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The Effects of Activated Sodium Bentonite on Distributed Three Types of Mycotoxins in Blood, Liver and Kidney of Broiler Chicks

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Abstract

This study have been don to know the effects of adding0.5% of Activated Sodium Bentonite (ASB)with 2.5 mg of aflatoxins (AF), 4 mg of ochratoxins (OCH), and 4 mg of T-2 toxin/kg of diets singly or in combination for each of OCH or T-2 toxin with AF on the distribution and levels of aflatoxin B1 (AFB1), aflatoxin M1 (AFM1), Ochratoxin A (OA), and T-2 toxin in blood, liver, and kidney of the broiler chicks fed from 1 to 21 days age. The results indicated that the residue's concentration of AFB1 and AFM1 in the samples of blood , liver, and kidney were significantly ($p<0.05$) decreased when we add the AF alone with ASB in the diet of chicks (as shown with treatment T2) which equal to(4.35 and 8.40 ng/ml), (18.50 and135.9 ng/g),and (12.25 and 125.0 ng/g) respectively. Also there are significant decrease in the concentrations of AFB1and AFM1 values with the occurrence of ASB with AF plus OCH in the chicks diet (as shown with treatment T6) which were equal to (5.55 and 12.35ng/ml), (16.25and 153.1 ng/g) and (11.65 and 189.8 ng/g) in the blood, liver and kidney samples respectively. Moreover, when the occurrence the ASB with AF in combination with T-2 toxin in the diet of chicks (as shown with treatment T10) there are also significant decreases between the values of (AFB1)&(AFM1) over the blood samples (6.15 and 12.45 ng/ml), the liver samples (19.30 and 164.2 ng/g) and the kidney samples (19.80 and 147.3 ng/g) respectively. However, there is no significance between OA& T-2 toxin values in both 6 and 10 treatments. All the above significant values were compared with the residue concentrations of AFB1 and AFM1 in the samples of blood, liver, and kidney from broiler chicks fed diets containing the same toxins without ASB (treatments T1, T5, and T9). The concentration of OA or T-2 toxin in samples of blood, liver and kidney from chicks fed diets containing OCH or T-2 toxin alone or in combination with AF and ASB (treatments T4, T6, T8, and T10) were not significantly effective when compared with the samples from chicks fed the diet containing the same toxins without ASB (treatmentsT3, T5, T7, and T9) respectively. From the results also show, the combination of AF with each OCH or T-2 toxin in the diets of broiler chicks causes a significantly increased in the residues of AFB1, AFM1, OA, and T-2 toxin in blood, liver, and kidney samples when compared with the same samples of chicks fed diets containing each toxin alone.

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تأثير بنتونايت الصوديوم المنشط في خفض متبقيات ثلاثة سموم فطرية في دم وكبد وكلى الأفراخ النامية

