THE HIGH INCIDENCE OF AFLATOXIN AND OCHRATOXIN COEXISTENCE IN BROILER RATIONS - A STUDY ON SOME POULTRY FARMS AT DAKAHLIA AND SHARKIA PROVINCES, EGYPT

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ABSTRACT

Twenty six samples of poultry rations were collected from private broiler poultry farms at Dakahlia and Sharkia provinces (thirty samples from each province) and analyzed for determination of aflatoxins and ochratoxin. The results indicated that all samples collected from Dakahlia province contain both aflatoxins and ochratoxin and 46% of these samples exceeded the permitted limit of each mycotoxin (5ppb). All the samples collected from Sharkia province contain ochratoxin and 92.4% contain aflatoxins and 7.4% of the samples were higher than the permitted limit for aflatoxins and 23% exceed the permitted limit for ochratoxin. The main conclusions of this study were that poultry rations may contain one or more mycotoxins at the same time which may have synergistic effects on production and resistance for diseases of broiler and may be considered hazards risk for the consumers especially these two mycotoxins which have decision of carcinogenic, immunosuppressive and teratogenic effects. So this synergistic effects must be considered during legalization for these mycotoxins in animal feeds.

INTRODUCTION

Mycotoxins are among the most common contaminants in animal feeds causing great economic loss in livestock (**Sharlin et al. 1981, Hafez et al. 1982**). No region of the world escapes the problem of mycotoxins contamination and according to the United Nations Food and Agriculture Organization (FAO) approximately 25% of the worlds grain supply is contaminated. Whether grain is produced in temperate, subtropical or tropical climates, if rainfall and humidity are experienced in the harvest season, infection of the grain by mould or fungi is likely. Where there is mould growth the likelihood of mycotoxins is significant although the presence of mould does not necessarily imply that mycotoxins can be found. Conversely, the absence of mould does not

necessarily mean the absence of mycotoxins **(Jon Ratcliff 2002)**. Mycotoxins are relatively stable compounds and are not destroyed by processing of feed. Due to the global import and export of raw materials, no country can be considered not to be at risk from Mycotoxins.

Aflatoxins are a group of secondary metabolites produced by *Asperagillus flavus* and *Aspergillus parasiticus* grown on foodstuffs during growing, harvest, storage and transportations **(Miller, 1995)**. It may caused death in people and animals, however, the greatest economic impact comes from reduced productivity, suppressed immune function, pathologic effect on the organs and tissues and altered the reproductive capability **(Cast 1989)**.

Ochratoxin A is a mycotoxin produced by mould fungi of the genus *Aspergillus* and *penicillium* which are found in contaminated food and feed **(Frohlich et al. 1991)**. It is a fungal metabolites that can be detected in a variety of improperly stored cereal and animal feed and has been shown to be nephrotoxic **(Schwerdt et al. 1996, Gekle and Sliberngal 1996,)**. Ochratoxin A increases the incidence of renal adenomas and carcinomas in several species and also suspected of causing Balkan endemic nephropathy in humans **(Petkova- Bocharova and Castegnaro 1985, Kane et al. 1986)**. In 1993, the International Agency on Research cancer (IARC) classified ochratoxin A as a possible human carcinogen (group B) based on the sufficient evidence for carcinogenicity in animal studies and inadequate evidence in humans **(IARC 1993)**. Mycotoxins in combination appear to exert greater negative impact on the health and productivity of livestock in comparison to their individual effects **(Smith and Sedden,1998)**. This is important when considering analysis of feed materials in terms of interpretation of the levels found and the mycotoxins to be tested.

The aim of this study was to evaluate the occurrence of two important mycotoxins (aflatoxin and ochratoxin) in poultry ration at two provinces produced a high ratio of broiler chickens in Egypt and the coexistence of these two toxins simultaneously.

