775/kvfd.2016.15618

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Effects of Thymoquinone on Cortisol Level, Blood Antioxidant Parameters and Capacity in Broiler Chickens under Oxidative Stress

Abdolhadi RASTAD [1] Ali Asghar SADEGHI [1]
Mohammad CHAMANI [1] Parvin SHAWRANG [2]

[1] Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran - IRAN
[2] Agricultural, Medical, and Industrial Research School, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran, Karaj - IRAN

Article Code: KVFD-2016-15618 Received: 09.03.2016 Accepted: 09.08.2016 Published Online: 10.08.2016

Abstract

This study was conducted to evaluate the effect of graded doses of thymoquinone (TQ) in broiler chickens exposed to oxidative stress on performance, antioxidant capacity and blood biochemical parameters. A total of 320 one-day-old broiler chicks was used in a completely randomized design. Chicks were divided to two groups, 160 chicks exposed to oxidative stress induced by tert-BHP injection (ip, 0.02 mmol per kg body weight) three in week from week 3 of age and 160 chicks as non-stressed. In each group, thymoquinone injection (ip) at 4 doses of 0, 5, 8 and 11 mg per kg body weight (40 chicks for each dose) were done three in week from week 2 of age. Results showed that TQ at dose of 8 mg per kg body weight increased total body gain and feed intake, but at dose of 11 mg per kg body weight improved feed conversion ratio in non-stressed chickens ($P<0.05$). Administration of TQ significantly increased the enzyme levels of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and the levels of total antioxidant capacity (TAC), and decreased cortisol, enzyme activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) and malondialdehyde (MDA) in both non-stressed and stressed chickens. Based on the results of this study, thymoquinone administration at dose of 8 and 11 mg/kg body weight could ameliorate the adverse effect of oxidative stress on growth and enhance the blood antioxidant capacity and antioxidant enzyme activity.

Keywords: *Antioxidant enzyme, Broiler, Oxidative stress, Performance, Thymoquinone*

Oksidatif Stres Altındaki Broiler Tavuklarda Timokuinon Uygulamasının Kortizol Seviyesi, Kan Antioksidan Parametreleri ve Kapasitesi Üzerine Etkileri

Özet

Bu çalışma, farklı dozlardaki timokuinon (TQ) kullanımının oksidatif strese maruz bırakılan broiler tavuklardaki performans, antioksidan kapasite ve kan biyokimyasal parametreler üzerine etkilerini araştırmak amacıyla yürütüldü. Toplam 320 adet bir günlük broiler civcivler rastgele düzen sistemi içerisinde gruplandırıldı. Civcivler iki gruba ayrılarak 160 civciv haftada üç kez ve 3 haftalıktan itibaren olmak üzere tert-BHP enjeksiyonu (ip. olarak 0.02 mmol her bir kg vücut ağırlık) ile oksidatif strese maruz bırakıldı. Diğer 160 civcive stres uygulanmadı. Her bir grup için timokuinon enjeksiyonu ip. olarak 4 doz halinde (her bir kg vücut ağırlığa 0, 5, 8 ve 11 mg dozlarında - her bir doz için 40 civciv olacak şekilde) haftada üç kez ve 2 haftalıktan itibaren olmak üzere uygulandı. Elde edilen sonuçlar her bir vücut ağırlığa 8 mg dozundaki TQ uygulamasının total vücut ağırlık kazanımını ve yem tüketimini arttırırken her bir vücut ağırlığa 11 mg dozundaki TQ uygulamasının ise yem dönüşüm oranını stres oluşturulmayan civcivlerde geliştirdiğini gösterdi ($P<0.05$). TQ uygulanması hem stres uygulanan hem de uygulanmayan civcivlerde anlamlı derecede süperoksit dismutaz (SOD), katalaz (CAT) ve glutatyon peroksidaz (GPx) enzim seviyeleri ile total antioksidan kapasitesini (TAC) artırırken kortizol, alanin aminotransferaz (ALT), alkalin fosfataz (ALP) ve aspartat aminotransferaz (AST) enzim aktiviteleri ile malondialdehit (MDA) seviyesini düşürdü. Çalışmanın sonuçları, her bir kg vücut ağırlığa 8 ve 11 mg dozlarındaki timokuinon uygulamasının büyüme üzerinde oksidatif stresin olumsuz etkilerini azalttığını, kan antioksidan kapasitesini ve antioksidan enzim aktivitesini ise arttırdığı gösterdi.

