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Original Article

Berberine Improves Meat Quality and Carcass Traits in Broilers Challenged with Mycotoxins

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Abstract

Background and Aim: Mycotoxin-contaminated feed causes significant concern in poultry production and public health because of serious economic losses and health problems caused by them. Berberine hydrochloride (Berberine), a natural plant alkaloid derived from Chinese medicine, is characterized by diverse pharmacological effects. This study is designed to evaluate the effects of different levels of Berberine (BBR) on carcass traits and meat quality of broilers fed diets contaminated with Aflatoxin B_1 (AFB) and Ochratoxin A (OCT).

Methods: A 42-day floor pen trial was performed with 288 Ross 308 broilers. A randomized design, with 4 replicates of 8 birds each, was conducted with the following 9 treatments: (1) negative control diet with no additives (NC); (2) NC + 2 ppm AFB (positive control AFB; PCAFB); (3) NC + 2 ppm OCT (positive control OCT; PCOCT); (4) PCAFB + 200 mg/kg BBR; (5) PCAFB + 400 mg/kg BBR; (6) PCAFB + 600 mg/kg BBR; (7) PCOCT + 200 mg/kg BBR; (8) PCOCT + 400 mg/kg BBR; and (9) PCOCT + 600 mg/kg BBR. At the end of the experiment, from every group, eight birds were selected, slaughtered, and subjected to analyses. The analyzed parameters carcass and cut yields and meat quality according to water-holding capacity (WHC) and breast level MDA.

Results: Both PCAFB and PCOTA diets decreased carcass relative weight, breast level MDA, and WHC values compared with the NC diet ($p<0.05$). Meat level of MDA in PCAFB and PCOTA treatments were higher than in the NC treatment ($p<0.05$), and supplementation with BBR at multiple levels partially these effects ($p<0.05$). The addition of 600 mg/kg BBR to PCAFB diets increased WHC value to levels not different from that in the NC group ($p>0.05$). Carcass efficiency in PCAFB and PCOTA treatments was lower than in the NC treatment ($p<0.05$), and supplementation with BBR at multiple levels partially or fully reversed these effects. The addition of 600 mg/kg BBR to PCAFB diet increased carcass relative weight compared to PCAFB alone ($p>0.05$).

Conclusion: These data provided supplementation of BBR (600 mg/kg) improves meat quality in broiler fed diet contaminated with mycotoxins.

Keywords: Berberine; Carcass; Malondialdehyde; Mycotoxin; Water Holding Capacity.

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Introduction

The products of lipid oxidation can also have a negative effect on the components of food. So that in food products, eliminating vitamins and essential fatty acids of the body can lead to adverse effects such as inflammatory diseases, cancer, and immunodeficiency in the human body (1). The ever-rising trend of poultry consumption shows the importance of controlling meat quality for the poultry industry (2). Besides, the technical quality of poultry meat is now of significant importance, since poultry meat is nowadays usually consumed as cuts or as processed products rather than as whole carcasses (3, 4). Poultry meat is subject to oxidative spoilage due to its high concentration of unsaturated fatty acids (5). One of the factors that affect the quality of meat is the oxidation of fats.

Free radicals are essential oxidizing agents in foods that cause the oxidation of fats and oils (1).

Mycotoxins are secondary metabolites of low molecular weight produced by a wide range of fungi, principally molds. There are over 200 species of molds that produce mycotoxins. Aflatoxin B1 (AFB), and ochratoxin A (OTA), are some of the mycotoxins that can significantly impact the health and productivity of poultry species (6-8). Mycotoxins cause changes in gene encoding enzymes' expression required for energy production, fatty acid metabolism, and antioxidant defense (9, 10). Moreover, mycotoxins, mainly AFB1 and OTA, may accumulate in edible products such as meat and eggs, suggesting public health concerns (11). It was described by many studies (12, 13) that AFB1 and OTA provoke reactive oxygen substances (ROS) formation and cause oxidative stress as one of the leading causes of its toxic effects. It has also been reported that alters intracellular antioxidant mechanisms, namely gene expression and protein synthesis of Nrf2, a redox-sensitive regulator of the antioxidant response element (ARE) gene cluster, and consequently, it controls the synthesis of superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione synthetase (GS), glutathione reductase (GR), and catalase (CAT) (14, 15). Low activity of antioxidant enzymes and the decreasing level of glutathione (GSH) (16) consequently provoke oxidative stress due to the imbalance between oxidants and antioxidants (17). For these reasons, several studies have been performed using antioxidants to counteract the adverse effects of oxygen radicals generated under toxin-treatment.

