

(RESEARCH ARTICLE)

Impact of dietary calcium tetraborate supplementation on the mineral content of egg and eggshell of laying quails

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Abstract

Boron (B) is a trace element that plays an important role in the mineral, cell membrane, hormone, and enzyme metabolism of animals and humans. The aim of this study was to examine the effect of dietary calcium tetraborate (CaB_4O_7) supplementation on the mineral composition of egg content and eggshell of laying quails. For this purpose, a total of 20 male and 40 female quails, 6-week-old, were equally divided into 2 groups (control and additive groups) in 5 replicates (6 birds/replicate) and given CaB_4O_7 300 mg/kg feed in additive group. The experiment was conducted for 56 days. The eggs were collected and the mineral composition [B, calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), potassium (K), manganese (Mn), copper (Cu), zinc (Zn)] of egg content and eggshell samples were determined at the end of the experiment in randomly collected 6 eggs per group. Results of this study indicated dietary supplementation of CaB_4O_7 increased Mg ($P<0.01$), Na ($P<0.01$), and K ($P<0.05$) contents of edible parts of eggs compared to the control group, but B concentration were not determined in edible parts of eggs in both groups. Moreover, B ($P<0.01$), Mg ($P<0.01$), Na ($P<0.01$), Fe ($P<0.01$), K ($P<0.05$), Cu ($P<0.05$) and Zn ($P<0.01$) of eggshell were higher in the additive group than control. There were also significant correlations between examined minerals both edible and eggshell parts of the eggs. It may be concluded that supplementing diets with CaB_4O_7 could improve Ca metabolism, producing eggs enriched in minerals, promoting B, Mg, Na, Fe, K, Cu, and Zn deposition in eggshells, and improving eggshells quality. The effective B supplementation doses for functional egg production could be determined and B could be advantageous in terms of beneficial physiological effects.

Keywords: Calcium tetraborate; Egg; Eggshell; Mineral; Quail

1. Introduction

In today's conditions where industrial food consumption is common, egg consumption is important for a healthy life, considering it is a natural nutrient and its rich vitamin and mineral content. Egg is a very valuable food source with high protein quality among animal products, rich in vitamins (A, D, E and B groups) and mineral substances [calcium (Ca), potassium (K), sodium (Na)] [1]. It also contains all essential trace elements, including copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), selenium (Se), and zinc (Zn), and the egg yolk provides the largest contribution to the Fe and Zn requirement [2]. The nutrient composition of the egg can be changed by various adjustments to the nutrient composition of the ration. For example; it has been reported that as a result of the consumption of legume forage plants such as alfalfa by free-range chickens, the obtained eggs and chicken meat can be rich in omega 3, 6,

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carotene and vitamin E [3]. In this context, vitamins (A, D₃, E and C), mineral substances (iodine, K, Se and Fe), omega 3, and conjugated linoleic come to the fore as functional feed additives that provide transition to the product. In addition, the enrichment of eggs with colour substances (carotenoid compounds) such as lutein and lycopene has been on the agenda recently. In recent years, table eggs enriched with vitamins (A and E), omega-3 and trace minerals (Se, I) have now taken their place on the market shelves [4, 5].

The food quality of eggs is largely dependent on the diet and the health of the animal [6]. Eggs can be enriched with different foods. It is possible to produce "functional eggs" enriched with one or more functional components according to the demands of the consumers [7]. Functional food should contain higher amounts of some compounds that positively affect human health. When naturally not present in sufficient quantities, functional foods should be further enriched with these compounds. The nutritional profile of eggs can be easily enriched with omega-3, minerals and vitamins by adding these compounds to the diet. These compounds are benefit health and also reduce the risk of some serious chronic diseases [8].

