



## RESEARCH ARTICLE

### Aflatoxin Residues in Tissues of Healthy and Sick Broiler Birds at Market Age in Pakistan: A One Year Study

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#### ABSTRACT

The objective of this study was to determine the levels of aflatoxin B1 (AFB1) and total aflatoxins (AF) in broiler meat at market age. During one year period a total of 264 samples from 199 healthy and 65 sick flocks were collected. AFB1 residues in liver, kidneys and muscles of healthy birds were detected in 75.9, 44.7 and 21.6% samples, and the highest individual levels were 2.0, 0.99 and 0.49 ng/g, respectively. AFB1 residues in liver muscle and kidneys of sick birds were detected in 89.2, 70.8 and 55.4% and the highest individual levels were 7.99, 3.99 and 1.99 ng/g, respectively. The AFB1 residue level in 0.5% liver samples of healthy birds were 2 ng/g whereas in sick birds 23.1 and 6.2% of liver and kidneys had AFB1 residues above 2.0 ng/g. AFB1 levels in the muscles of both healthy and sick birds were below 2.0 ng/g. Among healthy birds none of the tissues had AF residue levels above 4 ng/g whereas in sick birds, 24.6 and 7.7% liver and kidneys had AF residues above 4.0 ng/g.

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#### INTRODUCTION

Aflatoxins (AF) are common contaminants of many cereals, nuts, and spices (Khan *et al.*, 2010; Iqbal *et al.*, 2011; Zahoor-ul-Hassan *et al.*, 2011; Yunus and Bohm, 2011; Jubeen *et al.*, 2012; Saleem *et al.*, 2012; Anjum *et al.*, 2012). Among different subgroups of aflatoxins, Aflatoxin B1 (AFB1) is the most toxic. It induces hepatic cell injury, periportal parenchymal cell necrosis, hemorrhage and leads to hepatocellular carcinomas in human and animals (Luyendyk *et al.*, 2002; Rawal *et al.*, 2010). In food animals AFB1 induces toxic and pathological effects of economical significance (Wu *et al.*, 2004; Magnoli *et al.*, 2011; Hassan *et al.*, 2012; Zahoor-ul-Hassan *et al.*, 2012; Ul-Hassan *et al.*, 2012). Surveys conducted in different regions of Pakistan revealed that poultry feeds/ingredients were frequently contaminated with aflatoxins (Hanif *et al.*, 2005; Anjum *et al.*, 2011; Saleemi *et al.*, 2012; Rashid *et al.*, 2012). Broiler birds kept on AF contaminated feeds may have residues of these mycotoxins in their edible tissues. Previous studies have reported the presence of aflatoxins in poultry tissues

in Pakistan (Asim *et al.*, 1990; Begum *et al.*, 2001), Vietnam (Bintvihok and Davitayananda, 2002) and Greece (Eleftheriadou *et al.*, 2004). Information about the aflatoxin residues in poultry meat is scarce in relation to the similar data upon poultry feeds and its ingredients. There is a need to generate a data upon aflatoxin residues in the edible tissues of poultry marketed in Pakistan. The present study provides information collected in a year period about aflatoxin residues in liver, kidneys and breast muscles of the broiler chicks at market/depletion age in Pakistan.

#### MATERIALS AND METHODS

**Collection of samples:** Samples were collected from a total of 264 commercial broiler farms in Faisalabad region having broilers ready for marketing (around 5-6 weeks old) during one year period (November 2006 to April 2007). Samples from 133 farms were collected during winter-spring season (November-April) and 131 farms during summer-autumn season (May-October). Based upon the general demeanor and internal organs gross

appearance of birds, the farms were grouped as healthy and sick. Five birds from each farm were killed; liver, kidneys and part of breast muscles were placed in polythene bags at 4°C and transported on the same day to laboratory for storage. An almost equal portion of liver, kidneys or muscles from five birds was pooled to make a sample of each organ and kept at -20°C for analytical studies.

