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Influence of dietary boric acid and ascorbic acid on performance, egg traits, cholesterol and bone parameters of laying hens

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Summary: This study was conduct to investigate the impact of dietary boric acid (BA), includes 17.5 % boron, ascorbic acid (AA) and combined of boric acid and ascorbic acid (AABA) on performance, egg traits, blood serum and egg yolk cholesterol concentrations and some bone parameters of laying hens. In total, 160 Hyline-White 98 layers aged 59 weeks were allocated four treatments with ten replicates. Dietary treatments included the basal diets as control, AA-supplemented diets with an inclusion level of 200 mg/kg AA, and BA-supplemented diets with an inclusion level of 120 mg/kg BA and AABA- supplemented diets with an inclusion level of 200 mg/kg AA + 120 mg/kg BA. The experimental period lasted 16 weeks. Feeding ascorbic acid and boric acid diet did not significantly affect body weight, feed efficiency, egg weight, egg shell index, egg breaking strength, egg shell thickness, egg albumen index, egg yolk index, egg Haugh unit, egg yolk weight, tibia crude ash and phosphorus. Egg production increased ($P<0.05$) with ascorbic acid supplementation at only 11-12th weeks. There was a significant ($P<0.05$) increasing in feed consumption at first two weeks period. Hen serum cholesterol ($P\leq0.001$) concentrations decreased with ascorbic acid and boric acid supplementation single or together, while egg yolk cholesterol did not affect. Ascorbic acid and boric acid also significantly increased tibia calcium levels ($P<0.05$). Higher liver ($P<0.001$) and egg yolk ($P<0.001$) boron concentrations were detected in hens receiving BA and AABA diets. This study demonstrated that ascorbic acid and/or boric acid may have beneficial effects on Ca mobilization from the bone.

Keywords: Ascorbic acid, bone mineralization, boric acid, cholesterol, performance.

Yumurtacı tavuklarda borik asit ve askorbik asitin performans, yumurta kalitesi, kolesterol ve kemik parametreleri üzerine etkileri

Özet: Bu deneme, % 17.5 bor ihtiva eden borik asitin (BA) ve askorbik asitin (AA) tek başlarına ya da birlikte (AABA) kullanıldıkları zaman yumurta tavuklarında performans, yumurta kalitesi, kan serum ile yumurta sarısı kolesterol düzeyleri ve kemik parametreleri üzerine etkisini belirlemek amacıyla gerçekleştirilmiştir. Bu amaçla 59 haftalık 160 adet Hyline White 98 yumurta tavuğu 10 tekrarlı 4 ana gruba ayrılmıştır. Kontrol grubu rasyonuna herhangi bir ilave yapılmamıştır. AA ilaveli guba 200 mg/kg AA, BA ilaveli gruba 120 mg/kg BA, AABA ilaveli gruba ise 200 mg/kg AA + 120 mg/kg BA katkisi gerçekleştirilmiştir. Yemleme dönemi 16 hafta sürmüştür. Rasyonlara borik asit ve askorbik asit ilavesi canlı ağırlığı, yemden yararlanma oranını, yumurta ağırlığını, yumurta kirilma direnci, kabuk kalinligi, yumurta ak ve sari indeksi, Haugh birimi ile sari agirligini ve kemik kül ile P düzeyini etkilememiştir. Ancak 11-12. haftalarda askorbik asit ilavesi ile yumurta verimi ($P<0.05$), ilk 2 haftalık periyotta ise borik asit ilavesi ile yem tüketim değeri artmıştır ($P<0.05$). Kan serumu kolesterol düzeyleri rasyona borik asit ve/veya askorbik asit katkısı ile azalmıştır ($P\leq0.001$). Söz konusu yem katkı maddeleri tibia Ca düzeyini önemli düzeyde artırmıştır ($P<0.05$). En yüksek karaciğer ve yumurta sarısı bor konsantrasyonlarına ise BA ilaveli gruplarda rastlanmıştır ($P<0.001$). Sonuç olarak, askorbik asit/ ve/veya borik asit ilavesinin kemik Ca mobilizasyonuna yararlı olabileceği düşüncesine ulaşılmıştır.

