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(Research Article)

The effects of dietary calcium tetraborate on the ovalbumin levels and some egg quality traits in laying quails exposed to cadmium

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Abstract

Cadmium (Cd) is a toxic heavy metal and one of the most important environmental pollutant that is derived from agricultural and industrial sources. Boron (B) is a metalloid that has beneficial physiological effects on the metabolism of animals and humans. This research is aimed at investigating the effect of calcium tetraborate (CaB_4O_7) on the ovalbumin levels of quail's egg and some egg quality traits in laying quails exposed to Cd. For this purpose, one hundred twenty Japanese quail 6-week-old were randomly divided into 4 groups (5 replicates each). During the 8 weeks, quails were fed a basal diet (control) and basal diet supplemented with Cd (100 mg of $CdCl_2$), CaB_4O_7 (300 mg of CaB_4O_7), and Cd + CaB_4O_7 (100 mg of $CdCl_2$ and 300 mg of CaB_4O_7). A total of 160 eggs including 40 eggs from each group were collected for assessment of the quality of the egg and the ovalbumin level of the eggs. Compared with the Cd group, supplementation of Cd + CaB_4O_7 increased the egg weight ($P < 0.01$). Results of this study indicated that dietary supplementation with Cd or CaB_4O_7 was not affected significantly albumen pH, yolk ratio, and yolk color ($P > 0.05$). Regarding the egg quality, the significant effect of Cd or CaB_4O_7 ($P < 0.01$) was found in shape index, yolk weight, albumen weight, and yolk pH. The dietary combination of Cd and CaB_4O_7 led to the greatest increases in albumen ratio ($P < 0.01$). The Cd group had the highest ovalbumin level, while CaB_4O_7 and Cd + CaB_4O_7 groups had the same ovalbumin level. The findings of this study revealed that provided new insight into the toxicity of the Cd on ovalbumin level and some egg quality traits and CaB_4O_7 could be used as a potential strategy to ameliorate Cd toxicity.

Keywords: Calcium tetraborate; Egg; Ovalbumin; Egg quality; Lapanese quail.

1. Introduction

Cadmium (Cd) is one of the most toxic heavy metals that does not have a physiological function. Cd is an important environmental pollutant that is derived from natural, agricultural and industrial sources. It can cause Cd toxicity in humans, animals, and poultry through the air, water, and food chain. Feedstuffs may also contain Cd. Contamination of diets and the environment with heavy metals affects food safety and consumers' health. Contamination of poultry diets with chronic Cd exposure at low doses adversely affects growth, feed conversion efficiency, and egg production besides health, and this may lead to a huge economic loss. In poultry, Cd bioaccumulation occurs mainly in the liver, kidney, lung, and reproductive organs [1]. On the other hand, egg quality is an important factor and is affected by many factors, such as nutrition, environmental factors, and diseases [2]. Moreover, Cd residue may occur in the eggs after chronic exposure [1].

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Cadmium toxicity causes oxidative stress, an increase in metallothionein synthesis, as well as impairment in Zn, Ca, and Fe metabolism, enzyme functions, and plasma membrane integrity. Vitamins, minerals, and herbs have been studied to reduce the negative effects of cadmium, but few studies have been conducted on poultry [1, 3, 4, 5]. Based on these studies, it has been reported that some dietary supplements may be effective against cadmium toxicity.

Boron is a trace element that has an important role in cell membrane function, enzymatic reactions, mineral, and hormone metabolism. Boron affects mineral metabolism by interacting with Ca, Mg, P, and vitamin D [6]. Previous studies have indicated that boron increases the production of catalase, superoxide dismutase and glutathione in tissues, strengthens the antioxidant defense system, has an immunostimulating effect, and causes an increase in steroid hormones (plasma estradiol and testosterone) [7, 8].