#### Extraction and immunoaffinity cleanup of aflatoxins:

Extraction and cleanup of aflatoxin residues in the representative tissue samples of each farm was performed by a slightly modified method of Sizoo and Egmond (2005) using immunoaffinity column (Aflatest WB<sup>TM</sup>, VICAM, USA). The eluted tissue extract was derivatized (Trucksess, 2000) and injected into LC column in a HPLC system (Prominence<sup>TM</sup>, Shimadzu®, Japan) equipped with Binary pumps (LC-20AT® (Shimadzu, Japan), Mediterranean Sea18® 5µm 25cmx0.46cm (Teknokroma, Spain). The fluorescence detector (RF-10AXL® (Shimadzu, Japan) excitation and emission wavelengths were set at 360 and 440 nm. Acetonitrile, methanol and double distilled deionized water (22.5:22.5:55) was used as mobile phase. Limit of detection (LOD) of the HPLC system was 0.02 ng/ml. Concentrations of AF and AFB1 in tissue samples were computed with Shimadzu® LC solution software "Postrun Analysis" using a calibration curve prepared from AF standards.

## RESULTS

**Residues in liver:** Concentrations of AFB1 residues in liver tissue of total, healthy and sick broiler birds have been presented in Table 1. During Nov.-Apr., the highest concentration of AFB1 was 0.78 ng/g. During May-October, the highest concentration was 2.0 ng/g. In samples of sick birds collected from Nov.-Apr., the highest level of AFB1 was 7.91 ng/g while it was 7.77 in those collected during May-Oct.

Concentrations of AF residues in liver tissue of healthy and sick broiler chicks have been presented in Table 2. Among healthy birds during May-Oct., the highest concentration of AF residues was 2.22 ng/g whereas it was 3.71 in samples collected during Nov.-Apr. Among samples from sick birds collected during Nov.-Apr. and May-Oct., the highest AF levels were 10.41 ng/g and 13.83, respectively.

**Residues in kidneys:** Concentrations of AFB1 residues in kidney tissues of healthy and sick broiler birds have been presented in Table 3. In healthy birds, samples collected during Nov-Apr, the highest concentration was 0.23 ng/g. while it was 0.96 ng/g in those collected during May-Oct. Among sick birds during the same periods the highest concentrations were 3.98 and 2.87 ng/g, respectively.

Concentrations of AF residues in kidneys of broiler chicks have been presented in Table 4. During Nov.-Apr., AF residues in kidneys of healthy broiler birds were detected in 51.0% samples and highest concentration was 0.51 ng/g whereas in samples collected during May-Oct. the highest concentration was 1.97 ng/g. In sick birds during Nov.-Apr. and May-Oct., the highest AF levels were 3.99 and 5.67 ng/g, respectively.

**Table 1:** Distribution of Levels of Aflatoxin BI (AFB1) residues in liver tissue of healthy and sick broiler birds

AFB <sub>1</sub> levels (ng/g)	November-April	May-October	One year (November-October)
<b>Healthy birds</b>			
3.0-3.99	0		
2.0-2.99	0	1 (1.0)	1 (0.5)
1.0-1.99	0	4 (3.1)	4 (2.0)
0.5-0.99	2 (2.1)	3 (2.9)	5 (2.5)
0.1-0.49	30 (31.3)	19 (18.4)	49 (24.6)
0.05-0.09	21 (21.9)	21 (20.4)	42 (21.1)
0.02-0.049	21 (21.9)	29 (28.2)	50 (25.1)
ND <sup>a</sup>	22 (22.9)	26 (25.2)	48 (24.1)
Total	96	103	199
Mean±SD	0.318±0.916	0.477±1.233	0.370±1.085
<b>Sick birds</b>			
7.0-7.99	1 (2.7)	1 (3.6)	2 (3.1)
6.0-6.99	0	1 (3.6)	1 (1.5)
5.0-5.99	0	2 (7.1)	2 (3.1)
4.0-4.99	1 (2.7)	1 (3.6)	2 (3.1)
3.0-3.99	1 (2.7)	1 (3.6)	2 (3.1)
2.0-2.99	2 (5.4)	4 (14.3)	6 (9.2)
1.0-1.99	5 (13.5)	1 (3.6)	6 (9.2)
0.5-0.99	3 (8.1)	1 (3.6)	4 (6.2)
0.1-0.49	4 (10.8)	5 (17.9)	9 (13.8)
0.05-0.09	7 (18.9)	5 (17.9)	12 (18.5)
0.02-0.049	8 (21.6)	4 (14.3)	12 (18.5)
ND <sup>a</sup>	5 (13.5)	2 (7.1)	7 (10.8)
Total	37	28	65
Mean±SD	0.874±1.609	1.636±2.272	1.202±1.943

