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EFFECT OF ZINC ACETATE AND MAGNESIUM SULFATE DIETARY SUPPLEMENTATION ON BROILER THIGH MEAT COLOUR, NUTRIENT COMPOSITION AND LIPID PEROXIDATION VALUES UNDER CONTINUOUS HEAT STRESS CONDITION

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Abstract

Four hundred and fifty one-day-old male broiler chicks (Ross 308) in 9 groups of 50 each (3 × 3 factorial experiment) were randomly allocated to investigate the effects of different dietary levels of 0, 30 and 60 mg/kg Zinc (Zn) and 0, 300 and 600 mg/kg magnesium (Mg) on thigh meat colour, nutrient composition and lipid peroxidation value in broiler chickens under heat stress. The birds were kept under a high temperature (32±1°C) for 24 h/day for the first until the final day. At the end of the experiment (day 42), five birds per treatment were slaughtered. Then two pieces of the right thigh muscle per bird (upper part of the thigh muscle) were immediately collected for assessing the meat quality indices. The results of the present experiment showed that addition of Zn and Mg did not influence the ash, dry matter (DM) and ether extract (EE) contents of thigh meat (P>0.05), whereas the crude protein (CP) content of the thigh meat was increased when diet was supplemented with Zn (P<0.05). Addition of Zn and Mg in diet did not change the mean lightness (L) and redness (a) value in the thigh meat (P>0.05). Thigh yellowness (b) value was increased by inclusion of 30 mg/kg Zn alone or along with 600 mg/kg Mg (P<0.05). Furthermore, inclusion of 30 mg/kg Zn in diet (at any level of Mg) decreased the thigh thiobarbituric acid reactive substances (P<0.05).

Key words: thigh meat, thigh yellowness, crude protein, pH, thiobarbituric acid reactive substances

High ambient temperature is a major problem in many parts of the world such as Iran, especially during summer. Exposure to high temperature has been reported to cause undesirable changes in meat quality of broilers (Aksit et al., 2006). It results in a decreased growth rate, meat yield and breast protein content of broilers (Yalcin

et al., 2001). Heat stress during rearing is also one of the prominent antemortem stressors that results in a faster pH decline and pale colour of breast meat in birds such as turkey (McKee and Sams, 1997). McCurdy et al. (1996) reported that the mean lightness (L*) value in the breast muscle of turkeys was the highest during the summer. Petracci et al. (2004) observed higher lightness (L*) and lower redness (a*) and yellowness (b*) values for the breast muscle fillets of broilers reared in the summer than those reared in winter. Colour defects of raw and cooked poultry meat have been a problem in the poultry industry for many years. Increased chicken meat production and demand of food-store chains for standardized products caused the detailed evaluation of selected physical indicators, such as colour and tenderness of poultry meat (Abeni and Bergoglio, 2001). Nowadays, consumers pay special attention to meat colour and texture among the meat quality indices. Chicken meat contains an increased level of polyunsaturated fatty acids, which have a beneficial effect on the human health. A big demerit of this meat is the increased degradation of lipid fractions and its severity is positively correlated with the concentration of polyunsaturated fatty acids. Dietary unsaturated fat supplements increase the unsaturation degree and oxidation susceptibility of carcass fat in broilers (Daneshyar, 2012) leading to lipid peroxidation (LPO) caused by free radicals. The free radicals are involved in the uncontrolled chain reactions that primarily affect the phospholipids. The cell membrane phospholipids are particularly susceptible to the oxidative damage. It is widely established that a deficient as well as excessive intake of trace elements could cause an oxidative stress and lipid peroxidation in poultry (Surai, 2002), but the negative consequences of LPO can be overcome by the adequate supplementation of antioxidants to the diet. Some minerals such as zinc (Zn), and magnesium (Mg) are strongly associated with the antioxidant defence of the organism. Zn is a micronutrient that participates in the antioxidant defence system. The Zn deficiency stimulates the oxidative damage through produced free radical action and alters the status of antioxidant enzymes and substances (Sahin et al., 2006). In quails, $ZnSO_4$ or Zn picolinate supplementation (30 or 60 mg/kg) have improved the carcass quality under heat stress temperature (Sahin et al., 2005). Sahin et al. (2005) also reported decreased serum malondialdehyde (MDA) concentrations (an indicator of lipid peroxidation) as a result of dietary Zn picolinate supplementation in heat-stressed quail. Sahin and Kucuk (2003) reported that 30 or 60 mg/kg of Zn decreased serum and liver MDA levels in heat stressed chickens. Mg is the other required mineral for good health in humans and animals (Kucuk, 2008). The Mg deficiency is also related to oxidative stress. Mg is one of the most abundant divalent cations in living cells and plays a vital role in many cellular processes. D'Souza et al. (1998) reported that pigs supplemented with Mg-aspartate-hydrochloride had less pale meat with higher pH value and lower drip loss percentage compared to the control group. No report is available regarding the effect of Mg and Zn on meat quality and lipid peroxidation of broiler chickens reared under heat stress condition. The objective of the present study was, therefore, to determine the effect of the dietary supplementation with zinc acetate and magnesium sulfate on broiler thigh meat colour, nutrient composition and lipid peroxidation values under continuous heat stress condition.

