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Research Article

Effect of Diets with Various Calcium and Boron Levels in Prelaying Period on Growth, Bone Ash and Subsequent Performance and Shell Quality of Laying Hens

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Abstract: This experiment was conducted to determine the effects of diets with three different levels of calcium (% 0.8, 1.6, 3.2) and four different levels of boron (0, 75, 150, 300 mg kg-1) in prelaying period (14-20 weeks) on growth, bone ash and subsequent performance and egg shell quality of laying hens. A total 864 Super Nick pullets at 14 weeks of age were randomly allocated to twelve treatments arranged in a 3x4 factorial design with six replicates of 12 pullets each. Feed and water provided for ad-libitum access. The tibia samples were taken, three pullets and hens from every replicates at 20 and 42 weeks of age, respectively. Results of the present experiment showed that body weights and body weight gains were reduced in high prelay dietary Ca and B as the main factor. Feed intake of the pullets was not significantly influenced by the treatments. There were no detectable difference in feed intake, egg production, egg mass and feed conversion ratio (g, feed/ g, egg mass) and eggshell weight, but interactive effects of dietary Ca and B levels on eggshell thickness, egg specific gravity and shell breaking strength was significant. Shell thickness and breaking strength were significantly higher in hens fed pre-lay diet with 3.2 % Ca and no supplemental B than other treatments and egg specific gravity was significantly reduced by feeding 1.6 % Ca and 75 mg kg-1 B at 36 weeks of age. Bone ash (g or %) of hens at 20 and 42 weeks of age was not changed by Ca levels or interaction dietary Ca and B levels, but pullets receiving the 150 mg kg^{-1} B as the main factor had significantly higher in bone ash (g or %) values than other dietary B levels.

Keywords: Calcium, boron, egg shell, bone mineralization, hen.

1. Introduction

Calcium (Ca) is an essential mineral element for humans and animals. It plays an important role in a number of physiological and biochemical processes [1]. Although much research has been conducted to establish the Ca requirement of the laying hens and although Ca nutrition of pullets prior to laying period is important for the growth and skeletal development as well as for subsequent maximum reproductive performance, eggshell quality and skeletal integrity during the laying phase [2,3], not much importance has been given to Ca requirement of growing pullet at this period.

Ca storage of pullets prior to onset of egg production is crucial in terms of maintaining both egg production and eggshell quality, and it is desirable for the pullet to deposit as much Ca as possible in her skeletal store, since Ca balance is negative in pullets during early egg production phase and this situation can not be prevented by feeding high Ca diets [4, 5]. Also, it has been shown that eggshell quality and bone Ca and ash content increased when the pullet were fed with diets containing high Ca for varying times (2 to 6 weeks) during pre-laying period [4, 6].

Practical poultry diets, based on corn-soybean meal, are adequately high in boron (B) [7, 8]. Although there is a lack of information on the effect of dietary B on the reproductive performance in poultry, the results of the majority of experiments demonstrated that B significantly affected the performance characteristics [9, 10, 11, 12, 13], eggshell quality [14, 13], some properties of interior egg quality [12], mineral composition and biomechanical properties of bone [7, 9, 10, 11] and Ca level of plasma [14, 12]. While beneficial effects of B were demonstrated in poultry, how much boron is required in nutrition has not been firmly established, and the mechanism of action has not been clearly defined. Although it has been determined that B affects Ca metabolism [15, 16, 17], interactive effects of CaxB have been investigated in a limited amount of research [18].

This experiment was designed to determine the effect of dietary 3 levels of Ca (0.8, 1.6, 3.2 %) and 4 levels of B (0, 75, 150 and 300 mg kg-1) in pre-laying period (14-20 weeks) on growth, bone ash and subsequent performance and eggshell quality during the laying period (20-42 weeks).

2. Material and Method

Eight hundred and sixty four, 14-week-old pullets (Super Nick White) which were obtained from commercial firm were used in this experiment. The pullets were weighed in groups of four and placed in per cage in the four lower tiers of a five-tier battery cage system in a windowless, mechanically ventilated house. The pullets of three adjacent cages were considered an experimental replicate and each dietary treatment was fed with six replicates (72 pullets per treatment). At the start of the experiment, appropriate changes were made between pullets in cages for treatment means to be similar in terms of body weight. Replicates were equally distributed into upper and lower cages to minimize cage level effect.