Values in parenthesis indicate percentage. <sup>a</sup>Not detected.

**Table 2:** Levels of total aflatoxin residues in liver tissue of healthy and sick broiler birds

AF levels (ng/g)	November-April	May-October	One year (November-October)
<b>Healthy birds</b>			
4.0-4.99	0		
3.0-3.99	0	1 (1.0)	1 (0.5)
2.0-2.99	1 (1.0)	5 (4.9)	6 (3.0)
1.0-1.99	5 (5.2)	4 (3.9)	9 (4.5)
0.5-0.99	11 (11.5)	12 (11.7)	23 (11.6)
0.1-0.49	37 (38.5)	22 (21.4)	59 (29.7)
0.05-0.09	11 (11.5)	7 (6.8)	18 (9.1)
0.02-0.049	11 (11.5)	26 (25.2)	37 (18.5)
ND <sup>a</sup>	20 (20.8)	26 (25.2)	46 (23.1)
Total	96	103	199
Means±SD	0.291±0.404	0.321±0.650	0.306±0.544
<b>Sick birds</b>			
≥7.0	3 (8.1)	6 (21.4)	9 (13.9)
6-6.99	0	1 (3.6)	1 (1.5)
5.0-5.99	2 (5.4)	2 (7.1)	4 (6.2)
4.0-4.99	1 (2.7)	1 (3.6)	2 (2.1)
3.0-3.99	3 (8.1)	0	3 (4.6)
2.0-2.99	2 (5.4)	1 (3.6)	3 (4.6)
1.0-1.99	3 (8.1)	1 (3.6)	4 (6.2)
0.5-0.99	3 (8.1)	0	3 (4.6)
0.1-0.49	13 (35.1)	9 (32.1)	22 (33.9)
0.05-0.09	6 (16.2)	3 (10.1)	9 (13.9)
0.02-0.049	0 (0.0)	2 (7.1)	2 (2.1)
ND <sup>a</sup>	1 (2.7)	2 (7.1)	3 (4.6)
Total	37	28	65
Means±SD	1.826±2.703	3.602±4.962	2.591±3.909

Values in parenthesis indicate percentage. <sup>a</sup>Not detected.

**Residues in muscles:** Concentrations of AFB1 residues in muscle tissue of broiler chicks have been presented in Table 5. Mean AFB1 residue concentration in muscle samples from healthy birds during Nov.-Apr., was 0.01±0.02 ng/g with highest concentration of 0.12 ng/g. During May-Oct., mean AFB1 level was 0.025±0.057 with highest concentration of 0.45 ng/g. In samples of sick birds during Nov.-Apr. and May-Oct. the highest values were 1.85 and 0.97 ng/g, respectively.