Albumin is a globular protein that has non-enzymatic activity has some crucial roles in metabolism. One of these roles is binding activity. Due to restricted or non-enzymatic activity, albumin binds to other molecules and forms some bio compound. Ovalbumin is found abundantly in albumen (egg white) and referred to as egg albumin. Ovalbumin is an essential substance in the food sector. The bakery and wine industry is the most utilizing sector of ovalbumin due to its foaming property. Ovalbumin level determines the foaming capacity of albumen, therefore this property placed significantly in the food sector [9]. In this study, we hypothesized that dietary CaB_4O_7 may be potentially protective in ameliorating Cd-induced toxicity. The aim of this study was to investigate the effects of dietary supplementation of CaB_4O_7 on the ovalbumin levels and some egg quality traits in laying quails exposed to Cd.

2. Material and methods

2.1. Experimental design and diet

All experimental procedures were approved by Firat University, Animal Experiments Local Ethics Committee (2020/11). A total of 120 Japanese laying quails (6-week-old) were randomly divided into 4 groups (30 quails/each group), each group of quails was sub-divided into five replicates (4 females and 2 males/replicate). The experimental groups were assigned as follows: (i) control group (0 mg of Cd and CaB_4O_7 per kg of diet), (ii) Cd group (100 mg of $CdCl_2$ (Sigma-Aldrich (Cadmium chloride anhydrous)) per kg of diet), (iii) CaB_4O_7 group (300 mg of CaB_4O_7 per kg of diet, 22.14% elemental B/kg diet), (iv) Cd + CaB_4O_7 group (100 mg of $CdCl_2$ and 300 mg of CaB_4O_7 per kg of diet). The doses of Cd and CaB_4O_7 were chosen based on previous studies [1, 10].

Table 1 Ingredients and nutrient composition of experimental diet (%)

Experimental diet				
Ingredients	**%**		**Nutritional composition**	**%**
Maize	56.00		Dry matter, %	90.50
Soybean meal (44% CP)	26.80		Crude protein, %	17.50
Sunflower meal (28% CP)	1.20		Crude cellulose, %	3.65
Wheat bran	2.10		Ether extract, %	4.00
Sunflower oil	2.35		Crude ash, %	13.58
Sodium chloride	0.35		Phosphorus	0.35
L-Lysine hydrochloride	0.15		Lysine	1.00
L-Treonine	0.10		Threonine	0.74
Sodium bicarbonate	0.20		ME, kcal/kg	2750
DL-Methionine	0.10		Nutritional composition	90.50
Vitamin-Mineral premix	0.35		Dry matter, %	17.50
Ground limestone	8.00		Crude protein, %	3.65
Dicalcium phosphate	2.30		Crude cellulose, %	4.00
Total	100		Ether extract, %	13.58

Vitamin-mineral premix (per 1kg): vitamin A, 8000 IU; vitamin D3, 3000 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B12, 0.02 mg; biotin, 0.1 mg; folacin, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin, 3 mg copper (copper sulphate), 10 mg; iodine (ethylenediamine, dihydriodide), 1.0 mg; iron (ferrous sulphate monohydrate), 50 mg; manganese (manganese sulphate monohydrate), 60 mg; and zinc (zinc sulphate monohydrate), 60 mg, selenium (sodium selenite), 0.42 mg.

The quails were housed in a controlled environment with a 16 h light: 8 h dark/day program. Feed (17.5% crude protein and 2750 kcal/kg metabolizable energy) and water were offered ad libitum to the quails. The basal diet was prepared according to NRC [11] and shown in Table 1. The nutrient composition and the mineral content (Cd, B, etc.) of the diet used in the study was determined (Table 2) [12, 13, 14, 15, 16]. The duration of the experiment was 8 weeks.

Table 2 Mineral composition of diet

Mineral	B	Cd	Ca	Mg	Na	Fe	K	Mn	Cu	Zn
%	0.125	ND	6.335	0.280	0.428	0.023	0.919	0.016	0.001	0.020

ND: Not detected; Detection limit of: Cd < 0.0001.

