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Meat and Organs Quality of Broiler Chickens Fed Diet Contaminated with B1 Aflatoxin

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Abstract: The levels of aflatoxin B1 (AFB1) in breast, leg, liver, kidney and gizzard and in litter were studied in broilers chicks maintained for 6 weeks on AFB1 contaminated diets of 0.0(group 1,control group), 384.5 µg/kg AFB1 (group2,treatment 1), or 128.9 µg/kg AFB1(group3,treatment2). The highest AFB1 of 1.2 µg/kg was at the third week in liver tissues and 0.8 µg/kg in chicken legs fed diet contaminated with 374.53 ppb AFB1. Breast and gizzard showed lower AFB1 concentrations of 0.5 and 0.8 µg/kg, respectively, than treatment 1 at the end of the third week. The residual level of AFB1 were increased in liver and kidney of 2.1 and 1.9 µg/kg AFB1 at wk 6 and chickens breast and leg AFB1 levels also affected and increased to 0.93 and 1.64 µg/kg, respectively.

Key words: Broiler · Aflatoxin B1 · Organs · Litter

INTRODUCTION

Aflatoxins are naturally produced, toxic fungal metabolites identified in the 1960's and proved as cancer causing agents. These compounds are produced mainly by a *Aspergillus flavus, Aspergillus parasiticum* and to a lower extent *Aspergillus nomius*. Aflatoxins and their metabolites could be found in meat, milk and eggs or in feeds of grain origin and designated as hepatogenic and carcinogenic for human and animals [1- 6]. Many diseases associated with the presence of aflatoxins in foods and considered as a potential health risk for the community [7]. AFB1 is considered as the most potent toxins on monogastric animals such as poultry [8] and the toxicity level varies with the concentration and the exposure time [9- 11]. The mycotoxin limits are controlled by laws and legislation in most of the countries with a concentration of 10 µg/kg for total aflatoxins (AFB1, AFB2, AFG1 and AFG2) and 5 ug/kg for AFB1 [12]. Thus, based on the possibility of poultry products contamination with aflatoxins, this study was designed to determine the effect of contamination with aflatoxin B1 on its level in broiler muscles.

MATERIALS AND METHODS

Ninety 1-day-old commercial Hubbard chicks were purchased from commercial hatchery and divided into three groups of thirty birds with equal mean body weight. Regular feed free of AFB1 was provided to Group I and considered as the control group. Groups 2 and 3 were fed diets contaminated with 384.5 and 128.9 ìg AFB1/ kg. Chicks were fed for 6 weeks under with feed and water available *ad libitum*. The composition of diets is presented in Table 1.

Aflatoxin residues of the flesh were determined at 0, 3, 4, 5 and 6 weeks for a homogeneous representative sample of 5 birds chosen and slaughtered randomly.

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Table 1: Broiler experimental diet composition used in the study [1,2,3]

Ingredients	Starter %	Finisher %
Corn	58.5	67.05
Soybean meal	35.65	26.00
Vegetable Oil	1.69	3.00
Limestone	1.84	1.68
DCP	1.00	1.02
Salt	0.41	0.42
DL-Methionine	0.20	0.20
L-Lysine	0.11	0.13
Coccidiostat	0.10	0.00
Vitamin Premix	0.10	0.10
Mineral Premix	0.10	0.10
Choline chloride	0.10	0.10
Antioxidant	0.10	0.10
Antifungal	0.10	0.10
Analysis:		
Metabolizable energy (kcal/kg of dry matter)	2978.00	3156.00
CP (%)	22.30	18.20
NPP (%)	0.45	0.40
Ca (%)	1.03	0.95
Na (%)	0.18	0.18

[1]Each kg of vitamin premix contains 2.4x10^6 IU vitamin A; 3.2 x10^5 vitamin D3; 5.6 x10^3 mg vitamin E, 640 mg vitamin K3; 500 mg vitamin B1; 1120 mg vitamin B2, 3200 mg niacin; 1600 mg Ca-D-pantothenate; 800 mg vitamin C, 2.4 mg vitamin B12, 160 mg folic acid; 7.2 mg D-biotin; 8000 mg vitamin C; 20000 mg choline chloride

