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Determination the Toxicity of Aflatoxin and T-2 Toxin Induced Stress on Immune Response System with Feeding Activated Charcoal in Broiler Chicks

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Abstract: The activated charcoal (AC) 0.4% of feed were used with goal of providing further evidence as a factor for alleviation of toxicity and induced stress of aflatoxins (AF) and T-2 toxin on immune response system was examined in 160 male broiler chicks fed from 1 to 21 day of age. When adding 2.5 mg (AF) or 4 mg/kg T-2 toxin alone or in combination to the diet. At 21 day of age, 5 birds/pen were bled via cardiac puncture. Serum concentration of total protein, albumin, glucose, uric acid, triglycerides, cholesterol and alanine amino transferase (ALT), aspartate amino transferase(AST), lactate dehydrogenase(LDH) alkaline phosphatase(AP) and gamma glutamyl transferase(GGT), were determine. The other 5 birds/pen were intravenously injected with 4.0×10^6 *Escherichia coli* on day 21, then blood samples were collected at 60, 120 and 180 min post injection. Liver and spleen were collected after 180 min and colony forming units were determined. Results of the experiment indicated that AF or T-2 toxin alone or in combination caused a significant ($p<0.05$) decrease in total protein, albumin, glucose, uric acid, triglycerides and cholesterol. The presence of these toxin alone or in combination, also resulted in significantly decrease in enzyme activates of ALT, AST, LDH and AP except GGT was decreased in chicks fed AF or T-2 toxin only. There was significant increase in bacterial colonies counts in blood, liver and spleen compared with the control chicks group. The addition of activated charcoal at a concentration of 0.4% to the feed in this experiment was responsible for reduction the toxic effects on all parameters when AF or T-2 toxin present individually or in combination in the diets.

Key words: Aflatoxin · T-2 Toxin · Immune Response · Charcoal · Broiler

INTRODUCTION

Mycotoxins are structurally diverse secondary metabolites of fungi that grow on a variety of feed and foods that consumed by animals and human, respectively. Consumption of lesser amounts of fungal toxins may result in impaired immunity and decreased resistance to infection disease, indeed, it has long been recognized by veterinary clinic that marked immunosuppression is observed in livestock ingesting mycotoxins at levels below those that cause overt toxicity [1]. The aflatoxins (AF) are a group of closely related toxic metabolites produced in feedstuffs by *Aspergillus flavus* and *A. parasiticus*, plus related species *A. nomius* [2]. They commonly occur as natural contaminants of poultry feeds [3]. One of the myriad effects of mycotoxins is the ability to impair the immune system in fowl. Aflatoxin is the best-known mycotoxins for its ability to impair reticuloendothelial activity [4], primary immune response [5] complementary system [4], phagocytic activity of leukocytes and alveolar macrophages [6, 7]. The inhibitory effect of aflatoxin on antibody production has also been demonstrated in chicks [5] and turkeys [8]. The T-2 toxin is a naturally occurring mycotoxins produced by several species of fungi in the genus *Fusarium* [9]. Those are found in many cereals, feeds and vegetables. The T-2 toxin causes reduction in feed consumption and weight gain and severe and lesions in chickens [10, 11], altered feathering [12] and a coagulopathy [13]. Both AF and T-2 toxin are important to the poultry industry due to their toxicity and occurrence in feeds, the toxicity of individual mycotoxins can be

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enhanced when the occur as co-contaminates of feeds [10]. Practical methods for detoxifying mycotoxins containing feeds on a large commercial scale and in a cost-effective manner are not currently available. A new approach to this problem has been the addition of non-nutritive sportive materials to the diet known as sorbents in order to reduce the absorption of mycotoxins from the gastrointestinal tract. In recent years, the last adsorbent was reported that was effective in reducing toxic of AF and T-2 toxin in broiler chicks [14]. Therefore, the objective of this study was to evaluate the ability of AC when included in a diet to protect AF and T-2 toxin in response immune system in broiler chicks.

