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Short communication. Dietary supplementation effects of zinc acetate and magnesium sulfate on performance and antioxidant status of broilers under continuous heat stress

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Abstract

The aim of this study was to investigate the effects of different dietary levels of zinc acetate (Zn; 0, 30 and 60 mg kg^{-1}) and of magnesium sulphate (Mg; 0, 300 and 600 mg kg^{-1}) on performance and blood antioxidant status of broilers under continuous heat stress. For this purpose, four hundred and fifty one-day-old male chicks were used in a 3 × 3 factorial experiment from day 1 to day 42 of age. The inclusion of 30 mg Zn kg^{-1} increased the body weight gain and the average feed intake and declined the feed conversion ratio (FCR). Although there were no differences between the treatments for FCR, the supplementation with 30 and 60 mg Zn kg^{-1} decreased the FCR regardless the Mg level. The dietary supplementation with 30 mg Zn kg^{-1} decreased both blood glutathione peroxidase and superoxide dismutase activities regardless the Mg level. In addition, neither Zn nor Mg influenced the blood total antioxidant capacity content at the end of the experiment. Blood malondialdehyde (MDA) decreased as dietary Zn supplementation increased. The MDA reduction when diet was supplemented with Zn was not obvious at higher Mg level (interaction Zn × Mg, $p < 0.05$). In conclusion, dietary addition of Mg does not significantly influence either the performance or the antioxidant status of broiler at 42 d of age under heat stress but supplementation of 30 mg Zn kg^{-1} decrease the blood MDA concentration and improves the performance.

Additional key words: average feed intake; body weight gain; feed conversion ratio; glutathione peroxidase; malondialdehyde; productive results; superoxide dismutase**.**

Negative effects of high ambient temperature such as decreased body weight gain (BWG) and average feed intake (AFI), impaired feed efficiency and reduced nitrogen retention has been observed in poultry (Sahin K *et al.,* 2005). Increased mineral excretion is a major consequence of heat distress. Lower rates of phosphorus, potassium, sodium, magnesium, sulfur, manganese, copper and zinc (Zn) retention have been reported in broilers raised at high cycling ambient temperatures (24°C to 35°C) (Sahin K *et al.,* 2005). Several strategies are available to alleviate the negative effects of high environmental temperature on poultry performance. Recently, several studies have shown that antioxidant nutrient supplementation, especially vitamins C and E, are effective in preventing the deleterious effects of heat stress and their dietary inclusion have been proposed to avoid the negative effects of environmental stress (Sahin *et al.,* 2006). The Zn is a micronutrient that participates in the antioxidant defense system and is required for the activity of over 300 enzymes and participates in many enzymatic and metabolic functions in the body (Sahin *et al.,* 2009). Its positive effects on antioxidants absorption have been even reported in animals. Kim *et al*. (1998) showed that intestinal absorption of vitamin E was influenced by the Zn status of rats. In other experiment, Onderci *et al.* (2003) indicated the increase of levels of serum vitamins C and E but the decrease of malondialdehyde (MDA) concentration by supplemental Zn in laying hens. The Zn deficiency stimulates the oxidative damage through produced free radical action and alters the status of antioxidant enzymes and subs-

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Abbreviations used: AFI (average feed intake); BWG (body weight gain); FCR (feed conversion ratio); GPX (glutathione peroxidase); MDA (malondialdehyde); SOD (superoxide dismutase); TAC (total antioxidant capacity).

tances (Sahin *et al.,* 2006). Dietary Zn supplementation has improved the growth rate and feed efficiency of broiler under heat stress (Kucuk *et al.,* 2003).

The magnesium (Mg) is the other required mineral for good health in humans and animals (Kucuk, 2008). It is needed for more than 300 biochemical reactions in the body. The Mg helps maintain normal muscle and nerve function, keeps heart rhythm steady, supports a healthy immune system, and keeps bones strong (Kucuk, 2008). The Mg deficiency is also related to oxidative stress in chicks, rats, and humans (Boujelben *et al.,* 2006; Yang *et al.,* 2006). Consequently, the dietary requirements of both Mg and Zn increases in birds under heat stress and hence the dietary concentrations of these minerals should be higher under such circumstances (Kucuk, 2008). The NRC (1994) recommends the levels of 40 to 75 mg kg^{-1} for Zn and lower than 0.6 g kg^{-1} dry matter for Mg in poultry diets. The present experiment aimed to investigate the dietary supplementation effects of Zn and Mg on performance and antioxidant status of broiler chickens reared under continuous heat stress (32 ± 1°C).

