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Effects of Thymoquinone on Performance and Carcass Characteristics of Broiler Chickens under Oxidative Stress

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ABSTRACT: An experiment was conducted to determine the effect of thymoquinone under conditions with or without oxidative stress on the performance of broiler chickens. In this study 320 one-day-old broilers were randomly assigned to 8 groups, consisting of 4 replicates with 10 broilers. The treatments were assigned in a 4 × 2 factorial arrangement with 4 levels of thymoquinone (0, 5, 8 and 11 mg per kg of body weight) and 2 injection levels of tert-butyl-hydroperoxide (0 or 0.2 mmol/kg body weight). Body weight (BW), feed intake (FI), and feed conversion ratio (FCR) were measured at days 10, 21 and 42. At the end of the experiment, two birds from each pen were selected and slaughtered. Thymoquinone in amount of 8 mg per kg of body weight improved BW, FI and FCR, but under condition of oxidative stress only improved FCR (P<0.05). thymoquinone consumed in quantities of 5 and 11 mg per kg of body weight without oxidative stress and in quantity of 5 mg per kg of body weight increased the weight of gizzard (P<0.05) and injection of 5 mg of that under oxidative condition and 8 and 11 mg per kg of BW under both with and without oxidative stress increased liver weight (P<0.05). The use of thymoquinone in amount 8 mg per kg of body weight without oxidative stress and 11 mg per kg of body weight with oxidative stress increased abdominal fat than control group (P<0.05).

Key words: Thymoquinone, tert-BHP, Broiler, oxidative stress, Antioxidant

INTRODUCTION

Oxidative stress is a condition caused by an imbalance between the production of free radicals and antioxidant defense system. Primarily oxidative stress occurs due to a decreased activity of antioxidants such as gamma glutamyl-trans peptidase in the body (Uttara *et al.*, 2009). In this situation, the increase of reactive oxygen species causes malicious damage such as lipids peroxidation and damage to proteins and then the vital cell functions such as ion transports and movement of calcium which finally lead to poor performance of the body (Stark, 2005). There are two enzymatic and non-enzymatic systems used to protect the body against oxidative damages. Non enzymatic system consists of vitamin C, vitamin E, Coenzyme Q, glutathione, Ceruloplasmin, albumin and melatonin (Somogy, 2007), and enzymatic system consists of glutathione peroxidase, glutathione reductase, superoxidase dismutase (Kesavulu, 2000). There are many different antioxidants in the plants which are classified in the category of the natural antioxidants and show the effect of the preventing of oxidative stress. Carrots, tomatoes, garlic and black beans are a number of plants naturally contain a variety of antioxidants. These plants contain antioxidants such as vitamin E, vitamin C, saponins, alkaloids and carotene (Gupta and Sharma, 2006). *Nigella sativa* is an annual plant and a height of 40 cm, converted with tiny leaves. The seeds of this plant are black, angular and aromatic. The distribution of this plant is in Middle East, Iran and India. Medical history of this plant goes back to 2000 years ago. The medicinal properties of the seeds of this plant used to treat nasal congestion, toothache and intestinal worms. Seed of this plant contains 36 to 38 percent oil, significant amounts of proteins, alkaloids and saponins (melanin). Black seed extract has been used as a natural antioxidant to prevent oxidative stress. The major components of black seed extract are thymoquinone 28 to 57 percent and several alkaloids such as nijlicin, nijlidin and nijlimin-N- oxide. Among these components thymoquinone shows the highest antioxidant activity (Ahmad, 2013).

Broiler diets contain Black seed have shown the better performance and the better hematological and biochemical responses. It also reduced the mortality rate of these chickens (Khan, 2012). The use of different levels of Black seed showed anti-stress, anti-microbial, anti-tumor and immune system stimulant effects (Ahmad, 2013). The use of thymoquinone also decreased the production of eicosanoids and lipid peroxidation in rats (Houghton *et al.,* 1995). Heat stress is a major problem for growing broiler chickens in hot areas (Quinteiro-Filho *et al.,* 2012). Several studies have confirmed the positive effects of thymoquinone on rats and humans, so thymoquinone as a suitable antioxidant can prevents the negative effects of heat stress on broilers performance.

