Toxicity of aflatoxin B1 on laying Japanese quails (Coturnix coturnix japonica)

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ABSTRACT

This study was performed to evaluate the efficacy of a herbal mycotoxin binder to overcome the adverse effects of 1.5 mg/kg of aflatoxin fed to laying quails. Results showed that addition of aflatoxin altered the performance indices of quails in terms of feed intake, FCR, egg production, and egg weight. Intestinal morphology of quails was also manipulated by inclusion of aflatoxin into the diet. The immunity of quails fed aflatoxin declined significantly. However, it could increase the population of harmful bacteria collected from the ileum of quails. The results of this study established that using herbal mycotoxin binder could bypass these adverse effects significantly and can be used in laying quails as a suitable binder when mycotoxin is present in their feed.

1. Introduction

The poultry industry in Iran stands as the biggest private industry and is ranked for maximum revenue within all agricultural sections. Among different species of poultry, quail production has gained importance due to its very delicious taste and higher nutritional values. Normally, a quail egg has around 13% of crude protein and 140 μg of vitamin B1. Quail meat and eggs are richer in vitamin B12, choline, iron, potassium, calcium, and phosphorus than chicken eggs and so are rich in high-density lipoprotein (HDL). Their egg can be useful in treatment of anaemia, removal of toxins and blood heavy metals, has strong anti-cancer capability, and helps in inhibition of cancerous growth, nourishment of the prostate gland, sexual potency restoring, enhancement of good memory and brain activity, and strengthening the immune system. Other advantages of quail production are minimum requirement of floor space, low investment, early sexual maturity to lay eggs, and high rate of egg production (EP) (Hedayati et al. 2014a).

Aflatoxins (AFs) are mycotoxins that are produced by various Aspergillus species including A. flavus, A. parasiticus, and A. niger. As secondary metabolites of these fungi, AF may contaminate a variety of food and feedstuffs, especially corn, peanuts, and cottonseed.

Among the known AFs, AFB1 is most commonly encountered and considered the most toxic for animal/poultry (Yunus et al. 2011). AFB1 has been given considerable attention because of their demonstrated carcinogenic potential and hepatotoxic effects in both humans and animals. Aflatoxicosis in animals is accomplished by reduction in growth rate and feed efficiency, decreased EP and hatchability, and increased susceptibility to disease and mortality (Manafi et al. 2014). AFB1 toxicity in animal/poultry may be expressed by changes in biochemical and haematological parameters’ concentrations of blood serum (Basmacioglu, et al. 2005).

2. Materials and methods

2.1. Bird management and experimental design

Two hundred and twenty layer Japanese quails (Coturnix coturnix) at 45 weeks of age were divided into 4 treatment groups, with 5 replicates and 11 quails (8 females and 3 males).
in each replicate. The quails were fed a corn-soybean meal basal diet (control); control plus 1.5 ppm Aflatoxin B1 (AF); control plus 0.03% Niltox Mycotoxin Binder (NMB); and control plus AF and NMB (1.5 ppm Aflatoxin B1 and 0.03% NMB, respectively (added to the basal diet) for 45 to 50 weeks of age according to Malayer University-approved animal care rules and protocols. Niltox, the mycotoxin binder used in this study, is a unique composition of minerals (extra purified clay containing diatomaceous earth mineral), antioxidants (Curcuminoids extracted from Turmeric), and enzymes (Epoxidase and Esterase), a property product of Zeus Biotech Limited, Mysore, India. It is claimed that incorporation of this product in poultry diets would effectively prevent DNA adduct formation and cellular damages in the biological systems through degrading peroxides, amides, and lacto rings in non-polar toxins such as aflatoxins.

The basal commercial quail breeder diet was fed as mash. The diet was formulated as isonitrogenic and isoenergetic according to the NRC 1994 recommendations. The ingredients and chemical composition of the coccidiostat-free basal diets are shown in Table 1. The quails in each battery cage were exposed to 17 h light and 7 h dark during the experimental period.