Material and methods

Birds and housing

Four hundred and fifty day-old male broiler chicks (Ross 308) purchased from a commercial hatchery, were weighed on arrival and were randomly assigned to 9 treatment groups consisting of 5 replicate pens with 10 birds each in a 3×3 factorial design. The birds of different treatments had the same mean body weight at day one of age. The experiment was conducted under appropriate animal care regulations. The research proposal was reviewed and approved by the Animal Care Committee of Urmia University. The birds were kept under a continuous high temperature condition (32±1°C) for 24 h/day from the first until the final day. Average ambient relative humidity inside the house was 40%. Twenty-three hours of lighting and an hour dark was provided per day (Aviagen guidelines). The birds were fed a starter mash diet until day 21 of age followed by a grower mash diet afterwards (from day 22 to day 42 of age). The basal starter and grower diets were formulated according to the Ross requirements (Aviagen Company) guideline. Ingredients and chemical composition of the basal diet are shown in Table 1. Feed and water were provided *ad libitum*. The birds were fed either a basal diet supplemented with 0, 30 and 60 mg of Zn/kg or 0, 300 and 600 mg of Mg/kg of diet. Mineral premix already contained 85 mg Zn and no Mg per kg of feed. Hence three levels of 85, 115 and 145 mg Zn/kg were provided in the experimental diets. Source of Zn was zinc acetate ($C_4H_6O_4Zn$, Applichem, Germany) whereas magnesium sulfate ($MgSO_4$, Merck, Germany) was used as the source of Mg. Hence, small amounts of the basal diet were first mixed with the respective amounts of Zn and Mg as a small batch, and then with a larger amount of the basal diet until the total amount of the respective diets was homogeneously mixed. At the end of the experiment (day 42), five birds per treatment (one per replicate pen) were randomly selected and slaughtered. Two pieces of right thigh muscle per bird (upper part of the thigh muscle) were immediately collected in plastic bags and kept at 20°C for assessing the meat quality indices. One piece of thigh meat was used for determination of pH and nutrient composition (dry matter, ash, ether extract and crude protein). Thigh meat colour index was assessed from the surface area of the other piece.

