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### Incidence of aflatoxin and ochratoxin in some poultry ration with trial for control in Beni-Suef Governorate.

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#### ABSTRACT:

A total of 60 samples (30 poultry feed and 30 chicken liver samples) were collected randomly from some farms and localities in Beni-Suef Governorate in summer and winter seasons for monitoring mycotoxin contamination condition in such farms. Samples were examined for aflatoxin and ochratoxin levels. All samples (poultry feed and chicken liver samples) were found contaminated with both mycotoxins. Concerning , poultry feed samples in summer, aflatoxin levels ranged from 1.10 to 65 ppb with mean of  $8.79 \pm 4.11$ , one sample (6.66%) exceeded the permissible limit (20 ppb); While, ochratoxin levels ranged from 3.50 to 9.90 ppb with mean of  $5.51 \pm 0.52$ , eight samples (53.33%) exceeded the permissible limit (5 ppb). In winter, aflatoxin levels ranged from 0.15 to 7.70 ppb with mean of  $3.01 \pm 0.93$ , all samples were within the permissible limit (20 ppb); while, ochratoxin levels ranged from 2.50 to 11.90 ppb with mean of  $4.55 \pm 0.70$ , four samples (26.66%) exceeded the permissible limit (5 ppb). Chicken liver samples were found contaminated with aflatoxin levels ranged from 0.51 to 6.52 ppb with mean of  $2.15 \pm 0.72$ , all these levels were within FDA permissible limit (20 ppb); nine samples (30%) exceeded FAO permissible limits (4 ppb). While, ochratoxin levels ranged from 1.90 to 5.40 ppb with mean of  $2.55 \pm 0.32$ , three samples (10%) exceeded FAO permissible limits (5 ppb); all samples (100%) exceeded Italian permissible limits (1 ppb).

Such result was the motive to carry out a study aimed to reduce the toxicity of aflatoxin and ochratoxin in broiler chickens by using of Hydrated Sodium Calcium Aluminosilicate (HSCAS). Fifty, one-day-old broiler chicks were divided equally into five groups. Birds of group (1) kept as control, group (2) fed aflatoxin 100 ppb in ration, group (3) fed aflatoxin 100 ppb plus 1% HSCAS in ration, group (4) fed ochratoxin 50 ppb in ration and group (5) fed ochratoxin 50 ppb plus 1% HSCAS in ration. All groups were kept under close observation until 35 days of age; clinical sings, mortalities and body weight were recorded. At the end of the experiment, muscles and liver samples were obtained from all groups for mycotoxins estima-

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tion by using LC-MS/MS. Mycotoxins negatively affected the body weight of birds while, mortalities were increased. Addition of 1% HSCAS induced significant improvement on body weight reduction and mortalities. Mycotoxins residues were found in liver and muscles of birds fed contaminated rations, birds fed rations treated with HSCAS showed lower levels than those untreated. We concluded that HSCAS can be a useful approach to reduce the toxicity of aflatoxin and ochratoxin in broilers.

## INTRODUCTION

Nutrition plays a crucial role in poultry rearing. According to FAO reports, 25 to 50% of the crops harvested worldwide are contaminated with mycotoxins. The percentage is highest up to 80% in tropical regions. Mycotoxins are increasingly discussed issue; mycotoxins are toxic secondary metabolites of low molecular weight produced by naturally occurring fungi in different kinds of foods and feedstuffs. Nearly every food or feed commodity can be contaminated by fungal organisms and many of these fungi are capable of producing one or more mycotoxins, which are of concern to human and animal health (Brown et al. 2001; FAO, 2011 and Hassan et al. 2012a).

Among mycotoxins of greatest public health and agro economic significance are aflatoxins and ochratoxin A, which remain a worldwide problem and account for millions of dollars lost annually in terms of human health, animal health and condemned agricultural products (Zain, 2011).

Aflatoxins and ochratoxin A induce severe economic losses in poultry due to immunosuppression, poor growth and feed conversion, increased mortality and carcass condemnation (Chen et al. 2014 and Nedeljković-Trailović et al. 2015).

Consumption of aflatoxin and/or ochratoxin contaminated diet is potentially a health hazards for both humans and animals through induction of acute and chronic effects that have teratogenic, carcinogenic or immunosuppressive impact (Felizardo and Câmara, 2013). Aflatoxins are implicated in human hepatocellular carcinoma (Felizardo and Câmara, 2013), while ochratoxin A is highly nephrotoxic,

causing both acute and chronic lesions of the kidneys, and is implicated in urinary tract tumors (IARC, 1993).

