A PHYSIOLOGICAL APPROACH TO COUNTERACT AFLATOXICOSIS IN BROILER CHICKENS BY THE USE OF DIETARY LICORICE EXTRACT

Hazim J.Al-Daraji **I.A.Al-Ani** **J.K.Minati** **H.E.Al- Hiti**
Department of Animal Res. State Board for Agric. Res. , Ministry of Agriculture
College of Agric.
Univ. of Baghdad

ABSTRACT

This experiment was performed at the Animal Production farm / State Board of Agricultural Research to determine the mechanisms expected to be involved in alleviate the harmful effects of aflatoxicosis on broiler chickens by using dietary licorice extract . A total of 900 Fawbro broiler chicks , three weeks old were used in this study . Chicks were randomly allocated to 6 treatments of 3 replicates per each treatment . Birds in the first treatment (T1) were fed a basal diet and used as control group . Birds in T2 treatment fed a basal diet contaminated with aflatoxin , while birds in T3 treatment were fed a diet contaminated with aflatoxin and treated with mold killer . However , birds in T4 , T5 and T6 treatments were fed a diet contaminated with aflatoxin and supplemented with licorice extract at the levels of 150 , 300 and 450 mg / kg of diet , respectively .

Results of this study revealed that dietary aflatoxin (T2) significantly ($p < 0.05$) decreased erythrocyte counts (RBC) , haemoglobin concentration (Hb) , hematocrit (PCV) , thrombocyte counts (Thr) , leukocyte counts (WBC) , plasma glucose (Glu) , plasma protein (Pro) , plasma calcium (Cal) , plasma phosphorous (Pho) and alkaline phosphatase (ALP) activity , while significant ($p < 0.05$) increases were found in heterophil / lymphocyte ratio (H / L) , plasma uric acid (Uri) , plasma cholesterol (Cho) , and aspartate aminotransaminase (AST) activity compared with control group (T1) .

The addition of licorice extract (T4 , T5 and T6) or mold killer (T3) to an aflatoxin – containing diet significantly improved the adverse effects of aflatoxin on haematological and biochemical traits . However , results of the current study clearly show that licorice extract , especially at the level of 450 mg / kg effectively diminished the detrimental effects of aflatoxicosis on the general physiological status of broiler chickens .

مجلة العلوم الزراعية العراقية 36 (3) : 169 - 178 ، 2005 الدراجي وآخرون

التصور الفسلجي لتقليل آثار التسمم بالافلاتوكسين عن طريق اضافة مستخلص عرق السوس الى علائق فروج اللحم

حازم جبار الدراجي عماد الدين عباس العاني جاسم قاسم مناتي حاتم عيسى اهيتي
قسم الثروة الحيوانية الهيأة العامة للبحوث الزراعية ، وزارة الزراعة
كلية الزراعة ، جامعة بغداد

المستخلص

اجريت هذه التجربه في حقل الانتاج الحيواني التابع للهيأه العامه للبحوث الزراعيه / وزارة الزراعه لتحديد الميكانيكيات المتوقع مساهمتها في تخفيف التأثيرات الضاره الناجمه عن تسمم العلف بالافلاتوكسين عن طريق اضافة مستخلص عرق السوس لهذا العلف . واستخدم فيها 900 فرخ فروج لحم فاوبرو بعمر ثلاثة اسابيع ، اذ تم توزيعها عشوائيا على ست معاملات يتكون كل منها من ثلاث مكررات وبواقع 150 فرخ لكل معامله . وتم تغذية الطيور في المعامله الاولى (T1) على العليقه الاعتياديه واعتبرت كمجموعة مقارنه . وغذيت الطيور في المعامله T2 على العليقه الملوثه بالافلاتوكسين ، في حين ان طيور المعامله T3 غذيت على عليقه ملوثه بالافلاتوكسين ومعامله بماده قاتله للفطريات Mold killer. من ناحيه ثانيه ، فأن الطيور في المعاملات T4 و T5 و T6 غذيت على عليقه ملوثه بالافلاتوكسين ومضاف اليها مستخلص عرق السوس بمستويات 150 و 300 و 450 ملغم / كغم علف على التوالي .

اظهرت النتائج ان تلوث العلف بالافلاتوكسين (T2) ادى الى انخفاض معنوي ($P < 0.05$) في عدد كريات الدم الحمر وتركيز الهيموكلوبين وحجم خلايا الدم المرصوصه وعدد الصفيحات الدمويه وعدد كريات الدم البيض وتراكيز الكلوكوز والبروتين والكالسيوم والفسفور ونشاط انزيم الفوسفاتيز القاعدي في بلازما الدم ، والى ارتفاع معنوي ($P < 0.05$) في نسبة الخلايا المتغايره / الخلايا اللمفيه وتراكيز حامض البوليك والكولسترول ونشاط انزيم AST بالمقارنه مع مجموعة السيطره (T1) .

