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Effects of Zeolite and Mycosorb on Serum Biochemical and Hematological Parameters of Broilers Chicken Aflatoxicosis

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Abstract

Aflatoxins (AFS) are groups of toxin fungal toxins that are produced by different species of fungi such as *Aspergillus flavus* and *Aspergillus Parasiticus*. In this study Aflatoxin and two adsorbents (Zeolite and Mycosorb) was added to diet to evaluate some blood biochemical and enzyme activities in broiler chickens. Zeolite and Mycosorb added into the treatment basal diets at 3 g/kg and 1 g/kg levels which is contaminated level of 0.5 (AF0.5) or 1 ppm (AF1) aflatoxin/kg.

A total of 189 broiler chicks were divided into 9 treatment groups: T1) Control, T2) AF0.5, T3) AF1, T4) Zeolite + (AF0.5), T5) Zeolite + (AF1), T6) Mycosorb + AF0.5, T7) Mycosorb + AF1, T8) Zeolite + Mycosorb + AF0 and T9) Zeolite + Mycosorb + AF1. The results showed that AFS in T2 and T3 groups in comparison with control caused a significant decrease in red blood cell, haematocrit and lymphocyte counts while the monocyte cell and hetrophil counts increased at the end of experiment ($p < 0.05$). The Billirubin values, Aspartate Transaminase (AST), Gamma Glutamil Transferase (GGT) and Lactate Dehydrogenase (LDH) activities in diets containing AFs (T2 & T3) increased significantly compared with the controls ($p < 0.05$). The addition of Mycosorb and Zeolite to the AFs containing diet significantly improved haematolagy parameters, billirubin value, and AST, GGT, LDH activities. The protective effect of Mycosorb (T6 & T7) was higher than that of Zeolite (T4 & T5) against the toxic effects of AFs. The results of this study showed that the addition of both organic and inorganic adsorbents to the AFs containing diet reduced the adverse effects of AF and could be helpful in a solution of aflatoxicosis problem in poultry.

Keywords: Broiler; Biochemical parameters; Haematology; Mycosorb; Zeolite

Introduction

Aflatoxins (AF) are secondary metabolites and a class of mycotoxins produced by *Aspergillus flovus*, *Parasiticus* and *Nomius* [1,2].These toxins are worldwide in feeds and cause severe economic loses in the poultry and livesto ck industries in many cases [3]. Aflatoxin contamination may cause the difference between profit and loss to the poultry industry [4,5]. Studies have been related to the negative effects of aflatoxin in broiler chickens including decrease in Body Weight gain (BW), efficiency of feed utilization, liver damage, poor performance and immune responses. AF also caused pathologic alteration in important organs such as liver and kidneys [6,7]. The pathological signs in broilers are characterized by hepatic lesions such as enlarging, paleness, hydropic degeneration, bile duct hyperplasia and perportal fibrosis [8,9]. Decontamination of feed for AF is a major problem in the poultry industry. Producers and researchers desire to develop on effective de-contamination technology dealing with the feed-borne toxin [10]. Approaches used have included the physical and biological treatment of contaminated feed and feed stuffs. A successful detoxification process must be economical and must be capable of eliminating all traces of toxin without leaving harmful residues and also should not impair the nutritional quality of the commodity [6,11,12]. In the last decade several studies have been performed using adsorbent matter for detoxifying AF in contaminated feed and feed tuffs [11,13].

The natural and synthetic Zeolites [14,15], Bentonites [16,17], Mycosorb [18] and other adsorbents were preferred because of their binding capacities against AF and their reducing effect on AF adsorption from the gastrointestinal tract. The major advantages of adsorbents include cost, safety and easy administration through addition to animal feeds.