[2]Each kg of mineral premix contains 8 x10^4 mg manganese; 6 x10^4 mg iron; 6 x10^4 mg zinc; 200 mg cobalt; 100 mg iodine; 150 mg selenium

[3]DCP, dicalcium phosphate; CP, crude protein; NPP, non phytate phosphorous

Chemical and Reagent: Aflatoxins standards (98%) and methanol (99.5%), acetonitrile (99.5%), acetone (99%), anhydrous sodium sulphate (98.5%), NaCl (99.5%) were from Sigma (St Louis, MO, USA). Aflatoxoin AFB1 standard stock solutions were prepared in acetonitrile according to the AOAC method [13]. The cleanup procedure for the extracts was performed through using SPE-CN (Purchased from Varian and aflatoxin immunoaffinity columns (IAC) purchased from R-biofarm (R-biopharm, Darmstadt, Germany).

Test Protocol: A 2-g from a well mixed homogenized powdered feed samples was shaken with 10-ml methanol: water (70/30) mixture and shaken at 700 rpm for 50 min at room temperature (20-25°C) using a IKA shaker (IKA, Hamburg, Germany). A100-µL of the eluate was diluted with 600-µL (1+6) of the sample dilution Buffer (Phosphate buffer solution, pH 7.2) and the aflatoxins were determined using HPLC. Chicken muscles and organs samples were extracted using the method applied by Herzallah [14] with modifications, briefly; A 50g of were blended with 100 ml mixture of acetone and water (1:1)100 mL for 10 min then diatomaceous earth was added and stirred gently for 5-min and filtered through fast filtering Whatman No.1 filter paper. An aliquot of the filtrate was then mixed with 5% NaCl and Hexane (1:1) and shaken at 1200 rpm for 10 min using a mechanical shaker (IKA, Hamburg, Germany). The hexane layer was discarded and the AFB1 extracted with chloroform (3x50mL), chloroform was dried over anhydrous sodium sulfate and evaporated using rotary evaporator (IKA, Hamburg, Germany). The residues were redissolved in 1 mL chloroform and cleaned over IAC. The eluted aflatoxins were evaporated to dryness under a stream of N_2, redissolved in methanol and analyzed by HPLC.

HPLC Determination: Analysis of AFB1 was performed by Water HPLC (Waters Co., MA, USA) equipped with 1525 binary HPLC pump, column oven 5CH model and fluorescence detector (Model FL 2475) at wavelength 365 and 425 nm for excitation and emission, respectively. The column used was 250 x 4.6 mm Thermo LC-Si, kept in column oven at 40°C at flow rate of 2.0 mL/ min and the mobile phase was isocratic composed of toluene, ethyl acetate, formic acid and methanol (90:5:2.5:2.5, v/v/v/v). The results were confirmed by Agilent HPLC equipped with fluorescent detector and run under similar conditions.

Recovery of Aflatoxins: An AFB1 calibration curve was linear with correlation coefficient of 0.999 and the coefficient of variation was 1.32% for AFB1 with minimum detection limit of 0.05 µg/kg.

Statistical Analysis: Collected data was reduced for a significance difference (P<0.05) using CRD model with Duncan's Multiple Range Test. The B1 residues calculated were subjected to ANOVA using the general linear model (GLM) procedure in PC-SAS® version 9.0 [15]. Values with a significant difference in the least significant difference (LSD) procedure if P< 0.05.

RESULTS AND DISCUSSION

The residue level in flesh was dose dependent, as AFB1 level in feed increase the residue level in muscles and organs of broiler chicken were increased (P<0.05). Kidney of broiler chickens was significantly (P<0.05) higher in AFB1 at week 6 of 2.1 µg/kg and < 0.3 µg/kg at start. Liver from broiler also was higher (P<0.05) in AFB1 of concentration 1.2 and 0.762 for Group 2 fed 374.5 µg/kg and group 3 of 123.1 µg AFB1/kg feed, respectively.