Anahtar sözcükler: *Antioksidan enzim, Broiler, Oksidatif stres, Performans, Timokuinon*

İletişim (Correspondence)
+98 919 5579663
a.sadeghi@srbiau.ac.ir

INTRODUCTION

Oxidative stress is a condition caused by an imbalance between pro-oxidants and antioxidants. This imbalance is due to increased levels of reactive oxygen species, nitrogen species or decreased antioxidant defense system occurs [1]. An increase in reactive oxygen species causes lipids peroxidation and damage to proteins and then the vital cell functions which finally lead to poor performance in animals [2]. There are two enzymatic and non-enzymatic systems used to protect the body against oxidative damages [3]. Addition of antioxidant supplement to diet is a key non-enzymatic way to increase the antioxidant capacity of the animal body [4-6]. There are many different antioxidants in the plants which are classified in the category of the natural antioxidants and show the protective effects of oxidative stress [6]. Black seed (Nigella sativa) with the major active antioxidant component named thymoquinone (TQ) has been used as a natural antioxidant [4]. Supplementation of TQ in broiler diets showed the better performance, health and lower mortality rate [7]. The administration of TQ in rodents and other lab animals showed the anti-stress, anti-microbial, anti-tumor and immune system stimulant effects [4]. The effects of thymoquinone as an antioxidant on many biological parameters [2,4,8-10] in non-stressed condition have been studied, but its effect on growth and health parameters of broiler chicks in the stress condition remains also unclear. We hypothesized that thymoquinone is capable to increase blood antioxidant capacity and prevent the adverse effects of oxidative stress on performance and blood parameters in broiler chicks. Therefore, this experiment was carried out to evaluate the effects of TQ on performance, antioxidant capacity and blood biochemical parameters in non-stressed and stressed broiler chicks.

MATERIAL and METHODS

Chicks used in this study received human care and the experimental protocol was approved by the Research Committee of Islamic Azad University, Science and Research Branch (approval date: 17.05.2014; no: 18500).

Chemicals

Tert- butyl hydroperoxide (2-Methylpropane-2-peroxol, tert- BHP) was used to induce the oxidative stress. tert-BHP was purchased from Sigma–Aldrich Chemical Company (CAS Registry No. 75-91-2). Thymoquinone (2-isopropyl-5-methyl-1, 4- benzoquinone) was purchased from Sigma-Aldrich Chemical Company (CAS Registry No. 490-91-5). TQ diluted in dimethyl sulfoxide and pure sterile olive oil, and injected intraperitoneally.

Birds and Management

A total of 320 one-day-old broiler chicks (Ross 308) was used in a completely randomized design. Chicks were divided to two groups, 160 chicks exposed to oxidative stress induced by tert-BHP and 160 chicks as non-stressed. The birds belonging to oxidative stressed experimental groups intraperitoneally received 0.2 mmol tert-BHP per kg body weight (BW) intraperitoneally three in week from week 3 of age. A sham operation was conducted in birds belonging to non-stressed groups through injection of normal saline. Non-stressed or stressed chicks were divided to four subgroups with 40 chicks per each. Thymoquinone injected (ip) to subgroups at the levels of 0, 5, 8 and 11 mg per kg body weight three in week from week 2 of age. The study consisted of 8 treatments with 4 replicates of 10 birds per each. Treatments were as C, control; CTQ5, received TQ at 5 mg per kg BW; CTQ8, received TQ at 8 mg per kg BW; CTQ11, received TQ at 11 mg per kg BW; S, exposed to oxidative stress alone; STQ5, stressed chicks received TQ at 5 mg per kg BW; STQ8, stressed chicks received TQ at 8 mg per kg BW; and STQ11, stressed chicks received TQ at 5 mg per kg BW. The chicks were weighed and randomly assigned to experimental units. The rearing temperature was 32°C in the first week rearing to 21°C at the end of the trial. The birds were kept under a 23 h light: 1 h dark and had a free access to fresh water. Body weight and feed consumption were measured and feed conversion ratio (FCR) calculated for overall period.

