

# Reducing aflatoxin toxicity of contaminated diet fed to guinea pigs by addition of Egyptian Tafla

Nowar, M.S.<sup>(1)</sup>; El-Neshawy Saneya M.<sup>(2)</sup>; El-Meleigy Kh.M<sup>(3)</sup>.and Abdel - Salam A.F<sup>(3)</sup>.

<sup>(1)</sup>Dept of Anim. Prod. Fac. Agric. Zagazig University

<sup>(2)</sup> plant Pathology Research Institute. Agriculture Research Center.

<sup>(3)</sup> Regional Centre for Food and Feed Agriculture Research Centre

## ABSTRACT

Effect of Egyptian Tafla on reducing the toxicity of aflatoxin was examined by adding different levels of Tafla to an aflatoxin – contaminated diet fed to 30 growing male guinea pigs (average 133 gm). The animals divided into 5 groups equal in number and average body weight. Five diets were prepared uncontaminated diet (control), contaminated diet with aflatoxins B<sub>t</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> mixture (equals to 420 ppb aflatoxin B<sub>1</sub>), aflatoxins contaminated diet supplied with 1/2, 1 or 2% Tafla. Each of the 5 animal groups was fed with one diet only. The following measurement were recorded, daily feed intake, daily body weight gain, feed efficiency, aflatoxin intake (ppb).

On the 17<sup>th</sup> day of feeding period, three animals from each group were slaughtered. Blood samples were taken individually for biochemical study. Internal organs of each animal (liver, lung, kidney, heart and spleen) were clinically examined, weighed and samples for histopathological studies were taken.

The obtained results revealed that comparing to the uncontaminated (control) diet, feeding aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> mixture (equal to 420 ppb as B<sub>1</sub>) contaminated diet caused . Reduce in each of live body weight, body gain, feed intake and feed efficiency. Decrease in absolute weights of internal organs (significant only with liver and spleen) and significant increase in its relative weights. Decrease in serum total protein, albumin and globulin, meanwhile an increase in serum total lipids, total cholesterol, creatinine, urea – N, uric acid and GOT, GPT and alkaline phosphatase activities. The differences were significant (P<0.05) except in serum albumin, and urea - N.

Addition of 1/2, 1 and 2% Tafla to the aflatoxins diet caused, minimize the losses in body weight and increased daily feed intake and prolonged the survival period of tested animals. Minimize the changes in blood serum components. Where the significant (P<0.05) decrease in serum globulin, cholesterol, and GOT, GPT and alkaline phosphatase activities become insignificant. However the hazard effect of aflatoxins ingestion

was minimized by addition of Tafla (especially the 1/2% level). The differences in serum creatinine and uric acid still significant comparing to the control diet. Minimized the hazard effect on liver and kidney functions. The 1/2% level of Tafla was the highest efficient in detoxification of aflatoxins and reducing its toxicity.

## INTRODUCTION

Acute aflatoxicosis causes hepatitis, hemorrhage, immune suppression, genetic damage (carcinogenicity, teratogenicity and mutagenicity) and death. Growth impairment and lowering of reproductive performance are the most sensitive clinical signs of chronic aflatoxicosis. Scientific efforts were directed towards using physical, chemical and biological techniques for detoxification or inactivation of aflatoxins. Abdel hamid et al.,1986,1992a,b; Abdel hamid, 1993 and Abdel hamid and Mahomoud 1996/ These techniques have not been used on a commercial scale due to high costs, the need for special facilities, losses of important nutrients and the questionable safety of chemical degradation products of aflatoxins.

Many adsorbents as activated charcoal, bentonite, zeonite aluminosilicates and yeast cell wall were tested for binding of several mycotoxins both *in vitro* and *in vivo* the degree of adsorption in *in vitro* depends on the physical structure of the adsorbent, i.e. the total charge and distribution, the size of the pores and the accessible surface area. On the other hand, the properties of the adsorbate molecules (the mycotoxins) such as polarity, solubility, size, shape and in case of ionized compounds, their charge distribution (**Alexander et al., 2001**).

Recent information suggest that clay minerals and aluminosilicates added to mycotoxin – contaminated diets reduce the bioavailability of toxins and their hazardous effects in some animal species (**Philips et al., 1990; Harvey et al., 1993, Kubena et al., 1993; schell et al 1993 and Schiedeler, 1993**). The major advantages of adsorbent include low cost, safety and easy addition to animal feed. **Nowar, et al.(2002)** added bentonite at different levels to the Af-diet and proved the bentonite diminished to toxic effects of aflatoxins on all parameters of rabbit growth performance. **Abd El-Baki et al. (2002)** and **Abdel hamid et al.(2005)** found that addition of Tafla to Af-diet improved the growth performance daily body weight gain, feed consumption and feed efficiency in comparison with Af-diet. Yet, **Abdel hamid et al.(2002)** showed that the adsorbents (Antitox plus and Fix- a- tox) were less effective in reducing aflatoxin productivity in YES – media for

*Aspergillus flavus* , being 62.5 and 47.5%, respectively, but Tafla reduced the productivity of aflatoxin by 91.5% comparing with the controls

This study was carried out to investigate the effect of feeding aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub> mixture (equals to 420 ppb B<sub>1</sub>) contaminated diet on growing male guinea pigs and the effect of adding different levels of Tafla to such contaminated diet on reducing aflatoxins toxicity.

