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Effect of vitamin E and selenium supplement in reducing aflatoxicosis on performance and blood parameters in broiler chicks

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Abstract

This study was aimed to investigate the sufficiency of Vitamin E and Selenium Supplementation to Diets against containing aflatoxins on the relative organ weights and various structural blood parameters. One hundred and twenty unsexed Ross birds were used from 3 to 7 weeks of age. Birds were randomly distributed and subjected to five nutrition treatments as follows: (T1. Control Group: 0.0 AF + 0.0 Se +0.0 Vit. E, T2. 2.5 mg AF/kg diet, T3. 2.5 mg AF /kg diet + 0.18mg/kg Se +10 I.U Vit. E, T4. 2.5 mg AF/kg diet + 0.32mg/kg Se +30 I.U Vit. E, T5. 2.5mg AF /kg diet + 0.50mg/kg Se +50 I.U Vit. E) pollution by aflatoxins causes a significant increase in relative weights of liver, heart, gizzard, abdomen fat, and spleen. A significant decrease in total body and carcass weight gastrointestinal tract long. Moreover existence of aflatoxins caused a significant decrease in values of Packed cell volume (PCV), Red blood cells counts (RBC), Hemoglobin (Hb), mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC) significantly moreover, it has been noticed a significant increase occurrence in values of total white blood cell (WBC) and ration of Heterophills to lymphocytes in second treatment birds influenced by Aflatoxins. The addition of graded levels of Vit. E and Se to the AF containing diets (T4, T5) improvement total body and carcass weight and internal organs studied. The addition of both Vit. E and Selenium (T3, T4, T5) to the provender containing by aflatoxins, had no effects on blood parameters to their natural averages at control group. The existence of the two Vit. E and Selenium in polluted provender doesn't prevent a decrease in these values with a significant improvement (up to 10 I.U Vit. E and 0.18mg/kg Se) occurrence from birds group values which had been fed by polluted provender only.

Keywords: Vitamin E, Selenium, Aflatoxins, Broiler.
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تأثير اضافة فيتامين E وعنصر السلينيوم لتقليل تاثير سموم الافلا على الاداء ومعايير الدم في فروج اللحم

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الخلاصة

هدفت الدراسة إلى معرفة تأثير إضافة فيتامين E وعنصر السلينيوم إلى علائق فروج اللحم في الحد أو التقليل من تأثير سموم الافلا على ألأوزان النهائية والنسبية للأعضاء الداخلية ونسبة التصافي وصورة الدم الكاملة والعد التفريقي لكريات الدم البيضاء. استخدم في الدراسة (١٢٠) طائرا غير مجنسً من سلالة Ross بعمر ٣ أسابيع قسمت على خمس معاملات، وزعت الطيور عشوائيا بواقع (١٥) مكررا (ثلاثة لكل معاملة) وتضمن كل مكرر منها (٨) طيور. وكانت المعاملات التغذوية كالأتي (T1: معاملة السيطرة، T2: 2.5 ملغم سموم الافلا/كغم علف، T3: 2.5 ملغم سموم الافلا/كغم علف + 10 I.U فيتامين E+ 0.18 ملغم /كغم علف السلينيوم، T4: 2.5 ملغم سموم الافلا/كغم علف + 30 I.U فيتامين E +0.32 ملغم /كغم علف السلينيوم، T5: 2.5 ملغم سموم الافلا/كغم علف + 50 I.U فيتامين E + 0.50 ملغم /كغم علف السلينيوم). أشارت نتائج التحليل الإحصائي إلى أن تلوث عليقه الطيور بسموم الافلا 2.5 ملغم سموم الافلا/كغم علف أدى إلى انخفاض معنوي (P<٠,٠٥) في الوزن الحي ووزن الذبيحة وطول الأمعاء ونسبة التصافي وقيم خضاب الدم،