The experiment was conducted as prelaying (14-20 weeks of age) and laying period (20-42 weeks of age), including two phases. Pullets were fed twelve diets including combination of three levels of calcium (0.8, 1.6 and 3.2%) and four levels of boron (0, 75, 150 and 300 mg kg-1 boron as boric acid) during prelaying period. All nutrients of diets used during this period except Ca and B were similar and recommended practices for this strain [19] were followed with regard to feeding and lighting programs. A layer diet containing nutrient recommended level for this strain was used during laying period (Table 1). Feed in mash form and tap water were supplied as ad libitum.

Live body weight of pullets was determined in groups of all pullets in every replacement at 14, 20 and 42 weeks of age. Feed consumption of pullets for each replicate was obtained in groups during 14-20, 20-24, 24-28, 28-32, 32-36, 36-40 and 40-42 weeks of age. Daily feed intake and body weight gain per bird for pre-laying and laying periods were calculated from these data.

During the experiment, egg production (EP) and mortality were recorded daily and feed consumption (FC) was adjusted for mortality. Records of daily egg production were summarized in every period, which was mentioned above. Egg weight (EW) was measured and using all eggs produced during the last 3 consecutive days at 20, 24, 28, 32, 36, 40 and 42 weeks of age. Egg mass (EM) and feed conversion ration (FCR, g of feed/g of EM) were calculated from EP, EW and FC. After the measurement of EW, egg specific gravity (SG), eggshell strength (ES), shell weight (SW), percentage shell (PS) and shell thickness (ST) were determined on a random sample of 5 eggs per replicate. Egg specific gravity was measured by Archimedes' principle. Eggshell weight was measured after washing them using tap water to remove adhering albumen and drying them for 3 days at room temperature. To measure ST with membranes, three small pieces of shell were taken from the opposite locations of the equator and thick end of the egg, and their thickness were measured using caliper (Mitutoya,0.01 mm, Japan).

Table 1 Composition of experimental pre-lay diets and layer diet (%, as feed)

Ingredients	Calcium levels of pre-lay diets[1], %			Layer diet
	0.80	1.6	3.2	
Barley	28.08	22.00	14.35	-
Yellow corn	48.70	52.00	52.70	48.40
Soybean meal (47.4 % CP)	8.12	9.30	11.90	28.90
Sunflower meal (28 % CP)	10.00	9.00	7.50	3.00
Fishmeal (65 % CP)	2.00	2.00	2.00	3.50
Andgetable oil	-	0.50	2.10	4.50
Limestone	0.50	2.60	6.90	8.90
Dicalcium phosphate	1.70	1.70	1.70	1.80
Salt	0.40	0.40	0.40	0.40
Premix[2]	0.25	0.25	0.25	0.25
L-lysine	0.15	0.15	0.10	-
DL-methionine	0.10	0.10	0.10	0.35
TOTAL	100.0	100.0	100.0	100.0
Calculated analysis				
ME, kcal/kg	2816	2813	2812	2803
Crude protein, %	15.13	15.17	15.19	21.20
Lysine, %	0.76	0.76	0.76	0.96
Methionine, %	0.39	0.39	0.38	0.53
TSAA, %	0.66	0.65	0.63	0.82
Calcium, %	0.82	1.60	3.21	4.08
Available P, %	0.50	0.49	0.48	0.55

[1]Pre-lay diets with different leandls of calcium were supplemented with 0 ,75 ,150 and 300 mg boron/kg from boric acid (H_3BO_3), but layer diet was not supplemented with B.

[2]Similar premix was used in all diets, and it supplies the following per kg of feed: vitamin. A, 12,000 IU; vitamin. D3, 3,300 IU; vitamin E, 20 mg; vitamin. K3, 4 mg; vitamin.B1, 3.0 mg; vitamin. B2, 7.0 mg; vitamin B6, 5.0 mg; vitamine. B12, 0.015 mg; Niacin, 25.0 mg; D-calcium pantothenate, 10.0 mg; folic acid, 1.0 mg; D-biotin, 0.05 mg; cholin cloride, 175 mg; manganese, 100 mg; iron, 60 mg; zinc 60 mg; copper,

5 mg; cobalt, 0.5 mg; iodine, 2 mg; selenium, 0.15 mg; phytase, 300 units.

Three hens from each replacement were randomly selected at 20 and 42 weeks of age. After the slaughtering of hens, right tibia of each hen was removed and used to determine bone ash. Tibia were cleaned off soft tissue and dried at 105 °C over night and ash was obtained at 600 °C for 24 hours in muffle furnace. Data were subjected to analysis of variance for a completely randomized 3x4 factorial design that included the 3 levels of calcium and 4 levels of boron, using ANOVA-General Linear Model (MINITAB Reference Manual, Release 9.1). Significant differences among treatment means were identified by Duncan's multiple range test [20].