The feeding and collection protocols used in the present experiment were approved by the ethical committee of Malayer University under the guidelines of protection of animals used for experimental and other scientific purposes. The quails were also raised and cared based on the recommendation of the Iranian Council on Animal Care. All quails were fed ad libitum and housed in thermostatically controlled batteries (152.4 × 45.6 × 26.7 cm) with raised wire floors in an environmentally controlled building. The testing products were given to the quails 2 weeks prior to starting the experiment which was considered as adaptation period.

| Feed ingredients (%) | Corn | 65.3 |
|-----------------------|------|------|
| Soybean meal          | 19   |
| Shell powder          | 7.2  |
| Corn gluten           | 5.00 |
| Di-calcium phosphate  | 1.40 |
| Soybean oil           | 1    |
| dl-Methionine         | 0.31 |
| L-Lysine              | 0.09 |
| Mineral mixturea      | 0.25 |
| Vitamin mixtureb      | 0.25 |
| Salt                  | 0.20 |
| Analyzed chemical composition | 2950 |
| ME (Kcal/kg)          | 2.90 |
| Total protein (%)     | 18   |
| Total calcium (%)     | 3.10 |
| Available phosphorous (%) | 0.45 |
| Lysine (%)            | 0.85 |
| Methionine (%)        | 0.52 |
| Methionine + Cysteine (%) | 0.82 |
| Sodium (%)            | 0.18 |

*Mixture provided 500 mg of FeSO4, 65 mg of CuSO4, 100 mg of MnSO4, 0.5 mg of iodine, and 0.22 mg of selenium in each kilogram of feed.

1Vitamin mixture provided 11000 IU of vitamin A, 1800 IU of vitamin D3, 11 mg of vitamin E, 2 mg of vitamin K3, 4 mg of vitamin B1, 5.7 mg of vitamin B2, 2 mg of vitamin B6, 0.5 mg of folic acid, 250 mg of choline chloride, 0.125 mg of antioxidants, 0.03 mg of biotin, and 0.024 mg of vitamin B12 in each kilogram of feed.

2.2. Aflatoxin production

Aflatoxin (AF) was produced from Aspergillus parasiticus (NRRL 2999 culture (USDA, Agricultural Research Service, Peoria, IL) via fermentation of rice by the method of Shotwell et al. (1966). Successfully fermented rice was then steamed to kill the fungus, dried, and ground to a fine powder. The AF content in rice powder was analysed by the method of Shotwell et al. (1966) and measured by high-performance liquid chromatography (HPLC) (Camag-II, Basel, Switzerland).

2.3. Production rate

EP was recorded daily for each cage unit throughout the adaptation period, and an average EP rate (hen-day percent) was calculated. Feed conversion ratio (FCR) was calculated as gram feed per gram egg produced. Feed consumption was calculated after measuring the left-over feed and reported as gram per quail per day. EP was recorded daily on a cage basis and the same amount of feed was given to each replicate. Weekly EP was used for measuring EW. Egg mass was then calculated (egg weight × number of produced egg).

2.4. Egg quality indices

Egg component, Haugh unit scores, shell-quality test, and shell breaking strength measurements were done at the end of 50 weeks of age. Two eggs per replicate were chosen at random and eggshell breaking strength was measured with an eggshell force gauge (model-II, Robotmation Co. Ltd., Tokyo, Japan); eggshell thickness was measured with an ultrasonic thickness gauge (Echometer 1062, Robotmation Co. Ltd.), and Haugh unit was measured with automatic egg multi-tester equipment (EMT-5200, Robotmation Co. Ltd.). Shell weight was measured after depletion of internal contents placed at room temperature for 48 h by digital pan balance with 0.001 g accuracy.

2.5. Blood chemistry and antibody response

Two quails per pen (one male and one female) were selected from all groups, and individual blood samples were collected separately in non-heparinized tubes at 50 weeks of age. The sera were separated and stored at −20°C for further use. Each serum was analysed for glucose, cholesterol, low-density lipoprotein (LDL), HDL, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and triglyceride enzyme activities. Antibody titres against Newcastle disease (ND) and avian Influenza (AI) by ELISA technique were measured using commercial kits (Boehringer Mannheim Hitachi 704 automatic analyser, Japan).

