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Research Article

Effects of Dietary Boric Acid Supplementation on Performance, Eggshell Quality and Some Serum Parameters in Aged Laying Hens

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Abstract: The effects of dietary boric acid (BA) supplementation on feed consumption, feed efficiency, egg production, egg weight, eggshell thickness, eggshell breaking strength, and some serum parameters (Ca, P, and Mg) were examined in 100 layer hens at 60 weeks of age. Commercial corn-soy-based layer diets were formulated as basal diets for the control treatment. Basal diets were supplemented with 25, 50, and 100 mg/kg BA (H_3BO_3), respectively, in experimental groups I, II, and III. In total, 4 dietary treatments were employed in the experiment. The experiment lasted 75 days. Feed efficiency and egg production values were not affected significantly by dietary treatments during the experiment ($P > 0.05$). However, layer hens that received diets supplemented with BA had better feed consumption, egg weight, percentage of cracked eggs, and egg shell quality (eggshell thickness, eggshell breaking strength) than the control group. Additionally, serum Mg levels were significantly ($P < 0.01$) increased by BA supplementation. It was concluded that dietary boron supplementation has positive effects on mineral balance, and therefore reduces the percentage of cracked eggs by improving eggshell thickness and egg shell breaking strength in aged laying hens.

Key Words: Boric acid, layer hens, egg production, eggshell quality, serum mineral values

Borik Asit İlavesinin Yaşlı Yumurta Tavuklarında Performans, Yumurta Kabuğu Kalitesi ve Bazı Serum Parametreleri Üzerine Etkileri

Özet: Bu araştırma, borik asit ilavesinin yumurtacı tavuklarda yem tüketimi, yemden yararlanma oranı, yumurta verimi, yumurta ağırlığı, yumurta kabuğu kalınlığı, yumurta kabuğu kırılma mukavemeti ve bazı serum parametreleri (Ca, P, Mg) üzerine etkilerini incelemek amacıyla gerçekleştirildi. Araştırmada 60 haftalık yaştaki 100 adet yumurtacı tavuk kullanıldı. Araştırmada mısır-soya ağırlıklı bir temel rasyon ve bu rasyona 25, 50 ve 100 mg/kg düzeyinde borik asit ilave edilmesiyle oluşturulan farklı 4 deneme rasyonu değerlendirildi ve araştırma süreci 75. gün sonunda sonlandırıldı. Deneme süresince yemden yararlanma oranı ve yumurta verimi değerlerinin borik asit ilavesinden önemli düzeyde etkilenmediği tespit edildi ($P > 0,05$). Bununla birlikte, rasyonlarına farklı düzeylerde borik asit ilave edilen gruplarda yem tüketimi, yumurta ağırlığı, hasarlı yumurta oranı ve yumurta kabuğu kalite parametre değerleri (yumurta kabuk kalınlığı, yumurta kabuğu kırılma mukavemeti) kontrol grubuna göre daha üstün bulundu. Ayrıca rasyona borik asit ilavesinin serum Mg düzeyini de önemli ($P < 0,01$) düzeyde arttırdığı belirlendi. Bu araştırmadan elde edilen veriler ışığında rasyona borik asit ilavesinin yaşlı yumurtacı tavuklarda mineral dengeyi (Ca ve P mekanizmasını) olumlu yönde etkileyerek hasarlı yumurta oranında azalmaya ve yumurta kabuk kalitesinde iyileşmeye neden olduğu değerlendirilmiştir.

Anahtar Sözcükler: Borik asit, yumurtacı tavuk, yumurta verimi, yumurta kabuğu kalitesi, serum mineral değerleri

Introduction

Boron (B) is a light trace element that is turning out to be essential for humans and also for animals (1). B rarely occurs alone in nature and is typically found in combination with other elements; common combinations are boric acid (BA) and borates (2). Several studies have indicated that B is an important mineral for body weight, feed consumption, reduced mortality rate, normal cartilage, and bone formation in broilers; and for egg production, egg weight, and egg shell quality in laying hens (3-6).

B has been examined as a possible nutritional factor of calcium (Ca) and phosphorus (P) metabolism and their utilisation in human and animal models (7). Grain cereals

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that are widely used in poultry rations are low in B (1) and therefore poultry diets may be inadequate. Eggshell quality has always been a problem in the layer industry especially in the last stage of the laying period. Numerous studies have been conducted to solve the problems of poor shell quality. Many of these studies have focused on macro minerals, especially Ca and P, and trace minerals. Trace minerals such as manganese (Mn) and zinc (Zn) also play an important role in eggshell formation (8). Thus, the purpose of this study was to determine the effects of different doses of B on performance and particularly on eggshell formation and quality in aged laying hens.

