Nutrient Metabolism—Research Communication

# Zinc Supplementation Alleviates Heat Stress in Laying Japanese Quail

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**Kazim Sahin**[1] **and Omer Kucuk***

*Department of Animal Nutrition, Faculty of Veterinary Science, Firat University, Elazig Turkey and *Prevention Program, Barbara Ann Karmanos Cancer Institute, Wayne State University, Detroit, MI 48201*

**ABSTRACT The study was conducted to determine whether zinc supplementation could alleviate the detrimental effects of high ambient temperature (34°C) on egg production, digestibility of nutrients and antioxidant status in laying Japanese quail. Quail ($n$ = 180; 52 d old) were divided into six groups ($n$ = 30/group) and were fed a basal diet or the basal diet supplemented with 30 or 60 mg of zinc ($ZnSO_4 \cdot H_2O$)/kg diet. Birds were kept at 22°C and 58% relative humidity (RH). At 13 wk of age, the thermoneutral (TN) groups remained at the same temperature, whereas the heat-stress (HS) groups were kept in an environmentally controlled room at 34°C and 42% RH for 3 wk. Heat exposure decreased egg production in birds fed the basal diet ($P = 0.001$). Linear increases in feed intake ($P = 0.01$) and egg production ($P = 0.004$) and improved feed efficiency ($P = 0.01$) and egg quality variables ($P \leq 0.05$) occurred in zinc-supplemented groups reared under HS conditions. Heat exposure decreased digestibility of nutrients ($P = 0.001$), and these decreases were ameliorated by zinc supplementation ($P \leq 0.05$). Serum vitamin C ($P = 0.05$), vitamin E ($P = 0.01$) and zinc ($P = 0.001$) concentrations increased linearly, whereas malondialdehyde concentrations decreased ($P = 0.002$) as dietary zinc supplementation increased. No significant differences in any values were observed in the TN groups ($P > 0.05$). Results of the present study suggest that supplementation with 60 mg zinc/kg diet protects quail by reducing the negative effects of heat stress. J. Nutr. 133: 2808–2811, 2003.**

KEY WORDS: • zinc • egg • antioxidant status • heat stress • quail

High ambient temperature results in reduced feed intake, egg production and egg quality, and impaired antioxidant status in poultry (1–4). Environmental stress has been shown to increase mineral excretion (5,6) and elevate lipid peroxidation products in serum and liver, while decreasing serum and tissue levels of antioxidant vitamins (7–9). Antioxidants such as vitamin C, vitamin E and vitamin A, and the minerals, zinc and chromium, have been used to ameliorate the effects of environmental stress (9–13). Supplemental zinc is used in poultry diets because of its reported benefits to laying hens during periods of environmental stress (12,14). Serum, liver and spleen levels of zinc are reduced in stressed birds (9). Zinc has multiple important functions because it is a cofactor for >200 enzymes. One of its most important functions is its participation in the antioxidant defense system. Oxidative damage of the cell membrane by free radicals occurs during zinc deficiency (15–19), thus altering the status of antioxidant enzymes and substances (20–22). The mechanism by which Zn exerts its antioxidant action is not well defined. However, it has been suggested that it increases the synthesis of metallothionein, a cysteine-rich protein, which acts as a free radical scavenger (21,23). Another mode of action proposed for Zn as an antioxidant is its interaction with vitamin E, because vitamin E status is impaired in zinc-deficient animals (15,24). Furthermore, zinc can occupy iron and copper binding sites on lipids, proteins and DNA and thus exert a direct antioxidant action (19,21–25). Iron is a redox active metal that can catalyze the formation of the highly reactive hydroxyl radicals from $H_2O_2$ and decompose lipid peroxides to peroxyl and alkoxyl radicals, which favor the propagation of lipid oxidation (19,20). Anderson et al. (26) reported that rats supplemented with dietary chromium and zinc had a significant reduction in malondialdehyde (MDA)[2] levels in serum and tissues.

Dietary modifications are among the most preferred and practical ways to alleviate the effect of high environmental temperature on poultry performance; such methods have been used previously. The objective of this study was to evaluate the effects of zinc supplementation on egg production, digestion of nutrients, and antioxidant status in laying Japanese quail reared under heat stress (34°C).

