

Effect Of Aflatoxin On The Growth Performance And Some Hematological, Biochemical Alterations In Growing Lambs

Tarek H. Alam and Mona S. A. Al-Khalek

Animal Health Research Institute (Zagazig branch)

ABSTRACT

The objective of this study was designed to clarify the effect of aflatoxin contaminated feed on growth performance, hematological and some biochemical parameters in growing lambs. A total 12 growing lambs about 3 months old and 17 kg body weight, apparently healthy were divided into two equal groups (6 lambs in each). 1st group left without treatment as control, 2nd group was fed on a ration containing 1 mg aflatoxin /kg ration for 60 successive days, All lambs of each group were individually weighed at the beginning of the experiment and at 1st, 15th and 30th days post aflatoxin supplementation. Body weight gain, feed consumption, feed conversion were recorded. Two blood samples were collected from each animal by Jugular vein puncture at 1st, 15th and 30th days post aflatoxin supplementation. The first sample was taken in heparinized tube for haematological examination and the second one without anticoagulant to obtain serum for biochemical analysis.

The present work revealed that, aflatoxin supplemented lambs showed a significant decrease in total erythrocytic count, haemoglobin, packed cell volume, lymphocyte, body weight gain, gain percent, total protein, albumin, alpha, beta and gamma globulin, A/G ratio, calcium, inorganic phosphorus, magnesium, sodium and potassium associated with significant increase in leukocytic count, transferases (AST-ALT) alkaline phosphatase, lactic dehydrogenase, urea, creatinine, total fatty acid and cholesterol at 1st and 15th days post feeding on ration contain aflatoxin.

It could be concluded that aflatoxin induced many reversible alteration in growing lambs on blood picture, body weight and biochemical parameters as they returned to normal values 30 day after stopping aflatoxin addition.

INTRODUCTION

Inadequately stored products and agricultural by-products exposed to high humidity and high temperature facilitate the development of fungi. The presence of these microorganisms, in addition to spoiling the products, reduces their quality and favors the production of mycotoxins, fungal secondary metabolites. Which are responsible for serious health problems for animals and human (1).

Aflatoxins are difuranocoumarin derivatives produced via a polyketide pathway by many strains of *Aspergillus flavus* and *Aspergillus parasiticus* (2). Aflatoxins are produced in feedstuffs either in the field or during storage when there are appropriate conditions of humidity and temperature. Contamination of feedstuffs with aflatoxin in particular caused severe economic

problems for livestock producers and may create potential health risks by transmission of aflatoxin and /or their metabolites from livestock to human through consumption of meat, milk or eggs (3). Aflatoxin B1 is the most biologically active member of the aflatoxin family (4). The susceptibility of animals to aflatoxin varies considerably, depending on sex, age, species and nutrition (5). Aflatoxin bind to DNA and RNA resulting in adverse effects on metabolism, reduce protein synthesis, decrease cell mediated and humoral immunity (6). Acute aflatoxicosis caused a distinct clinical disease marked by hepatitis, icterus, hemorrhage and death (7). Aflatoxin induce anemia (8) poor digestion (9). Aflatoxicosis can significantly reduce the efficiency of food conversion and dramatically decrease growth rates (10).

MATERIAL AND METHODS

2-Animals

The present investigation was carried out on twelve growing Rahmani lambs aging about 3 months old and 17 kg body weight. Lambs were obtained from a private farms in Sharkia Province. Lambs were housed under hygienic conditions.

3-Experimental design

Lambs were divided into two equal groups, 6 lamb each. The first group was left without treatment as control, group, 2nd group was fed on a ration containing aflatoxins (1mg/kg ration) for 60 successive days then supplemented with ration free from aflatoxin. All lambs of each group were individually weighed at the beginning of the experiment and at 1st, 15th and 30th days post aflatoxin supplementation. Body weight gain, feed consumption, feed conversion were recorded.