2.6. Ileal morphology

At 50 wk of age, 4 birds of each treatment were randomly selected, stunned, and killed for evaluation of ileal morphology. The digestive tract with contents was removed aseptically and the ileum was separated from the Meckel’s diverticulum up to 1 cm proximal to the ileocecal junction and then dried with desiccant paper. A 2-cm section of ileum was taken from the
middle of the ileum and gently flushed with PBS (pH 7.2). Tissue sections were immediately fixed in 10% neutral buffered formalin; this formalin was changed three times to complete the fixation process. A single 0.5 cm sample was cut from each ileal section, dehydrated with increasing concentrations (70%, 80%, 95%, and 100%) of ethanol, cleared with xylene, and placed in polyfin embedding wax. Tissue sections (2 µm) were cut by microtome (Leitz-1512 Microtome, Leitz, Wetzlar, Germany), floated onto slides, and stained with haematoxylin (Gill no. 2, Sigma, St. Louis, MO) and eosin (Sigma). To measure villus height and crypt depth, images from samples and micrometre were taken using a digital camera that had light microscopy. Twelve images from 4 tissue sections of each ileal section were taken and 24 villus heights and crypt depths were measured by imaging software. Measurements for each villus length were taken from the tip of the villus to the valley and measurements for crypt depth were taken from the valley to the basolateral membrane.

### 2.7. Caecal microbial population

At 50 weeks, 2 quails from each cage were slaughtered by jugular vein for extraction of caecal contents and pooled individually for serial dilution. Microbial populations were determined by serial dilution (10−4–10−9) of caecal samples in anaerobic dilients before inoculation onto Petri dishes of sterile agar as described by Bryant and Burkey (1953). E. coli was grown on eosin methylene blue agar, Salmonella in *Salmo-nella Shigella* (Merck, Germany), and Coliforms on McConkey agar (Darmstadt, Germany). *E. coli* was incubated aerobically at 37°C. Plates were counted between 24 and 48 h after inoculation. Colony-forming units were defined as distinct colonies measuring at least 1 mm in diameter. Nine sterile test tubes with lids containing 9 mL of phosphate buffer solution as diluent were prepared and approximately 1 g of the caecal contents taken by sterile swab and homogenized for 3 min before transferring to a microbiology lab in cold condition (Bryant and Burkey 1953) and mixed employing aseptic technique. Then, 1 mL out of 10 mL of buffer plus caecal sample in the test tube was removed by 1000 µL sampler and transferred to tube no. 1 and mixed thoroughly. Similarly, it was transferred from tube no. 1 to tube no. 2 and this procedure was repeated until a dilution of 9 was completed. 1.0 mL of contents of each test tube was transferred to one of three selective media agars in a petri plate, respectively, and each petri plate was incubated at 37°C for 24 h. Finally, the intestinal bacterial colony populations formed in each plate was counted manually, adjusted to ×106, and reported.

### 2.8. Statistical analysis

Data were analysed in a completely randomized design manner using the GLM procedure of SAS (SAS Institute 2007) for those traits which were measured only once during the experimental period. The REPEATED statement of MIXED procedure of SAS (SAS Institute 2007) was used for analysis of traits that were measured repeatedly (EP parameters and internal egg quality characteristics) throughout the experimental period with age considered as a new main factor and initial values considered as a covariate effect in the model. Differences between treatment means were tested using Least Square Means (LSMEANS) for main effects and Tukey’s test for interactions. Statistical significance was declared at $P \leq 0.05$.

### 3. Results and discussion

A 2 weeks’ pre-experimental addition of probiotic to laying quails showed no significant ($P > 0.05$) differences between treatment means for EP and EW traits. Addition of AF significantly ($P \leq 0.05$) decreased EP (hen-day %) and NMB inclusion singly did not change the EP compared with the control group. Inclusion of binder into the AF-fed group significantly ($P \leq 0.05$) increased the EP when compared with AF-fed quails (Table 2). FCR of laying quails (g feed/g produced eggs) showed a significant ($P \leq 0.05$) increase in treatment and inclusion of NMB alone into the diet was improved the FCR (compared with control). In the AF + NMB group, FCR was not recovered, when compared with the AF group. Accordingly, feed intake (g/quail/day) was enhanced significantly ($P \leq 0.05$) by consumption of AF by quails and NMB when included into AF-contaminated diet could decrease the feed intake significantly ($P \leq 0.05$), compared with the control group (Table 2).

