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Effects of Clinoptilolite on Performance, Biochemical Parameters and Hepatic Lesions in Broiler Chickens During Aflatoxosis

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Abstract: This study was conducted to determine performance and biochemical and histopathological changes in Chicks that Clinoptilolite (CLI) was added to diets containing Aflatoxins (AF) from 1-42 days of age. A total of 480, One-day-old broiler chicks (Ross 308) was divided into 6 treatments: control; basal diet prepared with uncontaminated diet, control containing AF (0.5 mg kg^{-1}), control containing AF (1 mg kg^{-1}), control containing CLI (20 g kg^{-1}), control containing AF (0.5 mg kg^{-1}) + CLI (20 g kg^{-1}) and control containing AF (1 mg kg^{-1}) + CLI (20 g kg^{-1})) each consisting of 80 chicks. Chickens were monitored daily and then body weight and feed consumption were recorded. Plasma enzymatic and non-enzymatic parameters showed progressive changes in birds examined 21 or 42 days after dietary aflatoxin treatments. Compared to controls, the AF treatment significantly ($p<0.05$) decreased body weight gain, feed intake, serum total protein, albumin, cholesterol, uric acid, calcium, phosphorus, alkaline phosphatase (ALP) and from enzymatic parameters Lactate Dehydrogenase (LDH) and aspartate amino transferase (AST) increased. Decreases in serum parameters caused by AF was significantly ($p<0.05$) ameliorated by CLI. A similar increase was obtained in feed intake and body weight gain by adding CLI to the AF-containing diet ($p<0.05$). Histopathologic examination revealed severe fatty change, regeneration foci of liver cells, fibrosis of portal regions and bile ductule hyperplasia. The addition of CLI to the AF-containing diet decreased the severity of lesions (hepatic lipidosis, regeneration foci and bile-duct hyperplasia) in the livers. Prolonged exposure to low concentration of the toxin produce severe changes in fat metabolism and bile ductule proliferation. These results suggest that CLI (20 g kg^{-1}) was effective for the protection of AF-toxication in broiler chicks and addition of CLI effectively diminished the detrimental effects of AF on traits investigated.

Key words: Aflatoxin, clinoptilolite, biochemical, hepatic, broiler

INTRODUCTION

Mycotoxins are a group of extremely toxic and biologically active substances. Among them are the aflatoxins (AF: AFB_1, AFB_2, AFG_1, AFG_2) which are produced by the fungi *Aspergillus flavus* and *A. Parasiticus* (Wilson and Payne, 1994). *Aspergillus Flavus* is the most common contaminant of many grains used in human and animal nutrition (Abarca *et al*., 1994). Aflatoxins have been detected in the pre-harvest, post-harvest, transport, storage and after processing and packing of grains (Council for Agricultural Science and Technology, 1989). Animals that consume high levels of these toxins may develop various health problems, depending on their susceptibility. Aflatoxicosis in poultry is characterized by weakness, anorexia with lower growth rate, poor utilization, decreased weight gain, decreased egg production, increased susceptibility to environmental and microbial stresses and increased mortality (Bailey *et al*., 1998; Kubena *et al*., 1998; Oguz and Kurtoglu, 2000).

Aflatoxicosis is also associated with biochemical, haematological and pathological changes. Determination of biochemical toxic effects of AF is important for diagnosis of toxicosis in broilers (Rosa *et al*., 2001). AF toxicity in broilers may be manifested by decreased serum concentrations of total protein, albumin, total cholesterol (Kubena *et al*., 1998, Oguz *et al*., 2000a), uric acid (Kececi *et al*., 1998) and increased hepatic enzyme activities such as AST and ALT (Amer *et al*., 1998; Santurio *et al*., 1999). Liver is the target organ of aflatoxins and hepatobiliary damages are associated with alterations in liver function enzymes. The AF is a hepatotoxin, causing an excessive build-up of hepatic lipids, with enlargement of the liver, proliferation of biliar ducts (Adav and Godinwar, 1997) and hepatocelular carcinoma (Hamilton, 1978).