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عدد كريات الدم الحمر، حجم الخلايا المضغوطة، معدل حجم كرية الدم، معدل خضاب الكرية، معدل تركيز خضاب الكرية والعد التفريقي لكريات الدم البيضاء (وحيدة النواة واللمفية والقعدات والحمضة) مقارنة مع مجموعة السيطرة مع وارتفاع معنوي في الوزن النسبي للقانصة ووزن الكبد، الطحال، القلب، شحم البطن والعد الكلي لكريات الدم البيضاء ونسبة الخلايا العدلات الى اللمفية، ولم يسجل في نتائج المعاملتين الرابعة والخامسة وجود فروق معنوية في الوزن الحي ووزن الذبيحة وطول الأمعاء ونسبة التصافي والوزن النسبي للقانصة ووزن الكبد، الطحال، القلب، شحم البطن مقارنة مع السيطرة مع تحسن معنوي واضح في قيم خضاب الدم، عدد كريات الدم الحمر، حجم الخلايا المضغوطة، معدل خضاب الكرية، معدل تركيز خضاب الكرية بالمقارنة مع نتائج المعاملة الثانية لم يرتقي إلى نتائج طيور السيطرة عدا قيمة معدل حجم كرية الدم. و سجلت ذات المعاملتين (T4,T5) تحسن معنوي في عدد كريات الدم البيضاء والعد لوحيدة النواة واللمفية لم يصل الى مستوى المعنوية في مجموعة المقارنة في حين لم تسجل فروق معنوية للحمضية مقارنة مع نتائج مجموعة السيطرة.

Interoduction

Aflatoxins (AF) are a secondary fungal metabolites produced by some strains of *Aspergillus flavus* and *Aspergillus parasiticus.* Aflatoxin B1, B2, G1 and G2 are natural contaminants of many animal feed ingredients. AFB1 is the most abundant and toxic form of all naturally occurring aflatoxins also represents 75% of all aflatoxins found in contaminated food and feeds. It is hepatotoxic (1), hepa-tocarcinogenic (2), and teratogenic (3) to various animal species. They were classified as a group I carcinogen in humans (4) Aflatoxin B1 is biotransformed in the liver by monoxygenases and then transformed by cytochromo P450 into aflatoxin 8-9 epoxide and hydroxylated metabolites (AFM1, AFP1, AFQ1 and aflatoxicol) (5). Both Vitamin E and Selenium are essential nutrients for humans and animals. They are involved in the protection of biological membranes against lipid peroxidation and preventing the free radicals damage to phospholipids membranes and enzymes (6). Vitamin E is the major lipid soluble antioxidant that is present in biomembranes, scavenges free radicals in the early stages of lipid peroxidation and Selenium is essential to the activity of glutathione peroxidase (GSH – Px) which reduces H_2O_2 and lipid hydroxides to less reactive products (7). It is well understood that Selenium has a strong interaction with vitamin E in the protection of cell from important molecules (6). Alpha – tocopherol and glutathione peroxide protect tissues from oxidative damage associated with the free radicals generated during the respiratory burst of macrophages and neutrophils (experienced during immune response) (8) and improvement the bird health and immune status, Vitamin E and Se are antioxidants essential for cell survival in environments containing peroxides (9,10)

The response of broilers to Se supplementation could vary greatly with the level of Vit. E in the diet (6) Selenium is between 50 and 100 times more effective as an antioxidant than vitamin E. while Se supplementation rate of 0.1-0.5 mg/kg is satisfactory for most animals including poultry (11) Vitamin E was reported as an excellent biological chain – breaking antioxidant that protects cell and tissue from lipoperoxidative damage induced by free radicals (12,13). Chickens, however can not synthesize Vit. E such requirements must be met from dietary sources (14) Supplementation with 100 I.U of Vit. E /kg feed has been shown to decrease the concentration of malondialdehyde a product of lipid peroxidation in the livers of chickens fed ochratoxinin A and T-2 toxin compared to dietary level of 10 I.U of Vit. E /kg feed (15). A highly active electrophilic compound that is inactivated by conjugation with glutathione –s- transferase (GST) to the AFB – glutathione and excreted through urine and bile (16). High concentration of aflatoxin B_1-GSH-Px conjugate a nontoxic metabolite was produced in bile acid from rats fed diets containing aflatoxin with Se. Thus dietary addition of Vit. E and Se may alleviate the aflatoxicosis by increasing GSH-Px activity from 144.5 IU/g Hb to186.2 IU/g Hb and eventual changes of toxic substances to inert metabolites (6) The objectives of the present study was to evaluate the effect of dietary aflatoxin contamination alone or with the addition of Vitamin E and Se on broiler performance and blood parameters.

