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Effect of Naturally Contaminated Feed with Aflatoxins on Performance of Laying Hens and the Carryover of Aflatoxin B₁ Residues in Table Eggs

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Abstract: The aim of this study was to evaluate the effect of naturally contaminated feed with aflatoxin on performance of laying hens fed for 60 days and the carryover of AFB₁ residues in eggs as well as the stability of AFB₁ in naturally contaminated eggs to boiling process. Forty, 30 weeks old, White Leghorn laying hens were randomly assigned into four experimental groups and after 2 weeks were given naturally contaminated feed containing zero (control), 25, 50 and 100 µg aflatoxin/kg feed. Twenty eggs per treatment were collected on days (1-7); 10; 20, 30, 40, 50 and 60 and submitted to aflatoxin B₁ analysis using ELISA. Average egg production and egg weight were not affected by aflatoxin ($P>0.05$), while a significant decrease in feed intake ($p<0.05$) was appeared in the 2 groups fed on 50 and 100 aflatoxin µg/kg feed. Residues of aflatoxin B₁ were detected in eggs at levels that ranged from 0.02 to 0.09 with a mean value of 0.04, 0.05 and 0.07 µg/kg respectively. Aflatoxin B₁ was almost stable in naturally contaminated egg for up to 20 minutes of boiling, so avoiding aflatoxin B₁ transmission into egg appears to be the only practical way to ensure their safety for human consumption. Conclusively, the excretion of aflatoxin B₁ residues in hens' eggs might occur at relatively low concentrations under conditions of long term exposure of laying hens to low level of aflatoxin in naturally contaminated feed with reduction in feed intake started at 50 µg/kg.

Key words: Poultry feed, aflatoxin, laying hens, egg production

Introduction

Eggs have been an important commodity in international trade; however, it provides unique well balanced nutrients for persons of all ages. Their high nutrient content, low caloric value and ease of digestibility make egg valuable in many therapeutic diets for adults (Oliveira *et al.*, 2003; Heranz *et al.*, 2007; Ebubekir *et al.*, 2008).

Presence of fungi and their toxic metabolites (mycotoxin) in poultry ration is virtually inevitable particularly in tropic areas. Mycotoxins are unavoidable because they are naturally occurring compounds. They contaminate crops before harvest or invade feedstuffs of laying hen during processing, transport or storage (Liau *et al.*, 2007; Yaling *et al.*, 2008)

Aspergillus species are common soil fungi; they are recognized as major contaminant of many grains used for poultry diets. The aflatoxins are produced by two molds, *Aspergillus flavus* and *A. parasiticus*. Its specific forms are designated as B₁, B₂, G₁, G₂, M₁ and M₂. Aflatoxin B₁ is the most potent naturally occurring carcinogen known (Moss, 1991; Coulombe, 1993 and Binder *et al.*, 2007). Aflatoxin affects all poultry species. Although it generally takes relatively high levels to cause mortality, low levels can be detrimental if continually fed. As a general rule, growing poultry should not receive more than 20 ppb aflatoxin in the diet. However, feeding levels lower than 20 ppb may still reduce their resistance to disease, decrease their ability to withstand

stress and bruising and generally make them unthrifty. Laying hens generally can tolerate higher levels than young birds, but levels should still be less than 50 ppb (Jones *et al.*, 1994 and Yaling *et al.*, 2008). Aflatoxin contamination can reduce the birds' ability to withstand stress by inhibiting the immune system. This malfunction can reduce egg size and possibly lower egg production. In addition, one must pay special attention to the use of contaminated corn in layer rations because eggs are promptly used as human food and aflatoxin metabolites have been found in egg yolks (Barly and Vadehara, 1999 and Bray and Ryan, 2006).

The presence of aflatoxins in egg is a potential threat to the health of the consumer. Growing children are more sensitive than adults, as egg is one of their main sources of nutrients. Aflatoxin is known to be human carcinogens based on sufficient evidence of carcinogenicity in humans (IARC, 1987, 1993 and Yaling *et al.*, 2008).

Aflatoxins, especially B₁ have been tested extensively for gene-toxicity. It induces DNA damage, gene mutation, chromosomal anomalies and cell transformation in mammalian cells *in vitro*. For this type of carcinogen, it is generally felt that there is no threshold dose below which no tumor formation would occur. In other words, only a zero level of exposure will result in no risk (FAO/WHO, 2004). It is considered as the cause of acute hepatitis, immuno-suppression, plays a role in Kwashiorkor, increases neonatal susceptibility to

jaundice and may have relevance to hepatitis B and AIDS (Hsieh and Atkinson, 1999; Bintvihok *et al.*, 2002; Kuper-Goodman, 2003 and Kovacs, 2004).

