

## **Effect of using two types of local mineral mud (mohammed – torhan and Kirkuk) instead of activated sodium bentonite upon the haematological and biochemical parameters for female quails fed diet contaminated with aflatoxin**

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### **Abstract**

Two hundred and twenty four Japanese quail females ( 42 days old ) were randomly fed control diet and with thirteen diets contained two levels ( 0.4 and 0.8% ) of each local mineral muds: M – T, K and A. S. B with or without 2.0mg of AF/kg diet for 8 weeks. The statistical analysis showed significant decrease (  $P \leq 0.05$  ) the value of the haematological and serum biochemical parameters and some blood enzymes activity ( GOT, GPT and ALP ) of the quail females fed diet contaminated with 2.0mg of AF/kg diet. The addition of the natural adsorbents ( M–T,K and A. B. S ) led to significant correct the abnormality of the haematological, serum biochemical parameters and activity of some blood enzymes except the value of MCHC by adding 0.8% of M – T or K, mud noted there were no significant differences between the efficiency the three kinds of natural muds used in this study.

### **Introduction**

Aflatoxins ( AF ) are a class of mycotoxin produced by the *Aspergillus parasiticus*, *Aspergillus nomino* caused negative effects upon the performance of broilers, layers and quails ( Phillips et al, 1995, Miazzo et al, 2000, Phillips et al, 2002 and AL-Neemi, 2004). On the other hand these toxins cause abnormality of the blood physiological (haematological)and serum biochemical parameters value ( Huff et al, 1986b, 1988, Jindal et al, 1994, Abo – Norag et al, 1995, Kubena et al , 1995, 1997, Kececi et al, 1998 and Ibrahim et al, 2001).As well as the presence of the aflatoxin in the diet reduce the activity of Gamma Glutamy Transferase (GGT), Alkaline phosphatase (ALP) and Lactate Dehydrogenase (LDH) (Kubena et al, 1990, Huff et al, 1992). Feeding diet contaminated with 2.5mg of (AF) / kg diet led to decrease the activity of the Glutamic Oxaloa Transaminase (GOT) , (ALP) and (LDH) . Natural

adsorbents as muds the diets or feed stuffs contaminated with aflatoxin. The aim of this study was to compare the efficacy of the two kinds of local mineral mud (Mohammed – Torhan and Kirkuk) with (A.S.B) as adsorbent agent for aflatoxins in the females quail diets.

### **Material and Methods**

Aflatoxins were produced by fermentation of rice using *Aspergillus parasiticus* NRRL 2999 according to Shuttwell (1966) and determined spectrophotometrically (Nabney and Nesbit, (1965) as described by Wiseman *et al* (1967). Fermented rice then also was autoclaved and grounded. The rice powder was incorporated into basal diet and confirmed by High pressure liquid chromatography (HPLC) to provide the described level of 2.0mg of AF/kg diet (Davis and Diener,1980). At the age of 84 days, the study was terminated, 3 birds (females) from each replicate were bled by cardiac puncture for haematological and serum biochemical parameters. The haematological parameters determined according to Coles (1986) and the serum biochemical parameters determined according to Varley (1980). The birds were killed by cervical dislocation. The activity of the GOT and GPT were determined by using colourmetric reagent (Dinitrophenyl hydrazine 2.4) which were prepared from Randox Laboratories, U. K. while the activity of the ALP enzymes determined by using colourmetric reagent (Sodium Arsenate and Potassium Ferric Cyanide) which were prepared by Biomérieux, France. Four replicates per treatment (four females of 42 days old for each replicate) were kept under ideal conditions and fed the experimental diets. Birds fed basal diet (Table 1) and the levels of critical nutrients recommended by NRC (1994). The experiment diets (treatments) were treatment 1: control diet (basal diet) without addition (M-T mud), (K mud), (A. S. B) and 2.0mg (AF) /kg diet, Treatment 2: 2.0 mg (AF)/kg diet add to control diet, Treatment 3: 0.4% (A. S. B) add to control diet, Treatment 4: 0.8% (A. S. B) add to control diet, Treatment 5: 0.4% (M – T) mud add to control diet, Treatment 6: 0.8% (M – T) mud add to control diet, Treatment 7: 0.4% (K) mud add to control diet, Treatment 8: 0.8% (K) mud add to control diet, Treatment 9: 0.4% (A. S. B) add 2.0mg (AF) / kg diet add to control diet, Treatment 10: 0.8% (A. S. B) + 2.0mg (AF)/ kg diet add to control diet, Treatment 11: 0.4 % (M –T mud + 2.0mg (AF)/kg diet add to control diet, Treatment 12: 0.8% (M – T) mud + 2.0 mg (AF)/ kg add to control diet,

Treatment 13: 0.4 (K) mud + 2.0 mg (AF) /kg diet add to control diet,  
 Treatment 14: 0.8% (K) mud + 2.0mg (AF)/ kg diet add to control diet.

