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INCIDENCE AND CONCENTRATION OF AFLATOXIN B1 AND OCHRATOXIN A ON BROILERS RATION IN DAKAHILA GOVERNORATE EGYPT

ELALFY M MAHMOUD[1] and ABDEIN M MOHAMED[2]
[1] Lecturer of Forensic Medicine and Toxicology Faculty of Veterinary Medicine Mansoura University
[2] Researcher of National Agricultural Research Institute Cairo

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ABSTRACT

Mycotoxins consider as toxic secondary metabolites secreted from fungi which grow in certain condition of temperature and humidity. Now in our area, there is continuous change in climatic condition from year to another so we need to monitor level of mycotoxins in poultry ration regularly. Forty five samples of poultry rations collected from broiler chicken farms of Dakhalia Governorate and were analyzed by HPLC-UV. The present study showed that incidence of aflatoxin B1 (AFB1) and ochratoxin A were 64% and 50 % in starter ration respectively and in finishing one were 52 % and 45% for both aflatoxin B1 and ochratoxin A respectively. The concentration of aflatoxin B1 on poultry ration under analysis ranged from 0.5 to 62 ppb while ochratoxin A level ranged from 3 to 52 ppb. Additionally, the mean values of aflatoxin B1 and ochratoxin A in strater ration were 17.22 ±20 and 22.9 ±17.33 respectively and in finishing ration were 9 ± 10.04 and 13.8 ±13 respectively. In other word, these value of both mycotoxin is quite similar or low than the maximum european permissible limit of aflatoxin B1 (20 ppb) and for ochratoxin A (100 ppb).

Key words: *Aflatoxin B1, Ochratoxin A, Broilers Ration, Dakahila Governorate, Egypt.*

INTRODUCTION

Mycotoxins are toxic secondary metabolite of molds produced mainly by Fusarium, Aspergillus and Penicillium species found on feed stuff, particularly cereals. Food contamination with mycotoxins consider a matter of great concern as they may cause different harmful effects in animals varying from immune suppression, estrogenic or neurotoxin effects and may have lethal effect in severe cases Leung *et al.* (2006).

Mycotoxins have harmfull effect on both health and productivity in almost all species of domestic animals including poultry. In general, mycotoxicosis results in decrease feed intake, reduce feed conversion, decrease in production and subsequently increased susceptibility to various infections based on the type of toxins ingested (Xue *et al.*, 2010).

Striet *et al.* (2012) found that mycotoxins in combination may exert synergetic, additive or antagonistic effects. Additionally, co-occurrence of aflatoxin and ochratoxin exerts their toxic effects synergistically. These effects could be more lethal even at low concentration of mycotoxins (Boermans and Leung, 2007).

Corresponding author: ELALFY M MAHMOUD
E-mail address: dr_melalfym@yahoo.com
Present address: Lecturer of Forensic Medicine and Toxicology Faculty of Veterinary Medic Research Institute, Mansoura University.

Rajamalar and Ravikumar (2014) reported that the storage of animal feeds was responsible of increase microbial favorable conditions. So obligatory precautions of preventing contamination of dried and stored animal feeds must be carried out in order to reduce levels of mycotoxin occurrence on animals feeds.

The intake of feed contaminated by ochratoxin also represents a potential risk for animal health and a food safety issue due to the transfer of the toxin through the food chain to humans. (Battacone *et al.*, 2010).

European Union stated that no feed contained ochratoxin above the acceptable level on poultry finished feed. On the other hand, there was a number of samples contained aflatoxin above the acceptable limit (Ghulam *et al.*, 2014). Thus, restricted control measures should be considered to ensure safe poultry for human consumption.

Moreover, Ghulam *et al.* (2014) found that corn, cotton seed meal, sunflower meal, and cotton gluten meal were highly contaminated with aflatoxin. While ochratoxin was determined to be high in feed ingredients more than finished feed samples with an overall incidence of 50%. Maximum level of ochratoxin level was found to be more on corn gluten meal than other food ingredient for poultry feed.

The incidence of aflatoxin in analyzed samples of different feed ingredients varied from 0 to 94%. while ochratoxin A was present in 67% of samples in Sudan and in 100% of Nigerian samples. Additionally, the major levels found for B-trichothecenes, zearalenone and aflatoxin were 2786, 135 and 213 ng g^1, respectively and no ochratoxin was detected in a broiler feed sample from Egypt (Rodriguesa *et al.*, 2011).

Ali and Anwer (2009) found that the presence of aflatoxin B residues in hens' eggs might occur at relatively low level under conditions of long term exposure of laying hens to low level of aflatoxin in naturally contaminated ration at 50 µg/kg with reduction in feed intake.