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المستخلص

أجريت هذه الدراسة لمعرفة دور مركب بنتونايت الصوديوم المنشط عند اضافته بنسبة 0.5% مع كل من سموم الافلا بتركيز 2.5 ملغم وسموم الاوكرا بتركيز4 ملغم وسم T-2 بتركيز 4 ملغم/كغم في العلف منفردة او سوية في توزيع متبقيات سموم الافلا من كلا النوعين B1 و M1 وسم الاوكرا A وسم T-2 في دم وكبد وكلى أفراخ الدجاج النامية المغذاة من عمر يوم واحد لغاية 21 يوما. أشارت النتائج إلى إن تركيز متبقيات سموم الافلا B1 وM1 في نماذج دم وكبد وكلى الأفراخ المغذاة على عليقه أحتوت على بنتونايت الصوديوم المنشط مع سموم الافلا لوحدها قد انخفضت معنويا عند ($p<0.05$) الى (4.35 و 8.40 نانوغرام/مل) في الدم و(18.50 و 135.9 نانوغرام/غم) في الكبد و(12.25 و 125.0 نانوغرام/غم) في الكلى على التوالي. كذلك انخفضت معنويا متبقيات سموم الافلا B1 و M1 في نماذج الدم والكبد والكلى عند وجود مركب البنتونايت مع سموم الافلا والاوكرا سوية في علف الأفراخ اذ كانت تراكيزها (5.55 و 12.35 نانوغرام/مل) في الدم و(16.25 و153.1 نانوغرام/غم) في الكبد و(11.65 و 189.8 نانوغرام/غم) في الكلى على التوالي.وفي حالة خلط مركب البنتونايت مع سموم الافلا وسم T-2 في عليقة الأفراخ انخفض معنويا وجود هذه المتبقيات (M1,B1) اذ كانت تراكيزها (6.15 و12.45 نانوغرام/مل) في الدم و (19.30 و 164.2 نانوغرام/غم) في الكبد و (19.80 و 147.3 نانوغرام/غم) في الكلى على التوالي في حين لم تكن هنالك فروقات معنويه في قيم متبقيات سموم الأوكرا وسم T-2 كما في المعملات(6 و 10) .كل القيم ذي الفروقات المعنويه اعلاه قورنت مع وجود هذه المتبقيات في نماذج الدم والكبد والكلى للأفراخ المغذاة على علف قد احتوى على السموم أعلاه من دون وجود مركب البنتونايت المنشط وهي المعاملات (1 و 5 و 9). إن تركيز كل من سم الاوكرا A وسم T-2 في نماذج دم وكبد وكلى الأفراخ المغذاة على علف قد احتوى على سموم الاوكرا أو سم T-2 لوجدهما أو سويا مع سموم الافلا مع مركب البنتونايت المنشط لم تتأثر معنويا عند ($p<0.05$) عند المقارنة مع نماذج الدم والكبد والكلى المأخوذة من الأفراخ المغذاة على علف قد احتوى نفس السموم ولكن بدون وجود مركب البنتونايت المنشط. لوحظ كذلك من النتائج إن تواجد سموم الافلا مع كل من سموم الاوكرا أو سم T-2 في علف الأفراخ قد سبب زيادة معنوية في تركيز متبقيات كل من سموم الافلا B1 وM1 وسم الاوكرا A وسم T-2 في دم وكبد وكلى هذه الأفراخ عند مقارنتها مع النماذج الماخوذه من دم وكبد وكلى الأفراخ المغذاة على علف قد احتوى نفس السموم ولكن كل على حده.

Introduction

Mycotoxins are considered as fungal secondary metabolites which have presented health risks to human and animal populations. Mycotoxins are produced by molds that infect crops in the field and during storage and capable to resistant the milling and processing (1), and it can cause acute or chronic toxicological effects in the animals and humans who eat contaminated crops or foods and this effects depending on the quantities and consumed(2). The risk of mycotoxins on health may include immunological effects, nephropathy, organ toxicity, cancer, and, in some cases, death (3). Aflatoxins (AF) are difuranocoumarin derivatives which produce by a polyketide pathway by some strains of *Aspergillus flavus* and by most strains of *A. parasiticus*, plus related species *A. nomius* (4). Aflatoxin is cutely toxic, immunosuppressive, hepatotoxin in young broiler chickens (5). When mammalian animals consume AF-contaminated feeds, they metabolically biotransformation aflatoxin B1 into a hydroxylated form called aflatoxin M1 (6).Ochratoxin A (OA) is a widely distributed mycotoxin produced mainly by *Aspergillus ochraceus* and *Penicillium verrucosum* under diverse environmental conditions. OA has been detected in a variety of nutrients and in the majority of

both animals and human blood samples (7). The main target organ from OA is the kidney (8). Further, it may be implicated as a factor in the Balkan Endemic Nephropathy disease and the urinary tract tumours in humans (9, 10, 11), and chronic interstitial nephritis (12, 13, 14). The mycotoxins called T-2 are secondary metabolites produced by several species of the genus *Fusarium* (15). It was considered that one of the trichothecene toxin that has been detected in agricultural crops, especially in wheat and maize (16). T-2 toxin was shown to causes toxic syndrome in human and animals in concentrations as low as part per million or billion of food or feed (17, 16). It was causes reductions in weight gain and feed consumption and severs oral lesions in chickens (18, 19, 20) abnormal behavior (21) and a coagulopathy (22). Dsorbents clay products such as bentonites, zeolites and aluminosilicates have been found to be reduced negative effective of some mycotoxins by adsorbing it in intestinal of animals (23). In recent years, activated sodium bentonite was reported that was effective in reducing some mycotoxicosis in broiler chicks. (24, 25). Therefore, the aim this study was to investigate the role of feeding diets containing AF, OCH and T-2 toxin singly or in combination each OCH or T-2 toxin with AF and the effect of ASB in reducing the concentration of these mycotoxins in blood and liver and kidney tissues of male broiler chicks feeding the diet contaminated with above formula from mycotoxins to 3wk of age.