C-Blood and serum samples

Jugular vein puncture was used to collect blood samples at the 1st, 15th and 30th days post aflatoxin supplemented blood samples were collected in two portions, one portion was collected on heparin as anticoagulant for hematological studies (11) and the other one was centrifuged at 3000 r.p.m. for 15 min to separate serum, for determination of serum total proteins (12). Serum protein fractions were performed using cellulose acetate electrophoresis test (13). Serum samples were also used for estimation of aminotransferases (AST-ALT) (14), alkaline phosphatase (15), lactic dehydrogenase (LDH) (16), urea (17),

creatinine (18) serum cholesterol (19) total lipid (20), calcium (21), inorganic phosphorus (22), magnesium (23) sodium and potassium (24).

D. Statistical analysis

The obtained data were tabulated and statistically analysed (25).

RESULTS

It is clearly evident from Table 1 that exposure of lambs to aflatoxin resulted in a significant decrease in total erythrocytic count, haemoglobin, packed cell volume and lymphocyte but leucocytic count and neutrophils, monocyte and basophil significantly increased at 1st and 15th days after exposure to aflatoxin for two weeks.

Body weight and body gain was significantly decreased in lambs received contaminated ration with aflatoxin when compared with control at 1st and 15th days post aflatoxin supplementation in ration. (Table 2)

Table 3 showed that aflatoxin induce significant decrease in serum total protein, albumin and total globulin (alpha, beta and gamma globulin). Analysis of blood parameters revealed significant increase in aminotransferases (AST-ALT), alkaline phosphatase, urea, creatinine, total lipid and cholesterol values at 1st and 15th days post feeding on ration contain aflatoxin. (Table 4).

Serum calcium, inorganic phosphorus, magnesium, sodium and potassium were significantly decreased as a result of supplementation of aflatoxin in ration. (Table 5)

Table 1. Effect of aflatoxin (1 mg/kg BW) on haemogram and leukogram at 1st, 15th and 30th days post medication for two months in lambs (n=6).

Parameter Period post medication (days)	haemogram				leukogram				
	RBC's (x 10 ⁶ /ul)	Hb (g/dl)	PCV (%)	WBC's (x 10 ³ /ul)	Differential count(%)				
					Lymp.	Neutr	Mono.	Eos.	Baso.
control	11.84± 0.36	11.09± 0.25	31.13± 1.26	8.84± 0.43	72.10± 1.07	22.57± 1.06	4.12± 0.19	1.09± 0.14	0.11± 0.02
1	10.34± 0.31*	10.37± 0.19*	25.98± 1.06*	9.90± 0.31*	60.14± 1.12**	30.18± 0.79**	6.13± 0.69*	3.12± 0.25**	0.43± 0.09*
15	11.13± 0.30*	10.53± 0.26*	28.04± 1.29	9.00± 0.35	67.28± 1.38*	25.15± 1.05	5.11± 1.03	2.14± 0.69	0.23± 0.09
30	11.69± 0.36	10.89± 0.32	30.23± 1.46	8.95± 0.29	72.78± 1.46	21.47± 1.78	4.43± 1.23	1.12± 0.17	0.20± 0.08

*Significant at P < 0.05

** Significant at P < 0.01

Table 2. Effect of aflatoxin (1mg /kg BW) on body weight (kg), Weight gain(gm) and weight percent (%) at 1st, 15th and 30th days post medication for two months in lambs (n=6).

Parameter Period post medication (days)	Initial body weight	1 st day				15 th day			30 th day		
		body weight	Weight gain		body weight	Weight gain		body weight	Weight gain		
			Gm	%		Gm	%		Gm	%	
Control group	17.13± 0.32	26.82± 0.27	9.6± 0.12	56.57± 2.05	29.96± 1.02	3.14± 0.03	11.71± 0.06	33.09± 0.56	3.13± 0.02	10.45± 0.34	
Treated group	17.25± 0.64	23.45± 0.30*	6.2± 0.19*	35.94± 0.64**	25.03± 0.31*	1.58± 0.06**	6.74± 0.04**	27.17± 0.46	2.14± 0.03	8.55± 0.42	