Table 1: Ingridents of the basal (control) diet for quail male.

Feed Ingridents	%
Ground wheat	57.00
Soybean mael (44% protein)	30.00
Vegetable fat	5.50
Limestone	5.20
Dicalicum phos phate	1.90
Nacl	0.15
vitamins and minerals premix	0.10
DL-Methionine	0.15
Total	100
Chemical analysis calculated:	
Crude protein %	20.4
Metabolizable energy (kcal/kg diet)	2960
Methionine + cystine %	0.75
Calcium %	2.5
Avilable phos phourrs %	0.35

Bentonite is mean the added of mud material to the Poultry diets, but essentially this name is mean a type of clay stone that almost composed of Bentonite as a clay Mineral. The chemical composition of Bentonite is silicate, Oxygen, Aluminum, Hydroxide and a mount of Calcium or Magnesium or Sodium. In Iraq the abundance of Bentonite is rare (Babat, 1980) . So we tried to use another Rock that mineral different composition but gives the same effect and behavior with Poultry diets and that was Marl. Marl composition is mainly carbonat ( $CaCO_3$ ) with clay minerals (that mean mixing of different type of minerals (Carbonate – Clay Mineral – Quartz)).

Physical property of local Mud Marl:

The local Muds that we used is two type of Marl (Commercial name M. T. M. K. M) in fact it just gives some differences in grain size and a mount of Quartz.

Kind of Marl has:

Color stability.

No Radio activity.

Zero Salinity.

In the other hand the size analysis of the two types M. T. M), K. M is

mainly clay size with some of silt and fine sand size (Carroll, 1970) noted in the table (2).

Local Mud type	Fine sand	Silt	Clay
M. T. M	< 4 %	18 %	78 %
K. M	< 4%	30 %	66 %

### **Mineralogical Properties:**

By using of (X. R. D) X- Ray Diffraction Trechique (Brindly&Brown, 1980) for the local muds recognized several type of mineral and that were: Clay Mineral : Mainly Montmorillinite with some of chlorite and illite (Sperated among them by heating and Glicol athiline (Grim,1968)).

Carbonate.

Quartz.

The mount of Carbonate and Quartz was in small amount (A-1), fig(B-1).

Montmorillonite is in fact the best type of clay minerals in a kind of adsorption and absorption because of its shape and high ionic exchange capacity (Banat, 1980). This Mineral like the other clay minerals composed of two sub blocks. Tetrahedral (SiO<sub>4</sub>) as a symbol (T) fig (a-2). Octahedral Al (oH) 6-3 as a symbol (O) fig (B-2) these sub blocks (T,O) arranged in T-O-T as a sheet fig (3) (Dabbag, 1990) and make block so between every two block there is a gap and it is good trap for different material armored the clay mineral this material captured in this gap or by some exchanges between chemical element and some (a,Mg,Al). Data were statistically analyzed using the general Model procedure (SAS, 2001). Means for treatments showing significant differences in the analysis of variance were compared using Duncan's new multiple range procedure (1955). (Duncan, 1955).

### **Results**

The value of the biochemical serum parameters: Total protein, cholestrol, glucose, uric acid and triglyce rides (Table 4) and the active of the blood enzymes: ALP, GPT and GOT ( Table 5) significantly decreased for serum of quail females fed diet contaminated with 2.0mg of AF/Kg diet. On the other hand the values of the haematological parameters: PCV, RBC, WBC, HB and H/L were significantly lower for birds fed diet contained 2.0mg of AF/kg diet when compared that with another treatments (Table 3).