3. Results and Discussion

Prelayer diet Ca level or CaxB interaction did not affect tibia ash significantly at the ages of 20 and 42 weeks of age (in g and %). However, the growing diet B level as the main factor significantly affected tibia ash in pullets at the age of 20 weeks, and the tibia ash weight of the pullets fed with a diet containing 150 mg kg-1 B (g, data not shown) and its percentage (Figure 1) were found to be remarkably higher ($P<0.05$) than with diets containing other boron levels. The positive effect of boron on bone mineralization, probably may be due to that B modifies mineral balance by increasing the synthesis and/or action of sex hormones particularly estradiol [27]. However, the studies conducted on the effects of dietary boron levels on broiler chicks and laying hens presented inconsistent results regarding tibia ash. Wilson and Ruszler [8] reported that the addition of 50, 100 and 200 mg kg-1 B significantly increased tibia ash in the periods of 0-16 weeks and that the highest value was obtained with 50 mg kg-1 B. In another study in which layer hens were fed with the rations containing 0 to 400 mg kg-1 B from 16 or 18 weeks of age to 72 weeks of age, [11] reported that no B level used in the diet significantly affected tibia and radius at 35, 52 and 72 weeks of age. In another study in which broiler chicks were fed with a diet containing 30 and 60 mg kg-1 B in the form of boron trioxide and borax for 21 days, tibia ash was reported to decrease significantly compared with the control group fed with boric acid [7], while Eliot and Edwards [21] reported that an addition of 20, 40 and 80 mg kg-1 B to practical broiler diets did not affect tibia ash significantly. Kurtoğlu at al [22] reported that the addition of 5 and 25 mg kg-1 B to the broiler diets sufficient and deficient in cholecalciferol did not affect tibia ash significantly at 45 days of age (g and %).

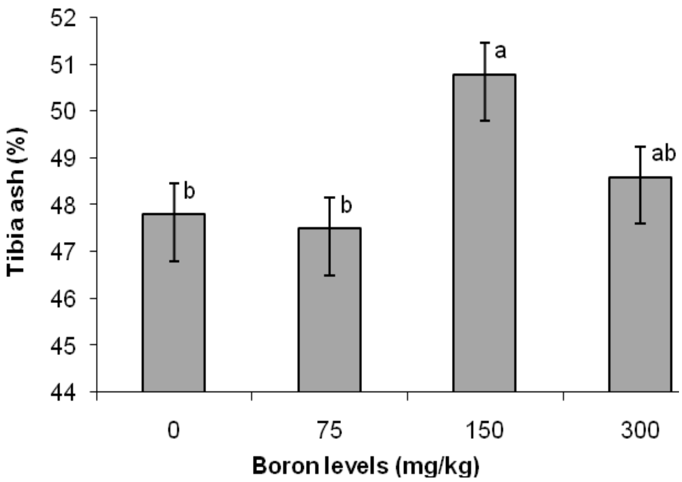

Figure 1. Effect of boron levels of prelayer diet on tibia bone ash of pullets at 20 weeks of age

These results show that B does affect mineral metabolism but that the effect may vary depending on the species, genetic make-up, dietary boron level and source and the duration of feeding with boron.

Table 2. The effect of prelaying calcium and boron levels on live body weight and live weight gain.

Treatments	Body weight, g 14 wk	Body weight, g 20 wk	Body weight, g 42 wk	Body weight gain, g 14-20 wk	Body weight gain, g 20-42 wk
Ca levels, %					
0.8	1008	1311^{a}	1628^{b}	303^{ab}	318
1.6	1027	1359^{a}	1702^{a}	332^{a}	343
3.2	1003	1247^{b}	1608^{b}	244^{b}	361
B, levels, mg kg-1					
0	1019	1341^{a}	1611	322^{a}	305
75	1014	1351^{a}	1680	336^{a}	329
150	1032	1258^{b}	1673	226^{b}	355
300	985	1273^{b}	1621	287^{ab}	348

a, b: means with no common superscripts within each column differ significantly ($P<0.05$)