*Significant at P < 0.05

** Significanhi P< 0.01

Table 3. Effect of aflatoxin (1mg /kg BW) on serum total protein and protein fractions at 1st, 15th and 30th days post medication for two months in lambs (n=6)

Parameter Period post medication (days)	Proteinogram							A/G ratio
	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)				Total	
			Alpha	Beta	Gamma	Total		
control	7.59± 0.52	4.51± 0.25	1.08± 0.06	0.94± 0.05	1.06± 0.04	3.08± 0.19	1.46± 0.26	
1	5.19± 0.31**	2.69± 0.21**	0.87± 0.08*	0.79± 0.04*	0.84± 0.07*	2.50± 0.14*	1.08± 0.21	
15	5.94± 0.21*	3.19± 0.29*	0.90± 0.08	0.82± 0.07	0.83± 0.03*	2.75± 0.11*	1.33± 0.18	
30	6.48± 0.42	3.41± 0.71	1.06± 0.10	0.93± 0.012	1.08± 0.24	3.07± 0.51	1.52± 0.21	

*Significant at P < 0.05

** Significanhi P< 0.01

Table 4. Effect of aflatoxin (1mg /kg BW) on some serum biochemical parameters at 1st, 15th and 30th days post medication for two months in lambs (n=6)

Parameter Period post medication (days)	AST (U/L)	ALT (U/L)	Alk.ph. (I.U/ml)	LDH (U/L)	Urea (mg/dl)	Creatinine. (mg/dl)	Cholesterol (mg/dl)	Total lipids, (mg/dl)
control	39.34± 1.59	12.51± 1.83	83.12± 2.56	283.10± 10.02	15.64± 1.94	1.46± 0.22	92.24± 2.89	358.85± 12.23
1	47.58± 2.38*	18.93± 1.76*	95.36± 1.83*	331.12± 9.91**	22.35± 1.02**	2.71± 0.63**	102.54± 3.06**	388.24± 9.27**
15	45.49± 1.42*	15.03± 1.11*	90.07± 1.85*	324.2± 8.73**	20.93± 1.01*	2.51± 0.51*	99.65± 2.61*	373.8± 8.93*
30	40.98± 2.89	13.95± 2.98	85.86± 1.98	291.43± 11.45	16.46± 2.76	2.04± 0.35	95.94± 2.98	361.98± 10.76

*Significant at P < 0.05

** Significanhi P< 0.01

Table 5. Effect of aflatoxin (1mg /kg BW) on some serum minerals at 1st , 15th and 30th days post medication for two months in lambs (n=6)

Parameter \ Period post medication (days)	Calcium (mg/dl)	Phosphorus (mg/dl)	Magnesium (mg/dl)	sodium (mEq/L)	potassium (mEq/L)
control	8.49±0.52	5.95±0.24	2.5±0.19	142.38±5.97	4.96±0.76
1	6.26±0.31**	3.71±0.34**	1.61±0.12**	133.87±2.81*	3.84±0.48*
15	6.83±0.24*	4.05±0.28**	1.92±0.10*	139.04±4.09	4.37±0.59
30	7.80±0.37	4.96±0.98	2.31±0.15	141.87±2.98	4.67±0.84

*Significant at P < 0.05

** Significant P < 0.01

DISCUSSION

Aflatoxins are group of toxic chemical compounds produced by certain strains of fungi when they grow under favourable condition on a wide variety of different substrate. This study was undertaken to investigate the effects of aflatoxin on the body weight humeral and cellular immunity as well as some liver and kidney functions in lambs.