Owing to the continuous consumer demand for fresh eggs, periodical assessment is required to offer good and safe eggs for consumption. Therefore, this investigation was planned to evaluate the effect of naturally contaminated feed with aflatoxin on performance of laying hens and evidence of AFB₁ residues in table eggs of laying chicken as well as the stability of AFB₁ in contaminated eggs to boiling process.

Materials and Methods

Experimental design: Forty, 30-wk-old, vaccinated White Leghorn laying hens, with a mean body weight of 2.2kg were randomly assigned into 4 experimental groups of 10 birds each. The birds were housed at average 22°C under 16 hr lighting in two batteries of eight wire cages each having linear feed troughs and V-shaped troughs for running water. Initially the hens were maintained for 2 weeks for adaptation and during this period they were fed a conventional maize and soybean meal basal diet previously screened for mycotoxins and formulated to meet or exceed all the nutritional requirement of laying hens.

Naturally contaminated feed: Layer's commercial feed contaminated with 100µg aflatoxin/kg feed (100ppb) was subjected for dilution with mycotoxin-free diet to adjust the required limits of treatment for this study. The dietary treatment was: zero (control), 25, 50 and 100 µg total aflatoxins/kg feed (ppb). The laying hens were exposed to the naturally contaminated feed at 32-wk-old of age and throughout the study (60 days) this contaminated feed and clean water were provided *ad libitum*.

Production performance: Egg production and feed intake/bird/gram was recorded daily. Eggs were identified for each group, dated, weighed and stored at 3°C until analyzed for AFB₁.

Statistical analysis was done according to Ingelfinger *et al.*, (1994). The results (cage mean) were subjected to one day ANOVA and treatment means were compared by the Tukey test. Statistical significant was accepted at P<0.05.

Determination of aflatoxins in feed: Total aflatoxin was detected in naturally contaminated layer's feed, using immunoaffinity method which is applicable for mycotoxins that have fluorescence (Trucksess *et al.*, 1991). Series-4 Fluorometer (VICAM) was used in this procedure which is summarized as follows:

Sample extraction: 50 g sample + 5 g. NaCl + 100 ml. methanol (80%), Blended at high speed (1 min.), Filtered with fluted filter paper. Ten ml. extract was diluted with 40 ml. distilled water and filtered with glass microfibre filter paper.

Column chromatography: Ten ml. (= 1g. sample equivalent) of filtered extract was passed through AflaTest-p affinity column with a rate of 1-2 drops/second. Column washed twice with 10 ml. distilled water. The toxin was eluted with 1 ml.HPLC methanol, to which 1 ml. of freshly prepared aflatoxin developer was added spontaneously. Reading of total aflatoxin was obtained after 60 second as part per billion (ppb).

Analysis of AFB₁ in eggs: The AFB₁ concentration was determined in the eggs collected from the hens in each experimental group (20 eggs/treatment) on day (1-7), 10, 20, 30, 40, 50 and 60 of the intoxication period according to the method recommended by the Association of Official Analytical Chemists (AOAC, 1995) including modifications described by Wolzak *et al.* (1985).

Each lot of eggs was pooled (egg white and yolk) to make approximately 100-g samples in a blender jar and blended for one minute at moderate speed. Analytical homogenate samples (50 ml) were transferred to another jar and mixed with 42 ml sodium chloride saturated solution. Samples were placed in water bath at 60 o for 25 min. Each sample was blended with 10g citric acid and 300 ml acetone and filtered. A lead acetate solution was added to the filtrate to remove interfering substances and then filtered again with the aid of sodium sulphate and celite powder. The filtrate was cleaned up by liquid-liquid extraction with hexane (discarded) and chloroform (collected). Further purification of chloroform extracts was performed using solid phase column from which aflatoxin B₁ was eluted with chloroform-acetone (9:1) according to AOAC (1995). The final extract was evaporated near dryness and diluted with 500 µl acetonitrile-benzene (98:2 v/v) for final quantification of aflatoxin B₁ by using indirect Enzyme Linked Immunosorbent Assay method (ELISA) according to the method applied by Riedel de Haen (1997). The detection limit was 0.01 µg/kg.

