Tikrit Journal for Agricultural Sciences (2022) 22 (4):45-53
https://doi.org/10.25130/tjas.22.4.7

IRAQI Academic Scientific Journals

ISSN:1813-1646 (Print); 2664-0597 (Online)
Tikrit Journal for Agricultural Sciences
Journal Homepage: http://www.tjas.org
E-mail: tjas@tu.edu.iq

TJAS
Tikrit Journal for Agricultural Sciences

Use the Iraqi Sodium Bentonite to ameliorate the impact of Fumonisine B1 and Aflatoxin B1 combination on some broiler physiological characters

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KEY WORDS:

Fumonisin B1and Aflatoxin B1, Iraqi Sodium Bentonite, complete blood picture, blood biochemistry parameter.

ARTICLE HISTORY:

Received: 24/04/2022

Accepted: 06/07/2022

Available online: 31/12/2022

ABSTRACT

The aim of this study was to investigate the efficiency of Iraqi Sodium bentonite into decrease the negative influences of Fumonisin B1(FB1) and Aflatoxin B1(AFB1) in some broiler physiological parameters, one hundred and twenty broiler chicks 1-day-old (Ross 308) unsexed were used. The chicks were randomly distributed into four treatment groups with three replicates for each treatment contain ten birds and to 35 day olds, the experimental treatments were conducted as follows - T1. Basil diet (BD) no other addition (Control). T2. BD containing 300 mg FB1/kg diet + 2 mg AFB1/kg diet; T3. BD + 0.4% Iraqi bentonite kg/diet containing 300 mg FB1/kg diet 2 mg AFB1/kg diet; T4. BD+ 0.8% Iraqi bentonite kg/diet containing 300 mg FB1/kg diet+ 2 mg AFB1/kg diet. Treatment two (T2) represent the effect of (FB1) and (AFB1) contaminated the broiler diets on some physiological characters, the statically results shows significant ($p \leq 0.05$) decrease in the RBC, PCV and Hb significantly while the WBC and H/L ratio were increased significantly ($P < 0.05$). The Heterophils (H) result shows that T2 was significantly higher ($P < 0.05$) than result of T1 (control group), in addition, T3 shows that However, T4 was shows no significant different result compare to control group. That mycotoxins reduced significantly ($P < 0.05$) the concentrations of Total protein, Albumen, Globulin, Glucose and Triglyceride in treatment in T2 compare to control group. Other results present in Cholesterol values and Uric acid were significantly increase. Treatment T4 that included adding Iraqi sodium bentonite illustrate no significant different compare to control group in most experimental parameters. © 2022 TJAS. College of Agriculture, Tikrit University

INTRODUCTION

The downstream handling for foodstuffs, grains and feeds such as poor harvesting applies, unsuitable storage and less than optimal circumstances during transportation, processing, and storage can may contribute to the growth of fungi and increase the threat of the major food spoilage agent caused by mycotoxin production (Khazaeli *et al*., 2017: Tardieu *et al*. ,2021).
Mycotoxins are fungal secondary metabolites that can accumulate in these resources leading to important economic losses, as well as to problems for livestock and human health. Some fungi can produce more than one mycotoxin, and the same mycotoxin can be produced by different fungi species (Mohd-Redzwan *et al*., 2013; Murugesan *et al*., 2015; Gott *et al*., 2018). However, little is known about how mycotoxins interact due to their complexity. Toxicity of some mycotoxins may

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increase in a synergistic, additive, or antagonistic way when they are combined in the diet of animals (Ogido *et al*., 2004). The natural co-occurrence of Aflatoxin B1 (AFB1) and Fumonisin B1 (FB1), especially in corn, is a worldwide problem and has been associated with a high incidence of human hepatocellular carcinoma (CAST, 2003).

Aflatoxin are secondary metabolites produced by toxigenic strains of *Aspergillus flavus, Aspergillu snomiusand* and *Aspergillus Parasiticus*. Which are known to be potent carcinogens and hepatoxic agents and pose serious hazards to human and animal health (Afzali and Devegowda, 2004;Sidhu *et al*., 2009). In addition, aflatoxin can cause serious economic loss by reducing grain nutritive value and animal production (Wu, 2004; Diaz *et al*., 2008). Aflatoxin B1 (AFB1), an active hepatocarcinogen and the most toxic AF, is known to depress performance and production, impair the immune system, and induce liver diseases and metabolic disorders in poultry species (Andretta *et al*., 2012; Somorin *et al*., 2012). The *Fusarium verticillioides* and *proliferatum* are the principal of Fumonisin (FB1) producing fungus in moldy grains (Tran *et al*., 2005 Tardieu *et al*., 2004 ;Wa´skiewicz *et al*., 2012).It is the most abundant of the fumonisin family and usually represent 70-80% of the total fumonisin content in Fusarium verticilioides cultures and naturally contaminated foods.(Migheli *et al* 2010).The decreased in serum protein concentration is a dependable biomarker of hepatotoxicity induced by AFB1 (Kubena *et al*., 1990; Kececi *et al*., 1998). Decreased serum glucose (Harvey *et al*., 1993; Zhao *et al*., 2010).