The present results displayed in Table 3 revealed that the body weight and weight gain significantly decreased at 1st and 30 days post supplementation of aflatoxin in ration for 2 month. These results run parallel with those previously obtained (26) reporting that aflatoxicosis can significantly reduce the efficiency of food conversion and dramatically decrease growth rates. The decrease in body weight is one of the earliest indicators of clinical aflatoxicosis in animals (27). Aflatoxin level greater than 0.5 mg/kg in the diet have been shown to decrease food intake and food conversion and retard growth (28). Also aflatoxicosis of rabbits dosed with 0.5 ppm/kg b.wt fed animals showed emaciation and a decrease in body weight (29). This decrease in body weight can be correlated to a reduction in their feed/ water intake or due to the effect of aflatoxin on digestibility of nutrients and consequently growth performance (30, 31) with growing rabbit and lambs. Similar results were previously recorded (32) with lambs fed

rations contained aflatoxin B1. Another explanation for decrease body weight and body gain was due to inhibition of protein synthesis, reduction feed intake (33). Also the decrease in body weight and body weight gain in the present study may be due to hypothyroidism produced by aflatoxin (34)

In the present study, it has been shown that in growing lamb, the application of aflatoxin in ration evoked a significant decrease in values of total erythrocytic count, hemoglobin, packed cell volume and lymphocytes. These results were in line with the work carried out by several investigators (3,33,35) and recorded decrease in hematological parameters as a result to aflatoxicosis. Aflatoxin induced immunosuppression and increased total leukocytic count (36). Which confirmed our finding, it has been proved that aflatoxin induce kidney and liver damage. significant increase in leucocyte, neutrophil, monocyte and basophil compared with the control group (37). Aflatoxin increased total leucocytic count, neutrophils, monocyte and basophil, but decreased lymphocytic count in lambs (38). Which nearly similar to our investigation.

The result obtained in this study revealed that aflatoxin produced severe change in proteinogram represented by hypoproteinemia associated with hypoalbuminemia and hypoglobulinemia. Several investigator (37, 39, 40) cited similar results. This can be explained

that aflatoxin inhibits the albumin biosynthesis due to its hepato-toxic effect. In addition to increase protein catabolism as a result of stress condition (41). This result could be due to the fact that aflatoxin molecule metabolically activated before exerting its effects by cytochrome P450 enzymes which convert aflatoxins to the reactive 8,9-epoxide form, which is capable of binding DNA and proteins to form covalent adducts and disturb DNA replication causing chromosomal aberrations. Moreover aflatoxin B1 suppresses messenger-RNA synthesis and affects protein synthesis in the living cell (5, 42). After absorption from the small intestine, aflatoxin B1 readily binds to plasma albumin which serves as the major transporter of aflatoxin B1 in blood (43). Given this background, it is tempting to suggest that the decline in protein concentration of aflatoxin B1 treated lambs could be due to a decline in the protein biosynthesis by forming adducts with DNA, RNA and proteins, and inhibition of RNA synthesis or DNA-dependent RNA polymerase activity, as well as degranulation of endoplasmic reticulum (44,45). In addition, oxidative DNA damage and lipid peroxidation are also manifestations of aflatoxin-induced toxicity (46). Oxidative stress may also result in the damage of critical cellular macromolecules, including DNA, lipids and proteins (47). Aflatoxin causes a failure in the acquired immunity system of animals (48) by decreasing antibody gamma globulin production and altering serum proteins profile. Decreased total proteins due to the aflatoxin inhibition of protein synthesis (49). Reduction of albumin values resulted in decrease in A/G ratio. It has been reported that aflatoxin interfer with carbohydrate oxidation, a process which is essential for providing energy needed for protein synthesis (48).

Electrophoretic separation of serum proteins in this work revealed a decrease in alpha, beta and gamma globulin percentage in lambs, which is inconsistent with previous investigation (8, 50). Decrease in gamma globulin after supplementation may be due to severe hepatitis and/or acute nephritis (51).

Aflatoxin bind to DNA and RNA resulting in adverse effects on metabolism, reduce protein synthesis and humoral immunity (6).

It is evident from the present study that aflatoxin induce a significant increase in aminotransferases (AST-ALT) and alkaline phosphatase at 1st and 30th days post feeding lambs on ration contain aflatoxin. The decline in transferases activity may be due to hepatotoxic effect of aflatoxin (52) in addition to alter permeability of hepatocytes (53).