## MATERIALS AND METHODS

***Animals, diets and experimental design.*** Japanese quail ($n$ = 180; 52-d-old) (*Coturnix coturnix japanica*) obtained from a commercial company (Uluova Quail Farm, Elazig, Turkey) were used in the study. The birds were fed a basal diet containing 16.8% crude protein (CP) and 11.82 MJ/kg metabolizable energy (ME) or the basal diet supplemented with 30 or 60 mg zinc/kg diet. The basal diet was formulated using NRC guidelines (27). $ZnSO_4 \cdot H_2O$ was used as the zinc source. Ingredients and chemical composition of the basal diet are shown in **Table 1**. Small portions of the basal diet were first mixed with the respective amounts of zinc; this small amount was then mixed with a larger amount of the basal diet until the total diet amounts were homogeneous.

Beginning at 52 d of age, the birds were divided into six groups in a 3 × 2 factorial design (10 replicates of 3 birds each per cage). Birds were housed at 22°C and 58% relative humidity (RH). Two groups received each of the three diets. At 13 wk of age, three groups (0, 30, and 60 mg zinc/kg of diet) were arbitrarily designated the thermoneutral (TN) groups and remained at the same temperature as at the beginning of experiment. The heat stress (HS) groups (also consuming 0, 30, and 60 mg zinc/kg of diet) were kept in an environmentally

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[1] To whom correspondence should be addressed.
E-mail: nsahinkm@yahoo.com.

[2] Abbreviations used: CP, crude protein; DM, dry matter; EE, ether extract; HS, heat stress; HU, Haugh unit; MDA, malondialdehyde; ME, metabolizable energy; OM, organic matter; RH, relative humidity; TN, thermoneutral.

## TABLE 1

*Ingredients and chemical composition of the basal diet fed to laying Japanese quail*

| Ingredient | *g/100 g* |
|---|---|
| Corn | 61.08 |
| Soybean meal | 25.88 |
| Corn oil | 2.0 |
| Limestone | 8.76 |
| Dicalcium phosphate | 1.30 |
| Vitamins[1] | 0.25 |
| Minerals[2] | 0.25 |
| DL-Methionine | 0.13 |
| NaCl | 0.35 |
| ME,[3] *MJ/kg* | 11.82 |
| Chemical analyses, dry matter (DM) basis | |
| Crude protein, *g/100 g DM* | 16.80 |
| Calcium, *g/100 g DM* | 3.60 |
| Phosphorus, *g/100 g DM* | 0.56 |
| Zinc, *mg/kg DM* | 36.0 |

[1] Mixture supplied per kg of diet: retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; dl-$\alpha$ tocopheryl acetate, 1.25 mg; menadione sodium bisulfite, 2.5 mg; thiamine-hydrochloride, 1.5 mg; riboflavin, 3 mg; D-pantothenic acid, 5 mg; pyridoxine hydrochloride, 2.5 mg; vitamin B-12, 0.0075 mg; folic acid, 0.25 mg; niacin, 12.5 mg.

[2] Mixture supplied per kg of diet: Mn ($MnSO_4 \cdot H_2O$), 50 mg; Fe ($FeSO_4 \cdot 7H_2O$), 30 mg; Zn (ZnO), 30 mg; Cu ($CuSO_4 \cdot 5H_2O$), 5 mg; I (KI), 0.5 mg; Se ($Na_2SeO_3$), 0.15 mg; Co ($CoCl_2 \cdot 6H_2O$), 0.1 mg; choline chloride, 125 mg.

[3] ME, metabolizable energy.

controlled room at 34°C and 42% RH for 3 wk. Water and diets were consumed ad libitum throughout the experiment. The bird house was lit for 17 h/d.

***Performance variables and egg quality.*** Body weights were recorded at the beginning and at the end of the study. Feed consumption was measured weekly. The number of eggs and egg weights were recorded daily. Egg quality measurements were conducted using all eggs of 1 d from all treatments. Parameters for egg quality measurement included egg shell thickness and Haugh unit (HU). The HU values were calculated using the HU formula (28) based on the height of albumen determined by a micrometer and egg weight (Saginomiya, TLM-N1010, Tokyo, Japan). Shell thickness was determined from measurements of the mean thickness at three locations on the egg (air cell, equator and sharp end) using a dial pipe gauge (Mitutoyo, 0.01–20 mm, Tokyo, Japan).

