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Research Article

The Role of Aflatoxin B1 And Fumonisin B1 In Fungal Contaminated Feed on Some Biochemical Parameters and Physiological Effects of Broiler Chickens in Nineveh Governorate

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Abstract:

Sixty pelleted broiler feed samples {15 samples of pre-starter feed No.0; 15 samples of starter feed No.1; 15 samples of grower feed No.2; 15 samples of finisher feed No.3}; were collected from 15 different broiler flocks in Mosul governorate during march 2024 till October 2024, for the estimation of the natural contamination Mycotoxins of these feeds with Aflatoxin B1(AFB1) and Fumonisin B1(FB1) using Enzyme linked immunosorbent assay (ELISA). Results revealed that 30% were positive for residual AFB1, with a mean of 5.0 µg/kg and 83.33% were positive for residual FB1, with a mean of 291.8 µg/kg. Four broiler flocks fed the following combinations of both mycotoxins (AFB1 5.2 µg/kg +FB1301.8 µg/kg); (AFB1 14.7 µg/kg +FB1301.8 µg/kg); (AFB1 11.1 µg/kg +FB1 393.3 µg/kg); (AFB1 19.0 µg/kg +FB1 486.0 µg/kg) were selected to study the toxins effect on serum levels of total proteins (TP), total cholesterol (Chol), triglycerides (Tri), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AP) activity and creatinine in serum of broilers at 42 days of age. Significant differences were not found concentrations of (TP), (Chol), (Tri), (ALT), (AST), and (AP) activity and creatinine in broilers serum of the groups.

Keywords: Aflatoxin B1, Fumonisin B1, Fungal contaminated feed, broiler chickens, Blood serum biochemical

Introduction:

Aflatoxins (AF), potent mycotoxins produced by Aspergillus flavus and Aspergillus parasiticus, are a major concern in the poultry production. The toxicity of AF in broiler chickens has been widely investigated by the determination of their carcinogenic, mutagenic, teratogenic (Magnoli and Monge 2011; Ahmed and Dong 2019) and growth inhibitory effects (Smriti and Anusmita 2023 Almashhadani and Qassim 2025). The biochemical–haematological (Seyyed and Jafar 2019), immunological (Peter and Andrew 2013) and pathological toxic effects (Júlia and Zsuzsanna 2024; Dorina et al. 2015) of AF have also been well described. Aflatoxins may contaminate many crops included in poultry feeds raw materials, like, corn, wheat and soybean with widespread contamination in hot and humid regions of the world. These mycotoxins occur in several chemical forms, designated aflatoxin B1, B2, G1, G2, and M1, among which the most important is the aflatoxin B1 (Seyed and Hasan 2023). Aflatoxin contamination of feed stuff has been reported to range as low as from 10-1500 ppb in commercially used feed ingredients and 34-115 ppb in mixed feed samples (Ananda and Shambulingappa 2010).

Fumonisins are mycotoxins discovered in 1988 and are produced by molds of genus Fusarium (Mariska and Ilze 2019). There are about 28 homologus compounds of fumonisins produced by a variety of Fusarium fungi, of these A1, A2, A3, B1, B2, B3, C1, C2, C3, P1, P2, P3, and others , are produced by *Fusarium verticillioides* (=moniliforme), *F. proliferatum, F. anthophilum, F. dlamini, F. napiforme, F. nygamai, F. globosum, F. oxysporum, F. polyphialidicum, F. subglutinans* and *F. thapsinum* in foods and feeds (Qassim et al. 2023; Deepa 2017; Matteo 2005). The most common cereal grain which is usually used in poultry feed formulation and affected by *Fusarium verticilliodes* is corn. It has been repeatedly proven that broilers have a high tolerance to fumonisins when fed the toxin in academic research conditions. The fungi *Fusarium verticilliodes* and *Fusarium proliferatum* have been found to cause fumonisin toxicosis in poultry. No differences in body weight gain and feed conversion when FB1 was given to broilers at concentration of 20, 40 ,60 and 80 ppm from 1 to 21 days of age compared to the control group. Several reports have been published showing that feed contaminated with *F. verticillioides*, containing fumonisins, causes losses to broilers (Deepa and Premila 2021). The clinical features of the disease of broiler chickens often include diarrhoea, weight loss, increased liver weight and poor performance (body weight [BW], feed intake [FI], and feed conversion rate [FCR]). Functional and morphological changes in broiler chicks, turkeys and ducklings observed at dietary concentrations greater than 150 mg/kg fumonisin (Munene and Youssef 2023). Fumonisin B1 is the most abundant and toxic of the fumonisins, representing about 70% of the total contamination of food and feed naturally contaminated.

