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LIVER LIPID PEROXIDATION AND LIVER ANTIOXIDANT PROFILE IN TURKEY POULTS (*Meliagridesgallopavo*) - BELTSVILLE SMALL WHITE VARIETY FED EXPERIMENTALLY WITH AFLATOXIN AND T-2 TOXIN

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Abstract: Experimental mycotoxicoses were induced singly and in combination in 48 newly hatched turkey poults (*Meleagridisgallopavo* - Beltsville small white) for a period of 0 to 13 weeks by feeding diets containing 100 ppb AF and 1 ppm T-2 toxin. The AF produced on rice and T-2 toxin on corn grits were estimated by TLC. Weighed amounts of powdered culture material were incorporated into the toxin free diet and were adjusted to 100 ppb AF and 1 ppm T-2 toxin. Liver tissue samples were collected from all control and toxin fed birds at the end of the 49th and 91st days of trial to estimate lipid peroxidation and antioxidants reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD). Feeding 100 ppb AF and 1 ppm T-2 toxin individually or in combination significantly increased TBARs levels. The study indicated that membrane damage occurred in the intoxicated hepatocytes. Significantly decreased GSH levels observed in turkey poults could be due to increase in lipid peroxidation while significantly decreased GPx, SOD and CAT except T-2 group while increased GST levels were observed in mycotoxin treated groups. The probable cause for the decrease in GPx might be attributed to the increase in oxidative stress induced by AF resulting in the formation of large quantities of hydrogen peroxide and the rapid depletion of GPx in combating oxidative stress. Despite the fact that in the present study we used sublethal doses of AF (100 ppb), the decrease in GPx would have caused more severe impairment of the glutathione redox system. These findings showed a reduction in antioxidants in the liver that could further aggravate lipid peroxidation. The induction of GST activity is a secondary response.

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INTRODUCTION

Mycotoxins are those secondary metabolites of fungi that have the capacity to impair animal health and productivity (D'Mello and Macdonald, 1998). The diverse effects precipitated by these compounds are conventionally considered under the generic term "mycotoxicosis"and include distinct syndromes as well as non-specific conditions.Aflatoxin (AF) and T-2 toxin are the most frequently encountered mycotoxins. AF is a potent hepatotoxin with dihydrofuran-coumarin moiety and is of importance in producing the biological effects and is produced by *Aspergillus flavus* and *A. parasiticus*. T-2 toxin is a 3 hydroxy 4, 15 diacetoxy 8 (3-methylbutryloxy), 12, 13 epoxy trichothec-9-ene metabolite. It is a potent irritant, inflammatory (dermotoxic, alimentary toxic, hepatotoxic and growth inhibitory agent) and radiomimetic agent produced by Fusarium species. The AF, by binding to both RNA and DNA blocks transcription whereas, T-2 toxin blocks initiation of translation. The studies on aflatoxicosis in turkey poults were limited owing to the potential sensitivity of the species, the same on T-2 was scant and there were none on AF-T-2 combined toxicity for a period of 91days. Hence, the present study was conducted.

MATERIALS AND METHODS

AF was produced on rice (Shotwell *et al*., 1966) by using *A. Parasiticus* NRRL 2999 strain. The T-2 toxin was produced on corn grits (Burmeister, 1971) by using *F. sporotrichoides* MTCC 1894 strain (Microbial Type Culture Collection, Chandigarh, India). The mycotoxin content in cultured material was analysed at Pharmacovigilance Laboratory for Animal Feed and Food Safety (PLAFFS), Centre for Animal Health Studies, TANUVAS, Madhavaram Milk Colony, Chennai, Tamil Nadu, India. Known amounts of AF and T-2 toxin containing powdered substrates were incorporated into turkey brooder mash both singly and in combination to yield 100 ppb AF and 1 ppm T-2 toxin. Forty-eight newly hatched unsexed turkey poults obtained from standard hatcheries were wing banded, weighed and housed in battery brooders with ad libidum supply of feed and water. They were randomly distributed into four groups of twelve chicks each.