MATERIAL AND METHODS

Samples :

Twenty six samples of poultry rations were collected from private poultry farms (broiler) at Dakahlia and Sharkia provinces (thirty samples from each province). The samples were collected randomly in plastic bags (1.0 kg.) and taken to the laboratory for aflatoxin and ochratoxin analysis.

Preparation, extracting and detection of the samples :

The samples were extracted, prepared and detected for aflatoxin and ochratoxin using Fluorometer according to **Truckess et al. (1991).**

Fluorometer protocol manual and its accessories (pure chemicals and columns) were supplied from Science Vicam Technology, USA.

Extraction of the samples :

50 g of ground sample were weighed, mixed with 5 g of sodium chloride and placed in blinder jar. Then 100 ml methanol: water (80:20 by volume) was added. Each sample was blinded for one minute. The extract was poured in fluted filter paper and the filtrate was collected in clean beaker.

Extract dilution :

Ten ml of filtrate extract was diluted with 40 ml deionized water, mixed well, then filtred through microfibre filter and the filtrate was collected in a clean beaker or directly into glass syringe barrel.

Column chromatography for aflatoxin :

Two ml of filtered dilute extract (0.2 g sample equivalent) was passed completely through AflaTest-p affinity column at a rate of about 1-2 drops/second until air comes through column. Five ml of deionzed water was passed through the column at a rate of 2 drops/second, this step was repeated once or more until air comes through column. The affinity column was eluted by passing 1.0 ml HPLC grade methanol through column at a rate of 1-2 drops/second, this elute was completely collected in a glass cuvette. One ml of AflaTest developer was added to the elute in the cuvette and mixed well and the cuvette was placed in a calliberated fluorometer. The reading of aflatoxin concentration was taken after 60 seconds.

Column chromatography for ochratoxin :

Ten mLof the filtered dilute extract (1.0 g sample equivalent) was completely passed through OchraTest affinity column at a rate of about 1-2 drops/second until air comes through the column. Ten mL of mycotoxin wash buffer was passed through the column at a rate of about 1-2 drops/second until air comes through column. Ten ml of deionzed water was passed through the column at a rate of 2 drops/second. The affinity column was eluted by passing 1.5 ml OchraTest eluting solution through column at a rate of 1-2 drops/second, this elute was completely collected in a glass cuvette, mix well and the cuvette was placed in a calibrated fluorometer. The reading of ochratoxin concentration was taken after 60 seconds.

RESULTS

The results indicated that all samples collected from the two provinces containing aflatoxins with different concentration except one samples collected from Sharkia province was aflatoxins free. All samples also containing ochratoxin with different concentration. Results are summarized in table (1). The results revealed that there is a relationship between the occurrence of aflatoxin and ochratoxin in rations mostly in all cases. Samples collected from Dakahlia provinces have a high concentration of both aflatoxins and ochratoxin. The percentage of samples contained an amount exceeded of the permitted limit was 46% of total samples for both the two toxins.

The results of toxicological analysis shwed that there was a correlation between the occurrence of aflatoxins and ochratoxin reached 0.943 which can be considered a high correlation. Samples collected from Sharkia province contain less concentration of aflatoxins and ochratoxin than Dakahlia province and the percentage of aflatoxin polluted samples reached 92.3% from the total examined samples. but the percentage of ochratoxin polluted samples reached 100% from total examined samples. The percentage of the samples exceeded the permitted limit was 7.6% for aflatoxins and 23% for ochratoxins and the correlation between the occurrence of aflatoxin and ochratoxin reach 0.717 which is also could be considered a high correlation. (table 2 and figures 1,2).