Blood Sampling and Measurements

On days 28 and 42, 2 chicks randomly selected from each replicate and blood samples were taken from wing's vein and then the blood serum was separated with centrifuge at 2000 × g for 15 min and stored at -20°C. Individual serum samples were analyzed for aspartate aminotransferase (AST), alkaline aminotransferase (ALT), alkaline phosphatase (ALP), catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and Plasma malondialdehyde level was determined using commercial kits (Pars Azmoon, Tehran, Iran) based on method described by Dropper et al.[11]. Antioxidant capacity was measured by Koracevic method [12] using an automated analyzer (Technicon RA-1000, Tarrytown, USA). Serum cortisol level was measured by EIA kit (Mono Bind Co., CA, USA).

Statistical Analysis

Statistical analyses were conducted with the general linear model procedure of SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC) to determine if variables differed between groups. The Kolmogorov-Smirnov test was used to test the normal distribution of the data before statistical analysis was performed. The data were compared between groups by Duncan test. Probability values of less than 0.05 ($P < 0.05$) were considered significant.

RESULTS

Total antioxidant capacity of broiler chickens was shown in *Table 1*. Chickens in CTQ8 and CTQ11 had the highest antioxidant capacities in both days 28 and 42 of

age. TQ increased total antioxidant capacity of serum in non-stressed chicks compared to control group, also in stressed chicks TQ increased this capacity as compared with S group, especially at level of 11 mg per kg BW.

Liver enzymes appeared in serum and MDA level of chicks at days 28 and 42 of age are presented in *Table 2* and *3*. Chicks in S group had the highest and those in CTQ8 and CTQ11 had the lowest levels of ALT, AST, ALP and MDA in both measurement days. In overall, administration of TQ resulted in decrease of liver enzyme and MDA level of serum in non-stressed chicks compared with the control group. Also, TQ decreased these measurements in stressed groups as compared with chicks in S group. The effect of TQ in decrease of ALT, AST, ALP and MDA levels in the serum was lower in stressed compared to non-stressed conditions.

Enzyme activities of SOD, GPx and CAT are presented in *Tables 4* and *Table 5*. Chicks in CTQ8 and CTQ11 had the highest and chicks in S and STQ5 had the lowest enzyme activity of SOD, GPx and CAT in 28 and 42 of age. TQ administration resulted in increase of these enzyme activity in non-stressed and stressed chicks; however, its effect on enzyme activity in non-stressed was higher than stressed conditions.

Table 1. *The effect of Thymoquinone on serum total antioxidant capacity of broilers (mmol per liter)*
Tablo 1. *Timukuinon uygulamasının broiler civcivlerde serum total antioksidan kapasite üzerine etkileri (mmol/L)*

Control Day	Treatments							
	C	CTQ5	CTQ8	CTQ11	S	STQ5	STQ8	STQ11
Day 28	0.64^{bc}	0.83^{b}	1.24^{a}	1.22^{a}	0.32^{d}	0.48^{cd}	0.51^{cd}	0.67^{bc}
Day 42	0.56^{c}	0.79^{b}	1.26^{a}	1.24^{a}	0.41^{d}	0.52^{c}	0.53^{c}	0.71^{b}

