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Effects of Dietary Zinc and L-Arginine Supplementation on Total Antioxidants Capacity, Lipid Peroxidation, Nitric Oxide, Egg Weight, and Blood Biochemical Values in Japanase Quails

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Abstract The aim of this study was to evaluate effects of dietary zinc and L-arginine supplementation on blood total antioxidant capacity (TAC), malondialdehyde (MDA), nitric oxide (NO), some blood chemistry parameters, and egg weights of laying quails. Three groups of Japanese quails were fed with a diet containing L-arginine (5 mg/kg), zinc (60 mg/kg), and normal basal diet (control) for 30 days. TAC, lipid peroxidation, and biochemical analysis were performed in the blood of animals. L-Arginine and zinc supplementation improved TAC and reduced MDA concentrations compared to the control ($P<0.05$). In comparison to the control, blood NO concentrations were increased by L-arginine ($P<0.01$) and zinc treatment ($P<0.05$). Both zinc ($P<0.001$) and L-arginine ($P<0.01$) supplementation significantly increased egg weight in laying quails. Some of the blood chemistry parameters were also altered by the treatment of L-arginine and zinc supplementation. No difference was found in blood albumin and creatinine levels among the groups. Blood glucose ($P=0.833$) and total protein ($P=0.264$) levels in control and L-arginine-treated groups were found to be similar. Glucose and total protein levels were decreased in zinc-supplemented animals compared to the control and L-arginine groups ($P<0.05$). No difference was found in triglyceride levels between control and zinc-applied groups ($P=0.197$). However, L-arginine treatment reduced the blood triglyceride levels compared to the control ($P<0.05$). In conclusion, L-arginine and zinc supplementation could be beneficial and effective for decreasing oxidative stress, boosting antioxidant capacity, and improving egg weight in the blood of the animals.

Keywords Zinc · L-Arginine · Total antioxidant capacity · Nitric oxide · Egg weight

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Introduction

Zinc is a hydrophilic metal which is found in the structure of more than 300 enzymes and molecules in the body. It is an essential element in many physiological functions including growth, immune response, reproduction, and antioxidant defense [1–4]. In addition, zinc plays an important role in pregnancy, bone growth, milk production, and egg production [5, 6]. Zinc was also reported to have antioxidant effect. It was shown that zinc supplementation could alleviate oxidative damage, and this could be due to the direct antioxidant effect of zinc by occupying the iron- and copper-binding locations in DNA, proteins, and lipids [7, 8]. Furthermore, zinc was shown to increase some antioxidant proteins such as metallothionein, which is an important metal-binding protein with antioxidant effect in iron-mediated oxidative stress and regulatory functions in redox status of the cells [9, 10]. Superoxide dismutase (SOD), which structurally contains zinc, protects the cells from oxidative stress by catalyzing the dismutation of the superoxide anion and is known to be increased following zinc treatment [11, 12].

L-Arginine is an essential amino acid which plays a number of important roles in metabolic activities. Nitric oxide (NO), a paramagnetic free radical, was synthesized by the oxidation of L-arginine through catalytic action of nitric oxide synthetase during citrulline formation. It is a versatile molecule with numerous functions in the body [13, 14]. Other than NO, L-arginine is also involved in the synthesis of creatine, ornithine, glutamate, polyamines, proline, glutamine, agmatine, and dimethylarginines having an important role in nutrition and physiology [1, 15]. Similar to zinc, supplementation of L-arginine was shown to alleviate oxidative damage and inflammation in the myocardium in response to exhaustive exercise in rats [16]. Arginine is also known to regulate pancreatic, pituitary, and placental hormones; hence, it affects metabolism of proteins, amino acids, glucose, fatty acids, and development [1].

In this study, it is aimed to evaluate the effects of zinc and L-arginine supplementation on egg weights, plasma total antioxidant capacity (TAC), and lipid peroxidation as well as some blood parameters including plasma NO, glucose, triglyceride, total protein, albumin, and creatinine concentrations in laying quails.

Materials and Methods

Sixty laying Japanese quails (*Coturnix coturnix japonica*) at 20 weeks of age with an average live body weight of 220±20 g were obtained from Animal Research Farm of Kafkas University. All animals were weighed before the experiment, and then the animals were allocated to three equal groups of 20 animals each. Animals were kept in cages in a temperature-controlled room at 22±2°C with a light period of 12 h light/12 h dark cycle. Animals were fed with a basal diet composed of 21.75% crude protein and 3,043 kcal kg^{-1} of metabolic energy (Table 1). The basal diets were formulated using National Research Council (NRC) [17]. Group 1 was fed basal diet and served as the control. Group 2 was fed with a basal diet and supplemental $ZnSO_4 \cdot 7H_2O$ equivalent of 60 mg elemental Zn per kilogram of diet. Group 3 was fed with a basal diet and supplemental L-arginine (5 mg/kg of diet) for 30 days. Control and experiment groups were allowed free access to feed and water. Egg weights were taken and recorded.