Berberine (BBR) is an isoquinoline alkaloid of the protoberberine type, which could be found in the root, rhizome, and stem bark of many plant species traditionally used for the treatment of hepatic disorders (Berberine is an isoquinoline alkaloid of the protoberberine type, which could be found in the root, rhizome, and stem bark of many plant species traditionally used for the treatment of hepatic disorders (18). Berberine possesses a wide range of pharmacological activities, including antioxidative (19), anti-inflammatory (20), and immunoregulatory (21) activities. Several studies demonstrated the inhibitory effects of Berberine on chemically induced cytotoxicity, lipid peroxidation, and oxidative stress in the liver (22), including CCl4-induced liver damage (23). BBR has been reported to act as a direct antioxidant and an indirect deleterious agent in the body, neutralizing free radicals and protecting the body from oxidative stress by increasing the production of SOD, CAT, GSH, and GPX (24). In the present study, we have investigated the protective effects of Berberine against oxidative damage from AFB and OTA and a possible mechanism for its antioxidant activity.

Methods

Toxin production

Standard vials of *Aspergillus flavus* (NRRL 2999) were used to produce AFB. Yeast extract medium, prepared as described by Shotwell et al. (25), was used to propagate the fungus, and after fermentation on rice, AFB was produced. Standard *Aspergillus ochraceus* (NRRL 3174) vials cultured on wheat were used to produce OTA (26). Toxin concentrations of AFB and OTA were measured by high-performance liquid chromatography (HPLC) at Mabna Veterinary Laboratory (Karaj, Iran).

Measurement of BBR antioxidant activity

BBR was purchased from Bulk Supplement Factory (USA), and based on the catalog, this product is Pure Berberine HCL powder and had no other ingredients. BBR was dissolved with 1% DMSO in EtOH, and serial dilutions of all compounds were carried out to give a suitable concentration (μM). A serial dilution of 3,5-di-tert-4-butylhydroxytoluene (BHT) was used as a positive control. All diluted compounds, 250 μL, were added to 250 μL of 33 μM DPPH in EtOH solution. After incubation at room temperature for 20 min, the absorbance was detected at 520 nm by UV spectrophotometer (Biotex-synergy-HT). The percentage of scavenged DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) was measured as % inhibition from the following equation (27).

$\%\ \text{inhibition} = ((A_{blank} - A_{compound})/A_{blank}) \times 100$

A_{blank}=absorbance of blank, and $A_{compound}$=absorbance of compound

Chickens and diets

The experiment was carried out in the test poultry station of the Agriculture Faculty, University of Birjand, Birjand, Iran. A total of 288 one-day-old Ross 308 broiler chickens were randomly divided into 9 treatments with 4 replicates each (n=4): (1) negative control diet with no additives (NC); (2) NC + 2 ppm AFB (positive control AFB; PCAFB); (3) NC + 2 ppm OTA (positive control OTA; PCOTA); (4) PCAFB + 200 mg/kg BBR; (5) PCAFB + 400 mg/kg BBR; (6) PCAFB + 600 mg/kg BBR; (7) PCOTA + 200 mg/kg BBR; (8) PCOTA + 400 mg/kg BBR; and (9) PCOTA + 600 mg/kg BBR. The diet was based on corn and soybean meal, containing or exceeding the nutritional requirements recommended by the NRC (1994), but the nutritional requirement of the chickens was based on Ross 308 company for starter (0-10 days) and grower (11-24 days) diets. The experiment lasted for 42 days. The broiler chickens were bred on the floor, in a temperature-controlled room; the temperature began at 32 °C and was decreased gradually to 24°C until the end of the experiment. The lighting time was steady during the feeding period. During the whole period of the experiment, the broiler chickens had ad libitum access to feed and water.