واشارت النتائج ايضا الى ان اضافة مستخلص عرق السوس (T4 و T5 و T6) او الماده القاتله للفطريات (T3) الى العلف الملوث بالافلاتوكسين ادت الى تحسن معنوي في التأثيرات السلبيه للتسمم بالافلاتوكسين في صفات الدم والصفات الكيميائيةويه لبلازما الدم . اضافه لذلك ، فأن نتائج الدراسه الحاليه اظهرت بوضوح ان اضافة مستخلص عرق السوس خصوصاً عند المستوى 450 ملغم / كغم علف ادت الى الحد وبدرجه كبيره من التاثيرات الضاره لتلوث العلف بالافلاتوكسين في الحاله الفسلجيه العامه لفروج اللحم .

(*)Accepted on 12/10/2004 - Received on 27/2/2005

Introduction

Mycotoxins are structurally diverse secondary fungal metabolites that occur worldwide as contaminants of grain . Among the various mycotoxins identified especially affecting poultry , aflatoxin , ochratoxin , T-2 toxin , are often encountered in feedstuffs at alarming concentrations in different parts of the world (4) . These mycotoxin contaminated feedstuffs when consumed , produce a range of sever devastating effects on the general well – being and productivity of farm animals and poultry (10) .

Aflatoxins , potent mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* , are a major concern in poultry production . Aflatoxins contamination causes reduced feed quality and reduced animal efficiency either through poor conversion of nutrients or problems such are reproductive abnormalities (23) . Aflatoxicosis in poultry also causes listlessness , anorexia with lowered growth rate , poor feed utilization , decreased egg production and increased mortality . Additionally , anemia (24) , reduction of immune function (25) , hepatotoxicosis and hemorrhage (26) are associated with aflatoxicosis . Huff et al. (18) found that aflatoxin treatment significantly decreased body weight , weight gain , increased relative weights of spleen , liver , kidney , gizzard , proventriculus , decreased activity of ALP , AST and lactic dehydrogenase and decreased levels of protein , albumin , triglycerides , calcium and phosphorous .Altered activities of enzymes, Viz., serum AST , ALT , ALP and gamaglutamyl transferase (GGT) have been noticed during aflatoxicosis (9). Bridane et al .(8) found that RBC , PCV , Hb , Thr , and lymphocyte counts were significantly reduced by aflatoxin treatment , while increases were seen in heterophil counts .

At the present time there dose not appear to be any way of preventing or ameliorating the disease other than avoiding conditions conductive to the formation of aflatoxin in feedstuffs and avoiding fed with performed aflatoxin . Because of the nature of the feed distribution system in the poultry industry , this avoidance is difficult . A more economical solution would be the development of a dietary additive or modification that would make the bird more resistant to aflatoxin .

The object of this investigation was to explore way that a broiler diet might be modified to enhance resistance to aflatoxin. This was done by studying the effect of the inclusion of different levels of licorice extract in the diet on the blood characteristics of broilers receiving diet which contaminated with aflatoxin .

Materials and Methods

This experiment was undertaken at the farm of Animal Production , State Board of Agricultural Research . Atotal of 900 Fawbro broiler chicks , three weeks - old were used . Birds were fed starter diet during the third week of age (starting date of experiment) (22.7 % crude protein and 2867.4 kcal / kg of diet) and finisher diet (20.6 % crude protein and 2922 kcal / kg of diet) till the marketing age (49 days of age) . Chicks were allocated at random to 6 treatment groups of 3 replicate per group , each replicate constitutes 50 chicks (150 chicks per treatment group) .

Birds in the first treatment fed a basal diet and used as control group (T1) . The second treatment (T2) was fed a basal diet contaminated with aflatoxin , while birds in the third treatment (T3) were fed a diet contaminated with aflatoxin and treated with mold killer . The type of mold killer used in this study was manufactured by Korean company that called Choong ang Biotech . However , birds in fourth , fifth , and sixth treatments were fed a diet contaminated with aflatoxin and supplemented with licorice extract . Licorice extract was added to the diet of birds throughout the total period of investigation at levels of 150 mg / kg (T4) , 300 mg / kg (T5) and 450 mg / kg of diet (T6) .