Exposure to fumonisin and aflatoxin may occur by the ingestion of contaminated feed, when contaminated cereals such as corn, wheat, soybean, as well as other raw materials, are used in the preparation of animal feed (Slim and Teresa 2023). Biomarkers are an important tool in analyzing the effects of mycotoxins in poultry.

Espada et al., 1994, , fed as low as 10 mg/kg of FB1 to broiler chicks for their first six days showed that chicks exhibit presence of petechiae, increasing coagulation time and decreasing albumin serum concentration.The same author .fed broilers diets containing pure FB1 (10 mg/kg) and FB1 (30 mg/kg) from *Fusarium verticillioides* (MRC 826) culture material found that there was an alteration in haematological parameters in broiler chicks. Increasing contamination level of FB1 to 50 mg /kg feed in broilers diet resulted in decreased serum calcium and increased serum chloride when compared to broilers fed on 25 mg FB1/kg in the study of (Munene and Youssef 2023). A specific biomarker of fumonisin is the increase of the sphingosine to sphinganine ratio due to the rise in concentration of free sphingoid bases occurring from the inhibition of ceramide synthase (Kenneth 2013). Serum enzymes released by tissues act as indicators that there is something wrong or cell death is occurring in tissues or organs such as the liver or kidney (Qassim et al. 2024).

Biochemically, aflatoxins affect energy, carbohydrates and lipids, nucleic acids and protein metabolism, and antagonism in the metabolism of vitamins, proteins and amino acids, lipids and carbohydrates, and damage to DNA. One of the most important effects of this toxin is the inhibition of protein synthesis, causing a marked reduction in the level of plasma protein, mainly α and β globulins, and albumin (Eliana and Estela 2010; Ahmed et al. 2023). Also, the activity of serum or plasma enzymes such as aspartate amino-transferase (AST) has been extensively used as a measure of aflatoxin toxicity in chickens.

Toxicity of some individual mycotoxins can be enhanced in a synergistic, additive or antagonistic manner when they occur as co-contaminants and are consumed by different animal models (Beatriz and Monika 2024). Weibking *et al.,* (1994) (Ogido 2004), concluded that the effects of AFB1 and FB1, in chickens and turkeys, when combined, can be more severe than when they are present alone. There is little information on the effects of simultaneous exposure to AFB1 and FB1 in broilers from commercial strains used in Iraq. The aim of this study was to detect AFB1 and FB1 natural contamination of broiler feeds and to evaluate sero-biochemical attractions in some of the broiler flocks fed diets contaminated with these mycotoxins.

Materials And Methods

Samples:

From march 2024 till October 2024, a total of sixty pelleted boiler feed {15 samples of feed No.0 (pre-starter, 1-5 days); 15 samples of feed No.1 (starter,6-20 days); 15 samples of feed No.2 (grower 21-30 days); 15 samples of feed No.3 (finisher> 30 days)}; were collected from different broiler farms in Mosul governorate (Kog-galy, Al-Hamdania, Al-Rashidia, and AL-Abasia) for detection of natural contamination with AFB1 and FB1 in these feed samples using ELISA (Enzyme linked immunosorbent assay).