Slaughter and measurements

At 42 days of age, two chickens weighing close to the pen average body weight (mean±1 SD) were selected from each of the four replicate pens for each dietary treatment, and blood samples were taken from the brachial vein to determine circulating levels of malondialdehyde (MDA). Activities were measured using commercial ELISA kits following the manufacturer's protocol (Pars Azmoon, Iran) in an autoanalyzer (Chem 200, Gesan, Italy). The relative weight of the carcass, thigh, and breast was then recorded from slaughtered birds.

Water-holding capacity (WHC)

WHC was estimated by determining expressible juice using a modification of the filter paper press method described by Wierbicki and Deatherage (28). A meat sample weighing between 200 and 400 mg was placed on an 11 cm diameter filter paper5 between plexiglass plates and pressed at 2,000 psi for 1 min. The outline area of the expressible juice and the meat film was traced, and the two regions were determined using a compensating polar planimeter.6 Expressible juice, as a percentage, was calculated as follows:

Expressible juice %= 100×(total juice area-meat film area)×water/square inch filter paper

Total moisture (mg) of the original sample (sample wt in (mg)×% moisture) Higher expressible juice percentage is related to decreased WHC.

Determination of lipid oxidation level in broiler meat

From the slaughtered birds, a 5 g sample of breast muscle was taken to calculate the degree of peroxidation. Oxidation products the levels of the lipid peroxidation product TBAR, main malondialdehyde, were determined spectroflourometrically at 515 nm excitation and 550 nm emission following isobutyl alcohol extraction. 1, 1, 3, 3-Tetraethoxypropane was used as the standard. The levels of conjugated dienes, another indicator of lipid oxidation, were measured spectrophotometrically. The content of protein-bound carbonyls, which is used to assess protein oxidation, was determined spectrophotometrically at 375 nm by the 2, 4-dinitrophenylhydrazine method (29).

Statistical analysis

Data were subjected to statistical analysis using the general linear model procedure of SAS (30). Parameters showing significant differences in the one-way analysis of variance were compared using Tukey's general linear model test. All the statements of significance were based on the 0.05 probability level.

Results

The antioxidant activity of Berberine (BBR)

The concentration of compound exhibited 50% inhibition (IC50) obtained from the dose-response curve was calculated and used to compare the scavenging ability of each compound. An antioxidant capacity of BBR was determined using DPPH assay and IC50 (Figure 1). IC50 values of BBR were 80.36 μM. Compound BBR demonstrated a better antioxidant activity than BHT standard antioxidant (IC50=62.36 μM).

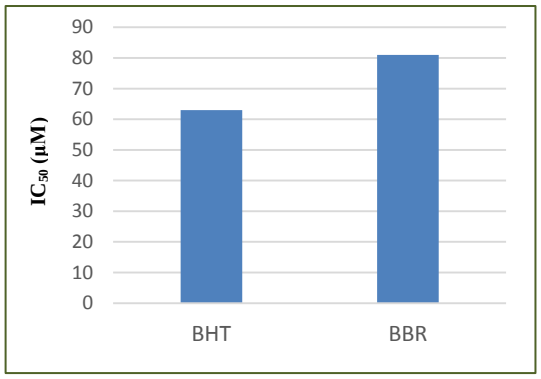

Figure 1. The IC50 of BBR and BHT

The relative weight of carcass characteristics

Effect of different treatments on the relative weight of carcass characteristics (carcass, breast, and thigh) in broiler chickens are shown in Table 1. Both PCAFB and PCOTA diets decreased carcass, breast, and thigh relative weight as compared with the NC diet ($p<0.05$). Supplementation of 600 or 400 mg/kg BBR to PCAFB diets reverse effect on the relative weight of carcass and breast muscle ($p<0.05$).

Adding 600 mg/kg BBR to PCOTA diets increased the relative weight of carcass from PCOTA alone ($p<0.05$). The lowest relative weight of thigh was in PCOTA diet as compared to NC diet, and there was no influence of BBR supplementation at any level on the relative weight of thigh in birds fed PCOTA or PCAFB diet ($p>0.05$).