Aflatoxin used in the present study was aflatoxin B1 which obtained from the Department of Plant Protection , College of Agriculture , University of Baghdad . Aflatoxin was prepared and incorporated into basal diet by method previously reported (32) . Aflatoxin was produced by growing *Aspergillus flavus* on rice . The moldy rice was dried and ground to a fine powder and analyzed spectrophotometrically for its total aflatoxin content by the method of Nabney and Nesbitt (21) . The moldy rice then added and well mixed with the yellow corn that involved in the basal diet . The final concentration of aflatoxin introduced to the birds was determined to be equal to 2 mg aflatoxin / kg of diet .

At the end of experiment (7 weeks of age) , blood samples were collected into a vacutainer (containing heparin) by vein puncture of brachial vein of 24 birds in each treatment (12 males and 12 females) . The haematological traits evaluated in the present investigation and which have been shown to indicate general physiological status of birds consisted of the following (method in parenthesis) : Erythrocyte counts – RBC and leucocyte counts – WBC (22) , haemoglobin concentration – Hb and plasma alkaline phosphatase activity – ALP (36) , hematocrit – PCV (5) , thrombocyte counts – Thr (3) , heterophil to lymphocyte ratio – H / L (15) , plasma glucose – Glu (6) , plasma protein – Pro (37) , plasma uric acid – Uri (17) , plasma aspartate aminotransaminase – AST activity (27) , plasma cholestero – Cho (14) , plasma calcium – Cal (19) , and plasma phosphorous – Pho (13) .

Significance of data was determined at the 5 % level of probability by analysis of variance (ANOVA) using the Statistical Analysis System (29) . Significance of the differences between treatment means was determined by Duncan's multiple range test (29) .

Results and Discussion

The influence of licorice extract as an ameliorating agent in broilers fed dietary aflatoxin B1 on various blood parameters have been presented in Tables 1 to 4 . Feeding aflatoxin alone (T2) caused significant ($p < 0.05$) decrease in RBC , Hb , PCV , Thr , and WBC , while significant increase was found in H / L ratio in both of males , females and both sexes (Tables 1 and 2) . These finding agree with the other reports that explain the suppressive effects of aflatoxin on hematopoiesis and immune response (25). Broiler chicks given 2.5 to 3.5 g aflatoxin / kg diet have shown not only decreased amount of Hb , PCV , Thr , and lymphocyte and monocyte counts (30) but also increased heterophils (24) . Aflatoxin might have affected the tissue of haemopoietic and immune system thereby the production of cells might have been affected . Various studies have reported that PCV , RBC and Thr counts were decreased by aflatoxin and aflatoxicosis caused lymphocytopenia and heterophilia in broiler chickens (28) . However , Gross and Siegel (15) reported a positive correlation between plasma corticosterone and H / L , and that H / L ratio is a good indicator of physiological stress . Therefore , increasing H / L ratio indicated that the birds were under acute stress .

Compared to control group (T1) , Glu , Pro , Cal , Pho , and ALP were significantly ($p < 0.05$) decreased and Uri , AST and Cho were significantly ($p < 0.05$) increased by aflatoxin treatment (T2 ; Tables 3 and 4) . Chronic and sub – clinical aflatoxicosis cases may be diagnosed by determining serum biochemical and haematological alterations before major symptoms became apparent (24) . Sehu et al. (31) indicated that aflatoxin toxicity in broilers may be manifested by decrease serum concentration of total protein , albumin , inorganic phosphorous and calcium . Afzali and Devegowda (2) concluded that the most important sequelae of aflatoxicosis in poultry is impairment of immune system resulting in high mortality . Aflatoxin inhibits protein synthesis , and resulting in lowered level of antibody production . The reduction in total serum protein is due to impairment of amino acid transportation at mRNA transcription level and then by inhibiting protein synthesis (34) . Bridane et al. (8) reported that inclusion of aflatoxin into the diet caused significant decreases in serum total Pro , albumin , triglyceride , Glu , inorganic Pho and creatinine values , while significant increases were recorded for Cho and AST activity . However , those authors concluded that the decrease in serum Pro , albumin and Glu values and increases Cho and AST activity are due to the hepatotoxic effects of aflatoxin characterized by inhibition of protein synthesis and the impairment of carbohydrate and metabolism . However , the decrease in serum inorganic Pho and the increase in Uri may be related to the nephrotoxic effects of aflatoxin in agreements with other studies (16) . Bailey et al . (7) found acute elevation in the levels of serum Uri , AST and GPT activities of broilers fed a diet contaminated with ochratoxin , and explained these changes by the large lesions occurred for heart , kidney and liver organs as a result of aflatoxicosis . Furthermore , altered activities of enzymes , *viz.*, serum AST , GPT and ALP have been noticed during aflatoxicosis (11 , 20) . Huff et al. (18) reported that aflatoxin treatment significantly decreased activity of ALP and decreased serum levels of Pro , albumin , Glu , Cal and Pho .