Further, the mycotoxin co-occurrence in grains (samples containing more than one mycotoxin) has been frequently reported and reviewed by multiple scientific publications (Benlasher *et al*., 2012; Gruber-Dorninger *et al*., 2019; Munkrold *et al*., 2019). However, with the growth improvements in modern broiler over the past 30 year, an increasing number of publications have demonstrated a close correlation between low doses of mycotoxin (AFB1 and FB1) and its influence on growth and health status in broiler chickens (Zain *et al*. 2011: Erdely *et al*.2018). Therefore, there is wide interest in the use of biological products to decrease mycotoxin availability. One of the alternatives is the use of adsorbents, such as natural clay mineral which bind to mycotoxins and prevent their absorption by the gastrointestinal tract, making them inert to animals (Basalan *et al*.2006: Zhang *et al*.2015).

Our study aims to detriment the ability of Iraqi Sodium Bentonite in case of synergistic mode of effect to AFB1 and FB1 on some broiler physiological parameters.

MATERIAL AND METHODS

This study was carried out at Animal department farm poultry farm section in Tikrit University for the period between (15/9/2021) to (19/10/2021). The experiments included, one hundred and twenty unvaccinated broiler chicks (Ross-308) were obtained from a commercial hatchery in Erbil province. The birds were placed in identical size batteries, and the experiment consisted four treatments, three replicates and thirty chicks of each, weighed chicks were divided into all of it at seven day of age. Birds assigned at randomly to the cages treatment groups. In an environmentally controlled room with 24-h day fluorescent lighting room temperature was maintained at 32ºC during the first week and gradually decreased to 22º C by the end of 3th week. Birds allowed to consume feed and water *ad-libitum* that were fed rations containing know concentration of (FB1) and (AFB1). The Iraqi sodium bentonite prepared by directly grinding and then give it to the birds to consume with the diet.

The experimental treatments received a corn – soybean meal basal diet (BD) and depending on the addition were labeled as follows: -

T1. BD no other addition (Control).
T2. BD containing a 200 mg FB1/kg diet+ 3 mg AFB1/kg diet
T3. BD containing a 200 mg FB1/kg diet + 3 mg AFB1/kg diet + Iraq Sodium Bentonite 0.4% diet.
T4. BD containing a 200 mg FB1/kg diet+3 mg AFB1/kg diet Irai Sodium Bentonite 0.8% diet.

The basal diet was also tested for possible residual fumonisin B1 and aflatoxin B1 before feeding. Diet Preparation mycotoxin test concentrations were obtained using Sun flower oil as the diluent and appropriate amounts of these solutions were added to the basal diet to obtain the required following procedures proposed by (Shenzhen Lvshiyuan Biotechnology Co., Ltd. Guangdong, China) sensitivity 0.1ppb.

Table (1): Composition of experiment diet %

Ingredient	Starter diet 1-10 day	Grower diet 11-24 day	Finisher diet 25-35 day
Corn	46.18	50.08	53.88
Wheat	9.92	10.22	10.4
Soybean meal 48%	36.9	33	29.5
Premix[1]	2.5	2.5	2.5
Dicalcium phosphate	1	0.5	0.2
Limestone	0.3	0.3	0.5
Lysine	0.3	0.2	0.12
Methionine	0.1	0.1	0.1
Sunflower oil	2.8	3.1	2.8
Total	100	100	100
Approximate analysis(calculated)			
Crude protein%	23.02	21.52	20.02
Metabolizable energy%	3028	3100	3202
Crude fiber%	3.761	3.489	3.354
Lysine%	1.48	1.28	1.12
Methionnine%	0.57	0.56	0.54
Cystine+ Methionnine%	0.92	0.89	0.85
Calcium%	0.97	0.84	0.83
Available phosphate%	0.61	0.52	0.45