DISCUSSION

How much aflatoxin can be tolerated in an animal's diet. this question has no easy answer because aflatoxin affects different animals in different ways. young animals are usually more sensitive than aged one **(newberne 1973)**. The question can be separated into two logical questions. First. what is the maximum aflatoxin content that will not affect production economically or result in unacceptable tissue residues. Second. what is the maximum aflatoxin amount that can be feed without inducing clinical symptoms of aflatoxicosis. For broiler chickens **Hamilton (1987)** claimed that economic threshold was below 10ug/kg. **Hamilton (1975)** concluded that the minimum effective dose for aflatoxin in broiler chickens was below 10ug /kg. Low concentration of aflatoxin have been shown to reduce the resistance of chickens to pasteurella, salmonella, coccidia and candida **(Pier and Heddleston 1970 and Richard et al. 1975)**. Regulation of mycotoxins differ from country to another and from time to time in some countries started from zero and other reached 50 ppb for aflatoxins and the regulation may be changed after a period of time according the toxicological evaluation of mycotoxins. Data on the occurrence of more than one mycotoxin together, with data on the toxicology help public heath officials to arrive a

decision, whether a hazard may exist and which commodities should be regulated **(Schuller et al.1982).** The present results indicated that there was high existence of ochratoxin and aflatoxins in the same ration and also there was correlation. This may be due to the fact that the fungi which produced these two toxins require nearly the same conditions for growth and toxin production as temperature, humidity and moisture contents. Different species of fungi as *Aspergillus flavus, Aspergillus ochraceus* and *Penicillium* spp. were isolated from the Egyptian poultry rations at the same time which capable of producing both aflatoxin and ochratoxin **(Abd-El-Hamid et al. 1989, Farah 1989 and Mossa 1992).** The difference in concentration of the two mycotoxins in two provinces may be due to the slight changes in temperature and humidity. The samples exceeded the permissible limit may not able to produce clinical signs or lesions but may decreases the resistance of chicken to some diseases and consequently lead to decreased the productivity and increases economic losses **(Pier and Heddlesn 1970 and Richard et al. 1975).** Also residues of these mycotoxins may remain in the poultry tissues and become hazard for human health involving carcinogenicity and/or nephrotoxicity sequences **(Schwerdt et al. 1996, Gekle and Silberngal 1996, IARC 1993).** The synergism between the different mycotoxins must be considered when deciding legalization and judgment and must not be ruled out.

The main conclusions of this study are that, the poultry feed may contain one or more mycotoxins which may decrease the productivity of the chicken, decreased the resistance to some diseases and may have hazard effect on consumers which eat the meat of these chicken. Although there is a permitted limit for each mycotoxin, the synergism between the different mycotoxins must be considered when deciding legalization.

Table (1) : Concentration of aflatoxins and ochratoxin in samples from broiler, rations Part per billion(PPb).

Samples number	Dakahlia province			Sharkia province		
	Total Aflatoxins*	Ochratoxin	Total detected mycotoxins	Total Aflatoxin*	Ochratoxin	Total detected mycotoxins
1	0.26	1.3	1.56	0.00	2.6	2.26
2	0.28	1.1	1.38	0.57	3.3	3.87
3	0.33	3.5	3.83	0.58	3.8	4.38
4	0.61	2.2	2.81	0.72	3.0	3.72
5	2.2	2.1	4.3	0.73	2.4	3.13
6	3.4	2.5	5.9	1.1	1.2	2.3
7	4.5	4.5	9.0	1.1	1.9	3.0
8	5.6	5.4	11.0	1.2	7.6	8.8
9	5.8	10.9	16.7	2.2	3.4	5.6
10	10	19	29	2.7	3.1	5.8
11	16	29	45	3.4	4.3	7.7
12	18	25	43	3.6	12	15.6
13	34	35	69	8.4	11	19.4

Total Aflatoxins* = (Aflatoxins B_1+B_2+ G_1+ G_2)

Table (2): Correlation, Numbers and percentage of samples containing aflatoxins and ochratoxin exceed the permitted limit collected from the two provinces.