A new approach to detoxify aflatoxin is to use an organic adsorptive in the diet of broiler chickens and one important organic adsorptive is Mycosorb. Mycosorb is a new product which acts by binding pathogens and mycotoxins without affecting gut bacteria. Several vitamins were first extracted and characterized from Mycosorb including bioton, niacin, pantothenic acid and thiamin. Chronic and sub-clinical aflatoxicosis cases may be diagnosed by determining changes in serum biochemical and haematological parameters before major symptoms become apparent [15]. Serum Gamma Glutamil Transferase (GGT) activity, which is a sensitive indicator of liver dysfunction, indicating liver inflammation, space occuying lesions or obstruction of the biliary tract, was significantly increased by feeding diets containing AFB1 to broilers

Zeolite is a crystalline aluminosilicate compounds that are classified according to common features of the framework structures. The Zeolite structure known as a type which is a specific arrangement in which the unite cell contains 24 tetrahedra, 12 AlO_4, and 12 SiO_4 [15].

The purposes of the present study were to evaluate the serum biochemistry and enzyme activities in broiler at two levels of aflatoxin (0.5 & 1 ppm) and long-term AF exposure and to determine the possible preventive role of dietary mycosorb and zeolite on investigated values.

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Materials and Methods

A Total of 189, one-day-old, unvaccinated broiler chicks (Ross strain) were obtained from a commercial hatchery. After one weeks, Zeolite and Mycosorb added into the treatment basal diets at 3 g/kg and 1 g/kg levels which is contaminated at the level of 0.5 (AF0.5) or 1 ppm (AF1) aflatoxin/kg.

Broiler chickens were divided randomly into nine groups. There were three replicates of 7 broiler chicks for each dietary treatments: Control(T1), AF0.5(T2), AF1(T3), Zeolite + (AF0.5) (T4), Zeolite + (AF1)(T5), Mycosorb + AF0.5(T6), Mycosorb + AF1(T7), Zeolite + Mycosorb + AF0.5 (T8) and T9-Zeolite + Mycosorb + AF1.

The chicks were housed in heated batteries under fluorescent lighting and were fed a commercial food starter (maize and soybean based with 230 gram protein, 13.8 Mj ME kg^{-1}) up to 21-days and a grower diet (215 gram protein, 13.6 Mj ME kg^{-1}) up to 42-days. Chickens consumed the food and water *ad libitum* and the lighting was continuous. The starter and grower basal diets were tested for possible residual AF before feeding [19] and there were no detectable levels present (detection limit 1 g AF kg^{-1} in food; recovery of the extraction method 95 per cent).

The mycosorb was provided from alltech, K.Y., USA. The Zeolite was provided by Incal Biotechnology and mining ltd., Izmir, Turkey; and the chemical formula was KNa_2ca_2 (Si_{29} Al_7) O72, $32H_2O$.

Aflatoxin Production Procedure

Aflatoxin was produced by methods described by Shotwell et al. [20] which were modified as by Demet et al. [21] from the strain of aspergillus NRLL-2999 (National center for Agricultural utilization Research, peorial, IL, USA) on rice. Immunoaffinity columns (Vicam Afla test-Affinity column) were used to analyze the aflatoxin content of the culture material and quantified via high performance liquid chromatography (HPLC) (Agillent 1100 series). The AF within rice powder consisted up to 76/40 percent AFB1, 16/12 percent AFB2, 6/01 percent AFG1 and 1/47 percent AFG, based on total AF in rice powder.

Serum Biochemical and Haematological Analysis

When the chicks reached to 6 weeks of age, the feeding trial was terminated and 9 broilers from each treatment were selected at random and blood samples were collected into heparinized test tubes from wing vein to determine biochemical and haematological parameters. Red Blood Cell (RBC), White Blood Cell (WBC) and heterophil counts were determined by a haemocytometer method using Natt-Herrick solution; haematocrit values were measured by the micro haematocrit method. The serum concentrations of total billirubin, directe billirubin and activity of aspartate amino transferase (AST), gamma gullotamil transferase (GGT) and lactate dehydrogenase (LDH) were determined on a clinical chemistry auto analyzer (Tokyo Boeki, TMS, 1024, Japan) with commercial test kits (Spinreact, Spain). Three chickens from each replicate were slaughtered on day 42, and lymphoid organs such as burs of fabricius, thymus and spleen were weighed and examined by veterinary.

