The efficacy of bentonite and zeolite in reducing aflatoxin B1 toxicity on production performance and intestinal and hepatic health of broiler chickens

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ABSTRACT
This research aimed to assess the influences of bentonite (BN) and zeolite (ZE) on reducing toxic influences of aflatoxin B1 (AFB1) in broilers by examining growth performance, carcass characteristics, serum indices, ileum morphology, apparent nutrient digestibility, and liver AFB1 residues. In total, 360 11-d-old straight-run broilers (Ross 308) were randomly allocated into 6 dietary treatments, with 10 replications of 6 birds each, in a 20-d experiment. The treatments were as follows: standard basal diet (negative control, NC); NC + 0.25 mg/kg AFB1 (positive control, PC); NC + 0.4% BN; NC + 0.4% ZE; PC + 0.4% BN; PC + 0.4% ZE. Compared to the NC diet, feeding the PC diet decreased daily feed intake (DFI) during the grower and overall periods (p < .01), reduced daily weight gain (DWG) and production efficiency factor (PEF) and increased feed conversion ratio (FCR) during grower, finisher, and overall periods (p < .001), lowered breast meat yield (p < .01), diminished dressing percentage, serum concentrations of total protein (TP), albumin (ALB), glucose (GLU), total antioxidant capacity (T-AOC), and total superoxide dismutase (T-SOD), villus height (VH), villus surface area (VSA), apparent digestibility of crude protein (CP) and ether extract (EE), apparent metabolisable energy (AME), and nitrogen-corrected AME (AMEn) (p < .001), and raised proportional liver weight, serum activities of glutamic oxaloacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT), and residues of AFB1 in the liver (p < .001). Compared to the PC diet, feeding the PC + 0.4% BN or PC + 0.4% ZE diets increased DWG and PEF and decreased FCR during finisher and overall periods, raised dressing percentage, serum levels of TP, GLU, T-AOC, and T-SOD, apparent CP digestibility, and reduced proportional liver weight and AFB1 residues in the liver (p < .001). Moreover, feeding the PC + 0.4% BN diet increased VH, VSA, apparent EE digestibility, AME, and AMEn, and decreased serum GOT and GPT activities when compared to the PC diet (p < .001). Whereas, feeding the PC + 0.4% ZE diet increased DFI during all experimental periods (p < .05) and DWG and PEF during the grower period (p < .001) as compared to the PC diet. To conclude, our findings demonstrate that dietary addition of 4 g/kg BN can deliver a better safeguard against the adverse influences of AFB1 in broiler chickens.

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Introduction
There are a considerable number of biological toxins existent in a natural environment, which would be hazardous for the well-being of farm animals. Mycotoxins, predominantly including aflatoxins, are presently regarded as amongst the most threatening ones in poultry (Murugesan et al. 2015). Aflatoxins are difuranocoumarin derivatives formed chiefly by strains of Aspergillus flavus - parasiticus and can infect plenty of different crops, especially corn, groundnut, and wheat (Abrar et al. 2013; Fountain et al. 2015). Crops contamination with these toxic metabolites adversely impacts the health status of humans and animals, the revenue of producers, and trading opportunities...
(Udomkun et al. 2017; Benkerroum 2020). Among the different kinds of aflatoxins, aflatoxin B1 (AFB1) is the most predominant and toxic one for poultry (Abudabos et al. 2017; Lauwers et al. 2019). The European Union has specified the maximum allowed concentration for AFB1 to be 0.02 mg/kg in the poultry feedingstuffs to safeguard these chickens from wellbeing dangers and to prohibit the transference of these toxic substances into their meat and egg products (Pappas et al. 2016).

Aflatoxin B1 is converted into secondary toxic metabolites chiefly in the liver, which can provoke liver injury via inducing apoptosis in hepatocytes and upsetting cellular enzymatic activity (Dohnal et al. 2014). Several studies have shown that the toxic metabolites of AFB1 are able to react adversely with various cell proteins, bringing about the suppression of carbohydrate and lipid metabolism and protein synthesis, and eventually cause apoptosis at a cellular and molecular level owing to elevated expression of death receptor-mediated pathways (Mughal et al. 2017; Wu et al. 2019). Numerous studies demonstrated the harmful influences of AFB1 in broiler chickens involving a decrease in growth responses (Liu et al. 2018a; Chen et al. 2022), changes in carcass quality (Bryden 2012), liver damage (Li et al. 2019), poor immune response (Li et al. 2014), enhanced susceptibility to infectious diseases (Ellakany et al. 2011), and augmented mortality (Pasha et al. 2007), causing considerable financial losses (Monson et al. 2015). Furthermore, AFB1 residue in the edible tissues is a possible risk to human health (Adegbeye et al. 2020).