Boron is a semiconductor element that has the symbol "B" in the periodic table and shows properties between metal and non-metallic [9]. It is now known that B is an essential nutrient component for humans and animals [10]. B meets most criteria as a basic nutrient. Because of its low atomic weight, it binds to organic compounds in ways that affect biological function [11]. B, which is not found in free form in the organism, generally exists as organic B compounds bound to sodium and oxygen elements in the form of boric acid (B(OH)₃) or borate (B(OH)₄). These compounds occur in human, animal and plant tissues [12, 13, 14]. The relationship of B, which has an important place in living metabolism, with other nutrients is also important. It has been stated that B has a role in the utilization of minerals effective in bone metabolism such as Ca, Mg, and interacts with phosphorus, Cu, methionine, arginine and vitamin D [14, 15]. The aim of the present study was to assess impact of dietary CaB₄O₇ supplementation on the mineral content of egg and eggshell of laying quails.

2. Material and methods

2.1. Experimental design and diet

The ethics committee for the animals used in this study was approved by Firat University, Animal Experiments Local Ethics Committee (2020/11). A total of 60 (6-week-old) quails were used. Quails were divided into 2 groups with 5 replications per group and 4 females + 2 males per repetition: (i) control group (0 mg of CaB₄O₇ per kg of diet), (ii) CaB₄O₇ group (300 mg of CaB₄O₇ per kg of diet, 22.14% elemental B/kg diet). The quails were housed in a controlled environment with 16 h light: 8 h dark/day program. Feed and water were given ad libitum to the quails. The basal diet was prepared according to NRC [16]. The nutrient composition of the diet used in the study was determined [17, 18, 19]. Diet compositions and nutrient contents were shown in Table 1. The experiment lasted 8 weeks. At the end of the study, 6 eggs were taken from each group together with the feed sample. Eggs were stored at +4°C until analysis.

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

Ingredients	%
Maize	56.00
Soybean meal (44% CP)	26.80
Sunflower meal (28% CP)	1.20
Wheat bran	2.10
Sunflower oil	2.35
Sodium chloride	0.35
L-Lysine hydrochloride	0.15
L-Treonine	0.10
Sodium bicarbonate	0.20
DL-Methionine	0.10
Vitamin-Mineral premix ^b	0.35

Ground limestone	8.00
Dicalcium phosphate	2.30
Total	100
Nutritional composition	
Dry matter, %	90.50
Crude protein, %	17.50
Crude cellulose, %	3.65
Ether extract, %	4.00
Crude ash, %	13.58
Phosphorus ^c	0.35
Lysine ^c	1.00
Threonine ^c	0.74
ME, kcal/kg ^c	2750

^aCaB₄O₇ (300 mg CaB₄O₇ per kg diet) was added to the basal diet. ^bVitamin-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. ^cCalculated.

Table 2 Mineral composition of diet

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

2.2. Preparation and characterization of calcium tetraborate

CaB₄O₇ as a powder form was synthesized by solid-state reaction method. For synthesis of the CaB₄O₇ was used CaCO₃ (98.5% pure, Merck), H₃BO₃ (99.5% pure, Merck), and CO(NH₂)₂ (99.5% pure, Merck). The obtained CaB₄O₇ samples were characterized by XRD method. Analysis of the crystalline structure was performed by using Rigaku MiniFlex X-ray diffractometer (XRD) with Cu-K α radiation ($\lambda = 1.54056 \text{ \AA}$, in the 2θ range of 3° – 60° at $2^\circ/\text{min}$ scan rate). The XRD of CaB₄O₇ was shown in Figure 1. According to the XRD results in Figure 1, the most intense peaks in the diffractogram overlap with the JCPDS (Card No: 31-0253) card. In this case, CaB₄O₇ compound has been synthesized successfully [20].