A variety of physical, chemical and biological approaches are used to counteract the aflatoxin problem have been reported in the literature on mycotoxins (Doyle *et al*., 1982; Samarajeewa *et al*., 1990; Ramos and Hernandez, 1991), but Large-scale, practical and cost-effective methods for detoxifying aflatoxin-containing foodstuffs are not currently available. One of the most recent approaches to the problem has been the use of nutritionally inert sorbents in the diet that sequester aflatoxins and reduce the absorption of these mycotoxins from gastrointestinal tract, avoiding toxic effects for livestock and the carry-over of the toxins to animal products. The natural and synthetic zeolites (Kececi *et al*., 1998; Oguz *et al*., 2000b), bentonites (Rosa *et al*., 2001; Santurio *et al*., 1999) and clinoptilolite (Ortatli and Oguz, 2001) were preferred because of their high binding capacities against AF and their reducing effect on AF absorption from the gastrointestinal tract. Zeolites are a chemically complex and diverse family of aluminosilicates with a variety of functional properties that are generally inert and nontoxic to animals. The impact of aflatoxins in feed on intensive animal production has been illustrated by studies in the poultry (Dalvi, 1986).

The objective of the present study was to evaluate the efficacy of commercial available zeolitic mineral ores (clinoptilolite (CLI), a proprietary product of Afrand Toska, an Iranian company) for protection against aflatoxin in broiler chickens by observing its effects on various serum biochemical components and histopathological changes and to determine the possible preventive role of dietary CLI on the production parameters in broilers.

MATERIALS AND METHODS

Aflatoxin production: High levels of aflatoxins were produced on rice as a natural substrate by toxigenic A. parasiticus (isolate #14) isolated from Pistachio nuts in our laboratory (Allameh *et al*., 2001). One-liter capacity flasks, each containing 150 g of rice, were inoculated with fungal spores (6.5×10^6-7×10^6) and then incubated at 28°C for 5 days. Further processing was done as per the procedure described by Shotwell *et al*. (1966). Successfully fermented rice was then steam heated to kill the fungi, the rice was then dried and ground to a fine powder. The AF content in rice powder was extracted and measured using High Performance Liquid Chromatography (HPLC) based on the procedure described by Wilson and Romer (1991), using Mycosep multifunctional cleanup column. The AF within the rice powder consisted of 81.7% AFB_1, 8.3% AFB_2, 9.9% AFG_1, 0.2% AFG_2 based on total AF in the rice powder (detection limit: 1 μg AF kg^{-1} rice powder). The powder was incorporated into the basal diet to provide the desired level of 0.5 and 1mg AF kg^{-1}.

Birds and diets: Four hundred and eighty one-day-old male commercial broilers (Ross 308), obtained from a commercial hatchery, were used in this study. Each group was housed separately in an open-sided deep litter pen house and reared under uniform managemental condition. The dimensions of each pen were 1.5×1.2 m. Food and water were allways available and lighting was continuous. Chickens were allowed free access to feed and tap water until they were 6 weeks old. These 480 chicks were divided into 24 pen (each group containing 20 chicks). The experimental design was a 2×3 factorial arrangement of treatments, consisting of 2 levels of clinoptilolite (0 and 20 g kg^{-1}) and three levels of aflatoxin (0, 0.5 and 1 mg AF kg^{-1}) in a completely randomized design. The composition of basal diet consisted of: a commercial starter diet (corn and soybean based, 210 g protein, 12.22 MJ kcal ME kg^{-1}) from 1-21 days and a grower diet (186 g protein, 12.47 MJ ME kg^{-1}) from 21-42 days. The starter and grower basal diets were both supplemented with amino acids, minerals and vitamins at levels recommended by the NRC (1994). The basal diet was tested for possible residual AF before feeding (Howel and Taylor, 1981) and there were no detectable levels present (detection limit 1 μg AF kg^{-1} food). Clinoptilolite, which is a member of natural zeolite, was provided from west region of Iran and its chemical formula is SiO_2 (66.1%), Al_2O_3 (11.5%), Fe_2O_3 (1.3%), CaO (3.1%), MgO (0.8%), TiO_2 (0.3%), P_2O_5 (0.1%), MnO (0.4%), SO_3 (0.1%), Na_2O (2.1%), K_2O (2.2%), Loss of ignition (L.O.I. 12.1% containing H_2O and CO_2). There were 6 experimental diets:

A : Basal diet prepared with uncontaminated diet (control).
B : Basal diet containing Aflatoxin (0.5 mg AF kg^{-1}).
C : Basal diet containing Aflatoxin (1mg AF kg^{-1}).
D : Basal diet containing clinoptilolite (20 g kg^{-1}).
E : Basal diet containing Aflatoxin (0.5 mg AF kg^{-1}) + clinoptilolite (20 g kg^{-1}).
F : Basal diet containing Aflatoxin (1mg AF kg^{-1}) + clinoptilolite (20 g kg^{-1}).