1 Each kilogram contains calcium, 196 g; phosphorous, 64 g; sodium, 30 g; magnesium, 6 g; copper, 400 mg; zinc, 1,200 mg; iron, 2,000 mg; manganese, 1,200 mg; cobalt, 20mg; iodine, 40 mg; selenium, 8 mg; vitamin A, 200,000 IU; vitamin D3, 80,000 IU; vitamin E, 1,600 mg; vitamin K3, 34 mg; vitamin C, 1,300 mg; vitamin B1, 35 mg; vitamin B2, 135 mg; vitamin B6, 100 mg; vitamin B12, 670 µg; nicotinic acid, 1,340 mg; calcium pantothenic acid, 235 mg; choline chloride, 8,400 mg; folic acid, 34 mg; biotin, 3,350 µg; and methionine, 30 g.

Fumonisin B1 and Aflatoxin B1 were produced in the lab via fermentation of milled rise and corn by *Aspergillus parasiticus* NRRL. and *Fusarium verticillioides* M. according to (Ledoux *et al.* 1992). For treatment rations were detected in the basal diet were determined by a monoclonal antibody-based ic-ELISA.

Samples of blood collected at the age of 5 weeks and at the end of the experiment six birds per treatment, both sexes equally and directly from the jugular vein at slaughter using two types of tube in order to conduct tests of cellular wares on the numbers of blood cells of both lymphatic (lymphocyte) and heterogeneous (Hetrophils) and the percentage of cells differentiated into lymphocytes (L / H) ratio, and the second contains no objection clotting , which put it in the refrigerator, then placed in a centrifuge at 3000 r / min for 15 minutes to separate the serum , which save off at a temperature of -18 ° m for the tests.

Total Protein, Albumin, Triglyceride, Glucose and Uric acid was measured concentration of each by using ready to use solutions record (Kit) is equipped with the company SPINREACT Spanish.

Data of the experiment were analyzed using the Complete Randomized Design (CRD) model and the (SAS ,2001) statistical program to study the effect of the transactions studied in different qualities and compared the differences morale among the averages using the test (Duncan,1955) according to the mathematical model the following:

$Yij= \mu + Ti + eij$

Yij = value attributable to the treatment of viewing I
μ = the overall average for the recipe studied
Ti = effect of treatment I
eij = random error which is normally distributed with an average equal to zero and variance equal amount² eσ.

RESULT AND DISCUSSION

The table (2) illustrate the hematological parameters in broilers challenged by FB1 and AFB1 synergistic impact in diet. Red blood cells (RBC), White blood cells (WBC), Packed cells volume (PCV), Hemoglobin (Hb), Heterophils (H), Lymphocyte (L) and Heterophils Lymphocyte Ratio (H/L). As these characters considerate are more sensitive than variables such as production performance, this purpose would signpost the intoxication in advance to other symptoms (Oğuz *et al*., 2000; Thieu *et al*., 2008; Khan *et al*., 2020).

Mycotoxins reduced ($P < 0.05$) the concentration of RBC, PCV and Hb significantly while the WBC and H/L ratio were increased significantly ($P < 0.05$) in the end of experiment. However, the RBC value were significantly decreased in T2 and T3 comparer to control group where T4 shows no significant ($P < 0.05$) difference compare to it. The results of WBC show significant increase in T2 compare to control group while T3 and T4 shows significant ($P > 0.05$) different compare to control group. The PCV and Hb values shows that T2 and T3 were less significant than control group, while T4 illustrated no significant ($P > 0.05$) different compare to control group.

The Heterophils (H) result shows that T2 was significantly higher ($P < 0.05$) than result of T1 (control group), in addition, T3 and T4 were also more significant result compare to control group. The result of H/L ratio value for T2 shows significant ($P < 0.05$) different compare to control group, the rest of treatments shows no significant differentiation.

Biochemical parameters in broilers challenged by Fumonisin B1 and Aflatoxins B1 are presented in Table 3. The results suggest that mycotoxins reduced significantly ($P < 0.05$) the concentrations of Total protein, Albumen and Globulin in treatment in T2 compare to control group T4 shows no significant ($P < 0.05$) different compare to it. The same variables were affected ($P< 0.05$) by the presence of aflatoxins B1 and fumonisin B1 in diets on Glucose in T2 while T3 and T4 shows no significant ($P < 0.05$) difference same results present in Cholesterol values, however with a reduction of Triglyceride and Uric acid, compared with those of the control groups. The concentration of urea was affected by the presence of mycotoxins in diets but the T4 shows no significant ($P > 0.05$) different compare to control group. This observation may be partially explained by the great variability among results in the database studies. Rauber *et al*., (2007) reported that Aflatoxins significantly affect feed consumption, total plasmatic proteins and cholesterol levels of turkeys. Furthermore, AFs lead to irreversible liver damage as indicated by decreased liver-to-body weight ratios, liver enzyme alterations, altered blood coagulation patterns, and histologic changes like hepatocellular necrosis and biliary hyperplasia in turkeys (Quist *et al*., 2000; Klein *et al*., 2002).