AFB1 detection and quantification:

A commercially available ELISA plate kit ,was applied for the extraction and quantification of AFB1. Mycotoxin extraction and testing were performed according to the manufacturer's instructions. Ten grams were weighed for each feed sample and extracted in 100 ml flask with 50 ml of methanol solution(1part methanol+1 part water). The process continued by shaking for 15 min, filtration and discarding 1/4 of the first filtrate. Remaining filtrate was collected. Separatory funnel was used for separation layers resulted from addition of 20 ml CHCL3 to 10 ml of the filtrate. The mixture was shacked for 3 minutes, then releasing the down-layer of CHCL3 after stratification through filter paper pre-filled with 5 g anhydrous Na2SO4 in 100 ml evaporating dish. The process of releasing the down-layer of CHCL3 was repeated into evaporating dish. Evaporating dish was blown to dry at 65C0 in water bath. After drying and cooling, 10 ml of methanol solution (1 part methanol+1 part water) were added to dissolve the coagulation in evaporating dish completely to get sample extract solution. Samples were then taken to test directly by tacking 0.1 ml extracted sample solution+0.3 ml sample dilution (Dilution factor: 20). The test kit is based on the competitive enzyme immunoassay method. The coupling antigen is pre coated on the micro-well stripes. The AFB1 in the samples and the coupling antigens pre-coated on the micro-well stripes compete for the anti-AFB1 antibodies. After the addition of the enzyme conjugate, the substrate is added for coloration. Software for result analysis of ELISA was used.

FB1 detection and quantification:

A commercially available ELISA plate kit, was applied for the extraction and quantification of FB1 according to the instructions of the manufacture. Mycotoxin extraction and testing were performed according to the manufacturer's instructions. Five grams portion of each sample was extracted with 25 mL of sample extracted solution (4 parts of methanol with one part of deionized water) centrifuged at above 4000 r/min for 5 min. The supernatant

(100ml) was taken and 300µl were added of 1XFB1diluent (provided with the kit). Fifty ml were taken for test. The test kit is based on the competitive enzyme immunoassay method. The coupling antigen is pre-coated on the micro-well stripes. The FB1 in the samples and the coupling antigens pre-coated on the micro-well stripes compete for the anti-FB1 antibodies. After the addition of the enzyme conjugate, the substrate is added for coloration. Software for result analysis of ELISA was used.

Blood Biochemical parameters

At 42 days of age, four broiler flocks elected among 15 broiler flocks fed different combinations of naturally contaminated AFB1 and FB1. Fifteen broilers from each flock were used for blood collection through the brachial vein into polythene tubes. Serum was obtained by first allowing the blood to clot, followed by centrifugation at 2000 x g per minute for 30 min after collection and serum was stored at −20 °C till biochemical analysis was initiated. According to the instructions of the manufacture, commercially available colorimetric kits were used to determine TP, Chol, Tri, ALT), AST, and AP. activity and 4 Creatinine using commercial kits (BIOLABO S.A, France) according to manufacturer's instruction. Total analysis was performed on spectrophotometer according to protocol.

Statistical analysis

Statistical analysis were carried out using a software package "SPSS' (Ogido et al. 2004).

Results

Mycotoxin's detection and quantification:

Table (1) presents the incidence of AFB1 contaminated pelleted broiler feed samples in Nineveh governorate. Of the 60 samples tested, 18 were positive (30.0%). Number and percentages of positive samples were as follows: 4 (26.66 %), 5 (33.33%), 5 (33.33%), 4 (26.66 %) for feed-0, feed-1, feed-2 and feed-3, respectively. The incidence of fumonisin FB1 contaminated pelleted broiler feed samples in Nineveh governorate is shown in table (1). Of the 60 samples tested, 50 were positive (83.33%). Number and percentages of positive samples were as follows: 13 (86.66 %), 11 (73.3%), 12 (80%), 14 (93.33%) for feed-0, feed-1, feed-2 and feed-3, respectively. Mean, median and range of AFB1 in these feed types is illustrated in Table (2). From this table it is clear that AFB1 mean of the total feeds were (4.646), (3.260), (6.096), (5.012) µg/kg for feed-0, feed-1, feed-2 and feed-3, respectively. Mean, median and range of FB1 in these feed types are illustrated in Table (2). From this table it is clear that FB1 mean in total feeds were (289), (269.13), (296.16), (291.89) µg/kg for feed-0, feed-1, feed-2 and feed-3, respectively.