Data collection

Dietary intake and body weight gain: Birds were inspected daily and their body weight and feed consumption was recorded weekly, respectively. Feed intake, mean body weight gain and feed conversion ratio (feed intake/gain) were calculated.

Serum biochemistry: Blood collection and other experiments were carried out in the experimental chickens at two time intervals. One set (n = 10) was sacrificed at the age of 21 days and other set was killed when they become 42 days of age. Blood samples were collected from the wing vein of all the birds at the age of 21 day (n = 240) and 42 day (n = 240), serum were separated and used for biochemical assays. Serum albumin, total proteins, triglyceride, cholesterol, uric acid, phosphor, calcium and enzymes, namely Lactate Dehydrogenase (LDH), Aspartate Amino Transferase (AST) and Alkaline Phosphatase (ALP) were measured using commercial kits on an auto-analyzer (Technicon RA-1000).

Histopathological examination: Three chickens from each pen randomly selected and sacrificed at the age of 21 and 42 days, each liver was weighed and processed for histological examinations. The weight of liver were adjusted to g 100 g^{-1} live weight and means were calculated. Tissue samples collected and fixed in neutral buffered 10% formalin, embedded in paraffin, sectioned at 5 μm and stained by haematoxylin and eosin (H and E).

Statistical analysis: Data were analyzed by the General Linear models procedure of SAS Institute (1994). Means for treatments showing significant differences in the analysis of variance were compared using Duncan's multiple range tests. All statements of significance are based on the probability level of 0.05.

RESULTS

Dietary intake and body weight gain: As shown in Table 1, chickens fed on diet prepared with adding clinoptilolite (groups E and F), were normal with respect to dietary intake, body weight gain and Feed Conversion Ratio (FCR). In contrast, in chickens fed AF-containing diet (groups B and C) there was a significant reduction ($p<0.05$) in feed intake concomitant with 20.7-27.3% reduction in body weight gain particularly during the second phase of rearing (42 days) and increased FCR as compared to control. The groups E and F showed relatively better feed conversion ratio. The group C exhibited lowest weight gain and growth rate.

Biochemical changes: The results of biochemical parameters presented in Table 2 show that the level of blood cholesterol significantly reduced at the age of 21 days and the decrease was intensified in birds after 42 days (17.2-24.7%; $p<0.05$) in groups B and C due to dietary aflatoxin. In chicken given CLI treatment containing aflatoxin, the level of cholesterol was within the normal range. There was no significant change in serum Triacylglycerol (TG) levels irrespective of different doses. As shown in Table 2, plasma total protein together with albumin levels was found to be significantly ($p<0.05$) inhibited in chickens fed aflatoxin-containing diet. As shown in Table 4, the serum uric acid level was found to be decreased in only chickens under high dose (group C)

Table 1: Production parameters and liver weight (g Kg^{-1}) of chicks fed different rations

	Experimental				
	Body weight gain (g day^{-1})	Dietary intake (g day^{-1})	Feed conversion ratio	Liver (g 100 g^{-1})	
Group	Day 42	Day 42	Day 42	Day 21	Day 42
Control	38.1±0.7^{a}	74.4±2.2^{a}	1.95±0.15^{a}	5.7±0.57^{a}	3.3±0.32^{a}
A*	30.2±2.2^{b}	69.2±3.2^{b}	2.22±0.08^{b}	7.12±0.35^{b}	5.3±0.3^{b}
AA**	27.3±1.02^{b}	64.1±3.1^{c}	2.35±0.09^{b}	7.45±0.46^{b}	5.9±0.33^{b}
CLI ***	39.5±1.2^{a}	78.8±3.7^{a}	1.99±0.15^{a}	5.9±0.45^{a}	3.35±0.4^{a}
A+CLI	35.5±1.3^{a}	71.8±2.0^{a}	2.05±0.56^{a}	6.3±0.3^{a}	3.5±0.3^{a}
AA+CLI	34.16±1.0^{ab}	71.2±1.4^{ab}	2.1±0.05^{ab}	6.87±0.6^{ab}	3.84±0.26^{a}