Materials and methods

The experiment was conducted at the poultry production farm, College of Agriculture, Tikrit University during the period from 4th March to2nd April -2009. The total number birds was 120 twenty one day-old Ross unsexed chicks were individually weighed, wing – banded, and housed in 15 pens under continuous lighting. Chicks divided at random into 5 treatment groups. Each one contains 3 replicates. Eight broiler chicks for each replicate group were fed corn – soybean meal based finisher diet was obtained from it contained 18.3 % crude protein, 1.30 % lysine and 0.52%methionine, 3100 Kcal /Kg metablizable energy. The feed and water were *ad-libitum*.Chicks were randomly assigned to the following treatment groups (T1. Control group: Corn – soybean basal diet+ 0.0 AF + 0.0 Se

+0.0 Vit. E, T2: basal diet+ 2.5 mg AF/kg diet, T3: basal diet+ 2.5 mg AF /kg diet + 0.18mg/kg Se +10 IU Vit. E, T4: basal diet+ 2.5 mg AF/kg diet + 0.32mg/kg Se +30 IU Vit. E, T5: basal diet+ 2.5mg AF /kgdiet+0.50mg/kgSe+50I.UVit. E) (Se and Vit. E were Obtained from the state company for drugs industry and medical appliances, Samarra). Aflatoxin (AF) was prepared through inoculation of rice by *Aspergillus parasiticus* NRRL 2999 (generously from the college of agriculture, Tikrit Univ., Salah Aldeen, Iraq) as described by (17) and modified by (18). Fermented rice was then autoclaved and ground, Aflatoxin concentration was measured by spectrophotmetric analysis (19,20) Later the rice powder was then mixed with the diet to obtain the level of 2.5 mg AF/kg. Six birds from each treatment groups were bled by cardiac puncture for determination of blood picture. The total erythrocyte count was measured according to (21). Hemoglobin was determined by the method of (22) Haematocrits were measured by the micro- haematocrits centrifugation method while the mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated (23) the same birds were then killed by cervical dislocation Abdomen fat, liver, kidneys, spleen, proventriculus, gizzard and heart were carefully removed to determine their relative weights and dressing percentage of carcasses. Data for all response variables were subjected to ANOVA. The results obtained were statistically analyzed using (24). Mean showing significant differences were compared by multiple range test (25). were considered significant based on the 0.05 level of probability.

Results

The effect of AF alone and when combination with graded addition of Vitamin E and Se on performance of growing chicks and the relative weight of internal organs from 21 day –old to the age of 46 day –old show in table (1). Chicks fed the diet containing 2.5mg AF/kg had a significantly ($P<0.05$) lower total body and carcass weight with length of the gastrointestinal tract. The results indicated that the presence of AF alone in the diet caused a significant enlargement in the size of internal organs like liver, kidney, spleen, gizzard, heart and abdomen fat. These findings agreed with those obtained previously (26-28) the liver is considered the principle target organ for AF it was evident from the data of the present study that feeding the diet containing AF alone resulted in a significant increase in the relative weight of liver as well as other internal organs including heart kidneys gizzards proventriculus spleen and pancreas. The results of third treatment differ significantly from that of the control group total body, carcass weight and gastrointestinal tract long with a significant ($P<0.05$) increased relative weights internal organs. The graded addition of Vit. E and Se at the two stated levels T4 and T5 ameliorated parameters mentioned above in comparison those of the control group or those of birds fed the diet containing AF alone. It is thought that the addition of Vit. E and Se (T4,T5) to the AF containing diets helped in restoring the relative weights of liver, kidney, spleen, proventriculus, gizzard and heart close to those of the control group. No significant differences were observed in relative weight of the above mentioned organs between those of the control group and those fed the AF containing diets amended with 0.32mg/kg Se +30 IU Vit. E and 0.50 mg/kgSe+50 IU Vit. E. The toxin fed birds treated with vitamin E and Se showed a significant gain of body weight as compared to toxin alone fed birds indicating the beneficial effect of vitamin E and Se on total body weight gain during aflatoxicosis.