Materials and methods

Two hundred, 1-day-old male broiler chicks (Faobro, an Iraqi broiler hybrid) were *individually weighed, wing banded and* housed in heated battery brooders under continuous fluorescent lighting with feed and water provided for *ad labium* consumption. Chicks were fed a corn-soybean meal-based starter diet obtained from a commercial mill; it contained 22% crude protein and 2950 K cal/kg metabolizable energy, without added antibiotics, coccidiostats, or growth promoters. The experimental design consisted of ten dietary treatments: T1) 2.5 mg AF/kg of diet; T2) 2.5 mg AF, 0.5% ASB; T3) 4 mg OCH/kg of diet; T4) 4 mg OCH, 0.5% ASB; T5) 2.5 mg AF, 4 mg OCH; T6) 2.5 mg AF, 4 mg OCH, 0.5% ASB; T7) 4 mg T-2 toxin/kg of diet; T8) 4 mg T-2 toxin, 0.5% ASB; T9) 2.5 mg AF, 4 mg T-2 toxin; T10) 2.5 mg AF, 4 mg T-2 toxin, 0.5% ASB; These were two replicates of ten broilers per dietary treatment and the chicks were maintained on these treatments to 3 wk of age. Aflatoxins were prepared through inoculation of rice by *Aspergillus parasiticus* NRRL 2999 by methods previously described by (19). Fermented rice was autoclaved and ground and the AF content measured by Spectrophotometric analysis (26, 27). The total AF content in the rice powder, 80% was AFB1, 14% was AFG1, 5% was AFB2 and 1% was AFG2. The T-2 toxin was produced by *Fusarium sporotrichioides* (Laboratory isolate) in cracked corn at 15 °c by method previously described by (28). T-2 toxin was extraction and determined under the conditions described by (29) and incorporated into the diet by dissolving the toxin in 95% ethanol and then mixing the appropriate quantities with 1 kg of the diet. After drying, the dissolved toxin was mixed with the basal diet to produce the treatments containing T-2 toxin. Ochratoxin (OCH) was produced from the static fermentation of wheat by *Aspergillus ochraceus* (Laboratory isolate) as described by (30). The OCH was extracted, purified and crystallized from benzene as described by (31). After incorporated the AF, OCH, and T-2 toxin into the basal diet then was conferment to provide the desired level of 2.5 mg AF and 4.0 mg from each OCH and T-2 toxin/kg of diet by (32).The basal diet was analyzed for mycotoxins and was found to be below detection limits for AF, DON, zearalenone, and cyclopiazonic acid as established by the methods described by (33). The activated sodium bentonite (ASB) was kindly provided by the state of Vegetable oil and

incorporated into the appropriate diets at the level of 0.5%. At 3 wk of age, 10 broilers (5 chicks from each replicate) from each treatment were bled by cardiac puncture for mycotoxins determinations determine in blood according to (32). The same chicks were then killed by cervical dislocation and the liver, and kidney was removed, and homogenized to quantitative the concentration of mycotoxins in it according to the (32).Data were analyzed by the ANOVA analysis, using the general linear model of the Statical Analysis System (34). Significant treatment differences were evaluated using Duncan's multiple-range test (35). All statements of significance are based on the 0.5 level of probability.

Results and Discussion

Data in table 1. show the effect of 0.5% ASB with or without AF, OCH and T-2 toxin singly or in combinations of each OCH or T-2 toxin with AF in the diets of chicks fed from 1 to 21 days of age, on the distribution of AFB1, AFM1, OA, and T-2 toxin in blood samples.

Table(1): The effects of 0.5% ASB on distribution of AFB1, AFM1, OA, and T-2 toxin in blood of male broiler chicks fed diets containing 4 mg OA or 4 mg T-2 toxin, singly or in combination from each toxins with 2.5 mg AF/kg diets for 1-21 days age.