In detail, AFB1 means in these feeds for each broiler flock of the 15 flocks are illustrated in (Table 3), they were distributed between 0.881 and 19.030 µg/kg.

In detail, FB1 means in these feeds for each broiler flock of the 15 flocks are illustrated in (Table 3), they were distributed between 144 and 486 µg/kg.

Distribution of positive samples to their AFB1 concentration is shown in Figure (1). From this figure, it is clear that 45 samples (90%) were occurred between 0 and 600 µg/kg, while the remaining 5 samples (10%) have concentrations between 601 and 1200 µg/kg. Distribution of positive samples to their FB1 concentration are evident in Figure (2). From this figure, it is clear that 12 samples (66.66%) were occurred between 0 and 20 µg/kg, while the remaining half (6 samples) (33.33%) have concentrations between 21 and 50 µg/kg.

parameters	AFB1				FB1			
	Broiler feed-0	Broiler feed-1	Broiler feed-2	Broiler feed-3	Broiler feed-0	Broiler feed-1	Broiler feed-2	Broiler feed-3
Samples analysed	15	15	15	15	15	15	15	15
Samples positive	4	5	5	4	13	11	12	14
Percentage (%)	26.66	33.33	33.33	26.66	86.66	73.3	80	93.33
Total	18/60				50/60			
Mean	30				83.33			

Table 1: Incidence of fumonisin B1 contaminated pelleted broiler feed samples in Nineveh governorate.

Mycotoxins	AFB1(µg/kg)				FB1(µg/kg)			
parameters	Broiler feed-0	Broiler feed-1	Broiler feed-2	Broiler feed-3	Broiler feed-0	Broiler feed-1	Broiler feed-2	Broiler feed-3
Means of positive samples	17.4	9.7	18.2	22.6	232.6	367.0	370.2	278.4
Means of all positives	17.0				337.0			
Means of total samples	4.6	3.2	6.0	6.0	289.0	269.1	296.1	291.8
Means of total all	5.0				286.5			
Median	14.3	7.1	12.4	19.9	252.0	375.5	336.0	194.2
Means of total median	13.4				289.4			
Range	7.4-37.4	5.4-14.8	3.5-43.3	6.2-44.4	102-915	114-624	165-630	156-1200
Range of all samples	3.5-44.4				102-1200			

Table 2: Positive samples, number, mean (µg/kg), median and range of samples contaminated by AFB1 and FB1.

Broiler farms	Types of broiler feed				mean AFB_1 (µg/kg)	Types of broiler feed				mean FB_1 (µg/kg
	Feed-0	Feed- 1	Feed- 2	Feed-3		Feed-0	Feed- 1	Feed- 2	Feed-3	
	AFB_1(µg/kg)					FB_1(µg/kg)				
Farm 1	21.3	ND	ND	ND	**5.3**	102	292.5	ND*	384	**194.6**
Farm 2	ND	7.118	ND	ND	**1.7**	217.5	ND	165	120	**125.62**
Farm 3	ND	ND	43.3	32.7	**19.0**	ND	114	630	1200	**486.0**
Farm 4	ND	14.830	ND	ND	**3.7**	223.5	375.5	267	202.5	**269.6**
Farm 5	7.4	ND	ND	ND	**1.8**	915	444	210	162	**432.7**
Farm 6	ND	14.451	ND	44.4	**14.7**	258	552	277.5	120	**301.8**
Farm 7	5.4	ND	ND	ND	**1.3**	285	147	276	252	**240.0**
Farm 8	ND	10.924	ND	ND	**2.7**	ND	525	360	156	**260.2**
Farm 9	ND	ND	ND	6.2	**1.5**	552	624	ND	120	**324.0**
Farm 10	ND	ND	12.4	ND	**3.1**	528	ND	462	186	**294.0**
Farm 11	3.5	ND	ND	ND	**0.8**	250	396	312	120	**262.5**
Farm 12	ND	ND	3.5	ND	**0.8**	250	ND	546	252	**262.0**
Farm 13	ND	7.048	ND	ND	**1.7**	225	243	435	300	**300.7**
Farm 14	37.4	ND	ND	7.2	**11.1**	267	ND	502.5	804	**393.3**
Farm 15	ND	ND	21.1	ND	**5.2**	252	324	ND	ND	**144.0**
Mean					**5.0**					**286.5**