Stability of aflatoxin B₁ in naturally contaminated eggs
Freshly laid eggs were collected and used within 48 hours after collection. Whole egg were manually separated from the shell and mixed to obtain a suspension. The suspension was heated up to 100°C for 5, 10, 15 and 20 minutes using controlled water bath Dual chamber 5/10 L Model 28 L, catalogue No. 040661 according to Fabien and Ulrich (2007). The suspension was previously tested for aflatoxin B₁ with a mean value of 0.06 µg/kg. The suspension was then extracted and quantified using ELISA.

Results and Discussion

Effect of aflatoxin-contaminated diet on performance of laying hens: Table 1 and Fig.1 showed that egg production and egg weight were not significantly affected (P > 0.05) by dietary treatment of 25, 50 and 100 aflatoxin

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Table 1: Effect of aflatoxin-contaminated diet fed to laying hens for 60 days on some production parameters (mean values + SD)

Aflatoxins in ration (ug/kg)	Egg production %	Egg weight/g	Feed intake (g/bird/day)
Zero (control)	74.66±1.8	59±3.2	104±11.5 ^b
25	74.9±8.8	59±4.2	102±10.4 ^b
50	74.0±6.6	58±3.1	85±8.6 ^a
100	74.7±9.4	58±3.7	83±9.9 ^a

^{a,b}Means within the same row with different letters are significantly different (P < 0.05).

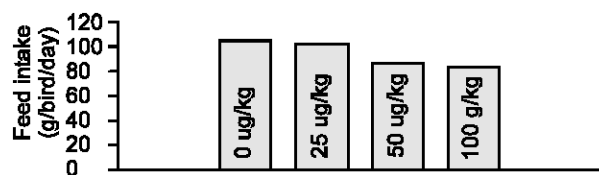


Fig. 1: Effect of aflatoxin contaminated feed on feed intake of laying hens

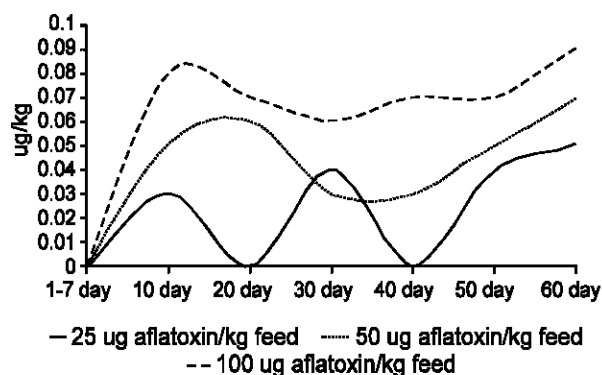


Fig. 2: AFB1 residue levels in laying eggs

ug/kg feed respectively. These results agree with Oliveira *et al.*, 2000 and Oliveira *et al.*, 2003, but with considering that the feed was contaminated with AFB₁. On another way, the results were disagree with Wolzak *et al.*, 1985; Kim *et al.*, 2003; Rizzi *et al.*, 2003; Zaghini *et al.*, 2005; Pandey and Chauhan, 2007; who found a significant decrease in egg production and egg weights of laying hens fed on AFB₁-treated ration. Regarding feed intake/bird/day, a significant decrease in feed intake (p<0.05) was appeared in the 2 groups fed on 50 and 100 aflatoxin ug/kg feed and this similar to results determined by Pandey and Chauhan, 2007.

Aflatoxin residue in eggs: As shown in Table 2 and Fig. 2, aflatoxin B₁ was detected in the eggs of all groups receiving aflatoxin contaminated rations after 10 days. The control egg pools showed no aflatoxin B₁ during 60 days of the experiment. The concentrations of aflatoxin B₁ in individual egg samples ranged from 0.03 to 0.09 ug/kg, during 60 days of treatment, with mean values of 0.04, 0.05 and 0.07 ug/kg for groups fed 25, 50 and 100

ug/kg aflatoxin respectively. Nearly similar findings were reported by Oliveira *et al.*, 2000 and Bintvihok *et al.*, 2002. However, previous studies reported relative lower level of aflatoxin B₁ in the examined egg samples (Fernandez *et al.*, 1994; Micco *et al.*, 1999; Wolzak *et al.*, 2004).

The average feed to egg transmission for aflatoxin B₁ after 60 day exposure to contaminated diets at levels of 25, 50 and 100 µg aflatoxin/kg feed were 625:1; 500:1 and 1428:1 respectively. These values are considerably below the ratio mentioned by Trucksess *et al.*, 1983, Wolzak *et al.*, 1985, Park and Pohland, 1986; Oliveira *et al.*, 2000. The variation of aflatoxin residue in the examined eggs confirm that only small quantities of the aflatoxins are likely to be deposited, while the majority of aflatoxins detoxified and/or stored in liver and other poultry tissues such as ovary, kidney, crops, breast muscles, thigh and excreted in excreta (Trucksess *et al.*, 1983; Micco *et al.*, 1988; Madden and Stahr 1995; Bintvihok *et al.*, 2002; Rizzi *et al.*, 2003; Rauber *et al.*, 2007).

