Effect of Dietary Inclusion of Mycodetox B₂ on Liveability, Immunity and Organ Pathology During Aflatoxicosis in Turkey Poults

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Abstract

To evaluate the efficacy of Mycodetox B₂ in ameliorating aflatoxicosis, day-old Turkey poults (n=200) were divided into five treatment groups, viz. T₁: control; T₂: T₁+100ppb aflatoxin B₁ (AFB₁); T₃: T₁+150ppb AFB₁; T₄: T₂+Mycodetox B₂; T₅: T₃+Mycodetox B₂. Each diet was fed to four replicates of 10 birds each for 42 days. The study revealed that overall liveability percent in T₃ was lower (P<0.05) than T₁. The CMI and HA titre values of T₂ and T₃ was lower (P<0.05) than T₁. The CMI and HA titre values in T₄ and T₅ was higher (P<0.05) than T₂ and T₃ and similar to T₁. The haemoglobin (Hb) value in T₂ and T₃ was lower (P<0.05) than T₁. The Hb value in T₄ and T₅ was higher (P<0.05) than T₂ and T₃; and similar to T₁. The haemoglobin (Hb) value in T₂ and T₃ was lower (P<0.05) than T₁. The Hb value in T₄ and T₅ was higher (P<0.05) than T₂ and T₃; and similar to T₁. Grossly, in T₂ and T₃ petechiae were seen in the kidneys and lungs; ecchymotic haemorrhages were also observed in liver and leg muscles; petechial haemorrhages were also recorded in thymus and bursa. The organs of T₁ and T₂ were normal as in T₁. Histopathologically, liver of T₂ and T₃ revealed congestion of central vein, mild degenerative changes in the hepatocytes and areas of necrosis associated with mild mononuclear (MNCs) cells infiltration especially in the portal areas. Hepatocytes also showed moderate fatty change, mild hyperplastic changes in the small bile ducts and lymphoid aggregation in the form of nodules. Haemorrhages in the liver parenchyma were quite marked. In T₁ and T₂, the architectural and cellular organization in the liver was normal as in T₁. It was concluded that dietary AFB₁ at 100 or 150ppb levels resulted in reduced liveability, immunity; decreased haemoglobin concentration, increased H/L ratio; and morphological and histopathological alterations in the internal organs. Moreover, the dietary incorporation of Mycodetox B₂ ameliorated the adverse effects of aflatoxicosis in Turkey poults.

Keywords: Aflatoxin B₁, Mycodetox B₂, Liveability, Immunity, Pathology, Turkey poults.

1. Introduction

Mycotoxins are ubiquitous in nature and has been reported a natural contaminants of various feed or fodder throughout the world (Katole et al., 2013). Mycotoxicosis, is characterized by its hepatotoxic, nephrotoxic, immunosuppressive, carcinogenic, mutagenic and teratogenic effects in animals and poultry (Patil et al., 2005, 2006, 2014, 2017a, b; Patial et al., 2013; Patil and Degloorkar, 2016a, b, 2018; Singh, 2019c, d, f; Singh et al., 2019a, b). Aflatoxicosis in poultry causes lowered performance in terms of reduced weight gain, feed intake and feed efficiency (Singh, 2019a, b, e; Singh and Mandal, 2013; Singh et al., 2013a; Singh et al., 2013b; Silambarasan et al., 2013; Patel et al., 2015; Singh et al., 2015; Singh et al., 2016), reduced nutrient utilisation (Silambarasan et al., 2013), increased mortality (Khatke et al., 2012b; Sharma et al., 2014), anemia (Singh et al., 2015; Singh et al., 2016), hepatotoxicosis and haemorrhage (Churchil et al., 2014; Singh et al., 2015; Singh et al., 2016; Pathak et al., 2017), altered biochemistry (Singh and Mandal, 2013; Singh et al., 2013a) and reduced
immunity (Khatke et al., 2012a; Patil et al., 2013; Sharma et al., 2016; Singh, 2019c; Singh, 2019d; Singh, 2019f; Silambarasan et al., 2016) leading to severe economic losses. Several approaches for detoxification of aflatoxins are available; however, they are very expensive and not applicable under practical conditions in animals and poultry (Patil et al., 2014). Therefore, a series of experiments was conducted. Among the various mycotoxin adsorbents, diatomaceous earth, sodium bentonite and zeolite either at 0.5% or 1% level were partially effective in ameliorating the adverse effects of AFB₁ in broiler chickens. Among these three mycotoxin adsorbents tested, diatomaceous earth was least effective. However, combination of the binders at a time was the most efficacious in ameliorating the ill effects of AFB₁ in broiler chickens (Silambarasan et al., 2013). Use of mannan oligosaccharide (MOS) and Saccharomyces cerevisiae (SC) (at the rate 0.05%, 0.1%, 0.2%) alone and their combination moderately ameliorated the adverse effects of 300 ppb AFB₁ (Khatke et al., 2012b). The 0.2% level of MOS and SC was found to be more effective in counteracting the 300 ppb AFB₁ in feed (Khatke et al., 2012c). Singh and Mandal (2013) reported that dietary supplementation of butylated hydroxyanisole at 1000 and 2000 ppm levels provided partial protection at 1 ppm aflatoxin toxicity. Singh et al. (2016) reported that supplementation of methionine (as DL-methionine) at 500 ppm or its analogue methionine hydroxy analogue at 769 ppm level in aflatoxin (500 ppb AFB₁) contaminated diet ameliorated the adverse effects in Japanese quails. In another study, Singh et al. (2013b) reported that inclusion of methionine at additional 0.025 and 0.05% levels over the prescribed requirements in the total aflatoxin contaminated diet at 1 ppm level provided partial protection against the aflatoxicosis in broiler chickens. Sharma et al. (2014) reported that supplementation of 40 mg Zn/kg ameliorated the ill effects of AFB₁ on the performance of the birds. Based on a decade’s research on mycotoxicosis in various avian species, Mycodetox B₂ was formulated and the objective of the present investigation was to test its efficacy in ameliorating aflatoxicosis in Turkey poult.s