Meat quality measurements

For thiobarbituric acid reactive substances (TBARS) determination, firstly 10 g of meat sample were weighed into a 50-ml test tube and 1 ml of 0.1% BHT was added. Then 35 ml of 5% trichloroacetic acid (TCA) were added and the meat samples were homogenized (Ultra-Turrax T-25, Janke & Kunkel IKA-Labortechnik, Staufen, Germany) at 13,500 rpm for 30 s. After filtering, 5 ml of the filtrate and 5 ml thiobarbituric acid solution (0.02 mM) were added to the test tube. Tubes were heated in a boiling water bath for 1 hour at 100°C, cooled and then absorbance was measured at 532 nm against a blank containing 5 ml of TCA and 5 ml of TBA solution. TBARS values were expressed as mg per kg of sample (Ulu, 2004).

The colour of thigh meat was measured by a chromameter (CR-400, Japan). Meat colour ranged from light, $L^*>53$; to normal, $48<L^*<51$; and dark, $L^*<46$. Colour categorization was based on 3 points of every meat in triplicate after slaughter.

For the pH determination, approximately 2.5 g of ground thigh meat was homogenized in 25 mL of an iodoacetate solution (5 mM sodium iodoacetate, 150 mM potassium chloride, and the pH adjusted to 7.0 with potassium hydroxide) for 30 s, and the pH of the homogenate was determined using a pH meter (TitroLine Easy, Schott Instruments, Mainz, Germany) that was calibrated at pH 4.0 and 7.0.

Table 1. Ingredients and calculated chemical analyses of the diets (g/kg, fresh basis)

Ingredients (g/kg)	Starter (0–21 d)	Grower (22–42 d)
Maize	319.0	328.7
Soya bean meal (CP 440 g/kg)	395.6	337.8
Wheat	200.0	250.0
Soya oil	38.0	42.0
Dicalcium phosphate	21.0	21.5
Limestone	11.0	8.6
Salt	3.7	3.4
DL-Methionine (98%)	3.8	0.8
L-Lysine (HCL)	2.9	2.2
Trace minerals premix[a]	2.5	2.5
Multi-vitamin premix[b]	2.5	2.5
Calculated chemical analysis		
Metabolizable energy (MJ/kg)	12.14	12.52
Crude protein (g/kg)	220	200
Calcium (g/kg)	10	9.0
Available phosphorus (g/kg)	4.5	4.5
L-Lysine (g/kg)	14.3	12.4
Threonine (g/kg)	8.5	7.7
Isoleucine (g/kg)	9.7	8.8
Valine (g/kg)	10.8	9.8
Tryptophan (g/kg)	2.9	2.6
Arginine (g/kg)	14.5	12.7
Methionine + Cystine (g/kg)	10.7	7.2
Sodium (g/kg)	1.5	1.5

[a]Provided per kg of ration; copper 10 mg, iron 50 mg, manganese 100 mg, zinc 85 mg, selenium 0.2 and iodine 1.0 mg.

[b]Provided per kg of ration; retinol 900 IU, cholecalciferol 2000 IU, tocopherol 18.0 IU, menadione 2.0 mg, thiamine 1.8 mg, riboflavin 6.6 mg, pyridoxine 3.0 mg, cyanocobalamin 0.015 mg, niacin 30 mg, pantothenic acid 10 mg, folic acid 1.25 mg, choline 500 mg and biotin 0.1 mg.

Meat moisture was determined using the vacuum-oven method according to the Association of Official Analytical Chemists (AOAC, 1999). The ground meat samples were dried for 48 h in a vacuum-oven (23 kPa) at 98°C and cooled to room temperature in a desiccator prior to taking final weights.

Statistical analyses

The data were analysed in a 3×3 factorial arrangement of treatments based on a completely randomized design with five replicates per treatment. The pen was used as the experimental unit. The results of the experiments were analysed by ANOVA using the GLM procedure of SAS software (SAS Company, version 9.1) to assess the effects of different levels of Zn, Mg and their interactions on thigh meat colour, nutrient composition and lipid peroxidation. The univariate test in SAS (SAS Institute Inc., Cary, NC, USA) was used to assess the normality of all data. Differences among treatments means were determined using Tukey multiple range test at $P<0.05$ significance level.

