



Problems of Some Mycotoxins in Broiler Farms in Egypt

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Abstract

In this study 223 samples (75 blood samples, 70 Litter & 78 Feed of broiler chickens) were collected from Dakahlia, Sharkia and Domiate Governorates, *Aspergillus* species (*Flaves* 87.4% and *Niger* 11.4%) was only detected in litter and ration. Also, estimation of aflatoxin and ochratoxin Out of examined samples 78 (31.39%) were positive for aflatoxin and ochratoxin. Positive aflatoxin in broiler feed (15.3%), litter (30%) and chicken sera (20%) while positive ochratoxin in broiler feed (11.5%), litter (18.57%) and in chicken sera (4%). One day old, Ross 308 chicks (125), weight 40 gm were obtained from Dakahlia Poultry Hatchery. All birds were vaccinated against Newcastle disease and gumboro disease. Broilers were divided into five groups (25 chicks/each), Gp (1) broilers feed ration free from mycotoxin all over the experimental period, Gp (2) broilers feed on ration contaminated with aflatoxin B1 (25 ppb) all over the experimental period, Gp (3) broilers feed ration contaminated with aflatoxin B1 (12.5ppb) all over the experimental period, Gp(4) broilers feed ration contaminated ochratoxin (5.5 ppb) all over the experimental period and Gp (5) broilers feed ration contaminated with (2.75 ppb) ochratoxin allover the experimental period. Broilers in all groups were weighted every week for determination body performance parameter. Mortality rate was recorded. blood sample were taken for estimation hematobiochemical parameters Chickens supplemented with ration contaminated with aflatoxin in tested dose showed mortality rate (32% Gp 2 and 16% Gp 3) while for ration contaminated with ochratoxin from 28% (Gp 4) and 20%(G5). Groups fed contaminated rations with Mycotoxin (aflatoxin and ochratoxin) induce significant decrease in body weight and body weight gain from the second week up to 5th week post treatment and increased FCR in broilers. In the other hand chicken supplemented with mycotoxin (aflatoxin and ochratoxin) showed significant decrease in total protein, albumin and heterophile phagocytic activity beside significant increase in serum AST, ALT, bilirubin, uric acid and creatinine all over the experimental period. In conclusion mycotoxin contamination in feed and liter in broiler farms cause high economic losses (significant decrease in body performance) as well as immunosuppression post vaccination and hepatorenal damage.

Keywords: Mycotoxins; Broilers; Egypt; Body performance, liver and kidney function

1. Introduction

Poultry industry has entirely shifted from conventional housing to environ-mentally controlled houses. For efficient and cost-effective poultry production good quality feed is basic need for progression of this industry (Khatoun et al., 2017). Origin of feedstuffs, processing, handling and storage, as well as many other factors, can affect at different levels both the quality and safety of feed (Oyekemi et al 2019).

Fungal growth and mycotoxin production depend on complex interaction parameters such as temperature, pH, water activity, oxygen and carbon dioxide levels, composition of substrate, competitive microorganism and prevalence of various fungal strains during the storage of grain and feed stuffs (Bennett and Klich, 2003). Fungi grow and utilize readily available nutrients in broiler feed causing nutrient loss and thereby imbalance nutrients in the feed which have serious effect in broilers performance (Binder et al., 2007). Different mould strains lead to various

secondary metabolites (Bryden, 2012). Mycotoxins induce immunosuppression (Ringot et al., 2006). The genes responsible for growth and development (insulin-like growth factor 1), antioxidant protection (glutathi-one S-transferase) and immune protection (interleukins) are also down regulate when the chickens ingest feed containing mycotoxins (De Oliveira et al., 2018).