ND= Not detected

Table 3: FB1analysis results of pelleted broiler feeds.

Figure 1: Distribution of feeds number on their AFB1concentration.

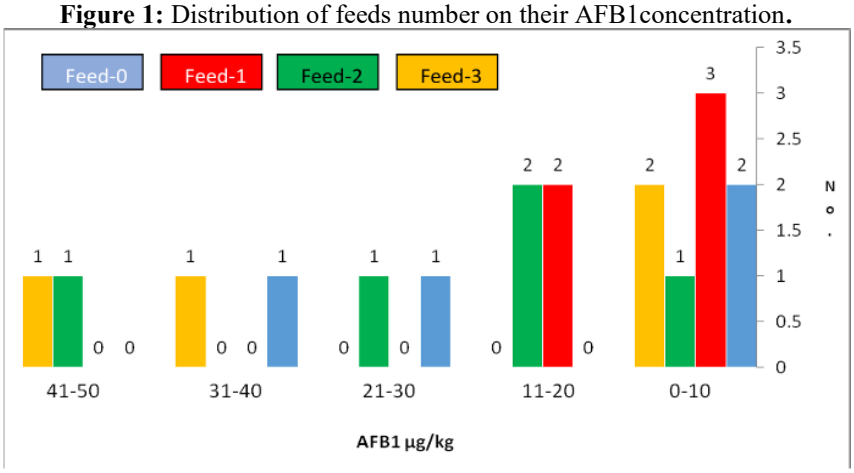
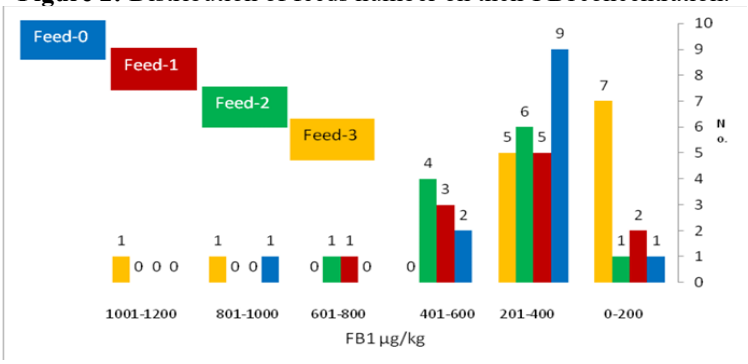


Figure 2: Distribution of feeds number on their FB1concentration.



Blood biochemicals:

Results from serum analyses are presented in Tables 4 to 6. Serum levels of TP, Creatinine, Glucose, Chol, and Tri, and serum activity of ALT, AST, and AP were not significantly ($p<0.05$) different in broilers of the four tested flocks receiving AFB1+FB1 contaminated feed for 42 days.

Broiler flock	Mycotoxins (µg/kg)		Total protein(g/dl)	Creatinine (mg/100ml)	Glucose (mg/100ml)
	AFB1	FB1			

Flock 1	5.2	144.0	2.6 ± 0.1	0.8 ± 0.1	230.3 ± 18.0
Flock 2	14.7	301.8	2.5 ± 0.2	1.2 ± 0.1	203.2 ± 17.0
Flock 3	11.1	393.3	2.9 ± 0.2	1.0 ± 0.1	204.4 ± 18.8
Flock 4	19.0	486.0	2.5 ± 0.1	1.2 ± 0.1	167.0 ± 5.5

Table 4: Serum levels of total proteins ,creatinine and glucose in broiler chickens fed AFB1+ FB1 contaminated feed during 42 days.

