Problems of Some Mycotoxins in Broiler Farms in Egypt

Ahmed, M. Hegazy, Mohamed A. El Sisi and Ehab, K. El Bendari*, Ehab, M; ABD-ALLAH, and Hala, M, N; Toliba

Avian and rabbit Medicine, Faculty of Vet. Medicine Zagazig university

*Correspondence: Ehab.elbendary@gmail.com

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 (Flavus 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 all over 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 Chicks 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 litter 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 (Khatoon 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 (Oykemi 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 struc-turally diverse group of mostly small molecular 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 contaminated 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 copmpared 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 absorpt-ion 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 reveald 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 creatine in broiler chickens. Aflatoxicosis induced degenerative in kidney tissue leading to kidney dysfunction and elevation of urea, creatine, 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, etal.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 vaccinatesed 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.

Acknowledgments

The authors would like to express their sincere gratitude to the staff of the Faculty of Veterinary Medicine, Zagazig University, Egypt to assist in the completion of this research

References

Abd El-Hamied S. and Hussein S. H. (2003): Some biochemical changes in broiler chickens during Aflatoxicosis and their reduction by using activated charcoal. Egypt. J. Agric. Res. 81 (2), P: 481-503.

Abd-El Hamid, A.; Sallam, A.; Abd Allah, G. and El-Samra, S. (2002): Effect of feeding male rats on aflatoxic diets without or with medicinal herbs (thyme, safflower, ginger).Proc.2nd Conf. Foodborne Contam. and Egyptian's Health, El-Mansoura, Egypt.

Anjum M.; Khan S.; Sahota A. and Sardar R. (2012) Assessment of Aflatoxin B1 in commercial poultry feed and feed ingredients. J. of Animal & Plant Sciences, 22(2):68-72

Allan, W. And Gough, R. (1974); Standard Haemagglutination Inhibition Test For Nd. Vet. Rec., 95(7):120-1 23.

Anwaar M.; Madian, K.; Eman, A. and Yahia M. (2016) The Effect of Mycotoxins on Immune Response of Broilers to the Live ND Vaccines Applied by Different Routes. Middle East J. of Applied Sci. 6 (1) 51-58

Applegate, T., Schatzmjr, K. Troche, G. and Z. Jiang. (2009) Effect of aflatoxin culture on intestinal function and nutrient loss in laying hens. Poult Sci.88:235–241

Artiss, J. (1981): Colorimetric deter uric acid. Clin. Chem. Acta.116:31-39

Bauer, J. (1982); Colormetric Determination of Serum albumin. Clinical Laboratory methods, 4th Ed., 495-496, 1121.

Barnett H and Hunter B. (1999). Illustrated Genera of Imperfect Fungi. The Amer. Phytopathol. Soc. St. Paul.

Bennett, J. and Klitch, M. (2003) Mycotoxins. Clinical Micro. Reviews, 16 (3) 497–516

Binder, E.; Handle, J. and Richard, J., (2007); worldwide occurrence of mycotoxins in commodities feed and feed ingredients. Animal Feed Science and Technology 137,65–82

Bryden W. (2012); "Mycotoxin contamination of feed supply chain: Implicat-ion for animal productivity and feed security". Animal Feed Sci. and Techno 173.1-2, 34-58.

Bryden, W. and Burgess, L. (1985) Plant pathogens and mycotoxins. Proce, Symposium 1985, pp. 35-56, The University of Sydney, February 1985.

Bundy, J.L., Grotk, Nc. And Mac Ci liary, M (1976); Nitro Blue tetrazolium test Med., 76:877-881.

Castegnaro M and Pfohl,A (2005)Further argument in favour of direct covalent binding of Ochratoxin after metabolic transformation. Food Add Cont.22(1)5-9

De Oliveira, H., Souto, C., Martins, P., Di Castro, I. and Mascarenhas, A. (2018); Mycotoxins in broiler production. Rev. Cienc. Agronet. 17: 292–299.

Donna, M.; David, R.; Lambert, F. and Coretta, A. (2017) A Limited Survey of Aflatoxins in Poultry Feed and Feed Ingredients in Guyana. Vet. Sci., 4, 60(2) 231-239

Doumas, B.; Baysa, D.; Carrier, R Peler, T. and Schaffer, R. (1981): Candidate reference for determination total protein in serum. I development and validation. Clin. Chem. 27:42-65

Duncan, D. (1955): Multiple ranges and multiple “F” test. Biometrics, 11:10.

Ebrahimi, M. and Shahsavandi S. (2008) Evaluation of antibody levels during simulta-neous aflatoxicosis and vaccination against infectious laryngotracheitis in pullets. Biologicals. 36 (5):327-329.

Elaroussi, M.; Mohamed, F.; Elgendi, M.; El Barkouky, M.; Abdou, A. and Hatab, M. (2008); Ochratoxinoa in broiler chickens: functional and histological changes in target organs. International J of Poultry Sci 7,414–422.

Elbayoumi, K.; Hoda M.; Zeinab M.; Eman R. and Bosila, A (2014) Effect of mycotoxin contaminated ration on performance parameters and immune response of broiler chickens against different doses of bivalent inactivated AI-ND vaccine.. World Applied Sci J. 32 (8) 1587-1594

El-Sheshawy, S.; El-Zoghby, A and Samak, D. (2021) Aflatoxicosis in Pekin duckling and the effects of treatments
with lycopene and silymarin, Vet World, 14(3): 788-793.

Folin, O. (1934); Colorimetric Determine Creatinine Phy. Chem. V 268:228.