The most important safety risks for feed industry are mycotoxins (Wu, 2015). Its stru-cturally diverse group of mostly small molecluar weight compounds which are produced by secondary metabolism of filamentous fungi and are toxic to mammals and poultry (Guillamont, et al. 2005). Mycotoxins were ubiquitously present in analyzed feed and feed materials in the different surveys (Paterson and Lima, 2010). Reduced unit weight of the small intestine, indicating reduced absorptive surface, at aflatoxin B1 levels as low as 0.02 mg/kg of diet (Applegate et al., 2009). Ochratoxin showed a 37% reduction in red blood cell count and decrease in the white blood

cell count (Elaroussi et al. 2008). The purpose of this study was to evaluate the harmful levels of mycotoxins in litter and feed of broilers; failure of vaccination with a special reference to body performance, immune response and some biochemical parameters.

2. Materials and methods

A total 223 samples (75 blood samples, 70 Litter and 78 Feed of broiler chickens) were collected from Dakahlia, Sharkia and Domiate governorates for estimation of aflatoxin and ochratoxin beside isolation and identification fungus from in litter and feed ration.

1-Isolation of fungi from litter and feed of broiler chickens

Isolation of fungi were done by plate dilution method according Roper and Funnel (1965), isolated fungi were identified morphologically by cultural basis according to (Moss and mcquown, 1953) and (Barnett and hunter, 1999)

2-Media for fungal isolation: Sabaroud dextrose agar and Malt extract agar.

3-Detection of mycotoxins in broiler feed, litter & chicken sera by ELISA test

Detection of mycotoxin in broiler feed, litter and serum by Helica aflatoxin and ochra- toxin assay. Phase competitive inhibition enzyme immunoassay intended for detection mycotoxin in grains and other commodities (Tang et al., 2014)

4-Mycotoxins used in the experiment

Isolated mycotoxins (aflatoxin and ochratoxin) were used in our experiment.

5-Vaccines:

- Nobilis ma5 + clone 30, Nobilis ND, colon 30 manufactured by intervet Boxmer holand (MSD)
- Nobilis Gumboro 228 E produced by intervet Boxmer holand (MSD)
- Inactivated vaccine (ND+AI) produced by intervet Boxmer holand (MSD)
- H9 inactivated vaccine (H9 Ai) produced by intervet Boxmer holand (MSD)

6-Experimental Birds:

One day old, Ross chicks (125), weight 40 gm were obtained from Dakahlia Poultry Hatchery. All birds were vaccinated against H9 at 3 day of age using killed vaccine H9 vaccine 0.5 ml SC, at 7 day of age by ND colon 30 eye drop and vaccinated ND &H5 by 0.5 ml injected SC, at 14 day of age vaccinated with gumboro E228 vaccine by eye drop.

7-Experimental design

Broiler chickens (125) were randomly divided into five equal groups (25 chicks/group) housed and maintained in separate cages in clean room, Gp (1) broilers feed on ration free from mycotoxin all over experimental period, Gp (2) broilers feed on ration experimentally contaminated with aflatoxin B1 (25 ppb) all over the experimental period, Gp (3) broilers feed

ration contaminated with aflatoxin B1 (12.5ppb) all over the experimental period, Gp (4) broilers feed ration contaminated ochratoxin (5.5 ppb) all over the experimental period and Gp (5) broilers feed ration contemned with (2.75 ppb) ochra-toxin all over experimental period.

Broilers were weighted every week for estimation body performance parameter. Mortality rate was recorded. At 7th, 14th, 21th and 28th day also two blood samples were collected every week from each group. 1st blood samples were collected in tube contains EDTA for estimation phagocytosis (Bundy et al.1976). the 2nd samples were taken for obtain serum for estimation AST and ALT (Reitman and frankel 1957), total protein (Doumas et al 1981), albumin (Bauer, 1982), uric acid Artiss (1981), creatinine (Folin, 1934).

8- Haemagglutination Inhibition test (Allan and Gough 1974)

9-Statistical analysis: The obtained data was analyzed by using computerized SPSS program version 16 according to (Tamhane and Dunlop, 2000).

3. Results

Out of 70 litter samples, 61 (87.4%) were positive for Aspergillus species beside out of 78 Feed samples 8 (11.4%) were positive for Aspergillus. Aflatoxin was present in broiler feed (15.3%), in litter (30%) and chicken sera (20%) but ochratoxin present in broiler feed (11.5%), in litter (18.57%) and chicken sera (4%) (Table 1).