While no treatment composed of differing Ca and B levels significantly affected LBW and LWG in chickens, dietary Ca and B levels had a significant effect on these parameters (Table 2). The LBW of the chickens fed with a diet containing 3.2 % Ca was found to be significantly higher than that of the chickens fed diets with composed of 0.8 and 1.6 % Ca ($p<0.05$) at the age of 20 weeks. At the same time, the LBW of the pullets fed with a growing diet composed of 3.2 and 0.8 % Ca was lower than that of the pullets fed a growing diet 1.6 % Ca at the age of 42 weeks ($p<0.05$). Besides, the LWG of the pullets fed diet with 1.6 Ca at 14-20 weeks was significantly higher than that of the chickens fed with a diet of 3.5 % Ca. Dietary Ca level did not have a significant effect on

LWG at the period of 20-42 weeks. In general, prelayer diets with low Ca favorably affected the growth of the chickens. These results are consistent with the results obtained by Miller and Sunde [23]. These researchers fed the chickens with diets containing 0.40 to 4.5 % Ca in the period between 18-22, and reported that the LBW of the chickens fed with a diet containing 3.0 % Ca was significantly lower at the age of 20 weeks ($p<0.05$).

This finding may be linked to increase the content of intestinal calcium binding-protein, CaBP, Bar and Hurwitz, [25] and the digestibility and utilization efficiency of Ca, when the chickens are fed with diets containing low Ca. However, there are studies reporting that the diets with high Ca (3.0-3.5 %) did not adversely affect LBW and LWG during growth (the period of 14 or 15-20 weeks) [3, 4]. The discrepancy in the results may be due to the differences in genetic make-up.

There was significant differences in average LBW or LWG between pullets fed the different dietary B levels, and increasing levels affected the growth adversely. LBW of pullets at 20 weeks of age was significantly lower with diet containing 150 and 300 mg kg^{-1} B than compared with diets containing 0 or 75 mg kg^{-1} B. Also, LWG of pullets fed diet with 150 mg kg^{-1} B was significantly lower than the other two diets with low levels of B (Table 2). Other researcher reported that diets containing B in medium or relatively higher levels reduced growth rate. Rossi [7] reported that LBW of female broiler chickens which fed with diet containing 120 mg kg^{-1} B decreased. Wilson and Ruszler [8] stated that 0-16 weeks layer chickens reached maximum LBW when they were fed with a diet containing 50 mg kg^{-1} B, while LBW started decreasing when diet contained 100 mg kg^{-1} B and significantly decreased when diet B level reached 400 mg kg-1 B.

No treatment used in the study affected FC of pullets, EP, EM and FCR significantly (Table 3). Results from this study generally agree with those of results obtained by Keshaverz [4] and Miller and Sunde [23]. They reported that prelaying diet with high Ca (3.5 or 4.5 %) with not significantly affect the EP, EW and FCR but Brooks [3] reported that EW of hens previously fed 2.0 and 3.0 % Ca diet was continuously higher and FCR values was better than hens fed 1.0 % Ca diet birds.

Table 3. The effect of prelaying calcium and boron levels on feed consumption, egg production, egg mass and feed conversion

Treatments		Feed consumption g/hen		Egg production %		Egg mass g		Feed conandrsion g/g
Ca.%	B.mg/kg	14-20 wk	20-42 wk	14-20 wk	20-42 wk	14-20 wk	20-42 wk	20-42 wk
0.8	0	59.1	102.6	5.2	73.8	2.47	49.88	2.06
0.8	75	64.5	104.9	4.3	82.5	2.09	52.80	1.99
0.8	150	67.3	104.1	2.9	85.4	1.30	51.41	2.02
0.8	300	63.9	99.3	0.5	77.2	0.21	49.41	2.01
1.6	0	68.1	99.6	5.0	79.5	2.30	50.16	1.99
1.6	75	61.5	106.2	6.4	84.0	2.92	52.92	2.01
1.6	150	62.0	103.1	5.7	78.6	2.59	49.83	2.07
1.6	300	61.2	105.8	6.2	83.7	2.82	53.23	1.99
3.2	0	70.2	101.4	3.5	82.9	1.60	52.31	1.94
3.2	75	68.6	104.5	5.2	85.9	2.62	54.03	1.93
3.2	150	59.8	98.5	4.0	80.5	2.15	50.39	1.95
3.2	300	61.5	104.2	4.0	83.3	1.89	52.15	2.00

Effect of prelaying B levels on the performance and bone mineralization of pullet only examined in one study [8]. This study was carried out in 0-16 week of period and effect of dietary B levels (50 to 400mg kg-1 B) on subsequent performance of laying hens has not been investigated. However, it has been reported that dietary B levels (50 to 250 mg kg-1) did not significantly affect the performance characteristic of laying hens [14] and diets with high levels of B decreased EP, FC and EW of laying hens [11, 12].