The addition of licorice extract to aflatoxin – containing diet (T4 , T5 and T6) completely ameliorated the adverse

effects of aflatoxin on haematological parameters included in this study . Supplementation of licorice to the diet significantly ($p < 0.05$) increased RBC , Hb , PCV , Thr , and WBC and reduced the H / L ratio compared with T2 group in both of males , females and both sexes (Tables 1 and 2) . However , T6 group surpasses other treatments as regards all of these blood characteristics . On the other hand , T4 and T5 were in general superior to T3 group in relation with these traits .

With incorporation of gradual levels of licorice extract (T4 , T5 and T6) or mold killer (T3) into the aflatoxin – contaminated diet , Glu , Pro , Cal , Pho and ALP significantly ($p < 0.05$) increased , whereas Uri , Cho , and AST significantly ($p < 0.05$) decreased in both males , females , and both sexes (Tables 3 and 4) . However , T6 recorded the best results in regard to these traits compared with all other treatments . Additionally , there were no significant differences between T4 , T5 and T3 regarding Glu , Uri , Pro , Cho , Cal and Pho in both of males , females , and both sexes .

The beneficial counteraction of licorice extract with aflatoxin molecules in the diet and gastrointestinal tract have been clearly observed in our study as predicted . The role of licorice extract in aflatoxin detoxification were attributed to two mechanisms , as we expected . First, it may have selective binding capacity for aflatoxin molecules in gastrointestinal tract , and the second that licorice shows some anti – infective properties . In laboratory and animal studies , it has stopped or slowed the growth of certain bacteria , fungi , and parasites . Several animal studies have also revealed a possibly strong antiviral and fungicide effects for true licorice . In these studies , true licorice component that belong to isoflavonoid class of chemicals , appear to have several anti – infective effects that include interference with oxygen utilization by infective – organisms . Additionally , true licorice may have some ability to improve functioning of the immune system (1 , 12 , 33) . However , by functioning as anti – fungal agent , this herb destroys or prevent the growth of fungi (35) .

In conclusion , haematological values and plasma biochemical were significantly affected by aflatoxin treatment . The addition of licorice extract to the aflatoxin – containing diet significantly recovered the adverse effects of aflatoxin on haematological parameters – plasma biochemical of broiler . The protective effect of 450 mg / kg licorice extract used in this study against the toxic effects of aflatoxin was greater than that of 150 and 300 mg / kg . Licorice extract was non – toxic and non – detrimental for broiler chicken and these improvements should contributes to a solution of aflatoxin problem in broiler chickens .

Table 1. The effect of different level of licorice extract on RBC , Hb and PCV of broiler fed a diet contaminated with aflatoxin .

Traits / Treatments	RBC ($X10^6$ / mm^3)			Hb (g / 100 ml)			PCV (%)		
	Males	Females	Both sexes	Males	Females	Both sexes	Males	Females	Both sexes
T1	B 2.49 ± 0.09	B 2.36 ± 0.10	B 2.43 ± 0.09	B 8.27 ± 0.48	B 8.39 ± 0.45	B 8.33 ± 0.39	B 24.41 ± 0.98	B 23.29 ± 1.03	B 23.85 ± 0.90
T2	E 2.11 ± 0.10	D 2.05 ± 0.13	D 2.08 ± 0.12	D 7.96 ± 0.35	E 8.01 ± 0.40	E 7.99 ± 0.42	D 20.13 ± 0.95	D 18.27 ± 0.90	E 19.20 ± 1.01
T3	D 2.34 ± 0.08	C 2.26 ± 0.11	C 2.30 ± 0.10	C 8.13 ± 0.46	D 8.21 ± 0.38	D 8.17 ± 0.44	C 23.17 ± 0.97	C 22.66 ± 1.02	D 22.92 ± 0.98
T4	CD 2.37 ± 0.10	C 2.28 ± 0.12	C 2.33 ± 0.11	C 8.18 ± 0.38	C 8.30 ± 0.39	C 8.24 ± 0.43	C 23.34 ± 1.0	BC 23.09 ± 0.95	C 23.22 ± 0.88
T5	C 2.41 ± 0.12	C 2.30 ± 0.12	C 2.36 ± 0.09	B 8.24 ± 0.40	BC 8.35 ± 0.36	B 8.30 ± 0.37	B 24.36 ± 1.02	C 22.12 ± 1.00	C 23.24 ± 0.89
T6	A 2.55 ± 0.08	A 2.48 ± 0.09	A 2.52 ± 0.09	A 8.34 ± 0.33	A 8.45 ± 0.35	A 8.40 ± 0.39	A 26.37 ± 0.87	A 24.29 ± 0.89	A 25.33 ± 1.0

T1= Birds fed a basal diet , T2= Birds fed diet contaminated with aflatoxin , T3=Birds fed diet contaminated with aflatoxin and treated with mold killer , T4= Birds fed diet contaminated with aflatoxin and supplemented with licorice extract at level of 150 mg / kg , T5= Birds fed diet contaminated with aflatoxin and supplemented with licorice extract at level of 300 mg / kg and T6= Birds fed diet contaminated with aflatoxin and supplemented with licorice extract at level of 450 mg / kg.