Statistical Analysis

The data on serum biochemical and hematological were grouped and expressed as mean pooled standard errors of mean. The obtained results were statistically compared using Duncan multiple range test [22]. Differences were considered to be significant based on the 5% level of probability.

Results and Discussion

The mean percentages of monocyte, lymphocyte and heterophil counts are presented in Table 1. The diet containing of AF1 caused a significant decrease on the lymphocyte counts over 42 days. A significant increase of heterophil counts obtained during the last week of the experiment. The results showed that AFS (0.5 or 1ppm) groups in comparison with control caused a significant decrease in red blood cell, haematocrit and lymphosyte counts while the monocyte cell and hetrophil counts increased at the end of experiment ($p < 0.05$) (Table 1).

The addition of Zeolite (3 g/kg) and Mycosorb (1 g/kg) to the AFs containing diets (group T5 to T9) caused a significant increased on the lymphocyte counts, while there was a significant decrease in monocyte counts and lymphocyte counts compared with controls. The addition of Zeolite or Mycosorb to the AF-containing diet (T7,T9) significantly improved RBC counts and haematocrit values compared with the(T3) AF1 group. Feeding AF caused significant increase in direct billirubin (0.11% to 0.28%) after 42 days (Table 2) while total billirubin on significant differences were found between control and AF-treated group. The researchers have directed towards the effective biological degradation process for AF.

Decontamination procedures have focused on degrading, destroying, in activating or removing AF by physical, chemical and biological methods recently. The adsorbent materials (Aluminosilicates, Bentonite, Silicas, Zeolite , Mycosorb, etc) have been evaluated for their ability to remove or diminish the adverse effect of mycotoxins in animal feed. These compounds must not be adsorbed from the

Treatments			Monocyte (%)	Lymphocyte (%)	Hetrophill (%)
AF	Zeolite	Mycosorb			
1) C.	-	-	3.80 ± 0.32^{a}	63.50 ± 0.50^{a}	28.50 ± 0.47^{c}
T2) A*	-	-	380 ± 0.34^{bc}	50.00 ± 0.36^{cb}	42.00 ± 0.51^{a}
T3) A**	-	-	3.90 ± 017^{a}	46.80 ± 1.1^{c}	40.50 ± 1.66^{a}
T4) A*	+	-	3.30 ± 0.21^{b}	60.00 ± 0.47^{a}	29.60 ± 0.53^{c}
T5) A**	+	-	2.70 ± 0.21^{c}	59.80 ± 0.3^{ab}	30.40 ± 0.37^{cb}
T6) A*	-	+	3.30 ± 0.30^{b}	56.60 ± 0.47^{b}	31.00 ± 0.60^{cb}
T7) A**	-	+	2.66 ± 0.21^{c}	63.88 ± 0.64^{a}	30.22 ± 0.68^{cb}
T8) A*	+	+	3.10 ± 033b^{c}	64.00 ± 0.33^{a}	30.00 ± 0.48^{cb}
T9) A**	+	+	3.70 ± 0.27^{ab}	55.60 ± 0.88^{b}	33.80 ± 0.49^{b}

Values within columns with different superscripts are significantly different ($p<0.05$).
A* :AF(0.5ppm), A** :AF(1ppm) C: control

Table 1: Effect of Zeolite and Mycosorb on Monocyte, Lymphocyte and Hetrophil Percentages In broiler chickens fed diets containing 0.5 or 1ppm Aflatoxins.