Four hundred and fifty one-day-old male broiler chicks (Ross 308, mean body weight = 43.4 ± 0.19 g) purchased from a commercial company (Behparvar, Urmia), were weighed on arrival to experimental facilities and randomly assigned to pens (floor with letter) with 10 birds each. The birds were kept under a continuous high temperature condition (32 ± 1°C) for 24 h d^{-1}. Average ambient relative humidity inside the house was 40%. Twenty-three hours of lighting and 1 hour dark was provided per day. The birds were fed a starter mash diet until 21 d of age followed by a grower mash diet upwards (from day 22 to day 42 of age) (Table 1). The basal starter and grower diets were formulated according the Ross requirements (Aviagen Company) guideline. Feed and water were provided *ad libitum* throughout the experimental period. The birds were fed either a basal diet supplemented with 0, 30 and 60 mg of Zn kg^{-1} of diet or 0, 300 and 600 mg of Mg kg^{-1} of diet. Zinc acetate [$(CH_3COO)\ 2Zn \cdot 2H_2O$] was used as the Zn source (Applichem, Germany) and magnesium sulfate ($MgSO_4$) as Mg source (Merck, Germany). Bioavailability of Zn acetate and Zn sulfate has been reported to be similar (Baker & Ammerman, 1995). However Cao *et al.,* (2002) indicated a higher bioavailability of Zn from Zn acetate as compared to Zn methionine for Metallothionein (a scavenger of free radicals) production. Hence, Zn acetate was used in current study. 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 were homogeneously mixed. The AFI and BWG were measured and the feed conversion ratio (FCR) was calculated for the entire period. At the end of the experiment (day 42), five birds per treatment (one per replicate) were selected randomly and killed by decapitation, then two series of blood samples were collected in anticoagulant tubes with EDTA. The blood samples were moved to laboratory and centrifuged at 5000 rpm for 5 min. The obtained plasma samples were kept in –20°C along with other series of blood samples for later analyses. Plasma total antioxidant capacity (TAC) was determined using Randox total antioxidant status test kit (Randox Laboratories Ltd, UK), blood superoxide dismutase (SOD) activity by Ransod spectrophotometric kit (Ransod, Randox Laboratories Ltd. UK), blood glutathione peroxidase (GPX) activity by Ransel spectrophotometric kit (Ransel, Randox Laboratories Ltd. UK) and plasma MDA concentration by MDA reaction with thiobarbituric acid followed by extraction with butanol (Kolahi *et al.,* 2011). The total mortality of each pen at the end of the experiment was divided by the number of birds at the beginning of the experiment (day 1), and transformed by *arcsin* $\sqrt{y}$ (Daneshyar *et al.*, 2012). The data were analyzed in a 3 × 3 factorial arrangement of treatments based on a completely randomized design with five replicates per treatment. The pen with 10 chicks was used as the experimental unit. The results were analyzed by ANOVA using the GLM procedure of SAS (2003) software. The model included the Zn and Mg levels as main effects and also their interaction. Differences among treatments means were determined using Tukey Multiple Range Test at $p < 0.05$ significant level.

Neither the dietary Zn nor the Mg affected the mortality during the entire experimental period ($p > 0.05$) (data not shown). Furthermore, BWG, AFI or FCR were not affected by dietary Mg supplementation at the end of the trail ($p > 0.05$) (Table 2). Lack of Mg supplementation effect on performance in the current study is consistent with unchanged blood antioxidant enzymes and even blood MDA concentration ($p > 0.05$). There are some reports which have shown that Mg can inhibit peroxidation in animal tissues (Fu, 1996; Guo *et al.,* 2003) but so far there is no clear antioxidant mechanism revealed. Sahin N

Table 1. Composition of the basal diets (g kg^{-1}, as fresh matter)

Ingredients	Starter (0-21 d)	Grower (22-42 d)
Maize (8% crude protein)	319.0	328.7
Soya bean meal (44% crude protein)	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-Lysin (HCl)	2.9	2.2
Trace minerals premix[a]	2.5	2.5
Multi-vitamin premix[b]	2.5	2.5
Chemical analysis		
Metabolizable energy (kcal kg^{-1})	2,900	2,990
Crude protein	220	200
Calcium	10	9.0
Available phosphorus	4.5	4.5
L-Lysine	14.3	12.4
Threonine	8.5	7.7
Isoleucine	9.7	8.8
Valine	10.8	9.8
Tryptophan	2.9	2.6
Arginine	14.5	12.7
Methionine + Cystine	10.7	7.2
Sodium	1.5	1.5