Anahtar sözcükler: Askorbik asit, borik asit, kemik mineralizasyonu, kolesterol, performans.

Introduction

Boron has a strong tendency to form complexes with organic molecules that have adjacent hydroxyl groups. Boron is able to interact with important biological substances, including polysaccharides, pyridoxine, riboflavin, dehydroascorbic acid, and the pyridine nucleotides (24). Boron is known to influence a variety of metabolic actions. It interacts with calcium, vitamin D, and magnesium, which are all important in bone metabolism. Boron accumulates in bone in concentrations dependent on the amount of boron consumed (3). An experiment (12) determined that boron provided significant improvements on serum Ca levels and damaged egg ratio but no significant difference between the controls and the boron supplemented groups (50, 100, 150, 200 and 250 ppm) about feed consumption, feed conversion ratio, egg production, body weight and egg weight. Boric acid compounds that are found different types which are carbohydrates (glucose and polisaccarides), nucleotides (adenosine monophosphate and niacineamide adenindinukcleotide), vitamins (ascorbic acid, pyridoxine, ryboflavine) in body structure. (15). So boric acid that contains boron was used with ascorbic acid in current study.

Ascorbic acid is a sugar acid with antioxidant properties. One form of ascorbic acid is commonly known as vitamin C. Normally ascorbic acid is synthesized from kidneys in laying hens, but some stress factors can obstruct this (31). A trial (4) determined that ascorbic acid supplementation can be effective in reducing laying hen mortality due to environmental stres and small influences on egg quality.

The present study was aimed at examining the effects of dietary ascorbic acid and boric acid supplementation on performance, egg traits, blood parameters and some bone parameters of Hyline-White 98 layers.

Materials and Methods

Layers, experimental design and diet: A total of 160 Hyline-White 98 layers aged 59 weeks were chosen at randomly. They were housed in cages (50 x 59 x 60 cm) and allocated randomly to four dietary treatments. Each treatment comprised ten replicates of four layers in groups of four. Therefore four groups each containing 40 hens were arranged.

The house was provided with programmable lighting and ventilation during the 16 wks experimental period. The temperature maintained at 20-22°C according to normal management practice. Chicks were maintained on 17 hours constant light schedule until the end of the experiment. Feed and water were provided for ad libitum consumption and the diets were presented in mash form.

The composition of the basal diet is given in Table 1. The diets were formulated to be isocaloric and isonitrogenous and to meet or exceed NRC (19) nutrient requirements for laying hens. 200 mg/kg ascorbic acid (AA), 120 mg/kg boric acid (BA) and 200 mg/kg ascorbic acid plus 120 mg/kg boric acid (AA+BA) respectively. Although the inclusion of at least 2 ppm boron in poultry feed was recommended by NRC (18), no matter which poultry category or type of production, such suggestions are not present in the requirements for poultry in NRC (19). Therefore, the only suitable suggestions are from experimental diets that were in previous studies (23; 24).

Feeds were analyzed moisture, ash, crude fibre, ether extract, crude protein, calcium and total phosphorus according to the reference methods AOAC (1).

Performance analysis: Hens were weighed individually at the beginning and end of the experiment. Eggs were collected daily. Eggs were weighed every week individually for 1 day of production. Feed consumption was recorded biweekly and calculated as g/day per bird. The value of feed efficiency was calculated as kg feed/ kg egg and kg feed per dozen eggs.

Throughout the experiment, 20 eggs were collected from each group (two eggs from each replicate) at 4 week intervals to determine egg traits. Individual eggs were weighed and their shape index, shell breaking strength (22) and shell thickness were measured. Then yolk height, albumen height, yolk width, albumen width and albumen length were determined. Using these values, yolk index, albumen index and Haugh unit were calculated **(**2). Egg and shell quality analyses were completed within 24 h of the eggs being collected.