Table 2: Aflatoxin B1 concentration of broiler flesh fed B1 contaminated diet [1,2,3]

	Treatment 1(T1)					Treatment 2(T2)				
	B1 (ppb)									
	Chicken Age (wk).									
Sample Description	0	3	4	5	6	0	3	4	5	6
Feed	384.51^{a} ± 4.12	374.53^{a} (± 3.11)	360.72^{a} ± 3.20	345.2^{a}(± 3.51)	350.20^{a} (± 2.82)	128.96^{a} (± 2.64)	123.08^{a} (± 2.03)	118.62^{a} (± 2.11)	117.31^{a} (± 2.07)	110.62^{a} (± 2.11)
Breast	<0.05	0.51^{c} (± 0.10)	0.73^{c} (± 0.10)	0.818^{d} (±0.11)	0.93^{d} (±0.19)	<0.05	0.38^{c} (± 0.02)	0.45^{c} (± 0.06)	0.71^{d} ±1.10	0.69^{d} (± 0.06)
Legs (drumstick + thigh)	<0.05	0.89^{c} (±0.11)	0.89^{c} (±0.10)	1.586^{c} (± 0.11)	1.64^{c}(± 0.11)	<0.05	0.51^{c} (±0.06)	0.63^{c} (± 0.10)	1.170^{c}(±0.11)	1.19^{c} (± 0.13)
Litter	<0.05	12.60^{b} (± 1.51)	18.30^{b}(± 1.82)	28.70^{b} (± 1.33)	37.60^{b} (± 2.01)	<0.05	8.30^{b} (± 1.23)	10.65^{b} (± 1.04)	14.10^{b} (± 1.71)	28.81^{b} (± 1.11)

1 HPLC aflatoxin detection limit was 0.05. [2] Values are mean of three readings ± SD

[3]Values of different superscript letter within column are significantly differ at P<0.05

Table 3: Aflatoxin B1 residues in broiler chicken organs fed AFB1 contaminated diet[1,2,3]

	T1					T2				
	B1 (ppb)									
	Chicken Age (wk)									
Sample Description	0	3	4	5	6	0	3	4	5	6
Kidney	<0.05	1.185^{a} (± 0.12)	1.28^{ab}(± 0.10)	1.68^{a} (± 0.11)	2.06^{a} (±0.11)	<0.05	0.67^{a}(± 0.10)	1.086^{a} (± 0.11)	1.28^{a} ± 0.11	1.32^{a} (± 0.10)
Liver	<0.05	1.23^{a} (±0.13)	1.34^{a}(± 0.17)	1.62^{ab} (±0.13)	1.88^{ab}(± 0.11)	<0.05	0.762^{a}(±0.11)	0.97^{a}(± 0.06)	1.05^{ab} ± 0.103	1.10^{ab} (± 0.09)
Gizzard	<0.05	0.83^{c} (±0.10)	0.98^{c}(± 0.12)	1.014^{c} (±0.1)	1.10^{c}(± 0.08)	<0.05	0.42^{a} (±0.05)	0.62^{b}(± 0.11)	0.78^{c} (± 0.11)	0.81^{c} (± 0.08)

[1] HPLC aflatoxin B1 detection limit was 0.05. [2] Values are mean of three readings ± SD

[3]Values of different superscript letter within column are significantly differ at P<0.05

The results found in this study for feed, breast, legs (Drumstick and thigh) and litter are presented in Table 2 and kidney, liver and gizzard, in Table 3.

The results showed an increase in AFB1 level in chicken muscles with significant (P< 0.05) difference from the control treatment (Group 1) of < 0.3 AFB1. The amount of AFB1 discarded in chicken waste was high as presented in Table 3.