2. Materials and Methods

2.1 Production and Analysis of Aflatoxin
Aflatoxin was produced using the fungal strain Aspergillus flavus NRRL 6513 that was obtained from U.S. Department of Agriculture, USA. To get the fresh spores, the culture was regularly subcultured on potato dextrose agar (PDA) medium slants and stored at 5°C. Aflatoxin was produced on maize substrate. Fermentations were carried out in batches as per

Shotwell et al. (1966). The extraction and estimation of AFB₁ was done as per Pons et al. (1966). Aqueous acetone was used for extraction of the toxin. Aflatoxin contents were finally quantified using a spectrophotometer.

2.2 Experimental Design
Experimental design was completely randomized design with five dietary treatments viz. T₁: control (Basal diet); T₂: T₁+100 ppb aflatoxin B₁ (AFB₁); T₃: T₁+150 ppb AFB₁; T₄: T₂+Mycodetox B₂; T₅: T₃+Mycodetox B₂. Each dietary treatment had 4 replicates and each replicate had 10 Turkey poult.s

The experiment was conducted for 6 weeks of age. The various dietary treatments were prepared by mixing mouldy maize to get the desired concentration of 100 and 150 ppb AFB₁ and the Mycodetox B₂ at the rate of 132 g per quintal of feed. The Mycodetox B₂ developed after a decade of intensive research efforts in mycotoxicosis consisted of sodium bentonite (30.30%), zeolite (30.30%), mannan oligosaccharide (15.15%), methionine (18.94%), butylated hydroxyanisole (3.74%) and Zinc (1.52%).

2.3 Birds and Diets
Two hundred, one-day-old Turkey poult.s (Belts Ville White) were used in this study. The Turkey poult.s were wing banded, weighed individually and divided into five treatment groups based on equal body weight in each group. The poult.s were housed in electrically heated compartments with continuous lighting. The feed for Turkey poult.s was formulated based on the nutrient requirements recommended by NRC (1994). The feed and water were provided ad libitum during the experimental period.

The birds were inspected daily. Mortality was recorded as and when occurred. The cell mediated immune response to PHA-P antigen was evaluated by the method described by Corrier and DeLoach (1990). The microtitre haemagglutination procedure as described by Siegel and Gross (1980) was followed to measure total HA antibody titres in chickens. At the end of the experiment, liver samples were collected and fixed in 10% formal saline.

The formal saline fixed samples were cut into pieces of 2-3 mm thickness and washed thoroughly in tap water overnight before dehydrating the tissues in ascending grades of alcohol (50%, 60%, 70%, 80%, 90%, absolute alcohol I and II). The dehydrated tissues were cleared in benzene and embedded in paraffin blocks. Serial sections of 5-micron thickness were cut and stained with hematoxyline and eosin (Culling, 1968) and examined under microscope for various histopathological changes, if any.
2.4 Statistical Analysis
The collected data was subjected to statistical analysis using Statistical Package for Social Sciences (SPSS Version 16.0). The recorded data were subjected to one-way analysis of variance with comparison among means was made by Duncan’s multiple range test with significance level of P<0.05.

3. Results and Discussion
The results on weekly percent liveability of Turkey poults fed on various dietary treatments are presented in Table 1. The data pertaining to cell mediated immune (CMI) response to PHA-P measured as foot web index and humoral immune response measured as haemagglutination titre (HA) against SRBCs, and haematological parameters in Turkey poults fed on various dietary treatments was statistically analyzed and presented in Table 2.