Tert-butyl-hydroperoxide (t-BHP) used to induce oxidative stress in several biological experiments. t-BHPis an organic peroxide. Since thymoquinone is the most effective antioxidant in the black seed, and its anti-oxidative effect has not been studied on broiler chickens and the experiments only have used several forms of black seed or its extract. This experiment was conducted to evaluate the effects of this material on body performance and carcass characteristics of broilers under oxidative stress.

MATERIAL AND METHODS

This experiment was conducted at the farm of Islamic Azad University of Darab, Fars, Iran. 320 one-old-day broiler chicks (Ros, 308) weighted on arrival and randomly assigned to 8 treatments and 4 replicates of 10 birds with factorial (4 × 2) arrangement were formulated in a completely randomized design. The study performed a 42- day trial with broilers housed on floor pens. Broiler chickens were fed by wheat-soybean based diets during rearing period. Food and water were supplied ad- libitum. Weighting of feed and chickens were made at days 10, 21 and 42 to evaluate body performance. At the end of experiment, two birds from each replicate of treatments were slaughtered for separation of carcass.

The composition of the experimental diets is given in Table 1. Treatments included: control group or group 1 (no thymoquinone and t-BHP), group 2 (5 mg per kg of body weight thymoquinone), group 3 (8 mg per kg of body weight thymoquinone), group 4 (11 mg per kg of body weight thymoquinone), group 5 (0 mg per kg of body weight thymoquinone + 0.2 mmol per kg of body weight t-BHP), group 6 (5 mg per kg of body weight thymoquinone + 0.2 ml per kg of body weight t-BHP), group 7 (8 mg per kg of body weight thymoquinone + 0.2 mmol per kg of body weight tert-butyl-hydroperoxide) and group 8 (11 mg per kg of body weight thymoquinone + 0.2 mmol per kg of body weight t-BHP)). t-BHP and TQ (2-isopropyl-5-methyl-1,4-benzoquinone) were purchased from Sigma-Aldrich. TQ diluted by 1mililiter of dimethyl sulfoxide and olive oil and daily injected intraperitoneally. Also t-BHP injected intraperitoneally at days 18, 21 and 24 in amount 0.2 mmol per kg of body weight. Antioxidant capacity was measured by novel method using dianisidinedihydrochloride (Erel, 2005).

Table 1: Composition of the basal diets.

Item	Starter (0 to 10 days)	Grower (11 to 21 days)	Finisher (22 to 42 days)
Ingredient, %			
Corn grain	60.50	55.86	63.45
Soybean meal (44% CP)	33.35	36.55	30.49
Di-Calcium phosphate	1.27	1.77	1.64
Salt	0.25	0.25	0.25
Vitamin premix	0.3	0.3	0.3
Mineral premix	0.3	0.3	0.3
DL-Met	0.77	0.43	0.36
Lys-HCl	0.00	0.00	0.00
Vegetable oil	2.27	3.61	2.49
Oyster shell	1.68	1.35	1.32
Calculated Composition			
ME, kcal/ kg	3010	3175	3225
CP, %	23	21	19
Ca, %	1	0.9	0.85
Available P, %	0.5	0.45	0.42
Met + Cys, %	0.81	0.78	0.69
Lys, %	1.16	1.05	0.88

Supplied the following per kilogram of diet: 11,025 IU of vitamin A; 3,528 IU of vitamin D3; 33 IU of vitamin E; 0.91 mg of vitamin K; 2 mg of thiamin; 8 mg of riboflavin; 55 mg of niacin; 18 mg of Capantothenate; 5 mg of vitamin B6; 0.221 mg of biotin; 1 mg of folic acid; 478 mg of choline; 28 µg of vitamin B12; 75 mg of zinc; 40 mg of iron; 64 mg of manganese; 10 mg of copper; 2 mg of iodine; and 0.3 mg of selenium; Available phosphorous.

