Research Article

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Study of Histopathological and Hematological Effects of Cysteine Added to The Broiler Diet contaminated With Aflatoxin B1

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Abstract

In this project, 270 broiler chickens one day old were used to demonstrate the effect of the addition of the amino acid cysteine added to the diets of birds contaminated with aflatoxin B1 on the liver and kidneys. The experiment was divided into 9 equal groups; & each group had 30 birds with 3 replicates, and each replicate had 10 birds. The control group was without addition. As for the treated groups, cysteine, and aflatoxin B1 were added to their diets at 40%, 80% & 160% cysteine, and aflatoxin B1 was added at 0 ml, 4 ml & 8 ml, respectively. The variables collected were liver & kidney histopathology, Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST) levels. When adding cysteine to a bird's diet contains Aflatoxin B1 not observed in blood ALT amount. The histopathological examination showed fibrosis in the liver and degeneration and dilatation of cortical tubules in the kidney. The amount of AST in the blood was greater at 28 days of age, specifically in G2 (Cysteine 80%) &G3 (Cysteine 160%) at Aflatoxin B1 0 ml, which caused significant damage to the liver. The giving of cysteine 40, 80& 160% in birds' feed contaminated with AflatoxinB1 0, 4& 8ml, which is intake by birds, has harmful effects on the health of the liver.

Keywords: Cysteine, Broiler, blood, histopathology.

Introduction:

Nutrition is one of the basic factors in the poultry industry. Broilers are among the birds characterized by a high speed of growth and high food conversion efficiency that converts kilograms of feed into kilograms of meat. Corn is an essential component of bird diets. It can be used in bird diets to 60% compared to the total diet. According to the hot weather in Iraq, corn cannot be stored for long periods because it affects the quality of the feed due to high heat and humidity that leads to the growth of molds and mycotoxins such as aflatoxin B1, which results in poor fodder. Aflatoxin B1 is produced by the fungus Aspergillus flavus, which is a major cause of cancers and diseases and affects birds, and this effect is reflected in human health. Moreover, taking low levels of aflatoxins B1 is possible and, for long periods, can lead to liver damage (1). (2) showed the effect of a low level of aflatoxin on biochemical performance parameters and broiler liver tissue. (3) observed the effect of dilatory afladetox on performance in the broiler. (4) demonstrated did aflatoxin in poultry. (5) showed an overview of the aflatoxicosis of poultry. (6) investigated the histology of the liver afflicted with Aflatoxins in broiler chicks of 42 days of age. The diagnosis criteria of aflatoxin depend upon clinical signs, mortality rate, and postmortem examination. (7) White Leghorn layer breeder hens, 30 weeks of age, the study was divided into 12 groups, one group was offered feed supplemented with 100,500,2500,5000 and 10000 µg/Kg aflatoxin B1, the experimental feeds for three weeks with two weeks recovery, body weight and relative weights of liver and kidneys of aflatoxin fed birds were significantly higher than the control group. The current study aims to show the effect of supplementing cysteine needs on the hematological and histology of birds' livers and kidneys after consuming meals containing Aflatoxin B1 in Iraq.

Materials & Methods

Two hundred- & seventy-one-day-old broiler chickens (Ross 308) were used in the project; birds were divided into 9 groups randomly, each group containing 3 replicates (10) bird / replicate. The feed treated given are: G1 = Cysteine 40% + Aflatoxin B1 (0 ml of 1ppm for every 250gm) of feed, G2 = Cysteine 80% + Aflatoxin B1(0 ml of 1ppm for every 250gm) of feed, G3 = Cysteine 160% + Aflatoxin B1(0 ml of 1ppm for every 250gm) of feed, G4 = Cysteine 40% + Aflatoxin B1 (4ml of 1ppm for every 250gm) of feed, G5 = Cysteine 80% + Aflatoxin B1(4ml of 1ppm for every 250gm) of feed, G6 = Cysteine 160% + Aflatoxin B1(4ml of 1ppm for every 250gm) of feed, G7 = Cysteine 40% + Aflatoxin B1(8 ml of 1ppm for every 250gm) of feed, G8 = Cysteine 80% + Aflatoxin B1(8ml of 1ppm for every 250gm) of feed. G9 = Cysteine 160% + Aflatoxin B1(8 ml of 1ppm for every 250gm) of feed. The duration of the experiment from start to finish was 5 weeks. At 21 &28 days old, samples were collected from birds. Fed and water *add libitum*. After killing 3 birds from each group, tissues were taken from the liver & kidneys. Tissue samples were taken and fixed with 10% neutral buffered formalin. Then, paraffin blocks were made and cut with a microtome at 5. Finally, slides were made and stained with H&E stain in the Anatomy Laboratory at the University of Basrah's Faculty of Veterinary Medicine (8)