Broiler chickens supplemented with aflatoxin in tested dose (25ppb-12.5ppb) showed mortality rate 32% &16% respectively. and for ochratoxin (5.5 ppb -2.75ppb) the mortality was 28% & 20% respectively (Table 2). chickens showed significant decrease in body weight and body weight gain from the second week up to 5th week post supplementation aflatoxin and Ochratoxin and increased FCR when compared with control (Table 3). Mycotoxin (aflatoxin and ochratoxin) showed Significant decrease in total protein, albumin and heterophile phagocytic activity in sera beside significantly increase in bilirubin, AST, ALT uric acid and creatinine all over the experimental period (Tables 4, 5 and 6)

4. Discussion

Toxic chemical products formed as secondary metabolites by a few fungi that readily colonise crops in the field or after harvest. These compounds pose a potential threat to human and animal health through the ingestion of food products prepared from these commodities. (Peterson, et al. 2001). Mycotoxins are recognized as common secondary fungal metabolites contaminant in feed stuffs as produced by mold and constitute a serious worldwide problem with high economic impact (Pappas et al. 2016)

The obtained result from survey of broiler feed samples from different localities and reveal detection Out of 70 litter samples, 61 (87.4%) were positive for Aspergillus species beside out of 78 Feed samples 8 (11.4%) were positive for Aspergillus species. Tarek, et al. (2019) found that most identified molds

from broiler feed samples was *Aspergillus flavus*

out of 78 samples revealed detection was 21 positive mycotoxin (26%), representing 12 samples positive (15.4%) aflatoxin and 9 samples positive (11.5%) ochratoxin. Nearly similar results were observed by Anjum et al. (2012) reported that the incidence of Aflatoxin B1 in poultry feed samples was 44%. Our results were agreed with Donna et al., (2017) reported that aflatoxin contamination in poultry feed was occurred with quantities range between 10 ppb and >100 ppb.

Our results revealed that most common clinical signs appeared on broilers exposed to mycotoxin (aflatoxin and ochratoxin) from 7 days to the end of experiment at 35 days were greenish diarrhea, depression, ruffled feather and high mortality rate. Similar clinical signs were observed by van Egmond and Joker (2004) who observed that chickens intoxicated with aflatoxin showed greenish diarrhea, leak of appetite, increased and high mortality. Similar clinical signs and mortality rate were reported by (Gholami, et al., 2016) found similar clinical signs in boiler received aflatoxin.

The obtained results in our study showed significant decrease in live body weight, body weight gain, feed consumption and increase in feed consumption rate in broiler chickens feed in ration contaminated with mycotoxin (aflatoxin and ochratoxin) when compared with healthy control broilers (table, 3). Reduction in body weight gain and elevation feed conversion rate may be due to anorexia, inadequate digestion and absorption due to damage of alimentary mucosa induced by mycotoxin (Mir and Dwivedi, 2010). Our results were reinforced with those recorded by Bryden and Burgess (1985) observed that retardation in body weight and weight gain with reduce feed intake in case of chronic mycotoxicosis. Recent investigators (Tessari, et al. 2006 1992) recorded that mycotoxin induced lower body weight and weight gain. The obtained data about the body weight agree with Wafaa, et al. (2013) who mentioned that aflatoxin induce significant decrease in body weight and weight gain. Our results coordinate with those reported by Elbayoumi, et al. (2014) reported that chicken fed on ration contaminated with mycotoxin revealed significant decrease in body weight, body weight gain, feed consumption and increase in feed conversion rate. Aflatoxin in feeds caused reduced feed intake and poor body weight among the treated birds (Mgbeah and Anthony, 2016). This finding fitted closely with the data previously obtained by Fouad, et al. (2017) mentioned that aflatoxin induces significant decrease in body weight and weight gain.