## MATERIALS AND METHODS

For producing aflatoxin the strain of *Aspergillus flavus* NRRL 3357 was grown in synthetic media, yeast extract — sucrose broth (YES) containing 2% yeast extract and 20% sucrose. The substrate was dispensed in conical flask. The flasks were then autoclaved for 15 minutes at 121 C°, then cooled and inoculated with spore suspension and incubated for 9 days at 25 — 29 C°. Aflatoxin concentrations determined using **A.O.A.C (1984)** and **Shih and Marth (1969)**. The media was found to contain a mixture of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>.

Thirty growing male guinea pigs (average weight 133 gm) were randomly assigned to one of five dietary treatments (6 animals for each), uncontaminated diet (control), diet contaminated with 0.42 µg aflatoxin /kg diet and aflatoxin contaminated diet provided with one of three levels of natural Egyptian Tafla 1/2, 1 and 2%. Tafla used in study contained (%) : 50.05 SiO<sub>2</sub>; 20.26 Al<sub>2</sub>O<sub>3</sub>; 9.74 Fe<sub>2</sub>O<sub>3</sub>; 2.02 CaO, 1.95 MgO; 2.19 Na<sub>2</sub>O; 1.05 K<sub>2</sub>O and 12.74 others.

On day 17 of feeding period, three animals from each group were slaughtered and internal organs of each one (liver, heart, lungs, spleen and kidneys) were removed from the body and subjected to the clinical and histopathological examination, according to **Bancroft et al.,(1990)**

At slaughter, blood sample from each animal was taken into dry glass tube and left to clot. Blood serum was separated by centrifugation at 3000 r.p.m. for 15 minutes. Serum was analyzed for total protein, albumin, total lipids, total cholesterol, glutamate—oxaloacetate transaminase (GOT), glutamate—pyruvate transaminase (GPT), urea — N, uric acid and creatinine. The biochemical determinations of blood serum parameters were performed colorimetrically by using commercial kits purchased from Egyptian — American Company for Laboratory Services, Egypt.

The data of all parameters were statistically analyzed as completely randomized design by using analysis of variance according to **Snedecor and Cochran (1982)**. Significant differences among treatment

means were statistically tested by using Duncan's New Multiple Rang Test (**Duncan, 1955**).

## RESULTS AND DISCUSSION

### **Body weight:**

Table (1) shows the body weight of guinea pigs fed diet contaminated with aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, & G<sub>2</sub> mixture (equals to 0.42 µg aflatoxin B<sub>1</sub>/ kg diet) alone or with 1/2, 1 and 2% Egyptian Tafla. Body weight was the highest (261.7 gm) in animals fed the control diet, the lowest (127.5 gm) in those fed the aflatoxins diet alone (without Tafla), and intermediate in animals fed aflatoxin diet provided with Tafla.

### **Survival period :**

Survival period (Tables 2) was the shortest (19 days) in animals fed aflatoxin diet alone, and it was 29, 25 and 22 days in animals fed aflatoxin diet supplied with 1/2, 1 and 2% Tafla, respectively.

### **Body gain :**

Table (2) shows that animals fed aflatoxin — diet alone or with 2% Tafla lost some of their initial body weight being 9.2 and 3.3 gm, respectively. Meanwhile animals fed aflatoxin diet supplied with 1/2 and 1% Tafla gained 16.7 and 5 gm, respectively.

### **Feed intake :**

Average daily feed intake (Table 2) was the highest (26.7 gm) in the control group, the lowest (11.39 gm) in group received aflatoxins diet alone and ranged from 13.10 to 15.03 gm in groups received Tafla with the aflatoxin diet.

### **Aflatoxin intake :**

Table (2) shows that total aflatoxin intake in group fed aflatoxin diet alone was 90.3 ppb/animal, and the survival period was 19 days. Aflatoxins intake increased to 183.1, 137.8 and 120.7 ppb/ animals and the survival period prolonged to 29, 25 and 22 days as a result of adding 1/2, 1 and 2% Tafla to this aflatoxins diet, in respective orders. These results might be, indicated that addition of Tafla to the aflatoxins diet, however, increased feed intake, and consequently increased aflatoxins intake, the survival periods were prolonged. This results cleared the role of Tafla in detoxification of aflatoxins. The level 1/2 Tafla was the highest efficient level in binding aflatoxins and reducing their toxicity.