Feeding started from the first day of housing and parameters calculated on days 21 and 42 days of age. Data presented as mean±S.D. Mean values in same column without common letters (a-c) differ significantly ($p<0.05$). * A = AF (0.5 mg kg^{-1}), **AA = AF (1 mg kg^{-1}), **CLI = 20 g kg^{-1}

Table 2: Comparison of serum biochemical parameters in chicks fed different rations

	Experimental						
	Phosphorous (mg L^{-1})		Calcium (mg L^{-1})		Uric acid (mg L^{-1})		Bone ash (%)
Group	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42	Day 42
A	55±2.6^{a}	65±3.2^{a}	84±7.2^{a}	96±5.9^{a}	83±10^{a}	77±7.4^{a}	37.5±0.6^{a}
B	46±3.8^{b}	52±2.5^{bc}	61±6.0^{b}	82±4.8^{b}	67±9.0^{b}	68±8.2^{ab}	34.2±1.2^{ab}
C	43±3.5^{b}	44±3^{c}	54±7.1^{b}	73±4.2^{c}	54±10^{b}	59±6.9^{b}	32.2±1^{b}
D	53±4.2^{a}	63±2.6^{ab}	82±6.8^{a}	94±5.8^{a}	79±11^{a}	75±7.8^{a}	37.8±0.8^{a}
E	52±5.3^{a}	61±4.2^{b}	79±5.9^{a}	93±6.2^{a}	77±12^{a}	75±9.3^{a}	37±0.5^{a}
F	50±5.4^{ab}	58±3.5^{b}	73±6.1^{ab}	89±5.4^{ab}	72±8.5^{ab}	71±8.0^{ab}	36.8±1.1^{a}

Experimental groups are as shown in Table 1. Data are mean±S.E.M. of 10 analyses carried out on samples obtained from 10 individual birds. Chickens were examined at two stages of growth when they were either 21 or 42 days old. Means in the same column without common letters (a-c) differ significantly ($p<0.05$)

Table 3: Serum levels of phosphorus, calcium, uric acid and bone ash in chickens fed different dietary rations

	Experimental					
	LDH (U L^{-1})		AST (U L^{-1})		ALP (U L^{-1})	
Group	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42
A	220.5±6.50^a	213.5±9.00^a	285±7.5^a	310.5±10.5^a	4750±225^a	4850±350^a
B	267±10.5^b	264±15.0^b	325±13^b	395.5±14.0^b	3950±370^b	3650±350^b
C	278±11.5^b	283±12.0^b	348±8.5^b	425.5±12.0^b	3600±300^b	3360±225^b
D	228±8.80^a	220±12.5^a	278±6.5^a	300.5±3.50^a	4820±320^a	4600±280^a
E	230±7.50^a	233±7.50^a	295.5±9.5^a	335±16.0^a	4450±260^a	4400±150^a
F	242±12.0^a	239±14.5^a	307±2.5^{ab}	347±2.50^{ab}	4300±370^a	4370±175^a

Experimental groups are as shown in Table 1. Results are mean±S.E.M. of 10 analyses obtained from 10 individual chickens. Means in the same column without common letters (a-c) differ significantly ($p<0.05$). Sample collected from each experimental group either at the age of 21 or 42 days after treatment

Table 4: Serum enzymes in chickens fed different dietary rations

	Experimental							
	Triglyceride (g L^{-1})		Cholesterol (g L^{-1})		Total Protein (g dL^{-1})		Albumin (g dL^{-1})	
Group	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42
A	89±7.0	84±6.0	148.5±15.6^a	127.5±8.5^a	2.48±0.26^a	3.78±0.3^a	0.72±0.05^a	1.39±0.2^a
B	78±5.0	67±6.0	112.5±13.2^b	105.5±8.0^b	1.75±0.27^b	2.37±0.27^b	0.53±0.07^b	0.95±0.1^b
C	76±6.2	63±8.0	107.5±12.8^b	96.0±5.4^a	1.62±0.19^b	2.25±0.21^b	0.42±0.08^b	0.86±0.12^b
D	86±5.5	85±7.0	144±14.0^a	125.5±7.2^a	2.43±0.12^a	3.7±0.12^a	0.75±0.08^a	1.42±0.18^a
E	83±6.0	71±3.5	138.5±14.5^a	118.5±11^a	2.12±0.11^a	3.58±0.25^a	0.67±0.09^a	1.27±0.14^a
F	81±4.5	68±5.0	136.0±11.5^a	114±8.0^{ab}	1.98±0.22^{ab}	3.5±0.25^{ab}	0.63±0.06^{ab}	1.23±0.22^{ab}