Statistical analysis was performed using the software SAS (2000). Means were compared using Duncan`s multiple-range test and significance was determined at $p \leq 0.05$ (Duncan, 1955).

RESULTS

A. Feed intake (FI)

Table 2 shows the effects of thymoquinone on feed intake, body weight gain (BW) and feed conversion ratio (FCR) of broiler chickens in different periods. There were no significant differences between the various groups in terms of their three body parameters during days 0 to 10 ($p>0.05$).

Experimental group 8 consumed the lowest feed in two periods of days 11 to 21 and 22 to 42. The highest feed intake was observed at intervals of days 11 to 21 in group 6 and within days 22 to 42 in group 7. Between days 11 to 21, groups 8 and 5 have made significant differences with other groups ($p<0.05$), so that the lowest feed intake observed in group 8, and group 5 was after that.

B. Body weight (BW)

The use of 8 mg per kg of body weight thymoquinone during days 11 to 21 showed the greatest amount of weight gain. The most significant difference of weight gain in this period was observed between group 3 with respectively groups 5 and 8.

So the use of thymoquinone at the level of 8 mg per kg of body weight has prevented the negative effects of t-BHP on growth performance in this period while the use of thymoquinone at the level of 11 mg per kg of body weight did not decrease the negative effects of t-BHP. Significant differences between the experimental groups 3 and 4 ($p<0.05$) show that the use of 8 mg per kg of body weight thymoquinone has created the better effect than 11 mg. Significant differences between control group with groups 8 and 5 ($p<0.05$) also show the effects of t-BHP on body performance of group 5 and lack of proper implementation of thymoquinone to prevent the negative effects of t-BHP in group 8.

The comparison also between the means of body weight gain, shows the significant differences between all groups except control group with group 4 ($p<0.05$). Results show that respectively use of 8 and 5 mg per kg of body weight thymoquinone induce stimulant effect on body weight gain and thus increase the growth of these two groups than control group. Raising the amount of thymoquinone from 8 to 11 mg per kg of body weight caused the inverse performance. The best performance of body weight gain between days 22 and 42 was respectively observed in groups 3 and 2 and these groups showed significant differences with groups 1, 4, 8 and 5 respectively ($p<0.05$). During of this period, group 5 showed significantly less body weight gain than all other experimental groups ($p<0.05$).

Table 2: Effects of thymoquinone on feed intake, daily weight gain and feed conversion ratio of broilers with and without oxidative stress.

	Body weight gain			**Feed consumption**			**Feed conversion ratio**		
Treatments	**0-10**	**11-21**	**22-42**	**0-10**	**11-21**	**22-42**	**0-10**	**11-21**	**22-42**
Control (1)	290 ± 4.86	673.22^{ab} ± 9.21	1207.56^{b} ± 21.64	403.55 ± 8.24	1050.22^{a} ± 16	508.82^{c} ± 8.18	1.39 ± .05	1.56^{ab} ± .07	2.53^{bc} ± .04
2	285.41 ± 5.21	683.89^{a} ± 25.04	1307.52^{ab} ± 37.42	392.66 ± 7.91	1072.86^{a} ± 23	535.69^{b} ± 11.24	1.37 ± .03	1.56^{ab} ± .09	2.46^{bc} ± .07
3	287.65 ± 5.11	700.08^{a} ± 7.34	1324.29^{a} ± 25.61	392.20 ± 9.05	1052.44^{a} ± 20	543.84^{a} ± 6.83	1.36 ± .04	1.50^{ab} ± .04	2.46^{bc} ± .04
4	287.25 ± 5.07	652.77^{bc} ± 13.45	1198.37^{b} ± 33.73	402.15 ± 6.34	1056.63^{a} ± 17	467.18^{f} ± 9.72	1.40 ± .05	1.62^{a} ± .07	2.34^{c} ± .11
5	284.32 ± 4.62	637.28^{c} ± 10.74	1093.74^{c} ± 44.33	392.36 ± 9.27	980.12^{b} ± 21	500.63^{d} ± 12.11	1.38 ± .05	1.56^{ab} ± .08	2.75^{a} ± .10
6	286.42 ± 4.92	672.22^{ab} ± 27.68	1246.67^{ab} ± 40.86	398.12 ± 8.72	1075.84^{a} ± 14	545.99^{a} ± 8.08	1.39 ± .06	1.60^{a} ± .03	2.63^{ab} ± .09
7	283.50 ± 5.72	680.21^{ab} ± 8.94	1286.43^{ab} ± 36.97	392.22 ± 6.97	1073.86^{a} ± 22	546.043^{a} ± 10.63	1.38 ± .04	1.58^{a} ± .05	2.55^{ab} ± .08
8	281.76 ± 5.33	641.27^{c} ± 11.33	1199.24^{b} ± 22.57	391.98 ± 10.13	917.02^{c} ± 17	472.26^{e} ± 7.51	1.39 ± .07	1.43^{b} ± .07	2.36^{c} ± .13