No. of Treatments	AF* mg/kg	OCH mg/kg	T-2 toxin mg/kg	ASB %	Toxins concentration in blood (ng/ml)			
					AFB1	AFM1	OA	T-2 toxin
T1	2.5	0	0	0	^{b}6.35±0.55	^{c}9.50±0.80	-	-
T2	2.5	0	0	0.5	^{c}4.35±0.25	^{d}8.40±0.30	-	-
T3	0	4.0	0	0	-	-	^{ab}10.35±0.75	-
T4	0	4.0	0	0.5	-	-	^{ab}10.30±0.70	-
T5	2.5	4.0	0	0	^{a}8.90±0.30	^{a}15.65±0.55	^{a}11.2±0.07	-
T6	2.5	4.0	0	0.5	^{bc}5.55±0.45	^{b}12.35±0.65	^{a}10.65±0.65	-
T7	0	0	4.0	0	-	-	-	^{b}8.10±0.40
T8	0	0	4.0	0.5	-	-	-	^{b}8.25±0.45
T9	2.5	0	4.0	0	^{a}9.55±0.65	^{a}14.75±0.75	-	^{a}9.35±0.45
T10	2.5	0	4.0	0.5	^{b}6.15±0.55	^{b}12.45±0.45	-	^{a}8.95±0.45

a-d Values within columns with no common superscript differ significantly ($p<0.05$).
* **AF** = Total aflatoxins. **OCH**= Total ochratoxin. **ASB**= Activated sodium bentonite.
OA=Ochratoxin A.
- means not detected.

From the results it was evident that ASB when occurrence with AF or AF when combination with OCH or T-2 toxin in the diets of chicks (T2,T6, and T10) were significantly ($p<0.05$) decreased the residue concentrations of AFB1 in the blood samples which becomes (4.35, 5.55and 6.15 ng/ml) and AFM1 (8.40,12.35and 12.45 ng/ml) respectively when compared with the residue concentration of same mycotoxins in blood samples of chicks fed diets containing the toxins without ASB (T1, T5 and T9) which AFB1 (6.35, 8.90 and 9.55 ng/ml) and AFM1 (9.50, 15.65 and 14.75 ng/ml) respectively.The level of distribution residue from each OA or T-2 toxin in blood samples of chicks fed diets containing each mycotoxins with ASB was not differ significantly when compared with the levels of it in blood samples of chicks fed diet containing the mycotoxins without ASB.The effects of ASB on the distributions of residue concentrations of AFB1, AFM1, OA, and T-2 toxin in the liver samples of male broilers chicks fed diets containing 4mg OCH or 4 mg T-2 toxin singly or in combinations from each one with 2.5 mg AF/kg diets are presented in table 2.

Table(2): The effects of 0.5% ASB on distribution of AFB1, AFM1, OA, and T-2 toxin in liver of male broiler chicks fed diets containing 4 mg OA or 4 mg T-2 toxin, singly or in combination from each toxins with 2.5 mg AF/kg diets for 1-21 days age.

No. of Treatments	AF* mg/kg	OCH mg/kg	T-2 toxin mg/kg	ASB %	Toxins concentration in liver (ng/g)			
					AFB1	AFM1	OA	T-2 toxin
T1	2.5	0	0	0	^{a}28.25±0.55	^{c}150.9±0.65	-	-
T2	2.5	0	0	0.5	^{bc}18.50±0.70	^{d}135.9±0.40	-	-
T3	0	4.0	0	0	-	-	^{b}140.8±1.40	-
T4	0	4.0	0	0.5	-	-	^{b}138.0±1.05	-
T5	2.5	4.0	0	0	^{b}22.70±0.40	^{b}163.1±1.20	^{a}149.0±1.60	-
T6	2.5	4.0	0	0.5	^{c}16.25±0.05	^{c}153.1±1.70	^{a}147.1±1.40	-
T7	0	0	4.0	0	-	-	-	^{b}123.1±0.65
T8	0	0	4.0	0.5	-	-	-	^{b}123.3±0.25
T9	2.5	0	4.0	0	^{b}21.30±0.60	^{a}173.9±0.65	-	^{a}125.8±0.05
T10	2.5	0	4.0	0.5	^{bc}19.30±0.30	^{b}164.2±1.25	-	^{a}126.6±0.65

a-d Values within columns with no common superscript differ significantly ($p<0.05$).
* AF = Total aflatoxins. OCH= Total ochratoxin. ASB= Activated sodium bentonite.
OA=Ochratoxin A.
- means not detected.