Biochemical analysis: At the end of the experiment, an egg per replicate were chosen randomly to determine yolk cholesterol. Eggs were boiled for 5 min. Cholesterol was extracted by using commercial kits (Teco Diagnostic) with spectrofotometric method according to the manufacturers' recommendations. Yolk weights were also determined for these eggs.

Table 1**.** Ingredients and chemical composition of the basal diets, g/kg.
Tablo 1. Bazal rasyonun içeriği ve kimyasal bileşimi, g/kg.

Ingredients	Laying Hens Diet
Corn	610.00
Meat bone meal	40.00
Soya bean meal	100.00
Full fat soybean	120.00
Limestone	100.00
Dicalcium phosphate	20.00
Salt	3.50
DL-Methionin	3.00
Vitamin/mineral premix[1]	3.50
ME, kcal/kg	2782
Crude protein	157.60
Ca	34.40
P	8.20

[1]:Supplies retinol 3,600 mg, cholecalcipherol 200,000 mg, tocopherol 50,000mg, menadione 10,000 mg, tiamine 4,000 mg, riboflavine 8,000 mg, Pyridoxine 5,000 mg, Cobalamine 25 mg, niacine 50,000mg, panthotenic acid 20,000 mg, folic acid 20,000 mg, biotine 250 mg, ascorbic acid 75,000 mg, choline 175,000 mg, apoester carot. 5,000 mg, canthaxanthin 250,000 mg, Mn 100,000 mg, Zn 150,000 mg, Fe 100,000 mg, Cu 20,000 mg, I 1,500 mg, Co 500 mg, Se 200 mg, Mo 1,000 mg, Mg 50,000 mg^{-1} diet.

[1]: retinol 3,600 mg, kolekalsiferol 200,000 mg, tokoferol 50,000mg, menadion 10,000 mg, tiyamin 4,000 mg, riboflavin 8,000 mg, Piridoksin 5,000 mg, Kobalamin 25 mg, niyasin 50,000mg, pantotenik asit 20,000 mg, folik asit 20,000 mg, biyotin 250 mg, askorbik asit 75,000 mg, kolin 175,000 mg, karoten. 5,000 mg, kantaksantin 250,000 mg, Mn 100,000 mg, Zn 150,000 mg, Fe 100,000 mg, Cu 20,000 mg, I 1,500 mg, Co 500 mg, Se 200 mg, Mo 1,000 mg, Mg 50,000 mg^{-1}

Blood samples were collected from the vena brachialis under the wing from 10 fed hens chosen randomly from each group (one from each replicate) at the end of the experiment and centrifuged at 3000 x g for 10 min. Serum was collected and stored at -20 °C for determination of serum parameters. Serum concentrations of cholesterol were determined by using commercial kits (Teco Diagnostic) with spectrofotometric method.

Bone parameters: Left tibias were collected from 10 fed hens chosen randomly from each group (one from each replicate) at the end of the experiment and their crude ash determined by litting in ash owen at 610˚C, calcium (Ca) and phosphorus (P) contents determined by spectrofotometrically after litted in microwave (BERGHOF, MWS-2, Germany) (1). Serum, liver and egg yolk boron levels were determined using the following method. Briefly; ultrapure HNO_3 (10 ml, Merck) was added to each sample in teflon tubes until it was completely dissolved; afterwards, all samples burned in microwave (BERGHOF, MWS-2, Germany). Subsequently, ash samples were completed with ultra pure water into the 25 ml flasks. The samples were filtered using WH 42 filter paper. The obtained solutions were diluted with ultra pure water to a final volume of 100 ml. The concentrations of minerals were measured at specific wavelengths for each element by an Inductively Coupled Plasma Mass Spectrometry (ICPMS, Thermo X-SERIES2). The calibrations for the B assays were conducted with a series of mixtures containing graded concentrations of standard solutions of each element (B ICP standard).

