

The Effects of Turmeric, Cinnamon, Ginger and Garlic Powder Nutrition on Antioxidant Enzymes' Status and Hormones Involved in Energy Metabolism of Broilers during Heat Stress

Research Article

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Received on: 8 Oct 2016

Revised on: 10 Apr 2017

Accepted on: 15 Apr 2017

Online Published on: Mar 2018

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Online version is available on: www.ijas.ir

ABSTRACT

This experiment was carried out to determine the effects of turmeric, cinnamon, ginger and garlic powder on mixed sex Cobb-500 broilers performance, serum antioxidant status, and serum thyroid hormones. Heat stresses were created by increasing ambient temperature to 32-34 °C from 12: a.m. to 16: p.m. during days 31-42 of the experiment. Blood serum samples were obtained from 6 broilers of each treatment at the end of experiment (day 42) to assess antioxidative activities of liver enzymes and thyroid hormones. Chicks fed diets supplemented with medical plants tended to have higher average daily gain and had greater spleen weight compared to the control. Supplementation of medical plants increased activities of total superoxide dismutase (SOD) in cinnamon, glutathione peroxidase (GPX) in ginger, catalase (CAT) in cinnamon and garlic powder, total antioxidant capacity (TAC) in cinnamon, alkaline phosphatase (ALP) in garlic powder and corticosteroid (CE) in cinnamon groups. But, the supplementation reduced concentrations of malondialdehyde (MDA) in cinnamon group ($P < 0.05$). Concentration of thyroid hormones (THs) in medical plant-supplemented broiler chicks tended to be higher at 42 d of age compared to the control ($P < 0.05$). Supplementation of turmeric and ginger caused significant reduction in feed conversion ratio (FCR), improvement in performance, and enhancement in antioxidant enzyme activity with the increase in cinnamon consumption. Also, turmeric and ginger increased THs of broilers.

KEY WORDS ambient temperature, health, immune system, medical plant, oxidative stress.

INTRODUCTION

Heat stress (HS) caused great economic losses in the tropical areas. Heat stress can yield the reactive oxygen species (ROS) which can inflict oxidative damage such as lipid peroxidation and oxidative damage to proteins and DNA (Tan *et al.* 2010) and can be considered as a major factor adversely affecting the performance of birds (Zhang *et al.* 2009). In the event of HS, T3 and T4 hormones play an important role in the regulation of bird's body metabolism and temperature. Previous studies have shown that ambient temperature decreases THs in poultry (Tao *et al.* 2006).

Therefore, in order to deal with the problems caused by HS, the use of plant extracts as natural antioxidants has been considered (Zhang *et al.* 2009).

Wang *et al.* (2008) reported that medical plant extracts supplementation, as a natural antioxidant against stressors, improved broilers' performance and immune system. Previous studies have shown that fast-growing broiler strains are more sensitive to HS than slow-growing strains. Also, the birds are more sensitive to HS with continued breeding and selection for fast-growing (Tan *et al.* 2010). Although there are few reports on the effect of the medicinal plants on liver enzymes and THs involved in energy metabolism

in broiler chickens, more research seems to be necessary in this area. Therefore, the aim of the present study was to determine the effects of medicinal plants on performance, antioxidative enzyme activity, and serum THs levels in broilers during HS.

MATERIALS AND METHODS

Broiler chicks used in this study received human care on the basis of the criteria outlined in the Guide for Care and Use of Laboratory Animals (Clark *et al.* 1997). Also, the experimental protocol was approved by the Research Committee of Islamic Azad University, Science and Research Branch (Approval date: 05.05.2013; No: 23874). Three hundred males and females 31-day mixed sex Cobb-500 broiler chickens were distributed in a completely randomized design with 5 treatments each replicated 6 times (10 birds for each replicate). They were placed in cages (1.5×1.5 m) and were fed with starter (1-14 days) and grower (15-30 days) diets adjusted according to the Cobb-500 recommendation (Table 1). On day 31 of age, chicks were weighed and randomly were assigned to dietary treatments. The dietary treatments included A) control (basal diet), B) basal diet + 0.5% turmeric powder, C) basal diet + 0.5% cinnamon powder, D) basal diet + 0.5% ginger powder and E) basal diet + 0.5% garlic powder. During days 31-42 of the rearing period, the chicks were exposed to environmental temperature (32-34 °C) daily from 12: a.m. to 16: p.m. to induce heat stress. Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and rectal temperature (RT) of the chicks were measured on a daily basis between 08:00 to 10:00 o'clock. Rectal temperature was measured by clinical thermometer placed in broilers rectum for 30 s. The chicks were fixed throughout this measurement.