At the current time, one of the more encouraging and functional techniques for averting or ameliorating the toxic influences of AFB1 is the usage of clay minerals or mineral adsorbents in animal feed such as aluminosilicate-based products involving phyllosilicates like bentonite (BN) and tectosilicates like zeolite (ZE) (Jard et al. 2011; Vila-Donat et al. 2018). These non-nutritive clay-based adsorbents, which originated from the decomposition of volcanic tuff, are capable to attach aflatoxin molecules in the alimentary canal of chickens, diminishing their bioavailability by forming non-resorbable complexes that are discarded via the excreta (Abidin et al. 2011; Fouad et al. 2019). The great surface area and the high ability to interchange cations give BN the potential to adsorb organic matters on the surface or within the interlayer space by penetrating the cations and polar molecules (Mil et al. 2015). In several in vitro and in vivo experiments, BN clays have demonstrated a significant capacity for absorption of AFB1 in broilers (Pappas et al. 2014; Shannon et al. 2017). Similarly, ZE with a great exterior surface area and a high cation-interchange ability can absorb polar molecules with elevated selectivity (Eroglu et al. 2017). Raj et al. (2021) showed that the inclusion of modified ZE into an AFB1-contaminated diet significantly heightened the production performance and diminished hepatic AFB1 residues in broilers. In addition, the efficiency of natural ZE in alleviating the impacts of aflatoxicosis has been reported in poultry feed under simulated gastric conditions (Moretti et al. 2018). Although several studies have been performed to determine the influences of supplementing these adsorbents on performance, biochemical indices, and residual levels of AFB1 in the liver, there is extremely limited information regarding their protective effects on intestinal morphology and nutrient digestibility in broiler chickens. Therefore, the current research was performed to evaluate the influences of dietary supplementation of BN and ZE on growth efficiency, carcass yields and visceral organs, serum biochemical parameters and enzyme activities, ileal histomorphology, apparent total tract digestibility of nutrients, and liver residual AFB1 level in broilers fed AFB1-contaminated diets.

**Materials and methods**

All practices utilised in this research were approved by the Animal Ethics Committee of King Saud University, Riyadh, Saudi Arabia.

**Birds and trial design**

A total of 360 one-day-old Ross 308 straight-run broiler chicks of similar weights were placed in battery cages (n = 6 chicks/cage; 30 kg BW/m²) in an environmentally controlled room and were fed a basal starter diet till 10 d of age. At 11 d of age, each cage was randomly assigned to 1 of 6 dietary treatments (10 replications each) in a completely randomised design. The treatments were as follows: standard basal diet (negative control, NC); NC + 0.25 mg/kg AFB1 (positive control, PC); NC + 0.4% BN; NC + 0.4% ZE; PC + 0.4% BN; PC + 0.4% ZE. The trial lasted for 20 d.

The PC diet was prepared using corn naturally contaminated with aflatoxins by replacing mycotoxin-free corn with naturally contaminated corn to provide the required AFB1 level as previously described by Yang et al. (2012). The contaminated diet was tested for the contents of AFB1 and other mycotoxins using a high-performance liquid chromatography system (Shimadzu Corp., Kyoto, Japan) with an appropriate method for
Table 1. Ingredients and nutrient composition of the basal starter (0-10 d) and grower-finisher (11-30 d) diets (% as-fed basis).

| Ingredients                  | 0-10 d | 11-30 d |
|------------------------------|--------|---------|
| Yellow corn                  | 48.0   | 60.5    |
| Soybean meal                 | 38.6   | 30.7    |
| Wheat bran                   | 7.00   | 2.00    |
| Choline chloride 60%         | 0.05   | 0.00    |
| Corn oil                     | 2.50   | 3.50    |
| Dicalcium Phosphate          | 1.94   | 1.58    |
| Ground Limestone             | 0.92   | 0.77    |
| Salt                         | 0.30   | 0.40    |
| DL-methionine                | 0.30   | 0.24    |
| Lysine-HCL                   | 0.16   | 0.11    |
| Vitamin-Mineral premix       | 0.20   | 0.20    |
| Total                        | 100    | 100     |