Blood samples were collected into the heparinized tubes from the wing vein at the end of the treatment. The samples were centrifuged for 10 min at 3,000 rpm. After centrifugation,

Table 1 Ingredients and Chemical Composition of the Diet Fed to Quails

Ingredients (g/100 g)	Percent	Analyzed contents of nutrients	Percent
Corn	61.25	Dry matter	90.07
Soybean meal	29.50	Crude protein	21.75
Fish meal	4.00	Crude extract	5.98
Limestone powder	3.30	Crude fiber	3.25
Sodium chloride	0.35	Crude ash	3.22
Dicalcium phosphate	0.90	N-free extract	55.87
DL-Methionine	0.10	ME (kcal/kg)	3,043
Lysine	0.10		
Vitamin–mineral premix[a]	0.50		

[a] Mixture supplied per kilogram of diet: all-trans-retinyl acetate, 6,000 IU; cholecalciferol, 800 IU; DL-α-tocopherol acetate, 1.25 mg; menadione sodium bisulfite, 2,5 mg; thiamine hydrochloride, 1.5 mg; riboflavin, 3 mg; D-pantotenic acid, 5 mg; pyridoxine hydrochloride, 2.5 mg; vitamin B_{12}, 0.0075 mg; folic acid, 0.25 mg; niacin, 12.5 mg; Mn ($MnSO_4 \cdot H_2O$), 50 mg; Fe ($FeSO_4 \cdot 7H_2O$), 30 mg; Cu ($CuSO_4 \cdot 5H_2O$), 5 mg; Zn ($ZnSO_4 \cdot 7H_2O$), 16 mg; I (KI), 0.5 mg; Se (Na_2SeO_3), 0.15 mg; Co ($CoCI \cdot 6H_2O$), 0.1 mg; choline chloride, 125 mg

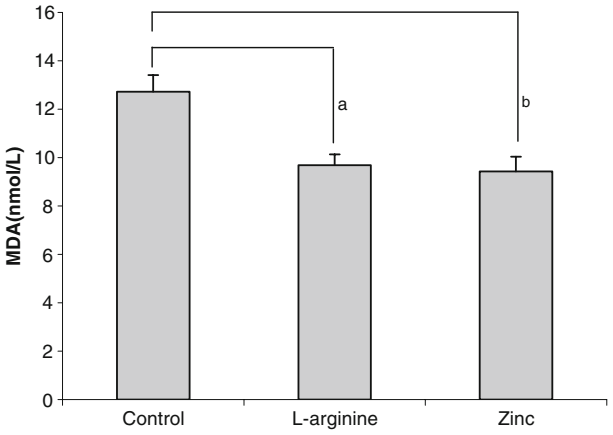
the plasma was collected and stored at −50°C until the day of assay. TAC, lipid peroxidation, and biochemical analysis were performed in the blood of animals. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) concentrations in plasma by the method of Mihara and Uchiyama [18]. The method is based on the reaction between MDA (an aldehyde of lipid peroxidation product) and thiobarbituric acid. MDA forms a pink-colored complex with thiobarbituric acid. The absorbance of the solution containing the complex was read at 532 nm using a spectrophotometer (UV-1201, Shimadzu, Japan) to estimate the lipid peroxidation.

TAC was determined colorimetrically using a commercially available kit (Rel Assay, Turkey) in plasma of the animals by the method of Erel [19]. Antioxidants in the sample reduce dark blue–green-colored 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample. NO concentrations were determined using a spectrophotometer (PowerWave XS, BioTek, Instruments, USA) in plasma samples by the method of Miranda et al. [20]. Initially, plasma samples were deproteinized with 10% zinc sulphate. Total NO concentrations (nitrate and nitrite) were determined calorimetrically by the acidic Griess reaction via reaction involving reduction of nitrate to nitrite by vanadium (III) chloride [20].

Plasma glucose, triglyceride, total protein, albumin, and creatinine concentrations were measured using a biochemical analyzer (Olympus AU-660, Japan).

Statistical Analysis

The data were initially tested for normal distribution by one-sample Kolmogrov–Smirnov. Following the confirmation of normal distribution ($P>0.05$), the data were analyzed by analysis of variance which is followed by post hoc Tukey test using SPSS Windows 10.0. All data were presented as mean±SE. Values were considered statistically significant if P value is <0.05.