Statistical analysis: All data were analyzed by ANOVA using SPSS 11.50 program (Inc., Chicago, II, USA). Significant differences among treatment were determined using Duncan's multiple range tests (6) with a 5% level of probability (27).

Results

Table 2 shows the result of dietary ascorbic acid and boric acid on performance. Over the whole experimental period, body weight, egg weight and feed conversion were not affected by supplemented or not with boric acid and/or ascorbic acid. However, at 1-2 wks, feed intake was increased (p<0.05) by addition of boric acid (BA) and combination of feed additives (AABA). In addition to this, there were significantly (p<0.05) increasing on hen day egg production at 11-12 wks in AA group. The egg quality parameters (shell thickness, breaking strength, and shape index) results were also summarized in Table 3. These parameters for performance were not significantly modified by the supplementation with boric acid and/or ascorbic acid combination over the whole experimental period. Blood

Table 2. Effects of ascorbic acid and boric acid on performance of laying hens.
Tablo 2. Askorbik asit ve borik asitin yumurtacı tavuklarda performans üzerine etkileri.

Parameters	Control	AABA	AA	BA	P
Initial body weight (g)	1781.00±29.63	1800.50±33.52	1799.35±39.75	1783.00±38.24	0.967
Final body weight (g)	1922.63±52.80	1903.13±23.63	1919.25±30.26	1892.25±32.19	0.927
Feed consumption (g/hen/day)	112.40 ± 1.44	109.19 ± 2.36	105.30 ± 2.35	117.29 ± 6.38	0.145
Feed consumption (g day^{-1} per bird) (1-2 weeks)	91.23 ± 1.83^{b}	95.89 ± 1.61^{ab}	91.63 ± 2.16^{b}	97.78 ± 1.63^{a}	0.038*
Egg production (%)	76.67± 2.32	78.06± 2.34	78.13± 1.50	77.85± 2.58	0.939
Egg production (%) (11-12 weeks)	76.07± 1.96^{b}	80.71± 2.09^{ab}	84.64± 1.31^{a}	75.54± 3.84^{b}	0.044*
Egg weight (g)	66.61± 0.53	67.37± 0.50	66.92± 0.92	67.11± 0.64	0.876
Feed conversion (kg feed per dozen eggs)	2.16± 0.07	2.14± 0.06	2.03± 0.05	2.24± 0.13	0.362

a, b: Means on the same line with different superscript differ significantly (*): p<0.05.
a, b: Aynı sırada farklı harf taşıyan ortalama değerler arasındaki fark istatistik bakımdan önemlidir (*): p<0.05.

Table 3. Effects of ascorbic acid and boric acid on egg traits of laying hens.
Tablo 3. Askorbik asit ve borik asidin yumurta özellikleri üzerine etkileri.

Parameters	Control	AABA	AA	BA	P
Shell index	77.33 ± 0.99	76.18 ± 0.57	76.48 ± 0.53	76.83 ± 0.66	0.696
Breaking strength (kg cm^2)	1.75 ± 0.15	1.73 ± 0.16	1.54 ± 0.14	1.65 ± 0.12	0.727
Shell thickness (mmx10^2)	38.16 ± 0.38	37.65 ± 0.43	37.53 ± 0.47	37.78 ± 0.39	0.739
Albumen index	93.38±3.86	89.68±3.53	88.72±3,10	95.34±4.13	0.546
Yolk index	399.94±5.05	419.64±6.07	401.74±6.58	410.35±5.33	0.071
Haugh unit	86.84±6.25	94.06±1.18	90.31±4.71	83.09±7.76	0.544
Yolk weight (g)	19.13±0.29	19.42±0.50	18.92±0.34	19.19±0.34	0.816

Differences between treatment groups is not statistically significant (p>0.05).
Gruplar arasındaki fark istatistik açıdan önemsizdir.