Nutrient composition

- Metabolisable energy (kcal/kg): 3000 – 3200
- Crude protein: 23.0 – 20.0
- Available phosphorus: 0.48 – 0.40
- Calcium: 0.96 – 0.81
- Digestible lysine: 1.28 – 1.03
- Digestible sulfur amino acids: 0.95 – 0.80
- Digestible threonine: 0.86 – 0.70

Vitamin–mineral premix provided the following per kg of the diets: vitamin A, 12,000,000 IU; vitamin D3, 5,000,000 IU; vitamin E, 8,000 IU; vitamin K3, 3,200 mg; vitamin B1, 1,200 mg; vitamin B2, 8,600 mg; vitamin B3, 65,000 mg; pantothenic acid, 20,000 mg; vitamin B6, 4,300 mg; biotin, 220 mg; vitamin B9, 2,200 mg; vitamin B12, 17 mg; antioxidant (BHA + BHT), 50,000 mg; copper, 16,000 mg; iodine, 1,250 mg; iron, 20,000 mg; manganese, 120,000 mg; selenium, 300 mg; and zinc, 110,000 mg.

Digestibility trial

At 25 d, 10 birds per treatment (1 bird/replicate) were selected randomly and housed individually in metabolic cages. After acclimatisation for 3 d, excreta from each cage were collected for 48 h using the total collection method (Ravindran et al. 2014). Feed consumption was recorded during the collection period. Afterward, excreta were oven-dried to constant weight and ground to pass through a 0.5 mm sieve prior to chemical examination. The diets and excreta were analysed for crude protein (CP) by the Kjeldahl procedure (method 984.13; AOAC 2019) and ether extract (EE) by the Soxhlet extraction procedure (method 920.39; AOAC 2019). The gross energy of feeds and excreta were quantified employing a bomb calorimeter (IKA Works, Wilmington, NC, USA) standardised with benzoic acid. The apparent digestibility of CP and EE and apparent metabolisable energy (AME) and nitrogen-corrected AME (AMEn) of the experimental diets were calculated according to De Marco et al. (2015).

Sampling and measurements

At the end of the feeding trial (30 d), 10 birds per treatment (1 bird/replicate) were randomly picked and blood specimens were gathered from the brachial vein into serum-separating tubes, which were centrifuged to acquire serum. The serum levels of total protein (TP), albumin (ALB), glucose (GLU), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), and glutathione reductase (GR) were quantified utilising commercial ELISA kits (MyBioSource, San Diego, CA, USA) following the manufacturer’s guidelines. Serum globulin (GLO) level was then calculated by subtracting ALB from TP.

After taking blood specimens, the birds were weighed, euthanized, plucked, processed, and eviscerated. The dressing was computed by dividing the hot carcase weight by pre-slaughter weight and expressed as a percentage. The weights of cut-up parts (breast muscles, leg quarters, and abdominal fat pad) and visceral organs (liver, spleen, bursa of Fabricius, and empty gizzard) were taken and expressed as a percentage of the pre-slaughter weight.

Approximately 2-cm long segments from the centre of the ileum were cut, flushed with phosphate buffer saline, and fixed in 10% neutral buffered formalin. Fixed sections were further dehydrated, cleared, embedded in paraffin, sectioned at 5-μm thickness, placed on glass slides, and stained with haematoxylin and eosin. The slides were photographed under a light microscope fitted with a digital camera (Olympus Corporation, Tokyo, Japan). Villus height (VH) and villus width (VW) based on at least 10 well-oriented villi per sample were measured utilising ImageJ software (National Institutes of Health, Bethesda, MD, USA). The villus surface area (VSA) was then computed from the VH and the VW at half-height (Al-Fataftah and Abdelqader 2014; Abudabos et al. 2019).
Residues of AFB1 in the liver tissues were extracted and purified following the procedure previously illustrated by Magnoli et al. (2011). A Shimadzu high-performance liquid chromatography system with fluorescence detection was used for the detection and mensuration of AFB1 in the final solution as formerly illustrated (Cui et al. 2017).

**Statistical analysis**

The experimental unit was the individual animal except for the performance measures where the pen of animals was the experimental unit. The data were checked for the homogeneity of variances with the Bartlett test and were analysed by one-way analysis of variance and Tukey test of multiple comparisons utilising SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). The statistical model involved treatment as a fixed factor and pen as a random factor. The significance level was specified at \( p < 0.05 \). Findings are presented as least square means with pooled standard error of the mean.