.Broiler flock	Mycotoxins (µg/kg)		AST(IU/L)	ALT(IU/L)	AP(IU/L)
	AFB1	FB1			
Flock 1	5.28	144.0	233.9 ± 1.5	24.5 ± 1.1	56.9 ± 2.8
Flock 2	14.735	301.87	237.6 ± 2.9	23.6 ± 1.5	61.3 ± 3.2
Flock 3	11.156	393.3	239.0 ± 2.4	24.6 ± 0.9	59.7 ± 3.2
Flock 4	19.030	486.0	242.1 ± 3.1	27.2 ± 1.0	61.4 ± 4.3

Table 5: Serum levels of AST ,ALT and AP in broiler chickens fed AFB1+ FB1 contaminated feed during 42 days.

Broiler flock	Mycotoxins (µg/kg)		Triglycerides (mg/100ml)	Cholesterol (mg/100ml)
	AFB1	FB1		
Flock 1	5.28	144.0	83.7 ± 5.6	170.0 ± 6.5
Flock 2	14.735	301.87	73.1 ± 4.0	182.9 ± 7.4
Flock 3	11.156	393.3	83.5 ± 6.0	175.2 ± 10.0
Flock 4	19.030	486.0	91.0 ± 6.1	183.6 ± 6.2

Table 6: Serum levels of Triglycerides and cholesterol in broiler chickens fed AFB1+FB1 contaminated feed during 42 days.

Discussion

From the results of this study, it is evident that all the 15 broiler flocks feeds (including feed types, 0,1,2 or 3 were contaminated with Aflatoxin and Fumonisins mycotoxins in different concentrations, which indicated clearly that these mycotoxins are unavoidable feed contaminants. They have been detected from broiler feeds intended for use in poultry. These mycotoxins are among the most significant mycotoxins in naturally contaminated feed like ochratoxins, T-2 toxins, Zearalenone, and deoxynivalenol (Murugesan and Ledoux 2015). aflatoxin B1 in this study, was recovered in 30% of the tested 60 broiler feed samples with a mean value of 5 µg/kg feed. These findings were in the same line of the survey program accomplished by Bio min company carried out all over the world about the presence of different mycotoxins in feeds through 2011. Aflatoxin was recovered in the mentioned worldwide study in 27% of the 2770 feed samples with an average of 16 µg/kg. Moreover and within the same geographical area, in the middle east(Egypt, Iran, Palestine and Saudia Arabia), aflatoxin was recovered in 37% of the tested feed samples with an average of 1 µg/kg (Naehrer 2012). So our results of aflatoxin recovery percentage and average aflatoxin concentration is very close to the percentage obtained by Bio min survey in Middle East. Another worldwide survey which also carried out by the same company, Bio min, through 2012 from January to December, a total of 4023 samples collected worldwide were analyzed for the presence of mycotoxins. A total, 14468 analyses were carried out for the most important mycotoxins, showed a varied percentages of aflatoxin positive results in each region in the world and were 30%, 19%, 0%,71%, 10%, 57%, 44%,73%, 35%, 18%, 80%, 2%, with an average Aflatoxin concentration ranging from 0-181 µg/kg, in North America, Central Europe, North Europe, Eastern Europe, North Asia, South-east Asia, Southern Europe, South Asia, Middle East, South America, Africa and Oceania, respectively (Naehrer 2012).

