Kafkas Univ Vet Fak Derg
20 (4): 599-604, 2014
DOI: 10.9775/kvfd.2014.10678

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Effects of Single or Combined Dietary Supplementation of Boric acid and Plant Extract Mixture on Egg Production, Egg Quality and Blood Cholesterolemia in Laying Hens

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Makale Kodu (Article Code): KVFD-2014-10678

Summary

This experiment was conducted to determine the effects of boric acid (BA) (60 mg/kg of feed) and plant extract mixture (Fitococci, F) (0.75 g/kg of feed), individually or in combination, on egg production, egg quality parameters and blood cholesterol concentration of laying hens in 23 wk of age. After wk 7 of the trial the level of boric acid in feeds were increased to 180 mg/kg. The feeding period lasted 14 wk. One hundred ninety-two pullets (Hyline) were randomly divided into control and 3 treatment groups each containing 48 birds and 6 replicates (each with 8 birds). At the end of the experiment there were no significant effects of dietary boric acid and plant extract mixture inclusion on feed efficiency, egg weights and egg production, egg quality parameters (shell thickness, breaking strength, and shape index) compared with control. But feed intake of hens which were supplemented with plant extract mixture and boric acid were increased ($P<0.01$). It was also determined that there were no effects of treatments on blood cholesterol levels. As a result, since hens which were fed with basal diet plus plant extract mixture showed better feed conversion ratio at 9-10 wks, this combination of feed additives can be added to diets without any adverse effect on laying hens. But inclusion of 60 mg/kg boric acid is considered better than 180 mg/kg with regard to feed efficiency in laying hens.

Keywords: *Boron, plant extract mixture, Laying hen, Egg production, Egg quality, Cholesterolemia*

Yumurtacı Tavuk Rasyonlarına İlave Edilen Borik Asit ve/veya Bitki Ekstraktı Karışımının Yumurta Verimi ve Yumurta Kalitesi ile Kan Kolesterolü Üzerine Etkileri

Özet

Bu deneme borik asit (BA) (60 mg/kg yem) ve bitki ekstraktı karışımının (Fitococci, F) (0.75 g/kg yem) tek başlarına ya da birlikte kullanıldıkları zaman 23 haftalık yumurta tavuklarında yumurta verimi, yumurta kalite parametreleri ve kan kolesterol düzeylerine etkisini belirlemek amacıyla yapılmıştır. Denemenin 7. haftasının ardından yemlerdeki borik asit düzeyleri 180 mg/kg'a yükseltilmiştir. Yemleme dönemi 14 hafta sürmüştür. 192 adet yarka (Hyline) rastgele kontrol ve 3 deneme grubuna ayrılmış olup bu grupların her biri 6 tekrarlı 48 hayvandan oluşmaktadır (her bir tekrar 8 hayvandan oluşmaktadır). Denemenin sonunda rasyonlara borik asit ve bitki ekstraktı karışımı ilave edilen deneme gruplarında kontrol grubuna göre yemden yararlanma, yumurta ağırlık ve verimleri, yumurta kalite parametreleri (kabuk kalınlığı, kırılma direnci ve şekil indeksi) bakımından önemli etki görülmemiştir. Ancak bitki ekstraktı ve borik asit karışımıyla beslenen deneme grubunda yem tüketimi önemli düzeyde artmıştır ($P<0.01$). Ayrıca rasyona borik asit ve bitkisel ekstrakt karışımı ilavesinin kan kolesterol düzeylerine istatistiki önemde etki etmediği tespit edilmiştir. Sonuç olarak, bitki ekstraktı karışımıyla beslenen tavuklarda yemden yararlanma oranının 9-10. haftalarda daha iyi olmasından dolayı, bu yem katkı maddelerinin karışımı yumurta tavuğu rasyonlarına olumsuz etki göstermeksizin ilave edilebilir. Ancak yumurta tavuklarında 60 mg/kg borik asit ilavesi 180 mg/kg borik asit ilavesine oranla yemden yararlanma açısından daha iyi sonuç ortaya koymaktadır.

