Effect of adding Toxisorb Premium, vitamin E, glucosamine, and Saccharomyces Cerevisiae to contaminated rations with aflatoxin B1- on aflatoxin B1 concentration in the liver and eggs of laying hen

M. H. Ali*, A. A. Y. Al-Hamdani 2 and M. A. Alwan 3

1 Ministry of Agriculture / Anbar/ Iraq
2 College of Agriculture/ University of Anbar/ Iraq
3 College of Veterinary Medicine/ University of Fallujah/ Iraq

Email: mus_hm_88@yahoo.com

Abstract. This study was conducted to demonstrate effect of adding Toxisorb Premium, vitamin E, glucosamine, and Saccharomyces Cerevisiae to contaminated rations with aflatoxin B1- on aflatoxin B1 concentration in the liver and eggs of laying hen. One hundred sixty Brown Lohman laying hens were used, all hens were distributed randomly into eight treatments, each treatment had 4 replicates, each replicator has 5 chickens were distributed in cages after dividing them into 32 pens. This experiment was conducted for twenty weeks (140 days), first treatment (T1) was an essential diet without addition, and it was considered as control group, (T2) was an essential diet with aflatoxin added at a concentration of 2 mg/ kg feed, (T3) was a standard diet with an Toxisorb Premium added at a concentration of 4 g/ kg feed + 2 mg aflatoxin, (T4) was a based diet with added vitamin E at a concentration of 300 mg/ kg feed  + 2mg aflatoxin, (T5) was a base diet with glucosamin 100 mg/ kg feed +2 mg aflatoxin, (T6) was a basic ration with added Saccharomyces Cerevisiae (SC) 4 g/ kg feed +2 mg aflatoxin, (T7) was a basic ration with added mixture of (Toxisorb Premium + vitamin E + (SC)+ aflatoxin) with concentration of 4 gm + 300 mg + 4 g + 2 mg / kg feed respectively, The second group was significant increase competed with ( first, third, fourth, fifth, sixth, seventh and eighth groups) in residual aflatoxin B1 in the liver and eggs, The residual aflatoxin toxins in the liver and eggs were (10.7 and 4.6 ppm) respectively. We concluded from this study that the feed additives reduced aflatoxin B1 concentration in the liver and eggs and increased the cells' resistance to eliminating these toxins.

1. Introduction
Mycotoxins are considered a very dangerous substance due to the lack of an immune response to them and because of their physiological effects on the cells of the bird's body, as they are considered immunosuppressive, mutagenic and carcinogenic [1]. In addition, the exposure of the gastrointestinal tract to mycotoxins, which leads to damage of the membranes lining the digestive system, and this in turn effects on the extent of digestion and absorption of nutrients by the intestine, The most important of them are aflatoxin the severity of which depends on the age and type of the bird, the amount of poison exposed, and the length of the exposure period, Many studies have been followed to find appropriate solutions to reduce the effects of mycotoxins on animal health, including the use of materials that absorb mycotoxins such as bentonite as this substance acts to absorb mycotoxins by forming a foam surrounding the fungal
toxin molecule and thus enlarging its size, which prevents its entry into the living cell. Flushing these toxins out of the body of the organism [2], or using antioxidants, the most important of them are vitamin E and glucosamine, which play a major role in maintaining cells and protecting them from oxidation, as mycotoxins cause fat oxidation, which increases free radicals, causing damage to those cells [3]. In addition, binders such as (SC) were used, as they bind to mycotoxins and excrete them outside the body, leading to a reduction or prevention of the toxicity of those mycotoxins [4].

2. Materials and methods

2.1. Experiment materials
This experiment was conducted in the animal field of College of Veterinary Medicine, University of Fallujah. All the birds were randomly divided to eight treatments each treatment had four replicates each. One had five laying hen, at age 24 weeks. Breeding system was floor system. Samples collecting at 40 weeks from liver and yolk. The following substances were added to the diet shown in Table (1) to see their effect on aflatoxin B1, as follows:

a- Anti-mycotoxins: TOXISORB PREMIUM, which contains Bentonite + Montmorillonite 100%, has been added, manufactured by the German company (CLARIANT).
b- Vitamin E: The synthetic vitamin E (CUXAVIT E 50%) was used by the German company (Kaesler).
c- Guanidino Acetic Acid from the Spanish company known by the brand name (Glucosamine).
d- Binder mater : Saccharomyces cerevisiae, manufactured by the French industrial company Lesaffre.

