

ENHANCING AVIAN HEALTH: ASSESSING THE EFFECTS OF BORIC ACID SUPPLEMENTATION ON SERUM ALBUMIN IN QUAIL DIETS

Maria Russo and Antonio Pereira

Department of Poultry Nutrition, Faculty of Veterinary Sciences, University of Lisbon, Portugal

Abstract: *Boric acid, a versatile inorganic acid known for its applications in pest control, fungistatic properties, ceramics, and cosmetics, has recently garnered attention for its potential medical uses. Studies have revealed its involvement in various physiological processes, including immune responses, endocrine regulation, mineral metabolism, and lipid homeostasis. Additionally, boric acid exhibits antioxidant properties, although the underlying mechanisms remain partially elucidated. Research suggests that boric acid treatment can mitigate oxidative metabolism inhibition resulting from mitochondrial degeneration. However, excessive boric acid usage may induce oxidative stress in the reproductive system. This abstract provides an overview of boric acid's multifaceted roles and underscores the need for further exploration of its medical applications and antioxidant mechanisms.*

Keywords: *Boric acid, medical applications, antioxidant effects, physiological processes, oxidative stress.*

1.0 Introduction

Boric acid is a white crystalline water-soluble inorganic acid that is used for pest control and as a fungistatic agent for many years. Beyond those usages, boric acid has taken an important place in the ceramic and cosmetic industries (De Seta *et al.*, 2009; Ozdemir Oge and Keskiner, 2018). In recent years, there has been an increase in studies on the use of boric acid for medical purposes. Boric acid has been determined to take a role in some physiological processes such as immune response, endocrine system, mineral, and lipid metabolism (Hakan *et al.*, 2012). On the other hand, boric acid is reported to have antioxidant effects (Sogut *et al.*, 2015; Cikler-Dulger and Sogut, 2020), but the underlying mechanism of the antioxidant action of

boric acid is still not fully clarified (Ali *et al.*, 2014). It has been observed that the inhibition of oxidative metabolism caused by mitochondria degeneration ameliorates by boric acid treatment (Ali *et al.*, 2014). In addition, boric acid also causes oxidative stress in the reproductive system depending on the at overdose usage (Yalcin and Abudayyak, 2020).

There are various feed additives used for many purposes in poultry feeding. The main goal of using those additives in poultry farming is to enhance production performance and improving feed conversion. Antibiotics, enzymes, microorganisms, some sort of yeasts, antioxidants, trace elements, plant-based, and animal originated additives are the most utilized ones (Yasar and Yegen, 2017; Ciftci *et al.*, 2018). Studies involved in these additives usage are planned at different doses for the determination of the most beneficial effects. Moreover, there is limited data on which dose is adequate for boron supplementation to animal diets. Quails are reared for meat and egg production and have an important place in the economy of the rural country because of they are fast-growing birds (maturity at 4-5 weeks) with requiring small space,

having a short incubation period and generation interval (Baykalir and Aslan, 2020). However, the effect of boric acid as an additive is not fully known in point of livestock (Hakan *et al.*, 2012).

Serum albumin is the most abundant protein amongst the other plasma proteins. Serum albumin is associated with varied essential physiological functions, such as maintaining osmotic pressure, cell regeneration, regulating metabolic and vascular functions (Chien *et al.*, 2017; Hovarthly *et al.*, 2017).

Moreover, serum albumin is the most potent in extracellular antioxidant defense components among transferrin, haptoglobin, and ceruloplasmin (Sitar *et al.*, 2013). Albumin has the low or no enzymatic activity so it can bind to several molecules such as copper, zinc, magnesium, calcium, and creates important biological compounds. The albumin concentration in plasma may alter in conditions that affect the liver such as malnutrition, absorption of some molecules, and drugs (Levit and Levit, 2016). Serum albumin is considered a biomarker in many clinical events, including the nutritional status of the individuals (Chien *et al.*, 2017; Smith, 2017).

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was first introduced by Laemmli in 1970 (Laemmli, 1970). With this method, proteins can be separated in a high resolution under the electrophoretically according to their molecular weights (Nowakowski *et al.*, 2014). In previous studies conducted on plasma proteins with SDS-PAGE, serum albumin was found to be approximately at a weight of 67 kDa (66.266.7 kDa) (Nurdiansyah *et al.*, 2016). Therefore, SDS-PAGE is the most utilized technique in such studies.

In the context of the mentioned information, the aim of this study was to investigate the effect of boric acid supplementation to feed and drinking water at different doses on serum albumin levels in Japanese quails (*Coturnix coturnix japonica*). With this study, it is also aimed to intend to provide a new perspective on the determination of the properties of feed additives used in livestock.