While Ca and B level of the growing diet did not significantly affect SW (data not shown), diet CaxB interaction significantly affected ST, ESG and breaking strength (Table 4). Eggshell thickness (0.543 mm) and breaking strength (4.1 kg) of the hens fed with a growing diet containing 3.2 % and no additional B were found to be significantly ($p<0.05$) higher than all the groups (0.420 to 0.431 mm and 2.7 to 3.3 kg) fed with other diets. Also, the ESG (1.079) of the hens fed with a diet containing % 1.6 % Ca and 75 mg kg-1 B was found to be significantly lower than all the other groups (1.081 to 1.083) (Table 4). Since the effect of the interaction on these characteristics was considered significant, the effect of the main factors was not taken into consideration. For the first time, the effect of prelaying diet CaxB interaction on eggshell quality was examined in this study, and the studies conducted regarding the effect of dietary B level on eggshell quality in laying hens yielded inconsistent results. For instance, Eren et al. [12] reported that the measured ST of the hens fed with a diet containing 200 mg kg-1 B was significantly ($P<0.01$) higher at 22 weeks of age than that of the hens fed with diets containing 0, 5, 50, 100 and 400 mg kg-1 B and that the rate of cracked/broken eggs significantly increased with the rations containing 200 and 400 mg kg-1 B. However, in a study in which the hens of 40 weeks of age were fed for 120 days with rations containing 0-250 mg kg-1 B, Kurtoğlu et al. [14] reported that the rate of cracked/broken eggs significantly ($P<0.05$) decreased with a ration containing 250 mg kg-1 B in a period of 60-90 days, while egg weight and ESG were reported not to be significantly affected in a study in which broiler breeders at 21 weeks of age were fed for 16 weeks with rations containing 250 mg kg-1 B in the form of boric acid or borax Rossi et al. [24]. These contradictory results might be due to difference in the genetic make up of experimental materials. Overall, these types of outcomes could be explained in the light of complex traits

dissection concept, with the aid of molecular genetic tools, which is an active research area of

nowadays. On the other hand, difference in species and age of animals, feeding period and B sources might also have affect on these contradictory results. For instance, Al-Batshan et al. [26] reported that SW as percent of EW and ST are affected by strain and age. Some strain, in comparison with other strains were more sensitive to nutritional treatments and/or boron toxicity.

Table 4. The effect of prelaying calcium and boron levels on shell thickness, egg specific gravity, and shell breaking strength of laying hens.

Treatments		Shell thickness, mm		Egg speficic gravity		Shell breaking strenght, kg	
Ca.%	B.mg/kg-1	36 wk	42 wk	36 wk	42 wk	36 wk	42 wk
0.8	0	0,421^{b}	0.400	1,082^{a}	1,082	2,7^{b}	2,8^{ab}
0.8	75	0,423^{b}	0.411	1,081^{a}	1,080	2,7^{b}	2,7^{abc}
0.8	150	0,422^{b}	0.402	1,081^{a}	1,080	2,8^{b}	2,7^{abc}
0.8	300	0,420^{b}	0.410	1,083^{a}	1,081	3,3^{b}	2,7^{abc}
1.6	0	0,422^{b}	0.405	1,083^{a}	1,083	3,1^{b}	2,9^{c}
1.6	75	0,431^{b}	0.404	1,079^{b}	1,080	2,9^{b}	2,7^{abc}
1.6	150	0,421^{b}	0.409	1,081^{a}	1,082	2,9^{b}	2,9^{a}
1.6	300	0,430^{b}	0.401	1,083^{a}	1,080	2,8^{b}	2,8^{abc}
3.2	0	0,543^{a}	0.407	1,081^{a}	1,081	4,1^{a}	2,9^{a}
3.2	75	0,425^{b}	0.405	1,083^{a}	1,082	2,8^{b}	2,9^{a}
3.2	150	0,424^{b}	0.408	1,083^{a}	1,083	3,1^{b}	3,0^{a}
3.2	300	0,430^{b}	0.404	1,081^{a}	1,082	3,2^{b}	2,9^{bc}

a-c: Means with no common superscripts within each column differ significantly ($P<0.05$)

Results from this study showed that dietary Ca and B levels in prelaying period did not affect the performance characteristics significantly. But growth of pullets and eggshell quality of laying hens were adversely influenced by high dietary Ca and B levels in this period.

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