2.2. Production of aflatoxin toxin B1
The first isolation of Aspergillus flavus was obtained from the College of Veterinary Medicine, Joint Diseases Branch, University of Baghdad, and the second isolation was from the Food Safety Branch at the Ministry of Science and Technology and followed the method of [5], modified by [6] and [7], in the development of mushrooms, as the fungus was activated by the cultivation medium Potato Dextrose Agar (PDA), and then the strain was grown on the grains of corn as a primary development medium, while the main development medium was rice.

2.3. Estimating the aflatoxin B1 concentration
The aflatoxin toxins were measured in the extract of each sample (liver and yolk) in two ways:

a- The first method was by means of (Enzyme-Linked Immune Sorbent (ELISA) according to the method of [6].
b- The second method is high-performance Liquid Chromatography (HPLC) adopting the method of [8].

2.4 Statistical analysis
Statistical analysis Complete Randomized Design (CRD) was performed in analyzing the effect of aflatoxin antagonists on the studied traits, using (SAS, 2004) program. Duncan test was performed to compare the mean parameters of the different traits.
Table 1. shows the percentages of the feed used in laying hens.

| Feed material                           | %  |
|-----------------------------------------|----|
| corn                                    | 40.5|
| wheat                                   | 24.0|
| Soybean meal (48% protein)              | 18.7|
| Protein Center                          | 8.0 |
| Limestone (calcium carbonate)           | 7.0 |
| Sunflower oil                           | 1.5 |
| salt                                     | 0.3 |
| Total                                    | 100|

Calculated chemical composition

|                          |     |
|--------------------------|-----|
| Metabolized energy (kcal / kg) | 2829|
| Crude protein (%)        | 18.85|
| Lysine%                  | 0.86 |
| Methionine + cysteine%   | 0.68 |
| Methionine%              | 0.41 |
| Calcium %                | 3.4 |
| Available phosphorous%   | 0.44|
| C/P ratio                | 150 |

3. Results and Discussion

Residual aflatoxin B1

3.1. In the liver of laying hens

Figure (1) shows the effect of The addition of aflatoxin B1, bentonite, vitamin E, GAA, and (SC) on the rate of residual aflatoxin/mg in the liver of laying hens. (T2) was a significant increase (P≤0.01) as compared with the (T1, T3, T4, T5, T6, T7 and T8) the aflatoxin concentration reached (10.7) compared with (0.12, 2.3, 1.7, 1.5, 1.2, 1.3 and 1.8) ppm respectively.

3.2. In the egg of laying hens

Figure 2 shows the effect the addition of aflatoxin B1, bentonite, vitamin E, GAA, (SC) in the rate of aflatoxin residue/ppm in eggs of laying hens. T2 was a significant increase (P≤0.01) as compared with the (T1, T3, T4, T5, T6, T7 and T8) the aflatoxin concentration reached (4.6) compared with (0.02, 0.6, 0.3, 0.2, 0.3, 0.16 and 0.11) ppm, respectively.
Figure 1. Effect of adding aflatoxin B1, Toxisorb Premium, vitamin E, glucosamine and Saccharomyces Cerevisiae aflatoxin B1 concentration in the liver/mg of laying hens

| Tretments | Residual aflatoxin in the liver/mg |
|-----------|-----------------------------------|
| T1: control (standard diet without adding aflatoxin B1) | 1.9 |
| T2: diet added (2 mg/kg diet) aflatoxin B1 | 1.3 |
| T3: standard diet added Toxisorb Premium 4 g/kg feed + 2 mg aflatoxin | 1.2 |
| T4: standard diet added vitamin E 300 mg/kg feed + 2 mg aflatoxin | 1.5 |
| T5: standard diet added glucosamine 100 mg/kg feed + 2 mg aflatoxin | 1.7 |
| T6: standard diet added Saccharomyces Cerevisiae 4 g/kg feed + 2 mg aflatoxin | 2.3 |
| T7: standard diet added mixture of (4 mg Toxisorb Premium + 300 mg vitamin E + 4 g (SC)+ 2 mg aflatoxin B1) / kg diet | 10.7 |
| T8: control (standard diet without adding aflatoxin B1) | 0.12 |

T1: control (standard diet without adding aflatoxin B1, T2: diet added (2 mg/kg diet) aflatoxin B1, T3: standard diet added Toxisorb Premium 4 g/kg feed + 2 mg aflatoxin, T4: standard diet added vitamin E 300 mg/kg feed + 2 mg aflatoxin, T5: standard diet added glucosamine 100 mg/kg feed + 2 mg aflatoxin, T6: standard diet added Saccharomyces Cerevisiae 4 g/kg feed + 2 mg aflatoxin, T7: standard diet added mixture of (4 mg Toxisorb Premium + 300 mg vitamin E + 4 g (SC)+ 2 mg aflatoxin B1) / kg diet.
4. Discussion