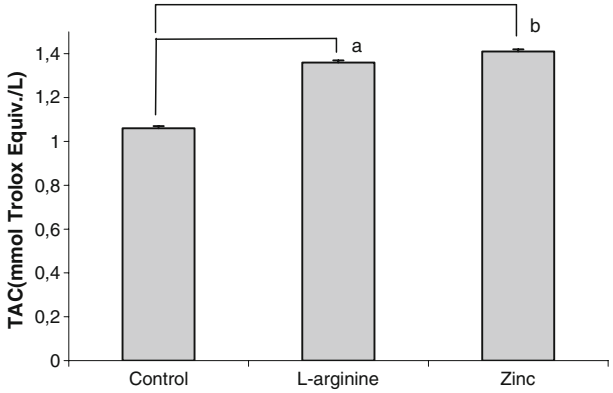


(a) P<0.003 versus control.
(b) P<0.002 versus control

Fig. 1 Effects of L-arginine and zinc treatments on blood MDA in Japanese quails. $P<0.003$ versus control. $P<0.002$ versus control

Results

At the end of the 30-day trial period, blood MDA concentration in L-arginine ($P<0.01$) and zinc ($P<0.01$) supplemented groups was significantly lower than in the control group (Fig. 1). Blood TAC concentrations of L-arginine-supplemented ($P<0.05$) and zinc-supplemented ($P<0.01$) groups were higher compared to that of the control (Fig. 2). However, there was no significant difference between arginine- and zinc-treated groups with respect to MDA ($P=0.952$) and TAC ($P=0.876$) levels. Blood NO concentrations were increased by L-arginine ($P=0.002$) and zinc treatment ($P=0.02$) compared to those of the control (Fig. 3). No difference was found in blood albumin and creatinine levels among the groups ($P>0.05$). Blood glucose ($P=0.833$) and total protein ($P=0.264$) levels in the control and L-arginine-treated groups were found to be similar. Glucose ($P<0.05$) and



(a) P<0.016 versus control.
(b) P<0.005 versus control

Fig. 2 Effects of L-arginine and zinc treatments on blood TAC in Japanese quails. $P<0.016$ versus control. $P<0.005$ versus control

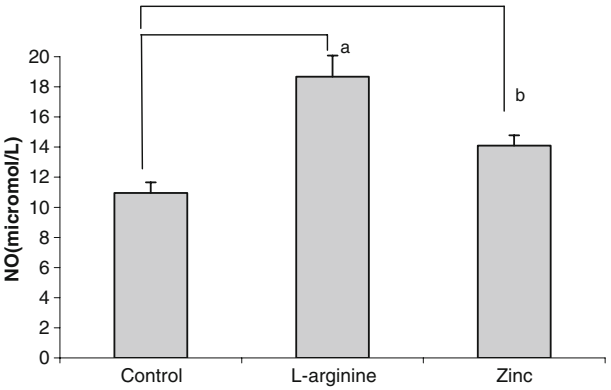


Fig. 3 Effects of L-arginine and zinc treatments on blood NO in Japanese quails. $P<0.002$ versus control. $P<0.020$ versus control

total protein ($P<0.001$) levels were decreased in zinc-supplemented animals compared to the control (Table 2). Glucose ($P<0.05$) and total protein ($P<0.01$) levels in zinc-supplemented animals were also lower than in the L-arginine group. No difference was found in triglyceride levels between control and zinc-applied groups ($P=0.197$). However, L-arginine treatment reduced the blood triglyceride levels compared to the control ($P<0.05$). In comparison to average egg weights among the groups, L-arginine-supplemented ($P<0.01$) and zinc-supplemented ($P<0.001$) quails had higher average egg weight than in the control. On the other hand, egg weights in L-arginine- and zinc-treated groups were similar (Table 2).

Discussion

In the present study, both L-arginine and zinc treatment increased TAC. It was reported that L-arginine treatment improves antioxidant status and decrease oxidative stress. These effects

Table 2 Effects of L-Arginine and Zinc Treatments on Some Blood Parameters and Egg Weight in Japanese Quails

Groups ($n=20$)	Glucose (mg/dL)	Triglyceride (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Creatinine (mg/dL)	Egg weight (g)
Control	368.78±2.78	134.57±9.41	5.44±0.20	2.15±0.01	0.18±0.00	11.45±0.23
L-Arginine	360.42±10.32	98.87±6.08	5.01±0.20	2.36±0.12	0.29±0.01	12.90±0.26
Zinc	323.51±14.13	113.26±9.50	4.07±0.16	2.35±0.01	0.21±0.00	13.68±0.40
Statistical comparisons (P)						
Control versus L-arginine	0.833	0.016	0.264	0.329	0.139	0.007
Control versus zinc	0.011	0.197	0.000	0.366	0.794	0.000
L-Arginine versus zinc	0.043	0.464	0.004	0.997	0.397	0.197