2.2. Preparation and characterization of calcium tetraborate

In this study, calcium tetraborate (CaB_4O_7) compound was synthesized by high temperature solid state synthesis methods. Initial materials were $CaCO_3$ (98.5% pure, Merck), H_3BO_3 (99.5% pure, Merck), and CO $(NH_2)_2$ (99.5% pure, Merck). Crystal structure of synthesized compound was characterized by X-ray Diffraction (XRD). XRD analysis was carried out by Rigaku MiniFlex X-ray diffractometer (XRD) with Cu-Kα radiation (λ = 1,54056 Å, in the 2θ range of 3°–60° at 2°/min scan rate). According to the XRD results in Figure 1 were matched with the standard data available JCPDS Card no: 00-031–0253. In this study, (CaB_4O_7) compound has been synthesized successfully [17].

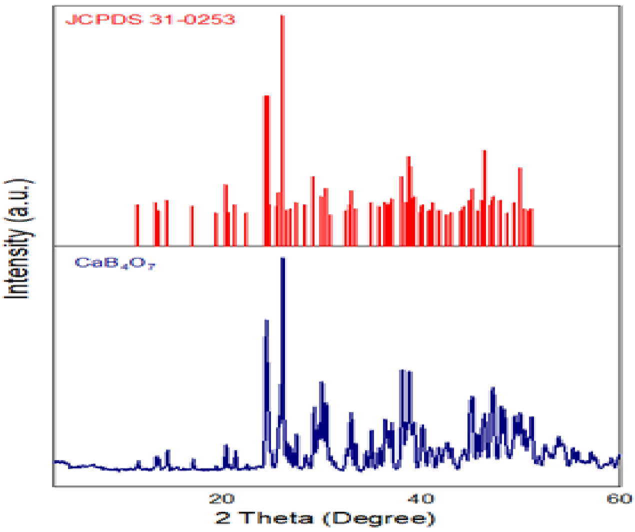

Figure 1 The X-ray pattern of synthesized calcium tetraborate

2.3. Egg quality traits

At the end of the experimental period, a total of 160 eggs including 40 eggs from each group were collected for egg quality parameters. All eggs collected in the last week of the study were examined. These eggs were kept at 4 °C and analyzed within 2 days after collection to determine egg quality traits. The collected eggs were evaluated for the pH and weights of albumen and yolk as well as yolk color and shape index. Yolks and albumens were separated and weighed. The length and width of egg were determined by using an electronic digital caliper (Tresna, 0-300 mm, USA). The egg-shape index was calculated with the formula width×100/length. Egg yolk color was scored using the Roche yolk color fan. The pH of egg yolk and albumen was measured using a pH meter (Hanna Instruments, HI99163, and USA).

2.4. SDS-PAGE analysis

Each albumen was homogenized with 25 mM Tris (pH=7.4) buffer 1/10 (v/v). The total protein amount of the albumen was determined by Nano drop spectrophotometer (Thermo Scientific™ NanoDrop™ 2000/2000c). The 25 µg/30 µL total protein was loaded into wells. Electrophoresis was carried out at a constant voltage of 135 V (Bio-Rad, Mini Protean Tetra, and USA). The gels removed carefully when migration completed and stained with the 1% coomassie brilliant blue dye solution for 1 hour. Thereafter, the dye was removed with the distaining solution (a mixture of pure methanol, glacial acetic acid, distilled H2O). Relative densitometric (RD, %) values of bands were analyzed by ImageJ (NIH image) software after grayscale calibration [9].

2.5. Statistical analysis

The data were subjected to the One-Way ANOVA test using the SPSS 21 package program after testing the normality with Shapiro-Wilk and homogeneity of variances with Levene's test. Tukey post hoc test was utilized for determining which group was different. The data were presented as the mean and standard error. The results were considered significant at $P \leq 0.05$ [18].