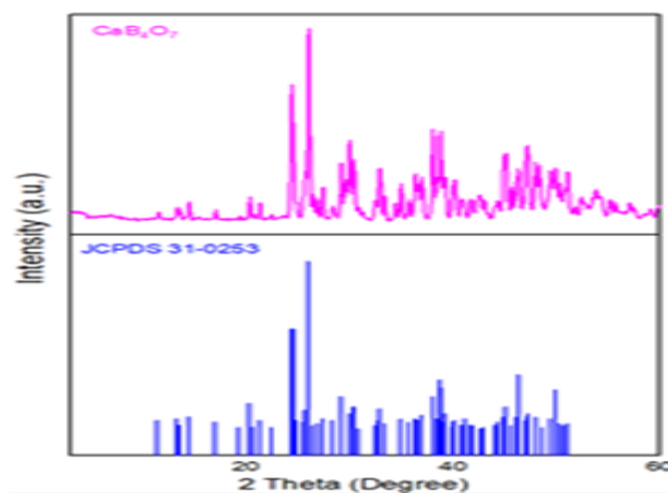


Figure 1 XRD patterns of CaB_4O_7 compound

2.3. Mineral determination

2.3.1. Sample preparation

Eggshells were dried at 100°C in a NÜVE-FN 500 brand oven until they reached a constant weight. The dehumidified eggshells were mixed in a mortar and powdered and homogeneous. Egg inside was placed in a 50 mL beaker and mixed in an IKA-RTO brand magnetic mixer at 300-400 rpm speed, and the yellow and white parts were mixed thoroughly and homogeneous. The feed samples were dried in a NÜVE-FN 500 brand oven at 100°C until they reached a constant weight. The value of metals in the homogenized eggshell, internal egg and feed samples were determined with the ICP-OES device after microwave acidic extraction pretreatment.

2.3.2. Inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis

Homogenized eggshell, internal egg and feed samples weighed approximately 0.1 g together with 9 ± 0.1 mL 65% (v/v) nitric acid (Merck) and 3 ± 0.1 mL 37% (v/v) hydrochloric acid (Merck) PTFE (Polytetrafluoroethylene) is taken into microwave containers. Acidic crushing process is performed at $175 \pm 5^\circ\text{C}$ with the CEM-Mars 6 microwave device. The EPA 3051A standard method was followed in the microwave acid digestion pretreatment. The same procedures as for the samples were done in the blank. After the samples coming out of the microwave were cooled down to room temperature ($22\text{-}23^\circ\text{C}$), all samples were completed to 50 mL with 1% HNO_3 . The metal values of the egg shell, egg and feed samples which were broken by microwave were analyzed by using the Thermo-Scientific-ICAP 7000 brand ICP-OES device according to the EPA 6010 D method. The calibration standards used in the analysis were prepared with 1% HNO_3 just like the blank and samples. In order to prevent contamination, all vessels and apparatus used in analyzes were kept in 10% HNO_3 for 1 night and then washed thoroughly with ultrapure pure water. Reagents of analytical purity were used in the analyzes and all solutions were prepared with ultrapure distilled water from the MES MP mini pure device.

The same analytical procedures applied to egg/feed samples to ensure the validity of the results of the analyzes are also applied to certified reference materials (NIST SRM 1573a Tomato Leaves, NIST SRM 1835 Borate Ore). The analysis results of the metals included in the certified reference materials were compared with the certificate values and it was seen that the results were within the certification range. The results of the enriched quality control samples were checked according to the % recovery value between 80-120% and it was seen that all results were within this range [21, 22].

2.4. Statistical analysis

The data were subjected to the Independent Sample T-test using the SPSS 22 package program after testing the normality with Shapiro-Wilk and homogeneity of variances with Levene's test. Moreover, Pearson's correlation analysis was used to assess the correlation between minerals. The results were considered significant at $P < 0.05$ [23].

3. Results

Table 3 is shown the effect of CaB_4O_7 on mineral contents of eggs. There were no significant differences in Ca, Fe, Mn, Cu and Zn minerals of egg content between the control and additive group ($P>0.05$). The CaB_4O_7 was significantly affected by the levels of Mg ($P<0.01$), Na ($P<0.01$) and K ($P<0.05$). The addition of 300 mg/kg CaB_4O_7 significantly increased egg Mg, Na and K content in layer quails. In addition, the Ca content of the additive group was higher than the control group, but not significant. The amounts of B was below the detection limit of the methods used.

Table 3 Mineral contents of whole edible parts of eggs.