**Table 3:** Levels of Aflatoxin B1 residues in kidney tissue of healthy and sick broiler birds

AFB <sub>1</sub> levels (ng/g)	November-April	May-October	One year (November-October)
<b>Healthy birds</b>			
Mean±SD	0.036±0.052	0.048±0.132	0.043±0.102
1.0-1.99	0	0	0
0.5-0.99	0	2 (1.9)	2 (1.0)
0.1-0.49	11 (11.5)	12 (11.7)	23 (11.6)
0.05-0.09	18 (18.8)	6 (5.8)	24 (12.1)
0.02-0.049	20 (20.8)	20 (1.5)	40 (20.1)
ND <sup>a</sup>	47 (49.0)	63 (61.1)	110 (55.3)
Total	96	103	199
Mean±SD	0.133±0.451	0.186±0.530	0.159±0.491
<b>Sick Birds</b>			
Means ± SD	0.385±0.805	0.693±0.973	0.517±0.88
4.0-4.99	0	0	0
3.0-3.99	1 (2.7)	1 (3.6)	2 (3.1)
2.0-2.99	1 (2.7)	1 (3.6)	2 (3.1)
1.0-1.99	3 (8.1)	7 (25.0)	10 (15.4)
0.5-0.99	1 (2.7)	2 (7.1)	3 (4.6)
0.1-0.49	9 (24.3)	3 (10.7)	12 (18.5)
0.05-0.09	1 (2.7)	5 (17.9)	6 (9.2)
0.02-0.049	7 (18.9)	4 (14.3)	11 (16.9)
ND <sup>a</sup>	14 (37.8)	5 (17.9)	19 (29.2)
Total	37	28	65

Values in parenthesis indicate percentage. <sup>a</sup>Not detected.

**Table 4:** Levels of total aflatoxin residues in kidneys of healthy and sick broiler birds

AF levels (ng/g)	November-April	May-October	One year (November-October)
<b>Healthy birds</b>			
Mean±SD	0.104±0.169	0.099±0.287	0.101±0.237
2.0-2.99	0	0	0
1.0-1.99	0	3 (2.9)	3 (1.5)
0.5-0.99	5 (5.21)	2 (1.9)	7 (3.5)
0.1-0.49	28 (29.17)	16 (15.5)	44 (22.1)
0.05-0.09	7 (7.29)	3 (2.9)	10 (5.0)
0.02-0.049	9 (9.38)	16 (15.5)	25 (12.6)
ND <sup>a</sup>	47 (48.96)	63 (61.12)	110 (55.3)
Total	96	103	199
<b>Sick birds</b>			
Mean±SD	0.693±1.141	1.282±1.741	0.947±1.448
6-6.99	0	0	0
5.0-5.99	0	1 (3.6)	1 (1.5%)
4.0-4.99	1 (2.70)	3 (10.7)	4 (6.2)
3.0-3.99	3 (8.11)	1 (3.6)	4 (6.2)
2.0-2.99	1 (2.70)	2 (7.1)	3 (4.6)
1.0-1.99	3 (8.1)	4 (14.3)	7 (10.8)
0.5-0.99	3 (8.1)	1 (3.6)	4 (6.2)
0.1-0.49	10 (27.0)	6 (21.4)	16 (24.6)
0.05-0.09	6 (16.2)	4 (14.3)	10 (15.4)
0.02-0.049	5 (13.5)	1 (3.6)	6 (9.2)
ND <sup>a</sup>	5 (13.5)	5 (17.9)	10 (15.4)
Total	37	28	65

Values in parenthesis indicate percentage. <sup>a</sup>Not detected.

Concentrations of AF residues in muscle tissues of broiler chicks have been presented in Table 6. In healthy birds, during Nov.-Apr., the highest value of AF was 0.60 ng/g whereas it was 0.68 ng/g during May-Oct. period. In muscle samples of sick birds the highest concentrations during Nov.-Apr. and May-Oct. were 2.20 and 2.63 ng/g, respectively.