Mycotoxins	Dakahlia province					Sharkia province					Permitted limit*(P.L.) (PPb)
	Positive samples		Exceed P.L		correlation	Positive samples		Exceed P.L.		correlation	
	No.	%	No.	%		No.	%	No.	%		
Aflatoxins	13	100	6.0	46	0.943	12	92.3	1.0	7.6	0.717	5.0(B1,B2,G1,G)
Ochratoxin	13	100	6.0	46		13	100	3.0	23		5.0

Permitted limit* according to EU. (2002)

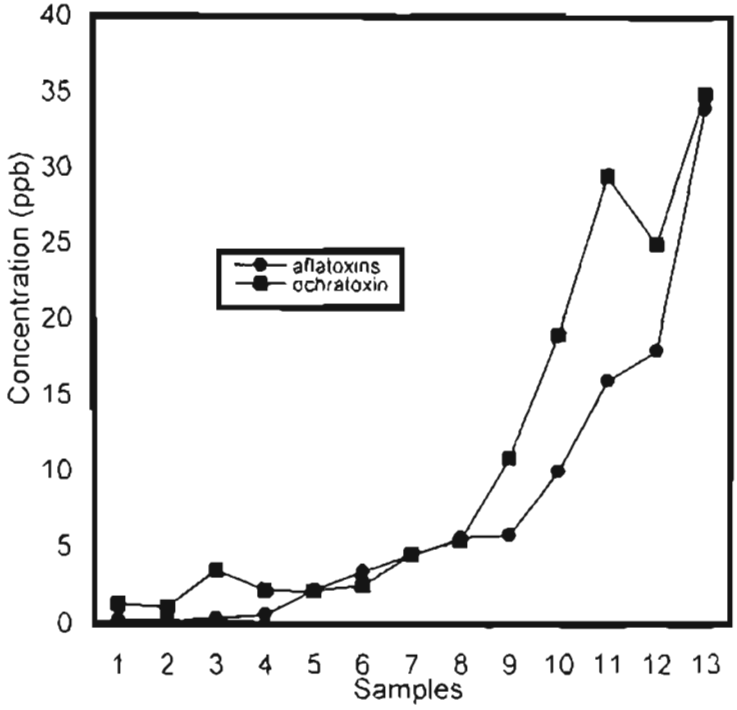
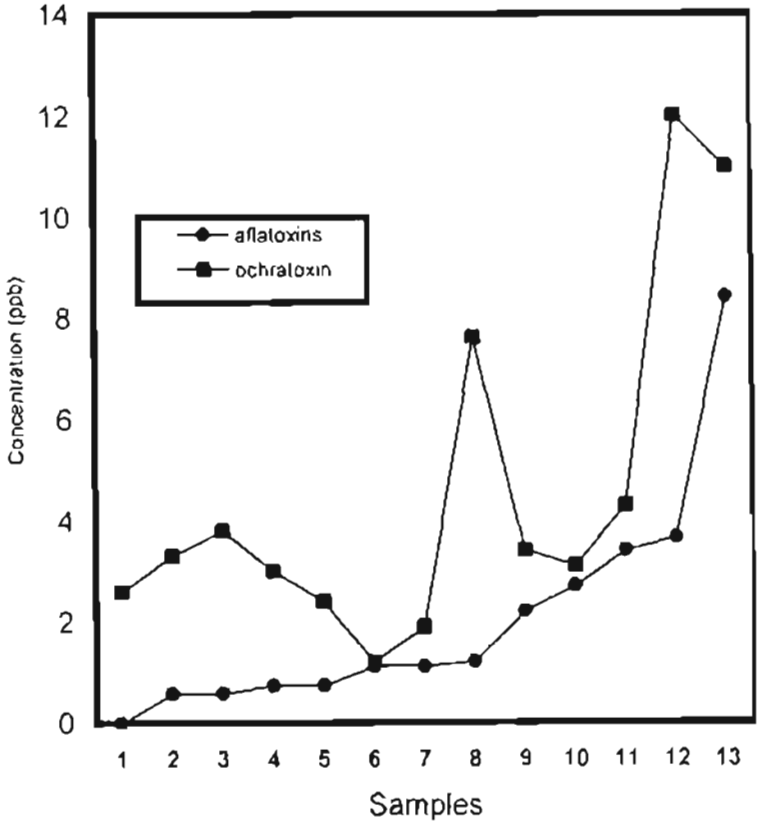


Fig. (1) : Correlation between the occurrance of aflatoxins and ochratoxin in poultry rations collected from Dakahlia province.