2.0 Material and Method

2.1 Birds management and experimental design

The current research related to animal use has complied with all the relevant national regulations and institutional policies for the care and use of animals (The Ministry of Agriculture and Forestry, Elazig Veterinary Control Institute, Animal Experiment Local Ethics Committee, Turkey. Approval No: 2018/5). A total of 35 days-old, 360 laying quails were used in this study. The birds reared in 5 tiered plastic cages that situated 3 compartments in each tier for 15 weeks. The five experimental groups consisted of: Control (fed with basal diet), 100 and 300 mg/kg boric acid (basal diet supplemented with boric acid), 100 and 300 mg/L boric acid (drinking water supplemented with boric acid). The extra pure $\geq 99.5\%$ boric acid was used (CAS No: 10043-35-3) in this study. The experimental design was formed with 20 females and 4 male quails in 3 repetitions of each group. The feed and drinking water have offered as *ad libitum*. Composition of the diets is presented in Table 1 (NRC, 1994). The light/dark period was 16/8 hours. The birds were kept under 22°C for 24 h. At the end of the trial, the 12 quails (6 females, 6 males), a total of the 60 quails were selected based on their same slaughter weight. Blood samples were collected into tubes after the slaughtered process of the birds. The blood serum was separated at 6000 rpm, 5-6 min by centrifugation. The serum aliquoted to 1.5 mL tubes and stored at -20°C until SDS-PAGE analysis.

Table 1: Composition of the diets

Feed composition	%	Nutritional composition	%
Maize	51.40	Dry matter	90.40
Soybean meal (44%)	22.00	Crude protein	18.00
Corn meal	2.00	Crude cellulose	4.40
Sunflower meal (45%)	4.30	Crude fat	5.35
Wheat bran	9.00	Crude ash	10.19

Vegetable oil	3.50	Calcium	2.50
Calcium phosphate	0.88	Available phosphorus	0.35
Calcium carbonate	4.50	Sodium	0.18
Limestone	1.50	Lysine	1.00
L-lysine hydrochloride	0.16	Methionine+cysteine	0.59
L-threonine	0.12	Threonine	0.76
Sodium bicarbonate	0.16	Tryptophan	0.25
Salt	0.20	Metabolic energy (ME), kcal/kg**	2800
Vitamin-mineral premix*	0.28		

* Per 1kg= Vitamin A: 15.000 IU; Vitamin D3: 3.500 IU.

Per 1kg= Manganese: 120 mg; Ferrous: 40 mg; Zinc: 100 mg; Copper: 16 mg; Cobalt: 200 mg; Iodine: 1.25; Selenium: 0.30 mg.

**It has been determined by calculation. ME (kcal/kg)= 53+38 B formula was used. B= (Crude protein %) + (2.25 X crude fat %) + (1.1 X starch %) + (sugar %)

2.2 SDS-PAGE analysis

The serum total protein amount was determined by nanodrop spectrophotometer (Thermo Scientific™ NanoDrop™ 2000/2000c). The 25 µg/30 µL total protein was loaded into wells. The bovine serum albumin was used as a control and to normalize the bands. SDS-PAGE analysis was performed according to Baykalir and Aslan (2020). Electrophoresis was carried out at a constant voltage of 135 V (Bio-Rad, Mini Protean Tetra, USA).

The gels removed carefully when migration completed and stained with the 1% coomassie brilliant blue dye solution for 1 hour. Thereafter, the dye was removed with the destaining solution (a mixture of pure methanol, glacial acetic acid, distilled H₂O). Relative densitometric (RD, %) values of bands were analyzed by ImageJ (NIH image) software after grayscale calibration. The albumin levels of the groups were determined by distributing the RD value of each group to 100% according to the software.

2.3 Statistical analysis

RD values (%) of the bands were subjected to One-Way ANOVA test using IBM®SPSS 22 (IBM, New York, USA). Tukey post hoc test was applied to determine the differences between the groups. The percentile data were presented as the group means. Statistical significance was considered when $P \leq 0.05$ (Petrie and Watson, 2013).