[a] Provided per kg of ration; 10 mg copper (cupric sulfate), 50 mg iron (ferrous sulfate), 100 mg manganese (manganese oxide), 85 mg zinc (zinc sulfate), 0.2 mg selenium (sodium selenite) and 1.0 mg iodine (calcium iodate). [b] Provided per kg of ration; 900 IU retinol, 2000 IU cholecaciferol, 18 IU tocopherol, 2 mg menadione, 1.8 mg thiamine, 6.6 mg riboflavin, 3.0 mg pyridoxine, 0.015 mg cyanocobalamin, 30 mg niacin, 10 mg pantothenic acid, 1.25 mg folic acid, 500 mg choline and 0.1 mg biotin.

Table 2. Effect of different dietary supplementation levels of zinc (Zn, 0, 30 and 60 mg kg^{-1}) as zinc acetate and magnesium (Mg, 0, 300 and 600 mg kg^{-1}) as magnesium sulfate supplementation on body weight gain (BWG, g d^{-1}), average feed intake (AFI, g d^{-1}), feed conversion ratio (FCR) and activity of glutathione peroxidase (GPX, units g^{-1} Hb) and superoxide desmotase (SOD, units g^{-1} Hb), malondialdehyde (MDA, nmol mL^{-1}) content and total antioxidant capacity (TAC, mmol mL^{-1}) of blood in broiler at day 42 of age under heat stress conditions.

Parameters	Mg 0			Mg 300			Mg 600			Pooled SEM[1]	*p-value*		
	Zn 0	Zn 30	Zn 60	Zn 0	Zn 30	Zn 60	Zn 0	Zn 30	Zn 60		Zn	Mg	Mg*Zn
BWG	1,793^{ab}	1,985^{a}	1,941^{ab}	1,708^{b}	1,944^{a}	1,894^{ab}	1,840^{ab}	1,938^{ab}	1,745^{ab}	21.9	0.010	0.27	0.27
AFI	3,515	3,737	3,562	3,581	3,734	3,513	3,619	3,638	3,375	30.9	0.010	0.73	0.43
FCR	1.96	1.88	1.84	2.05	1.92	1.84	1.96	1.86	1.91	0.0	0.010	0.28	0.70
GPX	39	35	42	37	36	40	41	36	40	0.6	0.003	0.57	0.68
SOD	1,384^{ab}	1,387^{ab}	1,476^{a}	1,386^{ab}	1,218^{b}	1,374^{ab}	1,443^{ab}	1,325^{ab}	1,450^{ab}	20.0	0.010	0.07	0.80
MDA	2.80^{ab}	1.48^{ab}	2.24^{ab}	3.35^{a}	1.22^{b}	1.98^{ab}	1.22^{b}	1.70^{ab}	2.68^{ab}	0.2	0.030	0.67	0.02
TAC	0.79	0.61	0.67	0.85	0.91	1.01	0.64	0.78	0.88	0.0	0.570	0.06	0.67

[1] Pooled standard errors of the mean. [a-b] Means with different superscripts in each row are significantly different ($p \leq 0.05$).

et al., (2005) reported lower serum MDA by either magnesium proteinate or magnesium oxide supplementation in the diet of quails reared under heat stress (34°C). They also reported an increase in AFI, BWG and feed efficiency of heat-stressed quails fed either 1 or 2 g of magnesium proteinate or magnesium oxide. The difference with our work could be related with the different bird species used, or the Mg source and level, or bioavailability (Firoz & Graber, 2001; Kucuk, 2008). Even, the higher unsaturated oils of diets in recent experiment without supplemented antioxidants (38 and 42 kg ton^{-1} soybean oil for starter and grower diets respectively) could be other reason for the mentioned difference. Dietary unsaturated fat supplements increase the unsaturation degree and oxidation susceptibility of carcass fat in broilers (Daneshyar, 2012).