Blood samples were collected from three birds slaughtered in the treated groups randomly. The samples were placed in a tube containing EDTA to obtain the blood plasma. Blood samples were tested by ALT & AST levels. By taking 5 ml of blood &then centrifuging it to get the plasma. Plasma was measured using a spectrophotometer. During the experiment, the basic diet provided the ratio to give all other nutrients except cysteine, following the NRC's dietary guidelines (9).

Bird feed ingredients and Chemical analysis of the components of the diet were indicated in table 1 and 2. **Analysis of Data**

According to (10) all data were analysed (ANOVA) using the SPSS program.

Table (1): Bird feed ingredients

(%) Ingredients	Diet from 21 to 28 day
yellow corn	63.50
Soybean meal 44%	24.86
Corn gluten meal	4.59
Plant oil	3.2
Dicalcium phosphate	1,5
calcium carbonate	1.13
Vitamins and minerals	0.3
Salt	0.3
Methionine	0.21
Lysine	0.23
The total (Kg)	100%

Table (2). Chemical analysis of the components of the diet

Parameter (%)	Diet from 21to 28 day
Crude protein	18.01
ME. cal/Kg feed	3176.00
C/P ratio	176.35
Ca	0.90
Phosphor (available).	0.47
Lys.	1.05
Methio.	0.51
Cyst.	0.40

. *NRC 1994 (9)

Results

In Table (3) the results showed no detection interference effect Between Cysteine and Aflatoxin B1 on ALT in birds' blood at 21 & 28 days of age. The results observed that AST values were the highest (P <0.05), with the highest level of cysteine obtained to diet at 28 days old. Also, the level of AST is highest (P <0.05) as Aflatoxin B1 is obtained at decreased levels at 21 &28 days old.

The interference impact of Cysteine & Aflatoxin B1 on AST birds for this experiment was significant (P<0.05) at 28 days old. In the table (4), the AST value in the blood of 28-day-old birds increased from 40% to 160% when the diet contained cysteine. At the age of 21 &28 days, At the lower level of Aflatoxin B1 for diet, AST value is higher. AST levels at 21days old were increased in Aflatoxin B1 4 ml & 0 ml at 28 days. The interference of Cysteine &Aflatoxin B1 at the highest AST levels was observed in G2 treated (Cysteine 80% &Aflatoxin B1 0 ml) &G3 (Cysteine 160% &Aflatoxin B1 0 ml).

Histopathology: Histopathological results did not show any effect on the liver & kidneys when the amino acid cysteine and aflatoxin B1 were given. Therefore, the liver & kidney functioned normally in all treated in fig (1&4) except for treated G1 & G3 where fibrosis & Parenchyma foci of the inflammatory cell of the liver were observed in fig (2&3) and degeneration & dilatation of cortical tubules of the kidney was observed in fig (5 &6) when given (Cysteine 40% &Aflatoxin B1 0 ml) & G3 (Cysteine 160% &Aflatoxin 0 ml). In this treatment, the lowest quantity of cysteine was 40%, and the maximum amount of aflatoxin B1 was 8 ml. The findings of treating G4 with 40% Cysteine and 4 ml of Aflatoxin B1 revealed that the liver would be affected by aflatoxicosis.

Table (3): ALT test for birds aged 21&28 days (µ/L).

Aflatoxin B1 (ml of 1ppm for every 250 gm.)	Amino acid (Cysteine)			Mean
	40%	80%	160%	
Aged (21 days)				
0 ml	3.70 ± 0.33	4.80± 1.74	5.10± 1.25	4.53 ± 1.10
4 ml	4.24 ± 0.37	5.20± 3.35	8.24± 2.66	5.89± 2.12
8 ml	4.27 ± 0.37	5.04± 1.28	4.94± 1.07	4.75 ± 0.90
Mean	4.07 ± 0.35	5.01± 2.12	6.09± 1.66	
Aged (28 days)				
0 ml	4.74 ± 1.70	4.74 ± 1.74	3.64± 0.56	4.37 ± 1.33
4 ml	3.47 ± 0.67	4.37 ± 0.67	3.20± 0.33	3.68 ± 0.55
8 ml	3.07 ± 0.55	3.57 ± 1.39	5.17± 0.13	3.93± 0.69
Mean	3.76 ± 0.97	4.22 ± 1.26	4.00 ± 0.34	

Table (4): AST test for birds aged 21&28 days (µ/L).