Recently, using of mycotoxin binders that can suppress or reduce absorption, promote the excretion of mycotoxins or modify their mode of action in feed has been officially allowed in the European Union as technological feed additives (Kolossova and Stroka, 2012). Mycotoxin binders such as hydrated sodium calcium aluminosilicate (HSCAS) decontaminates mycotoxins in the feed by binding them strongly enough to be unavailable for absorption from the gastrointestinal tract of chickens resulting in decrease of mycotoxin uptake and bioavailability (Bakutis et al. 2005). HSCAS proved to be effective against many mycotoxins such as aflatoxins, ochratoxin, Zearalenone and T-2 (Casarin et al. 2005; Abbès et al. 2006 and Wei et al. 2019).

Dietary HSCAS (up to 1%) doesn't impair manganese (Mn), vitamin A or riboflavin utilization (Chung et al. 1990) also apparent absorption of calcium (Ca), phosphorus (P), sodium (Na), and potassium (K) was not affected by HSCAS(2%) supplementation (Chestnut et al. 1992).

Liver and kidney are the main organs involved in the detoxification of aflatoxin B1 (AFB1) and ochratoxin also where the most residues accumulate as compared to muscles (Hussain et al. 2010 and Hassan et al. 2012a).

This search aimed to monitoring aflatoxin and ochratoxin contamination condition in Beni-Suef Governorate, and to evaluate efficacy of HSCAS as mycotoxin binder for mycotoxin control.

## 2. Materials and methods

### 2.1. Sampling:

A total of 60 samples (30 poultry feed and 30 chicken liver samples) were collected. Poultry feed samples were collected randomly in summer and winter seasons, while, chicken liver samples were collected in summer season from different localities in Beni-Suef Governorate, Egypt (levels of mycotoxins in poultry feed samples were higher in summer than in winter so samples of chicken liver were collected in summer season and each 3 liver tissues were mixed to represent one sample from each locality). Samples collected and analyzed for aflatoxin and ochratoxin using VICAM Series-4EX fluorometer according to the manufacturer's instructions (AOAC, 1991).

### 2.2. Chicks and experimental design:

Fifty, one-day-old, Cobb broiler chicks were obtained from Badrasheen Company for Poultry Industry, Giza, Egypt. Chicks were kept in clean, well ventilated cages under controlled temperature and hygienic conditions for 35 days (time of the experiment) with applying a vaccination program. Chicks were grouped into 5 equal groups. Chicks of group (1) act as control, while chicks in groups (2) and (3) fed ration contaminated with 100 ppb of aflatoxin. Chicks in groups (4) and (5) fed ration contaminated with 50 ppb of ochratoxin and chicks of groups (3) and (5) fed ration supplemented with 1% HSCAS plus the mycotoxin. Clinical signs, final body weight and mortalities were recorded.

Muscles and liver samples were collected from all experimental birds at the end of 35 days for detection of mycotoxins residues (aflatoxin and ochratoxin) by using LC-MS/MS 4000 QTRAP (Applied BioScience): Advanced Linear Ion Trap liquid chromatography technology was used for quantitative analysis of aflatoxin and ochratoxin according to European Commission (EC, 2002).

### 2.3. Mycotoxins:

Aflatoxin and ochratoxin were produced in the Mycology laboratory of Animal Health Research Institute, Giza, Egypt by using of *Asperigullus flavus* and *Asperigullus*

*ochraceous* strains respectively, according to **Wyllie and Morehouse (1978)**. Qualitative and quantitative estimation of toxins was performed according to **Schuller and Van Egmond (1981)**. Aflatoxin was used at a dose of 100 ppb in ration (**Bintrihok and Kositcharoenkul, 2006**) and ochratoxin was used at a dose of 50 ppb in ration (**El-Barkouky and Abu-Taleb, 2008**) for 35 days.

### 2.4. Binder:

**Sorbasafe** {100% Hydrated Sodium Calcium Aluminosilicate (HSCAS)}, was obtained from Kiotechagil, England. It was given at a dose of 10 g/kg ration (**Girish and Devegowda, 2006**).