The effect of aflatoxin with combination with Vitamin E and Se on selected blood parameters are presented in table (2). Values of hemoglobin, haematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were significantly ($P<0.05$) decreased in chicks fed the diet containing AF alone. Blood picture of treatment three was significantly decreased in Hb, RBC, PCV, MCH and MCHC value when compared with control group. Except MCV value which no significant differences were observed these findings are in agreement with those obtained previous (29,30). The addition of Vit. E and Se at the two stated levels (T4 and T5) was similarly effective in alleviating the negative effect of AF on all blood parameters Hb, RBC, PCV, MCH and MCHC significantly improved the values of when compared with those of birds fed the diet containing AF alone but they were still significantly lower then those of the control group. Data presented in table (3) show the effect of dietary treatment on the results indicated that the presences of Aflatoxin alone in the diet caused a significant ($P<0.05$) increase in leukocytes, heterophils and H/L ratio compared with those of birds from the control group. with significant ($P<0.05$) decrease in monocytes, lymphocytes, basophile and eosinophi same effect shown in treatment three birds result except eosinophi value These findings agreed with those obtained previous (31,32). The addition of Vit. E and Se to the diet had significant ($P<0.05$) effect on studied (T4 and T5) with those of control group. Furthermore levels of birds fed the AF containing diet amended with Se and Vit. E However although the addition of Vit. E and Se to the AF contaminated diets significantly improved the values of when compared with those of birds fed the diet containing AF alone but they were still significantly lower than those of the control group. The results of this study suggested that the Vit. E and Se in combination can partially ameliorate the toxicity of AF in broilers.

Table (1) Effect of graded addition of Vitamin E and Se on relative organ weights of chicks fed diets containing 2.5mg AF/kg diet.

Treatments	AF mg/kg	Vit.E IU	Se mg/kg	Final body weight	Carcass weight	Gastro-intestinal tract	Relative organ weight (g/100 body weight)							dressing percentage
							Proventriculus	Gizzard	Liver	Pancreas	Spleen	Heart	abdomen fat	
T1	0	0	0	2222.5 ± 28.7 a	1672 ± 21.7 a	180.5 ± 4.51 a	0.378 ± 0.02	1.180 ± 0.01 b	1.722 ± 0.02 b	0.210 ± 0.01 ab	0.112 ± 0.01 b	0.455 ± 0.03 b	1.442 ± 0.05 b	75.55 ± 1.08 a
T2	2.5	0	0	1682.5 ± 35.9 b	1182 ± 42.2 b	162.2 ± 2.05 c	0.379 ± 0.07	1.202 ± 0.01 a	1.857 ± 0.03 a	0.227 ± 0. 01 ab	0.185 ± 0.01 a	0.745 ± 0.09 a	1.655 ± 0.05 a	70.15 ± 1.26 b
T3	2.5	10	0.18	1773.8 ± 55.9 b	1250 ± 59.5 b	163 ± 2.58 c	0.376 ± 0.04	1.197 ± 0.01 ab	1.850 ± 0.04 a	0.242 ± 0.02 a	0.182 ± 0.01 a	0.637 ± 0.12 a	1.640 ± 0.10 a	70.80 ± 1.14 b
T4	2.5	30	0.32	2180 ± 167.9 a	1623 ± 126.9 a	170.7 ± 3.1 b	0.379 ± 0.02	1.185 ± 0.01 ab	1.772 ± 0.03 b	0.205 ± 0.01 b	0.122 ± 0.02 b	0.420 ± 0.02 b	1.490 ± 0.09 b	75.02 ± 0.73 a
T5	2.5	50	0.50	2344 ± 172.5 a	1756 ± 118.5 a	178.7 ± 4.91 a	0.376 ± 0.04	1.192 ± 0.01 ab	1.720 ± 0.01 b	0.207 ± 0.01 b	0.112 ± 0.01 b	0.417 ± 0.02 b	1.467 ± 0.05 b	74.92 ± 0.76 a