Materials and Methods

Chicks, experimental design, and diet

In this experiment, one hundred 60-week-old Hyline Brown hens were used. The hens were divided into 4 groups, consisting of 1 control and 3 experimental groups. The hens were randomly assigned to cages (40 × 40 × 46 cm) with 5 hens in each. The hens were placed in houses lit by a fluorescent source. During the experiment a lighting programme of 17 h a day was applied besides day light. Feed and drinking water were available ad libitum. The experimental period was 75 days. Data collection started after a 15-day adaptation period. The hens were fed the basal diet (control group) or basal diet supplemented with 25 mg/kg (group I), 50 mg/kg (group II), and 100 mg/kg (group III) BA (H_3BO_3, Merck, Germany/ BA 99.995%) for 75 days. As BA includes 17.5% B, diets of groups I, II, and III contained approximately 4.3, 8.7, and 17.5 mg/kg B, respectively. In order to have a homogeneous distribution, powdered BA was added by pre-mixing. For that purpose calculated amounts of BA were mixed with a small volume of the ration and then added to the main ration. Ingredients and chemical composition of the basal diet are shown in Table 1. The chemical compositions of the feed ingredients (dry matter, crude protein, ash, and ether extract) as dried samples were analysed using AOAC (9) procedures and metabolisable energy was determined by the methods of TSE (10). The levels of available P and total magnesium (Mg) in the diet were calculated via the amounts of these elements in feedstuff and additives. For each experimental group the vitamin-mineral premix and BA were weighed individually and supplemented to diets in a homogeneous form.

Table 1. Ingredients and chemical composition of the basal diet fed to laying hens.

Ingredients	%
Corn	54
Barley	11
Soybean Meal	23
Limestone	8.7
DCP	1.5
Vegetable oil	1
Vitamin- premix[1]	0.15
Mineral-premix[2]	0.15
Methionine	0.20
Salt	0.30
Nutrient contents[3], (dry matter basis)	
Dry matter, %	89.9
Crude protein, %	16.75
Ash, %	8.01
Calcium, %	3.50
Total phosphorus, %	0.60
Available phosphorus, %	0.36
Total magnesium, %	0.17
Metabolizable energy, kcal/kg	2770

1 2.5 kg of vitamin premix contains 3.6 mg of vitamin A, 0.05 mg of vitamin D3, 30 mg of vitamin E, 3 mg of vitamin K3, 3 mg of vitamin B1, 6 mg of vitamin B2, 5 mg of vitamin B6, 0.015 mg of vitamin B12, 25 mg of niacin, 0.04 mg of biotin, 8 mg of karotenoid, 1 mg of folic acid, 300 mg of choline chloride, 50 mg of vitamin C,

2 1 kg of mineral premix contains 80 mg of manganese, 35 mg of iron, 50 mg of zinc, 5 mg of copper, 2 mg of iodine, 0.4 mg of cobalt, 0.15 mg of selenium.

3 Calculated analysis

Performance and biochemical measurements

Feed consumption and feed conversion ratio were determined at 7-day intervals. Feed conversion ratio was calculated as kilograms of feed to kilograms of egg. The hen-day egg production was recorded daily. Collected eggs were classified as normal or cracked. Egg weights were recorded at 10-day intervals. Blood samples were collected by venipuncture from a sub-wing vein and placed into non-additive blood collection tubes for separating the serum, from 5 randomly selected laying hens for each treatment group at the end of the trial. Serum was separated by centrifuge at 3000 rpm, and subsequently analysed for serum Ca, P, and Mg levels (Architect- c8000 - Autolab Analyser).

Eggshell quality measurements

The eggs were subjected to determine characteristics of eggshell quality parameters (shell thickness and shell-breaking strength) at 10-day intervals. Egg shell breaking strength was measured using a cantilever system by applying increased pressure to the broad pole of the shell using an instrument (Imada, Newton) (11). Shell thickness was measured at 3 locations on the egg (air cell, equator, and sharp end) using a micrometer (Mitutoyo, 0.01 mm, Japan) (12).

Statistical analysis

Statistical analyses of the data were performed with SPSS version 10.0 for Microsoft Windows. One-way ANOVA (13) was used for the differences between groups. When the P-values were significant, Duncan's multiple range test was performed (14).