Mineral	Control	CaB_4O_7	SEM	P
B (mg/kg)	ND	ND	-	-
Ca (mg/kg)	687.49	722.82	50.24	0.638
Mg (mg/kg)	112.36	147.28	4.22	0.001
Na (g/kg)	1.47	1.75	0.05	0.004
Fe (mg/kg)	28.61	27.90	1.51	0.752
K (g/kg)	1.29	1.44	0.04	0.026
Mn (mg/kg)	0.79	0.74	0.12	0.760
Cu (mg/kg)	1.05	1.03	0.06	0.900
Zn (mg/kg)	10.56	10.19	0.86	0.775

CaB_4O_7 : Calcium tetraborate; ND: Not detected; Detection limit of: B < 1.66; SEM: Standard error of mean; $P<0.05$. Data are presented as mean and SEM.

Only Ca and Mn content measured in the eggshells were unaffected by the CaB_4O_7 supplementation (Table 4, $P>0.05$). The B content was higher in the eggshells of the animals in the additive group ($P<0.01$). Similarly, eggshells Mg ($P<0.01$), Na ($P<0.01$), Fe ($P<0.01$), Mn ($P>0.05$), Cu ($P<0.05$), K ($P<0.05$), and Zn ($P<0.01$) content was higher in the additive group. Zinc was higher in the additive group eggshells than in the control group eggshells.

Table 4 Mineral contents of eggshells.

Mineral	Control	CaB_4O_7	SEM	P
B (mg/kg)	16.33	51.02	4.31	0.001
Ca (mg/kg)	373.21	350.47	7.81	0.112
Mg (mg/kg)	6.86	8.87	0.34	0.004
Na (g/kg)	1.86	2.91	0.12	0.001
Fe (mg/kg)	9.45	17.34	1.36	0.003
K (g/kg)	1.15	1.45	0.06	0.019
Mn (mg/kg)	1.18	1.85	0.28	0.127
Cu (mg/kg)	2.08	2.84	0.19	0.020
Zn (mg/kg)	7.21	56.41	5.44	0.001

CaB_4O_7 : Calcium tetraborate; SEM: Standard error of mean; $P<0.05$. Data are presented as mean and SEM.

Table 5 presents the Pearson's correlation coefficients analyses among egg minerals. The results indicated that Mg had the highest correlation with Na ($r=0.750$; $P<0.01$) and the lowest correlation with Cu ($r=0.072$; $P>0.05$). Fe had the strongest correlation with Mn ($r=0.802$; $P<0.01$). There were positive correlations between Ca and Fe, Cu, Mn and negative correlation between Na and Ca, Fe, Mn.

Table 5 Pearson's correlation coefficients among whole egg mineral traits.

	Na	K	Ca	Fe	Mn	Cu
Mg	0.750**	0.734**	0.296	0.274	0.235	0.072
Na		0.522	-0.080	-0.025	-0.063	0.124
K			0.239	0.050	-0.081	0.113
Ca				0.593*	0.574	0.382
Fe					0.802**	0.413
Mn						0.338

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level.

The Pearson correlation coefficients between Ca, Mg, Na, Fe, K, Mn, Cu, Zn and B are shown in Table 6. Ca was negatively correlated with Mg ($r = -0.126$), Na ($r = -0.609$, $p < 0.05$), Fe ($r = -0.524$), K ($r = -0.555$), Mn ($r = -0.460$), Cu ($r = -0.385$), Zn ($r = -0.735$, $P < 0.01$), and B ($r = -0.648$, $P < 0.05$), respectively. Mg was positively correlated with Na, Fe, K, Mn, Cu, Zn, and B. Moreover, Fe, K, Mn, and Cu were positively correlated with Zn. Significant ($P < 0.01$) positive correlations were found between Zn and B ($r = 0.883$).

Table 6 Pearson's correlation coefficients among mineral deposition in eggshell.