were due to the increased level of glutathione (GSH) and glutathione/glutathione disulfide (GSSG) ratio as well as decreased lipid peroxidation in exercise-induced oxidative stress in rats [16]. L-Arginine is involved in the regulation of NADPH which is used in the production of GSH from GSSG [1]. It was shown that L-arginine has strong radical scavenging activity against oxygen radicals [21]. Wascher et al. [22] reported that L-arginine is capable of reducing copper-induced lipid peroxidation and also scavenges superoxide radical. NO is a known potential scavenger of superoxide radical leading to inactivation of superoxide radical [23]. It was suggested that the superoxide scavenging effect is due to the arginine-mediated increase in NO production [22, 23]. Accordingly, L-arginine treatment increased the NO concentration compared to the control in the present study.

Arginine plays an important role in many metabolic events via NO and its metabolites including ornithine, proline, glutamate, creatine, and agmatine. Furthermore, arginine is involved in the regulation of metabolism of protein, amino acids, glucose, and fatty acids and could affect the secretion of insulin and glucagon [1]. Most of the blood chemistry parameters including glucose, total protein, albumin, and creatinine levels (except for triglyceride level) were not affected by L-arginine treatment in this study. It was reported that arginine decreased blood glucose level in normal subjects [24]. In the present study, no effect was observed on blood glucose level following L-arginine treatment in quails. The beneficial effect of L-arginine on cardiovascular disease including atherosclerosis and vascular health was reported by several studies [25, 26], yet there are also several studies indicating no beneficial effect on vascular health [27, 28]. In the current study, blood triglyceride level was found to be lower in L-arginine-treated quails than in the control.

Our result indicated that mean egg weight was increased in L-arginine-treated quails compared to the control. NO has been postulated to regulate follicular development, ovulatory mechanisms and egg production [29, 30]. Manwar et al. [31] reported that there is an association between the size of ovarian follicles and NO metabolites, nitrite (NO_2) and nitrate (NO_3), levels in serum and hypothalamus of laying quails. Furthermore, L-arginine treatment increased the egg production and follicular NO_2 and NO_3 concentration without any change in egg weights.

Similar to L-arginine treatment, zinc-supplemented quails had reduced lipid peroxidation in the blood along with increased TAC concentrations. It is known that zinc have protective effects against free radical injury, lipid peroxidation, and cell membrane structure [4]. Zinc is a structural part of SOD which is an important element of antioxidant enzymes against ROS attack and lipid peroxidation. Superoxide radicals are scavenged by SOD in the presence of zinc cofactor [32]. Protection from the free radical damage by zinc is also related to another class of antioxidants known as metallothioneins which are major zinc-binding proteins playing an important role in protection against oxidative stress [8]. It was shown that zinc can protect from lipid peroxidation due to its direct antioxidant action by occupying iron- and copper-binding sites in lipids, proteins, and DNA [7, 8]. Shaheen and El-Fattah [12] reported that zinc deficiency resulted in increased lipid peroxidation in the blood, liver, and pancreas along with a decrease in antioxidant status. Supplementation of zinc reversed these alterations. It was suggested that zinc supplementation could reverse these alterations by increasing the metallothionein synthesis, inhibition of NADPH-cytochrome reductase, and stimulation of glutathione peroxidase. In the present study, TAC was increased in zinc-supplemented quails compared to the control. Improvement in antioxidant status following zinc treatment was shown in a number of studies including in heat-stressed quails [33], in Wilson's disease [34], in chlorpyrifos toxicity [9], and in zinc deficiency [33].

With regard to blood chemistry parameters, zinc supplementation had no effect on blood triglyceride, albumin, and creatinine levels. However, there was a reduction in blood glucose and total protein concentrations. It was reported that rats fed with zinc-deficient diet showed decreased protein levels in the brain of growing rats, and the decrease was attributed to the role of zinc in DNA synthesis and protein metabolism [32]. In another study, dietary zinc deficiency resulted in alterations in hematological parameters. Zinc-deficient rats had decreased total protein, globulin, glucose, and high-density lipoproteins but increased albumin, total lipids, cholesterol, and triglycerides. Urea and creatinine was unaffected in the rats [35].

In the present study, we have observed positive effects of zinc supplementation on egg weight. Dietary zinc increased egg weight in comparison to the control group. Zinc is an important element in reproductive functions due to its biological roles in many enzyme systems and proteins. Zinc deficiency could affect female reproductive functions adversely [3].

In conclusion, data from the current study demonstrate that L-arginine and zinc supplementation could be beneficial and effective for decreasing oxidative stress, boosting antioxidant capacity, and improving egg weight in Japanese quails. These two dietary factors are also able to alter some blood parameters in healthy quails.

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