Results

Thigh meat nutrient composition

The effects of dietary Zn and Mg supplementation on thigh meat nutrient composition of broilers are shown in Table 2. Dietary supplementation with different levels of Zn and Mg did not influence the thigh ash, DM and EE contents ($P>0.05$). Moreover, no significant interactions were observed between the Zn and Mg for the above mentioned parameters ($P>0.05$). Zn dietary supplementation had a significant effect on thigh meat CP value ($P< 0.05$). Supplementation with 30 mg/kg of Zn along with 600 mg/kg of Mg to diet increased the thigh meat CP content compared to control diet. Thigh meat pH value was affected by Mg dietary supplementation ($P<0.05$). Dietary supplementation with 600 mg/kg Mg without supplemental Zn increased thigh pH compared to the supplementation with lower levels of Mg (0 and 300 mg/kg) along with 30 mg/kg of Zn.

Thigh meat colour index and lipid peroxidation

Table 3 shows the supplementation effects of Zn and Mg on thigh meat colour index and lipid peroxidation values. Dietary addition of Zn or Mg did not change either the lightness (L) or the redness (a) of thigh meat ($P>0.05$). Moreover, no interactive effect was observed between the Zn and Mg for these two parameters ($P>0.05$). On the other hand, both the Zn and Mg affected thigh meat yellowness (b) value ($P<0.05$). Supplementation with 30 mg/kg Zn alone or along with 600 mg/kg Mg increased thigh yellowness (b) value as compared to the control treatment group ($P<0.05$). Thigh meat TBARS values were only affected by the Zn supplementation ($P>0.05$), as 30 mg/kg of Zn reduced the thigh TBARS content at any level of Mg in comparison to the control group.

Table 2. The effect of dietary supplementation levels of zinc (Zn) as zinc acetate and magnesium (Mg) as magnesium sulfate supplementation on nutrient composition[1] of thigh meat in broiler chickens at day 42 of age

Parameters	Mg 0 (mg/kg)			Mg 300 (mg/kg)			Mg 600 (mg/kg)			Pooled SEM[2]	P-value		
	Zn 0 (mg/kg)	Zn 30 (mg/kg)	Zn 60 (mg/kg)	Zn 0 (mg/kg)	Zn 30 (mg/kg)	Zn 60 (mg/kg)	Zn 0 (mg/kg)	Zn 30 (mg/kg)	Zn 60 (mg/kg)		Zn	Mg	Mg×Zn
Ash (g/kg)	8.6	8.6	8.0	8.9	9.0	9.6	8.4	8.8	8.8	0.2	0.77	0.07	0.39
Dry matter (g/kg)	312.5	282.0	312.0	284.0	302.0	270.0	26.0	280.0	310.0	6.8	0.57	0.45	0.17
Crude protein (g/kg)	166.7 b	210.2 ab	184.8 ab	177.6 ab	209.4 ab	198.2 ab	201.7 ab	220.2 a	175.6 ab	3.8	0.02	0.67	0.22
Ether extract (g/kg)	282.1	246.7	309.6	305.4	275.2	249.9	250.4	274.1	317.3	11.8	0.73	0.99	0.48
pH	6.00 ab	5.88 b	6.02 ab	5.94 ab	5.89 b	6.02 ab	6.84 a	6.04 ab	5.96 ab	0.04	0.07	0.03	0.08

a, b – means with no common superscript for each mineral and row are significantly different ($P<0.05$).
[1]Each value represents the mean of 5 cages with two chicks per cage.
[2]Pooled standard errors of the mean.