Means within a column with different letters differ significantly (P<0.05).

Discussion

There are many reports on the effects of various foods or nutrients and xenobiotics on AFB1-macromolecule adducts formation. Obviously the major objectives of these studies were to determine if and how those nutrients or xenobiotics could affect adducts formation especially DNA adduct. This study was undertaken to evaluated the effect of feeding AF containing diet amended with Vit. E and Se.Increasing Vit. E level in the diet from minimum requirement recommended by (33) 10 IU to 50 IU kg/ diet and Se from 0.18 to 0.50 mg/kg diet influence important production parameters such as performance of growing chicks, relative weight of internal organs, hemoglobin, haematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and the mean corpuscular hemoglobin concentration. (34) reported that the combined Se (0.2 mg/kg as Na2 SeO3)-VE (100 IU/kg) deficiency enhances activation or inhibits detoxification of aflatoxin B_1 1mg/kg (decreased AFB_1 binding to DNA, RNA, and protein) in white leghorn chicks which was completely effective in preventing oxidative diathesis and death. (35,36) reported that high concentration of aflatoxin B1-GSH – Px conjugate non toxic metabolite was produced in bile acid from rats red diets containing aflatoxin with Se. Thus dietary addition of Se may alleviate the aflatoxicosis by icreasing GSH – Px activity and eventual changes of toxic substances to inert metabolites. They also reported that the Vit. E reduced the formation of AFB_1 adducts in the liver. The observation that the rats fed AF free diet containing 120 IU VitE/kg showed significantly enhanced blood GSH – Px The feeding of AF diets containing 60 IU Vit. E/kg induced a significant decrease in the liver lipid contents (37).

Table (2) Effect of graded addition of Vitamin E and Se on hematological values of chicks fed diets containing 2.5mg AF/kg diet.

Treatments	AF mg/kg	Vit.E IU	Se mg/kg	Hemoglobin g/dl	R.B.C (10^6 / mm^3)	P.C.V%	MCV (μm^3)	MCH pg	MCHC g/dl
T1	0	0	0	9.62± 0.22 a	3.12± 0.12 a	35.75 ±0.95 a	114.65± 2.40 a	31.27± 0.97 a	26.87± 0.17 a
T2	2.5	0	0	5.05± 0.19 c	2.27±0.05 c	26.75± 0.95 c	90.82± 5.40 b	22.24± 0.34 c	18.82± 0.05 c
T3	2.5	10	0.18	5.12± 0.09 c	2.31± 0.04 c	26.50± 0.57 c	114.61± 1.87 a	22.16 ±0.38 c	19.32± 0.18 c
T4	2.5	30	0.32	8.12 ±0.66 b	2.78± 0.11 b	31.00± 1.41 b	111.51± 0.55 a	29.19± 1.18 b	26.12± 0.97 b
T5	2.5	50	0.50	8.13± 0.31 b	2.79 ±0.07 b	31.25± 0.95 b	112.19 ±1.31 a	29.16± 0.55 b	25.95± 0.26 b

Means within a column with different letters differ significantly (P<0.05).