AFB1 level in treatment T2 at the beginning of the experiment of chicks and feed control treatment were below the detection limit of the method (< 0.3) and increase with rearing time until the maximum reached by week 6 of values < 0.3, 1.2 and 2.1 ppb at wk 0, 3 and 6 for kidney, respectively. Liver and kidney showed higher residue level for AFB1 of significant (P<0.05) In the liver in T1 there was increase in AFB1 residue from 1.2 to 1.9 at week 3 and 6 respectively. Gizzard was also showed AFB1 residue but significantly (P<0.05) lower than Kidney and Liver of 0.8 ppb and 1.1 µg/kg at 3 and 6 wks in T1, respectively. Both organs (kidney and liver) were higher in AFB1 residue level with statistically insignificant (P>0.05) difference (Table 3). The increase in AFB1 in broiler organs, kidney, liver and gizzard followed an increase trend of dose response effect, which means as the concentration of the contaminant increase in animal diet the residue level increased in organs (Kidney and liver). The increase was insignificant for kidney and liver but significantly differs from the control of minimum detection limit MDL of less than 0.3 µg/kg for Group 2 and 3 (Tables 3 and 2). These results were in agreements with reported data of AFB1 residue in liver as a result of AFB1 contaminated diet [16- 20]. The results were also in agreement with the data reported by Denli *et al.* [16] who found that AFB1 were detected in liver of chickens fed AFB1 contaminated feeds of 0.2 µg/kg and affect the performance of broiler chicken through increasing the liver weight and reduction of animal weight [21].

Muscles of broiler chicken fed AFB1 contaminated diet showed also an increase of residue in chicken muscles breast of all Groups 1, 2 and 3 with less response as the AFB1 concentration in the feed decreased. The AFB1 residue in breast was lower than the residue in leg (Drumstick and thigh) the AFB1 concentration of Group 2 were < 0.3, 0.5 and 0.9 µg/kg at weeks 0, 3 and 6, respectively. Whereas, leg appeared to contain higher level of values 0.3, 0.9 and 1.6 µg AFB1 / kg at week 0, 3 and 6, respectively. These results were in agreement with Denli *et al*. [16] who found that breast muscles AFB1 residue was not detected using a method of detection limit of 0.1 µg/kg. The residue level also increase as the administration time and level increased along with the duration period of commercial broiler chicken production, for example chicken legs at week 3 contains 0.5 µg AFB1 / kg, while at week 6 the level was 1.19 µg AFB1 / kg for Group 3..The increase in AFB1 in organs and muscles of broiler chickens was significant (P<0.05) between chicken parts (Organs or muscles) and within treatments at week 5 and 6 for both groups 1 and 2 (Tables 2 and 3).

The results were in compliance with those found by Pasha *et al.* [20] who found that feeding a diet of 2.1 mg/kg for 35 days caused a residue level of less than 3 ppb in gizzard, liver and kidney of broiler chickens and with data reported by Zaghini *et al*. [17] who found the given range of AFB1 of chicken fed 2.1 mg a residual level ranged between 1.9 to 4.1 µg / kg in liver of 0.2 µg / kg in chicken liver. Broiler chicken litter was higher in AFB1 and increase with length of rearing; this could be due to the physiological stress of chicken organs at high level of AFB1. The chicken metabolism process was disturbed and withdrawal of AFB1 in chicken waste (Litter) was increased as rearing time increase, for example, the concentrations < 0.05, 12.6 and 37.6 was found for T1 and < 0.05, 8.3 and 28.81 for litter at 0, 3 and 6 week of production of treatment 2, respectively. The results were in agreement with the results reported by Zaghini *et al.* [17] who found that the use of mannanoligosaccharides increase adsorption of AFB1 to the polysaccharides and decrease the residue level in poultry muscles.

CONCLUSION

AFB1 residue level in liver, kidney, breast, legs and gizzard, expected and was increased with increase of AFB1 concentration in a given diet as will as the length of exposure to contaminated diet. Liver and kidney were highest in AFB1 residue levels whereas, breast was lowest in AFB1 concentration. Liver is known as the targeted organ of aflatoxin residue where the detoxification process, DNA and protein abduction occurred. The litter of chicken fed artificially contaminated feed is considered as an indicatory measure for aflatoxins contamination of chicken muscles and organs as well as raise awareness toward environmental risk.

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