The EW (g) was reduced in AF quails significantly ($P \leq 0.05$) and addition of NMB could restore its negative impacts, when compared with the control group. The same trend exists for egg shell (%) but inclusion of NMB could not increase the egg shell weight up to those of control group. Shell breaking strength (kg) and shell thickness parameters have not been influenced by any of the dietary treatments. The Haugh unit score was significantly ($P \leq 0.05$) suppressed when AF was included in the diet. The AF + NMB group could significantly ($P \leq 0.05$) enhance the Haugh unit score of quails’ eggs, when compared with AF and control groups (Table 3).

The effects of AF and mycotoxin binder on blood chemistry and antibody response of laying quails are shown in Table 4. Inclusion of AF into the basal diet significantly ($P \leq 0.05$) increased plasma cholesterol (mg/dl), triglyceride (mg/dl), HDL (mg/dl), LDL (mg/dl), AST (mg/dl), and ALP (106/µl), compared with the control group. When binder was added into AF-contaminated diet (treatment 4), the cholesterol, triglyceride, HDL, LDL, and ALP levels could nullify the adverse effects of AF significantly ($P \leq 0.05$); however, the AST level could not reach the control level. On the other hand, the phosphorous (%) content was not influenced by any dietary treatment and

### Table 2. Effects of dietary aflatoxin and mycotoxin binder on performance of laying quails.

| Experimental treatments | Egg production | FCR | Feed intake |
|-------------------------|----------------|-----|-------------|
| Control                 | 61.70a         | 3.82b | 226.12c     |
| AF                      | 49.20c         | 4.01a | 269.05c     |
| NMB                     | 61.90c         | 3.79a | 220.33c     |
| AF + NMB                | 54.30c         | 3.94b | 254.15c     |
| SEM                     | 1.08           | 0.07 | 3.54        |
| P-value                 | 0.001          | 0.001| 0.006       |

Notes: EP: (hen-day %); FCR: Feed conversion ratio (g feed/g egg produced); Feed Intake: (g/quail/day); AF: (Aflatoxin B1 @ 1.5 ppm); NMB: Niltox Mycotoxin Binder (@ 0.3%); AF + NMB: combination of Aflatoxin B1 with Niltox Mycotoxin Binder (1.5 ppm and 0.3%, respectively). SEM: pooled standard error of means.
The effects of different dietary treatments on ileal morphology and caecal microbial populations of laying quails are shown in Table 5. Villus height (µm), crypt depth (µm), number of goblet cells (in each 1 mm of villus length), villus height, and villus to crypt ratio were reduced significantly (P ≤ 0.05) by addition of AF into the basal diet. When NMB was added into AF-contaminated diet, the crypt depth and number of goblet cells were increased significantly (P ≤ 0.05); however, the binder could show its positive impact on villus height, and villus to crypt ratio when compared with AF-alone treatment. The populations of non-beneficial bacteria (Clostridium, Salmonella, and Escherichia coli) (log_{10} cfu/g of DM) of quail’s caeca were increased significantly (P ≤ 0.05) by inclusion of AF into the basal diet. Addition of mycotoxin binder could decrease the load of bacteria significantly (P ≤ 0.05) in laying quails.

Foods and feed when contaminated with AF increase the health risk to animals and consequently human beings, alongside severe economic losses. There has been always a demand for introducing effective decontaminants. Scientists have suggested the use of traditional herbal ingredients towards the effective AF biological decontamination process. The most relevant symptom and sign of aflatoxicosis in poultry and livestock is poor performance. In laying poultry, the reduction in EP leads to severe economic losses and also severe AF-dependent diseases within the flocks (Manafi et al., 2015). In the current study, quails consuming 1.5 mg/kg of AF-containing diet had significantly poor EP, FCR, and feed intake.