MATERIALS AND METHODS

In this experiment 160 day-old male broiler chicks (Lohman) were housed in heated starter batteries under continues fluorescent lighting. Chicks were fed a corn soybean meal based starter diet obtained from a commercial mill; it contained 22% CP and 2950 ME/kg. The diets were formulated without added antibiotics, coccidiosis or growth promoter. The chicks were randomly assigned to the following treatment groups: 1) Control with 0 mg AF, 0 mg T-2, 0% AC; 2) 0.04% AC; 3) 2.5 mg AF/kg of diet; 4) 2.5 mg AF/kg of diet plus 0.04% AC; 5) 4 mg T-2/kg of diet; 6) 4 mg T-2/kg of diet plus 0.04% AC; 7) 2.5 mg AF plus 4 mg T-2/kg of diet; 8) 2.5 mg AF plus 4 mg T-2/kg of diet plus 0.04% AC. Each treatment consisted of two replicates of ten chicks per replicate. Aflatoxin was prepared through inoculation of rice by *Aspergillus parasiticus* NRRL 2999 described by Shotwell *et al.* [15], as modified by West *et al.* [16]. Fermented rice was autoclaved and ground and the AF content measured by Spectrophotometric analysis [17, 18]. The total AF content in the rice powder, 80% was AFB1, 14% was AFG1, 5% was AFB2 and 1% was AFG2. The T-2 toxin was prepared through inoculation of cracked corn by *Fusarium sporotrichioides* (Laboratory isolate) at 15°C by method previously described by Cullen *et al*. [19]. T-2 toxin was extraction and determined under the conditions described by Lawrence and Scott [20] and incorporated into the diet by dissolving the toxin in 95% ethanol and then mixing the appropriate quantities with 1 kg of the diet. After drying, the dissolved toxin was mixed with the basal diet to produce the treatments containing T-2 toxin. The basal diet was analyzed for mycotoxins and was found to be below detection limits for AF, ochratoxin, zearalenone as established by the methods described by Clement and Phillips [21]. Feed and water were available for *ad labium* consumption. At 21 days of age, five birds per pen were bled via cardiac puncture. The serum was collected using corvac serum separator tubes. Serum concentration of total protein, albumin, glucose, uric acid, triglycerides, cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST). Lactate dehydrogenase (LDH), γ-glutamyl transferase (GGT) and alkaline phosphatase (AP) were determine using a clinical chemistry analyzer according to the manufacturer′s recommended procedure (obtained from Rondex Laboratories Ltd.). The other five birds were intravenously injected with 4.0×10^6 *E. coli*. The amount of *E. coli* used was based on pilot experiments, for this study, a dose was chosen that showed complete elimination of bacteria from circulation but did not cause severe homodynamic changes influencing clearance function. One milliliter blood was collected from the jugular vein into heparinized tubes at 60, 120 and 180 min post injection. Prior to *E. coli* inoculation, blood samples from each bird were collected and checked for the presence of *E. coli* in order to insure no preexisting infection. At 180 min post injection, poults were killed and tissue samples of liver and spleen were taken for quantitative bacterial determination. Whole blood samples were serially diluted with sterile PBS and 100 µl of each dilution at each time point was plated into MacConkey agar plates. Approximately one gram of tissue sample was homogenized with 5 ml of sterile PBS. Serial dilution of tissue suspension (100 µl) was plated onto MacConkey agar plates. Plates were then incubated at 37°C for 18 h and number of colonies on the plates were determined and expressed as *E. coli* colony forming units. Each samples run in duplicate plates. The final bacterial concentrations were calculated as the number of colonies per milliliters of blood and as colonies per gram of harvested tissue. The colonies grown on the plates were confirmed to be *E. coli* colonies by previously reported methods [22].

Data were analyzed by the ANOVA analysis, using the general linear model of the Statically Analysis System [23]. Significant treatment differences were evaluated using Duncan's multiple-range test [24]. All statements of significance are based on the 0.5 level of probability.

RESULTS

The effects of dietary 2.5 mg AF or 4 mg T-2 toxin/kg of diet singly or in combination with or without 0.4% AC on serum biochemical values are summarized in Table 1. When compared with the controls, serum total protein,

Table 1: Individual and combined Effects of AF and T-2 toxin with or without AC▪ on blood biochemistry in broiler chicks

AF	T-2	AC	TP*	Alb	Glu	UA	TG	Cho
----mg/kg----		%	---------- gm/100 ml ----------			---------- mg/100 ml ----------		
0	0	0	3 .14 ^{a}±0.01	1.19 ^{a}±0.01	269 ^{a}±3.78	6.17 ^{a}±0.1	102 ^{a}±1.5	174 ^{a}±1.5
0	0	0.4	3.12 ^{a}±0.01	1.13 ^{b}±0.02	270 ^{a}±3.98	6.19 ^{a}±0.01	103 ^{a}±1.2	176 ^{a}±1.1
2.5	0	0	2.40 ^{d}±0.01	0.79 ^{f}±0.01	249 ^{cd}±3.73	5.05 ^{c}±0.08	69 ^{c}±0.83	120 ^{c}±1.33
2.5	0	0.4	2.78 ^{c}±0.01	0.91 ^{e}±0.01	259 ^{b}±2.95	5.18 ^{c}±0.08	70 ^{c}±1.5	153 ^{c}±1.0
0	4	0	3.00 ^{b}±0.01	1.00 ^{c}±0.18	251 ^{c}±4.73	5.03 ^{c}±0.1	93 ^{b}±1.8	159 ^{b}±1.84
0	4	0.4	2.99 ^{b}±0.01	0.97 ^{d}±0.01	259 ^{b}±2.87	5.04 ^{c}±0.07	96 ^{b}±1.8	157 ^{b}±1.79
2.5	4	0	2.01 ^{f}±0.02	0.69 ^{g}±0.01	240 ^{e}±2.81	4.16 ^{d}±0.08	58 ^{d}±0.79	118 ^{e}±1.04
2.5	4	0.4	2.06 ^{e}±0.01	0.76 ^{f}±0.01	247 ^{d}±4.10	4.13 ^{d}±0.07	70 ^{c}±0.85	126 ^{d}±1.20