The present study illustrated that healthy broilers fed ration contaminated with mycotoxin (aflatoxin and ochratoxin) showed significant elevation in AST, ALT, uric acid and creatinine (table 4 and 5). Our results are parallel with those obtained in turkeys Abd El-Hamied and Hussein (2003) stated that chickens fed aflatoxin showed increase in liver enzymes,

urea and creatinine. Elevation liver enzymes most likely reflect damaging effect of mycotoxin on liver and leakage of enzymes into the blood stream (Sawarkar, et al 2011). Same data was previously obtained by Kalorey, et al (2005) observed that the mycotoxin (aflatoxin and ochratoxin) produce increase in liver enzymes, urea and creatinine in broiler chickens. Aflatoxicosis induced degenerative in kidney tissue leading to kidney dysfunction and elevation of urea, creatinine, and uric acid (Gowda and Ledoux 2008 The obtained results are agreed with that reported by (Gholami, et al., 2016) stated that aflatoxin induce significant increase in AST, ALT, Urea and creatinine.

In the other hand Broilers fed ration contaminated with mycotoxin (aflatoxin and ochratoxin) showed significant reduction in total protein and albumin (table 6). Reduction in total protein and albumin in broilers fed ration contained aflatoxin and ochratoxin may be due to inhibition of hepatic protein synthesis (Castegnaro and Pfohl 2005). Reduction in protein profile may be due to ochratoxin which induce liver damage leading to inhibition protein synthesis by liver (Prasanna, et al. 2007). Also, Elaroussi, et al. (2008) reported that ochratoxin induce decrease in protein synthesis. The above-mentioned result was supported by previous studies of Bryden (2012) stated that broilers received mycotoxin showed decrease in serum total protein and albumin. These results are clearly reinforced by Wafaa et al. (2013) and (Gholami, et al., 2016) mentioned that aflatoxin causes significant decrease in total protein and albumin. Furthermore, aflatoxicosis induced significant reduction in serum total proteins and albumin due to hepatic damage and impaired protein synthesis (Zhao, et al. 2010).

In the present study mycotoxin administration in feeds showed significant decrease in NDV Geometric mean antibody titer and phagocytosis (table, 7) these results agree with Raiwa Ebrahim (1994) detected reduce in Geometric mean of HI antibodies of ND. Also, when the same chicks vaccinated with IBD vaccine no immune response against vaccine could be detected even with low level of aflatoxin in feeds. The results come in accord with the result of (Ghosh and Chauhan 1991) had been detected the impairment in development of acquired humeral immunity against GD and ND as result of graded dose of aflatoxin in feeds. This result can be explained by that reported by Gabal and Azzam (1998) stated that aflatoxin in dose of 200 ppb induced significant decrease of antibody titers in young layer chicks vaccinated with commercial live vaccines against ND, IB and IBD. In addition, Ebrahimi and Shahsavandi (2008) stated that aflatoxin decreased antibody levels post vaccination. Our obtained results are in accordance with those recorded by Elbayoumi, et al. (2014) who observed that chicken fed on ration contaminated with mycotoxin showed significant decrease in hemagglutination inhibition (HI) antibody titer against ND inactivated vaccine. Aflatoxin induced decreased immunity and resistance to diseases (Gholami, et al., 2016). Serum NDV-HI antibody titers were decreased in broilers received mycotoxin (OIE Manual; 2016). Also, Anwaar, et al.

(2016) reported that aflatoxin with level of 16 PPB resulted in significant decrease in NDV-HI antibody titers. Aflatoxin toxin also reduced antibody titres against Newcastle disease and infectious bursal disease Fouad, et al. (2017). Another support of these results was recorded by Tarek, et al. (2019) who stated that aflatoxin B1 induce significant decrease in ND-HI antibody titers in vaccinated broilers.