These adverse effects are mainly due to anorexia, listlessness, inhibition of protein synthesis, and lipogenesis (Basmacoglu et al., 2005). In addition, liver malfunction and diminished protein/lipid utilization may also affect the performance and general health of poultry (Ortatati and Oguz, 2001). AFB1 is also regarded as causing macro nutrients’ malabsorption, hence reducing the digestive enzymes’ activities (Manafi et al., 2014). Although AF is known as hepatotoxic as well, it may affect poultry flocks through nutrient conversion demanding rate faster than hepatic metabolism. The unfavourable effects of AF on performance found in the current trial are in agreement with the previous reports of Miazzo et al. (2000). Impaired feed consumption in birds fed toxin has been reported by Raju and Devegowda (2000), Manafi and Khorasavinia (2013) and Sakamoto et al. (2018).

In the study of Oliveira et al. (2002), decrease in feed intake and FCR of laying quail fed 50 and 100 µg of AFB1/kg was observed. In contrast, Weibking et al. (1994) did not report significant difference in weight and shell thickness.

### Table 3. Effect of dietary Aflatoxin and Mycotoxin Binder on egg quality indices of laying quails.

| Experimental treatments | Egg weight (g) | Egg shell (%) | Shell breaking strength (kg) | Shell thickness (mm) | Haugh units |
|-------------------------|---------------|---------------|----------------------------|----------------------|-------------|
| Control                 | 15.22^a       | 7.17^a        | 0.24                       | 2.26                 | 92.43^a     |
| AF                     | 13.98^bc      | 5.89^bc       | 0.18                       | 2.17                 | 90.18^bc    |
| NMB                    | 15.60^d       | 7.23^d        | 0.23                       | 2.24                 | 92.54^d     |
| AF + NMB               | 14.12^e       | 6.78^e        | 0.19                       | 2.10                 | 91.34^eb    |
| SEM                    | 1.13          | 0.89          | 0.02                       | 0.41                 | 2.39        |

P-value: 0.001

Notes: AF (Aflatoxin B1 @ 1.5 ppm); NMB: Niltox Mycotoxin Binder (@ 0.3%); AF + NMB: combination of Aflatoxin B1 with Niltox Mycotoxin Binder (1.5 ppm and 0.3%, respectively). SEM: pooled standard error of means.

### Table 4. Effect of dietary Aflatoxin and Mycotoxin Binder on blood chemistry and antibody response of laying quails.

| Experimental treatments | Cholesterol (mg/dl) | Triglyceride (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | AST (mg/dl) | ALT % | ALP (10^5/µL) | Glucose (%N) | Protein (g/dl) | Phosphorus (%) | ND | AI |
|-------------------------|---------------------|---------------------|-------------|-------------|-------------|-------|---------------|--------------|----------------|----------------|----|----|
| Control                 | 325.18^a            | 563.29^a            | 91.33^a     | 138.25^a    | 288.4^a     | 2.2^a | 524^a         | 4.6^a        | 6.09^a         | 7.3^c          | 7.0^c |     |
| AF                     | 510.62^c            | 658.55^c            | 110.3^c     | 152.77^c    | 312.4^c     | 2.9^c | 618^c         | 5.12^c       | 8.7^c          | 4.6^c          | 4.9^c |     |
| NMB                    | 327.45^a            | 553.28^a            | 90.34^a     | 125.48^d    | 285.2^d     | 2.1^b | 532^a         | 4.6^c        | 6.03^b         | 7.1^c          | 6.6^c |     |
| AF + NMB               | 461.82^b            | 614.29^b            | 101.3^b     | 141.56^b    | 308.9^b     | 2.3^b | 587^b         | 4.5^b        | 6.02^b         | 5.8^c          | 5.3^a |     |
| SEM                    | 35.33                | 72.41               | 0.87        | 11.52       | 31.34       | 0.31  | 910           | 0.02         | 0.19           | 0.69           | 0.34 | 0.38 |

P-value: 0.001

Notes: AF (Aflatoxin B1 @ 1.5 ppm); NMB: Niltox Mycotoxin Binder (@ 0.3%); AF + NMB: combination of Aflatoxin B1 with Niltox Mycotoxin Binder (1.5 ppm and 0.3%, respectively). HDL: High-density lipoprotein; LDL: Low-density lipoprotein; AST: aspartate aminotransferase; ALT: Alanine Aminotransferase, ALP: Alkaline phosphatase; ND: Newcastle disease; AI: Avian influenza. Bacitracin (bacitracin methylene disalicylate): antibiotic growth promoter; SEM = pooled standard error of means.