Anahtar sözcükler: *Bitki ekstraktı karışımı, Bor, Kolesterol, Yumurta kalitesi, Yumurtacı tavuk, Yumurta verimi*

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INTRODUCTION

Plant extracts and herbs have been used for thousands of years by many cultures to enhance the flavour and aroma of foods. Scientific experiments since the late 19th century have documented the anti-microbial properties of some spices, herbs and their components [1]. Supplementation of feed additive (0.75 g/kg Fitococci) had no significant effects about egg weights, egg quality parameters and serum cholesterol whereas it had negative effects (P<0.05) on egg production and feed efficiency compare with the control [2]. Even though usage of plant extract in laying hen diets like as feed additive is already known subject addition of boron/boric acids into rations is substantially new issue in this area.

Boron is primarily obtained from boron mines, located in arid regions of Turkey [3]. Boric acid based on biochemistry of boron. Kurtoglu et al.[4] showed 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. Eren et al.[5] found that similar findings about B addition to laying hen diets at level of 400 ppm resulted negative values of live weight, feed consumption and egg productivity parameters.

This experiment was conducted to determine the effects of boric acid and plant extract mixture supplementation on egg production, egg quality, and serum cholesterol in laying hens.

Table 1. *Basal diet composition and analyzed results of feed ingredient for laying hens diets*
Tablo 1. *Bazal rasyonun bileşimi ve yem ham maddesi analiz sonuçları*

Ingredients	Percentage
Corn	40.00
Wheat	21.37
Soybean meal	15.20
Sun flower meal	5.00
Soybean, full fat	6.00
Oil	1.69
Limestone	8.28
Dicalcium phosphate	1.60
Common salt	0.35
DL-Methionine	0.24
DL-Treonine	0.06
Antioxidant	0.05
Vitamin and mineral premix*	0.16
Analyzed Results	
Crude protein (%)	16.60
ME (kcal/kg)**	2789
Calcium (%)	3.60
Phosphor (%)	0.50

** Supplied the following per kilogram/ton of diet:.Retinol (vit A), 12.000 IU; cholecalciferol, 8.000 IU; α-tocopherol acetate (vit E), 50 g; menadione (vit K_3), 10 g; thiamin (vit B_1), 4 g; riboflavin (vit B_2), 8 g; pyridoxine (vit B_6), 5 g; cyanocobalamin (vit B_{12}), 25 g; niacin, 50 g; cal. panthotenic acid, 20 g; folic acid, 20 g; biotin (vit H_2) 250 g; ascorbic acid (vit C) 75 g; choline cloride 175 g; Manganese (Mn), 100 g; zinc (Zn), 150 g; iron (Fe), 100 g; copper (Cu), 20 g; iodine (I)), 1.5 g; chobald (Co) 0.5 g; selenium (Se), 0.2 g; molybdenum (Mo), 1 g and magnesium (Mg), 50 g, ** The formula recommended by TSE (Turkish Standards Institute)* [6]

MATERIAL and METHODS

Layers, Experimental Design and Diet

This research was carried out on 23 wks old, totally 192 laying hens (Hyline White 98). The birds were randomly divided into 4 groups according to the dietary regimen and each group was constituted by 6 subgroups of 8 birds. The experimental protocol was approved by the local Ethics Committee of the Ankara University. In the control group, chickens were fed with basal standard diets based on corn and soybean meal, more specifically with a layer diets [16.5% crude proteins (CP) and 2750 kcal/kg metabolisable energy (ME)] for all experiment period (for 14 wks, *Table 1*). Birds from the groups 2 and 3 received standard diets supplemented with boric acid (BA) (60 ppm) or Fitococci (F) (0.75 g/kg) respectively whereas in the group 4, hens were supplemented with both of these additives (FBA) (60 ppm boric acid plus 0.75 g/kg humate). The dosage of boric acid from wk 8 to end of the trial was increased. Feeds were analyzed for nutrients *(Table 1)* according to the reference methods [7]. During the experiment feed and water were given to hens ad libitum. The birds were housed in battery cages with automatic drinker design during the 14 wks experimental period. The temperature maintained at 20-22°C according to normal management practice. Chicks were maintained on a 17 hours constant light schedule until the end of the experiment.

Plant extract mixture (Fitococci®) includes Origanum vulgare (thyme), Thymus vulgaris (oregano), thyme oil, garlic oil, anise oil and fennel oil. Active ingredients: 1,8-Cineole (0.24%), Allicine (0.24%), Alliine (0.12%), Alpha-Pinene (0.12%), Alpha-Terpineol (0.70%), Borneol (0.18%), Caffeic-Acid (2.28%), Camphene (0.08%), Carvacrol (4.48%), Eugenol (0.12%), Geraniol (1.04%), Limonene (0.56%), Linalool (0.96%), Myrcene (0.18%), P-Cymene (2.38%), Phenol (0.86%), Polyphenol (6.00%), Tannin (12.9%), Rosmarinic-Acid (7.60%), Terpinen-4-Ol (0.06%), Ursolic Acid (1.92%), Tymol (3.26%).