Results of the present study revealed that healthy broiler chickens vaccinated with ND vaccine then received mycotoxin (aflatoxin- ochratoxin) showed significant decrease in phagocytosis% (table, 7). large doses of aflatoxin are lethal while chronic exposure to low levels of aflatoxin can causing depression of cell-mediated immunity leading to poor vaccination response and decrease in phagocytic % (Sharma, 1993 These results are in a harmony with the results recorded by Jiang, et al. (2005) mentioned that aflatoxin induced strong reduction in phagocytosis %. These results agree with Meissonnier, et al. (2008) stated that aflatoxin B1 has Immuno-toxic effect and decrease in phagocytosis%. In addition, Anwaar, et al. (2016) reported that aflatoxin with level of 16 PPB resulted significant decrease in phagocytosis %.

In conclusion mycotoxin contamination in feed and liter in broiler farms cause high economic losses (significant decrease in body performance) as well as immunosuppression post vaccination and hepatorenal damage.

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Table (1): Aspergillus, Aflatoxin and Ochratoxin in examined broiler feeds, liter and serum

area	Aspergillus		Aflatoxin			Ochratoxin		
	+ve feed%	+ve litter%	+ve feed%	+ve Litter%	+ve sera%	+ve feed%	+ve Litter%	+ve sera%
Dakahlia	10	90	20	26	20	13.3	16	4
Sharkia	8.6	78.2	13	26	24	8.67	21.7	4
Dominate	17.6	94.21	12	41	16	12	17.6	4
Total	11.4	87.4	15.38	30	20	11.5	18.57	4

Table (2) Detection of mycotoxins in broiler feed using ELISA test

farm	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
aflatoxin	4.123	Zero	50 ppb	6.006	14.42g	Zero	8.18	25.004	8.18	4.517	12.434	3.492	22.219	8.18	19.122
ochratoxin	3.124	Zero	Zero	2.0033	4.449	11	2.564	4.875	2.564	6.2	2.182	1.219	2.221	2.576	3.422

Table (3): Effect of aflatoxin and ochratoxin on body performance of broilers (Mean + S.E)(n= 5).

groups	Body weight				Body weight gain			Feed consumption			FCR		
	7day	14day	21day	28 day	14day	21day	28day	14day	21day	28 day	14 day	21 day	28 day
Gp (1)	144± 2.21 a	485± 15 a	980± 45 a	1712± 106a	341 ± 11a	495± 40a	732± 85a	552± 13a	802± 60a	1193± 126a	1.43	1.62	1.36
Gp (2)	153± 2.85 a	356 ± 12c	670± 52c	1042± 85c	203 ± 12c	314± 52d	372± 66c	367± 14c	596± 65d	740± 105c	1.47	1.9	1.99
Gp (3)	195± 2.98 a	398± 15b	740± 44b	1340± 95b	203 ± 12c	342± 35b	600± 82b	435± 22b	664± 54b	1122± 98b	1.51	1.89	1.87
Gp (4)	148± 2.95 a	343± 12d	665± 61c	1105± 65b	195 ± 16d	322± 32c	440± 71d	360± 22c	618± 60c	885± 112d	1.48	1.92	1.95
Gp 5)	148± 2.79 a	406± 17b	745± 62b	1355± 104b	258 ± 21b	339± 44b	610± 53b	415± 24b	633± 62b	1146± 114b	1.51	1.82	1.88

Table (4):Effect of aflatoxin and ochratoxin supplementation on liver enzymes of broiler chickens (Mean + S.E)(n= 5).

groups	AST (U/L)				ALT (U/L)			
	7 day old	14 day old	21 day old	28 day old	7 day old	14 day old	21 day old	28 day old
Gp (1)	92.0 ±5.3d	93.2±5.3d	92.6 ± 5.1c	93.65 ± 6.2c	14.6 ±101d	14.2 ±102d	13.8 ±1.3c	14.1 ±1.1c
Gp (2)	125.4 ±12a	135.4 ±14a	147.8 ± 21a	148.8 ± 19a	22.4 ±201a	28.6 ± 2.3a	32.4 ±3.1a	37.5 ±2.8a
Gp (3)	104.8 ±11b	109.8 ±12b	112.4 ± 13b	116.4 ± 12b	16.4 ±102c	18.2 ± 1.8c	20.4 ±2.1b	20.8 ±2.2b
Gp (4)	102.64 ±8a	130.4 ±12a	148 ± 14a	146.8 ± 14a	23.8 ± 104a	25.5 ± 1.8a	31.6 ±2.3a	35.4 ±4.2a
Gp 5)	99.8 ±11c	104.4 ±12c	112.4 ± 8b	114 ± 9b	18.4 ±1.2b	20.4 ±2.1b	20.8 ±2.2b	21.6 ±1.9b

