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Effect of Aflatoxin B_1 on Different Body Tissues of *Gallus domesticus*

Azhar Saeed, Sibtain Afzal, Muhammad Wajid Hussain, Syed Yassir Abbas Bokhari,
[1]Muhammad Saqib Shahzad, [2]Abdul Qayyum and M. Hashim Raza
University of Arid Agriculture, Rawalpindi, Pakistan, [1]University of Agriculture,
Faisalabad, Pakistan, [2]Bahauddin Zakariya University. Multan, Pakistan

Abstract: Aflatoxins are of serious concern for poultry industry and human beings as well. The deleterious effects of aflatoxins beyond the level of 20 ppb significantly inhibit the growth and productive performance of poultry. The present project was designed to study the effect of Aflatoxin B_1 in various edible tissues of *Gallus domesticus* at the stage of their marketing. For this purpose liver, kidney, dressed meat and poultry feed of the representative flocks were collected, oven dried, their moisture contents and Aflatoxin B_1 were determined through Thin Layer Chromatography. The data thus collected were statistically analyzed and the results showed that the Aflatoxin B_1 level was significantly different in liver with respect to kidney and meat with minimum ratio of Aflatoxin B_1.

Key words: Aflatoxin B_1, different body, tissues, *Gallus gomesticus*

Introduction

Feed constitute about 70 percent of the total cost of poultry production. Its quality significantly affects the growth and productive performance of chickens. Poultry feed is a compound of different macro and micro ingredients. These ingredients are mainly contributed by various agricultural and industrial by-products/wastes. During the last two decades the number of poultry has increased up to the extent that it is very difficult to maintain the growth rate of 15 percent per annum. In this regard the main problem faced by the industry is the availability of quality feed resources. Moreover the proper storage facilities are inadequate, which results the fungal contamination of poultry feed ingredients (Bhatti, 1999-2000).

The fungi produce various secondary metabolites termed as mycotoxins. Aflatoxins are mycotoxins, which are of serious concern. These are produced by the specific strains of fungi in (*Flavus parasiticus*) group of Aspergillus (Van der Merve *et al.,* 1965*).* Aflatoxins are extremely toxic when fed to the birds. It is primarily a hepatotoxin and its diagnosis can be made based on enlarged friable and pale liver observed in birds (Moorthy *et al.*, 1985).

The discoloration of liver results from the inhibition of lipid transport and resultant lipid accumulation (Donaldson *et al.*, 1972). It damages kidney also and thus disturbs the entire metabolism in poultry (Huff *et al.*, 1974). Symptoms of aflatoxins in chickens include depressed weight gain and feed consumption (Singh and Panda, 1988).

The most important negative effect of aflatoxin is on immunological system. The inhibition of immune response invites attack of various viral, bacterial and protozoan diseases. The deleterious effects of aflatoxin are more pronounced beyond the level of 20 ppb.

The toxin residues in various tissues of *Gallus domesticus* are of serious concern for human beings as well. The present study was designed to examined the residual effects of aflatoxin in various edible tissues of *Gallus domesticus*. For this purpose liver, kidney and dressed meat were selected as test samples.

Materials and Methods

The study was conducted in Feed Testing and Nutrition Division of Poultry Research Institute Rawalpindi. The samples of poultry feed, liver, kidney and dressed meat from the different *Gallus domesticus* farms were collected. The samples were properly processed in polythene bags. All the samples were oven dried at 65oC in hot air oven for 48 hours. These samples were subjected to Thin layer Chromatography for the determination of Aflatoxin B_1 according to the following procedure. (A.O.A.C., 1990).

Thin Layer Chromatography: 25 gm of sample was taken and blended for three minutes with 250 ml of acetone water (25+15). It was filtered out. 150 ml of filtrate was taken out and 3gm of CuCO3 (Cupric Carbonate) added. A slurry gel was prepared consisting of 170 ml 0.2 N NaOH and 30 ml FeCl3 (6.67%) and celite powder. Contents of step three were mixed with that of step two, it was shaken and filtered. 150 ml of 0.03% (H2SO4) Sulphuric acid and 10 ml of chloroform was added 150 ml filtrate in step four. It was shaken well and allowed it to stand for 15

minutes (separating funnel was used).
The lower chloroform layer was separated in an other separating funnel and 100 ml of KOH-KCl solution (1 gm KOH and 100 gm KCl in a liter of water) were added to it. It was shaken gently and allowed to stand it for 15 minutes. The lower chloroform layer was
separated in step six in a beaker filtrating through anhydrous Sodium sulphate to remove moisture. By using chloroform 10 ml filtrate volume was taken.

Qualitative Test for Aflatoxin B_1.: 2 ml filtrate obtained at step seven was added in activated mini column and allowed to drain it. Then 3 ml of chloroform acetone solution (9:1) was added to it and allowed to drain it. A blue band was appeared indicating the presence of Aflatoxin B_1 when it was seen under the UV light.