### Table 5. Effect of dietary Aflatoxin and Mycotoxin Binder on ileal morphology and caecal microbial populations of laying quails.

| Experimental treatments | Villus height (µm) | Crypt depth (µm) | Villuscrypt ratio | Number of goblet cells | Coliforms (log_{10} cfu/g of DM) | Salmonella (log_{10} cfu/g of DM) | E. coli (log_{10} cfu/g of DM) |
|-------------------------|-------------------|-----------------|-------------------|------------------------|---------------------------------|---------------------------------|-------------------------------|
| Control                 | 510^d             | 92              | 5.54^d            | 10.34^b                | 3.38^b                          | 6.3^c                           | 3.51^d                        |
| AF                     | 385^d             | 81              | 4.75^d            | 8.93^c                 | 5.12^d                          | 8.7^c                           | 5.36^d                        |
| NMB                    | 405^d             | 93              | 5.32^d            | 10.29^a                | 3.62^b                          | 6.1^d                           | 3.49^d                        |
| AF + NMB               | 405^d             | 87              | 4.65^d            | 9.26^d                 | 4.35^d                          | 7.9^d                           | 4.61^d                        |
| SEM                    | 1.28               | 0.76            | 0.22              | 0.93                   | 0.04^a                          | 0.16^a                          | 0.02^a                        |

P-value: 0.003

Notes: Number of goblet cells in each 1 mm of villus length.

Means with different letters within the same column are significantly different (P < 0.05).

AF (Aflatoxin B1 @ 1.5 ppm); NMB: Niltox Mycotoxin Binder (@ 0.3%); AF + NMB: combination of Aflatoxin B1 with Niltox Mycotoxin Binder (1.5 ppm and 0.3%, respectively). SEM: pooled standard error of means.
any changes in these parameters on turkeys fed with mycotoxin. Difference among studies in length of exposure to the mycotoxins was undoubtedly a contributing factor in these contrasting results.

In the current study, quails fed AF showed a decrease in EP and EW. Ogido et al. (2004) reported a similar decline in EP of laying quails fed aflatoxin. Other reports by Kubena et al. (1999) and Oliveira et al. (2002) exist on laying hens, and they did not observe any effect on EP and EW. It can be assumed that the effect of mycotoxins on egg quality characteristic is dependent on mycotoxin dosage (Butkeraitis, 2003). Egg shell, shell breaking strength, shell thickness, and Haugh unit scores are frequently used in the evaluation of egg quality in case of laying birds. This characteristic is directly related to eggshell percentage. Galkate and Rokde, (2010) reported the higher eggshell thickness in layers fed diets containing aflatoxin. In quails intoxicated with 50 mg/kg FB1, (Butkeraitis 2003) has verified higher eggshell thickness. The result of the current study is consistent with the previous reports of Devegowda and Ravikiran (2008), who found a linear correlation between reduction in egg quality and AF. Oliveira et al. (2001) reported that eggshell percentage was not affected by AFB1 treatments, whereas Zaghini et al. (2005) observed reduced eggshell weight when birds were fed AFB1. Washburn et al. (1985) found higher eggshell strength in layers intoxicated with aflatoxins.

Haugh unit scores were significantly reduced in this study. Contrary to our findings, Oliveira et al. (2001), Galkate and Rokde (2010) and Manafi et al. (2012) observed no changes in Haugh unit in layers and breeders fed with varying levels of AF. The reduction of Haugh unit scores found in the current experiment may be due to the fact that aflatoxins may affect egg formation through an impairment of fat normal mobilization from the liver to the ovary (Leeson et al. 1995). Also, Sawhney et al. (1973) reported that albumen height is adversely affected when Japanese quails are exposed to AFB1.

In the current trial, the biochemical parameters and liver enzymes function have been altered negatively by feeding AF to the laying quails. The sensitive indicators of aflatoxicosis on the target organs are changes in serum biochemical and hematological parameters before major symptoms of chronic and sub-clinical symptoms become apparent (Kececi et al. 1998), which were also clearly observed in the present study.