Experimental groups are as shown in Table 1. Data are mean±S.E.M. of separate analyses carried out on 10 samples obtained from individual chickens. Enzymes measured at two stages of growth, viz. 21 and 42 days after treatment. U, one unit is equivalent to 1µmole of lactate, oxaloacetate or inorganic phosphate released per minute at 25°C for LDH, AST and ALP, respectively

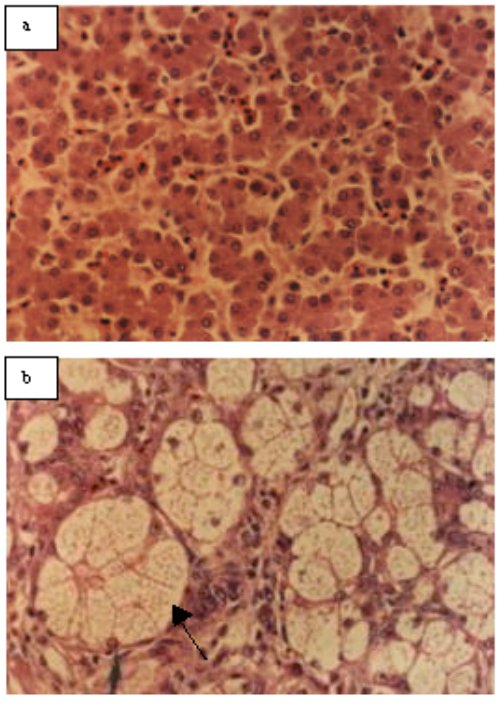

Fig. 1: Histopathology of liver. (A) Liver section (×400) of normal chicken (group A) stained by H and E. (B) Section (×200) shows hepatic tissue of chickens given dietary aflatoxin at a concentration of 1000 ppb

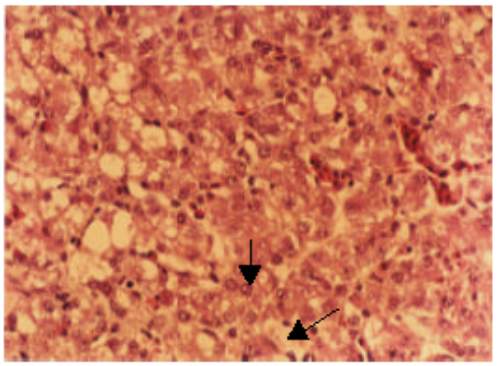

Fig. 2: Liver from 1000 ppb AF -treated plus CLI group. slight granular degeneration (arrowes) and fatty change H and E×400

of dietary aflatoxin. It was observed that in chickens of groups B and C given dietary aflatoxins, there was a significant depletion ($p<0.05$) in serum phosphorus and serum calcium. These changes were more significant in chickens fed diet with higher aflatoxins levels (diet C). The weight of bone ash was reduced significantly (~10-12%, $p<0.05$) in chickens maintained for 42 days on aflatoxin contaminated feed (Table 4).

Serum lactate dehydrogenase as well as aspartate amino transferase activities were found to be elevated ($p<0.05$) in chickens fed aflatoxin contaminated feed (Table 3). These changes were dependent on aflatoxin

concentration in diet and the duration of exposure. Nevertheless, serum alkaline phosphatase was found to be inhibited in aflatoxin treated chickens. The addition of CLI on contaminated diet, resulted in compensation of toxic effects of aflatoxin on these enzymes.