Means within columns with different superscript differ significantly (P<0.05); ***C,*** *control;* ***CTQ5,*** *received TQ at 5 mg per kg BW;* ***CTQ8,*** *received TQ at 8 mg per kg BW;* ***CTQ11,*** *received TQ at 11 mg per kg BW;* ***S,*** *exposed to oxidative stress alone;* ***STQ5,*** *stressed chicks received TQ at 5 mg per kg BW;* ***STQ8,*** *stressed chicks received TQ at 8 mg per kg BW; and* ***STQ11,*** *stressed chicks received TQ at 5 mg per kg BW*

Table 2. *The effect of thymoquinone on level of liver enzymes and MDA in serum of broilers at day 28 of age*
Tablo 2. *Timukuinon uygulamasının 28 günlük broiler civcivlerde karaciğer enzim seviyesi ve serum MDA üzerine etkileri*

Treatments	ALT (U/L)	ALP (U/100 mL)	AST (U/L)	MDA (mol/L)
C	9.42±1.14^{d}	758.51±27^{f}	39.11±3.79^{e}	4.11±0.37^{e}
CTQ5	8.26±1.20^{f}	749.95±23^{f}	38.72±3.17^{e}	3.64±0.29^{f}
CTQ8	7.25±1.39^{g}	684.94±24^{f}	33.40±3.52^{f}	3.32±0.33^{g}
CTQ11	8.83±1.18^{e}	928.84±26^{e}	31.56±3.66^{g}	3.28±0.3
S	17.11±1.36^{a}	2451.26±36^{a}	74.28±5.91^{a}	6.89±0.56^{a}
STQ5	12.15±0.97^{c}	2289.13±43^{b}	61.35±5.27^{b}	4.47±0.41^{c}
STQ8	13.91±1.39^{b}	2108.54±36^{c}	57.14±4.37^{c}	4.24±0.31^{d}
STQ11	13.85±1.41^{b}	1962.73±39^{d}	53.54±4.78^{d}	4.27±0.39^{d}

Means within columns with different superscript differ significantly (P<0.05); ***C,*** *control;* ***CTQ5,*** *received TQ at 5 mg per kg BW;* ***CTQ8,*** *received TQ at 8 mg per kg BW;* ***CTQ11,*** *received; TQ at 11 mg per kg BW;* ***S,*** *exposed to oxidative stress alone;* ***STQ5,*** *stressed chicks received TQ at 5 mg per kg; BW;* ***STQ8,*** *stressed chicks received TQ at 8 mg per kg BW; and* ***STQ11****, stressed chicks received TQ at 5 mg per kg BW*

Table 3. *The effect of thymoquinone on level of liver enzymes and MDA in serum of broilers at day 42 of age*
Tablo 3. *Timukuinon uygulamasının 42 günlük broiler civcivlerde karaciğer enzim seviyesi ve serum MDA üzerine etkileri*

Treatments	ALT (U/L)	ALP (U/100 mL)	AST (U/L)	MDA (mol/L)
C	12.41±1.06^{d}	1278.62±41^{e}	36.61±3.46^{f}	5.97±0.81^{d}
CTQ5	11.22±0.87^{e}	1224.08±38^{f}	37.45±3.52^{e}	5.66±0.74^{d}
CTQ8	10.13±1.21^{f}	1127.58±28^{g}	33.36±2.87^{g}	4.76±0.58^{e}
CTQ11	11.40±1.34^{e}	1005.77±34^{h}	32.95±3.13^{g}	3.71±0.92^{f}
S	16.71±1.33^{a}	2187.27±39^{a}	63.05±3.68^{a}	9.38±0.75^{a}
STQ5	11.53±0.98^{e}	1962.68±36^{b}	59.30±4.05^{b}	7.86±1.03^{b}
STQ8	13.64±1.18^{b}	1884.76±43^{c}	54.10±3.79^{c}	6.87±0.92^{c}
STQ11	13.26±1.27^{c}	1665.42±29^{d}	52.82±4.14^{d}	6.79±0.67^{c}