### 2.5. Vaccines:

Chicks were vaccinated on the 7th day of age with Pestikal HI, Genera, Croatia against Newcastle Disease Virus (NDV), Hitchner strain, on the 14th day with Bursine Plus, Pfizer Animal Health, USA against Bursal Disease Virus (BDV) and on the 18th day with Pestikal La Sota, Genera, Croatia against Newcastle Disease Virus (NDV) La Sota strain.

### 2.6. Statistical analysis:

Collected data were statistically analyzed for the mean and standard error of the mean using t-test, One-way ANOVA, Graph Pad InStat Software (Prism version 7, IFF-Italy), (**Graph Pad InStat, 2017**).

## RESULTS

In present study, 60 samples of poultry feed and liver samples were analyzed for incidence of aflatoxin and ochratoxin, the results have shown that 100% of samples were contaminated with various levels of both mycotoxins **Table (1)**. Poultry feed samples, in summer, aflatoxin levels ranged from 1.10 to 65 ppb with mean of  $8.79 \pm 4.11$ , one sample (6.66%) exceeded FAO permissible limit; while, ochratoxin levels ranged from 3.50 to 9.90 ppb with mean of  $5.51 \pm 0.52$ , eight samples (53.33%) exceeded the permissible limit (5 ppb). In winter, aflatoxin levels ranged from 0.15 to 7.70 ppb with mean of  $3.01 \pm 0.93$ , all samples were within the permissible limit (20

ppb); while, ochratoxin levels ranged from 2.50 to 11.90 ppb with mean of  $4.55 \pm 0.70$ , four samples (26.66%) exceeded FAO permissible limit.

Chicken liver samples, aflatoxin levels ranged from 0.51 to 6.52 ppb with mean of  $2.15 \pm 0.72$ , all these levels were within FDA permissible limit (20 ppb); nine samples (30%) exceeded FAO permissible limits (4 ppb). While, ochratoxin levels ranged from 1.90 to 5.40 ppb with mean of  $2.55 \pm 0.32$ , three samples (10%) exceeded FAO permissible limits (5ppb); all samples (100%) exceeded Italian permissible limits (1ppb).

In the present experiment, no clinical signs were seen in control birds (group1). Clinical signs of depression, diarrhea and decreased body weight observed in birds received aflatoxin (group 2). Changes in body weight of experimental birds and mortalities are shown in **table (2)**.

Ochratoxin consumption caused high significant reduction in the body weight (group 4).

No mortalities were found in control group and group (5), mortality rate was 20% in group

(2) and 10% in both (3 and 4) groups all over the experiment.

Dietary supplementation with HSCAS to aflatoxin contaminated feed improved body weight reduction and mortality rate produced by aflatoxin as birds of group (3) showed body weight improvement to be within normal and mortality rate less than those of group (2). Addition of HSCAS to ochratoxin contaminated diet minimized the negative effect of ochratoxin on birds body weight, also improved the mortality rate as no mortalities were found in birds of group (5).

Mycotoxins residues analysis in muscles and liver of birds of control group was negative (not detected), results of aflatoxin and ochratoxin residues in mycotoxin fed groups are shown in **table (3)**, aflatoxin residues were found in liver and muscles of groups (2 and 3) and ochratoxin residues were found in liver and muscles of groups (4 and 5).

Aflatoxin and ochratoxin residues were found in liver at levels higher than those in muscles, levels of mycotoxins were lower ( $P < 0.05$ ) in liver and muscles of birds fed mycotoxin plus HSCAS compared with birds fed mycotoxin alone.

Table 1. Incidence of aflatoxin and ochratoxin in some poultry feed and chicken liver.

	Poultry feed in summer		Poultry feed in winter		Chicken liver	
	Aflatoxin	Ochratoxin	Aflatoxin	Ochratoxin	Aflatoxin	Ochratoxin
Minimum /ppb	1.10	3.50	0.15	2.50	0.51	1.90
Maximum/ppb	65	9.90	7.70	11.90	6.52	5.40
Mean±SE	8.79±4.11	5.51±0.52	3.01±0.93	4.55±0.70	2.15±0.72	2.55±0.32
No. of +ve samples	15	15	15	15	30	30
% of +ve samples	100%	100%	100%	100%	100%	100%
No. of samples exceeded MPL of FAO	1	8	---	4	9	3
% of samples exceeded MPL of FAO	6.66%	53.33%	---	26.66%	30%	10%

ppb: part per billion.

MPL: Mean Permissible Limit.

FAO: Food and Agriculture Organization.