**Results**

**Growth performance**

The impacts of dietary treatments on broiler growth performance for grower (11–20 d), finisher (21–30 d), and overall (11–30 d) phases are summarised in Table 2. Broilers fed the PC diet had decreased DFI for the grower and overall phases \((p < 0.01)\) and reduced DWG and PEF and increased FCR in the grower, finisher, and overall phases \((p < 0.001)\) compared with those fed the NC diet.

In contrast, the inclusion of 0.4% BN or 0.4% ZE in the PC diet increased DWG and PEF and decreased FCR for the finisher and overall phases \((p < 0.001)\) compared to the PC diet. The increase in BWG has reached to the similar levels as in the NC groups. Moreover, adding 0.4% ZE to the PC diet increased DFI in all experimental phases \((p < 0.05)\) and DWG and PEF for the grower phase \((p < 0.001)\) compared to the PC diet.

**Carcase parameters**

The impacts of dietary treatments on carcase yield and components of broilers are summarised in Table 3. Broilers fed the PC diet had decreased breast meat yield \((p < 0.01)\) and reduced dressing percentage and increased proportional liver weight \((p < 0.001)\) compared with those fed the NC diet.

In comparison with the PC diet, the inclusion of 0.4% BN or 0.4% ZE in the PC diet increased dressing percentage and decreased proportional liver weight \((p < 0.001)\) to the similar levels as in the NC groups. No differences were detected in the relative weights of leg meat yield, abdominal fat, bursa, spleen, and empty gizzard among the treatments \((p > 0.05)\).

**Blood constituents**

The impacts of dietary treatments on serum analyses of broilers including biochemical indexes, liver function enzymes, and antioxidant status of broilers are given in Table 4. Broilers fed the PC diet had decreased levels of TP, ALB, GLU, T-AOC, and T-SOD \((p < 0.001)\) compared with those fed the NC diet.

In contrast, the inclusion of 0.4% BN or 0.4% ZE in the PC diet increased levels of TP, GLU, T-AOC, and T-SOD \((p < 0.001)\) compared to the PC diet. The increase in the levels of TP, T-AOC, and T-SOD has reached to the similar levels as in the NC groups. Moreover, adding 0.4% BN to the PC diet decreased GOT and GPT activities \((p < 0.001)\) compared to the PC diet. No differences were observed in GLO and GR levels among the treatments \((p > 0.05)\).

Table 2. Effect of adding bentonite (BN) and zeolite (ZE) as aflatoxin adsorbents to aflatoxin B1 (AFB1)-contaminated diets on growth performance of broilers during grower (11–20 d), finisher (21–30 d), and overall (11–30 d) phases.

| Treatments | 11-20 d | 21-30 d | 11-30 d |
|------------|---------|---------|---------|
|            | DFI g    | DWG g   | FCR g   | PEF g | DFI g    | DWG g   | FCR g   | PEF g | DFI g    | DWG g   | FCR g   | PEF g |
| NC         | 67.3a    | 49.4a   | 1.36b   | 282a  | 119ab   | 79.6a   | 1.49c   | 340a  | 92.0a    | 64.5a   | 1.44c   | 360a  |
| NC + 0.4% BN | 66.6a    | 48.4ab  | 1.38b   | 279a  | 121bc   | 79.8a   | 1.52bc  | 347a  | 93.5a    | 63.8a   | 1.47bc  | 343a  |
| NC + 0.4% ZE | 65.7ab  | 47.8ab  | 1.38b   | 279a  | 112b    | 64.3a   | 1.74a   | 257a  | 85.8a    | 51.6a   | 1.67a   | 287a  |
| PC         | 59.9a    | 38.8a   | 1.55a   | 200b  | 123c    | 77.2a   | 1.57a   | 314a  | 91.7a    | 60.4a   | 1.52bc  | 324a  |
| PC + 0.4% BN | 62.5ab  | 43.8bc  | 1.44ab  | 233bc | 123b    | 76.3a   | 1.61b   | 311b  | 94.6a    | 61.1a   | 1.55b   | 323b  |
| PC + 0.4% ZE | 66.4a   | 45.9ab  | 1.45ab  | 251ab | 123b    | 76.3a   | 1.61b   | 311b  | 94.6a    | 61.1a   | 1.55b   | 323b  |
| SEM        | 1.48     | 1.24    | 0.03    | 10.17 | 2.23    | 1.83    | 0.02    | 8.68  | 1.62     | 1.18    | 0.02    | 7.25  |
| p Value    | .008     | .001    | .001    | .001  | .001    | .001    | .001    | .001  | .001     | .001    | .001    | .001  |

\( ^a-c \)Means within the same column with different superscripts differ \((p < 0.05)\).