3.0 Results and Discussion

The RD values of the albumin bands of the groups are presented in Table 2. According to Table 2, the highest albumin level appears to be in the F300 group (24.94%) and the F100 group (22.46%), respectively. The RD value of the W100 group was 18.63%, and the W300 was 17.20%. The Control group resulted in the lowest

RD value (16.77%). The statistically significant difference was observed between Control, W100, and W300 with F300 ($P < 0.05$). However, the F100 and the F300 groups were similar ($P > 0.05$). It is important to understanding albumin interaction with chemical compounds and molecules. The secondary and tertiary structure of albumin is influenced by such interactions. It is crucial to reveal such interactions in terms of understanding the metabolism and physiological functions of albumin (Sohrabi *et al.*, 2017). There are several studies conducted on food additives and serum albumin interactions (Xu *et al.*, 2012; Mohammadzadeh-Aghdash *et al.*, 2017; Sohrabi *et al.*, 2017). Food additives that using in the food industry common such as olaquinox, sodium acetate, and ascorbyl palmitate bind to albumin and form a biomolecule complex. This binding has been shown to be occurred by hydrophobic forces. It is concluded that the formation of this bio complex as a result of the binding of food additives to albumin can lead to the

conformational change of albumin (Mohammadzadeh-Aghdash *et al.*, 2017). In addition, such additives can transfer into the bloodstream via albumin (Sohrabi *et al.*, 2017). In livestock, the usage of feed additives is very common. However, studies mostly focused on yield performance in determining the effect of feed additives and their effective dose. In the current study, the F300 group exhibited more RD value of albumin band than the other groups. This result can be attributed to the formation of the bio complex by binding of boric acid to albumin. In addition, this bio complex could be transferred to the bloodstream and might be increased the level of albumin (Sohrabi *et al.* 2017). On the other hand, serum albumin levels correlated with various nutritional parameters (Smith, 2017; Keller, 2019). Under poor food intake, prealbumin levels decrease but this case also related to zinc deficiency, age, and other diseases (Beck and Rosenthal, 2002). In the deficiency of boron as an element like zinc, albumin levels may also decrease. In this study, supplementation of boric acid into both feed and drinking water increased the albumin levels when compared to the Control group (Table 2). In Figure 1, serum albumin levels of the groups are presented.

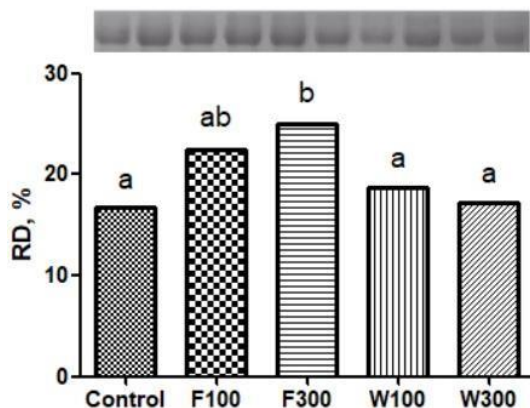
Table 2: The RD values of the albumin bands of the groups

Groups	RD (%)	P value
Control ^a	16.77	
F100 ^{ab} F300 ^b	22.46	
	24.94	0.046
W100 ^a	18.63	
W300 ^a	17.20	

RD: Relative densitometry. F100: boric acid supplemented as 100 mg/kg into the feed, F300: boric acid supplemented as 300 mg/kg into the feed, W100: boric acid supplemented as 100 mg/L into the drinking water, W300: boric acid supplemented as 300 mg/L into the drinking water. Superscripts in the "Groups" column show the difference between the groups.

Baykalir, Aslan & Simsek

17



Different letters on the bars show the statistical differences between the groups.

Figure 1: Serum albumin levels of the groups Conclusion

In this study, the novel data is presented about boric acid supplementation as a feed additive in quail farming. According to the results of the current study, supplementation of boric acid into quail feed was more effective than adding it to drinking water. The supplementation of 300 mg/kg dose of boric acid into the quail diet has offered the highest result. It is recommended to investigate the effect and dose determination of further feed additives commonly used in livestock with the SDS-PAGE method, too.

