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# The Effects of Boron, Zinc and Their Combination with Cadmium on the Mineral Contents of the Tibia and Eggshell of Quails

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**ABSRACT**

This study was conducted to determine the effects of dietary boron, zinc and supplementation in combination with cadmium on the mineral concentrations of eggshell and bone in quails. One hundred and twenty, twenty-week-old female quails were randomly distributed among six equal diet groups. Diet I consisted of a control group and diet II contained 20 mg/kg cadmium; diet III contained 20 mg/kg cadmium and 60 mg/kg boron; diet IV contained 20 mg/kg cadmium and 50 mg/kg zinc; diet V contained 20 mg/kg cadmium, 30 mg/kg boron and 25 mg/kg zinc and diet VI contained 20 mg/kg cadmium, 60 mg/kg boron and 50 mg/kg zinc. The effect of mineral addition treatments on the manganese content of tibia and eggshells was not significant (P>0.05). The tibia cadmium content increased when animals were fed with diets III, IV, V and VI (P<0.05). The addition of boron (alone or combined) to the diets containing cadmium increased the boron concentration in the tibia. The tibia zinc concentration was the highest when quails were fed with diet IV (P<0.01). The addition of boron or zinc or their combination to diets containing cadmium decreased the concentrations of calcium, phosphorus and magnesium in the tibia. The cadmium content of eggshells was reduced when both boron and zinc were added to the diet (P<0.01). The results of the present study show that the addition of boron and zinc to diets containing cadmium decreased the cadmium accumulation in the bone and eggshells of quails.

## 1. Introduction

The toxic element cadmium (Cd) is potentially an environmental pollutant that is non-essential for animal nutrition and interacts with essential minerals such as calcium (Ca), phosphorus (P) and zinc (Zn), which are required for the formation of bone and eggshell (Brzoska and Moniuszko-Jakoniuk 2004; 2005; Al-Waeli et al. 2012). The presence of Cd in animal products is an issue for some countries and in some cases, the concentration in manure and feed products can exceed 100 mg/kg (Li et al. 2010; Al-Waeli et al. 2012).

Many researchers have demonstrated that nutrients such as Ca, P and magnesium (Mg), which are necessary for bone health, are affected by Cd in animals (Brzoska and Moniuszko-Jakoniuk 2004; 2005; Brzoska et al. 2005). Brzoska et al. (2001) showed that 50 µg/mL Cd added to the water, decreased the concentrations Ca, Zn, and iron in the tibia of rats, but did not affect the Mg or copper contents. Korenekova et al. (2007) and Skalicka et al. (2008) demonstrated that exposure to Cd increased the Cd content of eggshells in quails.

It is necessary to know the degree of Cd pollution in the environment and to develop new methods to eliminate its adverse effects on the environment and to living organisms. The essential element Zn plays an important role in reducing the detrimental effects of Cd (Brzoska et al. 2001; Korenekova et al. 2007; Nad et al. 2012). Previous studies reported that Zn can reduce Cd absorption, accumulation, and prevent or decrease the detrimental action of Cd (Dorian and Klaassen 1995; Nolan

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and Brown 2000; Nad et al. 2012). In contrast to the negative effect of Cd on bone, boron (B) has positive effects on mineral metabolism and bone. It was shown that dietary B supplementation affected minerals of bone (Wilson and Ruszler 1996; 1997; Olgun et al. 2012) in poultry. Therefore, the adverse effects of Cd on bone health could be eliminated by the addition of B and Zn to the diet.

The aim of this study was to investigate the effects of B, Zn and combinations of Cd supplementation on the mineral content of bone and eggshell in quails.

## 2. Material and Methods

The criteria specified by the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals were obeyed during the experiments carried out on animals. A total of 120, 20-week-old Japanese quails were randomly assigned to six equal groups according to their dietary treatment. Each experiment group consisted of five replicates and each treatment contained four female birds. The six experimental groups (diets) were fed as following for 70 days: Diet I was the control group; diet II contained 20 mg/kg Cd as Cd-sulphate; diet III, 20 mg/kg Cd + 60 mg/kg B as boric acid; diet IV, 20 mg/kg Cd + 50 mg/kg Zn as Zn acetate; diet V, 20 mg/kg Cd + 30 mg/kg B + 25 mg/kg Zn; diet VI, 20 mg/kg Cd + 60 mg/kg B + 50 mg/kg Zn. The basal diet (Table 1, containing 0.34 mg/kg Cd, 13.58 mg/kg B and 94.77 mg/kg Zn by analysis) was formulated to meet or exceed the nutrient requirements of quails (NRC 1994) except for Cd, B and Zn. The experimental conditions complied with the requirements for the ethical standards of welfare and animal treatment. The quails were housed in a room with controlled ventilation and lighting (16 hours/day). Feed and water were provided *ad-libitum*.