\(^b\)NC (negative control), standard basal diet; PC (positive control), NC + 0.25 mg/kg AFB1.

\(^c\)DFI: daily feed intake; DWG: daily weight gain; FCR: feed conversion ratio; PEF: production efficiency factor.
The impacts of dietary treatments on ileal histomorphometry, nutrient apparent digestibility, and AFB1 concentration in liver tissue of broilers are summarised in Table 5. Broilers fed the PC diet had decreased VH, VSA, apparent digestibility of CP and EE, AME, and increased AFB1 residues in the liver (p < .001) compared with those fed the NC diet. In contrast, the inclusion of 0.4% BN or 0.4% ZE in the PC diet increased apparent CP digestibility and decreased AFB1 residues in the liver (p < .001) compared to the PC diet. Moreover, adding 0.4% BN to the PC diet increased VH, VSA, apparent EE

**Ileal morphology, nutrient digestibility, and hepatic AFB1 residues**

The impacts of dietary treatments on ileal histomorphometry, nutrient apparent digestibility, and AFB1 concentration in liver tissue of broilers are summarised in Table 5. Broilers fed the PC diet had decreased VH, VSA, apparent digestibility of CP and EE, AME, and AFB1 residues in the liver (p < .001) compared with those fed the NC diet. In contrast, the inclusion of 0.4% BN or 0.4% ZE in the PC diet increased apparent CP digestibility and decreased AFB1 residues in the liver (p < .001) compared to the PC diet. Moreover, adding 0.4% BN to the PC diet increased VH, VSA, apparent EE
digestibility, AME, and AMEn ($p < .001$) compared to the PC diet. The increases in VH, VSA, EE digestibility, and AME have reached to the similar levels as in the NC groups. No differences were detected in VW between the treatments ($p > .05$).

Discussion

Aflatoxin B1 can lead to enormous financial losses in the broiler industry by lowering growth rate and feed efficiency and heightening the occurrence of diseases, therefore rising mortality (Rawal et al. 2010). The toxic influence of dietary AFB1 (0.25 mg/kg) and the ameliorative efficacy of dietary adsorbents (0.4% BN or 0.4% ZE) on the growth performance, carcase characteristics, ileum morphology, apparent nutrient digestibility, and liver AFB1 residues of broilers were evaluated. Several experiments have demonstrated the detrimental impact of AFB1 on growth performance (Sarker et al. 2021; Tavangar et al. 2021) and carcase characteristics (Arif et al. 2020; Mesgar et al. 2022) of broilers. Our results are in agreement with the aforementioned reports and showed that broilers fed diet naturally contaminated with AFB1 had significantly reduced FI, BWG, and EPEF and augmented FCR during the experimental period, along with lowered dressing and breast meat yields as compared to those provided with the un-contaminated diet. In addition, previous researchers revealed that AFB1 adversely influenced intestinal morphology (Sarker et al. 2021) and diminished the digestibility of nutrients (Liu et al. 2018b), bringing about lowered growth efficiency of broilers. Likewise, feeding the AFB1-contaminated diet in this study reduced VH and VSA in the ileal mucosa, apparent digestibility of CP and EE, AME, and AMEn. This decrease might be related to the impairments in epithelial cell proliferation and protein synthesis in the small intestine, which could, in turn, lower the nutrient digestibility (Han et al. 2008; Sarker et al. 2021).