Table 4. Effects of ascorbic acid and boric acid on blood serum and egg yolk cholesterol concentrations of laying hens (mg/dl).
Tablo 4. Yumurtacı tavuklarda askorbik asit ve borik asitin kan serumu ve yumurta sarısı kolesterol konsantrasyonu üzerine etkileri (mg/dl).

Parameters	Control	AABA	AA	BA	P
Initial serum	142.63±9.10^{bc}	181.21±8.16^{a}	162.51±8.88^{ab}	135.85±9.17^{c}	0.004***
Final serum	141.04±6.63^{ab}	152.59±6.67^{a}	128.11±6.30^{bc}	112.56±6.02^{c}	0.001***
Egg yolk	40.25±1.86	43.09±1.73	46.18±1.67	42.38±1.53	0.120

a, b, c: Means on the same line with different superscript differ significantly, (***)P≤0.001
a, b, c: Aynı sırada farklı harf taşıyan ortalama değerler arasındaki fark istatistik bakımdan önemlidir (***)P≤0.001.

Table 5. Effects of ascorbic acid and boric acid on tibia ash, Ca and P (%) levels.
Tablo 5. Askorbik asit ve borik asitin kemik kül, Ca ve P düzeyleri üzerine etkileri (%).

Parameters	Control	AABA	AA	BA	P
Ash	63.03±0.28	62.92±0.28	63.13±0.24	62.77±0.28	0.795
Ca	15.27±0.75^{b}	17.95±0.78^{a}	17.10±0.62^{ab}	18.38±0.72^{a}	0.021*
P	11.15±0.16	11.05±0.14	11.22±0.13	11.13±0.14	0.863

a, b: Means on the same line with different superscript differ significantly. (*): p<0.05.
a, b: Aynı sırada farklı harf taşıyan ortalama değerler arasındaki fark istatistik bakımdan önemlidir (*): p<0.05.

Table 6. Effects of ascorbic acid and boric acid on liver and yolk boron levels (ppm).
Tablo 6. Askorbik asit ve borik asitin karaciğer ve yumurta sarısı bor seviyesi üzerine etkileri (ppm).

Parameters	Control	AABA	AA	BA	P
Liver	14.20±0.51^{b}	18.59±1.19^{a}	14.10±0.95^{b}	20.04±1.76^{a}	0.000***
Egg yolk	16.44±0.79^{b}	20.40±0.97^{a}	15.57±0.71^{b}	23.26±1.51^{a}	0.002**

a, b: Means on the same line with different superscript differ significantly. (**): p<0.01, (***): p<0.001.
a, b: Aynı sırada farklı harf taşıyan ortalama değerler arasındaki fark istatistik bakımdan önemlidir (**): p<0.01, (***): p<0.001.

serum cholesterol concentration was decreased (p≤0.001) by supplemented or not with boric acid and/or ascorbic acid compared with control group in laying hens while egg yolk choleaterolemia was not affected (Table 4). At the end of trial it was observed that there were no effects of dietary additions on tibia ash and P levels compare the control (Table 5). However, tibia Ca levels increased with BA supplementation to the laying hen diets. In same way, liver and egg yolk boron contents were significantly affected by boric acid (Table 6). Higher liver (p<0.001) and egg yolk (p<0.002) boron concentrations were detected in hens receiving BA and AABA diets. Additionally of this, serum boron concentration was not determined for laying hens.