These changes are probably associated with the impairment of functions, such as amino acid transport and protein synthesis, usually related to aflatoxicosis (Meissonnier *et al*., 2006). The decrease observed in uric acid levels can be explained by changes in the efficiency of amino acid utilization, in enzyme systems, in renal filtration, or in reabsorption rates or by unknown aspects (Swamy *et al*., 2002). However, in several studies of the database, the level of uric acid appeared increased as evidence of injury in hepatocytes, where the substance is produced. Aflatoxins can induce important macroscopic and microscopic changes in various organs, such as the kidneys, spleen (Bilgic and Yesildere, 1992), and liver the latter being the main target organ of aflatoxicosis (Diaz *et al*., 2010).

Broilers challenged by aflatoxins in this study presented a reduction ($P < 0.05$) the plasma concentration of glucose. Animals challenged by mycotoxins showed lower plasma levels ($P < 0.05$) of triglycerides, albumin, globulin, and total cholesterol. Aflatoxins reduced ($P < 0.05$) the concentration of triglycerides, albumin, globulin, and total cholesterol.

The interference on the plasma levels of albumin may be due to damage in hepatocytes and impairment of protein synthesis in challenged broilers (Faixova *et al*., 2007).
The transport and plasma profile of lipids may also be influenced by liver damage caused by mycotoxins, particularly aflatoxins. Thus, serum cholesterol increase caused by aflatoxin can be related to inhibition of the biosynthesis of the compound to liver damage and changes in concentration of circulating cholesterol to the liver (Kubena *et al*., 1993).

Little is known about the mechanism involved in aflatoxin removal, an understanding of the binding mechanism is required to allow the optimization and safe dietary application of this methodology. In addition, it is highly probable that AFB1 in contaminated foods (Oliveira *et al.*, 2006; Pietri *et al*., 2009; Rocha *et al*., 2009).

Table (2): Effects of contaminated diet and detoxification ability of Iraqi sodium bentonite on some blood characters for broiler

Treatment	RBC(10^6cell/ ml^3)	WBC (10^3cell/ml^3)	PCV%	Hb(gm/100ml)	H (10^3/ml^3)	L(10^3/ml^3)	H/L ratio
T1	3.04±0.08 ^{a}	22.87±0.30^b	33.79±0.59 ^{a}	11.26±0.32^a	25.31±0.21 ^{c}	55.87±0.06 ^{ab}	0.453±0.04^b
T2	2.30±0.085^b	26.33±0.26^a	28.76±0.37^c	9.58±0.27^c	27.94±0.20^a	55.26±0.02 ^{b}	0.505±0.02^a
T3	2.18±0.04^b	26.17±0.36^c	28.73±0.20^c	9.57±0.17^c	27.25±0.20^b	55.28±0.04 ^{ab}	0.492±0.02^a
T4	2.64±0.034^a	24.50±0.74^c	31.10±0.32^b	10.36± 0.43^b	25.32±0.23^b	55.62±0.05 ^{a}	0.455±0.04^b

T1. BD (Basil diet) no other addition (Control). T2. BD containing a 300 mg FB1/kg diet+2 mg/ AFB1/kg diet T3. BD +Iraq calcium bentonite 0.4% diet +300 mg FB1/kg diet+2 mg/ AFB1/kg diet. T4. BD + calcium bentonite 0.8% diet +300 mg FB1/kg diet+2 mg/ AFB1/kg diet. Within the same row means with different letter are significantly different p≤0.05. results are reported as means ±SEM.