	Mg	Na	Fe	K	Mn	Cu	Zn	B
Ca	-0.126	-0.609*	-0.524	-0.555	-0.460	-0.385	-0.735**	-0.648*
Mg		0.759**	0.623*	0.626*	0.396	0.564	0.408	0.517
Na			0.817**	0.863**	0.323	0.667*	0.715**	0.672*
Fe				0.829**	0.303	0.510	0.746**	0.588*
K					0.212	0.516	0.560	0.352
Mn						0.551	0.468	0.675*
Cu							0.517	0.622*
Zn								0.883**

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level.

4. Discussion

Nutrition affects egg quality, so differences in the mineral composition of the egg can be associated with the mineral composition of the diet. Minerals in the animal body affect the egg quality. Poor quality eggs caused by poor eggshell formation, softshells, and cracked shells cause great economic loss. The economic loss arising from the low eggshell quality is the main problem in poultry farming. Mineral contents of eggs reflect the nutrition and health status of laying. The higher the serum calcium level, the higher the calcium level in the eggshell. The aim of this study was to investigate the effects of dietary supplementation with CaB_4O_7 (300 mg/kg) on the mineral content of egg and eggshell in layer quails [24, 25].

B plays a role in calcium metabolism and seems to improve the absorption of Ca and Mg [13]. CaB_4O_7 addition (300 mg/kg) to a basal diet did not affect edible parts of eggs mineral of Ca, Fe, Mn, Cu, and Zn in laying quails. Fe had the strongest correlation with Mn in whole egg. CaB_4O_7 supplementation at 300 mg/kg, to a diet, increased Mg, Na, and K contents of whole edible parts of eggs. In the present study, whole edible parts of eggs B concentration were not determined for laying quails. However, Sizmaz and Yildiz [26] reported that supplementation of 120 mg/kg boric acid (includes 17.5% B) significantly affected liver and egg yolk B contents. Moreover, Kucukyilmaz and Erkek [27] observed a significant increase in B content of edible parts of eggs in laying hens fed diets supplemented with B. There is no study investigating the effect of B addition to the diet on other minerals except B in the edible part of the egg.

Minerals such as Ca, P and vitamin D are the primary factors affecting eggshell quality of layers [24]. Zinc has been associated with carbonic anhydrase enzyme and in improving eggshell quality. Also, a deficiency of copper can cause shell abnormalities [28]. These all minerals improve the quality of the shell. These results suggested that CaB_4O_7 supplementation positively affects eggshell mineral content. The correlation between B and Zn was high and positive in eggshell. In the current study, B concentration was detected in eggshell, while it was not detected in the whole eggs of both groups. B, Mg, Na, Fe, K, Cu and Zn was higher in eggshells of the additive group than in control. In contrast, Bintas ve Ozdogan [29], who observed that supplementation of B (100 mg/kg) to laying hens diet did not affect the Ca, P, and B levels of eggshell. Our results were consistent with and in accordance with those of Arslan Kaya and Macit [30], who reported that supplementation of B (orthoboric acid, 150 mg/kg) into the diet caused an increase in B and Ca contents of eggshells, and a decrease in Fe contents of eggshells. Also, the Zn, Mg, Mn, and Na contents of eggshells were not affected by the B addition. Similarly, El-Saadany et al., [31] stated that the dietary B supplementation significantly increased eggshell calcium and boron concentrations. These results are in correspondence with the finding of Mizrak et al., [32] and Mizrak and Ceylan [33], they reported that the supplementation of B increased Ca and B in eggshell. This may be due to B effects on metabolism Ca, P, Mg and vitamin D which improves eggshell quality [31]. These results can be associated with animal age, mineral level of basal ration, B dose, source, and form [30].

5. Conclusion

This study showed that Ca, Mg, Na, and K contents of edible parts of eggs were affected by CaB_4O_7 supplementation. Moreover, CaB_4O_7 supplementation in layer diets might be a positive effect on calcium metabolism, producing eggs enriched in minerals, promoting B, Mg, Na, Fe, K, Cu and Zn deposition in eggshells, and improving eggshell quality. Further study should be conducted to evaluate the effective B supplementation doses for functional egg production and usage of B could be advantageous in terms of beneficial health effects.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors report no conflicts 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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