On day 42, serum samples of six chicks were used to determine the activity of liver antioxidant enzymes and serum THs levels. Blood samples were taken from the wing of the birds using a medical syringe. Superoxide dismutase (SOD) enzyme was measured by Flohé and Otting (1984) method in which cytochrome c reacts with the enzyme in competition with superoxide ion. Catalase (CAT) enzyme was measured by Aebi (1984) method in which activity was evaluated at the amount of hydrogen peroxide decomposition. Glutathione peroxidase activity was determined by Hopkins and Tudhope (1973) method in which t-butyl hydroperoxide was used as a substrate. The method for measuring the total radical-trapping capacity (to determine total antioxidant capacity, TAC) of biological fluids was the method introduced by Wayner *et al.* (1985). For spectrophotometric determination of MDA-TBA, an aliquot of plasma (50 µL) was transferred into a 5 mL tube followed

by successive additions of 5 ml 0.2% butylated hydroxytoluene (dissolved in ethanol) and 250 µL 15% aqueous trichloro acetic acid. The mixture was then centrifuged at 4000 g for 15 minutes at 4 °C. From that stock, an aliquot of 1 mL was transferred into a 5 mL tube and 250 µL TBA (0.375% in 0.25 M HCl) was added. It was then heated at 100 °C for 15 minutes. After cooling, the solution was analyzed by spectrophotometry using 1 cm absorption cell (Hitachi, Japan) at 535 nm. (Normal values: 3-4.5 µmol/L). Alkaline phosphatase and corticosteroid (CE) measured use of stated methods by Tietz (1986). The triiodothyronine (T3) and thyroxine (T4) concentrations were measured by radioimmunoassay Decuypere *et al.* (1994) and Hassanzadeh *et al.* (2004). On day 42, six broilers from each pen were slaughtered and weights of spleen and bursa of fabricius (BF) were measured by accurate digital scale. The statistical model employed in this study was:

$$Y_{ij} = \mu + A_i + e_{ij}$$

Where:

Y_{ij} : value of each observation.

μ : mean of the studied attribute.

A_i : effect of dietary treatment.

e_{ij} : impact of experimental error.

Data analysis was done using the general linear model (GLM) procedure of SAS software (SAS, 2001). After the analysis of the variance, the means were compared with Duncan multiple range test at significant level of 0.05.

RESULTS AND DISCUSSION

All broilers appeared healthy and no mortality occurred throughout the entire experimental period. Overall body weight (BW) of the birds was significantly affected by the addition of ginger. However, the addition of turmeric, cinnamon and garlic powder did not have significant effects on growth of broilers. Average daily gain of birds that fed with diets supplemented with turmeric and ginger were significantly higher than control, cinnamon and garlic powder birds during the experimental phase (Table 2). All birds had similar feed consumption and efficiency except those fed turmeric and ginger-supplemented diets that were significantly higher than the other treatments. However, birds in the cinnamon-supplemented group had higher FCR ($P < 0.05$). Broilers in turmeric-supplemented group had a higher 42-day-old RT than the control, and birds fed diet containing ginger had a significantly higher 42-day-old SW compared to the control group ($P < 0.05$). However, no significant differences were observed in BF weights of 42-day-old chicks in different dietary treatments (Table 2).