Table 2. Changes of body weight and mortalities of experimental chicken groups at the end of experiment.

Group	Body weight / gram	Mortality	
		No.	%
I	1293 ± 58.12 <sup>A</sup>	--	--
II	861.66 ± 20.37 <sup>B</sup>	2	20
III	1130 ± 57.74 <sup>A</sup>	1	10
IV	815 ± 17.08 <sup>B</sup>	1	10
V	966.70 ± 54.57 <sup>b</sup>	--	--

Group I Control birds.

Group II Aflatoxin receiving birds.

Group III Aflatoxin receiving birds with addition of 1% HSCAS.

Group IV Ochratoxin receiving birds.

Group V Ochratoxin receiving birds with addition of 1% HSCAS.

Data expressed as Mean ± SE. (N =10).

Means with different superscripts (<sup>b</sup>) are significantly different between groups at P < 0.05 and means with different superscript (<sup>A-B</sup>) are highly significantly different between groups at P < 0.01.

Table 3. Residues of aflatoxin and ochratoxin in liver and muscles of experimental chicken groups.

<b>Liver</b>		
II		--
III	6±0.57 <sup>a</sup>	--
	1.2±0.13 <sup>b</sup>	--
<b>Muscles</b>		
II		--
III	3±0.26 <sup>a</sup>	--
	0.38±0.11 <sup>b</sup>	--
<b>Liver</b>		
IV		2.99±0.14 <sup>a</sup>
V	--	0.52±0.02 <sup>b</sup>
	--	
<b>Muscles</b>		
IV		0.96±0.05 <sup>a</sup>
V	--	0.15±0.01 <sup>b</sup>
	--	

ppb: part per billion.

Group II Aflatoxin receiving birds.

Group III Aflatoxin receiving birds with addition of 1% HSCAS.

Group IV Ochratoxin receiving birds.

Group V Ochratoxin receiving birds with addition of 1% HSCAS.

Data expressed as Mean±SE. Means with different superscripts (<sup>a-b</sup>) are significantly different at P < 0.05.

## DISCUSSION:

In this study, poultry feed samples analyzed for aflatoxin and ochratoxin, showed high incidence of aflatoxin, agreed with **Torky, et al. (2003)** and **El-Alfy and Abdein (2016)** who recorded a high incidence of aflatoxin in poultry ration in Assiut and Dakhalia Governorates, respectively. Ochratoxin high incidence agreed with **Torky, et al. (2003)** and **Hassan, et al. (2012b)** who reported incidence of ochratoxin in poultry ration samples in Assiut and Ismailia Governorates, respectively. The current results revealed that levels and incidence of samples exceeded permissible limits of aflatoxin and ochratoxin in ration during summer season were comparatively higher than in winter season which coordinate with previously reported by **Abdou, et al. (2017)**.

The most important factors that influence growth and mycotoxin production are environmental temperature, substrate water activity, relative humidity, gas composition, substrate composition, inoculum concentrations, microbial interactions and mechanical or insect damage (**Guynot et al., 2003** and **Giomi et al., 2007**). In particular, it is the interaction between some or all of these factors that determines whether contamination increases and mycotoxins are produced. Interactions between available water and temperature are fundamental because fungi may be able to germinate, grow and actively compete for the allocation of the available resources (**Samapundo et al., 2005** and **Marín et al., 2012**).

Results of mycotoxin residues in chicken liver samples coordinate to those by **Iqbal et al. (2014)** who analyzed broiler liver for aflatoxin and ochratoxin in Pakistan.

In the present experiment, no clinical signs were seen in control birds (group1). Clinical signs of depression, diarrhea and decreased body weight observed in birds received aflatoxin (group 2) coordinate with previous reports by **Abd El-Ghany et al. (2013)** and **Chen et al. (2014)**.

Stunted growth upon feeding aflatoxin could be attributed to reduced protein synthesis as aflatoxins interfere with normal metabolic

pathway through the inhibition of protein synthesis and enzyme system that is involved in carbohydrate metabolism and energy release (**Ahsan-ul-Haq et al., 2000**).

Ochratoxin consumption caused high significant reduction in the body weight (group 4) resembles previous reports by **Joo et al. (2013)** and **Nedeljković-Trailović et al. (2015)**. The reduction in body weight is most likely associated with inhibition of protein synthesis.