Dietary supplementation of Zn affected the BWG at the end of the trail ($p < 0.01$) (Table 2). Broiler fed diets supplemented with 30 mg Zn kg^{-1} and with lower Mg levels (0 and 300 mg kg^{-1}) had greater BWG than broiler fed diets with 300 mg Mg kg^{-1} and no Zn addition. Also, AFI and FCR were influenced by dietary Zn ($p < 0.05$). Although there were no differences between Zn levels for FCR, chicks fed 30 and 60 mg Zn kg^{-1} had better FCR regardless of the Mg level. Furthermore no interaction was found between the Zn and Mg addition with regard to BWG, AFI and FCR ($p > 0.05$). Also, GPX and SOD activities were affected by Zn supplementation ($p < 0.01$). No significant differences were observed between the treatments for GPX activity, but broiler fed 30 mg Zn kg^{-1} showed a decrease in blood GPX activity regardless of the Mg level. Chicks fed diets supplemented with 30 mg Zn kg^{-1} and 300 mg Mg kg^{-1} had lower blood SOD activity as compared to the birds fed 60 mg Zn kg^{-1} without supplemental Mg. Moreover, Zn did not influence the blood TAC content ($p > 0.05$). The MDA decreased as dietary Zn supplementation increased ($p < 0.05$).

At medium Mg level (300 mg kg^{-1}), dietary supplementation of 30 mg Zn kg^{-1} decreased the MDA content as compared to lower Zn level (0 mg). Consistent with our results, Sahin & Kucuk (2003) documented the improved AFI and growth rate in quail by dietary Zn supplementation. The better performance results of broilers fed 30 mg kg^{-1} Zn in our experiment were simultaneous with lower blood MDA. It is not clear why the higher Zn level (60 mg kg^{-1}) does not affect the blood MDA in a same way. But, parallel with our results, decreased plasma MDA concentrations upon Zn supplementation (30 ppm) were reported in quail reared under heat stress (35°C) (Kucuk *et al.,* 2003). According to Shaheen & Abd El-Fattah (1995), Zn deficiency causes increased lipid peroxidation and this can be inhibited by Zn supplementation. The Zn acts in different distinct mechanisms in antioxidant system. The first mechanism is the protection of proteins and enzymes against free radical attack, or oxidation. The Zn is a cofactor of the main antioxidative enzyme CuZnSOD and probably plays an important role in suppression of free radicals and in inhibition of the nicotinamide adenine dinucleotide phosphate (reduced form) dependent lipid peroxidation (Prasad, 1997). The second mechanism by which Zn involves in antioxidant system is through the prevention of free radical formation by other metals, such as iron and copper (Kucuk, 2008). Furthermore, induced metallothionein production may be other mechanism for Zn exerting its antioxidant property. Metallothionein is a cysteine rich protein that scavenges the free radicals such as hydroxyl (Sahin *et al.,* 2006). According to the role of Zn in CuZnSOD enzyme, we expected higher activity for this enzyme in blood, but lower blood SOD activity was observed for this enzyme. This phenomenon possibly is related to diminished expression of this enzyme due to lower peroxidation (lower blood MDA). It has been documented that high amounts of peroxidation increases the antioxidant enzyme expression. For example, higher expression of GPX enzyme has been reported in lung mitochondria of broiler chickens with pulmonary hypertension syndrome connected to its response to greater hydrogen peroxide production (Iqbal *et al.,* 2001). Hence low blood MDA content of 30 mg Zn kg^{-1} fed birds possibly resulted in lower SOD enzyme expression of these birds. So, MDA reduction of 30 mg Zn kg^{-1} fed birds has been exerted by the second mechanism of Zn as an antioxidant and not SOD activity. The decreased MDA content of 600 mg Mg kg^{-1} (without supplemental Zn) could be due to the important role of Mg in the activation of catalase which is involved in redox reaction (Guo *et al.,* 2003). We expected lower MDA by combination of Zn and Mg in diet but MDA reduction by 600 mg Mg kg^{-1} was not detected at higher Zn level (30 and 60 mg kg^{-1}, interaction Zn × Mg, $p < 0.05$). The underlying mechanisms of this interaction are not revealed in current experiment and more research works are needed in this field.

Based on the results of present study, dietary addition of Mg does not influence either of performance or plasma antioxidant status in broiler chickens under heat stress. Moreover, supplementation of 30 mg kg^{-1} Zn decreases the blood MDA concentration and hence improves the performance of broiler at the end of the trail. The mechanism of Zn for prevention of MDA production is not exerted by SOD activity but is through the prevention of free radical formation by other metals, such as iron and copper or induced metallothionein production.

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