Flg.(2): Correlation between the occurrance of aflatoxins and ochratoxin in poultry rations collected from Sharkia province

REFERENCES

Abd-El-Hamid H. S., Torky H. A. Ezzat M. and Hassieb M. M. (1989) : Studies on contaminated poultry feed stuffs with special reference to the effect of their metabolic products on chicken embryos. Proc. 1st scientific symposium of the Egypt. poultry science Assoc. 128-136.

CAST, (Council for Agriculture Science and Technology) (1989) : Mycotoxins.: Economic and health risk. Taskforce Rep. NO. 116. Ames. Iowa.

(EU) European Union legalization limits. Commission Regulations 446/2001, 257/2002 and 472/2002.

Farah A. E. (1989) : Studies on the nutritional value and microbiological status of poultry feed in Egypt. M.V.SC. Thesis, Faculty of Vet. Med. Alexandria University. Egypt.

Frohlich A. A. Marquardt R. R. and Ominski K. H. (1991) : Ochratoxin A as a contaminant in the human food chain: a Canadian perspective. In: M. Castegnaro, R. Plestina, G. Dirheimer, I. N. Chernozemisky and H. Bartsch (Eds), Mycotoxins, Endemic Nephropathy and urinary tract Tumors, IARC Sci.Publ. No. 115, Lyon.

Gekle M. and Silberngal S. (1996) : Renal toxicodynamics of ochratoxin A: a pathophysiological approach. Kidney Blood Press. Res.19: 225-235.

Hafez A. M., Megalla S. E., Abdel-Fattah H. M. and kamel Y. Y. (1982) : Aflatoxin and aflatoxicosis II. Effects of aflatoxin on ovaries and testicles in mature domestic fowls. Mycopatholgia, 77: 137-139.

Hamilton P. B. (1975) : Determining safe levels of mycotoxins. J. Food prot.47: 570-575.

Hamilton P. B. (1987) : Aflatoxicosis in farm animals. In Aflatoxin in maize : A proceeding of the workshop) M. S. Zuber, E. B. Lillehoj and B. L. Renfro, eds.) pp. 51- 57 CIMMYT. Mexicocity, Mixico.

IARC (1993) : IARC Monographs on evaluation of carcinogenic risks to humans. Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. 56: 489-521.

Jon Ratcliff (2002) : The role of mycotoxins in food and feed safety. Animals and feed Manufactures Associations (AFMA's) symposium on "The role of animal feed in food safety" on 16 August 2002

Kane A., Creppy E. E., Roschenthaler R. and Dirheimer G. (1986) : Biological changes in kidney of rats fed subchronically with low doses of ochratoxin A. Dev. Sci. Toxicol. Environ.

14 : 241-250.

Miller J. D. (1995) : Fungi and mycotoxins in grain implication for stored product. J. Stored product Research. 31: 1- 16.

Mossa M. I. (1992) : Epidemiology of mycotic affections in poultry with special emphasis on metabolic products. M.V.SC. Thesis. Faculty of Veterinary Med. Zagazig University. Egypt.

Newberne P. M. (1973) : Chronic aflatoxicosis. J.Am. Vet. Med. Assoc. 163: 1262- 1269.

Petkova- Bocharova T. and Castegnaro M. (1985) : Ochratoxin A contamination of cereals in an area of high incidence of Balkan endemic nephropathy in Bulgaria. Food Addit. Contam.2: 267-270.