Performance Analysis

During the experimental period, the egg production of the hens was evaluated by daily recording whereas feed consumption recording weekly. Egg samples were collected, stored (at room temperature for one day) and

weighed biweekly. Feed conversion ratio was calculated as kilograms of feed consumed per kilogram of egg produced. An additional sample of 6 eggs was randomly collected from each experimental group every 14 days to assess egg quality parameters which were shape index, breaking strength and shell thickness.

Biochemical Analysis

At the end of the trial, blood samples (5 mL) were obtained from each one bird from every cage by the brachial vein for determine blood cholesterol level of hens. These samples were allowed to clot at room temperature for 6 hours and then they were centrifuged at 1500 g for 10 min at room temperature. Serum were carefully harvested and stored at -20°C until analysis. The serum cholesterol concentrations were analyzed using commercial kits (Teco Diagnostic, 1286 Anaheim, CA 92807).

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 [8] with a 5% level of probability.

RESULTS

Table 2 shows the result of dietary boric acid and plant extract mixture on feed consumption. Over the whole experimental period, feed consumption was increased (P<0.01) by addition of boric acid (BA) and combination of feed additives (FBA). Feed convertion ratio (FCR) was not affected by supplemented or not with boric acid and/or plant extract mixture *(Table 3)*. However at 9-10th wks of treatment, FCR for hens was significantly decreased in 1st trial group (F) while BA significant increased of it compare the control group (P<0.01). The egg yield and weight results were also summarized in *Table 4*. These parameters for performance were not significantly modified by the supplementation with boric acid and/or plant extract mixture combination over the whole experimental period. At the end of trial it was observed that there were no effects of dietary additions on egg quality parameters (shell thickness, breaking strength, and shape index) compared with control *(Table 5)*. In same way, blood cholesterol levels were not affected by boric acid and/or plant extract mixture *(Table 6)*.

Table 2. *Effects of plant extract mixture and boric acid on feed consumption of laying hens (mean ± standard error)*
Tablo 2. *Bitki ekstraktı karışımı ve borik asidin yem tüketimi üzerine etkileri (ortalama ± standart hata)*

Week	Control	F	BA	FBA	P
1-2	98.44±1.15	101.80±2.52	100.74±3.41	104.35±1.50	0.367
3-4	102.01±1.38 ^{b}	109.74±1.63 ^{a}	109.64±1.32 ^{a}	108.30±1.03 ^{a}	0.002**
5-6	106.61±1.75	109.78±1.82	102.48±4.32	112.97±1.52	0.058
7-8	107.73±1.23	106.18±1.88	108.09±1.54	112.05±1.46	0.078
9-10	109.22±0.90 ^{bc}	106.10±2.08 ^{c}	114.23±1.98 ^{ab}	119.64±3.37 ^{a}	0.002**
11-12	109.10±0.65	111.37±2.85	114.20±0.98	113.78±1.40	0.154
13-14	102.37±2.14 ^{a}	96.12±2.61 ^{b}	107.58±1.08 ^{a}	107.02±1.70 ^{a}	0.002**
1-14	105.07±1.01 ^{b}	105.87±1.44 ^{b}	108.14±0.91 ^{ab}	111.16±1.14 ^{a}	0.005**

a, b, c: *Means on the same line with different superscript differ significantly, ** P<0.01*

Table 3. *Effects of plant extract mixture and boric acid on FCR of laying hens (mean ± standard error)*
Tablo 3. *Bitki ekstraktı karışımı ve borik asidin yemden yararlanma oranı üzerine etkileri (ortalama ± standart hata)*