***Sample collection and laboratory analyses.*** During the last week of the experiment, 60 birds (10 birds from each group; one per replicate) were placed in individual battery cages for collection of excrement to measure the nutrient digestibilities of dry matter (DM), organic matter (OM), CP and ether extract (EE). The composite excrement samples were oven-dried at 60°C for 48 h and then ground and subsampled (1 g) for chemical analysis. Digestibility of nutrients was measured using $Cr_2O_3$ as described by Petry and Rapp (29). Chemical analyses of the diets and excrement samples were conducted using international procedures of AOAC (30). To estimate protein digestibility, excrement N was chemically analyzed according to the method of Terpstra and De Hart (31). At the end of the experiment, serum samples from 10 birds (one per replicate) randomly chosen from each group were collected. Lipid peroxidation, assessed as TBARS in serum and liver, and serum vitamins E and C were determined as described (9). Serum concentrations of Zn were measured at specific wavelengths using an atomic absorption spectrometer (Shimadzu AA-660, Kyoto, Japan). Calibrations for the zinc assay were made with a series of mixtures containing graded concentrations of standard solutions. For Zn content analysis, triplicate basal diet samples were wet-digested as described by Chang et al. (32) and were read using an atomic absorption spectrometer.

***Statistical analyses.*** The data were initially analyzed by two-way ANOVA using the General Linear Models procedure of SAS (33) for the factors of temperature and effects of zinc. Duncan's post-hoc test was used to identify means that differed at $P < 0.05$. Linear and quadratic orthogonal contrasts were used to evaluate the effects of different levels of zinc.

## RESULTS

Zinc supplementation did not affect performance or egg quality in birds kept at thermoneutral conditions. Body weight, feed intake, egg production and feed efficiency were reduced by heat stress ($P = 0.001$). Final body weights of birds were not affected by dietary Zn level ($P = 0.96$). However, zinc supplementation of 30 and 60 mg/kg of diet increased feed intake ($P = 0.01$), egg production ($P = 0.004$), and improved feed efficiency ($P = 0.01$) in quail reared under HS conditions. Similarly, egg weight ($P = 0.001$), eggshell thickness ($P = 0.001$) and HU ($P = 0.05$) were positively affected by Zn supplementation (**Table 2**). Heat exposure decreased digestibility of DM ($P = 0.001$), OM ($P = 0.001$), CP ($P = 0.001$) and EE ($P = 0.001$), which were elevated by supplemental zinc ($P = 0.05$) (**Table 3**). Serum vitamins C ($P = 0.05$), E ($P = 0.01$) and zinc ($P = 0.001$) concentrations increased linearly, whereas MDA ($P = 0.002$) concentrations decreased as dietary zinc concentration increased (**Table 4**). An interaction between dietary zinc and temperature for these variables ($P \leq 0.05$) was detected. None of the variables differed among the three TN groups.

## TABLE 2

*Effects of zinc supplementation on performance and egg quality in laying Japanese quail reared under conditions of heat stress*[1]

| | Supplemented dietary zinc, *mg/kg* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 30 | | 60 | | | *P*-value | | |
| Item | TN[2] | HS[3] | TN | HS | TN | HS | SEM | T[4] | Zn | T × Zn |
| Final body weight, *g* | $176^{a}$ | $166^{b}$ | $178^{a}$ | $168^{b}$ | $177^{a}$ | $167^{b}$ | 2.1 | 0.001 | 0.97 | 0.68 |
| Feed intake, *g/d* | $28.0^{a}$ | $26.4^{bc}$ | $28.2^{a}$ | $26.8^{b}$ | $28.0^{a}$ | $27.0^{b}$ | 0.4 | 0.001 | 0.02 | 0.05 |
| Quail-day egg production, *%* | $88.3^{a}$ | $69.6^{d}$ | $89.5^{a}$ | $73.2^{c}$ | $89.0^{a}$ | $78.0^{b}$ | 1.7 | 0.001 | 0.01 | 0.01 |
| Feed efficiency, *g feed: g egg* | $2.37^{d}$ | $2.52^{a}$ | $2.36^{d}$ | $2.47^{b}$ | $2.36^{d}$ | $2.43^{c}$ | 0.04 | 0.001 | 0.01 | 0.05 |
| Egg weight, *g* | $11.8^{a}$ | $10.5^{d}$ | $11.9^{a}$ | $10.8^{c}$ | $11.9^{a}$ | $11.2^{b}$ | 0.2 | 0.001 | 0.01 | 0.01 |
| Eggshell thickness, *$\mu m$* | $22.2^{a}$ | $20.8^{c}$ | $22.1^{a}$ | $21.3^{b}$ | $22.2^{a}$ | $21.6^{b}$ | 0.4 | 0.001 | 0.01 | 0.05 |
| Haugh unit[5] | $89^{a}$ | $79^{c}$ | $90^{a}$ | $83^{bc}$ | $90^{a}$ | $85^{b}$ | 1.6 | 0.001 | 0.05 | 0.08 |

[1] Values are means, $n = 10$. Means in a row without a common letter differ, $P < 0.05$.