* Values in a column with different superscripts differ significantly ($p < 0.05$) .

The Iraqi Journal of Agricultural Sciences, 36(3): 169 - 178, 2005

Table 2. The effect of different level of licorice extract on Thr , WBC and H / L ratio of broiler fed a diet contaminated with aflatoxin .

Traits / Treatments	Thr ($X10^3$ / mm^3)			WBC ($X10^3$ / mm^3)			H / L ratio		
	Males	Females	Both sexes	Males	Females	Both sexes	Males	Females	Both sexes
T1	B 24.17 ± 0.83	B 22.81 ± 0.92	B 23.49 ± 0.95	B 25.97 ± 0.90	B 24.04 ± 0.87	B 25.00 ± 0.96	C 0.23 ± 0.009	C 0.22 ± 0.008	C 0.23 ± 0.010
T2	E 19.0 ± 1.13	E 18.36 ± 1.11	E 18.68 ± 1.11	D 20.11 ± 0.98	E 19.63 ± 0.95	E 19.87 ± 0.93	A 0.29 ± 0.011	A 0.28 ± 0.012	A 0.29 ± 0.013
T3	D 20.96 ± 0.90	D 20.03 ± 0.93	D 20.50 ± 1.0	C 23.85 ± 1.0	D 22.80 ± 0.91	D 23.33 ± 0.88	C 0.23 ± 0.010	B 0.25 ± 0.010	C 0.24 ± 0.012
T4	C 22.09 ± 0.88	C 21.78 ± 1.01	C 21.94 ± 0.90	C 24.36 ± 0.84	C 23.51 ± 0.79	C 23.94 ± 0.90	B 0.26 ± 0.009	B 0.24 ± 0.007	BC 0.25 ± 0.010
T5	B 24.33 ± 0.95	B 22.82 ± 0.89	B 23.58 ± 0.86	B 25.71 ± 0.88	B 24.83 ± 0.92	B 25.27 ± 0.96	C 0.23 ± 0.008	B 0.24 ± 0.009	C 0.24 ± 0.007
T6	A 25.51 ± 0.80	A 24.07 ± 0.91	A 24.79 ± 0.89	A 26.42 ± 0.86	A 25.79 ± 0.77	A 26.11 ± 0.90	D 0.20 ± 0.006	C 0.19 ± 0.008	D 0.20 ± 0.010

T1= Birds fed a basal diet , T2= Birds fed diet contaminated with aflatoxin , T3=Birds fed diet contaminated with aflatoxin and treated with mold killer , T4= Birds fed diet contaminated with aflatoxin and supplemented with licorice extract at level of 150 mg / kg , T5 = Birds fed diet contaminated with aflatoxin and supplemented with licorice extract at level of 300 mg / kg and T6= Birds fed diet contaminated with aflatoxin and supplemented with licorice extract at level of 450 mg / kg.

* Values in a column with different superscripts differ significantly ($p < 0.05$) .

Table 3. The effect of different level of licorice extract on Glu , Uri , Pro and GOT of broiler fed a diet contaminated with aflatoxin .

