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ORIGINAL ARTICLE

Effect of Dietary Inclusion of Mycodetox B_2 on Liveability, Immunity and Organ Pathology During Aflatoxicosis in Turkey Poults

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Abstract

To evaluate the efficacy of Mycodetox B_2 in ameliorating aflatoxicosis, day-old Turkey poults (n=200) were divided into five treatment groups, viz. T_1: control; T_2: T_1+100ppb aflatoxin B_1 (AFB_1); T_3: T_1+150ppb AFB_1; T_4: T_2+Mycodetox B_2; T_5: T_3+Mycodetox B_2. Each diet was fed to four replicates of 10 birds each for 42 days. The study revealed that overall liveability percent in T_3 was lower ($P<0.05$) than T_1. The CMI and HA titre values of T_2 and T_3 was lower ($P<0.05$) than T_1. The CMI and HA titre values in T_4 and T_5 was higher ($P<0.05$) than T_2 and T_3 and similar to T_1. The haemoglobin (Hb) value in T_2 and T_3 was lower ($P<0.05$) than T_1. The Hb value in T_4 and T_5 was higher ($P<0.05$) than T_2 and T_3; and similar to T_1. The H/L ratio of T_1 was lower ($P<0.05$) than T_2 and T_3. The H/L ratio of T_4 and T_5 was lower ($P<0.05$) than T_2 and T_3 and similar to T_1. Grossly, in T_2 and T_3 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 T_4 and T_5 were normal as in T_1. Histopathologically, liver of T_2 and T_3, 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 T_4 and T_5, the architectural and cellular organization in the liver was normal as in T_1. It was concluded that dietary AFB_1 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 B_2 ameliorated the adverse effects of aflatoxicosis in Turkey poults.

Keywords: Aflatoxin B_1, Mycodetox B_2, Liveability, Immunity, Pathology, Turkey poults.

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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). Mycoxicosis, 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 AFB_1 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 efficaceous in ameliorating the ill effects of AFB_1 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 AFB_1 (Khatke *et al*., 2012b). The 0.2% level of MOS and SC was found to be more effective in counteracting the 300 ppb AFB_1 in feed (Khatke *et al*., 2012c). Singh and Mandal (2013) reported that dietary supplementation of butylated hydroxylanisole 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 AFB_1) 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 AFB_1 on the performance of the birds. Based on a decade's research on mycotoxicosis in various avian species, Mycodetox B_2 was formulated and the objective of the present investigation was to test its efficacy in ameliorating aflatoxicosis in Turkey poults.

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 AFB_1 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. T_1: control (Basal diet); T_2: T_1+100 ppb aflatoxin B_1 (AFB_1); T_3: T_1+150 ppb AFB_1; T_4: T_2+Mycodetox B_2; T_5: T_3+Mycodetox B_2. Each dietary treatment had 4 replicates and each replicate had 10 Turkey poults.

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 AFB_1 and the Mycodetox B_2 at the rate of 132 g per quintal of feed. The Mycodetox B_2 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 poults (Belts Ville White) were used in this study. The Turkey poults were wing banded, weighed individually and divided into five treatmemt groups based on equal body weight in each group. The poults were housed in electrically heated compartments with continuous lighting. The feed for Turkey poults 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 per cent 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 T_3 to 95.00 in T_1. The liveability per cent of group T_3 was lower ($P<0.05$) than that of T_1. The liveability percent of groups T_4 was T_5 did not vary significantly from that of control. The results of present study revealed that 150 ppb AFB_1 in the diet of Turkey poults resulted in significantly ($P<0.05$) higher mortality compared to that of control, whereas, inclusion of AFB_1 at 100 ppb did not cause heavy mortality, which might be due to the low level of dietary AFB_1, however, numerically lower liveability was reported in T_2 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 AFB_1 (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 AFB_1 in feed. Khatke *et al*. (2012b); Silambarasan *et al*. (2015) also reported significantly ($P<0.05$) increased mortality due to 300 ppb AFB_1 in broiler chickens. Reduced liveability percent due to AFB_1 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 T_4 and T_5 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 T_2 and T_3 was lower ($P<0.05$) than that of T_1. The CMI values between groups T_2 and T_3 did not differ significantly. The present study revealed that dietary AFB_1 at 100 and 150 ppb levels decreased ($P<0.05$) the CMI response compared to that of control. The CMI value in T_4 and T_5 was higher ($P<0.05$) than those of T_2 and T_3 and statistically similar to that of T_1. The CMI value between groups T_4 and T_5 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 AFB_1 in broiler chickens. Ghosh and Chauhan (1991) observed that 300 ppb AFB_1 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 AFB_1 at 400 ppb level in the diet of broiler chickens. Giambrone *et al*. (1978) indicated that AFB_1 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 AFB_1 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 AFB_1 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 B_2 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 T_2 and T_3 was lower ($P<0.05$) than that of control group (T_1). This result showed that dietary AFB_1 at 100 and 150 ppb levels significantly ($P<0.05$) decreased the HA titre as compared to that of T_1. The HA titre value in T_4 and T_5 was higher ($P<0.05$) than those of T_2 and T_3 and statistically similar to that of control. The HA titre value between groups T_4 and T_5 did not differ significantly. This result showed that AFB_1 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 AFB_1 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 AFB_1 at 300 ppb