[2] Thermoneutral (22°C) (6 wk).

[3] Heat stress (34°C) (3 wk).

[4] Temperature.

[5] Haugh unit, $100 \times \log (H + 7.57 - 1.7 \times W^{0.37})$ where H is the albumen height (mm) and W is the egg weight (g).

## TABLE 3

*Effects of zinc supplementation on apparent digestibility of nutrients in laying Japanese quail reared under conditions of heat stress*[1]

| | Supplemented dietary zinc, *mg/kg* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 30 | | 60 | | | *P*-value | | |
| Item | TN[2] | HS[3] | TN | HS | TN | HS | SEM | T[4] | Zn | T × Zn |
| | *g/100 g* | | | | | | | | | |
| Dry matter | $64^{a}$ | $60^{c}$ | $65^{a}$ | $62^{b}$ | $64^{a}$ | $62^{b}$ | 0.6 | 0.001 | 0.05 | 0.05 |
| Organic matter | $71^{a}$ | $67^{b}$ | $70^{a}$ | $69^{ba}$ | $71^{a}$ | $69^{ba}$ | 0.5 | 0.001 | 0.04 | 0.05 |
| Crude protein | $79^{a}$ | $74^{c}$ | $78^{a}$ | $75^{cb}$ | $78^{a}$ | $76^{b}$ | 0.8 | 0.001 | 0.02 | 0.05 |
| Ether extract | $84^{a}$ | $75^{d}$ | $83^{a}$ | $78^{c}$ | $84^{a}$ | $80^{b}$ | 1.5 | 0.001 | 0.01 | 0.05 |

[1] Values are means, $n = 10$. Means in a row without a common letter differ, $P < 0.05$.
[2] Thermoneutral (22°C) (6 wk).
[3] Heat stress (34°C) (3 wk).
[4] Temperature.

## DISCUSSION

Zinc is an important component of biological antioxidant systems and it is required for optimum performance, growth and modulation of the immune system; this is due in part to its role as a cofactor of various enzymes (15,20,21). Growth rate, feed intake and feed efficiency decrease, but excretion of minerals increases when the ambient temperature is above the TN zone, which is between 18 and 22°C for Japanese quail (4). The reduction in feed consumption and increase in zinc excretion adversely affect poultry performance, health status and antioxidant system. Moreover, stress causes an accumulation of zinc in the liver, decreasing plasma zinc concentration; thus, it may exacerbate a marginal zinc deficiency or an increased zinc requirement (34). In the present study, although zinc supplementation did not affect the variables measured in quail housed at TN temperatures, feed intake, egg production and feed efficiency as well as egg quality of laying Japanese quail reared under HS conditions (34°C) were improved by supplementation (Table 2). The reduced egg production, quality and feed efficiency in heat-exposed quail might be due to the reduction in feed intake and impairment in utilization of nutrients. Zinc deficiency has been shown to decrease alkaline phosphatase, resulting in depression in bone and egg shell formation (35–37). Zinc supplementation improved the egg quality variables in the present study. Moreng et al. (38) reported that dietary zinc supplementation significantly improved shell breaking strength, shell weight and percentage of shell defects. Similarly, Sahin et al. (12) reported that zinc and chromium supplementation improved egg production and quality in laying hens under conditions of low temperature.

Results concerning the apparent digestibility of nutrients in the present study also support the benefits of supplementation by dietary zinc (Table 3). High ambient temperatures were reported to suppress nutrient digestibility in poultry (4). Wallis and Balnave (39) found that the digestibility of amino acids was decreased by high environmental temperature in broilers. Hai et al. (40) reported that the activities of trypsin, chymotrypsin and amylase decreased significantly at a high temperature of 32°C. In the present study, supplementation of dietary zinc increased digestibility of nutrients, reversing the negative effects of the stress in Japanese quail. Because zinc has a protective effect on pancreatic tissue against oxidative damage (14,41), it may help the pancreas to function properly, including secretion of digestive enzymes, thus improving digestibility of nutrients. Onderci et al. (14) reported that supplemental chromium and zinc ameliorated the decrease in digestibility of DM, CP and EE in laying hens reared under a low temperature.