Table (5): Effect of aflatoxin and ochratoxin supplementation on kidney function of broiler chickens (Mean + S.E)(n= 5).

groups	Uric acid			Creatinine		
	14day old	28 day old	35 day old	14day old	28 day old	35 day old
Gp (1)	3.9 ± 0.38b	3.8 ± 0.23d	3.7 ± 12c	17.8 ± 1.7d	18.2 ± 1.8d	17.5 ± 2.0d
Gp (2)	3.8 ± 0.24b	4.9 ± 0.8a	5.6 ± 0.7a	22. 0 ± 2.4a	30.1 ± 3.1a	33.4±3.5c

Gp (3)	3.8 ± 0.5b	4.2 ± 0.2c	5.7 ± 0.52b	19.8 ± 2.2c	21.3 ± 3.1c	24.4±3.1a
Gp (4)	4.2 ± 5.6a	5.9 ± 0.9a	6.8 ± 1.2a	23.7 ± 3.1a	32.3 ± 4.5a	35.4±4.1c
Gp 5)	4.1 ± 1.1a	4.7 ± 0.6b	5.3 ± 0.7b	20.2 ± 2.2b	24.2 ± 4.9b	30.7±5.1b

Table (6):Effect of aflatoxin and ochratoxin on Serum total protein, albumin and serum bilirubin of broiler chickens (Mean + S.E)(n= 5).

groups	Total Protein`				Albumin				Bilirubin			
	7 day old	14 day old	28 day old	35 day old	7 day old	14 day old	28 day old	35day old	14dayold	21day old	28day old	35day old
Gp (1)	6.42±0.3a	6.62±0.4a	6.54±0.3a	6.8±0.4a	3.25±0.4a	3.4±0.3a	3.14±0.4a	3.06±0.4a	1	1c	1c	1e
Gp (2)	4.32±0.4c	3.95±0.3d	3.64±0.4d	3.59±0.4c	2.1±0.2c	1.98±0.3c	2.01±0.5c	1.94±0.4c	2	3a	5a	8b
Gp (3)	5.8±0.4b	5.2 ±0.3b	5.2±0.4b	4.95±0.2b	2.5 ±0.4b	2.4±0.5b	2.3±0.4b	2.2 ±0.5b	1	2b	3b	3d
Gp (4)	4.22±0.12c	4.12±0.5c	3.8±0.4d	2.52±0.6c	2.08±0.2c	1.88±0.4c	1.92±0.5c	1.94±0.4c	2	3a	5a	9a
Gp 5)	5.4±0.3b	4.9±0.3c	4.8±0.4c	4.91±0.3b	2.56±0.2b	2.21±0.5b	2.1±0.4b	2.06±0.2d	1	2b	3b	4c

Table (7): effect of Aflatoxin and Ochratoxin on mortality rate and Immune response (Mean + S.E)(n= 5).

groups	Mortality %	Immune response 21 P V			phagocytic activity%			
		GMT ND	GMT H5	GMTH9	7 day old	14 day old	21 day old	28 day old
Gp (1)	00	5.4a	4a	4a	52.6a	53.4a	52.2a	52.4a
Gp (2)	32%	3c	2c	2c	22.4c	21.2c	22.4c	20.6c
Gp (3)	16%	4b	3b	3b	38.6b	36.6b	37.4b	36.4b
Gp (4)	28%	3c	2c	2c	23.4c	20.8c	20.4d	19.6d
Gp 5)	20%	4b	2b	2b	36.6b	37.2b	36.8b	37.2b