## **Discussion**

The present hematological parameters (Table 3) indicate the significant ability of the three kinds of adsorbents ( M – T, K and A. B. S) to correct the abnormality values which caused by adding 2.0mg of (AF) / Kg diet(T<sub>2</sub>).These findings agree with the result of pervious studies (Ibrahim et al, 1998, Maizzo et al, 2002, Al- Neemi, 2004 and Al-Neemi and Al-Mufti,2006).On the other hand the values of the haematological parameters (Table 4) refered to the ability of natural muds (M – T, K and A. B. S) to correct the abnormality of the values PCV, RBC, WBC, HB and H / L except the values of the MCHC which were declined for all treatments contaminated with 2.0mg of AF / kg diet inspite of containing those diets 0.4 or 0.8 % of natural muds (M– T, K and A. B. S).These findings agree with the previous studies (Weibking et al, 1994, Kubena et al, 1995,Shareef et al, 1998, Phillips et al, 2002 and Al-Neemi, 2004).The cause of abnormality of the haematological parameters values of the birds fed diet contaminated with 2.0mg of AF / kg diet because the significant decline or shortage of the iron availability in the blood which is very necessary for hemoglobin synthesis and some important blood proteins (Tung et al, 1975, Lanza et al, 1979).

Table(3): Effect of the addition 2.0mg AF/kg diet and local mineral mud M-T, K and A. B. S upon Some haematological parameters (Mean. ± S. E).

Blood parameters						
Treatments	PCV%	RBC (10 <sup>6</sup> /mm <sup>3</sup> )	WBC (10 <sup>6</sup> /mm <sup>3</sup> )	HB g/1000ml	MCHC g/di	HL
T1	39.25 ± 0.47 ab	0.03 ± 3.05 a	0.05 ± 24.48 d	0.04 ± 8.60 ab	0.25 ± 21.84 a	0.02 ± 0.24 d
T2	28.75 ± 0.47 d	0.01 ± 2.62 h	0.06 ± 33.96 a	0.30 ± 4.75 f	0.30 ± 16.52 b	0.03 ± 0.58 a
T3	39.25 ± 0.47 ab	0.02 ± 2.99ab	0.08 ± 24.49 d	0.04 ± 8.67 a	0.19 ± 22.09 a	0.02 ± 0.28 c
T4	40.00 ± 0.40 ab	0.01 ± 2.95c	0.02 ± 24.47 d	0.04 ± 8.55 b	0.19 ± 21.55 a	0.04 ± 0.29 c
T5	40.5 ± 0.28 a	0.01 ± 2.96bc	0.03 ± 24.45 d	0.04 ± 8.57 ab	0.08 ± 21.37 a	0.02 ± 0.28 c
T6	39.00 ± 0.40 b	0.01 ± 2.93 c	0.03 ± 24.46 d	0.06 ± 8.55 b	0.23 ± 21.32 a	0.04 ± 0.28 c
T7	39.25 ± 0.47 ab	0.01 ± 2.86 d	0.02 ± 24.44 d	0.06 ± 8.67 a	0.23 ± 22.15 a	0.02 ± 0.28 c
T8	39.75 ± 0.75 ab	0.02 ± 2.87 d	0.02 ± 24.49 d	0.04 ± 8.62 ab	0.41 ± 21.84 a	0.04 ± 0.28 c
T9	31.25 ± 0.47 c	0.09 ± 2.73 f	0.01 ± 28.44 b	0.02 ± 4.87 ef	0.29 ± 15.61 c	0.06 ± 0.40 b
T10	31.30 ± 0.40 c	0.01 ± 2.74 f	0.02 ± 28.42 bc	0.06 ± 4.95 e	0.17 ± 15.96 bc	0.02 ± 0.39 b
T11	32.00 ± 0.40 c	0.08 ± 2.79 e	0.01 ± 28.31 c	0.04 ± 5.00 de	0.25 ± 15.88 bc	0.04 ± 0.40 b
T12	31.5 ± 0.28 c	0.01 ± 2.74 f	0.01 ± 28.43 bc	0.04 ± 5.17 c	0.07 ± 16.39 b	0.06 ± 0.39 b
T13	31.75 ± 0.25 c	0.01 ± 2.71 fg	0.06 ± 28.50 b	0.04 ± 5.11 cd	0.08 ± 16.04 bc	0.08 ± 0.40 b
T14	30.75 ± 0.25 c	0.01 ± 2.67 g	0.02 ± 28.53 b	0.04 ± 5.14 cd	0.26 ± 16.57 b	0.05 ± 0.39 b