Means within columns with different superscript differ significantly (P<0.05); ***C,*** *control;* ***CTQ5,*** *received TQ at 5 mg per kg BW;* ***CTQ8,*** *received TQ at 8 mg per kg BW;* ***CTQ11,*** *received TQ at 11 mg per kg BW;* ***S,*** *exposed to oxidative stress alone;* ***STQ5,*** *stressed chicks received TQ at 5 mg per kg BW;* ***STQ8,*** *stressed chicks received TQ at 8 mg per kg BW; and* ***STQ11,*** *stressed chicks received TQ at 5 mg per kg BW*

Cortisol levels in the serum of chickens are presented in *Tables 4* and *Table 5*. There were differences for cortisol level among treatment at day 28 of age, but a significant difference was found between chicks in S group and other treatments at days 28 and 42 of age. The highest cortisol level in both days was for S group. Administration of TQ decreased serum cortisol level in stressed condition at days 28 and 42 of age.

Table 6 shows the effects of TQ on body weight gain, feed intake and feed conversion ratio of broiler chickens in overall period. The lowest body weight gain was for

Table 4. *The effect of thymoquinone on Cortisol and activities of antioxidant enzymes in serum of broilers at day 28 of age*
Tablo 4. *Timukuinon uygulamasının 28 günlük broiler civcivlerde kortizol ve antioksidan enzim aktiviteleri üzerine etkileri*

Treatments	Gpx (U/mL)	CAT (U/mL)	SOD (U/mL)	Cortisol (ng/mL)
C	476.83±17.5^{d}	13.15±1.24^{c}	114.34±5.64^{c}	1.52±0.06^{cd}
CTQ5	522.12±23.6^{c}	14.64±1.49^{b}	139.81±5.71^{b}	1.47±0.04^{d}
CTQ8	571.48±31.3^{b}	15.76±1.62^{a}	151.58±6.84^{a}	1.57±0.05^{cd}
CTQ11	596.39±29.4^{a}	15.65±1.34^{a}	144.62±4.94^{a}	1.43±0.06^{d}
S	363.26±28.8^{h}	6.17±0.62^{f}	76.28±4.19^{f}	3.16±0.05^{a}
STQ5	422.63±26.4^{g}	8.92±0.77^{e}	99.41±4.26^{g}	1.97±0.07^{b}
STQ8	438.78±30.3^{f}	9.56±1.07^{d}	113.28±5.53^{e}	1.77±0.04^{bc}
STQ11	458.35±24.1^{e}	9.84±0.86^{d}	107.24±4.76^{d}	1.63±0.07^{cd}

Means within columns with different superscript differ significantly (P<0.05); ***C,*** *control;* ***CTQ5,*** *received TQ at 5 mg per kg BW;* ***CTQ8,*** *received TQ at 8 mg per kg BW;* ***CTQ11,*** *received TQ at 11 mg per kg BW;* ***S,*** *exposed to oxidative stress alone;* ***STQ5,*** *stressed chicks received TQ at 5 mg per kg BW;* ***STQ8,*** *stressed chicks received TQ at 8 mg per kg BW; and* ***STQ11,*** *stressed chicks received TQ at 5 mg per kg BW*

Table 5. *The effect of thymoquinone on Cortisol and activities of antioxidant enzymes in serum of broilers at day 42 of age*
Tablo 5. *Timukuinon uygulamasının 42 günlük broiler civcivlerde kortizol ve antioksidan enzim aktiviteleri üzerine etkileri*