Table 1 Ingredients and chemical compositions of the experimental diets

Ingredients (%)	Control diet	Turmeric diet	Cinnamon diet	Ginger diet	Garlic diet
Corn (8.8% CP-3300 kcal ME)	38.73	38.73	38.73	38.73	38.73
Soybean meal (44% CP-2550 kcal ME)	28.40	28.40	28.40	28.40	28.40
Wheat grain	25.00	25.00	25.00	25.00	25.00
Soybean oil	3.18	3.18	3.18	3.18	3.18
Dicalcium phosphate	2.15	2.15	2.15	2.15	2.15
Limestone	0.86	0.86	0.86	0.86	0.86
Salt	0.34	0.34	0.34	0.34	0.34
Vitamins premix ¹	0.25	0.25	0.25	0.25	0.25
Mineral premix ²	0.25	0.25	0.25	0.25	0.25
DL-methionine 98%	0.14	0.14	0.14	0.14	0.14
L-lysine	0.20	0.20	0.20	0.20	0.20
Wheat bran	0.5	-	-	-	-
Turmeric powder	-	0.5	-	-	-
Cinnamon powder	-	-	0.5	-	-
Ginger powder	-	-	-	0.5	-
Garlic powder	-	-	-	-	0.5
Total percentage	100	100	100	100	100
Calculated composition (%)					
Dry matter	85.83	85.83	85.83	85.83	85.83
Metabolizable energy (ME) (kcal/g)	2.98	2.98	2.98	2.98	2.98
Crude protein (CP)	17.99	17.99	17.99	17.99	17.99
Crude fat	5.33	5.33	5.33	5.33	5.33
Crude Fiber	3.34	3.34	3.34	3.34	3.34
Calcium	0.89	0.89	0.89	0.89	0.89
Available phosphorus	0.44	0.44	0.44	0.44	0.44
Potassium	0.79	0.79	0.79	0.79	0.79
Chlorine	0.29	0.29	0.29	0.29	0.29
Sodium	0.15	0.15	0.15	0.15	0.15
Methionine	0.41	0.41	0.41	0.41	0.41
Lysine	1.09	1.09	1.09	1.09	1.09
Arginine	1.22	1.22	1.22	1.22	1.22
Met + Cys	0.74	0.74	0.74	0.74	0.74
Tryptophan	0.23	0.23	0.23	0.23	0.23
Tyrosine	0.81	0.81	0.81	0.81	0.81
Threonine	0.69	0.69	0.69	0.69	0.69

¹ Vitamin in 10 g of premix consisted of: vitamin A (500000 IU): 24.0 mg; vitamin D₃ (100000 IU): 6.0 mg; vitamin E (500 IU): 60.0 mg; vitamin K₃ (purity, 22.7%): 6.6 mg; vitamin B₁₂ (purity, 0.1%): 100.0 mg; Biotin (purity, 0.01%): 2000.0 mg; Choline chloride (purity, 50%): 1100.0 mg; Folic acid (purity, 90%): 1.1 mg; Nicotinic acid (purity, 100%): 65.2 mg; D-pantothenate (purity, 92%): 16.3 mg; vitamin B₆ (purity, 100%): 4.5 mg; Riboflavin (purity, 80%): 12.5 mg and vitamin B₁ (purity, 100%): 2.5 mg.

² Mineral in 10 g of premix consisted of: CuSO₄·5H₂O: 32.00 mg; FeSO₄·H₂O: 333.20 mg; MnO: 166.80 mg; Na₂SeO₃·5H₂O: 1.0 mg; ZnSO₄·H₂O: 220.00 mg; CoSO₄·7H₂O: 4.80 mg; KI: 0.56 mg; Ethoxyquin: 100.00 mg and Corn meal as carrier: 5742.94 mg.

Supplementation of cinnamon significantly increased activities of SOD, CAT, TAC and CE, but it reduced MDA content in the serum at the age of 42 d (Table 2). In contrast, activity of MDA in the serum of birds fed turmeric-supplemented diet was significantly increased at 42 d of age whereas CE ($P < 0.05$) was decreased. Ginger powder had more significant effect on GPX at 42 d of age. Serum of broilers in garlic powder-supplemented groups appeared to contain the highest ALP activity. Birds in cinnamon-supplemented groups had higher ($P < 0.05$) serum concentration of CE compared to the other.