\* Mean in each column bearing different letters are significantly different ( P ≤ 0.05 )

Table(4): Effect of the 2.0mg (AF)/kg diet and local mineral/mud (M-T, K) and A. B. S upon Some blood biochemical parameters

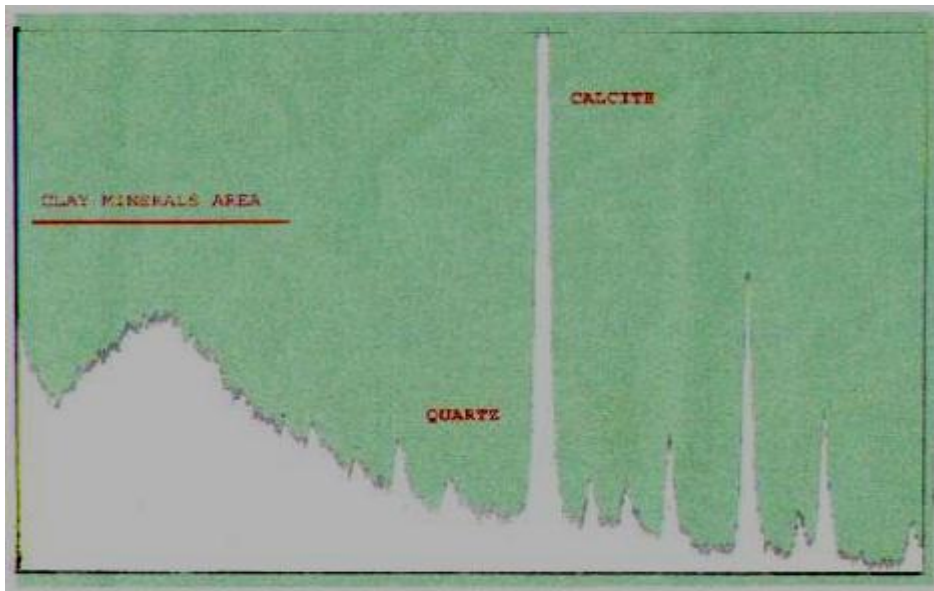
Treatments	100 gm/100	100 ml/mg			
	Total protein	Cholesterol	Glucose	Uric acid	Triply cerides
T1	0.01 ± 3.36 a	0.87 ± 179.25 ab	1.37 ± 190.25 a	0.01 ± 4.59 a	0.02 ± 3.18 cd
T2	0.01 ± 2.54 g	1.87 ± 128.00 h	0.95 ± 156.50 g	0.04 ± 4.25 g	0.05 ± 2.60 f
T3	0.07 ± 3.37 a	0.62 ± 180.75 a	1.10 ± 181.25 c	0.02 ± 4.61 a	0.07 ± 3.47 a
T4	0.08 ± 3.34 a	0.81 ± 181.00 a	1.44 ± 186.50 b	0.02 ± 4.61 a	0.04 ± 3.27 bc
T5	0.01 ± 3.37 a	0.64 ± 176.50 bc	0.75 ± 183.25 c	0.05 ± 4.69 a	0.04 ± 3.30 bc
T6	0.01 ± 3.35 a	0.81 ± 178.00 abc	0.86 ± 188.50 ab	0.01 ± 4.58 a	0.02 ± 3.32 b
T7	0.09 ± 3.37 a	0.47 ± 175.25 c	0.94 ± 187.25 ab	0.01 ± 4.54 b	0.04 ± 3.20 bc
T8	0.09 ± 3.30 b	0.95 ± 171.50 d	0.62 ± 182.25 c	0.08 ± 4.60 a	0.02 ± 3.12 d
T9	0.02 ± 2.84 c	0.75 ± 154.25 ef	1.58 ± 166.00 ef	0.02 ± 4.32 ef	0.07 ± 2.77 e
T10	0.01 ± 2.79 de	1.47 ± 157.00 e	1.37 ± 169.25 de	0.01 ± 4.30 f	0.02 ± 2.55 f
T11	0.01 ± 2.82 cd	1.10 ± 156.25 e	0.62 ± 167.25 def	0.02 ± 4.36 de	0.04 ± 2.62 f
T12	0.01 ± 2.79 de	0.81 ± 155.00 e	0.95 ± 169.50 d	0.01 ± 4.47 c	0.02 ± 2.57 f
T13	0.07 ± 2.76 e	1.43 ± 151.75 fg	0.95 ± 164.50 f	0.08 ± 4.32 ef	0.02 ± 2.62 f
T14	0.01 ± 2.74 f	1.43 ± 150.25 g	0.62 ± 167.75 de	0.01 ± 3.37 d	0.02 ± 2.67 e