Results

The addition of BA (25, 50, 100 mg/kg) to the diets caused a significant ($P < 0.01$) increase in feed consumption compared to the control group, while there was no significant ($P > 0.05$) effect on feed conversion ratio or egg production (Table 2). Average egg weight increased significantly ($P < 0.01$) in the experimental groups, which contained BA. Average percentage of cracked eggs was significantly decreased by the addition of BA ($P < 0.05$) (Table 2). Eggshell thickness increased significantly with the addition of 50 mg/kg and 100 mg/kg BA ($P < 0.001$) (Table 3). Eggshell breaking strength was also significantly ($P < 0.001$) increased in the experimental groups. Serum Mg levels significantly ($P < 0.01$) increased, especially in group III, which received 100 mg/kg BA, while there were no significant differences in serum Ca or P values between the control and BA supplemented groups.

Discussion

In this study, feed consumption was significantly ($P < 0.01$) increased by supplementation of 25, 50, and 100 mg/kg of BA. Eren et al. (15) reported that B supplementation at doses of 250 mg/kg had no effect on feed consumption in laying hens. Contrary to that, feed consumption was depressed by 400 mg/kg B in laying hens (4,5,15) and by 300 mg/kg B and 320 mg/kg B in broilers (16,17). It can be postulated that a high amount of BA supplementation may reduce feed consumption while causing an increase in low amounts.

In the present study, supplementing BA to the diet did not affect ($P > 0.05$) egg production in aged laying hens. Similarly, Kurtoğlu et al. (18) have reported that egg production did not show any significant difference with 50-250 mg/kg B addition. Rossi et al. (16) and Wilson and Ruszler (4,5,19) found no significant increase in egg production in younger laying hens by supplementing B at the levels of 0-250 mg/kg and 0-200 mg/kg, respectively. Furthermore, it is worth noting that an increase up to approx. 6% was recorded in the egg production of BA supplemented groups.

Although there was a slight improvement when compared to the control group, BA dietary regimens had

Table 2. Average feed consumption, egg production, feed conversion ratio, egg weight, and percentage of cracked eggs of control and boric acid supplemented groups in aged laying hens.

		Boric acid supplementation (mg/kg)				
Parameters	n	0 (control)	25	50	100	P
Feed consumption (g/hen per day)	5	105.65 ± 3.38^b	117.66 ± 3.04^a	117.90 ± 1.12^a	116.46 ± 2.46^a	**
Average egg production (%)	5	72.37 ± 2.82	78.22 ± 0.62	77.30 ± 1.66	76.70 ± 2.88	-
Feed conversion ratio (kg feed/kg egg)	5	2.17 ± 0.004	2.11 ± 0.003	2.18 ± 0.004	2.14 ± 0.008	-
Average egg weight (g)	20	67.67 ± 0.49^b	71.05 ± 0.82^a	69.95 ± 0.48^a	70.14 ± 0.69^a	**
Average cracked eggs (%)	5	3.88 ± 0.11^a	2.49 ± 0.37^b	2.92 ± 0.41^{ab}	1.85 ± 0.64^b	*

[a-b] The mean values within the same row with different superscripts differ significantly ; -: not significant * : $P < 0.05$ ** : $P < 0.01$ *** : $P < 0.001$

Table 3. Eggshell thickness and eggshell breaking strength of control and boric acid supplemented groups according to sampling period (days).

Parameters	Experimental period, days	*Boric acid* supplementation (mg/kg) 0 (control)	25	50	100	p
Egg shell thickness (mm × 10^2)	15-25	35.65 ± 0.52	34.06 ± 0.65	35.17 ± 0.61	33.68 ± 1.13	-
	25-35	32.33 ± 0.89	32.97 ± 0.71	34.10 ± 0.71	33.11 ± 0.52	-
	35-45	32.90 ± 0.70[b]	35.37 ± 0.67[a]	36.24 ± 0.78[a]	35.04 ± 0.99[ba]	*
	45-55	32.11 ± 1.09[c]	35.46 ± 0.91[b]	37.69 ± 0.54[ba]	39.29 ± 0.61[a]	***
	55-65	31.80 ± 0.71[c]	33.69 ± 0.60[cb]	35.34 ± 0.60[b]	39.47 ± 0.95[a]	***
	65-75	31.79 ± 0.43[c]	33.57 ± 0.76[b]	36.57 ± 0.47[a]	37.28 ± 0.40[a]	***
	Total	32.97 ± 0.35[b]	33.97 ± 0.44[b]	35.84 ± 0.19[a]	36.01 ± 0.45[a]	***
Egg shell breaking strength (Newton)	15-25	32.24 ± 2.14	34.68 ± 1.86	37.00 ± 2.12	32.08 ± 1.85	-
	25-35	32.30 ± 1.44[c]	36.02 ± 1.14[b]	40.85 ± 1.14[a]	35.10 ± 0.78[bc]	***
	35-45	33.25 ± 1.68[b]	36.89 ± 1.24[a]	37.14 ± 0.90[a]	39.78 ± 1.28[a]	**
	45-55	35.26 ± 1.71[b]	41.36 ± 1.15[a]	42.07 ± 1.32[a]	41.75 ± 1.11[a]	**
	55-65	31.57 ± 2.12[c]	34.50 ± 1.10[bc]	36.83 ± 1.37[ab]	40.77 ± 1.04[a]	***
	65-75	33.52 ± 1.58[b]	38.26 ± 1.53[a]	40.50 ± 1.61[a]	39.45 ± 0.76[a]	**
	Total	32.94 ± 0.87[a]	36.91 ± 0.54[b]	38.95 ± 0.81[b]	37.81 ± 0.68[b]	***