Pier A. C. and Heddleston K. L. (1970) : The effect of aflatoxin on immunity in turkey 1.Impairment of actively of acquired resistant to bacterial challenge. Avian disease 14: 797- 809.

Richard J. L., Thurston J. R. and Pier A.C. (1975) : Mycotoxin induced alteration of immunity. In Microbiology (D. Sschlessinger, ed.) pp. 388- 396 Ammerican society of Microbiologists. Washington, D.C.

Schuller P. L., Van Egmond H. P. and Stoloff L. (1982) : Limits and regulation of mycotoxins. Proceeding of the International symposium on Mycotoxins. 6-8 September 1981. National Recearch Center .Cairo. Egypt. -

Schwerdt G., Bauer K., Gekle M. and silbernagl S.(1996) : Accumulation of ochratoxin A in rat in vivo and in cultivated renal epithelial cells in vitro. Toxicology. 114: 177-185.

Sharlin J. S, Howarth B., Thompson F. N and Wyatt R. D. (1981) : Decreased reproductive potential and reduced food consumption in mature white leghorn males fed aflatoxin. Poultry Science, 60: 2701-2708.

Smith, T. K. and Sedden, I. R., (1998) : Toxicological synergism between Fusarium mycotoxins in feeds. In: Biotechnology in the Feed Industry. Ed. Lyons. T.P. and Jacques. K.A. Nottingham University Press. Loughborough. Leics. UK. 257-269.

Truckess M. W., Stack M. E., Nesheim S., Page S. W., Albert R. H., Hansen T. J. and Donahue K. F. (1991) : Immunoaffinity column coupled with solution fluorometry or liquid chromatography postcolumn derivatization for determination of aflatoxins in corn, peanut and peanut butter: collaborative study. Journal of the Association of the Official Analytical Chemistry. 74 (1): 81- 88.

الملخص العربى

دراسات المعدل العالى لمعايشة كلا من الأفلاتوكسين والأوكراتوكسين فى علائق بدارى التسمين بمحافظتى الدقهلية والشرقية - مصر

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السموم الفطرية هى أحد النواتج الثانوية ينمو الفطريات على الأغذية وعلائق الحيوان وتؤدى إلى خسائر فادحة فى الثروة الحيوانية خصوصاً مزارع الدواجن حيث تسبب نفوق الطيور أو قلة الإنتاج أو تقل المقاومة لكثير من الأمراض أو تنتقل إلى المستهلك عبر لحوم هذه الدواجن مما يؤدى إلى حدوث أمراض مثل السرطان.

تم جمع ست وعشرون عينة من بعض مزارع دجاج التسمين من محافظتى الدقهلية والشرقية لفحص تواجد الأفلاتوكسين والأوكراتوكسين باستخدام جهاز فلوروميتر. كشفت النتائج عن وجود الأفلاتوكسين والأوكراتوكسين فى جميع العلائق التى جمعت من محافظة الدقهلية وتعدت ٤٦٪ من هذه العلائق الحد المسموح به بالنسبة للأفلاتركسين والأوكراتوكسين. وفى محافظة الشرقية كانت نسبة تواجد الأفلاتوكسين ٣ر٩٢٪ من العلائق المفحوصة وتعدت ٦ر٧٪ من هذه العلائق نسبة الحد المسموح به بينما كانت نسبة تواجد الأوكراتوكسين ١٠٠٪ من العلائق المفحوصة وتعدت ٢٣٪ نسبة الحد المسموح به.

من هذه الدراسة يتضح تزامن تواجد الأفلاتوكسين والأوكراتوكسين فى علائق الدواجن مما يؤدى إلى نقص الإنتاج وضعف المناعة كما أن هذا التزامن قد يؤدى إلى تضاعف التأثير السمى لهذين المركبين ولذا يجب أن يؤخذ هذا فى الاعتبار عند تقنين الحد المسموح به.