Table 1. Effect of aflatoxin B_1 (AFB) or ochratoxin A (OTA) contamination with or without Berberine (BBR) supplementation on carcass characteristics

Treatment	Carcass (%)	Breast (%)	Thigh (%)
NC*	66.67^{a}	27.51^{a}	19.56^{a}
PCAFB*	53.51^{b}	17.32^{c}	16.53^{ab}
PCOTA*	58.18^{ab}	18.19^{c}	15.04^{b}
PCAFB+200 BBR**	58.89^{ab}	19.00^{c}	17.08^{ab}
PCAFB+400 BBR**	65.68^{a}	24.52^{ab}	17.86^{ab}
PCAFB+600 BBR**	66.63^{a}	25.77^{ab}	18.65^{ab}
PCOTA+200 BBR**	58.93^{ab}	20.73^{bc}	17.78^{ab}
PCOTA+400 BBR**	59.79^{ab}	19.03^{c}	17.95^{ab}
PCOTA+600 BBR**	61.30^{ab}	25.54^{ab}	18.51^{ab}
P-Value	0.0001	0.0001	0.0001
SEM	2.22	1.08	0.81

$^{a-c}$Superscripts within columns indicate significant differences between treatments ($p<0.05$).
*NC: negative control diet with no additives; PCAFB: NC + 2 ppm AFB (positive control AFB); PCOTA: NC + 2 ppm OTA (positive control OTA) **BBR supplementation (mg/kg diet).

Table 2. Effect of aflatoxin B_1 (AFB) or ochratoxin A (OTA) contamination with or without Berberine (BBR) supplementation on breast muscle malondialdehyde (MDA) levels at 0, 15m 30, and 45 days after slaughter

Treatment	Day 0	Days 15	Days 30	Days 45
NC*	1.90^{d}	2.05^{d}	2.22^{d}	2.38^{c}
PCAFB*	2.20^{a}	2.41^{a}	2.53^{a}	2.73^{a}
PCOTA*	2.17^{ab}	2.36^{ab}	2.46^{ab}	2.64^{ab}
PCAFB+200 BBR**	2.10^{abc}	2.30^{ab}	2.42^{abc}	2.60^{ab}
PCAFB+400 BBR**	2.06^{abcd}	2.25^{abc}	2.30^{bcd}	2.48^{bc}
PCAFB+600 BBR**	1.98^{bcd}	2.10^{cd}	2.23^{cd}	2.40^{c}
PCOTA+200 BBR**	2.12^{abc}	2.31^{ab}	2.42^{abc}	2.57^{abc}
PCOTA+400 BBR**	2.00^{abcd}	2.19^{bcd}	2.36^{abcd}	2.51^{bc}
PCOTA+600 BBR**	1.93^{cd}	2.18^{bcd}	2.30^{bcd}	2.45^{bc}
P-Value	0.0001	0.0001	0.0001	0.0001
SEM	0.036	0.031	0.038	0.041

$^{a-c}$Superscripts within columns indicate significant differences between treatments ($P<0.05$).
*NC: negative control diet with no additives; PCAFB: NC + 2 ppm AFB (positive control AFB); PCOTA: NC + 2 ppm OTA (positive control OTA). **BBR supplementation (mg/kg diet)

Meat malondialdehyde

Table 2 presents the result of the effect of experimental treatments on breast muscle malondialdehyde (MDA) in slaughter day (Days 0) and 15, 30, and 45 days after slaughter. Meat levels of MDA in PCAFB and PCOTA treatments were higher than in the NC treatment ($p<0.05$). The addition of higher levels of BBR to PCAFB diets reduced levels of MDA in all periods (0, 15, 30, and 45 days) ($p<0.05$). Only 600 mg/kg BBR addition to PCOTA diets decreased levels of MDA in slaughter day as compared to PCOTA only diet ($p<0.05$). Birds fed PCOTA diets with all levels of BBR still had higher levels of MDA than NC birds, and these were no different than birds fed PCOTA alone in 15, 30, and 45 days after slaughter ($p>0.05$).