Week	Control	F	BA	FBA	P
1-2	2.32±0.12	2.45±0.12	2.36±0.15	2.26±0.14	0.767
3-4	1.92±0.04	2.13±0.05	1.81±0.33	2.03±0.04	0.593
5-6	1.96±0.03	2.08±0.08	1.90±0.09	2.10±±0.04	0.129
7-8	1.97±0.24	2.02±0.06	2.00±0.05	2.05±0.06	0.774
9-10	2.06±0.52 ^{ab}	1.94±0.03 ^{b}	2.15±0.05 ^{a}	2.19±0.06 ^{a}	0.009**
11-12	1.98±0.03	2.01±0.04	2.07±0.03	2.03±0.02	0.245
13-14	1.85±0.05	1.78±0.09	1.88±0.02	1.95±0.05	0.240
1-14	2.01±0.34	2.06±0.03	2.03±0.03	2.09±0.05	0.417

a, b: *Means on the same line with different superscript differ significantly, ** P<0.01*

Table 4. *Effects of plant extract mixture and boric acid on egg weight and egg yield of laying hens (mean ± standard error)*
Tablo 4. *Bitki ekstraktı karışımı ve borik asidin yumurta ağılığı ve yumurta verimi üzerine etkileri (ortalama ± standart hata)*

Week	Control	F	BA	FBA	P
Egg weight (g)					
1-2	53.88±0.35	54.28±0.36	54.92±0.77	55.74±1.85	0.626
3-4	55.27±0.55	55.20±0.55	54.98±0.54	54.96±0.56	0.972
5-6	57.06±0.46	56.99±0.68	57.15±0.40	56.94±0.59	0.994
7-8	57.95±0.80	57.81±0.61	57.47±0.49	57.60±0.59	0.951
9-10	58.50±0.68	59.61±0.40	58.24±0.43	58.29±0.76	0.340
11-12	60.03±0.74	60.30±0.44	59.96±0.43	60.27±0.29	0.949
13-14	61.29±0.79	60.98±0.47	60.49±0.68	60.03±0.56	0.533
1-14	57.71±0.57	57.88±0.36	57.60±0.42	57.69±0.46	0.977
Egg yield (%)					
1-2	79.76±3.94	77.23±3.68	78.87±4.92	84.23±3.55	0.658
3-4	96.43±0.40	93.75±2.29	91.96±1.93	97.17±0.78	0.100
5-6	95.39±0.84	93.30±2.66	94.49±1.95	94.49±0.54	0.863
7-8	94.35±1.38	91.37±3.10	94.05±1.43	95.24±0.95	0.540
9-10	90.92±1.62	91.96±2.69	91.52±1.36	93.75±1.49	0.737
11-12	91.96±1.11	91.82±2.14	92.27±0.88	93.15±1.14	0.907
13-14	90.63±0.82	89.29±2.25	94.49±1.21	91.67±1.23	0.114
1-14	91.35±0.72	89.82±1.87	91.09±1.00	92.81±0.96	0.405

Differences between treatment groups is not statistically significant (P>0.05)

DISCUSSION

The experiment has shown that after dose escalation of boric acid the feed consumption and feed conversion ratio of hens fed boric acid were significantly different from those of the control. Yesilbag and Eren [9] found that similar findings like as Olgun et al.[10] about supplementation of boric acid to the laying hens diet significantly increased the feed consumption while it did not affect the feed efficiency. These results are in agreement with the results of studies involving hens on supplementations of boric acid. At the same time there is a negative correlation with other study [11] which tried to determine different level of boron (25, 50, 75 ppm) effects on feed consumption and feed convertion ratio in laying hens. They did not find any significant differences about performance parameters of hens with supplementation of boric acid. Also, while Elkin et al.[12] reported that 1-sterilboronic acid had no effect on feed consumption and feed efficiency, Koksal et al.[13] reported that boric acid (90 ppm) supplementation to the hen diet decreased the feed convertion ratio. This differences was quantitatively accounted for by a difference level of the boron and different boron sources.

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 [9,14,15], which have shown that egg performance and egg quality were not affected by different level of boric acid in the diet. Moreover, Kurtoglu et al.[4] reported similar performance in laying hens when 50, 100, 200 and 250 ppm boric acid was added to the diet. Koksal and Kucukersan [2] indicated that plant extract mixture had no significant effect on egg production and egg quality in laying hens. Present study in a harmony with the other studies [16-22] that supplemented plant extract and essential oils to the diets. In contrast, Qin and Klandorf [15] reported that first two wks of spawning period, supplementation of 100 ppm boron, and the remaining three wks supplementation of 60 ppm boron decreased egg production in the level of boron addition of both.