Treatments	Gpx (U/mL)	CAT (U/mL)	SOD (U/mL)	Cortisol (ng/mL)
C	428.57±19.4^{b}	13.87±1.35^{c}	110.24±6.32^{b}	1.55±0.04^{b}
CTQ5	442.18±21.6^{b}	14.92±1.52^{b}	135.42±5.15^{a}	1.42±0.07^{b}
CTQ8	483.74±18.7^{a}	16.76±1.11^{a}	147.27±5.24^{a}	1.51±0.04^{b}
CTQ11	478.36±23.4^{a}	16.84±1.27^{a}	139.36±4.86^{a}	1.39±0.03^{b}
S	291.44±27.6^{e}	9.63±1.77^{f}	85.70±5.55^{c}	2.64±0.06^{a}
STQ5	325.73±20.9^{d}	9.76±1.61^{f}	107.28±7.09^{b}	1.71±0.05^{b}
STQ8	353.72±18.7^{c}	10.28±1.74^{e}	119.13±6.31^{b}	1.64±0.04^{b}
STQ11	488.91±24.2^{b}	11.43±1.43^{e}	111.51±7.51^{b}	1.58±0.06^{b}

Means within columns with different superscript differ significantly (P<0.05); ***C,*** *control; CTQ5, received TQ at 5 mg per kg BW;* ***CTQ8,*** *received TQ at 8 mg per kg BW;* ***CTQ11,*** *received TQ at 11 mg per kg BW;* ***S,*** *exposed to oxidative stress alone;* ***STQ5,*** *stressed chicks received TQ at 5 mg per kg BW;* ***STQ8,*** *stressed chicks received TQ at 8 mg per kg BW; and* ***STQ11,*** *stressed chicks received TQ at 5 mg per kg BW*

Table 6. *Effects of thymoquinone on feed intake, daily weight gain and feed conversion ratio of broilers with and without oxidative stress*
Tablo 6. *Timokuinon uygulamasının oksidatif stres uygulanan ve uygulanmayan civcivlerde yem tüketimi, günlük ağırlık kazanımı ve yem dönüşüm oranı üzerine etkileri*

Treatments	Body Weight Gain	Feed Consumption	Feed Conversion Ratio
C	2320±41.2^{b}	4508±63.2^{b}	1.94±0.09^{ab}
CTQ5	2426±38.4^{ab}	4682±51.8^{a}	1.93±0.11^{ab}
CTQ8	2461±51.2^{a}	4702±48.9^{a}	1.91±0.06^{bc}
CTQ11	2388±62.9^{b}	4502±71.7^{b}	1.88±0.08^{c}
S	2165±52.2^{c}	4380±61.5^{c}	2.02±0.07^{a}
STQ5	2373±48.3^{ab}	4752±56.6^{a}	2.00±0.04^{a}
STQ8	2400±38.4^{ab}	4746±59.5^{a}	1.98±0.03^{ab}
STQ11	2327±54.8^{ab}	4539±67.3^{b}	1.95±0.08^{ab}

Means within columns with different superscript differ significantly (<0.05); ***C,*** *control;* ***CTQ5,*** *received TQ at 5 mg per kg BW;* ***CTQ8,*** *received TQ at 8 mg per kg BW;* ***CTQ11,*** *received TQ at 11 mg per kg BW;* ***S,*** *exposed to oxidative stress alone;* ***STQ5,*** *stressed chicks received TQ at 5 mg per kg BW;* ***STQ8,*** *stressed chicks received TQ at 8 mg per kg BW; and* ***STQ11,*** *stressed chicks received TQ at 5 mg per kg BW*

stressed chicks (S) and the highest was for CTQ8. Chicks in stressed groups received thymoquinone (STQ5, STQ8 and STQ11) had higher body weight gain than chicks in S group. Chickens exposed to oxidative stress had the lowest feed intake. Administration of TQ at doses of 5 and 8 mg/kg BW increased and at dose of 11 mg/kg BW had no significant effect on feed intake in non-stressed chicks. The lowest FCR was for chicks received 11 mg TQ per kg BW in non-stressed group. Chicks in S and STQ5 groups had the highest FCR. Administration of TQ at 5, 8 and 11 mg/kg BW in stressed chicks had no significant effect on FCR.