Results of the present study revealed that aflatoxin induced a significant elevation in serum urea and creatinine values concentrations in lambs which may be attributed to the inflammatory changes of the kidneys and renal pelvis (33). The significant increase in urea and creatinine values in the present study indicate renal insufficient or nephropathy (54).

The present investigation revealed that aflatoxin induce significant increase in cholesterol and total lipid at 1st and 15th days post intoxication. Similar findings are cited previously by (38, 55).

The present study illustrated that the lambs fed on ration contained aflatoxin revealed significant decrease in calcium, inorganic phosphorus, magnesium, sodium and potassium. The decrease in serum potassium concentration may be attributed to the decrease food intake during aflatoxicosis syndrome (56). Also the decreased serum magnesium, levels may be a result of decrease reabsorption from the inflamed renal tubules that lead to increased losses (57).

Accordingly it could be concluded that the aflatoxins induce severe adverse effect on body weight, blood picture as well as impairment of liver and kidney function so it is very important to feed lambs on aflatoxin free ration.

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الملخص العربي

تأثير الأفلاتوكسين على كفاءة النمو وبعض الوظائف البيوكيميائية في الحملان النامية

طارق حسن علام ، منى صلاح عبد الخالق

قسم الكيمياء الحيوية والسموم والنقص الغذاء والباثولوجيا الاكلينيكية

معهد بحوث صحة الحيوان بالزقازيق

أجريت هذه الدراسة على الحملان النامية بهدف دراسة تأثير إضافة الأفلاتوكسين العلائق على معدل أداء الحملان وصورة الدم وبعض الوظائف البيوكيميائية، وقد تم البحث على مجموعتين متساويتين من الحملان النامية ٦ بكل مجموعة (المجموعة الأولى غذيت على عليقة خالية من الأفلاتوكسين (مجموعة ضابطة)، المجموعة الثانية غذيت على عليقة مضاف إليها الأفلاتوكسين بجرعة (١ مجم لكل كجم عليقة) لمدة شهرين بعد ذلك يتم تغذية الحملان على عليقة خالية من الأفلاتوكسين. تم أخذ عينتين دم من كل حيوان عند اليوم الأول، ١٥، ٣٠ يوم من نهاية استخدام الأفلاتوكسين على العليقة الأولى على هيبارين وذلك لدراسة التأثيرات على صورة الدم والأخرى لفصل مصل الدم وذلك لقياس بعض المؤشرات البيوكيميائية .

وقد أظهرت النتائج الى حدوث نقص معنوي في العدد الكلي لكرات الدم الحمراء، تركيز الهيموجلوبين، حجم خلايا الدم المرصوصة و الخلايا الليمفاوية مصحوبة بزيادة في العدد الكلي لكرات الدم البيضاء و الخلايا المتعادلة ، الخلايا الملتهمة الكبيرة و الخلايا الحامضية

تشير نتائج الدراسة أن الأفلاتوكسين ادى الى وجود نقص معنوي في وزن الجسم، البروتين الكلي الزلال ، الجلوبيولين (الفابيتا-جاما) والنسبة بين الالبومين والجلوبيولين، الكالسيوم، الفسفور، البوتاسيوم، الصوديوم والماغنسيوم وزيادة معنوية في معدل كلا من الترانس امينيزسس (ALT-AST)، والفوسفاتيز القاعدي، الكتك دى هيدروجينيز ،اليوريا والكرياتين الدهون الكلية والكلوستيرول في مصل الدم .

نستخلص من هذه الدراسة أن الأفلاتوكسين أحدث تأثيرات عكسية على صورة الدم، وزن الجسم و بعض القياسات البيوكيميائية في الحملان النامية. وتلك التأثيرات عادت لوضعها الطبيعي بعد ٣٠ يوم من نهاية اعطاء الأفلاتوكسين للحملان لذلك ينصح باستخدام علائق خالية من الأفلاتوكسين في تغذية قطعان الاغنام.