Histopathological observation: The macroscopic appearance of livers from chickens fed aflatoxin contaminated feed showed a gross appearance with hypertrophy, friable and yellowish coloration. The livers from other treatments had a normal appearance (Fig. 1, A). Relative weights (g 100 g^{-1} BW) of the livers in intoxicated chicks significantly increased as compared with the control ($p<0.05$), which was compensated in birds fed contaminated diet plus CLI. Histopathological examination revealed accumulation of large fat droplets that displaced the nucleus in chicks given dietary aflatoxin (Fig. 1, B). Nevertheless, fatty liver was not the endpoint of toxicity, since higher dietary aflatoxins caused fibrosis of portal regions and bile ductile hyperplasia, necrosis of hepatocytes, cellular atypia of hepatocytes. As shown in Fig. 2 (B), the lesions were very severe in 42-day-old chickens and had comparatively lesser severity in 21-day-old chickens. Chickens fed ration containing aflatoxin plus CLI showed no sign of liver injury as judged by light microscopic observations. Nevertheless at higher dose AF (group F) observed a slight fatty change and granular degeneration (Fig. 2).

DISCUSSION

Aflatoxins (AF) are important to the poultry industry because of their frequent occurrence in feedstuffs which produces severe economic losses and health problems in poultry (Santurio *et al*., 1999). Diagnosis is rather difficult and medical treatment may be almost impossible. Unfortunately, discontinuing the feeding of aflatoxin contaminated grain is not allways practical, especially when alternative feedstuffs are not readily available or affordable. Thus, these toxins frequently are detected in animal feed (Allameh *et al*., 2005). Various classes of sorbent materials (aluminosilicates, bentonite, silicas, zeolite, etc) have been evaluated for their ability to remove or diminish the adverse effects of mycotoxins in animal feed. One compound in particular, clinoptilolite (natural zeolite) was found to have an affinity for aflatoxin (Parlat *et al*., 1999). The major advantages of these absorbents include expense, safety and easy administration through addition to animal foods (Ledoux *et al*., 1999). Shane (1994) has reviewed the relationship between commercial performance of broilers and aflatoxin contamination of diets. Dersjant-Li *et al*. (2003) reported that the rate of body weight gain in pigs and broilers is reduced depending on the levels of aflatoxin in the diet. They suggested that with each mg kg^{-1} increase of aflatoxin in broiler diet would depress the growth rate by 5%.

The broilers consumed AF (0.5 and 1 mg kg^{-1} diet) containing diet showed a significant poor body weight ($p<0.05$); 30.2 and 27.3 g day^{-1} versus 38.1 g day^{-1} and also AF caused significant suppression on food intake (FI) ($p<0.05$). The FCR values were negatively affected by AF ($p<0.05$); 2.22 and 2.35 versus 1.95, respectively. These detrimental effects of AF on FI, BWG and FCR are due to anorexia, listlessness and the inhibitory of AF on protein synthesis and lipogenesis (Oguz and Kurtoglu, 2000c; Kiran *et al*., 1998). Impaired liver functions and protein/lipid utilization mechanisms may have also affected the growth performance and general health (Kececi *et al*., 1998). The results of this study agree with the other reports of AF studies performed in broiler chickens (Kubena *et al*., 1991; Stewart *et al*., 1998; Allameh *et al*., 2005).

The most important effect of aflatoxicosis is poor body weight gain since this directly affects profit in the poultry industry. In this study, CLI (20 g kg^{-1}) addition to the AF-containing diet significantly reduced the adverse effect of AF on FI and BWG in broiler chickens ($p<0.05$). These improvements agree with previous results on the protective effects of CLI against an AF-containing diet in broiler chicks ($p<0.05$) (Oguz and Kurtoglu, 2000c). And those showing that adding natural zeolites such as phillosilicate (Kubena *et al*., 1998; Ledoux *et al*., 1999; Abo-Norag *et al*., 1995), perlite and zeobrite (Scheideler, 1993) clinoptilolite (Parlat *et al*., 1999) significantly diminished the toxicity of AF to broiler chicks ($p<0.05$). Harvey *et al*. (1993) found no beneficial effect on broiler chicks of adding CLI (5 g kg^{-1}) to an AF-containing diet (3.5 mg kg^{-1}) for 3 weeks. The reason for these conflicting results may be attributable to differences in type or physical characteristics of CLI, concentration of AF in the diet or broiler strains. In this study, the toxic effects of AF on the biochemical and pathological base and the ameliorative efficacy of dietary adsorbent (CLI) on the detrimental effects of AF, which can lead to improvement in production rates, were investigated. This was achieved by comparing different parameters related to aflatoxicosis as well as the production efficiency of broilers. Data presented in this study show that most of the plasma parameters were found to be affected by aflatoxin but remained within normal range in chickens given CLI. Unlike the non-enzymatic parameters, marker enzymes particularly lactate dehydrogenase and aspartate amino transferase showed more dependency on