3. Results and discussion

Egg quality characteristics of the experimental groups are shown in Table 3. The shape index resulted in 78.28%, 80.81%, 78.81% and, 76.09% in control, Cd, CaB_4O_7 and, Cd + CaB_4O_7 groups, respectively. Egg shape index has an important effect in determining some egg quality parameters. More round or elongated eggs do not fit in the egg cartons properly. These eggs are susceptible to breaking during transportation and, chicks are unable to hatching from such eggs [2]. The present study showed that Cd had a significant impact on shape index ($P < 0.01$) however, the shape index had not affected by the boron supplementation compare with control ($P > 0.05$). Consistent with our results, a previous study showed that shape index was not affected by boric acid (60 and 180 mg/kg) supplementation to the diets [19]. Similar result also was presented by Koksal et al. [20]. They observed that there were no significant effects of dietary 90 mg/kg boric acid on shape index. In another study, no significant effect of 75 and 150 mg/kg boron supplementation on shape index was observed [21]. In this study, the more rounded eggs were obtained from Cd group. This fact is related to that Cd caused uterus oedema in birds [22]. It is observed that CaB4O7 ameliorated the rounded shape index. On the other hand, it is determined that Cd accumulation were reduced by boron and calcium in organisms [1]. Similarly, in the current study, the boron and calcium could be helped the normalization of the shape index values.

There are only a few studies on egg quality parameters of cadmium. Abou-Kassem et al. [4] reported that ascorbic acid or natural clay decreases the negative effects of Cd and improves some egg quality traits of laying Japanese quails. Korénekova et al. [23] found that Cd (0.12 mg/day per quail) had adverse effects on the quality parameters of eggs. Zhu et al. [5] found that dietary supplementation with Cd at 60.67 mg/kg decreased egg quality and disrupted the endocrine system in the eggshell gland of laying hens. Olgun and Bahtiyarca [24] demonstrated that 0, 60, and 120 mg/kg boron added to the basal diet failed to prevent adverse effect of cadmium (15 or 45 mg/kg) on egg quality.

Abou-Kassem et al. [1] observed that the egg weight was significantly decreased in quails fed by Cd polluted diets. In the present study, the Cd group had a lower egg weight than the control group. This may explain by the adverse effects of cadmium in the egg formation pathway. Compared with the Cd group, supplementation of Cd + CaB_4O_7 increased the egg weight. These results indicated that CaB_4O_7 may show a positive effect and may be prevented the suppression of calcium metabolism of cadmium [25].

As seen in Table 3, there is no significant difference was found among the control, Cd, and the Cd + CaB_4O_7 group on albumen weight, yolk weight, and yolk pH ($P > 0.05$). Similarly, Vodela et al. [26] reported that no difference in pH of albumen and yolk were observed in broiler breeder hens exposed to a chemical mixture (arsenic, 8 mg/kg; benzene, 13 mg/kg; cadmium, 51 mg/kg; lead, 67 mg/kg; and trichloroethylene, 6.5 mg/kg) in drinking water. Moreover, Sizmaz and Yildiz [27] also reported that egg yolk weight and shape index were not affected by boric acid supplementation (120 mg/kg).

In the present study, when compared with the control group, Cd or CaB_4O_7 supplementation had no impact on albumen pH and yolk color ($P > 0.05$). Similarly, El-Saadany et al. [28] reported that there was no significant effect in the shape index, yolk color score, albumen, and yolk ratios of laying hens fed diet supplemented with 100, 200, and 300 mg boron/kg feed. Additionally, our results revealed that the dietary combination of Cd and CaB_4O_7 led to the greatest increases in albumen ratio and albumen ratio increased in proportion to ovalbumin. However, there was no difference detected for the yolk ratio among the groups. The results of the current study were supported by Chen et al. [3], who reported that sub-chronic exposure to Cd (50 mg/L to water) in hens for 8 weeks was decreased yolk color and no effect in shape index. The different results between studies may be explained by the dose and exposure period of Cd.