In the United States, the Food and Drug Administration, has established a tolerance of 20 ppb of aflatoxin for human foods other than milk, but European markets are striving for a lower Codex importation standard of 2 ppb (Abbas, 2005).

In one of egyptian record of mycotoxins in Gharbia Governorate, Fahmy *et al.* (2015) found that aflatoxin were higher than the maximum permissible limit in most samples (20 ppb) while the mean concentration of ochratoxins in feed samples was 43.58 ppb, and 2.22% of samples were higher than the MPL (100 ppb).

The rational of this study to explore incidence and the concentration of aflatoxin B1 and ochratoxin A in broiler poultry feeds.

MATERIALS and METHODS

Forty five samples of broiler chicken starter and finishing rations were collected from private poultry farms at Dakahlia Governorate during summer season 2014. The samples were collected randomly in special bags from broiler poultry farm which kept separately until taken to laboratory for aflatoxin B and ochratoxin A extraction and analysis.

Extraction of the samples

10 gm of each sample was extracted and prepared for detection of aflatoxin and ochratoxin using a mixture of acetonitrile: water (80:20) and cleaned up by dispersive liquid–liquid microextraction which is a very economical, fast and sensitive method which described by Ansarin and Mahboob (2015). The samples were blinded at high speed for one minute. The extract was poured in fluted filter paper and the filtrate was collected in clean beaker.

Extract dilution

The filtrated extract was diluted with 40 mL diionized water, mixed well, then filtration through microfibre filter and the filtrate was collected in a clean beaker or directly into glass syringe barrel.

Column chromatography for aflatoxin

The diluted extract was passed (2 mL = 0.2 g sample equivalent) completely through AflaTest-p affinity column at a rate of about 1-2 drops/second until air comes through column. 5 mL of diionzed water (Sigam-aldrich company) was pass through the column at a rate of 2 drops/second, this step was repeated once or more until air comes through column. Elute affinity column by passing 1.0 mL HPLC grade methanol through column at OchraTest affinity column at a rate of about 1-2 drops/second until air comes through column.10 mL of mycotoxin wash buffer were pass through the column at a rate of about 1-2 drops/second until air comes through column. 10 mL of diionzed water was pass through the column at a rate of 2 drops/second. Elute affinity column by passing 1.5 mL OchraTest eluting solution through column at a rate of 1-2 drops/second, this elute must be completely collected in a glass cuvette, mix well and the cuvette was placed in a calibrated fluorometer. Ochratoxin was quantified by HPLC-UV (mo1el 1100-21) without need for any complex derivatisation in samples to enhance the detection (Amirkhizi *et al.*, 2015).

RESULTS

The present survey (Table 1) found that incidence of both aflatoxin and ochratoxin were 64% and 50 % for both aflatoxin and ochratoxin respectively in starter ration and in finishing one were 52% and 45% respectively.

Moreover, the mean values of aflatoxin B1 and ochratoxin A in strater ration were 17.22 ±20 and 22.9 ±17.33 respectively and 9 ± 10.04 and 13.8 ±13 in finishing ration respectively (table 2).

Finally, the level of aflatoxin on poultry ration under experiment was ranged from 0.5 ppb to 62ppb with the mean value of 17.22 ppb and ranged from 5.4 to 40 ppb with the mean value of 9 ppb in finishing ration. While ochratoxin level was ranged from 3 to 52ppb with the mean value of 22.9 ppb in starter ration and ranged from 5.4-35 ppb with the mean value of 13.8 ppb in finishing ration (table 1and table 2).

Table1: Show the incidence and range of aflatoxin B1and ochratoxin A on broilers ration.

	Incidence		Range (ppb)	
	Aflatoxin B1	Ochratoxin A	Aflatoxin B1	Ochratoxin A
Starter ration	64.00%(16/25)	52(13/25	0.5 to 62	3-52
Finishing ration	50.00%(10/20)	45(9/20)	5.4 to 40	5.4-35

Table 2: Show mean of concentration (ppb) of aflatoxin B1 and ochratoxin A ppb on broilers ration.

Type of ration	Aflatoxin B1	Ochratoxin A
starter	17.22 ±20	22.9 ±17.33
Finishing	9 ± 10.04*	13.8 ±13*

DISCUSSION

The aflatoxin and ochratoxin on poultry ration could be pass to human food through chicken meats or eggs and consider hazardous on both poultry production and human health (Bryden, 2012 and Zafar *et al.*, 2014).

As mycotoxins are one of the major risk element on poultry productivity and also product quality, control of their impact is serious (Oguz, 2011). The need of continuous detection of these toxins in chicken ration, meat and eggs consider important.