Table (3) Effect of graded addition of Vitamin E and Se on Differential count of normal leukocytes chicks fed diets containing 2.5mg AF/kg diet.

Treatments	AF mg/kg	Vit.E IU	Se mg/kg	Leukocytes (10^6 / mm^3)	Monocytes %	Lymphocytes %	Heterophil %	Basophile %	Eosinophil %	H/ L ratio
T1	0	0	0	24.40 ±0.07 c	6.51 ± 0.09 a	61.32 ± 0.30 a	25.17 ± 0.21 c	4.93 ± 0.13 a	2.04 ± 0.15 a	0.40 ± 0.05 c
T2	2.5	0	0	32.29 ± 0.50 a	3.97 ± 0.16 c	47.14 ±0.12 c	44.98 ± 0.12 a	3.64 ± 0.36 b	0.24 ±0.20 b	0.95 ± 0.05 a
T3	2.5	10	0.18	32.12 ± 0.48 a	4.02 ± 0.11 c	47.06 ± 0.18 c	43.71 ± 0.54 a	4.06 ± 0.14 b	1.13 ±0.37 a	0.92 ± 0.01 a
T4	2.5	30	0.32	29.34 ± 0.38 b	6.09 ± 0.16 b	52.98 ± 0.22 b	36.72 ± 0.81 b	3.33 ± 0.22 b	0.87 ± 0.42 ab	0.69 ±0.02 b
T5	2.5	50	0.50	28.73 ± 0.43 b	6.25 ± 0.09 b	52.77 ± 0.41 b	35.62 ± 0.41 b	3.70 ± 0.55 b	1.48 ± 0.51 a	0.66 ± 0.01 b

Means within a column with different letters differ significantly (P<0.05).

The inhibition of body growth and the decline in carcass muscles weight, muscle protein and RNA synthesis appear to be solely the result of the profound effect of AF. Inhibition of cellular processes the most prevalent symptom of aflatoxicosis in poultry is reduced growth rate and poor performance. These adverse effects of AF due to anorexia, listlessness, inhibition protein and DNA synthesis and lipogenesis (38) AF appears to exert its negative effect on birds chiefly by depressing DNA and RNA synthesis and protein synthesis which eventually reflected in the final body weight and later carcass yields (49). (40) find out the influences of 2 mg/kg of aflatoxin B_1 and immunomodulators on the performance of broiler, aflatoxin B_1 alone had significantly reduced the body weight feed efficiency carcass yield immune development against Newcastle disease in broilers supplementation of lactobacilli, Vit. E and Se had improved the body weight, feed efficiency, carcass yield and immune against Newcastle disease. Feeding of aflatoxin B_1 (1 mg/kg) to 2-week old Japanese quail for a period of 8 weeks indicating that supplementation of selenium selentie 5 mg/kg had some protective action against the toxic effect microscopic changes in the liver heart and bursa of fabricius lymphoid aggregation in liver aflatoxin B_1 (41). An improved antioxidant status was observed in chicks of second group with α-tocopherol and selenium supplementation including higher concentration vitamin E, increased activity of glutathione peroxidase and lower levels of lipid peroxidation (42).