The second Mycotoxin in our study, Fumonisin B1, was recovered in a percentage of 83.33%, (50 positive samples out of 60 tested samples), with an average of 286.5µg/kg. These findings are close to the findings obtained by Bio min survey about the feed contamination with fumonisin in Middle East which revealed 67% of Fumonisin B1contamination of tested samples with an average of 298 µg/kg. Another worldwide survey which also carried out by the same company, Bio min, through 2012 from January to December, a total of 4023 samples collected worldwide were analyzed for the presence of mycotoxins. A total, 14468 analyses were carried out for the most important mycotoxins, showed a varied percentages of Aflatoxin positive results in each region in the world and were 78%,23%,0%,54%, 60%, 53%, 84%,45%, 65%, 64%, 100%, 12%,with an average Aflatoxin concentration ranging from 0-1501 µg/kg, in North America, Central Europe, North Europe, Eastern Europe, North Asia, South-east Asia, Southern Europe, South Asia, Middle East, South America, Africa and Oceania, respectively (Rodriguse 2013). These figures are not surprising, since The Food and Agriculture Organization (FAO) estimated that 25% of the world's food crops are affected with mycotoxins and the main causes of mycotoxins contamination in feeds are high temperature, humidity, physical and chemical damage caused by insects and unfavorable storage conditions which could result in the generation of toxins . Aflatoxin B1 and Fumonisin B1 ranges in this study (0-50 µg/kg and 0-1200 µg/kg) were lower than those reported in the two surveys done by Bio min company during 2011-2012 (0-181 µg/kg and 0-1501 µg/kg). The main cause may be related to the addition of mold inhibitors to the broiler feeds which could aid in the lowering of the mycotoxins

ranges.

The effect of feeding AFB1 and FB1 at four combinations of AFB1 and FB1 naturally (AFB1 5.2 µg/kg + FB1301.8 µg/kg); (AFB1 14.7 µg/kg + FB1301.8 µg/kg); (AFB1 11.1 µg/kg + FB1 393.3 µg/kg); (AFB1 19.0 µg/kg + FB1 486.0 µg/kg) contaminated four broiler flock diets on serum biochemicals of broilers at 42 days of age (Tables 4, 5 and 6) showed no significant differences between these broiler flocks in all studied parameters and the total serum protein in the birds of four flocks occur within the normal physiological levels of (2.5-4.5g/dl) (Agag 2004). It is an expected findings, since other studies revealed that AFB1 at a rate of ≥300 µg/kg and FB1 at a rate of 75 mg/kg were reported to decrease TP in broiler chickens (Thrall 2004). founded that plasma TP was lower ($p<0.05$) at six days post feeding only in groups fed 200 µg AFB1/kg alone or in combination with 200 mg/kg FB1. Other studies confirmed these results, among them (Eliana et al. 2010), fed broilers 50 and 100 µg AFB1/kg diet and also reported no differences in serum total protein at day 33 post feeding. In contrast (Oguz et al. 2002), fed broilers 0.5 and 1 mg AFB1/kg diet and reported decreased serum total protein concentrations at both days 21 and 42 of age. Similarly (Safameher 2008), fed broilers 1 mg and 2 mg AFB1/kg diet and also reported decreased serum total protein concentrations at both days 21 and 42 of age. Results of the current study and those cited appear to justify the conclusion by (Eliana et al. 2010) that concentrations of AF≥300 µg/kg are required before serum total proteins are affected.

Aflatoxin contamination of feed causes significant loss of nitrogen and amino acids and inability of the intestine to absorb, leading to a decrease in the level of total protein (Chen and Naehrer 2016; Davide 2022; Qassim et al. 2023). As for other species considered more sensitive to the effects of mycotoxins, (Ogido 2004) fed turkey poults at levels similar to the present study (200 µg AFB1/kg and 75 mg FB1/kg), and observed a reduction in serum total proteins in the groups fed AFB1 alone and in combination with FB1 by day 21 of age**.**