Aflatoxin B1 (ml of 1ppm for every 250 gm.)	Amino acid (Cysteine)			Mean
	40%	80%	160%	
Aged (21 days)				
0 ml	168.2± 38.2	245.3± 40.8	245.9± 40.8	219.8 ± 39.9^{f}
4 ml	252.5 ± 56.1	260.5± 88.2	272.4± 49.0	261.8± 64.4^{e}
8 ml	205.1 ± 24.8	209.7± 17.4	181.9± 29.4	198.9 ± 23.8^{f}
Mean	208.6 ± 39.7	238.5± 48.8	233.4± 39.7	
Aged (28 days)				
0 ml	207.7± 53.0^{wx}	331.3 ± 23.4^{v}	220.0± 19.4^{v}	253 ± 31.9^{e}
4 ml	166.4 ± 67.5^{x}	200.4 ± 50.8^{wx}	268.3± 13.7^{vw}	211.7 ± 44.0^{f}
8 ml	163.4 ± 10.6^{x}	215.8 ± 11.4^{wx}	176.9± 12.6^{wx}	185.3± 11.5^{f}
Mean	179.1 ± 43.7^{b}	249.1 ± 28.5^{b}	221.7 ± 15.2^{a}	

Small litters when different means a significant difference between groups

Fig. (1): Liver normal stain.10x.

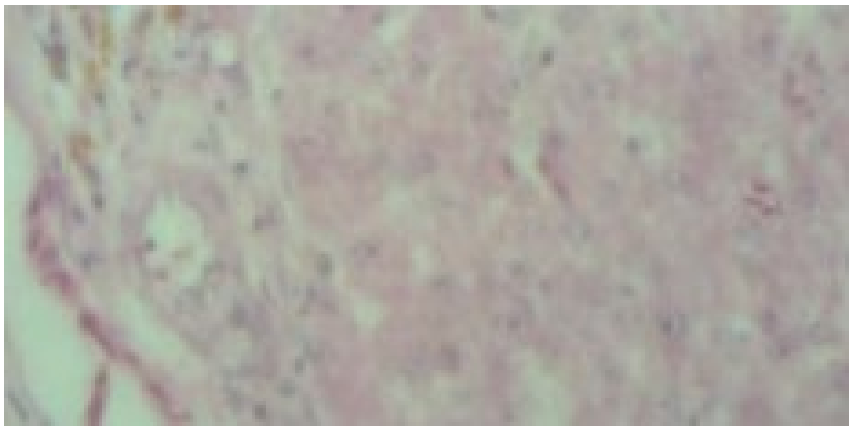

Fig. (2): Liver with fibrosis. H&E stain.10x.

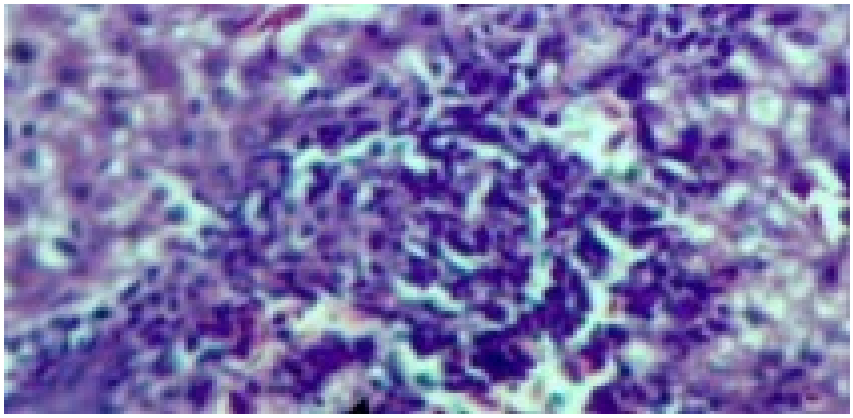

Fig. (3): Liver with Parenchyma foci of inflammatory cell. H&E stain.40x.