It was clear that the presence of residues from AFB1 and AFM1 in the liver samples were significantly ($p<0.05$) reduced in the chicks group fed diets containing ASB with the AF alone (T1) which was become (18.50 and 135.9 ng/g) respectively or AF with each OCH or T-2 toxin (T6) which was become (16.25 and 153.1 ng/g) respectively and (T10) which was become (19.30 and 164.2 ng/g) respectively, when compared with the liver samples from chicks groups fed diets containing the toxins without ASB which means (T1, T5, T9) that contained from AFB1 and AFM1 (28.25 and 150.9 ng/g) ; (22.70 and 163.1 ng/g) and (21.30 and 150.9 ng/g) respectively. While the residue concentrations of OA or T-2 toxin in the liver samples from chicks groups fed diets containing ASB with each OCH (T4) or T-2 toxin (T8), or AF combined with each OCH (T6) or T-2 toxin (T10),were not affected significantly when compared with the residue concentrations of same toxins in the liver samples from chicks fed diets containing the toxins without ASB which means (T3, T7, T5, and T9) respectively.From the table 3. Was observed the levels of residue concentrations of AFB1, AFM1, OA, and T-2 toxin in kidney samples from chicks fed diets containing ASB with or without AF, OCH, or T-2 toxin alone or in combination for each OCH or T-2 toxin with AF from 1 to 21 days age.

Table(3): The effects of 0.5% ASB on distribution of AFB1, AFM1, OA, and T-2 toxin in kidney of male broiler chicks fed diets containing 4 mg OA or 4 mg T-2 toxin, singly or in combination from each toxins with 2.5 mg AF/kg diets for 1-21 days age.

No. of Treatments	AF* mg/kg	OCH mg/kg	T-2 toxin mg/kg	ASB %	Toxins concentration in kidney (ng/g)			
					AFB1	AFM1	OA	T-2 toxin
T1	2.5	0	0	0	^{a}22.35±0.25	^{e}136.6±1.20	-	-
T2	2.5	0	0	0.5	^{c}12.25±0.95	^{f}125.0±0.55	-	-
T3	0	4.0	0	0	-	-	^{b}121.3±1.40	-
T4	0	4.0	0	0.5	-	-	^{b}118.1±0.75	-
T5	2.5	4.0	0	0	^{b}19.55±0.95	^{a}199.7±2.15	^{a}138.1±1.05	-
T6	2.5	4.0	0	0.5	^{c}11.65±0.85	^{b}189.8±1.90	^{a}136.1±1.0	-
T7	0	0	4.0	0	-	-	-	^{b}15.75±0.15
T8	0	0	4.0	0.5	-	-	-	^{b}14.90±0.20
T9	2.5	0	4.0	0	^{a}21.10±0.40	^{c}158.6±1.85	-	^{a}23.20±0.30
T10	2.5	0	4.0	0.5	^{b}19.80±0.60	^{d}147.3±1.15	-	^{a}22.75±0.65

- a-f: Means in each column bearing different letters differ significantly ($p<0.05$).
* AF = Total aflatoxins. OCH= Total ochratoxin. ASB= Activated sodium bentonite. OA=Ochratoxin A. - means not detected.

AFB1 and AFM1 residue concentrations were significantly ($p<0.05$) reduced in kidney samples from each treatments of chicks fed diets containing ASB with toxin which means (T2) that contained (12.25 and 125.0 ng/g) ; (T6) that contained (11.65 and 189.8 ng/g) and (T10) that contained (19.80 and 147.3 ng/g) respectively, when compared with the residue concentrations of toxins in kidney from chicks groups fed diets containing the toxins only which means (T1, T5, and T9) respectively. While the OA or T-2 toxin residue concentrations in the kidney samples from chicks groups fed diets containing ASB with each toxins which means (T4, T6, T8, and T10) were not significantly effective when compared with samples of kidney from chicks groups fed diets containing the toxins only which means (T3, T5, T7 and T9) respectively.

From this study was observed from all results that the combinations of AF with each OCH or T-2 toxin in the diets of chicks caused significantly increased in the residue concentrations of AFB1, AFM1, OA, and T-2 toxin in blood, liver, and kidney samples when compared with the level of residue of toxins in the blood, liver and kidney samples of male broiler chicks fed diets containing the each toxin alone.There are a little study in this field, and the ameliorative effect of dietary ASB in the reduced AFB1 and AFM1 concentrations in the blood, liver and kidney samples of chicks fed diets containing AF with ASB could be attributed to the role of ASB as a sequestering agent against AF present in the diet through reducing its bioavailability in the gastrointestinal tract , which agreement with (36) for the role of ASB.

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