Traits / Treatments	Glu (mg / 100 ml)			Uri (mg / 100 ml)			Pro (g / 100 ml)			GOT (IU / Liter)		
	Males	Females	Both sexes	Males	Females	Both sexes	Males	Females	Both sexes	Males	Females	Both sexes
T1	B 172.39 ± 17.11	B 170.11 ± 18.52	B 171.25 ± 19.90	C 7.55 ± 0.47	C 7.69 ± 0.46	C 7.62 ± 0.49	B 5.20 ± 0.30	B 5.15 ± 0.25	B 5.18 ± 0.24	B 95.26 ± 9.17	B 97.12 ± 8.45	B 96.19 ± 9.36
T2	D 158.26 ± 20.72	C 160.54 ± 19.93	C 159.40 ± 21.12	A 7.83 ± 0.50	A 8.01 ± 0.48	A 7.92 ± 0.53	D 4.92 ± 0.28	D 4.88 ± 0.33	D 4.90 ± 0.31	A 96.02 ± 10.42	A 100.11 ± 11.37	A 98.07 ± 11.63
T3	C 168.18 ± 17.65	B 172.04 ± 18.47	B 170.11 ±18.29	B 7.67 ± 0.39	B 7.79 ± 0.42	B 7.73 ± 0.40	C 5.08 ± 0.30	C 5.03 ± 0.21	C 5.06 ± 0.27	B 95.14 ± 9.41	B 96.90 ±8.52	B 96.02 ± 10.32
T4	B 171.9 ± 20.06	B 170.43 ± 19.36	B 170.81 ± 18.45	B 7.69 ± 0.44	B 7.75 ± 0.50	B 7.72 ± 0.47	C 5.05 ± 0.28	C 5.00 0.30	C 5.03 ± 0.23	B 95.30 ±8.97	B 97.18 ± 9.35	B 96.24 ± 9.41
T5	B 172.67 ± 17.82	B 171.44 ± 18.05	B 172.06 ± 18.74	B 7.64 ± 0.36	B 7.82 ± 0.40	B 7.73 ± 0.42	C 5.86 ± 0.25	C 5.02 ± 0.27	C 5.04 ± 0.20	B 95.19 ± 9.11	B 96.84 ± 8.99	B 96.02 ± 9.27
T6	A 181.94 ± 20.55	A 177.18 ± 19.36	A 179.56 ± 18.07	D 7.27 ± 0.40	D 7.29 ± 0.38	D 7.28 ± 0.45	A 5.29 ± 0.29	A 5.25 ± 0.22	A 5.27 ± 0.24	C 94.38 ± 9.21	C 95.15 ± 8.78	C 94.77 ± 9.05

T1= Birds fed a basal diet , T2= Birds fed diet contaminated with aflatoxin , T3 =Birds fed diet contaminated with aflatoxin and treated with mold killer , T4= Birds fed diet contaminated with aflatoxin and supplemented with licorice extract at level of 150 mg / kg , T5 Birds fed diet contaminated with aflatoxin and supplemented with licorice extract at level of 300 mg / kg and T6= Birds fed diet contaminated with aflatoxin and supplemented with licorice extract at level of 450 mg / kg.

- Values in a column with different superscripts differ significantly ($p < 0.05$) .

Table 4. The effect of different level of licorice extract on Cho , Cal , Pho and ALP of broiler fed a diet contaminated with aflatoxin .

Traits / Treatments	Cho (mg / 100 ml)			Cal (mg / 100 ml)			Pho (mg / 100 ml)			ALP (King Armstrong unit)		
	Males	Females	Both sexes	Males	Females	Both sexes	Males	Females	Both sexes	Males	Females	Both sexes
T1	C 128.14 ± 15.46	C 128.25 ± 16.17	C 128.20 ± 16.34	B 7.21 ± 0.39	B 7.10 ± 0.43	B 7.16 ± 0.40	B 3.47 ± 0.21	B 3.26 ± 0.27	B 3.37 ± 0.19	B 43.02 ± 4.63	B 39.91 ± 4.52	B 41.47 ± 4.44
T2	A 141.49 ± 17.15	A 138.12 ± 16.33	A 139.81 ± 16.92	C 6.79 ± 0.50	C 6.77 ± 0.55	C 6.78 ± 0.49	C 3.21 ± 0.25	C 3.07 ± 0.25	C 3.14 ± 0.23	C 39.06 ± 4.81	D 36.30 ± 4.65	D 37.68 ± 5.11
T3	B 135.75 ± 15.99	B 133.65 ± 14.82	B 134.70 ± 15.53	B 7.23 ± 0.38	B 7.16 ± 0.45	B 7.20 ± 0.41	B 3.51 ± 0.20	B 3.30 ± 0.21	B 3.41 ± 0.18	B 42.36 ± 4.49	C 38.14 ± 3.91	C 40.25 ± 5.03
T4	B 136.08 ± 16.27	B 133.11 ± 15.73	B 134.60 ± 16.01	B 7.17 ± 0.42	B 7.11 ± 0.51	B 7.14 ± 0.46	B 3.44 ± 0.17	B 3.26 ± 0.29	B 3.35 ± 0.19	B 42.30 ± 5.07	C 38.11 ± 4.57	C 40.21 ± 4.33
T5	B 131.21 ± 15.93	B 129.84 ± 14.72	B 130.53 ± 15.57	B 7.20 ± 0.44	B 7.14 ± 0.43	B 7.17 ± 0.48	B 3.52 ± 0.2	B 3.29 ± 0.20	B 3.41 ± 0.20	B 42.98 ± 5.0	B 39.94 ± 4.13	B 41.46 ± 4.20
T6	D 124.52 ± 14.84	D 119.94 ± 15.06	D 122.23 ± 15.18	A 7.40 ± 0.40	A 7.33 ± 0.47	A 7.37 ± 0.47	A 3.75 ± 0.17	A 3.67 ± 0.20	A 3.71 ± 0.18	A 49.15 ± 3.96	A 46.23 ± 4.19	A 47.64 ± 4.27