\* Mean in each column bearing different letters significantly ( P ≤ 0.05 )

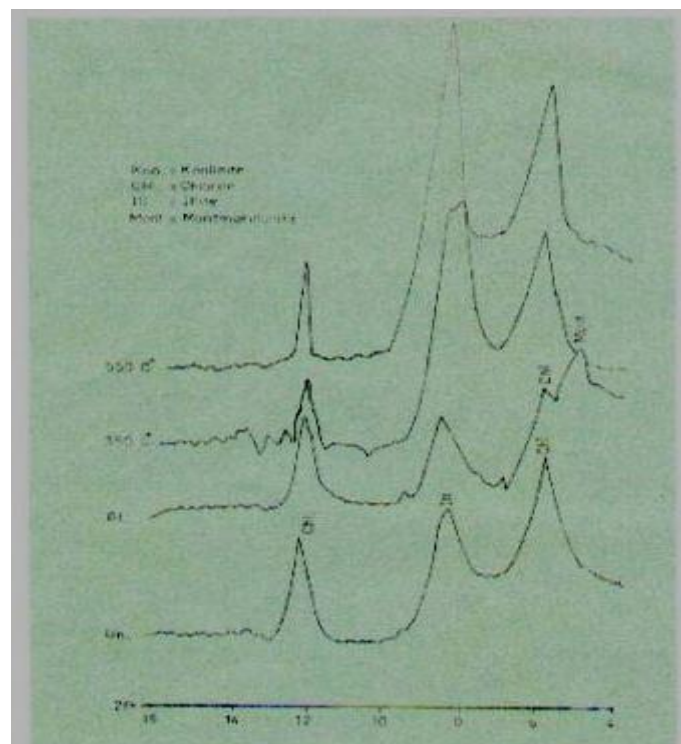
Table(5): Effect of the addition 2.0mg AF / kg diet and local mineral mud M-T, K and A. B. S upon activity of some blood enzymes(Mean.±S.E).

Treatment	Activity of blood enzymes		
	GoT ( IU/mol)	GPT(IU/mol)	ALP(IU/mol)
T1	108 ± 0.25 a	0.05 ± 9.18 a	0.41 ± 41.00 c
T2	72.50 ± 1.19 f	0.10 ± 6.53 g	0.40 ± 32.00 g
T3	108.00 ± 0.40 a	0.05 ± 8.82 b	0.63 ± 43.80 ab
T4	107.50 ± 0.29 a	0.04 ± 8.72 bc	0.25 ± 44.80 a
T5	108.03 ± 0.25 a	0.03 ± 9.07 a	0.25 ± 42.25 bc
T6	107.00 ± 0.40 a	0.06 ± 8.78 bc	0.40 ± 43.00 b
T7	105.00 ± 0.40 b	0.04 ± 8.63 c	0.29 ± 43.50 ab
T8	105.25 ± 0.25 b	0.02 ± 8.42 d	0.62 ± 44.75 a
T9	85.75 ± 1.44 de	0.07 ± 7.97 f	0.63 ± 38.75 d
T10	84.00 ± 1.47 e	0.04 ± 8.13 ef	0.70 ± 37.00 e
T11	89.25 ± 0.85 c	0.07 ± 8.67 bc	0.58 ± 37.00 e
T12	87.25 ± 0.95 cd	0.08 ± 8.13 ef	0.74 ± 35.75 ef
T13	84.75 ± 0.47 e	0.02 ± 8.17 e	0.62 ± 35.25 f
T14	83.75 ± 0.25 e	0.07 ± 8.00 f	0.28 ± 35.5 ef