Both aflatoxin and ochratoxin increased the mortality rate, such result coordinates previous report of **Hedayati et al. (2014)** and **El-Aroussi et al. (2006)**, respectively. Aflatoxins cause mortalities via reducing feed intake, altering protein metabolism, altering enzymatic activity and decreasing nutrient utilization and absorption (**Hedayati et al., 2014**). Ochratoxin A had been suggested by various researchers to mediate its toxic effects via induction of apoptosis and disruption of mitochondrial respiration (**O'Brien and Dietrich, 2005**).

Aflatoxins are known to be potent hepatotoxic, mutagenic, carcinogenic, teratogenic, immunosuppressive and also inhibit several metabolic systems (**Li et al., 2012**).

**O'Brien and Dietrich (2005)** stated that ochratoxin A had been implicated in a diverse range of toxicological effects, including renal toxicity, mutagenicity, teratogenicity, neurotoxicity and immunosuppression.

Dietary supplementation with HSCAS to aflatoxin contaminated feed improved body weight reduction and mortality rate produced by aflatoxin as birds of group (3) showed body weight improvement to be within normal and mortality rate less than those of group (2). Similar results were obtained by **Zhao et al. (2010)** and **Abd El-Ghany et al. (2013)**. Addition of HSCAS to ochratoxin contaminated diet minimized the negative effect of ochratoxin on birds body weight, also improved the mortality rate as no mortalities were found in birds of group (5).

Mycotoxins residues analysis in muscles and liver of birds showed that, aflatoxin residues were found in liver and muscles of groups

(2 and 3). Aflatoxin residues were found in liver at levels higher than those in muscles, levels of aflatoxin were lower ( $P < 0.05$ ) in liver and muscles of birds fed aflatoxin plus HSCAS (group 3) compared with birds fed aflatoxin alone (group 2).

**Herzallah (2013)** and **Darwish et al. (2016)** reported that the highest concentration of aflatoxin was in liver, kidneys, gizzard, while the lowest concentrations were in muscles, which resembles results of this study.

Results of ochratoxin residues, higher levels of mycotoxin were in liver followed by muscles such trend agree with previous observation by **Hassan et al. (2012b)**.

Hydrated sodium calcium aluminosilicate treatment led to reduction in aflatoxin residues in liver similar to previously mentioned by **Neeff et al. (2013)**. According to **Phillips et al. (2002)** who explained the mode of action of the protective effect of hydrated sodium calcium aluminosilicate (HSCAS) as it binds rapidly to aflatoxin in the gastrointestinal tract of chickens resulting in decrease of aflatoxin uptake, bioavailability and normal distribution to the liver.

Hydrated sodium calcium aluminosilicate treatment reduced the ochratoxin residues in liver and muscles, in addition to minimize the negative effect on body weight and mortality rate, this reduction of ochratoxin toxicity also mentioned by **Casarin et al. (2005)** who reported that commercial HSCAS (Myco-Ad®) at 0.25% was effective in preventing the toxic effects of ochratoxin in broiler chickens.

## CONCLUSION

**S**urveyed samples of poultry feed and chicken liver were contaminated with both aflatoxin and ochratoxin with levels exceeded the permissible limits which of concern for birds and human health hazard, so this study aimed to reduce mycotoxicity by using mycotoxin binder (HSCAS). Feeding broiler chicks with aflatoxin and ochratoxin contaminated diets from 1 to 35 days old caused reduction of birds body weight, high mortalities and high mycotoxins residues in liver and muscles of birds received mycotoxins, 1% die-

tary HSCAS supplementation caused a significant improvement in body weight reduction, mortalities and mycotoxins residues levels. Therefore, HSCAS can be considered as a useful approach for control of aflatoxicosis and ochratoxicosis in broilers.

## RECOMMENDATIONS

- 1- Proper storage conditions of temperature, humidity, ventilation and storage duration for cereals and cereal products should be considered to avoid mycotoxin formation and contamination.
- 2- Analysis of poultry feed for mycotoxins and getting rid of contaminated feed to avoid economic losses and public health hazard.
- 3- Using of mycotoxin binders (for example HSCAS) in ration factories and in poultry farms to control mycotoxicosis, as HSCAS is already used in ration factories as anti-caking agent so by increasing HSCAS level (up to 0.5%) it will be a dual purposed substance (mycotoxin binder and anti-caking agent) in addition to its safety for birds as HSCAS up to 1% doesn't impair absorption of most of important minerals.

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