Ortattali and Oguz (2001), Kececi et al. (1998), Madden et al. (1999) and Sakamoto et al. (2018) have reported similar results concerning the biochemical parameters of birds fed AF. Liver and kidney are regarded to be target organs for AF encounters. The decreased serum biochemistry observed in this trial was due to the hepatotoxic effects of AF, characterized by the inhibition of protein synthesis and impairment of carbohydrate and lipid metabolism (Rosa et al., 2001).

AF is supposed to be transformed into its epoxide form and this derivative produces DNA adducts which will carry to DNA stand-breaks and finally gene mutation (Manafi and Khosravinia 2013). For this, antioxidants are known to inhibit the AF-induced DNA adduct formation (Sakamoto et al., 2018).

The natural mycotoxin binder fed to laying quails of the current trial is composed of curcumin, an active ingredient of turmeric which is famous for being a natural antioxidant, minerals from clay sources, and enzymes (Shannon et al., 2017). Plant compounds like curcuminoids have inhibitory action on biotransformation of AF to their active epoxide byproducts. Turmeric (Curcuma longa), a medicinal plant, is known to possess antimicrobial and antioxidant properties (Hedayati et al. 2014b). Curcumin has been already shown to scavenge the free radicals and act as suitable antioxidants. Also, due to its non-toxicity, the use of turmeric in preventing AF-induced liver damage in the duckling has been reported (Tran, et al. 2005). In previous studies, it is reported that curcumin addition in the AF-contaminated diet improved the activities of HDL and ALT in chicken (Hedayati et al. 2014a). Practically, curcumin will reduce AFB (1)-N (7)-guanine adduct excretion within the urine, DNA adduct in the liver, and albumin adduct in the serum (Gowda et al. 2008). Turmeric powder is reported to alter the total serum proteins and albumin, AST, cholesterol, and triacylglycerol significantly in broilers and ducklings fed. It may be due to the antioxidant properties of turmeric against AF (Manafi and Khosravinia 2013).

AFB1 is metabolized in the liver which is having a high level of metabolizing enzymes and induces damage to this organ even leading to hepatocarcinogenesis (Sakamoto et al., 2018). In the presence of AF, the altered live functional enzymes may not be effective to do their normal functions. In this situation, addition of useful enzymes through toxin binder will reduce the harmful effects of AF (Gowda et al., 2008).

On the other hand, enzymes are believed to break the functional atomic group of the mycotoxin molecule and thereby render them nontoxic, Kumar and Balachandran (2009). Enzymes, viz. carboxylesterase, present in the microsomal fraction of the liver, esterase, and epoxidase are being tried for their practical applicability in the field conditions, which are being included in the studied toxin binder of the current trial (Kraieski et al. 2017).

It is believed that medicinal plants owe their antimicrobial properties mostly to the presence of alkaloids, phenols, glycosides, steroids, essential oils, coumarins, and tannins (Yin et al., 2017). All antioxidants are not suitable for adding to diets due to stability concerns, solubility, and interaction with other feed components (Monson et al. 2015). It is also postulated that the antioxidant system in the body mainly involves reducing agents (tocopherol, ascorbic acid, glutathione, and carotenoids), peroxidases (glutathione peroxidase, catalase), enzymes (peptidases, proteases, vitamin A), and superoxide dismutase (SOD). The most common functional chemical groups which have radical scavenging properties are hydroxyl (phenolics), sulfhydryl (cysteine, glutathione), and amino groups (uric acid, spermene). Antioxidative phenolics include tocopherol, catechins, ubiquinone, and synthetic compounds like Butylated hydroxyanisole and Butylated Hydoxytoluene (Manafi et al. 2011).

There is sufficient evidence to suggest that antioxidants ameliorate oxidative stress during mycotoxicosis by reducing the level of free radicals. Several natural (vitamins, pro-vitamins, carotenoids, polyphenols, and micronutrients) (Yin et al., 2017) and synthetic compounds seem to be chemo protective against common mycotoxins (Manafi and Khosravinia 2013).

It is believed that clay materials have the capability to bind molecules of certain sizes and configurations and have been reported to effectively alter the negative impacts of AF in
poultry. It is thought that the clays form a complex with the toxin thus preventing the absorption of AF across the intestinal epithelium. Due to their montmorillonite content, clay minerals swell and form thixotropic gels as a result of their ion exchange capabilities and are widely used as mycotoxin sequestering agent (Shannon et al., 2017). Manafi et al. (2009) reported the effectiveness of sodium bentonite in relieving the damages due to the presence of aflatoxins in broilers.