Means with different superscripts in the same column are significantly different at $p<0.05$

Therefore the groups that respectively received thymoquinone at levels 8 and 5 mg per kg of body weight have shown the best performance in the absence of oxidative stress and also while creating oxidative stress by t-BHP, the most appropriate use of thymoquinone was respectively shown in groups 7 and 6 that show the greatest effects on inhibition effects of oxidative stress. Another important point in this period is the performance of experimental group 4 that is showed the worst result after group 5 so that even the body weight performance of groups 7 and 6, significantly ($p<0.05$) and body weight performance of groups control and 8, no significantly ($p>0.05$) increased than group 4.

C. Feed conversion ratio (FCR)

During days 11 to 21 of experimental period, the best FCR is obtained in group 8 that used thymoquinone in amount of 11 mg per kg body weight with t-BHP. This group has shown the significant differences among groups 4, 6 and 7 ($p<0.05$). Between the all experimental groups, respectively groups 2 and 3 in contrast to group 4 showed the better FCR performance than the control group.

These results show that the amount of the thymoquinone used in group 4 was too much to create the best appropriate FCR. The feed conversion ratio in the third stage of the experimental period (days 22-42) than in the second stage (days 11-21) shows completely different performance.

In this stage groups 4 and 8 have shown the best performance of FCR respectively with 2/34 and 2/36. Group 4 has the best FCR in this period. So the best FCR has occurred by use of 11 mg thymoquinone in both situations with and without oxidative stress. In this stage the experimental group 5 that received only tert-butyl-hydroperoxide has shown the worst FCR which has created significant effects with all other experimental groups ($p<0.05$). Between experimental groups received different levels of thymoquinone, only groups 6 and 7 have shown the worse FCR than control group while groups 2, 3, 4 and 8 have created better performance than control group but these differences were not statistically significant ($p>0.05$). In this period, the most significant differences were observed between group 5 with groups 4 and 8 ($p<0.05$) while there were no oxidative stress impose to birds of group 4, but the chickens of group 8 that used similar amount of thymoquinone were under oxidative stress. This result indicates the thymoquinone role to improving FCR in both conditions, with and without oxidative stress. The thymoquinone role against oxidative stress can also be seen from the comparison of experimental group 5 that received only t-BHP with other groups because group 5 has created the worst FCR. There were no significant differences between some average weights of carcass parts contain wings, breast, heart and neck ($p<0.05$) but the average weights of gizzard, spleen, liver and abdominal fat showed several significant differences ($p<0.05$).

Table 3: Effect of Thymoquinone on various slaughter traits of broilers with and without oxidative stress (g/kg pre-slaughter live body weight) (0 to 42 d of age).