T1= Birds fed a basal diet , T2= Birds fed diet contaminated with aflatoxin , T3= Birds fed diet contaminated with aflatoxin and treated with mold killer , T4= Birds fed diet contaminated with aflatoxin and supplemented with licorice extract at level of 150 mg / kg , T5= Birds fed diet contaminated with aflatoxin and supplemented with licorice extract at level of 300 mg / kg and T6= Birds fed diet contaminated with aflatoxin and supplemented with licorice extract at level of 450 mg / kg.

Values in a column with different superscripts differ significantly ($p < 0.05$).

References

1.Adam , L .1997 . *In vitro* antiviral activity of indigenous glycyrrhizin , licorice , glycyrrhizic acid (sigma) on Japanese encephalitis virus .J.Commun . Dis . 29(2) : 91 – 99 .

2.Afzali , N . and G . Devegowda .2004 . The effect of graded levels of dietary aflatoxin on certain biochemical parameters in broiler breeders . WPC 2004 , XXII World's Poultry Congress . Istanbul , Turkey .

3.Al-Daraji , H.J., I.A.Abdul-Hassan , K.M.Abdul-Latif and F.A.Al-Obaidi.2001.AN evaluation of dietary nettle *Urtica urens* supplementation on some physiological traits of broilers . The Veterinarian 11(1) : 14 – 26 .

4.Aravind , K.L., V.S.Patil , G.Devegowda , B.Umakantha and S.P.Ganpule .2003. Efficacy of modified glucomannan to counteract mycotoxicosis in naturally contaminated feed on performance , serum biochemical and haematological parameters in broilers . Poultry Sci . 82 : 570 – 576 .

5.Archer , R.K.1965 . Haematological Techniques for Use on Animals . Oxford , Blackwell Scientific Puplications .

6.Asatoor , A.M. and E.J.King .1954 . Simplified colorimetric blood sugar method .Biochem J.56 : 44 – 46 .

7.Bailey , C.A., R.M.Gibson , L.F.Kubena , W.E.Huff and R.B.Harvey .1989 . Ochratoxin and dietary protein .2. Effects on haematology and various clinical chemistry measurements . Poultry Sci. 68 : 1664 – 1671 .

8.Birdane , Y.O , R Col , H.Basmacioglu and H.Oguz . 2004 . Effect of esterfied glucomannan on aflatoxicosis in broilers : II . Serum biochemical – haematological and bone parameters . WPC 2004 ,XXII World's Poultry Congress .Istanbul , Turkey .

9.Devegowda. G.,B.I.R.Aravind, K.Raienra, M.G.Morton,A. Babutrathna. 1994. Abiological approach to counteract aflatoxin in broiler chickens and ducklings by the use of *Saccharomyces cerevisiae* cultures added to feed. Biotechnology in Feed Industry, Proc. Ailtech Technical Publication, Nicholasville, Kentucky, USA : 235 – 245 .

10.Devegowda , G., M.V.L.N.Raju and H.V.L.N.Swamy .1998 a . Mycotoxins : novel solution for counteraction . Feedstuffs 70 : 12 – 15 .

11.Devegowda , G., M.V.L.N.Raju , N.Afzali and H.V.L.N.Swamy .1998 b . Mycotoxin picture worldwide : novel solutions for their counteraction . In : Biotechnology in the Feed Industry , Proceeding of the 14 th Annual Symposium (T.P.Lyons and K.A.Jackues eds.) , Nottingham University Press .pp.241 – 255 .

12.Duke , J.A.1985. CRC Handbook of Medical Herbs .Boka Raton , Florida : CRC press .

13.Fiske , C.H. and Y.Subbarow . 1982 . Determination of inorganic phosphor in serum and urine .In : Fundamentals of Clinical Chemistry . Ed. Tietz , N.W., W.B.Saunders company , London .

14.Franey , R.J. and A.Elias . 1968 . Serum cholesterol measurement based on ethanol extraction and ferric chloride – sulphuric acid . Cli. Chem . Acta . 2 : 255 – 263 .

15.Gross , W.B. and H.S.Siegel .1983 . Evaluation of the heterophil / lymphocyte ratio as a measure of stress in chickens . Avian Dis. 27 : 972 – 979 .

16.Harvey , R.B. , L.F.Kubena , M.H.Ellisalde and T.D.Phillips . 1993 . Efficacy of zeolitic ore compounds on the toxicity of aflatoxin to growing broiler chickens . Avian Dis . 37 : 67 – 73 .