Discussion and Conclusion

Diets supplemented with arrange of 60 to 120 ppm boron was shown increment effect for poultry feed efficiency (23). Altough, many experiments were conducted by several researches that the effects of boron on performance, the results were frequently variable. Yesilbag and Eren (32) found that similar findings like as Olgun *et al.* (20) about supplementation of boric acid to the laying hens diet significantly increased the feed consumption while it did not affect the feed efficiency, whereas different level of boron (25, 50, 75 ppm) didn't effect on feed consumption and feed convertion ratio in laying hens (14). Sizmaz and Yildiz (26) showed that additional ascorbic acid to the broiler diets increased feed intake at 35-42 days of the trial, while Skrivan *et al.* (25) indicated that feed intake significantly reduced by additional ascorbic acid to laying hens rations. Also, while Elkin *et al.* (7) reported that 1-stearylboronic acid had no effect on feed consumption and feed efficiency, Koksal *et al.* (11) reported that boric acid (90 ppm) supplementation to the hen diet decreased the feed efficiency. This diversities may be due to the act of boron in regulating enzymatic activity in metabolism, since boron affects at least 26 enzymes in animals and plants (9).

For the entire of experimental period, egg weight, egg yield and egg quality were similar for the present additives, results that agree with previous reports (21; 29; 31), which have shown that egg performance and egg quality were not affected by different levels of boric acid in the diet. Moreover, Kurtoglu *et al.* (12) reported similar performance in laying hens when 50, 100, 200 and 250 ppm boric acid was added to the diet. Present study that didn't show significant differences about egg production in whole period in a harmony with the other investigation (28) that supplemented vitamin C and aminolevulinic (ALA) acid to the diets. They didn't find that significant effect on egg production in ascorbic acid additional group. Skrivan *et al.* (25) also reported that ascorbic acid improved the laying performance and influenced some traits and egg quality of hens. In addition to this, Qin and Klandorf (21) reported that first two weeks of laying period, supplementation of 100 ppm boron, and the remaining three weeks supplementation of 60 ppm boron decreased egg production in the level of boron addition of both. This unsignificant effect was most likely due to the unchanged feed intake in laying hens fed with ascorbic acid and/or boric acid and the role of boron in energy metabolism has not been defined. As boron regulates the enzymatic activity in pathways and also the antioxidant activity, it may be modify the energy (16; 24).

In the current study, the beneficial attribute of boron was down regulation in serum cholesterol concentration in hens. This results agree with the reports of Sizmaz and Yildiz (26) who fed broilers at concentration of 175 ppm boric acid. However there is negative correlation with the study (10) which determined the level of 60 ppm boric acid had no effect on serum cholesterol concentration in broilers. Observably, in present study the reduction in concentration of serum cholesterol was not associated with the measured egg yolk cholesterol content. This could be caused from the role of boron in lipid metabolism that the reduction effects on LDL, cholesterol and triglyceride by lowering the synthesis (8).

It is well known that boron serves on body as a regulator in mineral metabolism and it could be beneficial for optimal calcium metabolism resulting in bone metabolism (13). In present study, tibia ash and P contents didn't differ significantly between dietary treatments, whirst tibia Ca level was increased with supplementation of boric acid to the laying hen diets. Sizmaz and Yildiz (26) found semi parallel results about supplementation of boric acid and ascorbic acid to the broiler diet didn't affect the tibia ash level, while boric acid increased the bone P content. Results of the present study are in agreement with findings of several researchers (30; 5), with respect to dietary boric acid supplementation for bone boron content of poultry. Thereby, it may be thought that boron could be have an important role in metabolism, because it also affects the histologically bone composition and physical characteristics by stimulating the formation and maturation of organic matrix on which calcification occurs (17).

We conclude that there is no deterioration in feed intake, feed efficiency, egg production and egg quality was observed in laying hens fed with boric acid and ascorbic acid. However, the present study findings recommend that dietary boric acid might be beneficial in calcium metabolism through different way of mechanisms in poultry. The marked decreasing in serum cholesterol concentration is not precisely in response to feed additives. Therefore, further research testing of boric acid and/or ascorbic acid are warranted to investigate their potential as poultry feed additive, and further studies need to support if the beneficial effect of that on feed efficiency by affecting energy metabolism, cholesterol concentration and bone mineralization are consistent when they are supplemented with single or combination to different diet types.

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