In this study, the Cd supplemented group resulted in the highest ovalbumin level (78.16%). The CaB_4O_7 (47.13%) and Cd + CaB_4O_7 (42.19%) groups had the highest ovalbumin levels following Cd group (Figure 2). The control group had the lowest ovalbumin level (26.26%). These results agree with those by Baykalir et al. [29], who found that supplementation of boric acid into feed (300 mg/kg) increased the serum albumin levels when compared to the control group. These results may be explained by albumin binding capacity. Cadmium, calcium and boron may be bound by albumin in circulation. Thus, it formed the active biological compounds that could be passed into the eggs. In addition, ovalbumin synthesis in the oviduct is controlled by steroid hormones in birds [30]. It is proved that boron increases the synthesis of steroid hormones [7].

Table 3 Effect of cadmium and/or boron compound on some egg quality traits of the experimental groups

Traits	**Control**	**Cd**	**CaB_4O_7**	**Cd + CaB_4O_7**	**P**
Egg weight, g	11.71 ± 0.17^a	11.07 ± 0.21^b	10.62 ± 0.15^b	11.77 ± 0.16^a	**
Yolk weight, g	3.81 ± 0.07^a	3.62 ± 0.12^{ab}	3.38 ± 0.05^b	3.77 ± 0.09^a	**
Yolk ratio, %	32.53±0.30	32.58±0.67	32.02±0.50	32.30±0.50	NS
Albumen weight, g	6.02 ± 0.11^{ab}	5.88 ± 0.11^{ab}	5.61 ± 0.12^b	6.38 ± 0.09^a	**
Albumen ratio, %	51.38 ± 0.45^b	53.12 ± 0.57^{ab}	52.78 ± 0.66^{ab}	54.52 ± 0.44^a	**
Shape index, %	78.28 ± 0.41^b	80.81 ± 0.39^a	78.81 ± 0.33^b	76.09 ± 0.70^c	**
Albumen pH	8.85±0.02	8.72±0.05	8.79±0.08	8.73±0.06	NS
Yolk pH	5.86 ± 0.03^b	6.19 ± 0.03^a	5.84 ± 0.04^b	6.22 ± 0.07^a	**
Yolk color	8.33±0.19	7.77±0.20	7.76±0.19	8.23±0.19	NS

Cd: Cadmium; CaB4O7: Calcium tetraborate; a, b, c: Mean values with different superscripts within a row differ significantly; NS: non-significant; **: P < 0.01; the data are presented as mean ± SE

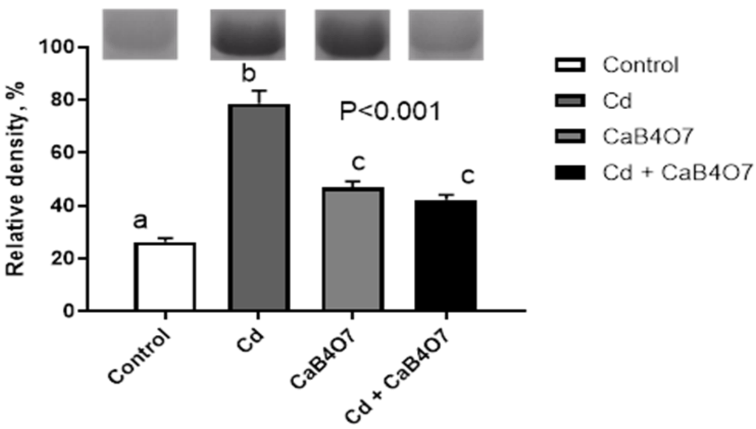

Figure 2 Effect of cadmium and/or boron compound on albumin levels of the experimental groups

4. Conclusion

Our findings indicated that there was no negative effect of the CaB_4O_7 supplementation on the egg quality traits and the ovalbumin level and CaB_4O_7 can be effective in the management of the adverse effects of Cd. We evaluated egg quality and ovalbumin levels in the eggs of Japanese quails. The present study has novel data regarding mentioned traits. Further investigation should be conducted to determine the effects of CaB_4O_7 on egg quality traits in cadmium exposure. Moreover, the CaB_4O_7 can be utilized to increase ovalbumin levels and improve albumen quality in quail eggs.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict of interest.

Statement of ethical approval

This study was approved by the Local Ethics Committee of Firat University, Elazig, Turkey (2020/11).

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