In the current study, no significant variations in cholesterolemia were observed among groups. This result is positive correlation with the study [22] which determined the level of 60 ppm boric acid and Koksal and Kucukersan [2] which determined the level of 0.75 g/kg plant extract mixture had no effect on serum cholesterol concentration in broilers and laying hens, respectively. In addition, Sizmaz and Yildiz [23] reported similar result in broilers when 175 ppm boric acid added to the diet. Results that agree with earlier studies in rabbits [24], in broilers [25], which have shown that cholesterolemia were not affected by boric acid supplementation to the drinking water and to the diets, respectively. In contrast, previous studies [15,21,26] showed that plant extract reduced the serum cholesterolemia in layings. The differences about results between other

Table 5. *Effects of plant extract mixture and boric acid on egg quality parameters (mean ± standard error)*
Tablo 5. *Bitki ekstraktı karışımı ve borik asidin yumurta kalite parametreleri üzerine etkileri (ortalama ± standart hata)*

Week	Control	F	BA	FBA	P
Shell thickness					
1-2	39.36±0.45	38.64±0.45	39.89±0.44	39.14±0.63	0.372
3-4	38.11±1.35	35.88±1.67	36.69±1.26	35.75±1.19	0.606
5-6	34.14±0.83	35.89±0.68	35.67±0.86	35.25±0.77	0.413
7-8	35.28±0.78	32.56±1.06	33.33±0.73	35.19±0.86	0.074
9-10	38.25±0.36	37.61±0.47	38.06±0.60	38.03±0.31	0.783
11-12	36.71±0.49	37.13±0.59	35.74±0.55	36.14±0.56	0.301
13-14	36.97±0.41	36.28±0.53	36.56±0.34	36.59±0.43	0.733
Breaking strength					
1-2	1.89±0.25	1.94±0.17	2.14±0.21	1.76±0.18	0.605
3-4	1.88±0.17	1.85±0.21	1.71±0.18	1.84±0.17	0.917
5-6	1.96±0.10	2.06±0.12	1.87±0.15	1.99±0.14	0.779
7-8	2.38±0.18	1.97±0.14	2.06±0.15	2.40±0.16	0.132
9-10	2.31±0.10	2.03±0.12	2.31±0.13	2.15±0.09	0.244
11-12	1.81±0.14	2.03±0.17	1.91±0.13	1.75±0.13	0.562
13-14	2.04±0.07	1.98±0.09	2.00±0.06	2.00±0.04	0.935
Shape index					
1-2	77.27±0.52	77.23±0.59	78.17±0.57	77.50±0.46	0.591
3-4	77.83±0.67	78.83±0.62	77.58±0.73	78.08±0.43	0.508
5-6	78.58±0.55	78.46±0.45	79.08±0.73	77.33±0.72	0.252
7-8	77.38±0.46	77.96±0.69	77.58±0.79	76.79±0.66	0.654
9-10	78.32±0.69	77.46±1.15	78.90±0.77	77.86±0.36	0.613
11-12	77.50±0.53	78.42±0.52	77.33±0.66	77.58±0.65	0.576
13-14	77.81±0.27	78.06±0.34	78.11±0.29	77.53±0.27	0.487
Differences between treatment groups is not statistically significant (P>0.05)					

Table 6. *Effects of plant extract mixture and boric acid on blood cholesterol concentration (mean ± standard error)*
Tablo 6. *Bitki ekstraktı karışımı ve borik asidin kan kolesterol düzeyi üzerine etkileri (ortalama ± standart hata)*

Parameters	Control	F	BA	FBA	P
End of 7th wk	154.20±24.57	171.47±14.16	171.87±16.93	146.89±6.42	0.655
End of 14th wk	107.28±15.43	164.92±19.38	128.44±7.05	124.13±23.31	0.168
Differences between treatment groups is not statistically significant (P>0.05)					

studies and ours can be caused by using of different composition and different levels of plant extracts.

We conclude that boric acid and plant extract mixture can be used successfully in laying hen diets. The effects of supplementation of plant extract mixture and boric acid during the laying period on egg production and egg quality parameters were investigated, and their effectiveness was compared. Supplemental plant extract mixture and boric acid combination had linear effects on production parameters including increased feed intake (during the trial) and feed convertion ratio (9-10 wks). However, they had no specific effects on egg quality parameters.

Acknowledgements

We express our thanks to Farmavet for supplying the plant extract mixture (Fitococci) used in the experiments.

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