So our data indicate that the carry over of aflatoxin B₁ residues is relatively most probable to occur in laying hen when the birds are continuously exposed for long period of low level of aflatoxin in the diet. This fact may be related to the lower capacity of laying hen in detoxifying aflatoxin B₁ (Mathes, 1984; Wolzak *et al.*, 1985; Hassan, 1995; Del Bianchi *et al.*, 2005). Aflatoxins incorporated into the feed of laying hens may cause relevant lesions in liver and in kidneys, heart and ovaries. The ovaries showed follicular atresia which has a detrimental effect on egg production (Hafez *et al.*, 1982; Del Bianchi *et al.*, 2005; Pandey and Chauhan, 2007). Results also, indicated that prolonged administration of aflatoxins, may cause economic losses to egg producers, besides aflatoxins in egg even in small amounts may cause public health problems due to its cumulative effects for egg consumers as concluded by Chowdhury and Smith, 2004; Ogido, *et al.*, 2004.

Stability of AFB₁ in eggs: Table 3 showed high stability of AFB₁ in contaminated eggs after boiling for 5,10,15 and 20 minutes, with a negligible mean reduction %, ranged from 0.2 -1.0%. Aflatoxin B₁ was almost stable in egg for up to 20 minutes of boiling. Nearly similar findings were reported with Samarajewa, *et al.* (1990) Rustom (1997) and Soliman (2002). Heat processing is a common procedure in egg cooking, as regards the safety and nutritive value of egg. It appears to be an effective method for controlling, or even eliminating, contamination with *Salmonella* and *Escherichia coli* (Tony *et al.*, 2008). Thermal processing was not effective for detoxification of aflatoxin B₁ in egg. Therefore, avoiding aflatoxin B₁ transmission to egg appears to be the only practical way to ensure the safety of egg for

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Table 2: The mean aflatoxin B1 in eggs during 60 days exposure of laying hen to different level of total aflatoxins in ration ($\mu\text{g}/\text{kg}$)

Aflatoxins in ration	1st -7th day	10th day	20th day	30th day	40th day	50th day	60th day	Mean \pm SD	Feed to eggs AFB1 ratio
0 (control)	ND	ND	ND	ND	ND	ND	ND	ND	
25	ND	0.03	ND	0.04	ND	0.04	0.05	0.04 \pm 0.01	625:1
50	ND	0.05	0.06	0.03	0.03	0.05	0.07	0.05 \pm 0.01	500:1
100	ND	0.08	0.07	0.06	0.07	0.07	0.09	0.07 \pm 0.01	1428:1

ND: not detected (determination limit of the analytical method: 0.01 $\mu\text{g}/\text{kg}$ for aflatoxin B1)

Table 3: Stability of aflatoxin B1 in contaminated egg samples (0.06 $\mu\text{g}/\text{kg}$) after heat treatment.

Treatments	Mean aflatoxin B1 reduction%
A (Boiling for 5 min.)	0.2 \pm 0.03
B (Boiling for 10 min.)	0.5 \pm 0.06
C (Boiling for 15 min.)	0.8 \pm 0.01
D (Boiling for 20 min.)	1.0 \pm 0.03

human consumption (Wood, 1989 and Bong *et al.*, 2007). So laying hens, should not receive more than 20 ppb aflatoxin in the diet (Ayesh *et al.*, 1997; Dhand *et al.*, 1998 and Bray and Ryan, 2006).

The aflatoxin B₁ levels used in the experimental rations in the present investigation were in the range of natural occurrence of the toxin in the contaminated grains and cereals in Egypt. Thus the carryover of the aflatoxin metabolites into hens' eggs is possible. Therefore, the control of aflatoxin B₁ contamination in rations of laying hens is recommended in order to avoid the occurrence of aflatoxin B₁ in hen eggs intended for human consumption.

In conclusion, food safety has become and increasingly important issue for all sectors of the poultry industry. For eggs, the food safety focus has been on mycotoxins, especially aflatoxins, which cause human illness. To promote safety, a growing number of egg producing companies are adopting egg quality assurance programs, which stimulate actions for all aspects of egg production to reduce the risk of egg becoming contaminated with aflatoxins. These actions include making efforts to ensure that poultry feeds are mycotoxin free.

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