DISTRIBUTION Of AFLATOXIN B_1 FROM POULTRY FEED TO DIFFERENT BODY TISSUES OF BROILERS

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ABSTRACT

This study was carried out to know the distribution of aflatoxin B_1 in various edible tissues of broilers from poultry feed at the stage of marketing. For this purpose liver, kidney, dressed meat and poultry feed of the representative flocks were collected and oven dried. The aflatoxin B_1 contents of the samples were determined through thin layer chromatography. The data thus collected were statistically analyzed and the results showed that the aflatoxin B_1 level was higher ($P<0.01$) in liver as compared to kidneys and meat.

Key words: Broilers, Body tissues, aflatoxin B^1 distribution.

INTRODUCTION

Feed constitutes 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 result 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. Aflatoxins are extremely toxic when fed to the birds. It is primarily a hepatotoxin and its diagnosis can be made on the basis of enlarged friable and pale liver (Smith and Hamilton, 1970).

The discoloration of liver results from the inhibition of lipid transport and resultant lipid accumulation (Donaldson *et al.*, 1972). It damages kidney and thus disturbs the entire metabolism in poultry (Huff *et al.*, 1974). Symptoms of aflatoxicosis 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 protozoal diseases. The maximum tolerance level of aflatoxin in feed has been set as 20 ppb. (Peckham, 1984). The Aflatoxin B and G were detected by (Asim *et al.*, 1990) in 82 percent morbid poultry livers. The level of aflatoxin varied greatly (12.30 to 493.0 ppb) and 56 percent samples contained Aflatoxin less than 200 ppb. Highest aflatoxin contents (>300 ppb) were seen in 7.22 percent of the samples.The toxin residues in various tissues of broilers are of serious concern for human beings as well. The present study reports the residual aflatoxin in various edible tissues of broilers i.e., liver, kidney and dressed meat.

MATERIALS AND METHODS

The study was conducted in Feed Testing and Nutrition Division of Poultry Research Institute, Rawalpindi. The samples of poultry feed, from five different broilers farms were collected. The liver, kidneys and dressed meat of five broilers from each farm were collected at random. The samples were stored in polythene bags.

All the samples were weighed initially and dried at 65°C in hot air oven for 48 hours till the final weight was constant to determine moisture content of the sample (Anonymous, 1990) as per following formula.

$$\text{Moisture \%} = \frac{\text{Difference in weight of sample}}{\text{Initial weight of sample}} \times 100$$

These samples were subjected to thin layer chromatography for the determination of aflatoxin B_1 according to the following procedure (Anonymous, 1990).

Thin layer chromatography

1) 25 gm of sample was blended for three minutes with 250 ml of acetone water (25:15) and filtered.
2) To 150 ml of filtrate 3 gm of $CuCO_3$ (Cupric Carbonate) was added.
3) A slurry gel was prepared consisting of 170 ml 0.2 N NaOH and 30 ml $FeCl_3$ (6.67%) and celite powder.
4) Contents of step three were mixed with that of step two, it was shaken and filtered.
5) 150 ml of 0.03% (H_2SO_4) 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 litre of water) were added to it.
6) It was shaken gently and allowed to stand it for 15 minutes.
7) 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 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 3/4 of the plate. The plate was taken out and dried and seen under the UV light.

The volume used for unknown sample was resembling closely with the volume of standard spot used for the standard estimation by using the following formula.

$$\text{Aflatoxin} = \frac{\text{Standard Spot x Conc. Of Standard. Spot x Dilution}}{\text{Effective Wt. x Sample Spot (µl)}}$$

RESULTS

The results of aflatoxin contents are given in Table 1. The average moisture percentage of poultry feed was 8.1 ± 1.30 having level of AFB_1 varied from 19-26 ppb, the average being 21.8 ± 3.83. It was found that liver contained moisture 75.10 ± 2.30 and 19-39 ppb level of AFB_1. The average being 32.4 ± 9.37. Similarly the kidney of the birds under observation showed similar trend as in case of poultry feed being offered to the birds.

Table 1: Distribution of aflatoxin B_1 (pbb) in poultry feed and different body tissues

Sr. No.	POULTRY FEED	LIVER	KIDNEY	MEAT
1	19	19	19	19
2	19	39	26	19
3	26	39	19	19
4	26	39	26	19
5	19	26	19	19
Av	21.80a	32.40 b	21.80a	19ca
±S.E	± 3.83	± 9.37	± 3.83	± 0.00

Figures bearing different letters differ (P<0.01).

Out of 5 samples of poultry feed, 3 contained 19 ppb AFB_1 while2 contained 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 meat against the intake of poultry feed. AFB_1 level is significantly different in liver with respect to all other body tissues with maximum ratio of AFB_1.

The results indicated that concentration of aflatoxin in various body tissues varied with respect to their metabolic functions.

DISCUSSION

The statistical analysis of the data indicated 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. The maximum tolerance level of aflatoxin in feed has been reported to be 20 ppb (Peckham, 1984). 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 (Smith and Hamilton, 1970). 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 affected organ was liver resulting in fatty changes, cellular dissociation, necrosis, cellular infiltration 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 kidny contain higher level when compared with meat. Kidneys are mainly excretory organs, therefore, a comparatively high level in kidney can be justified.

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 from various countries of the world in chronic toxicity. It is also obvious from the results that the level of AFB_1 in liver may increase as in present case (32.40 ± 9.37 ppb) on account of accumulation of AFB_1 through constant feeding of aflatoxin contaminated feed. 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 avoided. The liver of such birds is a matter of concern. The aflatoxin content has been reported to be REDUCED with proper withdrawal time before slaughtering (Duarte *et al.*, 1997).

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