Quantitative Test for Aflatoxin B_1.: Filtrate obtained in step seven was dried and one ml of chloroform added in beaker containing aflatoxin contents. This solution was used to spot the pre-activated silica gel plate spotting of standard was also done for the comparison with standard. After spotting the plate was dipped in the chloroform and allowed to reach it ¾ of the plate the plate was removed and dried and it was seen under UV light. The volume used for unknown sample was resembling closely with the volume of standard spot used for the standard estimation buy using the following formula.

$$\text{Aflatoxin ppb} = \frac{\text{St. Spot x Conc. Of St. Spot x Dilution}}{\text{Effective Wt. x Sample Spot (ul).}}$$

Results

Samples were analyzed by Thin Layer Chromatography and their results are given in Table 1 and 2. The AFB_1 level in poultry feed varied from 19 to 23 ppb, the average being 21.8 ppb. Out of 5 samples 3 samples had 19 ppb while 2 samples contained 26 ppb AFB_1.
The distribution of AFB_1 from poultry feed to liver, kidney and meat was recorded. It was found that liver contained 19-39 ppb AFB_1. The average being 32.4 ppb. Out of 5 samples 3 showed maximum level of AFB_1 that is 39 ppb. The remaining two samples contained 19 and 26 ppb. Similarly the kidney of the birds under observation showed similar trend as in case of poultry feed being offered to the birds.
Out of 5 samples 3 samples contained 19 ppb AFB_1 while rest two samples had 26 ppb. All the five samples of poultry meat contained the same level of AFB_1 that is 19 ppb. There was no variation in the level of toxin of poultry against the intake of poultry feed. The results indicated that the concentration of aflatoxin in various body tissues varied with respect to their metabolic function.

Table 1: Moisture contents and aflatoxin B_1

Poultry Feed		Body Tissues					
		Liver		Kidney		Meat	
% M	AFB_1 ppb	% M	AFB_1 ppb	%M	AFB_1 ppb	%M	AFB_1 ppb
1	6.9	19	78.38	77.48	19	73.90	19
2	7.3	19	75.00	79.72	26	76.04	19
3	7.7	26	74.07	79.06	19	74.44	19
4	8.4	26	75.91	79.00	26	73.70	19
5	10.2	19	72.15	77.57	19	75.35	19
AVE.	8.1	21.80	75.10	78.56	21.80	74.68	19.00

Table 2: Anova Table

S. O. V	d.f.	S. S.	M. S.	F. Cal	F. tab	Results
Treatments	3	524.95	174.98	5.97	F. 05 (3, 16) = 3.24	Highly
Error	16	468.80	29.30		F. 01 (3, 16) = 5.29	Significant
Total	10	993.75				

LSD = 7.26
Results: AFB_1 level is significantly different in liver with respect to all other body tissues with maximum ratio of AFB_1

Discussion

The statistical analysis of the data in dictated that AFB_1 in liver with respect to all other body tissues was significantly high ($P < 0.01$). AFB_1 was also distributed in kidney and meat but their level was not beyond the aflatoxin content of poultry feed. It is obvious from the results that AFB_1 introduced through poultry feed mainly

attracted liver because it is primarily a hepatotoxin and its diagnosis can be made on large friable and pale liver observed in birds (Moorthy *et al.*, 1985).

The accumulation of AFB_1 in liver can be attributed to the main detoxifying function of the liver. Arshad *et al.* (1992) also observed that the main effected organ was liver resulting in fatty changes, cellular dissociation, necrosis, cellular filtration and fibrosis of liver tissues.

Quantitative recovery of AFB_1 from chick liver was also recorded by Espada *et al.* (1991) through the extraction of AFB_1 from liver of birds fed on contaminated feed.

The kidney and meat samples of the birds indicated significantly low level of AFB_1 as compared with liver while kidney contained higher level when compared with meat. Kidneys are mainly excretory organ, therefore the comparatively high level in kidneys can be justified. The comparative high level of AFB_1 could be due to the congestion of the organ with AFB_1 along with other deposits of wastes (Glahn *et al.*, 1991). Thereby disturbing the excretory function of the kidney and further accumulation of aflatoxin in kidney. The varying levels of aflatoxin in various samples of kidneys might have been affected with respect to their water intake and extend of nephritic disfunctioning.

However it is obvious from results that the consumption of liver, kidney and meat is of great significance from human beings point of view. The principal organ affected by AFB_1 is liver as liver cancer has been reported form various countries of the world in chronic toxicity. Such changes have also been reported in other organs like pancreas, kidney, lungs, stomach, brain, heart, intestines, adrenal glands, spleen and testes etc. (Salunkhe *et al.*, 1987).

It is therefore advisable that liver and kidneys in particular of the flocks reared on aflatoxin contained feed must be voided. Moreover the liver of such birds can not recommended for human consumption even the birds are allowed with drawl time before slaughter (Duarte *et al.*, 1997).

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