A biological trial was conducted with a total number of 48 newly hatched turkey poults. The turkey poults were wing banded, weighed and housed in battery brooders with ad-libitum supply of feed and water. They were randomly allotted to four groups namely group I control, group II aflatoxin, group III T-2 toxin and group IV combined AF-T-2 of 12 birds each. The control and toxin mixed diets were fed to different groups for 91 days from the day of hatch. A detailed post mortem was conducted on each sacrificed bird at the end of experimental

period. Liver tissue samples were collected from all control and toxin fed birds at the end of the 49th and 91st days of trial and stored at -20°C till the assays were carried out. Non-enzymatic antioxidant, reduced glutathione (GSH) was estimated by the method of Moron *et al*. (1979).Enzymatic antioxidants such as Glutathione peroxidase (GPx) was estimated by the method of Rotruck*et al*. (1973), glutathione-S-transferase (GST) by the method of Habig*et al*. (1974), catalase (CAT) by the method of Caliborne (1985) and superoxide dismutase (SOD) by the method of Marklund and Marklund (1974). The data generated from the experimental study were subjected to statistical analysis, as per Snedecor and Cochran (1989). The results of the study were subjected to two-way analysis of variance (ANOVA).

RESULTS

LIVER LIPID PEROXIDATION

The mean (± SE) liver TBARs levels in turkey poults fed AF and T-2 toxin singly and in combination are presented in Table 1. Comparison of overall means revealed significant (P<0.05) differences between the control and mycotoxin treated groups. The difference was significant (P<0.05) among the toxin fed groups. There was a significant (P<0.05) increase in liver lipid peroxidation in the toxin treated groups when compared to the control group.

LIVER ANTIOXIDANT PROFILE

Reduced glutathione

Mean (± SE) liver GSH levels in turkey poults fed AF and T-2 toxin singly and in combination are presented in Table 2. Comparison of overall means revealed significant (P<0.05) differences between the control and mycotoxin treated groups. No significant differences were observed among the toxin fed groups. The GSH levels significantly (P<0.05) decreased in the toxin treated groups when compared to the control.

Enzymatic antioxidants

Mean (± SE) liver GPx, SOD, CAT and GST levels in turkey poults fed AF and T-2 toxin singly and in combination are presented in Tables 3-6 respectively. Comparison of means revealed significant (P<0.05) differences between the control and mycotoxin treated groups. The mean liver GPx, SOD and CAT levels except T-2 group significantly (P<0.05) decreased, while GST level significantly (P<0.05) increased in turkey poults fed AF and T-2 toxin singly and in combination. Among the toxin fed groups GPx and GST showed significant (P<0.05) differences in all, SOD in AF-T-2 group and catalase in the AF and AF - T-2 group.

DISCUSSION

LIVER LIPID PEROXIDATION

Feeding 100 ppb AF and 1 ppm T-2 toxin individually or in combination for 13 weeks significantly increased TBARs levels in mycotoxin treated groups when compared to control. In AF fed birds significantly increased TBARs levels were observed between control and mycotoxin treated groups. Eraslan *et al.* (2004) found a significant increase in LPO level in the liver tissues in broiler chicken exposed to 5.0 g/kg with 1 ppm of AF for 45 days. Ajith Jacob George (2007) reported increased liver lipid peroxidation in broiler chicken fed 50, 150 and 300 ppb AF from 0 to 42 days of age. The results for T-2 toxicosis are in accordance with Babu Prasath (2008) who reported a significant increase in liver lipid peroxidation in 3 ppm T-2 toxin fed birds. Similar increase in liver lipid peroxidation was reported in experimental T-2 toxicoses in ducks (Mezes *et al.,* 1998), broiler chicks (Narayanaswamy, 1998; Leal *et al.,* 1999) and in Japanese quails (Arun Prasath, 2006).