The mean values of serum T3, T4, and T3/T4 levels were different among the medical plant-supplemented groups, but the differences were significant ($P < 0.05$). Supplementation of ginger powder significantly increased blood serum T3, T4 and T3/T4, so garlic powder increased T4 content in the serum ($P < 0.05$) at the ages of 42 d (Table 2).

In agreement with the results of this study, improvement of FCR and performance of broilers under high ambient temperatures via medical plant-supplementation have been reported by Zhang *et al.* (2009) and Wang *et al.* (2008).

Thus, the increase of broiler ADG and BW with turmeric and ginger supplement in this study could be due to the effects of biological compounds of medical plants on improving antioxidant status and the metabolism of protein and fat.

Medical plant-supplementation causes better absorption of nutrients in the diet and ultimately leads to higher BW, which causes improvements in the growth and FCR of broiler chickens (Windisch *et al.* 2008). Also, heat stress reduces feed intake which results in reduced secretion of digestive enzymes and ultimately reduces nutrient digestibility in broiler chickens.

Although digestibility and nutrient absorption were not examined in this study, many researchers have reported that medicinal herbs affect the broiler gastrointestinal tract, especially the small intestine, and increases the secretion of amylase and chymotrypsin and the absorption of intestinal villi. Thus, it increases the amount of nutrients and ultimately improves the performance (Pirmohammadi *et al.* 2016). The outgrown of spleen increased safety and more body struggle with toxins.

Table 2 Body weight, feed intake, feed conversion rate, rectal temperate, bursa of fabricius weight, spleen weight, liver enzymes, total antioxidant capacity, T3 and T4 hormones concentration of 42-day-old broilers exposed to HS fed diets with or without medical plants-supplementation

Treatments	A	B	C	D	E	SEM
BW (g)	1.543 ^b	1.516 ^b	1.636 ^b	1.836 ^a	1.480 ^b	0.0831
ADG (g)	63.6 ^b	73.6 ^a	61.5 ^b	70.3 ^a	64.6 ^b	0.0246
ADFI (g)	128 ^b	141 ^a	127 ^b	137 ^a	128 ^b	0.0039
FCR (g:g)	2.031 ^{ab}	1.914 ^d	2.067 ^a	1.951 ^{cd}	1.997 ^{bc}	0.0318
CRTM (°C)	41.466 ^b	42.033 ^a	41.313 ^b	41.633 ^{ab}	41.633 ^{ab}	0.2599
CBFWM (g)	2.492	2.400	2.764	3.288	2.497	0.6170
CSWM (g)	1.421 ^c	2.042 ^b	1.453 ^c	2.379 ^a	1.305 ^c	0.1182
SOD (%)	1.660 ^d	2.136 ^e	3.183 ^a	2.840 ^b	2.440 ^c	0.1670
GPX (%)	0.960 ^d	1.040 ^d	1.656 ^b	1.883 ^a	1.413 ^c	0.1050
CAT (%)	1.880 ^c	1.780 ^e	2.596 ^a	2.203 ^b	2.836 ^a	0.1491
MDA (%)	11.100 ^a	10.440 ^a	6.060 ^d	7.166 ^c	8.560 ^b	0.4835
TAC (%)	24.430 ^c	26.620 ^c	37.597 ^a	33.473 ^b	31.443 ^b	1.9402
ALP (%)	38.500 ^c	40.200 ^c	47.220 ^b	45.443 ^b	51.477 ^a	2.2450
CE (%)	1.960 ^c	2.840 ^d	4.466 ^a	3.676 ^b	3.336 ^c	0.1842
T3 (%)	2.360 ^c	2.890 ^{ab}	2.716 ^{bc}	3.163 ^a	2.480 ^{bc}	0.2369
T4 (%)	8.626 ^b	9.366 ^{ab}	9.400 ^{ab}	9.730 ^a	10.190 ^a	0.5445
T3/T4 (%)	0.273 ^{ab}	0.308 ^a	0.288 ^{ab}	0.327 ^a	0.244 ^b	0.0295