Water-holding capacity (WHC)

The effect of experimental treatments on water-holding capacity (WHC) at four periods, one day after slaughter (WHC 0), 15 days after slaughter (WHC 15), 30 days after slaughter (WHC 30), and 45 days after slaughter (WHC 45) are presented in table 3. In all of the periods, NC-fed birds had higher WHC as compared to PCAFB-fed or PCOTA-fed birds ($p<0.05$), while both PCAFB and PCOTA had the lowest WHC as compared to NC in all periods ($p<0.05$). Higher amounts of BBR supplementation to PCAFB groups increased WHC in all periods ($p<0.05$). Adding 600 mg/kg BBR to PCOTA group increased WHC at 30 and 45 days after slaughter compared to PCOTA ($p<0.05$).

Table 3. Effect of aflatoxin B_1 (AFB) or ochratoxin A (OTA) contamination with or without Berberine (BBR) supplementation on water-holding capacity (WHC) (%)

Treatment	WHC 0*	WHC 15*	WHC 30*	WHC 45*
NC**	79.00^{a}	73.20^{a}	65.37^{a}	59.73^{a}
PCAFB**	65.25^{c}	58.35^{c}	50.52^{c}	44.88^{d}
PCOTA**	66.25^{c}	59.35^{bc}	50.88^{c}	44.36^{d}
PCAFB+200 BBR***	69.50^{bc}	63.70^{bc}	55.87^{bc}	50.23^{bc}
PCAFB+400 BBR***	69.75^{bc}	63.95^{bc}	56.12^{bc}	50.48^{bc}
PCAFB+600 BBR***	71.75^{b}	64.72^{b}	56.89^{b}	53.04^{b}
PCOTA+200 BBR***	66.45^{bc}	60.45^{bc}	52.62^{bc}	46.98^{cd}
PCOTA+400 BBR***	66.75^{bc}	60.95^{bc}	53.12^{bc}	47.48^{cd}
PCOTA+600 BBR***	70.50^{bc}	64.70^{b}	56.87^{b}	51.23^{bc}
P-Value	0.0001	0.0001	0.0001	0.0001
SEM	1.14	1.18	1.22	0.99

$^{a–c}$Superscripts within columns indicate significant differences between treatments ($P<0.05$).

* One days after slaughter (WHC 0), 15 days after slaughter (WHC 15), 30 days after slaughter (WHC 30), and 45 days after slaughter (WHC 45). ** NC: negative control diet with no additives; PCAFB: NC + 2 ppm AFB (positive control AFB); PCOTA: NC + 2 ppm OTA (positive control OTA). ***BBR supplementation (mg/kg diet)

Discussion

Berberine antioxidant activity

From DPPH assay, BBR exhibited a good antioxidant activity with IC50 values lower than the standard antioxidant, BHT. BBR antioxidant activity probably due to the presence of 1,2 hydroxyl groups in its molecule, as mentioned in the previous report (31).

Most antioxidant tests have indicated the high antioxidant activity of Berberis species BBR (31, 32).

Carcass characteristics

The percentage of carcasses due to contamination of feed with aflatoxin and ochratoxin toxins can be attributed to reduced performance and protein synthesis in chickens (33-35). The aflatoxin binds and interferes with enzymes and substrates that are needed in the initiation, transcription, and translation processes involved in protein synthesis. They interact with purines and purine nucleosides and impair the process of protein synthesis by forming adducts with DNA, RNA, and proteins (36). Aflatoxin also inhibits RNA synthesis by

interacting with the DNA-dependent RNA polymerase activity and thus causes degranulation of the endoplasmic reticulum. Also, the reduction in protein content in body tissues like skeletal muscle, heart, liver, and kidney could be due to increased liver and kidney necrosis (37). The regulation of protein synthesis is an important part of the regulation of gene expression. Regulation of mRNA translation controls the levels of particular proteins that are synthesized upon demand, such as synthesis of the different chains of globin in hemoglobin, or the production of insulin from stored insulin mRNAs in response to blood glucose levels, to name a few. The control of the cell cycle and cell proliferation also involves the regulation of protein synthesis, and malignant transformation of cells involves loss of certain translational regulatory controls (38).

BBR could upregulate uncoupling protein 2 (UCP2). UCP2, which is found in many tissues, affects body weight gain, resting metabolic rates, and food intake, which is all involved in energy balance (39). Nevertheless, it can be concluded that BBR increased the expression of UCP2 mRNA in skeletal muscle (40). Accordingly, BBR probably increased protein synthesis and protein tissue and ultimately increased the relative weight of carcasses and breasts in contaminated diets.