Table 1
Basal composition of diet (diet I)

| Ingredients | g/kg | Chemical composition | |
|---|---|---|---|
| Corn | 570.0 | Metabolisable Energy, kcal/kg ME | 2912 |
| Soybean meal | 320.0 | Crude Protein, g/kg | 199.9 |
| Sunflower oil | 34.4 | Lysine, g/kg | 10.63 |
| Limestone | 56.0 | Methionine, g/kg | 4.55 |
| Dicalcium Phosphate | 11.8 | Methionine + Cystine, g/kg | 8.27 |
| Salt | 3.5 | Calcium, g/kg[2] | 24.67 |
| Premix[1] | 2.5 | Total phosphorus, g/kg[2] | 6.48 |
| DL methionine | 1.8 | Non-phtate phosphorus, g/kg | 3.51 |
| Total | 1000.0 | Crude Ash, %[2] | 10.68 |
| | | Cadmium, mg/kg[2] | 0.34 |
| | | Boron, mg/kg[2] | 13.58 |
| | | Zinc, mg/kg[2] | 94.77 |

[1]Premix provided/kg of diet; Manganese: 60 mg; Iron: 30 mg; Zinc: 50 mg; Cupper: 5 mg; Selenium: 0.1 mg, Vitamin A, 8.800 IU; Vitamin $D_3$, 2.200 IU; Vitamin E, 11 mg; Nicotine acid, 44 mg; Cal-D-Pan, 8.8 mg; Riboflavin 4.4 mg; Thiamin 2.5 mg; Vitamin $B_{12}$, 6.6 mg; Folic acid, 1 mg; D-Biotin, 0.11 mg; Coline: 220 mg

[2] Analyzed value as feed

At the end of the study, 10 quails from each treatment group (n = 2) were killed by cervical dislocation and the left tibias were dissected out for subsequent analysis. Approximately 0.20 g dried sample from the tibia (bone with marrow removed) and eggshell (eggshell without membrane) were placed into a burning cup and 5 mL nitric acid and 3 mL perchloric acid were added. The sample was incinerated in a MARS 5 Microwave Oven (CEM Corp., NC, USA) at 190ºC and 175 Psi pressure and diluted to 50 mL with distilled water. The concentrations of Cd, B, Zn, Ca, P, Mg and manganese (Mn) in the tibias and eggshells were determined by MarsXpress Technology Inside and Inductively Coupled Plasma Atomic Emission Spectrometry (Vista AX CCD Simultaneous ICPAES) (Skujins 1998).

Data were subjected to One-Way ANOVA in MINITAB (2000). Duncan's multiple range tests were applied to separate the means (Duncan 1955). Statements of statistical significance are based on a probability of $P < 0.01$ or $P < 0.05$.

## 3. Results

The results for the tibia mineral concentrations are shown in Table 2. The treatments had no significant effect on the Mn concentration in the tibia samples ($P > 0.05$). The content of Cd in the tibia was significantly ($P < 0.05$) lower in the group fed with diet I (control group), than in groups fed with diets III, IV, V or VI. Furthermore, the tibia B content was significantly ($P < 0.01$) higher in diets III, V or VI than in diets without B (I, II or IV diets). The tibia Zn content was higher in quails fed with diet IV, than in those fed with diets II or III (Cd or Cd+B supplemented groups). Compared to quails fed with diets I and II, the Ca tibia level was significantly ($P < 0.01$) lower when animals were fed with diets IV and

V. The tibia P content was significantly ($P < 0.01$) reduced by diets III, IV, V and VI. Similarly, the Mg concentration of the tibia was lower for quails fed with diets IV, V or IV, than those fed with diets I and II ($P < 0.05$).

The concentrations of Cd, B, Zn, Ca, P, Mg and Mn in eggshells are presented in Table 3. There were no significant differences in Zn, Ca, P, Mg and Mn concentration in eggshells between treatment groups ($P > 0.05$). The eggshell Cd concentration was clearly lower with the addition of high levels of B and Zn (diet VI) to quail diets containing Cd ($P < 0.05$). The B concentration in eggshells was significantly lower ($P < 0.01$) with diet IV, than with diets III, V and VI (B supplemented groups), but increased with diet V compared to quails fed with diets I, II or IV (no added B groups).