Treatments			heamatocrite(%)	RBC(10^6mm^3)	billirubin(mg/dl)
AF	Zeolite	Mycosorb			
T1)C	-	-	38.90 ±0.43^{a}	6.46 ± 0.12a	0.8770 ± 0.340a
T2)A*	-	-	30.60 ± 0.40^{bc}	3.12 ± 0.19bc	0.8600 ± 0.024a
T3)A**	-	-	29.40 ± 0.30^{c}	2.93 ± 0.12c	0.8950 ± 0.060a
T4)A*	+	-	34.50 ± 0.44^{b}	4.88 ± 0.14ab	0.3930 ± 0.028bc
T5)A**	+	-	33.40 ± 0.58^{b}	3.82 ± 0.09b	0.6240 ± 0.051b
T6)A*	-	+	34.80 ± 0.37^{b}	4.05 ± 0.09b	0.5840 ± 0.032b
T7)A**	-	+	33.44 ± 0.63^{b}	6.10 ± 0.12 a	0.6811 ± 0.013ab
T8A*	+	+	39.20 ± 1.16^{a}	5.76 ± 0.21a	0.2390 ± 0.028c
T9)A**	+	+	33.90 ± 0.32^{b}	3.91 ± 0.14b	0.4400 ± 0.007bc

Value within columns with different superscripts are significantly different ($p<0/05$).
A* =AF(0.5ppm), A** =AF(1ppm)

Table 2: Effects of Zeolits and Mycosorb on heamatocrite, RBC and billirabin in broiler chickens fed diet containing 0.5 or 1ppm AF during experiment.

gastrointestinal tract and must have the ability to bind physically with chemical substances, precluding their adsorption [23,24].

The addition of Zeolite and Mycosorb to the 1 ppm AF-containing diet provided a partial improvement in the adverse effects of AF on total billirubin and direct billirubin values. In T3 group the AST, GGT and LDH enzyme activities increased compared with control (Table 3), while the addition of Zeolite to the AF-containing diet (groups T2 and T3) caused significant increase on the AST activities compared with control (Table 3).

Bilgic et al. [25] showed broiler chicks had pethecial hemorrhages in liver and kidneys. This was in agreement with results obtained by Celik et al. [26]. Our results agreed with those reported by Kubena et al. [6] who found significant decreases in the biochemical parameters at exposure level of AFB1 ranging from 2.5 to 5 ppm.

Treatments (T5 to T9) caused a significant decrease in enzymes LDH, GGT activities compared with AF-containing diet (T2, T3). The results showed that AST levels in blood samples of control and aflatoxin groups were 128.80 and 191.90 unit/l respectively (Table 3). The elevation of AST may be due to disruption of hepatic cell as a result of necrosis or a consequence of altered membrane permeability [27]. In our study the decreases in the mean values of haematocrit, RBC and percentage of lymphocyte counts in AF-fed chicks indicated the depressing effect of AF hemopoietic tissue and immune responses as reported by others [6,15,28]. Immune suppressive effects associated with AF feeding in chicks reduced T lymphocyte counts [29], suppression of cell-mediated immunity and reduced immunoglobulin production [30]. In addition, AF exposure has been shown to reduce resistance to various bacterial, viral, and protozoan diseases in poultry [31,32]. In the present study the reduction of Lymphocyte counts in chicks given AF (0.5 or 1 ppm) is consistent with others but heterophil percentage significantly increased ($p < 0.05$).

The immune suppression in broilers exposed to aflatoxin was described by Qureshi et al. [33], that suggesting the aflatoxin interference with protein metabolism as the cause of this damage. The presence of Mycosorb in diet of birds fed with 1ppm of aflatoxin ameliorated in lymphocyte counts and heterophil percentage when compared with controls.

Many studies have been recently made to determine whether Zeolite in the diets of chickens have a beneficial effect on immune response [34]. Our results seem to differ from those observed by Kececi et al. [15]. Others concluded that AST and ALT are the serum enzymes which are sensitive specific indicator of liver damage. The LDH activity