Table 3. The effect of dietary supplementation levels of zinc (Zn) as zinc acetate and magnesium (Mg) as magnesium sulfate supplementation on thigh meat color and lipid peroxidation[1] in broiler chickens at day 42 of age

Parameters	Mg 0 (mg/kg)			Mg 300 (mg/kg)			Mg 600 (mg/kg)			Pooled SEM[6]	P-value		
	Zn 0 (mg/kg)	Zn 30 (mg/kg)	Zn 60 (mg/kg)	Zn 0 (mg/kg)	Zn 30 (mg/kg)	Zn 60 (mg/kg)	Zn 0 (mg/kg)	Zn 30 (mg/kg)	Zn 60 (mg/kg)		Zn	Mg	Mg×Zn
L* value[3]	49.07	54.19	50.58	56.35	55.35	48.38	53.82	48.70	54.87	0.90	0.20	0.96	0.43
a* value[4]	6.47	4.52	6.19	6.51	6.05	6.82	6.43	6.64	6.38	0.19	0.16	0.11	0.28
b* value[5]	6.73 b	10.51 a	7.86 ab	8.71 ab	9.22 ab	8.83 ab	9.53 ab	10.12 a	9.43 ab	0.24	0.01	0.05	0.07
TBARS[2] (mmol/L)	0.29 a	0.19 b	0.22 ab	0.23 ab	0.18 b	0.22 ab	0.20 ab	0.19 b	0.20 ab	0.03	0.04	0.21	0.26

a, b – means with no common superscript for each mineral and row are significantly different ($P<0.05$).
[1]Each value represents the mean of 5 cages with two chicks per cage.
[2]Thiobarbituric acid reactive substances.
[3]Lightness.
[4]Redness.
[5]Yellowness.
[6]Pooled standard errors of the mean.

Discussion

In the current experiment, supplementation with 30 mg of Zn per kg of the basal diet (providing 115 mg/kg) enhanced the thigh meat CP. One possible reason of the increased thigh CP of these birds could be the improved digestibility of nutrients, especially proteins, due to Zn supplementation. It has been recognized that heat stress increases the excretion of minerals such as zinc, copper, and manganese (Belay and Teeter, 1996). The Zn positively affects feed utilization through its action in the metabolism of carbohydrates, lipids and proteins (MacDonald, 2000). The digestibility of proteins, fats, and starch decreased with exposure of broiler chickens to high temperatures. In addition, activities of trypsin, chymotrypsin and amylase are decreased at a temperature of 32°C (Hai et al., 2000). Zn has a protective role on pancreatic tissue against oxidative damage and it may help the pancreas to function properly including secretions of digestive enzymes, thus improving digestibility of nutrients. Higher performance of Zn dietary supplemented birds (especially feed intake) could be the other reason for higher thigh CP compared to the control group. Better body weight gain, feed intake and feed conversion ratio for broiler chickens fed 30 mg/kg of Zn have been reported elsewhere (Nourozi et al., 2013). The Zn deficiency is characterized by decreased feed intake, decreased growth, low circulating levels of growth hormone (GH) and insulin-like growth factor-I, and decreased hepatic production of insulin-like growth factor-I, GH receptor, and GH binding protein in animals (MacDonald, 2000).

Furthermore, Mg inclusion in diet had an effect on the thigh pH in the current study. Generally, pH value is a direct reflection of muscle acid content, and affects the shear force, drip loss and colour in meat. Muscle pH variation is also related to glycogenolysis, and increased catecholamine secretion in response to an acute stressor just prior to slaughter increases glycogen breakdown and the rate of pH decline post-slaughter while the carcass temperature is still high, resulting in pale, soft, exudative (PSE) meat (Briskey and Wismer-Pedersen, 1961). D'Souza et al. (1998) stated that the sedative function of Mg reduced the stress-induced catecholamine secretion and then inhibited glycogen breakdown and glycolysis. Guo et al. (2003) reported the highest thigh meat pH after the dietary supplementation with 2.0 g/kg proteinate of Mg in broiler diets. Holm and Fletcher (1997) observed that broilers held at 29°C during crating had lower ultimate pH compared to broilers held at 7 and 18°C. Stress before slaughter can lead to increased muscle glycogen breakdown and glycolysis after slaughter, and then the increased muscle lactic acid lowers the pH of meat.