A: control; B: turmeric; C: cinnamon; D: ginger; E: garlic powder; BW: body weight; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio; CRTM: 42-day-old chickens rectal temperature mean; CBFWM: 42-day-old chickens bursa of fabricius weight; CSWM: 42-day-old chickens spleen weight; SOD: superoxide dismutase; GPX: glutathione peroxidase; CAT: catalase; MDA: malondialdehyde; TAC: total antioxidant capacity; ALP: alkaline phosphatase; CE: corticosteroid; T3: triiodothyronine and T4: thyroxine.

The means within the same column with at least one common letter, do not have significant difference ($P>0.05$).

SEM: standard error of the means.

In the current study, the use of medicinal plants increased spleen weight that reflects the positive effects of turmeric and ginger on the improvement of body safety.

High environmental temperature increases the risk of HS in poultry. In previous studies (Tan *et al.* 2009), increasing ambient temperature led to the significant increase of rectal temperature which is consistent with the results of the current study. These observations suggest that the high environmental temperature can cause significant HS and increasingly intensifies the HS with gradual increase in ambient temperature.

Zhang *et al.* (2009) reported that diet supplementation with ginger powder significantly increased total SOD and GPX activity, but reduced the contents of MDA in serum in the ages of 21 and 42 days. These results are consistent with the findings of the present study. More activity of SOD, GPX, CAT, TAC, ALP and CE in the serum of chickens fed with cinnamon, ginger and garlic powder-supplements compared with control treatment showed that this medical plants improve serum antioxidant activity. Antioxidative enzymes increased activity which means improvement in the capacity of chickens for inhibition of oxygen free radicals. It is proven that in the serum of chickens fed with ginger, SOD activity and GPX concentration increased while the concentration of MDA decreased (Zhang *et al.* 2009). Malondialdehyde is formed as an end product of lipid peroxidation and so, extent of lipid peroxidation by ROS can determined according to MDA levels (Sumida *et al.* 1989).

Decrease in MDA and increase in TAC indicate decreased oxidative stress or increase in total antioxidant defense. Accordingly, reduction in the level of MDA in treatments containing cinnamon, ginger and garlic powder compared to control, proved that lipid peroxidation reduced by consumption of medical plants and increasing the antioxidative activities. Besides, decrease in the concentration of MDA in serum can be related to increase in the activity of antioxidant enzymes because of the use of medicinal herbal supplement.

In support of the current study, the results of Zhang *et al.* (2009) experiment showed that medical plant-supplementation improve antioxidant statuses in broilers.

Also, Kota *et al.* (2008) observed that ginger-supplementation significantly improved SOD and GPX activity and reduced liver and kidney MDA in rat. These findings are consistent with the results of the present experiment.

Improvement in antioxidant status in chicks fed with medical plants-supplementation could be due to these plants' antioxidant compounds (Zhang *et al.* 2009). Tan *et al.* (2010) reported significant difference in serum and liver SOD activities among control and treatments exposed to HS after 3 h of applying high environmental temperature. Also, they stated that serum and liver CAT activities showed increase-trend dependent on temperature increase. They reported that treatments exposed to the highest temperature (38 °C) had significant differences with control. These findings confirm the results of this study.

These results show that high environmental temperature could be the cause of compensatory increases in antioxidative enzymes activities. High environmental temperature significantly stimulates the MDA production and this index is the increasing-trend that is dependent on the temperature. Significant increase in MDA content was observed in broilers exposed HS.

Therefore, it can be concluded that high temperature stimulates oxidative damage to lipids and proteins, and the extent of damage depends on the temperature (Tan *et al.* 2010).