Meat Malondialdehyde

MDA is recognized as being a carcinogenic substrate because it reacts with DNA to induce mutations, which can lead to cancer, especially hepatic cancer. Two of the most common assays used for the evaluation of the lipid damage are based on the measurement of thiobarbituric acid reactive substances (TBARSs) or MDA by the thiobarbituric acid (TBA) test and conjugated dienes (41).

An inhibitory effect of AFB and OTA on protein synthesis may decrease the synthesis rate of ceruloplasmin and transferrin in the liver, which may lead to an increase in free copper and iron, respectively. The higher levels of these cations may impair the defense system against lipid peroxidation. Iron plays a particularly important role in the Fenton reaction, which is one of the most important phases in lipid peroxidation (42). A growing number of in vitro and in vivo studies has been collected and described evidence compatible with a role for oxidative stress in AFB and OTA toxicity and carcinogenicity. For these reasons, several studies have been performed using antioxidants to try to counteract the adverse effects of oxygen radicals generated under AFB or OTA-treatment. In line with the present study, previous studies have shown that BBR significantly reduced lipid peroxidation products with its antioxidant properties (43). Protecting the ability of berberine against Fe2+-induced lipid peroxidation has been already shown (44). Thus, BBR prevents the damage to antioxidant enzymes against oxygen radicals and hydrogen peroxide. Also, it has been shown that BBR reduces the damage caused by oxidation in the brain (45).

Water-holding capacity (WHC)

WHC determines the juiciness, flavor, and tenderness of the meat (46). Lactic acid accumulation and pH decline in postmortem result in protein denaturation and an overall decrease in muscle WHC.

It was shown (47) that depressed skeletal development affected muscle development, where the embryo weight, and the weights of leg and breast muscle, decreased significantly at different stages of embryo development after the injection of AFB1; these were a consequence of suppressed cell proliferation and reduced number of myotube nuclei, thereby explaining the depressed muscle development. Based on this, it can be said that feeding chickens from diets contaminated with toxins have reduced water storage capacity and thus reduced meat quality.

The addition of different levels of Berberine to chickens challenged with aflatoxin and ochratoxin reduced the negative effect of this toxin on broiler WHC. According to various studies, Berberine, with its antioxidant properties (48, 49), has prevented the negative effects of aflatoxins on the cell cycle, as well as lipid peroxidation, which has prevented the loss of moisture in breast tissue and improved meat quality in berberine-receiving chickens. Also, this improvement is probably due to the ability of BBR in scavenging ROS within the cell membranes, as well as their capacity to form

complexes with iron and copper redox-active forms (50). BBR could reduce oxidative stress by attenuating the expression level of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which was a major source of ROS production in cells (51).

NADPH oxidase was able to be upregulated by high levels of fatty acids, glucose, or advanced glycation end products (AGEs), resulting in the overproduction of ROS (52). Among various NADPH oxidase isoforms, BBR was reported to suppress the overexpression of NADPH oxidase 2/4 and decrease ROS production in macrophages and endothelial cells upon stimulation with inflammatory stimuli (53).

Conclusion

In this study, we aimed to investigate mycotoxin decontamination methods by using Berberine. Our data demonstrated that adding BBR to contaminated diets positively affected the carcass characteristics and meat quality, reduced MDA and improved WHC in broiler chickens. Thus, the antioxidant function of BBR is able to counteract the deleterious effects of chronic consumption of AFB and OTA and confirm the potential effectiveness of dietary strategies to counteract mycotoxin toxicity. Also, berberine treatment induced a significant enhancement of meat quality in broiler as revealed by reducing oxidative damage biomarkers with great activation of antioxidant biomarkers. Further studies are needed to determine the optimal levels of untreated and treated BBR that can be included in diets for broiler chickens reared under different circumstances, thus answering some essential questions for a better understanding and improvement of these methods.

Conflict of Interest

The authors declared that they have no conflict of interest.

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Ethics

We have included the required statement that animal procedures were approved by the appropriate committee. The animal ethical number is not available.

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