DISCUSSION

Body weight gain of chicks exposed to oxidative stress reduced compared to control and it reflects the effects of free radicals on chicken's body. Free radicals produced by t-BHP particularly peroxyl radical disrupt many body functions including inter- and intra-cellular enzymes and receptors by splitting the peptide chain, changing electric charges and increasing the sensitivity of proteins to proteolysis enzymes [13-16]. In addition, these products lead to membrane lipids peroxidation and affect to cell membrane and increase of cell membrane permeability and ion transfer from inappropriate places of cell surfaces and their mitochondria [14]. These events lead to wasting a lot of produced energy [20] and disruption of cellular function and body weight in chickens receiving tert-BHP. Most of the free radicals produced by oxidative stress play a secondary role to the occurrence of these events could lead to lower growth and feed intake and worse FCR.

Thymoquinone is a free radical scavenger that makes cleanup superoxide, hydroxyl radical and single oxygen molecule [9]. As seen in *Table 1*, thymoquinone increased total antioxidant capacity of serum. This means that TQ acts as free radical scavenger.

Thymoquinone crosses morpho-physiological barriers and its easy access to subcellular compartments could facilitate the radical scavenging effect [21]. Also, thymoquinone influence on intracellular signaling pathways and increased the secretion of insulin [22], catabolism of glucose and energy production in both conditions with and without received t-BHP [23-25]. These two properties, free radical scavenger and improvement in energy production, resulted in increased body weight gain and feed intake; as observed in our study.

At the time of stress, cortisol releases from the adrenal gland and protects the body against stress by increasing the gluconeogenesis and glucose production and the metabolism of fats, proteins and carbohydrates to produce energy and ultimately the protection of the body against stress [26]. Increase of cortisol level in S group shows the effects of free radicals to create oxidative stress. Differences in cortisol levels between S group and STQ groups are due to the antioxidant effect of TQ on the neutralization of a part of free radicals.

The level of AST, ALT, and ALP increased in S group. These enzymes called liver enzymes, because their levels are high in the liver cells and are low in other tissues. Damages to liver cell membranes as a result of oxidative stress disrupt their performance [18], and thus cause to release these three enzymes into the blood [13,27]. High levels of these enzymes in chickens of S group related to the free radicals come from tert-BHP. The neutralization of free radicals in the groups received TQ prevented the damages to liver cells. The destruction of liver cells is reduced by TQ as antioxidant and then a less amounts of these enzymes released into the blood.

The enzyme activities of glutathione peroxidase and catalase increased in both conditions with and without oxidative stress by increasing the level of TQ. There are a lot of findings [7,10,27] that showed free radicals are able to neutralize these two enzymes. Thus the loss of these enzyme activities in groups under oxidative stress is due to the injection of tert-BHP and the production of free radicals. TQ removed free radicals and thereby prevented its negative effect on the enzyme activities of glutathione peroxidase and catalase. A study [10] showed that TQ prevents the glycosylation of superoxide dismutase enzyme by reducing glucose level in the blood. Glycosylation of this enzyme reduces its activity; therefore, TQ can effects on pancreatic beta cells and reduces the glucose level which leads to increase the activity of SOD. This enzyme cooperates with glutathione peroxidase to create the first barrier to defense of the cells, thus the use of TQ can decreases free radicals and damage to the cells.

Based on the results of this study, in non-stressed condition 8 and 11 mg thymoquinone per kg body weight could enhance the antioxidant capacity and activity and performance of broiler chicks and in stressed condition ameliorate the adverse effect of oxidative stress on growth and enhance the blood antioxidant capacity and antioxidant enzyme activity.

Acknowledgement

The authors are grateful to the Islamic Azad University for research funding support. We also thank all staffs in the poultry unit, for the assistance in the care and feeding of chicks used in this research.

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