Table 1: The percent liveability in Turkey poults as influenced by various dietary treatments

Treatment	Identification	I wk	II wk	III wk	IV wk	V wk	VI
T_1	Basal diet (Control)	100.00±0.00	100.00±0.00	97.50±2.50	95.00±2.88	95.00±2.88^{b}	95.00±2.88^{b}
T_2	T_1+100 ppb AFB_1	100.00±0.00	100.00±0.00	95.00±2.88	90.00±4.08	87.50±2.50^{ab}	87.50±2.50^{ab}
T_3	T_1+150 ppb AFB_1	100.00±0.00	100.00±0.00	90.00±4.08	87.50±2.50	85.00±2.88^{a}	85.00±2.88^{a}
T_4	T_2+Toxin binder	100.00±0.00	100.00±0.00	97.50±2.50	97.50±2.50	95.00±2.88^{b}	95.00±2.88^{b}
T_5	T_3+Toxin binder	100.00±0.00	100.00±0.00	97.50±2.50	95.00±2.88	95.00±2.88^{b}	95.00±2.88^{b}

Values bearing different superscripts in a column differ significantly (P<0.05)

Table 2: Cell mediated and humoral immunity; and haematological parameters of Turkey poults fed on various dietary treatments

Treatment	Identification	CMI (mm)	HA Titre Log_2 value	Haemoglobin (g/dl)	H/L Ratio
T_1	Basal diet (Control)	0.50±0.01^{b}	10.25±0.08^{c}	9.14±0.05^{b}	0.58±0.01^{a}
T_2	T_1+100 ppb AFB_1	0.33±0.01^{a}	7.05±0.03^{b}	6.39±0.14^{a}	1.06±0.02^{b}
T_3	T_1+150 ppb AFB_1	0.31±0.01^{a}	6.66±0.21^{a}	6.30±0.15^{a}	1.05±0.02^{b}
T_4	T_2+Toxin binder	0.51±0.01^{b}	10.28±0.09^{c}	9.32±0.05^{b}	0.58±0.01^{a}
T_5	T_3+Toxin binder	0.51±0.01^{b}	10.32±0.11^{c}	9.29±0.05^{b}	0.59±0.02^{a}

Values bearing different superscripts in a column differ significantly (P<0.05)

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 T_2 and T_3 was lower (P<0.05) than that of T_1. The Hb value in T_4 and T_5 was higher (P<0.05) than those of T_2 and T_3; and statistically similar to that of control. The Hb value in T_2 was statistically similar to that of T_3. The Hb value of T_4 was statistically similar to that of T_5. The results revealed that dietary AFB_1 at 100 and 150 ppb levels resulted in reduced (P<0.05) Hb concentration in Turkey poults. 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 AFB_1, respectively in broiler chickens. In the case of heterophil/lymphocyte (H/L) ratio, the H/L ratio of T_1 was lower (P<0.05) as compared to those of T_2 and T_3. The H/L ratio between groups T_2 and T_3 was statistically similar. The H/L ratio of groups T_4 and T_5 was lower (P<0.05) than those of T_2 and T_3 and statistically similar to that of control. The present study revealed that dietary AFB_1 at 100 and 150 ppb levels resulted in increased (P<0.05) H/L ratio in Turkey poults. Sharma (2013) also reported an increase (P<0.05) in the H/L ratio during 250 ppb AFB_1 in the feed of broiler chickens. Also, Basmacioglu *et al*. (2005) reported elevated H/L ratio in broilers due to feeding of 2 ppm AFB_1. This result was also in agreement with those of Huff *et al.* (1986); Oguz *et al*. (2003) wherein the suppressive effects of AFB_1 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 T_2 and T_3 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 poults. In groups T_2 and T_3, livers were enlarged, fatty and pale in appearance and the atrophy of bursa was also observed. The organs of T_4 and T_5 groups were normal in appearance as that of T_1 group. The present study revealed that 100 and 150 ppb of aflatoxin contamination in feed resulted in altered gross pathology of organs. Similar gross legions 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 B_2 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 T_1 was normal and this group served as a reference standard for comparison with other groups. In AFB_1 fed groups T_2 and T_3, 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 T_4 and T_5 the architectural and cellular organization of liver was normal as that of control.

4. Conclusion

It was concluded that dietary AFB_1 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 B_2 ameliorated the adverse effects of aflatoxicosis on liveability, immunity, haematological parameters; and gross and histopathology of internal organs in Turkey poults.

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