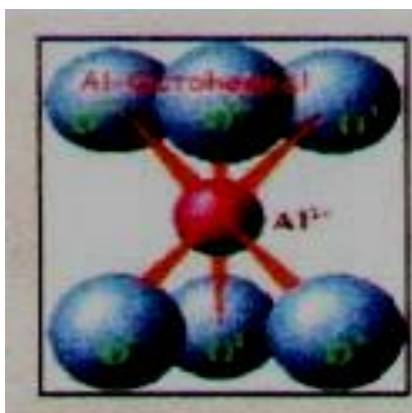
\* Mean in each column bearing different letters are significantly different ( P ≤ 0.05 )



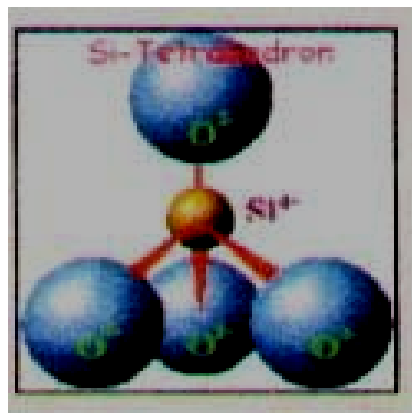
Fig(1-A)X-Ray diffraction chart of the bulk sample



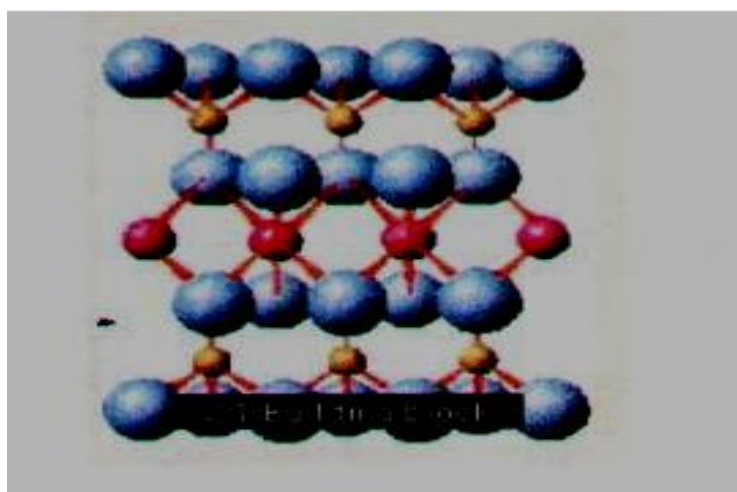
Fig(1-B)X-Ray diffraction chart of clay minerals identification



Fig(2-B) Crystal structure of the octahedral layer in clay mineral



Fig(2-A) Crystal structure of the tetrahedral layer in clay mineral



Fig(3) Mineral structure of montmorillonite











تأثير استخدام نوعين من الطين المعدني المحلي (T - M) و (K) بدلا من بنتونايت الصوديوم الفعالة على المقاييس الفسلجية والبايوكيمياوية لدم إناث السمان المغذاة على عليقة ملوثة بالافلاتوكسين

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الخلاصة

مئتان واربعة وعشرون من اناث السمان الياباني بعمر (٤٢) يوم وزعت عشوائيا وغذيت عليقة مقارنة وثلاثة عشر عليقة بمستويين (٠،٤ و ٠،٨) % لكل من الاطيان المعدنية المحلية: (K, M-T) و بنتونايت الصوديوم المنشط (A. S. B) مع او بدون اضافة ٢ ملغم من الافلاتوكسين كغم من العليقة لمدة ٨ اسابيع. التحليل الاحصائي اثبت انخفاض معنوي ( $P \leq 0.05$ ) في القياسات الدموية والبايوكيمياوية لمصل الدم وبعض فعاليات انزيمات الدم (GpT, GoT و AIP) لاناث السمان المغذاة على عليقة تحتوي ٢ ملغم من الافلاتوكسين / كغم من العليقة. اضافة هذه المميزات الطبيعية (K), (M-T) و (A. S. B) ادت الى تعديل معنوي في مقاييس الدم ومقاييس المصل البايوكيمياوية وفعالية بعض انزيمات الدم ما عدا قيمة (MCHC) باضافة (٠،٨) % من طين (M-T) او (K) . لوحظ انه لا يوجد اختلاف معنوي بين كفاءة الاطيان الثلاثة المستخدمة في الدراسة من حيث الحد من التأثيرات السلبية لسوم الافلاتوكسين في الاداء الانتاجي لاناث طائر السمان.