Table (3): Effects of contaminated diet and detoxification ability of Iraqi sodium bentonite on some blood biochemistry characters for broiler

Treatment	Total protein gm/100ml	Albumen gm/100ml	Globulin gm/100ml	Glucose U/L	Cholesterol U/L	Triglyceride mg/100ml	Uric acid mg/100ml
T1	4.52±0.03 ^{a}	1.53± 0.07^{a}	2.99±0.06 ^{a}	189.84±1.92^{a}	173.31±1.13^{b}	159.29±2.53^{a}	6.15±0.03^{c}
T2	3.42±0.08^{b}	0.83±0.04^{c}	2.59±0.12^{b}	160.78±0.60^{b}	179.143±3.28^{a}	129.04±1.91^{c}	9.78±0.27^{a}
T3	3.44±0.06^{b}	0.78±0.05^{c}	2.66±0.10^{b}	190.48±2.61^{a}	137.15±2.12^{b}	117.17±3.23^{d}	7.46±0.26^{b}
T4	4.38±0.08^{a}	1.32±0.02^{b}	3.06±0.316^{a}	190.72± 3.67^{a}	134.10±1.20^{b}	144.22±3.19^{b}	6.30±0.05^{c}

T1. BD (Basil diet) no other addition (Control). T2. BD containing a 300 mg FB1/kg diet+2 mg/ AFB1/kg diet T3. BD +Iraq calcium bentonite 0.4% diet +300 mg FB1/kg diet+2 mg/ AFB1/kg diet. T4. BD + calcium bentonite 0.8% diet +300 mg FB1/kg diet+2 mg/ AFB1/kg diet. Within the same row means with different letter are significantly different p≤0.05. results are reported as means ±SEM.

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أستخدام بنتونايت الصوديوم العراقي لمنع التأثير التأزري للفيومنسين B1 والافلاتوكسين B1 في بعض الصفات الفسلجية لفروج اللحم

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الخلاصة

الكلمات المفتاحية:
االفيومنسين B1, ألافلاتوكسين B1 , بنتونايت الصوديوم العراقي ,فروج اللحم ,صورة الدم ,كيموحيوية الدم

هدفت هذة الدراسة الى التحري عن كفاءة بنتونايت الصوديوم العراقي الخام على القدرة على تقليل الاثر السلبي للفيومنسين B1 و ألافلاتوكسين B1 في بعض الصفات الفسلجية لفروج اللحم . مئة و عشرون فرخ من فروج اللحم الهجين Ross 308 غيرمجنس وبعمر يوم واحد ,وزعت عشوائيا على أربع معاملات تضمنت كل واحدة ثلاث مكررات أحتوت كل وادة منها عشرة طيور وحتى العمر 35 يوم من التربية ,معاملات التجربة كانت كالاتي:-المعاملة الاولى معاملة السيطرة عليقة أساسية بدون أي أضافة : المعاملة الثانية عليقة أساسية ملوثة بالفيومنسين B1 300 ملغرام /كغم علف مع الافلاتوكسين 2 ملغرام/كغم علف : المعاملة الثالثة عليقة أساسية ملوثة أساسية ملوثة بالفيومنسين B1 300 ملغرام /كغم علف مع ألافلاتوكسين 2 ملغرام/كغم علف + 0.4% بنتونايت الصوديوم العراقي الخام :المعاملة الرابعة عليقة أساسية ملوثة بالفيومنسين B1 300 ملغرام /كغم علف مع ألافلاتوكسين 2 ملغرام/كغم علف + 0.8% بنتونايت الصوديوم العراقي الخام, المعاملة الثانية تمثل تأثير الفيومنسين B1 و ألافلاتوكسين B1 التأزري على الصفات الفسلجية وقد أوضح التحليل الاحصائي حدوث أنخفاض معنوي في عدد الخلايا الحمر و الحجم المضغوط للخلايا وخضاب الدم في حين أرتفع وبشكل معنوي عدد الخلايا البيض و نسبة الخلايا الهيتروفيل الى اللمفوسايت حيث كانت نسبة خلايا الهيتروفيل هي ألاعلى في المعاملة الثانية وكانت قد أظهرت المعاملة الثالثة نتائج لم تختلف معنويا عن هذه المعاملة لكن المعاملة الرابعة لم تسجل فروق معنوية مقارنة مع مجموعة السيطرة .

أن التلوث بهذة السموم مجتمعة تسبب بأنخفاض معنوي في تركيز البروتين الكلي و الالبومين و الكلوبيولين و الكلوكوز و الكليسريدات الثلاثية في المعاملة الثانية مقارنة مع مجموعة السيطرة , النتائج ألاخرى تمثل قيمة الكوليسترول و حامض اليوريك التي أرتفعت معنويا .المعاملة الرابعة التي تضمنت أضافة بنتونايت الصوديوم العراقي أظهرت عدم وجود فروق معنوية مقارنة مع نتائج مجموعة السيطرة في أغلب الصفات الفسلجية التي درست.