The result of this study indicates that the residual aflatoxin in the liver and eggs shown in in study indicates a significant deterioration for the second treatment, as the residual aflatoxin toxin content in the liver and eggs was (10.7, 4.6 ppm) respectively, and this indicates damage to liver tissue cells by aflatoxin. The epoxide substance worked to break down the cell walls, which caused the inability of the liver cells to purify the blood from toxins and excrete them to the outside, or the reason may be due to the oxidative action caused by aflatoxin, which causes damage to unsaturated fatty acids, which led to the breakdown of cell membranes and the exit of their content to Blood, offset by impairment of the endogenous antioxidant system leading to an imbalance in the oxidative/antioxidant system [9]. The reason may be due to the direct inhibitory effect of free radicals resulting from oxidative stress by the action of aflatoxin on the activity of various endogenous antioxidant systems, PX-GSH, CAT, which are responsible for expelling free radicals and raise the level of MDA [10].

5. Conclusions

We concluded from this study that the feed additives reduced aflatoxin B1 concentration in the liver and eggs and increased the cells' resistance to eliminating these toxins.

![Figure 2. Effect of adding aflatoxin B1, Toxisorb Premium, vitamin E, glucosamine and Saccharomyces Cerevisiae aflatoxin B1 concentration in the eggs/mg of laying hens.]

T1: control (standard diet without adding aflatoxin B1, T2: diet added (2 mg/ kg diet) aflatoxin B1, T3: standard diet added Toxisorb Premium 4 g/ kg feed + 2 mg aflatoxin, T4: standard diet added vitamin E 300 mg/ kg feed + 2mg aflatoxin, T5: standard diet added glucosamine 100 mg/ kg feed +2 mg aflatoxin, T6: standard diet added Saccharomyces Cerevisiae 4 g/ kg feed +2 mg aflatoxin, T7: standard diet added mixture of (4 mg Toxisorb Premium + 300 mg vitamin E + 4 g (SC)+ 2 mg aflatoxin B1) / kg diet.
References

Althagafi, I. I., Ahmed, S. A., & El-Said, W. A. 2019. Fabrication of gold/graphene nanostructures modified ITO electrode as highly sensitive electrochemical detection of Aflatoxin B1. *PloS one*, 14(1), e0210652. https://doi.org/10.1371/journal.pone.0210652.

Gul, H., Khan, S., Shah, Z., Ahmad, S., Israr, M., & Hussain, M. 2017. Effects of local sodium bentonite as aflatoxin binder and its effects on production performance of laying hens. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 23(1), 31-37. DOI: 10.9775/kvfd.2016.15714

Liu, C., Chaudhry, M. T., Zhao, D., Lin, T., Tian, Y., & Fu, J. 2019. Heat shock protein 70 protects the quail cecum against oxidant stress, inflammatory injury, and microbiota imbalance induced by cold stress. *Poultry Science*, 98(11), 5432-5445. https://doi.org/10.3382/ps/pez327

Bovo, F., Franco, L. T., Kobashigawa, E., Rottinghaus, G. E., Ledoux, D. R., & Oliveira, C. A. F. 2015. Efficacy of beer fermentation residue containing Saccharomyces cerevisiae cells for ameliorating aflatoxicosis in broilers. *Poultry Science*, 94(5), 934-942. https://doi.org/10.3382/ps/pev067

Shotwell, O. L., Hesseltine, C. W., Stubblefield, R. D., & Sorenson, W. G. 1966. Production of aflatoxin on rice. *Applied Microbiology*, 14(3), 425-428. https://doi.org/10.1094/phyto-64-17

West, S., Wyatt, R. D., & Hamilton, P. B. 1973. Improved yield of aflatoxin by incremental increases of temperature. *Applied Microbiology*, 25(6), 1018-1019.

Al Warshan, Salem Hassan Saleh. 2006. Comparison of some probiotics and two enhancers in reducing the negative effects of toxin AFLA B1 and improving the productive performance of broilers. PhD thesis, College of Agriculture, University of Baghdad.

Seitz, L. M. and Mohr, H. E. 1977. A new method for quantitation of Aflatoxin in corn, *Cereal Chemistry*, 54: 179-183.

Ayala, A., Muñoz, M. F., & Argüelles, S. 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative medicine and cellular longevity, 2014. https://doi.org/10.1155/2014/360438

He, L., He, T., Farrar, S., Ji, L., Liu, T., & Ma, X. 2017. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cellular Physiology and Biochemistry*, 44(2), 532-553. https://doi.org/10.1159/000485089