Table 2
The effects of supplementation boron, zinc and their cadmium combinations on tibia mineral concentration in quails

| Combinations | | | | Tibia mineral composition | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cd mg/kg | B mg/kg | Zn mg/kg | Ca g/kg | P g/kg | Mg g/kg | Mn mg/kg |
| Diets | Cd | B | Zn | | | | | | | |
| I | 0 | 0 | 0 | 0.137[b] | 4.18[c] | 393.8[ab] | 270.5[a] | 112.4[a] | 5.06[a] | 22.13 |
| II | 20 | 0 | 0 | 0.850[ab] | 5.42[c] | 314.8[b] | 269.7[a] | 113.4[a] | 5.05[a] | 21.89 |
| III | 20 | 60 | 0 | 0.980[a] | 14.52[a] | 299.7[b] | 239.5[ab] | 97.4[b] | 4.35[ab] | 26.93 |
| IV | 20 | 0 | 50 | 1.504[a] | 5.09[c] | 430.4[a] | 227.9[b] | 93.4[b] | 4.18[b] | 30.95 |
| V | 20 | 30 | 25 | 1.410[a] | 9.92[b] | 350.5[ab] | 227.0[b] | 90.1[b] | 4.12[b] | 25.27 |
| VI | 20 | 60 | 50 | 1.080[a] | 14.22[a] | 408.6[ab] | 233.4[ab] | 93.1[b] | 4.16[b] | 21.91 |
| | | | SEM | 0.220 | 0.44 | 23.3 | 8.2 | 3.3 | 0.23 | 3.67 |
| | | | P Value | 0.012 | 0.000 | 0.006 | 0.002 | 0.000 | 0.034 | 0.558 |

Table 3
The effects of supplementation boron, zinc and their cadmium combinations on eggshell mineral in quails

| Combinations | | | | Eggshell mineral composition | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cd mg/kg | B mg/kg | Zn mg/kg | Ca g/kg | P g/kg | Mg g/kg | Mn mg/kg |
| Diets | Cd | B | Zn | | | | | | | |
| I | 0 | 0 | 0 | 0.311[a] | 1.057[cd] | 4.32 | 349.4 | 2.67 | 6.29 | 0.320 |
| II | 20 | 0 | 0 | 0.295[ab] | 1.178[bcd] | 3.13 | 352.9 | 2.66 | 6.91 | 0.484 |
| III | 20 | 60 | 0 | 0.217[abc] | 1.641[abc] | 3.31 | 345.5 | 3.24 | 6.60 | 0.407 |
| IV | 20 | 0 | 50 | 0.221[abc] | 0.725[d] | 4.17 | 354.5 | 3.01 | 6.95 | 0.356 |
| V | 20 | 30 | 25 | 0.188[bc] | 2.109[a] | 3.16 | 349.1 | 3.04 | 6.75 | 0.320 |
| VI | 20 | 60 | 50 | 0.156[c] | 1.731[ab] | 3.98 | 353.4 | 2.46 | 6.39 | 0.307 |
| | | | SEM | 0.033 | 0.123 | 0.48 | 2.2 | 0.22 | 0.17 | 0.046 |
| | | | P Value | 0.045 | 0.000 | 0.461 | 0.106 | 0.202 | 0.061 | 0.113 |

## 4. Discussion

The Cd concentration of the tibia was significantly ($P < 0.05$) higher in the groups fed with diets III, IV, V or VI compared to with diet I. However, these differences were insignificant in comparison with the diet II group (only added Cd group). This result disagrees with that previously reported by Brzoska et al. (2001) and Brzoska and Monioszko-Jakoniuk (2005). Brzoska et al. (2001) showed that 50 µg/mL Cd added to the water increased the Cd content in the bone of rats, and the addition of Zn (240 µg/mL) to the water did not prevent Cd level in the tibia. Similarly, Brzoska and Monioszko-Jakoniuk (2004, 2005) indicated that Cd levels in the bone of rats increased following the supplementation of Cd (1, 5, and 50 mg/L) to the water. In this study, the addition of B (diet III), Zn (diet IV) or B + Zn (diets V and VI) to diets containing Cd slightly enhanced the accumulation of Cd in the tibia compared with diet II. In this study, the B concentration in the tibia was significantly ($P < 0.01$) increased by the addition of B (diets III, V or VI). This increase was independent from added Cd or Zn. These result agree with those of the previous studies of Wilson and Ruszler (1997; 1998), Armstrong et al. (2002) and Olgun et al. (2012).