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References

1. O'Brien K, Moss E, Judah K & Neal G. Metabolic basis of the species difference to aflatoxin B1 induced hepatotoxicity. Biochem Biophy Res Commun.1983. 114: 813-821.
2. Adamson RH, Correa P, Seiber, SM, McIntire, K & Dalgard D.W. Carcinogenicity of aflatoxin B1 in Rhesus monkeys: two additional cases of primary liver cancer. J Natl Cancer Inst. 1979;57:67-68.
3. Bassir O & Adekunle A.Teratogenic action of aflatoxin B1, palmotoxin, B0 and palmotoxin G0 on the chick embryo. J Pathol.1970;102: 49-51.
4. International Agency for Research on Cancer (IARC).IARC Monographs on the evaluation of carcinogenic risks to human. some naturally occurring substances: Food Items and Constituents, Hetrocyclic Aromatic Amines and Mycotoxins.1993;56: 362-395.
5. Monroe D H,. Eaton D L. Comparative effects of butylated hyroxyanisole on hepatic in vivo DNA binding and in vitro biotransformation of aflatoxin B1 in the rat and mouse. Toxicol Appl. Pharm.1987;90: 401-409.
6. Choct, M. and A. J. Naylor. The effect of dietary Selenium Source and vitamin E levels on performance of male broilers. Asian –Aust. J AnimSci. 2004; 17:1000-1006.
7. Hoekstra W C. Biochemical function of Selenium and its relation to vitamin E. Fed Proc.1975;34:2083-2089.
8. Aseltine M S. Critical role of Selenium and vitamin E nutrition and immunity:The potential for improving Selenium availability by microbial accumulation.Biotechnology in the feed Industry.proc. Alltech's 8th Annal sym. Nottingham Untied press Nottingham,UK.PP.1992.23-31.
9. Mitsumoto M R N, Arnold D M., Schaefer and R G. Cassens. Dietary vitamin E supplementation shifted weight loss from drip to cooking loss in fresh beef longissimus during display J Anim Sci.1995;73:2289-2294.
10. Surai P F. Selenium in poultry nutrition: A new look at an old element.1.Antioxidant properties, deficiency and toxicity. Worlds Poult Sci.. 2002;58B:333-347.
11. Mervyn L. The dictionary of minerals,the complete Guide to minerals therapy.Lothian publishing,Melbourne. Australia .pp.1985.173-177.
12. Halliwell B, M. C. Gutteridge. Lipid peroxidation A radical chain reaction.Pages 188-218in:Free radicals in biology and medicine.2nd ed Oxford Univ. press, New York,NY.1989.
13. Yu B P. Cellular defenses against damage from reactive oxygen species. Physiol Rev.1994;74:139-162.
14. Chan K M. and E A. Decker. Endogenous skeletal muscle antioxidants. Crit Rev Food Sci Nutr.1994;34:403-426.
15. Hoehler,D.,and R. R. Marquardt,..Influence of vitamin E and C on the toxic effects of Ochratoxin A and T-2 toxin in chicks. Poult Sci.1996; 75:1508-1515.
16. Eaton D L, Gallagher E P. Mechanisms of aflatoxin carcinogenicity. Ann Rev Pharmacol. Toxicol.1994; 34: 135-172.
17. Shotwell O L, C W Hessettine, R.D. Stubblefield and W.G. Sorenson. Production of aflatoxin on rice. Appl Microbiol.1966; 14:425-428.
18. West S, Wyat R D, P B. Hamilton.Increases yield of aflatoxin by incremental increases of temperature. Appl Microbiol.1973;25:1018-1019.
19. Nabney J, Nesbitt B F. A spectrophotometric method of determining the aflatoxin Analysis.1965;90:155-160.
20. Wiseman H. G, W C. Tacobson and W.E. Harmeyer. Note on removal of pigments from chloroform extracts of aflatoxin cultures with copper carbonate. J Asso Agric Chem. 1967;50:982-983.
21. Natt. M.P, Herrick C A.. A new blood diluent for counting the erythrocytes and leucocytes of the chicken. Poult Sci.1952;31: 735–738.
22. Sunderman F W, MacFate D A, McFayden G F, B C Copeland.. Symposium clinical haemoglobinometry. Am J Clin path. 1953;23:519-598.
23. Jain, N C. Schalam's Veterinary Haematology.4 th ed.,Lea and Febiger. Philadelphia. 1986, pp..35-36.
24. SAS Veraion, Statistical Analysis System .SAS Institute Inc., Cary, NC.27512-8000,USA. 2001.
25. Duncan D B. Multiple range and F., test Biometric. 1955;11:42.
26. Huff W E, Kubena L F, Harvey R B, Phillips T D. Efficacy of hydrated sodium calcium aluminosilicate to reduce the individual and combined toxicity of aflatoxin and ochratoxin A. Poult Sci. 1992.71:64-69.
27. Kubena L F, Harvey R.B, Huff W E, M.H. Elissalde, Yersin A G,. Philips T D, Rottinghous G E. Effect of hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and diacetoxyscripnol. Poult Sci. 1993. 72: 51 – 59.
28. Kubena,L. E.;T.S. Edrington;C. Kamps-ToHzapple, ; R.B.,Harvey;M.H.Elissalde and G.E.Rottinghaus.Individual and combined effects of fumonisin B1 present in fusarium moniliformine culture material and aflatoxin in broiler chicks. Poult. Sci.1997. 16:265-270.
29. Kubena,L.F., ;R.B.Harvey ;W.E.Huff ;D. E.Corri ;T.D. Phillips and G.E. Rottinghans. Efficacy of ahydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin.Poult. Sci.1990. 69:1078-1096.
30. Shareef, A.M. ; K.M.T., Al-Jubory, and M.G., Hassan. Effect of activated charcoal in reducing dietary aflatoxin – induced stress in broiler chicks. Iraqi Journal of Veter. Sci. 1998. Vol. (11). No. (1).
31. Ibrahim,I.K.,;A.M.shareef and K.M.T. Al-Jubory. Efficiency of sodium bentonite in Reducing aflatoxicosis in growing chicks effects on blood parameters and aflatoxin induced stress.Iraqi J. Vet. Sci.,2001. Vol.14,No.2,(211-218).
32. 33.Al-Jubory,K. MT., Shareef and A.M.,Ibrahim,I.K. Efficiency of sodium bentonite in reducing aflatoxicosis in growing chickns effect on performance and blood chemistry.Iraqi J. Vet. Sci. 2001;2:223-230.
33. NRC, National Research Council. Nutrient Requirement of Poultry National Academy Press. Washington, DC. 1994.