a-d Values within columns with no common superscript differ significantly at 0.05.

▪AC=Activated charcoal. * TP=Total protein, Alb=Albumin, Glu=Glucose, UA=Uric acid, TG=Triglycerides, Cho=Cholesterols.

Table 2: Individual and combined Effects of AF and T-2 toxin with or without AC▪ on serum Enzyme activities in broiler chicks.

AF	T-2	AC	ALT*	AST	LDH	GGT	AP
--- mg/kg-----		%	---------- IU/ml ----------				
0	0	0	20.5 ^{a} ±0.33	145 ^{a}±1.42	444 ^{a}±5.8	10.4 ^{d}±0.20	5.99 ^{a}±0.01
0	0	0.4	20.4 ^{a}±0.17	146 ^{a}±1.40	443 ^{a}±4.8	10.4 ^{d}±0.14	6.03 ^{a}±0.01
2.5	0	0	10.5 ^{d}±0.16	87 ^{e}±1.46	357 ^{c}±4.1	13.4 ^{a}±0.13	4.11 ^{c}±0.01
2.5	0	0.4	14.0 ^{c}±0.20	121^{d}±0.92	392 ^{b}±4.0	11.6 ^{b}±0.23	4.85 ^{b}±0.01
0	4	0	18.1 ^{b}±0.10	134 ^{c}±0.67	320 ^{d}±3.1	13.0 ^{a}±0.11	3.91 ^{d}±0.01
0	4	0.4	17.9 ^{b}±0.10	137 ^{b}±0.68	384 ^{f}±5.0	11.0 ^{c}±0.11	3.85 ^{e}±0.01
2.5	4	0	9.1 ^{e}±0.10	83 ^{f}±1.46	320^{d}±3.95	11.5 ^{b}±0.20	3.79 ^{f}±0.01
2.5	4	0.4	10.3 ^{d}±0.05	85 ^{f}±0.74	302 ^{e}±5.40	10.6 ^{cd}±0.15	3.78 ^{f}±0.01

a-d Values within columns with no common superscript differ significantly at 0.05.

▪AC=Activated charcoal. *ALT = Alanine amino transferase. GGT = γ-glutamyl transferase. AST = Aspartate amino transferase. AP = Alkaline phosphatase. LDH = Lactate dehydrogenase.

Table 3: Individual and combined Effects of AF and T-2 toxin with or without AC▪ on *Escherichia coli* clearance from blood and tissue in broiler chicks

			Time post injection (log10cfu/ml blood)			(log10cfu/gm tissue after 180 min post injection)	
AF	T-2	AC	----------			----------	
----- mg/kg ------		%	60 min	120 min	180 min	Liver	Spleen
0	0	0	2.80 ^{e} ±0.03	2.27 ^{e} ±0.01	1.02 ^{e} ±0.04	4.55 ^{e} ±0.04	4.88 ^{f} ±0.03
0	0	0.4	2.81 ^{e} ±0.01	2.29 ^{e} ±0.01	1.02 ^{e} ±0.01	4.56 ^{e} ±0.01	4.90 ^{f} ±0.01
2.5	0	0	3.28 ^{b} ±0.01	3.26 ^{b} ±0.01	3.18 ^{b} ±0.01	5.02 ^{a} ±0.01	6.02 ^{b} ±0.01
2.5	0	0.4	3.07 ^{c} ±0.01	3.11 ^{c} ±0.09	2.88 ^{c} ±0.01	4.80 ^{c} ±0.01	5.85 ^{d} ±0.01
0	4	0	2.90 ^{d} ±0.01	2.50 ^{d} ±0.01	1.40 ^{d} ±0.01	4.59 ^{d} ±0.01	5.14 ^{e} ±0.01
0	4	0.4	2.90 ^{d} ±0.01	2.48 ^{d} ±0.01	1.39 ^{d} ±0.01	4.57 ^{de} ±0.01	5.12 ^{e} ±0.01
2.5	4	0	3.40 ^{a} ±0.01	3.37 ^{a} ±0.01	3.29 ^{a} ±0.01	5.02 ^{a} ±0.01	6.08 ^{a} ±0.01
2.5	4	0.4	3.28 ^{b} ±0.01	3.24 ^{b} ±0.01	3.19 ^{b} ±0.01	4.89 ^{b} ±0.01	5.90 ^{c} ±0.01

a-d Values within columns with no common superscript differ significantly at 0.05.