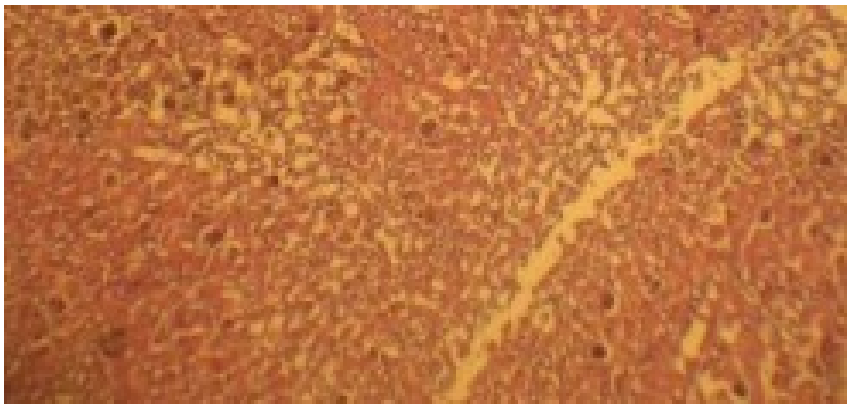

Fig. (4): Kidney normal. H&E stain.10x.

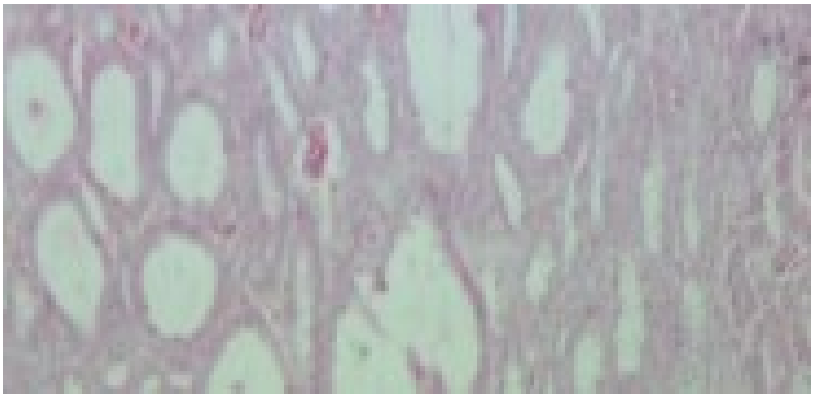

Fig. (5): Kidney with degeneration & dilatation of cortical tubules. H&E stain.10x.

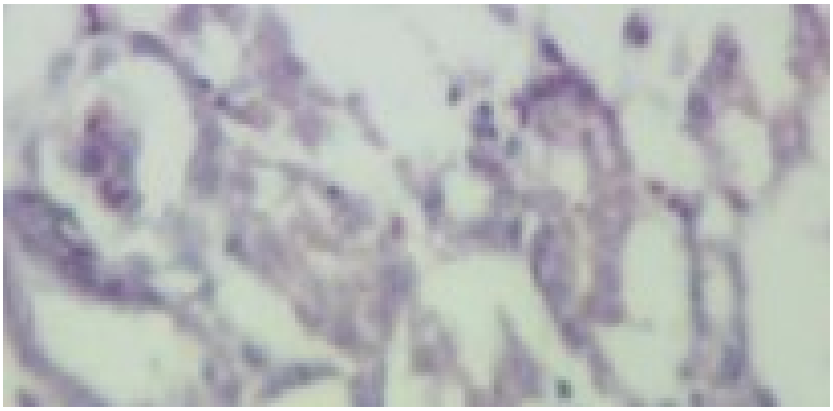

Fig. (6): Kidney with degeneration & dilatation of cortical tubules. H&E stain.40x.