3.1 Liveability Percent
No mortality was recorded during first and second weeks. During third and fourth weeks, the liveability percent did not differ significantly due to different dietary treatments. During fifth and sixth weeks, the liveability per cent varied from 85.00 in T2 to 95.00 in T1. The liveability per cent of group T1 was lower (P<0.05) than that of T2. The liveability percent of groups T4 was T3 did not vary significantly from that of control. The results of present study revealed that 150 ppb AFB1 in the diet of Turkey poults resulted in significantly (P<0.05) higher mortality compared to that of control, whereas, inclusion of AFB1 at 100 ppb did not cause heavy mortality, which might be due to the low level of dietary AFB1, however, numerically lower liveability was reported in T2 compared to that of control. Similar results were also reported by Singh et al. (2011) wherein significantly reduced liveability per cent (P<0.05) during AFB1 (300 ppb) toxicity in Turkey poults. Sharma et al. (2014) also reported reduced liveability per cent but did not produce heavy mortality in broiler chickens at 250 ppb AFB1 in feed. Khatke et al. (2012b); Silambarasan et al. (2015) also reported significantly (P<0.05) decreased mortality due to 300 ppb AFB1 in broiler chickens. Reduced liveability percent due to AFB1 contamination in feed has also been reported by earlier researchers (Sharma et al., 2015; Shamsudeen et al., 2013; Singh et al., 2011; Singh, 2019c; Singh, 2019d; Singh, 2019f; Gopi, 2006; Denli et al., 2009). In this study, the overall liveability per cent of groups T4 and T3 was statistically similar to that of control.

3.2 Cell Mediated and Humoral Immunity
Aflatoxicosis impairs the humoral and cellular immune responses and increase susceptibility to some environmental and infectious agents (Azzam and Gabal, 1998). In the present study, the CMI value of groups T2 and T3 was lower (P<0.05) than that of T1. The CMI values between groups T2 and T3 did not differ significantly. The present study revealed that dietary AFB1 at 100 and 150 ppb levels decreased (P<0.05) the CMI response compared to that of control. The CMI value in T4 and T5 was higher (P<0.05) than those of T2 and T3 and statistically similar to that of T1. The CMI value between groups T4 and T5 was statistically similar. Silambarasan et al. (2016); Khatke et al. (2012a) also reported significant decrease in the CMI response at 300 ppb level of dietary AFB1 in broiler chickens. Ghosh and Chauhan (1991) observed that 300 ppb AFB1 in broiler feed caused immune suppression with no apparent clinical effects, but can result in flock morbidity and/or mortality caused by secondary infections. Yunus et al. (2011) also reported ill effects of AFB1 at 400 ppb level in the diet of broiler chickens. Giambonne et al. (1978) indicated that AFB1 had marked effect on CMI in chicken, as measured by graft-versus-host and delayed hypersensitivity reaction. Since CMI plays a major role in resistance coccidiosis, a reduction in this immunologic function by AFB1 could make chicks more susceptible to this disease. Suppression of CMI response may be due to impaired lymphoblastogenesis (Chang et al., 1976) and impairment of lymphokine production (Ghosh et al., 1991). Decreased CMI response in chickens due to AFB1 feeding was also earlier reported by several researchers (Patil et al., 2013; Sharma et al., 2016; Singh, 2019c; Singh, 2019d; Singh, 2019f; Kadian et al., 1988; Deo et al., 1998; Bakshi, 1991). In this study, inclusion of Mycodetox B2 to the aflatoxin contaminated feed ameliorated the ill effects of aflatoxin on cell mediated immune response in Turkey poults. With regard to humoral immunity, the HA titre value of T2 and T3 was lower (P<0.05) than that of control group (T1). This result showed that dietary AFB1 at 100 and 150 ppb levels significantly (P<0.05) decreased the HA titre as compared to that of T1. The HA titre value in T4 and T5 was higher (P<0.05) than those of T2 and T3 and statistically similar to that of control. The HA titre value between groups T4 and T5 did not differ significantly. This result showed that AFB1 at 100 and 150 ppb levels in feed decreased (P<0.05) the humoral immune response compared to that of control. This result was in agreement with Oguz et al. (2003) who also reported decreased humoral immunity at 50 ppb AFB1 in feed. Thaxton et al. (1974) also reported reduced antibody production following injection of SRBCs in chickens experiencing aflatoxicosis. Silambarasan et al. (2016); Khatke et al. (2012a) also reported a significant decrease in the humoral immune response of dietary AFB1 at 300 ppb.
level in broiler chickens. Aflatoxin depresses protein synthesis via inhibition of RNA polymerase, which results in suppression of specific immunoglobulin synthesis (Giambrone et al., 1985). Aflatoxin is an immunosuppressant by virtue of its ability to stimulate lysosomal degradation of immunoglobulins (DeDuve and Wattiaux, 1966). Non-specific factors such as complement, interferon and nonspecific serum protein concentrations also decrease due to liver damage (Tung et al., 1975). During experimental aflatoxicosis, reduced humoral immune response has also been reported by earlier researchers (Patil et al., 2013; Sharma et al., 2016; Singh, 2019c; Singh, 2019d; Singh, 2019f; Virdi et al., 1989; Bakshi, 1991).