▪AC=Activated charcoal

albumin, glucose, uric acid, triglycerides and cholesterol was significantly ($p<0.05$) decreased in chicks fed diets containing AF or T-2 toxin singly or in combination. The addition of AC alone was not differing significantly when compared with the control group. Activated charcoal when added to the diet containing T-2 alone causes no change in these parameters except Albumin and glucose concentration that were ameliorative the negative effect of toxin. Also the negative effects which obtained in serum biochemical parameters caused when dietary AF was ameliorative by the addition of 0.5% AC to the chicks diets. The impact of adding the AC to chicks fed did not influence in complete protection from the two toxins negative effects. But they were became intermediately effects between the toxicants treatment and control group.

Data presented in Table 2. Show the effects of dietary treatments on serum enzyme activities. The activity of alanine aminotransferase (ALT), aspartate amino transferase (AST) lactate dehydrogenase(LDH) and alkaline phosphatase (AP), was significantly ($p<0.05$) decreased and the activity of γ-glutamyl transferase was increased in chicks fed diets containing AF or T-2 toxin individually or in combination, when compared with the

control group. Adding AC to the diet containing AF alone or in combination with T-2 was acting in ameliorate the effects of the toxins on the enzyme activities. Whereas the added of AC to the diet containing T-2 alone did not effect on the activity of ALT enzyme.

In comparison with the results in the control, the delayed systemic bacterial clearance in chicks fed 2.5 AF and 4 mg T-2 toxin/kg diet singly or in combination were associated with significantly higher numbers of viable bacteria in blood system and organs (Table 3). The accounts of *E. coli* colonies in blood system, liver and spleen was associated with significantly higher accounts in chicks fed AF or T-2 toxin individually or in combination. The addition of AC did not effect in complete protection as evidenced by all values which determined that were appear intermediate between those of control and AF and T-2 toxin singly or in combination.

DISCUSSION

The aflatoxins and T-2 toxin are important mycotoxins due to their toxicity and occurrence in feedstuffs that are used in poultry and livestock diets. In the present study, the toxic effects of AF or T-2 toxin singly or in combination were expressed as decreased concentration of serum total protein, albumin and activity of alkaline phosphatase are most likely associated with inhibition of protein synthesis by AF through conversion to a 2, 3 epoxide binding to DNA and inhibiting RNA synthesis [25, 26]. T-2 toxin also acted in inhibits the protein synthesis through the inactivation and termination process, possibly through its binding to ribosome's [27]. Therefore, aflatoxin primarily effects on protein synthesis during transcription and T-2 toxin effects on protein synthesis during translation process, which may partially account for their synergistic toxicity. The reduction in concentration of serum cholesterol is most likely due to inhibition of cholesterol biosynthesis and the reduction in concentration of serum triglycerides might be associated with impaired lipid transport which causes from AF or T-2 toxin, as suggested by Manafi *et al* .[28] and Kubena *et al.* [29]. The γ-glutamyl transferase activity is a sensitive indicator of liver disease, furthermore with other enzyme checked in this study, whether the disorder involves liver inflammation, a space occupying lesion, or obstruction of the biliary tract.

Reduction in systemic bacterial clearance observed in the current study may also be a result of dysfunction of the mononuclear phagocytic system [30, 31]. In addition to the delayed bacterial clearance in the blood, higher accounts of bacterial colonies in the tissue cultures of chicks fed AF or T-2 toxin singly or in combination also suggest that the ability to engulf bacteria is impaired. It is speculated that macrophages in mycotoxins fed chicks were unable to digest bacteria within macrophages, leading to higher accounts of bacteria present in the tissues. As a result, systematic bacterial clearance was delayed. The diminished systemic bacterial clearance was associated with increased of bacterial colonies accounts in tissues, indicating that the phagocytic and lytic capacity of the mononuclear phagocytic system was compromised [32, 33].

The results indicate that the AC tested was marginally effective in reducing some of the toxic signs of chronic toxicosis in growing broilers fed AF, but of little benefit when T-2 toxin was fed. Any protective effect probably involves the sequestration of the toxic molecules in the gastrointestinal tract and chemisorption to the charcoal.

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