The mechanism that associates dietary Mg with post mortem pH could be explained by the roles of Mg in some enzymes involved in glycogen metabolism, such as pyruvate kinase and ATP-Mg-dependent phosphatase. Pyruvate kinase is unique among kinases in its requirement for enzyme-bound cations, normally Mg^{2+} and K^{+}. The enzyme is found in all cells and tissues during glycolysis and is of particular importance for controlling the flux from fructose-1, 6-bisphosphate through to pyruvate (Muirhead et al., 1986). ATP-Mg-dependent phosphatases have broad substrate specificity, responsible for the major dephosphorylation reactions of the en-

zymes involved in the hormonal control glycogen metabolism (Vandenheede et al., 1981).

In addition, the results of our study indicated that dietary supplementation with 600 mg/kg of Mg and 30 mg/kg of Zn increased the yellowness (b) value in the thigh meat. Colour is a major criterion that can be used by consumers to judge meat quality. Hot weather conditions (such as summer) have been reported to increase the lightness (L) and to decrease the redness (a) and yellowness (b) in the breast muscle fillets of broilers (Petracci et al., 2004). Barbut (1993) reported that lightness (L) had the highest correlation of the L*, a*, b* colour parameters with PSE-like conditions. The yellowness (b) of meat colour during heat stress is related to the reactive oxygen radicals (ROS) and lipid peroxidation. Heat stress increases ROS, possibly by the disruption of the electron transport assemblies of the membrane. A moderate increase of ROS promotes cell proliferation and differentiation, whereas excessive amounts of ROS due to an elevated ROS production or to a decline of ROS-scavenging capacity can cause severe oxidative damage to lipids, proteins and DNA (Boonstra and Post, 2004). Consistent with the results of the current experiment, Liu et al. (2011) detected increased b* value of thigh muscles after 60, 120 and 180 mg/kg of Zn dietary supplementation. Saenmahayak et al. (2007) have shown that Zn supplementation affects the meat quality through reduced cooking loss and darker fillets, which may be correlated with a lower incidence of pale, soft and exudative meat. This effect may be due to the ability of Zn to bind myoglobin and increase its oxygenation. The Zn also inhibits mitochondrial respiration and decreases the production of free radicals by acting as an antioxidant, thus facilitating the maintenance of meat colour (Powell, 2000).

The thigh TBARS was reduced by the dietary supplementation with 30 mg/kg Zn in the present study. Oxidation of lipid components in muscle tissues is the major cause of quality deterioration and short shelf life after slaughter (Guo et al., 2003). TBARS or malondialdehyde (MDA) are the soluble degraded product of lipids that can be widely used to reflect the extent of lipid oxidation in meat (Raharjo and Sofos, 1993). Heat stress causes oxidative stress, and TBARS production is increased (Halliwell and Gutteridge, 1989). Sahin and Kucuk (2003) reported that 30 or 60 mg/kg of Zn decreased serum and liver MDA levels in heat-stressed birds. In heat-stressed quail, Kucuk (2008) also showed that both Zn and Mg supplementations decreased plasma MDA levels. Zinc may play a key role in suppression of free radicals because it is a cofactor of the main antioxidative enzyme Cu-Zn-SOD and it also inhibits the NADPH-dependent lipid peroxidation (Prasad and Kucuk, 2002). It prevents lipid peroxidation via inhibiting glutathione depletion as well. Due to the ability to replace Fe and Cu from binding sites, Zn can compete with these transition metals to bind to the cell membrane and decrease the production of free radicals and thus exert a direct antioxidant action (Oteiza et al., 1996).

Based on the results of the present study showing the highest yellowness (b value) and CP, and the lowest TBARS content in thigh meat of 30 mg/kg Zn fed birds, it was concluded that inclusion of 30 mg/kg Zn in diet can improve the meat quality of broiler chickens under heat stress.

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