Treatments	carcass	heart	gizzard	spleen	neck	breast	Abdominal fat	liver	wings	Thigh
1	72.71 ±4.56	0.40 ±.03	1.15^{ab} ±.15	0.058^{c} ±.02	5.34 ±.35	24.40 ±1.44	1.10^{b} ±.08	1.53^{b} ±.20	7.35 ±.42	22.75 ±.84
2	75.69 ±3.54	0.44 ±.09	1.26^{a} ±.26	0.080^{bc} ±.04	5.24 ±.42	26.69 ±1.29	1.69^{ab} ±.17	2.29^{a} ±.14	7.42 ±.43	23.14 ±.92
3	75.85 ±4.15	.440 ±.05	1.13^{ab} ±.22	0.122^{a} ±.03	5.31 ±.28	24.88 ±1.19	2.15^{a} ±.23	1.93^{b} ±.28	7.37 ±.38	23.13 ±1.03
4	74.39 ±5.01	0.41 ±.04	1.27^{a} ±.09	0.112^{ab} ±.03	5.12 ±.32	24.06 ±1.47	1.08^{b} ±.09	2.08^{ab} ±.30	7.43 ±.47	23.24 ±1.22
5	74.91 ±4.84	0.42 ±.06	1.12^{b} ±.14	0.103^{ab} ±.05	5.21 ±.27	25.28 ±1.77	1.15^{b} ±.19	1.91^{b} ±.18	7.45 ±.50	22.97 ±1.21
6	75.63 ±4.23	0.42 ±.07	1.24^{a} ±.23	0.100^{ab} ±.04	5.15 ±.39	24.73 ±1.66	1.19^{b} ±.11	2.15^{ab} ±.26	7.36 ±.36	22.86 ±1.08
7	72.31 ±3.64	0.41 ±.03	1.16^{ab} ±.18	0.101^{ab} ±.06	5.18 ±.44	26.04 ±1.34	1.27^{b} ±.14	2.58^{a} ±.34	7.37 ±.48	22.80 ±1.15
8	74.23 ±3.72	0.43 ±.08	1.01^{b} ±.11	0.101^{ab} ±.03	5.23 ±.38	25.37 ±1.73	1.96^{a} ±.18	2.30^{a} ±.23	7.42 ±.52	22.95 ±.94

Means within columns with no common superscript differ significantly ($P<0.05$).

The highest and lowest average weights of spleen respectively caused by the experimental groups 3 and control (1). There were two significant differences between experimental group 3 with respectively groups control and 2. The greatest average weights of gizzard respectively showed in groups 4 , 2 and 6 created significant differences with experimental group 8 ($p<0.05$). Groups 3 and 8 respectively show the highest average weight of abdominal fat relative to live body weight and they have created significant differences respectively with groups 4, control, 5 and 7 ($p<0.05$). Therefore the use of thymoquinone at the level of 11 mg per kg of body weight in the absence of t-BHP showed the greatest effects to prevent accumulation of abdominal fat, while the use of thymoquinone at all other levels, both with and without oxidative stress increased the percentage of abdominal fat than control group. Respectively experimental groups 7, 8 and 2 showed the highest average weight of liver tissue. There were significant differences between these three groups with respectively groups control (1), 3 and 5 ($p<0.05$). The control group created the lowest and group 7 in the presence of t-BHP by using of thymoquinone in amount of 8 mg per kg body weight created the highest proportion of liver weight ($p<0.05$).