Fouad, A.; Tawfeek, R. and Hassan, Y. (2017) Evaluation of Antimycotoxin Effects of Humate and Hydrated Sodium Calcium Alumino-silicate on Broilers Toxicated with Aflatoxin. AJVS. 2017; 54(1): 45-56

Gholami, M.; Rangsaz, N. and Azizi, S (2016) Evaluation turmeric (Curcum longa) effect on biochemical parameters of liver and kidney in chicken aflatoxicosis. Pharm Biol.;54(5): 780-787

Gabal M. and Azzam, A. (1998). Interaction of Aflatoxin in the feed and immunization against selected infectious diseases in poultry. II. Effect on One-day-old Layer Chicks Simultaneously Vaccinated against Newcastle Disease, Infectious Bronchitis and infectious Bursal Disease. Avian Pathol.; 27(3): 290–295.

Gowda N and Ledoux D. (2008) Use of antioxidants in broiler chicks. World Mycotoxin J. 10: 255–262.

Guillamont,E.;Lino,C. and Vinuesa, J. (2005) Acomparative study of extraction appa-ratus in HPLC analysis of ochratoxin A in muscle. Anal Bioanal Chem.383(4):570-575.

Jiang,Y.;Jolly,P.;Ellis,W. and Williams, J. (2005): Aflatoxin B1 albumin adduct levels and cellular immune status in Ghanaians .international immunology. 17:807-814.

Kalorey, D, Kurkure, J and Nigot, N (2005) Effect of polyherbal feed supplement “Growell” during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers. Asian- australas. J. Anim. Sci. 18:375–383

Khatoon, A.; Khan M.Z.; Abidin Z.U.; Khan A.; Saleemi M.K. (2017); Mitigation potential of distillery sludge (DS) against ochratoxin A induced immunological alterations in broiler chicks, World Mycotoxin J. 10: 255–262.

Meissonnier, G.; Pinton, P.; Laffitte, J. and Oswald, I. (2008): Immunotoxicity of aflatoxin B1: Impartment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. Toxicology and Appl. Pharmacol.; J.231: 23-30.

Mgbeah, U. and Anthony C. (2016) Aflatoxin contamination of poultry feeds in Nigerian feed and effect on performance of broilers. Animal Res Inter. 13(2) 2436 –2445

Mir, M. and Dwivedi, P. (2010) Immunopathology of Ochratoxosis - A in New Zealand White Rabbits. Vet Scan J. 5(1): 54.

Moss, E.S. and Mcquown, A. L. (1953).Atlas of medical mycology 3rd Ed., Williams and Wilking Co., Baltimore.

OIE terrestrial Manual (2016) Chapter.2.3.14.Newcastle Disease (infection with Newcastle disease virus).
Wu, F. (2015); Global impacts of aflatoxin in maize: Trade and human health. World Mycotoxin J., 8: 137–142.

Zhao, J., Shirley, R., Dibner, J., Kitchell, M., Vazquez-Anon, M. and Knight, C.D. (2010) Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler chicks. Poult. Sci., 89(10): 2147-2156

Table (1): Asperigillus, Aflatoxin and Ochratoxin in examined broiler feeds, litter 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         | aflatoxin | ochratoxin |
|--------------|-----------|------------|
|              | +ve       | +ve        | +ve       | +ve       | +ve       | +ve       | +ve       | +ve       | +ve       | +ve       | +ve       | +ve       | +ve       | +ve       | +ve       | +ve       |
|              | feed%     | feed%      | feed%     | Litter%   | sera%     | feed%     | Litter%   | sera%     | feed%     | Litter%   | sera%     | feed%     | Litter%   | sera%     | feed%     | Litter%   | sera%     |
| Dakahlia     | 10        | 90         | 20        | 26        | 20        | 13.3      | 16        | 4         | 11.4      | 87.4      | 15.38     | 30        | 20        | 11.5      | 18.57     | 4         |
| Sharkia      | 8.6       | 78.2       | 13        | 26        | 24        | 8.67      | 21.7      | 4         | 11.4      | 87.4      | 15.38     | 30        | 20        | 11.5      | 18.57     | 4         |
| Dominate     | 17.6      | 94.21      | 12        | 41        | 16        | 12        | 17.6      | 4         | 11.4      | 87.4      | 15.38     | 30        | 20        | 11.5      | 18.57     | 4         |
| Total        | 11.4      | 87.4       | 15.38     | 30        | 20        | 11.5      | 18.57     | 4         | 11.4      | 87.4      | 15.38     | 30        | 20        | 11.5      | 18.57     | 4         |

30
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           | 28 day  | 14 day | 21 day | 28 day |
| Gp (1)  | 144±2.21 a  | 485±15a         | 980±45a         | 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 supllementation 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 | 93.6±6.2c | 14.6±101d | 14.2±102d | 13.8±13.3c | 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 supllementation 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 |
|--------|---------------|---------|-----------|
| Gp (1) | 6.42±0.3a     | 5.4a    | 4a        |
| Gp (2) | 4.32±0.4c     | 3.8±0.3c| 3.8±0.3c  |
| Gp (3) | 5.8±0.4b      | 5.2±0.3b| 5.2±0.3b  |
| Gp (4) | 4.22±012c     | 4.12±0.5c| 4.12±0.5c |
| Gp 5)  | 5.4±0.3b      | 4.9±0.3c| 4.9±0.3c  |

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 % |
|--------|-------------|-----------------|--------|-----------------------|
| Gp (1) | 00          | 5.4a            | 4a     | 52.6a                 |
| Gp (2) | 32%         | 3c              | 2c     | 22.4c                 |
| Gp (3) | 16%         | 4b              | 3b     | 38.6b                 |
| Gp (4) | 28%         | 3c              | 2c     | 23.4c                 |
| Gp 5)  | 20%         | 4b              | 2b     | 36.6b                 |