### 3.3 Haematological Examination

The haemoglobin (Hb) value in T2 and T3 was lower (P<0.05) than that of T1. The Hb value in T2 and T3 was higher (P<0.05) than those of T2 and T3, and statistically similar to that of control. The Hb value in T2 was statistically similar to that of T3. The Hb value of T4 was statistically similar to that of T5. The results revealed that dietary AFB1 at 100 and 150 ppb levels resulted in reduced (P<0.05) Hb concentration in Turkey pouls. Sharma (2013) also reported that aflatoxin contamination at 250 ppb level resulted in reduced Hb level in broiler chickens. This finding was also in agreement with that of Kececi et al. (1998); Basmacioglu et al. (2005) who reported reduced Hb level at 2.5 and 2.0 ppm AFB1, respectively in broiler chickens. In the case of heterophil/lymphocyte (H/L) ratio, the H/L ratio of T4 was lower (P<0.05) as compared to those of T2 and T5. The H/L ratio between groups T2 and T3 was statistically similar. The H/L ratio of groups T1 and T2 was lower (P<0.05) than those of T2 and T3 and statistically similar to that of control. The present study revealed that dietary AFB1 at 100 and 150 ppb levels resulted in increased (P<0.05) H/L ratio in Turkey pouls. Sharma (2013) also reported an increase (P<0.05) in the H/L ratio during 250 ppb AFB1 in the feed of broiler chickens. Also, Basmacioglu et al. (2005) reported elevated H/L ratio in broilers due to feeding of 2 ppm AFB1. This result was also in agreement with those of Huff et al. (1986); Oguz et al. (2003) wherein the suppressive effects of AFB1 on haematopoiesis and immune responses were reported. The increase in heterophil counts suggested that the toxin elicited the inflammatory response (Kececi et al., 1998).

### 3.4 Gross and Histopathological Studies

Morphologically, the organs of control group, receiving basal feed were normal in size, colour and border marking, and served as reference standard. In groups T4 and T5, petechiae were seen in kidneys and lungs; ecchymotic haemorrhages was observed in liver and leg muscles; petechial haemorrhages were also recorded in thymus and bursa of Fabricius of Turkey pouls. In groups T4 and T5, livers were enlarged, fatty and pale in appearance and the atrophy of bursa was also observed. The organs of T4 and T5 groups were normal in appearance as that of T1 group. The present study revealed that 100 and 150 ppb of aflatoxin contamination in feed resulted in altered gross pathology of organs. Similar gross lesions on morphology of liver due to aflatoxicosis were also...
reported by Singh et al. (2015); Singh et al. (2016). The results of the present study revealed that addition of Mycodetox B₂ to the aflatoxin contaminated feed ameliorated the adverse effects of aflatoxicosis on morphology of organs in Turkey poults. Histopathological alterations were observed in the liver parenchyma as liver is the main target organ of aflatoxicosis. With regard to histopathology, the architectural and cellular organization of liver in the birds of group T₁ was normal and this group served as a reference standard for comparison with other groups. In AFB₁ fed groups T₂ and T₃, the histopathological alterations were congestion of central vein, mild degenerative changes in the hepatocytes and areas of necrosis associated with mild MNCs infiltration in the portal areas. Hepatocytes showed moderate fatty change, mild hyperplastic changes in the small bile ducts and lymphoid aggregation in the form of nodules.

Similar histopathological lesions were also reported in other studies (Singh, 2019c; Singh, 2019d; Singh, 2019f). In groups T₂ and T₃, the architectural and cellular organization of liver was normal as that of control.

4. Conclusion
It was concluded that dietary AFB₁ at 100 or 150 ppb levels resulted in reduced liveability, suppression of immunity, decreased haemoglobin concentration, increased heterophil/lymphocyte ratio; and morphological and histopathological alterations in the internal organs. However, the incorporation of Mycodetox B₂ ameliorated the adverse effects of aflatoxicosis on liveability, immunity, haematological parameters; and gross and histopathology of internal organs in Turkey poults.

References
Singh...Effect of Dietary Inclusion of Mycodetox B2 on Liveability, Immunity and Organ Pathology During Aflatoxicosis in Turkey Poults