the concentration and duration of the exposure to aflatoxins. These data suggest that serum enzymes particularly AST and LDH can be used as indices for investigating the performance of chickens challenged with aflatoxins. Decreased serum ALP occurred because of aflatoxin intoxication, whereas it was unaffected in chickens fed diet containing CLI plus AF (Table 3). Hence, in intoxicated broilers, addition of CLI is probably responsible for recovering the biochemical alterations in chickens. As shown in Table 4, serum phosphorus and calcium levels were also depleted due to aflatoxin contaminated diet, but addition of CLI to contaminated diets compensate this changes.

Gross and microscopic lesions as well as biochemical changes in chicks fed toxic diet observed in our study were more or less similar to those reported in the literature. Liver is considered the main target organ for aflatoxins (Cysewski *et al*., 1977). The increase in the relative weight of livers induced by AF is attributed to an accumulation of lipid in the liver, which produces the characteristic, enlarged, friable, fatty livers associated with aflatoxicosis in broilers (Kubena *et al*., 1993; Ibrahim *et al*., 1998). The increase in liver weight could be attributed to increased lipid deposition on liver due to impaired fat metabolism (Tung *et al*., 1972). Other workers have reported similar increase in liver weight when AF- containing diets are fed (Smith and Hamilton, 1970; Reddy *et al*., 1984; Verma, 1990; Beura *et al*., 1993).

An extensive accumulation of lipid droplets (fatty liver) after 21 or 42 days of treatment and degenerative cell bodies were found to be dependent on the duration of exposure to aflatoxins. Prolonged exposure to low concentration of the toxin may produce merely reduced growth rates and significant hepatic signs. The enlargement may be partly due to hypertrophy of hepatocellular smooth endoplasmic reticulum and some degree of fatty change (Jones *et al*., 1993). Accumulation of large lipid droplets in liver tissue was associated with changes in plasma lipid profile, suggesting the impairment of the release of hepatic triglyceride to the plasma. Changes in plasma lipid and protein profile as shown in Table 2 were more obvious in chickens under dietary aflatoxins. Depletion in plasma cholesterol is probably due to accumulation of bile acids because of destruction in bile canaliculi together with focal accumulation of bile. Impaired protein biosynthesis and lipid transportation observed in chickens fed with aflatoxin contaminated diet were in accordance with the data published in several papers (Kececi *et al*., 1998; Kubena *et al*., 1993; Ortatatli and Oguz, 2001). Protein and RNA synthesis are inhibited at higher dose rates, which probably accounts for the necrotizing effects and fatty change seen at these rates (Jones *et al*., 1993). The hepatotoxic effects of AF caused impairments in protein, carbohydrate and lipid metabolism, decreases in serum protein, uric acid and cholesterol levels (Kubena *et al*., 1993, 1998; Ledoux *et al*., 1999) and inhibition of haematopoiesis (Oguz *et al*., 2000a). The reduction in total serum proteins could be due to impairement of amino acid transport and m-RNA transcription, there by inhibiting DNA and protein synthesis an observed by Thaxton *et al*. (1974).

The effects of long-term exposure (6 weeks) of broilers to dietary aflatoxin on production parameters was corroborated with a significant decrease (10-12%) in bone ash levels, which was recovered in broilers fed diets with containing CLI plus AF. The adverse effects of aflatoxin on bone mineralisation may be through interference with vitamin D_3 metabolism (Glahn *et al*., 1991). The basic mechanism for protection against the toxicity of AF appears to involve sequestration of AF in the gastrointestinal tract and chemisorption (i.e., tight binding) to the adsorbent, which reduces the bioavailability of AF (Davidson *et al*., 1987).

The present data and previous data clearly demonstrate that specific adsorbents can greatly diminish the toxicity of AF in young growing chicks.

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