In the present study, the Zn, Ca, P and Mg concentrations of the tibia were significantly affected by all dietary treatments (Table 3). However, the addition of Cd alone (diet II) in the diet did not affect these parameters compared with the control group (diet I). The addition of B and Zn (alone or in combination) in the diets significantly reduced the content P in the tibia. A similar result was found for the Ca and Mg concentration in bone. A study performed on rats reported that the addition of 50 µg/mL Cd + 240 µg/mL Zn in water had a marked effect on the Zn and Ca levels in the tibia, but did not affect the Mg level (Brzoska et al. 2001). Boron is a trace element that plays an important role in mineral metabolism and interacts with Ca, P and Mg (Küçükyılmaz et al. 2014). Olgun et al. (2012) noted that the additional of B (60, 120 and 240 mg/kg) to the diet did not affect the P concentration of the tibia, but it reduced the Zn, Ca and Mg concentrations in the tibia compared to the control group in laying hens. Nevertheless, Küçükyılmaz et al. (2014) reported that supplementation of the diet of hens with 75 and 150 mg/kg B did not affect the mineral (Ca, P, Mg or Zn) concentration of bone. Many researchers have reported that increased Zn plays a very important role in the growth and maintenance of bone, and Zn intake prevents the accumulation of Cd in bone (Suzuki et al. 1990; Iwami and Moriyama 1993; McClung et al. 2006; Brzoska et al. 2001). Brzoska et al. (2007) demonstrated that the Cd accumulation in the bone of rats was reduced by supplementation with Zn. These findings differ from those in this study, which showed that the addition of Zn to quail diets did not prevent Cd content in the tibia. The differences between these results might be due to the species of experiment animals studied, and the method of administration of Cd (oral or injection), and to the levels of Cd and/or Zn used. However, Al-Waeli et al. (2012) showed that Zn was not correlated with Cd in broilers. Similarly, Ohta and Cherian (1991) found that oral pretreatment with 40 mg Zn sulphate did not affect the intestinal uptake of Cd in rats.

The content of Cd in eggshells was lower in groups fed with diet VI than with diets I and II ($P < 0.05$). Some researchers have noted that the Cd concentration in eggshells from Zn- or chromium supplemented groups was significantly lower than that of the Zn-Cd or chromium-Cd groups after 50 or 58 days (Korenekova e al. 2007; Skalicka et al. 2008). However, no significant differences between the groups that received Cd and the control group were observed in either study. In this study, the Cd level in eggshells was significantly lower with diets V and VI (B+Zn added groups), than with diet I (control group), but this effect was not observed following the addition of B (diet III) or Zn (diet IV) alone. The highest B concentration in eggshells was obtained with diet V and the lowest concentration was obtained with diet IV. In previous studies, Cd supplementation of the diets did not affect the B concentration of eggshells. Mas and Arola (1985) showed that the concentration of copper, iron, nickel, and Zn in eggshells was not influenced by the injection of 100 µg Cd in twelve-day incubated eggs, but this treatment did affect the concentration of nickel and Zn in eggshells The results of this study partially agree with those of the current study.

Many studies have shown that Cd toxicity adversely affects bone health (Kjellström 1986; Miyahara et al. 1988; Brzoska and Moniuszko-Jakoniuk 2004; 2005) and eggshell quality (Kottferova et al. 2001; Sant'ana et al. 2005; Rahman et al. 2007; Skalicka et al. 2007). The Cd interacts with macro-elements such as Ca and P, which are the main component of bone and eggshell, and trace elements such as Zn and Mn, which are involved in the formation and the maintenance of bone and eggshell, and thereby inhibit their function (Brzoska and Moniuszko-Jakoniuk 2004; 2005; Brzoska et al. 2001; 2005). However, in the present study, the mineral concentrations of bone and eggshell were not affected by the addition of 20 mg/kg Cd in the diet (diet II) compared with the control group (diet I). Therefore, a potential protective effect of B and Zn in the diet against Cd toxicity was not significant. In conclusion, the supplementation of B and Zn to diets containing Cd caused a decrease in Cd accumulation in the tibia. However, the addition of B and Zn in combination decreased the Cd concentration in the eggshells of quails.

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