Treatments			AST(IU/l)	GGT(IU/l)	LHD(IU/l)
AF	Zeolite	Mycosorb			
T1)C	-	-	128.80 + 2.89^{cde}	16.30 + 1.22^{cd}	677.00 + 21.01^{cd}
T2)A*	-	-	179.70 + 3.75^{ab}	21.10 + 0.90^{b}	992.90 + 35.7^{a}
T3)A**	-	-	191.90 + 5.10^{a}	30.10 + 2.44^{a}	1045.50 + 46.06^{a}
T4)A*	+	-	121.00 + 7.36^{e}	15.10 + 1.46^{de}	613.60 + 30.49^{d}
T5)A**	+	-	165.5 + 6.25^{b}	19.20 + 1.35^{c}	815.90 + 30.65^{b}
T6)A*	-	+	138.30 + 6.45^{cd}	11.30 + 1.74^{e}	830.60 + 41.58^{b}
T7)A**	-	+	127.89 + 4.05^{cde}	19.44 + 1.25^{bc}	803.70 + 76.70^{b}
T8)A*	+	+	123.80 + 7.23^{de}	11.90 + 1.42^{e}	593.50 + 27.58^{d}
T9)A**	+	+	143.3 + 3.04 c	21.4 + 0/95^{b}	789/30 + 34/15^{c}

Value within columns with different superscripts are significantly different ($p<0/05$).
A* =AF(0/5ppm), A** =AF(1ppm)

Table 3: Effect of Zeolite and Mycosorb on enzyme activities (AST, GGT and LHD) in broiler chickens fed diet containing 0.5 or 1ppm Aflatoxin.

Treatments			Average Daily Weight gain	
AF	Zeolite	Mycosorb	1-21days	21-42days
T1)C	_	_	404.82^{a}	955.42^{a}
T2)A*	_	_	358.42^{b}	725.58 ^{c}
T3)A**	_	_	347.19^{b}	722.09 ^{c}
T4) A*	+	_	391.73^{a}	830. 30^{b}
T5) A**	+	_	359.00^{bc}	748. 48^{c}
T6)A*	_	+	397.56^{a}	920.56 ^{a}
T7)A**	_	+	389.38^{a}	830.40 ^{b}
T8) A*	+	+	395.74^{a}	889. 90^{ab}
T9)A**	+	+	383.34 ^{ab}	788.78 ^{bc}
SEM			27.32	15.91

Value within columns with different superscripts is significantly different ($p<0/05$). A* = AF(0.5ppm), A** =AF(1ppm)

Table 4: Effect of Zeolite or Mycosorb on mean daily gain weight in Broiler chickens fed diet containing 0.5 or 1ppm Aflatoxin.

was 677 and 1045.5 units/Lin the untreated control and experimentally infected birds. The increase in LDH activity due to my- cotoxicosis was found to be significant ($p < 0.05$) as Benjamin [35] stated that LDH may be elevated in many disease processes in which there is cell necrosis. The addition of both adsorbents (T8 & T9) to the AFs containing diet reduced the adverse effects of AF and improves immune response.

The feeding of AF containing diets caused a significant decrease on the cumulative body weight gain over 42 days and a decrease on weight gain during the first 3 week of the experiment (Table 4). The birds that received AFB1 at 1ppm level in T3 had a significantly lower average body weight than others ($p < 0.05$). By adding Zeolite or Mycosorb to diets, feed intake increased ($p < 0.05$).

Previous study have reported that the addition of Zeolite to the AF (2.5 mg/kg) containing diet significantly reduced the growth inhibitory effects, biochemical-haematological toxic effects [14] in comparison with organic adsorbent of AF in broiler chicks for 21 days. In this study chickens consuming 1 ppm AF- containing diet showed a significant poor body weight gain ($p < 0.05$) (Table 4). The adverse effects of AF on BWG are due to anorexia, listlessness, inhibition of protein synthesis and lipogenesis [29,36]. Body weight reduction caused by intake of feed contaminated has already been reported in previous studies [17]. The effects of supplemental Cr on weight of lymphoid organs are shown in Table 4. The weights of lymphoid organs were not affected in broiler chicks fed different treatments diets.

Conclusion

The addition of Mycosorb (1 g/kg) and Zeolite (3 g/kg) to the AF-containing diet significantly recovered the adverse effect of AF on performance and biochemical-haematological values of broilers. The protective effect of Mycosorb was higher than that of Zeolite against the toxic effects of AFs. The results of this study showed that the addition of adsorbents to the AFs containing diet reduced the adverse effects of AF and could be helpful in a solution of aflatoxicosis problem in poultry.

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