17.Henry , R.J. , C.Sobel and J.Kim . 1982 . Determination of uric acid . In : Fundamentals of Clinical Chemistry . Ed . Tietz , N.W. , W.B. Saunders company , London .

18.Huff , W.E. , L.K.Kubena , R.B.Harvey , W.M.Hagler , Jr . , S.P.Swanson , T.D.Phillips and C.R.Creger . 1986 . Individual and combined effect of aflatoxin and deoxynivalenol (DON , Vomitoxin) in broiler chickens . Poultry Sci . 65 : 1291 – 1298 .

19.Kramer , B. and F.F. Tisdall . 1982 . Determination of serum calcium by oxalate precipitation and redox titration . In : Fundamental of Clinical Chemistry . Ed . Tietz , N.W. , W.B.Saunders company , London .

20.Kubena , L.F. , R.B.Harvey , T.D.Phillips and B.A.Clement . 1993 . Effect of hydrated sodium calcium aluminosilicate on aflatoxicosis in broiler chicks . Poultry Sci . 72 : 651 – 657 .

21.Nabney , J. and B.F.Nesbitt. 1965 . A spectrophotometric method of determining the aflatoxins . Analyst 90 : 155 – 160 .

22.Natt , M.P. and C.A.Herick . 1952 . A new blood diluent for counting the erythrocytes and leucocytes of the chicken . Poultry Sci . 31 : 735 – 738 .

23.Oguz , H. and V.Kurtoglu . 2000 . Effect of clinoptilolite on fattening performance of broiler chickens during experimental aflatoxicosis .Br. Poultry Sci . 41 : 512 – 517 .

24.Oguz , H. , T.Kececi , Y.O. Birdane , F. Onder and V.Kurtoglu . 2000 . Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during experimental aflatoxicosis . Res . Veter . Sci. 69 : 89 – 93 .

25.Oguz , H. , H.H.Hadimil , V.Kurtoglu and O.Erganis . 2003 . Evaluation of humeral immunity of broilers during chronic aflatoxin (50 and 100 ppb) and clinoptilolite exposure . Rev . Med . Vet. 154 : 483 – 486 .

26.Ortatatli , M. and H . Oguz. 2001 . Ameliorative effects of dietary clinoptilolite on pathological changes in broiler chickens during aflatoxicosis . Res . Veter . Sci . 71 : 59 – 66 .

27.Reitman , S. and S.Frankel . 1957 . A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase .Am.J.Clin.Path. 28 : 56 – 63 .

28.Safameher , A.R. , A.Allameh , M.Shivazad and A.Mirhadi . 2004 . The performance and haematological characters in broiler chicks fed ammonia – treated aflatoxin contaminated feed . WPC 2004 , XXII World's Poultry Congress . Istanbul , Turkey .

29.SAS . 1989 . SAS User's Guide : Statistics (Version – 5 ed) . SAS . Inst . Inc . Cary .NC . USA .

30.Scheideler , S.E. 1993 . Effect of various types aluminosilicates and aflatoxin B1 on aflatoxin toxicity , chick performance , and mineral status . Poultry Sci . 72 : 282 – 288 .

31.Sehu , A. , S. Cakir and D.Essiz . 2004 . Effect of hydrated sodium calcium aluminosilicate on aflatoxicosis in quails (*Coturnix coturnix Japonica*) . WPC 2004 , XXII World's Poultry Congress . Istanbul , Turkey .

32.Shatwell , O.L. , C.W.Hesse and W.G.Sorenson . 1995 . Production of aflatoxin on rice . Appl . Microbial . 14 : 425 – 428 .

33.Shibata , S.A. 2000 . Drug over the millennia : Pharmacognosy , chemistry , and pharmacology of licorice [review]. Yakugaku Zasshi . 120 (10) : 849 – 862 .

34.Thaxton , J.P. , H.T. Tung and P.B.Hamilton . 1974 . Immunosuppression in chicken by aflatoxin . Poultry Sci . 53 : 721 – 725 .

35.Utsunomia , T. , M.K.Kobayashi , D.N.Herndon , R.B.Pollard and F.Suzuki . 1999 . Effects of glycyrrhizin , an active component of licorice root on *Candida albicans* infection in thermally injuried mice . Clin . Exp . Immunol . 116 : 291 – 298 .

36 Varley , H. , A.H.Gowenlock and M.Bell . 1980 . Practical Clinical Biochemistry . 5 th ed. William Heinamann Medical Books Ltd . London .

37 Wotton , I.D.P. 1964 . Micro Analysis in Medical Biochemistry . 4 th ed . Churchill Livingston , London .