DISCUSSION

The best body weight gained between days 22 to 42 showed in group 3 used only thymoquinone in amount 11 mg per kg of body weight and it was not under oxidative stress. Improvement of body Weight gained in this group shows the effects of free radicals on broilers under oxidative stress. Oxidative stress occurs when free radicals production is greater than antioxidant capacity to remove them (Uttara *et al.,* 2009). Since free radicals have at least one unpaired electron, they can combine with other substances in the body and impair their activities (Lobo, 2010). Also free radicals are able to combine with proteins and affect on their activities. Enzymes have protein structure and like other proteins are affected by free radicals (Rahman, 2007). On the other hand free radicals can attack to DNA and RNA into cells and indirectly influence on protein synthesis (Marcus *et al.,* 2003) and ultimately can affect on their body weight gain. Effects of free radicals on cell membrane lipids especially unsaturated fatty acids make cellular leakage (Guerzoni, 2001). Ion leakage that comes from inappropriate places of cells and mitochondria would be wasting a lot of energy (O'Rourke, 2007) and shows the negative effects on the cell functions and body weight performance. Thymoquinone effects on intracellular signaling pathway and increases the insulin secretion (Wessam and Wahab, 2013). Thymoquinone also increases the catabolism of glucose and energy production which ultimately lead to improve the growth (Passos and Von Zglinicki, 2006). The other things can help to improve the body weight, is the thymoquinone antibacterial activities that will lead to consume less energy by immune system. The use of black seed extracted from broiler diets has shown antibacterial, antifungal and improved body weight gain (Saeid and Mohamed, 2013). The best FCR observed in groups 3 and 8 in final period (d 22-42) which suggests thymoquinone by 11 mg per kg body weight is the best amount to make the best FCR in both situations with and without oxidative stress, but group 8 was involved with oxidative stress. Free radicals increase susceptibility of body proteins to proteolysis enzymes and in turn they affect on many protein enzymes, membrane transports, cell receptors and many other functions (Davies, 2006). Effects of free radicals on unsaturated fatty acids and produce lipid peroxidation also make up a series of reactions that produce other kinds of free radicals. Some of free radicals produced, have a secondary role to occurrence of oxidative stress, therefore, these events can be reduced body growth and thereby effective on FCR (Gavazza and Catala, 2006). Thymoquinone enhances the glucose and other nutrients absorption but an inverse function happened by overusing it. Excessive thymoquinone creates pancreatitis and hypocalcaemia which lead to reduction of feed intake and growth performance. Poisoning symptoms and hypocalcaemia was observed by excessive injection of thymoquinone in more than 50 percent of rats (Farah *et al.,* 2005).

Gizzard weight loss shows the effects of ROS on body protein production in chickens that had been under oxidative stress which probably due to a disruption of protein synthesis by ROS and also reduce the amount of energy production in the body. ROS impairs protein synthesis through the influence on DNA and RNA. Another reason can be expressed is ROS attack to cell membrane which leads to lipid peroxidation, impact on protein-protein and protein- lipid bonds and impairs the activity of intracellular organelles which ultimately reduces protein synthesis and energy production within the cells (Slimen *et al.,* 2014). The lack of oxygen can lead to slow down the kerbs cycle and reduce the energy production (Dean *et al.,* 2013). These reasons are also effective in reducing liver weight. So that the average weight of gizzard and liver had been less in group 5 than other groups.

The use of thymoquinone reduces the negative effects of oxidative stress and then increases the average weights of gizzard and liver in the groups that received thymoquinone and even the average weight of these organs (liver and gizzard) were increased in thymoquinone groups than control group.

There was an exception in this trend. That is the average weight of gizzard in group 8 that shows less average weight than groups 2, 4 and 6 which is caused by overuse of thymoquinone.

The use of thymoquinone at the level of 11 mg per kg of body weight increased abdominal fat store in both conditions with and without oxidative stress respectively in groups 3 and 8. Effects of excessive thymoquinone on lowering blood glucose lead to move the storage fats and increase the blood triglyceride and abdominal fat.

The use of thymoquinone increased spleen weight than control group in this experiment ($p<0.05$). Thymoquinone causes an increase in the systemic immune response. There were clearly increased differentiation of spleen cells, macrophage and NK anti-tumor activity while using *Nigella sativa*. The use of thymoquinone reduced the number of neutrophils and increased the number of lymphocytes and monocytes in rat. Lymphocytes migrate to spleen to full maturation and specialization. The cells migrate to spleen called transitional lymphocytes. Thymoquinone effects in increasing the number and proportion of lymphocytes, and their migration to the spleen increase the activity of its tissue and therefore its weight (Khan *et al.,* 2012).

CONCLUSIONS

The use of thymoquinone at the levels of 8 and 11 mg per kilogram of body weight in the presence and absence of oxidative stress improve body weight gain and feed conversion ratio. Level of 11 mg per kilogram of body weight thymoquinone leads to accumulating the abdominal fat and we can show that by the presence of oxidative stress it can lead to weight loss of gizzard. Thymoquinone is a powerful antioxidant and it can neutralize free radicals that lead to increase antioxidant capacity.

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