Discussion

Hematology :Liver damage detected by ALT & AST test. Toxins that enter the liver & are excreted in serum are neutralized by the enzymatic reactions carried out by the enzyme ALT. The levels of ALT enzyme showed no similarity and apparent changes in the blood of birds fed with different concentrations of Cysteine and Aflatoxin B1, & no effect was shown to interfere between the two additions. In this study, the levels of the ALT enzyme were within the normal level, & the reason may be due to being less than 10 µ/L (11). In contrast, the levels of aflatoxin B1 decreased relatively, making the liver healthy without any damage due to the body's ability to remove toxins. Unlike (11) where ALT levels were high in birds treated with 1000 & 1500 ppb & Aflatoxin B1.This is due to the difference in the ability of birds to adapt to different 4ml & 8 ml levels of Aflatoxin B1. The current study found that ALT did not affect lowering liver damage, which might be related to necrosis and hepatitis infection. We know that liver damage occurs when the amount of ALT&AST in plasma secreted by cells increases (12) Aflatoxin detoxification process it will lead to increased levels of both ALT & AST. The dsease is caused by liver injury, which causes a rise in ALT at the expense of AST levels [13&14]. At 28 days old, a high AST value meant the liver was damaged. This liver damage was caused by giving a lot of cysteine, which changed methionine into homocysteine and caused it to stick together. The lack of aflatoxin caused much homocysteine to be stored. The feed's lowest level of aflatoxin B1 will lead to an elevated AST value at age 21 or 28 days.

Histopathology

We did a study on the effect of the amino acid Cysteine in broiler chickens that were fed a diet contaminated with Aflatoxin because it is essential, as was supported by (15) in his histopathology of the liver affected with aflatoxin in broiler chicks reported hyperplasia, congestion, necrosis, sorosis & accumulation red blood cells & inflammatory cells around the central vein, the present study also found histopathological lesions in the liver fibrosis & foci inflammatory cells. (16) reported

biochemical & histopathological lesions in the liver & kidney of rats induced by aflatoxin. The present paper also found histopathological lesions in the liver & kidney in broiler chickens fed with aflatoxin. (17) found changes in serum protein, cholesterol & liver enzymes. Histopathological, they found a lesion in vital organs such as the gizzard, liver, and kidney. In gizzard, there was erosion and ulceration. The present paper was mainly on the pathological lesion in the liver & kidney. (18) investigated histopathology alterations in the liver and kidney; the current investigation likewise discovered histological changes in the liver and kidney. (19) found microscopic lesions in liver as congestion & vacuolation of hepatocytes & renal tubular necrosis. The current investigation also discovered histological abnormalities such as liver fibrosis, degeneration, and cortical tubules in the kidney in broiler chicken feed containing aflatoxin B1.

Conclusion

Through the results we obtained in this study, we concluded that adding cysteine at a concentration of 40, 80 & 160% in broiler rations contaminated with Aflatoxin B1 0, 4, and 8 ml could not improve the performance of the liver & kidneys.

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دراسة التأثيرات النسيجية المرضية والدموية للسيستايين المضاف إلى علائق فروج اللحم الملوثة بالأفلاتوكسين B1

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الخلاصة

في هذا البحث تم استخدام 270 فروج لحم عمرها يوم واحد لإثبات تأثير إضافة حمض السيستين المضاف إلى علف الطيور الملوثة بالسموم الفطرية ب1على الكبد والكلى. تم تقسيم التجربة إلى 9 مجاميع متساوية. وكان لكل مجموعة 30 طائرا بثلاث مكررات، ولكل مكرر 10 طيور. كانت المجموعة الضابطة بدون إضافة. أما بالنسبة للمجموعات المعالجة، فقد أضيف السستين والسموم الفطرية ب 1 إلى وجباتهم الغذائية بنسبة 40٪، 80٪ و160٪، وأضيف الأفلاتوكسين عند 0 مل، 4 مل ،8 مل على التوالي. المتغيرات التي تم جمعها هي أمراض الكبد والكلى، ألانين امينوترانسفريز واسبارتيت امينوترانسفريز عند اضافة السيستين الى النظام الغذائي للطائر بحتوي على السموم الفطرية ب 1 الذي يتم ملاحظته في كمية الالنين امينوتراسنفريز في الدم. واظهر الفحص التشريحي المرضي تليف الكبد وتضخم وتوسع الانابيب القشرية في الكلى. كانت كمية اسبارتيت امينوترانسفريز في الدم أكبر عند عمر 28 يوما وتحديدا في المجموعة الثانية (سيستين 80%) والمجموعة الثالثة (سيستين 160%) مما يتسبب بأضرار جسيمة للكبد. إن إعطاء السيستين 40 و80 و160٪ في علف الطيور الملوث بالسموم الفطرية ب 1 (8,4,0) مل والذي تتناوله الطيور له اثار ضارة على صحة الكبد.

الكلمات المفتاحية: السيستين، الفروج، الدم، النسيج المرضي.