[a-c] The mean values within the same row with different superscripts differ significantly ;
-: not significant * : P < 0.05 ** : P < 0.01 *** : P < 0.001

no significant effects (P > 0.05) on feed conversion ratio. Some results consistent with our data have previously been collected from broiler chicks fed with B supplementation. Kurtoğlu et al. (20), who used 5-25 mg/kg B in broiler diets including 2000 IU Vit D3, and Elliot and Edwards (21), who added 80 mg/kg B to diets, reported insignificant effects on feed conversion ratio.

B supplementation significantly (P < 0.05) affected the percentage of cracked eggs (Table 2). These percentages in the control group and 25, 50, and 100 mg/kg B supplemented groups were 3.88%, 2.49%, 2.92%, and 1.85%, respectively. Kurtoğlu et al. (18) reported that B supplementation significantly (P < 0.05) affected the percentage of damaged eggs at 60 and 90 days of the study period, despite there being no significant differences over the whole period (1-120 days) of the study. It is thought that poor shell quality in aged laying hens is associated with a loss of the ability to absorb minerals (3). Because there is a preferable balance in mineral absorption in young hens, it should not been expected to obtain significant variations with dietary B usage. Hence, the dramatic decrease in the percentage of cracked eggs detected in the present study is probably related to the improving effect of B on Ca and P absorption.

In this study, eggshell thickness was significantly (P < 0.001) increased (35-75 days) especially in group III, which included 100 mg/kg BA supplementation (Table 3). Qin and Klandorf (3) reported that eggshell thickness was not affected in aged broiler breeder hens by 100 mg/kg B supplementation, while Eren et al. (15) stated that eggshell thickness was significantly (P < 0.01) increased in 10 and 200 mg/kg B supplemented groups at week 22. This positive effect on eggshell thickness in aged laying hens is thought to result from the effects of BA as a nutritional factor in Ca metabolism and utilisation (7). Supplementation of BA significantly (P < 0.001) increased the eggshell breaking strength in aged laying hens (Table 3). Total eggshell breaking strength was 32.94 (Newton) in the control group and 36.91, 38.95, and 37.81 in the experimental groups including 25, 50, and 100 mg/kg B, respectively. Eren et al. (15) found that 0-400 mg/kg B supplementation had no effect on eggshell breaking strength in younger laying hens between 22 and 28 weeks. The increase in eggshell breaking strength detected in the present study may be related to the increase in eggshell thickness.

B seems to have a regulatory role in mineral metabolism but the mechanism of this role is not completely understood. Some recent studies have shown that B affects Ca, P, and Mg metabolism (3,21,22). In

Table 4. Serum mineral levels of control and boric acid supplemented groups in laying hens.

		Boric acid supplementation (mg/kg)				
Parameters	n	0 (control)	25	50	100	p
Ca (mg/dl)	5	25.63 ± 0.46	25.80 ± 0.30	26.93 ± 0.42	26.43 ± 0.33	-
P (mg/dl)	5	6.49 ± 0.18	6.49 ± 0.008	7.03 ± 0.14	7.42 ± 0.39	-
Mg (mEq/l)	5	3.26 ± 0.14^a	3.62 ± 0.13^a	3.34 ± 0.009^a	4.33 ± 0.23^b	**

[a-b] The mean values within the same row with different superscripts differ significantly ; -: not significant **: P < 0.01

this study, slight increases in serum Ca, P ($P > 0.05$), and Mg ($P < 0.01$) (Table 4) levels in all treatment groups were consistent with the results reported by Hunt (6), Nielsen (7), and Kurtoğlu et al. (18). The increases in the levels of these minerals may result from either the improvement of absorption or the reduction of urine losses because of the B supplementation in the ration.

From these results it is concluded that dietary BA supplementation may positively affect on mineral balance and therefore reduces the percentage of cracked eggs by improving eggshell thickness and eggshell breaking strength in aged laying hens. The other performance parameters were not negatively affected.

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