In the present study, birds in the AFB1 group had a higher liver relative weight that is similar to the results of other investigators who reported that relative liver weights were significantly augmented in broilers after exposure to AFB1 (Fowler et al. 2015; Rajput et al. 2017). Furthermore, feeding AFB1 to the PC group significantly reduced the contents of serum TP, ALB, and GLU, which is in agreement with the results of Bagherzadeh Kasmani et al. (2012) and Chen et al. (2014). The toxicity of AFB1 has been demonstrated to trigger suppression of hepatic protein, carbohydrate, and lipid metabolism and might therefore lead to liver enlargement and serum biochemical changes (Sakamoto et al. 2018; Zabiulla et al. 2021). The present study showed that serum activities of GOT and GPT and residual levels of AFB1 in the liver were significantly elevated in broilers fed with the AFB1-contaminated diet. These findings are in accordance with former studies on broiler chickens regarding transaminase enzyme activities (Liu et al. 2018b; Elwan et al. 2021) and AFB1 accumulation in the liver (Salem et al. 2018; Śliżewska et al. 2019), illustrating that the accumulation of AFB1 in the liver could trigger apoptosis and inflammation in broiler hepatocytes. During aflatoxicosis, AFB1 is chiefly metabolised in the liver and transformed to its reactive metabolite (AFB1-8,9-epoxiodeoxyfuroladin) that can attach to cellular macromolecules like proteins, lipids, and nucleic acids, bringing about hepatocyte cancerisation and liver damage thereafter (Ismail et al. 2020). When hepatocyte permeability increased following liver injury, transaminases could be liberated from the infected hepatocyte into the bloodstream and resulted in elevated serum activity of transaminases, including GOT and GPT (Rashidi et al. 2020). Similar to the results of Rajput et al. (2017), our results also showed that broilers given the AFB1-contaminated diet had significantly lower levels of T-AOC and T-SOD in the serum than those provided with the uncontaminated feed. Reduced protein biosynthesis can be accountable for lowering the activity of antioxidant enzymes (Elwan et al. 2021). Besides, the metabolites of AFB1 induce cellular oxidative stress through increasing lipid peroxidation reactions, possibly leading to a disturbance in the antioxidant/oxidant system balance (Muhammad et al. 2018). Comprehensive, AFB1-induced impairments in both liver function and intestinal integrity are probably responsible for the reduced growth rate and carcase yield of broilers.

A variety of mineral clay products have been experimented and shown to have the potential for sequestering aflatoxins by reducing their absorption from the gastrointestinal tract, hence evading the toxic influences on animals and the transmission of toxins into their products (Di Gregorio et al. 2014). Among these adsorbents, BN and ZE that are derived from the weathering of volcanic ash have been tested due to their adsorptive properties, high availability, and low cost (Elliott et al. 2020). Based on our findings, dietary supplementation of 0.4% BN was more efficient in mitigating the toxic influences of AFB1 on growth efficiency by improving BWG, FCR, and EPEF during the finisher and overall periods, dressing yield, biochemical metabolites by increasing TP and GLU levels, antioxidant capacity by increasing the activities of T-AOC and
T-SOD, liver health by lowering relative liver weight, GOT and GPT activities, and AFB1 residues, ileal morphometry by improving VH and VSA, and apparent nutrient digestibility by enhancing CP, EE, AME, and AMEn retentions of broilers, showing its high binding capacity to AFB1 molecules. The high adsorption capability of this clay for binding AFB1 could be attributed to its high surface area, ion exchange capacity, and swelling or water-holding capacity (Manafi 2012). In addition, silicate clay mineral has been reported to decline the rate of feed passage across the intestinal canal and might subsequently result in improved nutrient metabolism (Safaeikatouli et al. 2012). These results are in accordance with the former research reports. Zabiulla et al. (2021) concluded that the addition of smectite clay to broilers’ diet that was contaminated with AFB1 heightened growth performance and lowered toxicological influences on the liver, indicating its protecting impact against aflatoxicosis. Amer et al. (2018) found that BN supplementation enhanced growth efficiency and nutrient digestibilities and reduced liver weight and histopathological lesions in rabbits provoked by AFB1-contaminated feed.

**Conclusion**

In conclusion, our results indicate that dietary AFB1 at a dose of 0.25 mg/kg gave rise to lowered growth efficiency, deteriorated carcass yield, altered serum biochemical metabolites, liver injury, depressed antioxidant capacity, impaired ileal architecture, and diminished nutrient digestibility of broilers. On the other hand, dietary supplementation of 4 g/kg BN provided better protection against the detrimental influences of AFB1 on the productive and health indicators of the birds. Therefore, BN can be considered as a safe and cost-effective feed additive in poultry nutrition and can be employed as an effective aflatoxin adsorbent to improve the production performance and health status of broilers. Accordingly, we can recommend using it as a feed additive for assisting in the prevention of aflatoxicosis in broiler flocks.

**Ethical approval**

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of King Saud University, Riyadh, Saudi Arabia (KSUSE-20-22).

**Disclosure statement**

No potential conflict of interest was reported by the author(s).
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