Heat stress in birds happens with increasing ambient temperature above the comfort zone of thermal. In this case, physiological changes take place in acidity and blood metabolites. Reduction in the consumption of feed efficiency, weight loss, loss of defense and the immune system are the most vulnerable to heat stress damages. Heat stress, through the influence of the sympathetic nervous system releases catecholamine and, as a result, leads to the production of free radicals (ROS) in the blood and heart tissue cells. Free radicals damage cell membranes of the body by attacking the structure of unsaturated fats (Pirmohammadi *et al.* 2016). Mujahid *et al.* (2005) reported that acute heat stress is causes oxidative stress in broilers. High ambient temperature leads to oxidative stress and weak antioxidant defense system.

Increasing temperature improves many chemical and biochemical reactions. Therefore, there is a great possibility that increased body temperature causes the production of ROS by accelerating the metabolic reactions of cells and tissues. Also, respiratory chain complex activity was significantly blocked after applying HS in the range of 32-38 °C. As a result of this restriction, ROS production increased and led to unlimited oxidative damage (Tan *et al.* 2010). The activity of serum and liver basic antioxidant enzymes upregulated significantly with the increase in ambient temperature. The increase in antioxidant enzymes activities is considered as a protective response against oxidative stress (Thomas, 2000). Therefore, it has been shown that the balance can be disrupted by acute HS.

The intestinal isoenzyme makes the largest contribution to serum ALP activity in birds (Campbell and Coles, 1986). In contrast, the increases of serum ALP are most related to liver diseases, even though the level of ALP activity in this organ is low (Viveros *et al.* 2002). Quantification of ALP in medical plant-supplement diets may explain the source and the mode of action of these enzymes. The results of some studies show that salvia root-extract hampered liver fibrosis in mice and affected the changes in the level of ALP (Nan *et al.* 2001). Therapeutic effect of topical CE is justified based on their anti-inflammatory activity (Katzung *et al.* 2011), which their anti-inflammatory and anti-proliferation

effects are the reasons for their effectiveness (Hengge *et al.* 2006). Corticosteroids make the regulation of immunomodulatory (Hughes and Rustin, 1997).

Two picks have in steroid hormones secretion in the broiler chicks exposed HS and the event of pick time is influenced by the neutral thermal status (Tao *et al.* 2006). High temperature affects the amount of THs. The results of a study showed that daily average of T3 decreased significantly compared to first day, but T4 response was relatively less when broiler chickens were exposed to HS (Tao *et al.* 2006). It was shown that T4 concentration decreased after 1h of exposure at temperature of 35 °C, but T3 concentration did not change (Rudas and Pethes, 1984). Sinurat *et al.* (1987) proved that high temperature decreases T3 and increases T4 in plasma. A recent study (Tao *et al.* 2006) showed no significant differences in T3 and T4 levels at different times for each sample and their average for the entire period of the birds under thermo natural in broilers. These results are against the findings of the present study.

Tao *et al.* (2006) stated that there was no significant difference in the means of T3, T4 or T3/T4 in 3 days placement at HS, and daily means of T3 and T4 decreased by increasing ambient temperature. The findings of this study are in odds with the results of the current study. These differences with the present study could be due to differences in the environmental impact of different scenarios. Tao *et al.* (2006) presented that applying HS reduces T3 and T4 hormones. According to the results of this study, it can be stated that it can be used for medicinal herbal supplements to increase the amount of THs in terms of HS and improve safety.

CONCLUSION

In conclusion, supplementation of cinnamon powder at the level of 0.5% to diet tended to improve FCR of broilers. Inclusion of cinnamon in the diet also enhanced oxidative stability, increased SOD, CAT, TAC, and CE but lowered CE concentrations. Ginger supplementation increased serum T3, T4 and T3/T4 concentrations broiler chicks. The beneficial effects of cinnamon is related to the composition and medicinal characteristics and its antioxidant properties on the current experimental broiler chicks.

ACKNOWLEDGEMENT

The authors are grateful to the Atomic Energy Organization, Islamic Azad University and Tehran National Elites Foundation for research funding support. We also thank all staffs in the poultry unit in Nuclear Agricultural Research Institute located in Alborz Research Complex, for the assistance in the care and feeding of chicks used in this research.

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