Elevation of hepatic lipid peroxidation in the toxin treated groups could be correlated with histological changes observed in toxin treated groups.The study indicated that membrane damage occurred in the intoxicated hepatocytes. Liver lipid peroxidation is one of the responsible factors for the damage and necrosis of liver, induced by chemical compounds like T-2 toxins. Because biological membranes are rich in unsaturated fatty acids, the susceptibility of membranes to peroxidative attack was not rare (Leal *et al*., 1999).

LIVER ANTIOXIDANT PROFILE

Reduced glutathione

Feeding 100 ppb AF and I ppm T-2 toxin and their combination for 13 weeks significantly decreased GSH levels in turkey poults. The findings of elevated GSH level for T-2 toxicosis are in accordance with BabuPrasath (2008) who found significant reduction in GSH by feeding diets containing 1 and 3 ppm T-2 toxin from 0 to 28 days of age to turkey poults. However, Elisângela Aparecida Guaiume (2005) reported no significant changes in the values of GSH in turkey poultsfed diets containing 2 mg T-2/kg diet singly or in combination with 0.15 mg/kg AFB1 from first week onwards in a 21-day trial. The reduction in the GSH levels in all toxin treated groups could be due to increase in lipid peroxidation.

Enzymatic antioxidants

Feeding 100 ppb AF and 1 ppm T-2 toxin individually or in combination for 13 weeks significantly decreased GPx, SOD and CAT except T-2 group while GST levels increased in mycotoxin treated groups in turkey poults. Similar observations were made by Ajith Jacob

George (2007) who reported that feeding 50, 150 and 300 ppb AF in broiler chicken from 0 to 42 days of age decreased GPx, SOD and CAT and increased GST in AF fed groups. A decrease in GPx level was reported by Eraslan *et al*. (2004) in broiler chicken fed 1 ppm AF for 45 days. Eraslan *et al.* (2005) by conducting trial with 0.05, 0.1, 0.5 and 1 ppm of AF concluded that GPx is one of the important antioxidants in the assessment of the severity of aflatoxicosis in poultry naturally intoxicated with AF. The probable cause for the decrease in GPx might be attributed to the increase in oxidative stress induced by AF resulting in the formation of large quantities of hydrogen peroxide and the rapid depletion of GPx in combating oxidative stress. Despite the fact that in the present study we used sublethal doses of AF (100 ppb) the decrease in GPx would have caused more severe impairment of the glutathione redox system (Mezes *et al*., 1998).

Babu Prasath (2008) also reported that feeding diets containing 1 and 3 ppm T-2 toxin from 0 to 28 days of age to turkey poults revealed a significant reduction in GPx and SOD and increased GST. Though no more comparable studies were available in antioxidant profile in turkey poults fed T-2 toxin, similar findings were reported in chicken, ducks, geese and Japanese quails (Mezes *et al.,* 1998; Leal *et al.,* 1999; Arun Prasath, 2006). These findings showed a reduction in antioxidants in the liver that could further aggravate lipid peroxidation. The induction of GST activity is a secondary response. The T-2 toxin caused elevated GST in broiler chicks fed 1.5 mg T-2 toxin/kg body weight/day from 7 to 21 days (Leal *et al.,* 1999). No comparable literature could be cited for the combined toxicity.

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TABLE 1
Mean (± SE) liver TBARs levels (mmol/g tissue) of turkey poults fed aflatoxin and T-2 toxin singly and in combination

(n=6)

Groups	TBARs levels (mmol/g tissue)		Overall means
	7th week	13th week	
Control	433.52±10.53	441.40±9.91	437.45^{d}±6.99
Aflatoxin (100 ppb)	473.80±6.07	515.40±8.66	494.60^{c}±8.05
T-2 toxin (1 ppm)	528.60±5.09	579.70±6.93	554.15^{b}±8.73
Aflatoxin (100 ppb) + T-2 toxin (1 ppm)	591.80±6.96	648.70±8.18	620.25^{a}±9.98

Overall means bearing same superscripts within a column do not differ from each other (P>0.05)