34. Chen J, Goetchius M.P, Campbell T C and Combs G F Jr. Effects of dietary selenium and vitamin E on hepatic mixed-function oxidase activities and in vivo covalent binding of aflatoxin B1 in rats. J Nutr.1982.; 2: 324:331.
35. Degan,G.H. and H.G. Newman. The major metablites of aflatoxin B1in the rat is aglutathinon conjygate.Chem.Biol.Intactt.1978;22:239-255.
36. Weiss W P, Hogan J S, Smith K L, Hobiet K H. Relationship among selenium, vitamin E, and mammary gland health in commercial dairy herds. J Dairy Sci.1990;73:381-390.
37. 38.Choi,Y.K.,K.K.J.jung,K.Y.Chae,I.Jang,B.D.Lee.and K.H.Nahm..Effects of vitamin E and Selenium supplementation to diet containing aflatoxin B1on the contents of liver lipids and various blood parameters in rats. AJAS.1995;8 :329-385.
38. Oguz H., Kurtogh V. Effect of clinoptilolite on fattening performance broiler chickens during experimental aflatoxincosis. Brit Poult Sci.. 2000; 4:512-517.
39. Afzal N, Devegowda. G D. The effect of graded levels of dietary aflatoxin on certain biochemical parameters in broiler breeders.WPC 2004 XXII World's poultry Congress, Istanbul, Turkey.2004.
40. Mani,K K, Sundaresan and Viswanathan K. Effect of immunomodulators on the Performance of broilers in aflatoxicosis. Indian Vet J 2001; 78:1126-1129.
41. Jakhar K K, Sadana J R. Sequential pathology of experimental aflatoxicosis in quail and the effect of Selenium supplementation in modifying the disease process. Mycopathologia. 2004;157(1):99-109.
42. Sodhi, S. A. Sharma A.P S. Brar R. S. Effect of α tocopherol and Selenium on antioxidant status, lipid peroxidation and hepatopathy induced by malathion in chicks. pesticide Biochemistry and physiology.2007; 90:82-86.