Environmental stress causes increased free radical production (7) and lowers the concentrations of antioxidants such as vitamins E, C and A and minerals such as Zn and Cr in serum (9,34,42). Shaheen et al. (43) reported that dietary zinc deficiency caused increased lipid peroxidation, and this was inhibited by zinc supplementation. In the present study, significantly higher serum concentrations of vitamins C and E and

## TABLE 4

*Effects of zinc supplementation on antioxidant status in laying Japanese quail reared under conditions of heat stress*[1]

| | Supplemented dietary zinc, *mg/kg* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 30 | | 60 | | | *P*-value | | |
| Item | TN[2] | HS[3] | TN | HS | TN | HS | SEM | T[4] | Zn | T × Zn |
| Serum MDA,[5] *μmol/L* | $2.10^{d}$ | $3.43^{a}$ | $2.00^{d}$ | $2.93^{b}$ | $2.00^{d}$ | $2.75^{c}$ | 0.09 | 0.001 | 0.009 | 0.05 |
| Liver MDA, *μmol/g* | $4.2^{d}$ | $5.8^{a}$ | $4.2^{d}$ | $5.3^{b}$ | $4.0^{d}$ | $5.3^{b}$ | 0.3 | 0.001 | 0.01 | 0.05 |
| Serum vitamin C, *μmol/L* | $59.0^{a}$ | $31.6^{d}$ | $60.5^{a}$ | $35.6^{c}$ | $60.6^{a}$ | $39.3^{b}$ | 2.3 | 0.001 | 0.05 | 0.05 |
| Serum vitamin E, *μmol/L* | $2.07^{a}$ | $1.38^{d}$ | $2.13^{a}$ | $1.53^{c}$ | $2.15^{a}$ | $1.80^{b}$ | 0.08 | 0.001 | 0.04 | 0.01 |
| Serum zinc, *μmol/L* | $31.3^{a}$ | $24.9^{d}$ | $30.4^{a}$ | $26.8^{c}$ | $31.2^{a}$ | $28.9^{b}$ | 1.6 | 0.001 | 0.001 | 0.01 |

[1] Values are means, $n = 10$. Means in a row without a common letter differ, $P < 0.05$.
[2] Thermoneutral (22°C) (6 wk).
[3] Heat stress (34°C) (3 wk).
[4] Temperature.
[5] MDA, malondialdehyde.

Zn occurred in birds receiving zinc supplementation. Zinc supplementation decreased serum and liver MDA levels in stressed birds. Similar to our results, Onderci et al. (14) reported that Zn and chromium supplementation decreased serum MDA concentrations and increased the concentrations of vitamins C, E and A in cold-stressed laying hens.

The mode of action of zinc in antioxidant defense system in vivo is yet to be elucidated (20,44). The reduced lipid peroxidation in Zn-supplemented birds might be due to the multifunctional roles of zinc, which include the induction of metallothionein, modulation of the transition elements and its relationship with the antioxidant vitamins such as vitamin A and E (15). Zinc is a cofactor of the main antioxidative enzyme CuZn-superoxide dismutase; it may play a key role in suppressing free radicals and in inhibiting NADPH-dependent lipid peroxidation (16,45) as well as in preventing lipid peroxidation via inhibition of glutathione depletion (46). One of the proposed mechanisms of zinc's action is its capacity to displace transition metals (Fe, Cu) from binding sites. Zinc can compete with iron and copper to bind to the cell membrane and decrease the production of free radicals, thus exerting a direct antioxidant action (16,25,47). Zinc induces the production of metallothionein, an effective scavenger of hydroxyl radicals and it has been suggested that Zn-metallothionein complexes in the islet cells provide protection against immune-mediated free-radical attack (16,43).

It is apparent from the results of the present study that dietary zinc supplementation offers a feasible way to reduce the losses in performance of Japanese quail reared under conditions of heat stress; supplementation with 60 mg zinc/kg diet appeared to be the most efficacious dose.

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