This result indicated that Aflatoxin B1 and ochratoxin A were of high incidence and its were significantly increase in starter ration when compared with finishing one (Table 1, 2). This result agree with Rodriguesa *et al.* (2011) who found that the presence of aflatoxin B1 in monitored poultry feed samples varied from 0 to 94% while Ochratoxin A was present in 67% of samples in Sudan and in 100% of Nigerian samples. Moreover, only on Egypt for aflatoxin our result agree with Rodriguesa *et al.* (2011) who reported that aflatoxin and no ochratoxin were detected in a broiler feed sample collected from Egypt. Additionally, our result agree with Thirumala-Devi *et al.* (2002) who found that 38 % of the samples were contaminated with aflatoxins and 6% with ochratoxin A. The incidence scores of aflatoxin contamination in excess of 10 microgram /kg were 41 of 95 for maize, 18 of 30 for mixed feeds of Indian poultry.

Notably, our result agree with value of both mycotoxin maximum permissible limit of aflatoxin B1 (20 ppb) and for ochratoxins (100ppb) for European standard. And disagree with Fahmy *et al.* (2015) who found that aflatoxin in Gharbia Governorate were higher than the maximum permissible limit in most broiler feed samples (20 ppb) while the mean concentration of ochratoxins in feed samples was 43.58 ppb, and 2.22% of samples were higher than the MPL (100 ppb).

Aflatoxin and ochratoxin are the most common mycotoxins in poultry feed (Pattison *et al.*, 2008). We need to monitor the amount of mycotoxins in our area regularly in order to avoid hazardous effect of these mycotoxins on poultry production. Dietary exposure of broiler hens to 10 ppm of AFB resulted in embryonic mortality and reduced the immunity in the progeny chicks. Embryonic exposure with aflatoxins resulted in long-term depression of the immune function in chickens (Resanovic R *et al.*, 2009). Growth inhibition is linked with malabsorption syndrome, as confirmed by the presence of hypocarotenoidemia. The minimum amount of ochratoxin also causes reduced bone firmness and poor pigmentation. Acutely intoxicated birds are depressed, dehydrated and often polyuric and die in acute renal failure. Survivors will be poorly feathered, have delayed sexual maturity, increased clotting times, anaemia and immunosuppression.(Resanovic R *et al.*, 2009). The interaction between aflatoxin B and ochratoxin A was noted experimentally on broiler chickens as both mycotoxins were decreased significantly the growth rate and increased the mortality through induced nephropathy even at very low levels (Huff and Doerr, 1981 and Raja and Balachandran, 2009).

Finally, the present study showed that aflatoxin B1 and ochratoxin A were detected in poultry ration with low level as similar as European standard but of high incidence in starter ration than finishing ration of broiler chicken.

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نسب تواجد وتركيز الأفلاتوكسين ب١ والاكراتوكسين أ في علائق الدواجن بمحافظه الدقهليه

محمود محمد الالفي الحفناوي ، محمد محمد عابدين

Email: dr_melalfym@yahoo.com Assiut University web-site: www.aun.edu.eg

تفرز السموم الفطريه من الفطريات التي تنمو في ظروف خاصه من درجه الحراره والرطوبه ونتيجه التغير المستمر في ظروف الطقس من سنه الي اخري فيلزم تحديد مستوي هذه السموم في علائق الدواجن وتهدف هذه الدراسه الي تحديد مستوي الافلاتوكسن و الاوكراتوكسين خاصه في علائق دواجن التسمين في محافظه الدقهليه. تم تجميع عدد ٤٥ عينه من علائق دواجن التسمين البادى والناهى فى صيف ٢٠١٤ وتم تحليل العينات وتحديد نسبه الافلاتوكسين ب١ واوكراتوكس١. ولقد خلصت هذه الدراسه الى ان نسبة تواجد الافلاتوكسين ب١ والاوكراتوكسين ١ فى علائق البادى لدواجن التسمين ٦٤% و ٥٠% فى علائق البادى لدواجن التسمين و٥٢% و ٤٥% فى علائق الناهى لدواجن التسمين على الترتيب. ولقد وجد ان تركيز الافلاتوكسين فلا علائق الدواجن يتراوح من ٠.٥ الى ٦٢ ميكروجرام اما اوكراتوكس ١ فيتراوح من ٣ الى ٥٢ ميكروجرام. واخيرا متوسط مستوى سم الافلاتوكسين واوكراتوكس ١ فى علائق البادى تكون اعلى منها فى علائق الناهى بصوره معنويه.