The decrease in serum glucose and protein which reflects an injury to the liver cells may be associated with the nephrotoxic effects of AF in agreement with other studies (Harvey et al., 1993). AFB1 can lower the activity of SOD and peroxidase resulting in damage at the histological level to the liver and kidney. The findings of this study about the suppressed immunity are in agreement with the other reports on haematopoiesis and immune responses due to AF eliciting inflammatory response to birds (Öguz et al., 2003).

The decreased antibody titres seen in the current study could be due to DNA inhibitory action of mycotoxin and protein synthesis through impairment of amino acid transport and m-RNA transportation leading to less antibody production. The possible mechanism of the counteraction of AF by minerals from clays (present in NMB) was binding of AF in GIT leading to excretion of AF without affecting the immune system. Improvement of antibody titres against ND and AI diseases on clay-product supplementation was earlier reported by Rosa et al. (2001), Tran et al. (2005), and Kraieski et al. (2017).

It is assumed that AF inhibits protein synthesis which is also the basic mechanism that underlies the immunosuppressive effect of the toxin. This immunosuppressing effect may be established as depressed T- or B-lymphocyte activity, suppressed antibody production, and impaired macrophage/neutrophil-effector activities. The immune system is primarily responsible for defense against attacking organisms. Inhibited immune activity by mycotoxins may ultimately decrease resistance to infectious diseases, reactivate chronic infections, and/or decrease vaccine and drug efficacy (Surai and Dvorska, 2001). Medicinal plants and their direct effects on birds’ immunity rely on the innate immunity of each cell and on systemic signals emanating from infection sites. This is how the herbal plants and their bioactive ingredients show their efficacy to alter the harmful effects of mycotoxins in poultry.

It is a general observation that the size of lymphoid organs is not normal in birds exposed to AFB1. In such animals, lymphoid cell depletion in thymus, spleen, and bursa of Fabricius has been described (Shivachandra et al., 2003). Thus, one explanation of immune-toxicity of AFB1, as also proposed by Azzam and Gabal (1998), could be inhibition of antibody production through the toxin’s effects on lymphocytes leading to enhanced turnover of serum antibodies and consequently to decreased antibody half-life (Carrillo et al., 1985).

In this study, AF has reduced the villus height, crypt depth, number of goblet cells, and villus height to crypt depth ratio significantly. In agreement with our findings, a linear increase in the crypt depth in distal jejunum with the increasing levels of AFB1 in the diet is noted by Kana et al. (2010), Yunus et al. (2011), and Kumar and Balachandran (2009). However, the specific chicken type employed in various studies may also affect the reaction of the intestine towards chronic aflatoxicosis.

This hypothesis is supported by the observations regarding aflatoxicosis in Hyline W36 layers (Applegate et al., 2009) and in broilers (Liu et al., 2018).

In the current study, the addition of AF increased the population of Coliforms, salmonella, and _E. coli_. This is mainly due to the fact that aflatoxins act as debilitating intestinal mucosal immunity leading to reduce overall immunity and increase the presence of the harmful bacteria in the upper intestinal tract which consequently increase their excretion rate through the faeces (Grenier and Applegate, 2013). Aflatoxin may destroy the beneficial bacteria and cells by peroxidation and making free radicals (Monson et al., 2015). On the other hand, reduction in villus height and absorption surface may lead to decrease the beneficial intestinal bacteria like _Bifidobacteria_ cause increase in the population of harmful bacteria in the absence of beneficial ones (Park et al., 2015).

### 4. Conclusion

Laying quails fed with aflatoxin at 1.5 mg/kg showed the impairment on most of the studied parameters. Addition of herbal mycotoxin binder containing antioxidants, enzymes, and diatomaceous earth minerals could partially enhance the performance and biochemical parameters, gut physiology, and immunity of quails in the current study. Considering the use of cost-effective and natural feed additives, this mycotoxin binder can be used while layer flocks encounter aflatoxicosis.

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