TABLE 2
Mean (± SE) liver GSH levels (mg/g of tissue) of turkey poults fed aflatoxin and T-2 toxin singly and in combination

(n=6)

Groups	GSH levels (mg/g of tissue)		Overall means
	7th week	13th week	
Control	379.00±8.13	387.60±9.04	383.25[a]±5.94
Aflatoxin (100 ppb)	253.70±5.77	308.90±7.83	281.25[b]±9.52
T-2 toxin (1 ppm)	264.70±6.96	289.60±6.88	277.15 [b]±5.99
Aflatoxin (100 ppb) + T-2 toxin (1 ppm)	295.60±5.75	273.90±8.61	284.70[b]±5.92

Overall means bearing same superscripts within a column do not differ from each other (P>0.05)

TABLE 3
Mean (± SE) liver GPx levels of turkey poults fed aflatoxin and T-2 toxin singly and in combination

(n=6)

Groups	GPx levels		Overall means
	7th week	13th week	
Control	805.00±5.84	822.20±7.56	813.63[a] ±5.24
Aflatoxin (100 ppb)	784.60±6.33	793.50±8.49	789.03[b]±5.22
T-2 toxin (1 ppm)	749.90±4.63	736.80±5.08	743.35[c]±3.82
Aflatoxin (100 ppb) + T-2 toxin (1 ppm)	707.90±6.00	687.50±8.49	697.68[d]±5.83

Overall means bearing same superscripts within a column do not differ from each other (P>0.05)

GPx expressed as µm of glutathione utilized/min/mg protein

TABLE 4
Mean (± SE) liver SOD levels of turkey poults fed aflatoxin and T-2 toxin singly and in combination

(n=6)

Groups	SOD levels		Overall means
	7th week	13th week	
Control	1.28±0.02	1.33±0.03	1.31^{a}±0.02
Aflatoxin (100 ppb)	1.06±0.04	1.21±0.05	1.13^{b}±0.04
T-2 toxin (1 ppm)	1.06±0.06	1.24±0.04	1.15^{b}±0.04
Aflatoxin (100 ppb) + T-2 toxin (1 ppm)	0.94±0.05	1.09±0.04	1.01^{c}±0.04

Overall means bearing same superscripts within a column do not differ from each other (P>0.05)
Enzyme required for inhibiting 50% pyrogallol auto oxidation/min/mg protein

TABLE 5
Mean (± SE) liver catalase levels of turkey poults fed aflatoxin and T-2 toxin singly and in combination

Groups	Catalase levels		Overall means
	7th week	13th week	
Control	1.04±0.06	1.21±0.87	1.13^{a}±0.04
Aflatoxin (100 ppb)	0.90±0.04	1.01±0.73	0.95^{b}±0.05
T-2 toxin (1 ppm)	0.97±0.03	1.09±0.78	1.02^{ab}±0.03
Aflatoxin (100 ppb) + T-2 toxin (1 ppm)	0.77±0.02	0.83±0.60	0.81^{c}±0.02

Overall means bearing same superscripts within a column do not differ from each other (P>0.05) Catalase required for decomposing µm of H_20_2/min/mg protein

TABLE 6
Mean (± SE) liver GST levels of turkey poults fed aflatoxin and T-2 toxin singly and in combination

(n=6)

Groups	GST levels		Overall means
	7th week	13th week	
Control	1.26±0.91	1.37±0.04	1.32^{d}±0.03
Aflatoxin (100 ppb)	2.53±1.82	2.73±0.05	2.63^{c} ±0.06
T-2 toxin (1 ppm)	2.70±1.94	3.10±0.07	2.89^{b}±0.07
Aflatoxin (100 ppb) + T-2 toxin (1 ppm)	3.16±2.28	3.44±0.09	3.29^{a}±0.07

Overall means bearing same superscripts within a column do not differ from each other (P>0.05). GST levels expressed as µm CDNB-GSH conjugate formed/min/mg protein