Creatinine values were not different significantly the four broiler flocks, since in all the mycotoxin combinations in these flocks did not reach the effective level in each mycotoxin alone or in combination to increase creatinine level in plasma of broilers. Matur et al., stated that AFB1 at a rate of 100 mg/kg had no effect on creatinine level, but a level of 3.5 mg AFB1/kg feed was effective in reducing creatinine level in broiler chicks (Allameh et al. 2005). Weibking et al., 1993 (Agag 2004), fed turkey 100 and 200 mg FB1 /kg feed observed no increase in creatinine level compared with control in spite of more sensitivity of turkey to FB1 than broilers. Levels of AFB1 and FB1 in all four flocks in this study were far lower than the effective concentrations to increase creatinine level in broiler chicks, which means no inflammatory or degenerative changes in the kidney. No significant differences were also found in serum glucose levels of broiler chicks fed mycotoxin combinations in four broiler flocks like that of creatinine and TP. Zhao et al., 2010 (Zhao et al. 2010), found that AFB1 was not less than 200 µg/kg and could reduce glucose level in broilers serum compared to that of the control. Broomhead et al.,2002 (Ogido 2004), showed that no reduction in glucose level was obtained when broilers or turkey fed 25 and 50 mg FB1 /kg feed.

Liver is the prime target organ during aflatoxicosis and Fumonisins toxicosis. Increased activities of serum ALT, AST, ALP and LDH in broiler chicks are well known diagnostic indicators of hepatic injury. Wherever there is Liver disorder like inflammation of liver, a space occupying lesion or obstruction of biliary tract, so the activity of AST and ALT found to be altered. Alanine amino transferase and AST activities were within the normal physiological levels (not more than 275 IU/L for AST, and between 19-50 IU/L for ALT (Agag 2004). So they showed a non-significant difference among treatment groups. This indicates that the combination of these mycotoxins at their concentrations had no detrimental effect on the liver. However, altered concentrations of AST but not ALT enzyme was noted upon feeding 200, 400, 600 µg/kg (Zhao et al. 2010) and 1 mg AFB1/kg diet (Ananda 2010; Surai and Dvorska 2005). Alanine amino transferase activity could not be true indicator of liver damage.

Broomhead et al., 2002 (Ogido 2004), did not find significant affect on serum AST, ALP, or GGT in broiler chicks or turkey fed Fumonisin B1 at a rate of 25 and 50 mg/kg. ALP was also not affected in previous experiments with turkeys (Sefa et al. 2005) and laying hens (Kenneth 2013) fed 200 mg FB1/kg or broilers fed 20, 40, or 80 mg/kg purified FB1 (Munene and Youssef 2023). An increase in AST and liver lesions and a decrease in ALP have been observed in turkeys fed 100 mg FB1/kg (Thrall 2004). Increases in the serum enzyme AST that occurred in broilers fed 30 or 300 mg FB1/kg from cultural material were suggested to be linked to liver damage.

Serum cholesterol and triglycerides in all broiler flocks fed naturally contaminated AFB1 and FB1 in the concentrations listed in Table 6 were within the normal physiological levels in birds (Agag 2004), and were not different significantly in the four flocks. Cholesterol and triglycerides were reduced by adding 250 µg/kg aflatoxin B1 to broiler diet, and was also reported by (Bermudez et al. 1997) who found a reduction in serum triglyceride in broiler chicken received aflatoxin at a rate of 1 mg/kg feed. The lowest AFB1and FB1 that could lower cholesterol level in broiler serum were given by (Thrall 2004), who found that Serum cholesterol levels of poults fed 100 mg/kg dietary FB 1 were lower than those of controls and who stated that cholesterol levels of broilers fed 300 µg/kg dietary FB1 were lower than those of controls. The decrease in total cholesterol and triglycerides is due to increased expression of protein kinase and an increase in lipolysis genes in addition to a decrease in fatty acid absorption genes (Hao and Ruitao 2023).

No significant Differences in serum chemical substances of broiler chicks in these flocks indicated that there was no interaction between aflatoxin B1 and Fumonisin B1 in these naturally contaminated levels which could not lead to potentiation or suppression of the toxicity of either of the feed toxicants.

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