References

- Ali, S. E., Thoen, E., Evensen, O., Wiik-Nielsen, J., Gamil, A. A. A., & Skaar, I. (2014). Mitochondrial dysfunction is involved in the toxic activity of boric acid against *Saprolegnia*. *PLoS ONE*, 9(10) Article e110343.
- Baykalir, Y., & Aslan, S. (2020). Phenotypic correlations between egg quality traits, albumen pH and ovalbumin levels in four varieties of Japanese quail (*Coturnix coturnix japonica*). *GSC Biological and Pharmaceutical Sciences*, 10(3), 69-75.
- Beck, F. K., & Rosenthal, T. C. (2002). Prealbumin: A marker for nutritional evaluation. *American Family Physician*, 65(8), 1575-1579.
- Chien, S-C., Chen, C-Y., Lin, C-F., & Yeh, H-I. (2017). Critical appraisal of the role of serum albumin in cardiovascular disease. *Biomarker Research*, 5, Article 31.
- Ciftci, M., Simsek, U. G., Dalkilic, B., Erisir, Z., IflazogluMutlu, S., Azman, M. A., Ozcelik, M., Yilmaz, O., Baykalir, Y., & Tonbak, F. (2018). Effects of essential oil mixture supplementation to basal diet on fattening performance, blood parameters and antioxidant status of tissues in japanese quails exposed to low ambient temperature. *The Journal of Animal & Plant Sciences*, 28(2), 421-430.
- Cikler-Dulger, E., & Sogut, I. (2020). Investigation of the protective effects of boric acid on ethanol induced kidney injury. *Biotechnic&Histochemistry*, 95(3), 186-193.
- De Seta, F., Schmidt, M., Vu, B., Essmann, M., & Larsen, B. (2009). Antifungal mechanisms supporting boric acid therapy of candida vaginitis. *The Journal of Antimicrobial Chemotherapy*, 63(2), 325-336.
- Hakan, K. B., Gultekin, Y., & Ozge, S. (2012). Effects of boric acid and humate supplementation on performance and egg quality parameters of laying hens. *Brazilian Journal of Poultry Science*, 14(4), 233-304.
- Hovarth, D. B., Simon, M., Schwarz, C. M., Masteling, M., Vacz, G., Hornyak, I., & Lacza, Z. (2017). Serum albumin as a local therapeutic agent in cell therapy and tissue engineering. *Biofactors*, 43(3), 315-330.
- Keller, U. (2019). Nutritional laboratory markers in malnutrition. *Journal of Clinical Medicine*, 8(6), 775-785.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685.
- Levitt, D. G., & Levitt, M. D. (2016). Human serum albumin homeostasis: a new look at the roles of synthesis, catabolism, renal and gastrointestinal excretion, and the clinical value of serum albumin measurements. *International Journal of General Medicine*, 9, 229-255.
- Mohammadzadeh-Aghdash, H., Dolatabadi, J. E. N., Dehghan, P., Panahi-Azar, V., & Barzegar, A. (2017). Multispectroscopic and molecular modeling studies of bovine serum albumin interaction with sodium acetate food additive. *Food Chemistry*, 228, 265-269.

- Nowakowski, A. B., Wobig, W. J., & Petering, D. H. (2014). Native SDS-PAGE: High resolution electrophoretic separation of proteins with retention of native properties including bound metal ions. *Metallomics* 6(5), 1068-1078.
- NRC (1994). Nutrient requirements of poultry. (9th rev. ed.). National Academy Press, Washington, DC: USA. pp: 44-45.
- Nurdiansyah, R., Rifa'I, M., & Widodo, M.R. (2016). A comparative analysis of serum albumin from different species to determine a natural source of albumin that might be useful for human therapy. *Journal of Taibah University Medical Sciences*, 11(3), 243-249.
- Ozdemir Oge, T., & Keskiner, A. U. (2018). Experimental and DFT calculation studies on boric acid and salicylic acid-boric acid-ethanol solution. *Journal of Boron*, 3(2), 118-125.
- Petrie, A., & Watson, P. (2013). Statistics for veterinary and animal science. (3rd ed.). Wiley-Blackwell Publishing, New Jersey, NJ: USA.
- Sitar, M. E., Aydin, S., & Cakatay, U. (2013). Human serum albumin and its relation with oxidative stress. *Clinical Laboratory*, 59(9-10), 945-952.
- Smith, S. H. (2017). Using albumin and prealbumin to assess nutritional status. *Nursing*, 47(4), 65-66.
- Sogut, I., Oglakci, A., Kartkaya, K., Kusat Ol, K., Savasan Sogut, M., Kanbak, K., & Erden Inal, M. (2015). Effect of boric acid on oxidative stress in rats with fetal alcohol syndrome. *Experimental and Therapeutic Medicine* 9, 1023-1027.
- Sohrabi, Y., Panahi-Azar, V., Barzegar, A., Dolatabadi, J. E. N., & Dehghan, P. (2017). Spectroscopic, thermodynamic and molecular docking studies of bovine serum albumin interaction with ascorbyl palmitate food additive. *Bioimpacts*, 7(4), 241-246.
- Xu, T., Guo, X., Zhang, L., Pan, F., Lv, J., Zhang, Y., & Jin, H. (2012). Multiple spectroscopic studies on the interaction between olaquinox, a feed additive, and bovine serum albumin. *Food and Chemical Toxicology*, 50(7), 2540-2546.
- Yalcin, C. O., & Abudayyak, M. (2020). Effects of boric acid on cell death and oxidative stress of mouse TM3 Leydig cells in vitro. *Journal of Trace Elements in Medicine and Biology*, 61, 126506.
- Yasar, S., & Yegen, M. K. (2017). Yeast fermented additive enhances broiler growth. *Brazilian Journal of Animal Science*, 46(10), 814-820.