This results agreed with those of **Nowar et al., 2001; Shehata 2001 and Abd El-Baki et al (2002)** on rabbit, **Shehata et al., (2003)** and **Abdel hamid et al (2004)** on Tilapia fish, **Schell et al (1993)** and **Harvety et al (1993)** on growing pigs and **Abdel hamid et al (2005)** on rats.

### **Internal organs :**

Animals fed aflatoxins diet alone or with Tafla were lower in absolute weight of internal organs (liver lungs, kidneys, heart and spleen) than the control group (Table 3). The relative weight of internal organs showed an opposite trend being significantly higher in animal fed aflatoxin diet alone or with Tafla than those fed the control diet. These results agreed with those of **Nowar *et al*, 1996 and 2000**.

#### **Blood serum analysis :**

Table (4) shows blood serum components of guinea pigs fed aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> & G<sub>2</sub> mixture (equals to 0.42 ppm as B<sub>1</sub>) diet alone or with different level of Tafla. Comparing to the control diet, feeding aflatoxin diet decreased serum total protein, albumin and globulin and increased serum total lipids, total cholesterol, creatinine, urea - N, uric acid and GOT, GPT, alkaline phosphatase and acid phosphatase activities. The differences were significant .

Addition of Tafla especially the 1/2 % level to the aflatoxins diets minimized the changes in serum components. However, the differences than the control animals were still significant (P<0.05) in serum total lipids, creatinine, uric acid and alkaline and acid phosphatase activities.

The significant differences in serum globulin, total cholesterol, GOT and GPT activities resulted from aflatoxins ingestion become insignificant as a result of adding Tafla to the aflatoxins contaminated diet.

#### **Liver function:**

Feeding aflatoxins diet alone or with Tafla as prementioned insignificantly decreased serum total protein and globulin and significantly increased GOT, GPT and alkaline phosphatase activities than the corresponding figures of animals fed the control diet. These results indicated that liver function affected by ingestion of aflatoxins alone or with Tafla. But, this hazard effect was minimized by ingestion of Tafla especially the 1/2 % level, where there were no significant differences between the control animals and those fed aflatoxins diet supplied with 1/2% Tafla in the values of each of serum total protein, albumin, globulin, and GOT and GPT activities.

#### **Kidney fuction :**

As previously mentioned (Table 4) serum creatinine, urea — N and uric acid of animals fed aflatoxins diet alone or with Tafla 1/2% were higher than those of the control animals. The difference were significant (P<0.05) except in serum urea - N. This results indicated that kidney function affected by ingestion of aflatoxins alone or with tafla. But the hazard effect was minimized by ingestion of Tafla especially the % level, however the differences in serum creatinine and uric acid than the control animals were still significant.

The obtained results confirmed that addition of Tafla at a rate of 1/2% to aflatoxins (0.42 ppm as B<sub>1</sub>) diet was functioned in binding aflatoxins and reducing its toxicity. These results agreed with those of **Abdel hamid et al. (2005)** on rats, **Nowar et al (1996)** and **Abdel Baki et al (2002)** on growing male NZW rabbits, and **EI — Zahar et al. (1996)** on mature rabbits, and **schell et al (1993)** and **Harvey et al (1993)** on growing pigs.

### **Pathological examination:**

#### **I-The kidneys:**

Grossly, the kidneys are slightly enlarged, grey in colour and slightly firm in consistency. Microscopically the kidneys revealed that their glomerul; shirnk with small intrarenal empty spaces The renal tubules are suffered from various degenerative changes while appeared as cloudy swelling and vacuolation in other renal tubules. The renal blood vessels are also congested.

#### **2- The liver:**

Grossly, the liver appeared enlarged and congested. The cut surface appeared also redish in color and freely blood oozed. Microscopically the hepatic blood vessels are severely enlarged. Some hepatic cells suffered from variable degenerative changes and other .hepatic cells are reversed.

The histopathological aleration observed in sections of kidney and livers from guinea pigs fed on Af-diets are in agreement with those found by **Nowar et al.,2001**, **Soliman et al 2001** **Vinita et al 2003** and **Abdelhamid et al 2004a** on different animal.

### **CONCLUSION AND PRACTICAL APPLICATION**

It could be concluded from the obtained results that feeding growing male guinea pigs on aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> & G<sub>2</sub> mixutre (equals to 420 ppb) diet, decreased each of body gain, feed intake, feed efficiency, survival period, internal organs, and liver and kidney functions. Supplying such diet with Egyptian Tafla (especially at a rate of 1/2%) minimized the deleterious effect of aflatoxins, which indicate the role of Tafla in binding aflatoxins and consequently- reducing their toxicity. Therefore, it could be recommended the addition of Tafla at a rate of 1/2% to the aflatoxins (420 ppb as B<sub>1</sub>) diet, can substantially provide a simple, effective, cheap and safe practical technique for prevention or reduction of aflatoxicosis in growing guinea pigs.

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