## DISCUSSION

A vast data is available upon the aflatoxin contamination of different agriculture based foods and feeds. Chicken fed aflatoxin contaminated feeds are likely to have residues in their tissues and eggs. However, few reports described the aflatoxin residues in the tissues of

**Table 5:** Levels of Aflatoxin B1 residues in Muscle tissue of healthy and sick broiler birds

AFB <sub>1</sub> levels (ng/g)	November-April	May-October	One year (November-October)
<b>Healthy Birds</b>			
Mean±SD	0.011±0.023	0.015±0.057	0.013±0.044
0.5-0.99	0	0	0
0.1-0.49	1 (1.0)	2 (1.9)	3 (1.5)
0.05-0.09	6 (6.3)	5 (4.9)	11 (5.5)
0.02-0.049	18 (18.8)	11 (10.7)	29 (14.6)
ND <sup>a</sup>	71 (73.9)	85 (82.5)	156 (78.4)
Total	96	103	199
<b>Sick birds</b>			
Mean±SD	0.146±0.348	0.244±0.310	0.188±0.334
2.0-2.99	0	0	0
1.0-1.99	1 (2.7)	0	1 (1.5)
0.5-0.99	1 (2.7)	7 (25.0)	8 (12.3)
0.1-0.49	10 (27.0)	5 (17.9)	15 (23.1)
0.05-0.09	3 (8.1)	1 (3.6)	4 (6.2)
0.02-0.049	3 (8.1)	5 (17.9)	8 (12.3)
ND <sup>a</sup>	19 (51.4)	10 (35.7)	29 (44.6)
Total	37	28	65

Values in parenthesis indicate percentage. <sup>a</sup>Not detected.

**Table 6:** Levels of total aflatoxin (AF) residues in muscle tissue of healthy and sick broiler birds

AF levels (ng/g)	November-April	May-October	One year (November-October)
<b>Healthy birds</b>			
Mean±SD	0.036±0.088	0.023±0.087	0.030±0.088
1.0-1.99	0	0	0
0.5-0.99	1 (1.0)	1 (1.0)	2 (1.0)
0.1-0.49	12 (12.5)	5 (4.9)	17 (8.5)
0.05-0.09	5 (5.2)	4 (3.9)	9 (4.5)
0.02-0.049	9 (9.4)	9 (8.7)	18 (9.1)
ND <sup>a</sup>	69 (71.9)	84 (81.6)	153 (76.9)
Total	96	103	199
<b>Sick birds</b>			
Mean±SD	0.270±0.485	0.518±0.735	0.377±0.613
3.0-3.99	0	0	0
2.0-2.99	1 (2.7)	2 (7.1)	3 (4.6)
1.0-1.99	3 (8.1)	3 (10.7)	6 (13.9)
0.5-0.99	2 (5.4)	5 (17.9)	7 (10.8)
0.1-0.49	10 (27.0)	3 (10.7)	13 (20.0)
0.05-0.09	2 (5.4)	1 (3.6)	3 (4.6)
0.02-0.049	3 (8.1)	4 (3.9)	7 (10.8)
ND <sup>a</sup>	16 (43.2)	10 (35.7)	26 (40.0)
Total	37	28	65

Values in parenthesis indicate percentage. <sup>a</sup>Not detected.

broiler birds in different regions of the World. Eleftheriadou *et al.* (2004) reported AF residues in 64.4% of 120 liver samples collected from West Thessaloniki (Greece) with highest value of 10.74 ng/g which was close to the corresponding value of the present study. Bintvihok and Davitiananda (2002) from Thailand reported 55.11% of 900 liver tissues of broiler birds contaminated with AF with a mean concentration of 0.6092±0.0994 ng/g. In Pakistan, Begum *et al.* (2001) reported 19-39 ng/g aflatoxin B1 levels in liver tissue of broiler birds at marketing stage. These levels were too high than those observed in the present study. These authors used thin layer chromatography compared with more sensitive HPLC-FD technique used in the present study for determination of aflatoxin B1.

In the present study the levels of AFB1 and total AF were determined separately in tissues of healthy and sick birds. No report from other regions described the aflatoxin residues in healthy and sick birds. Asim *et al.* (1990) from Pakistan reported 12.30-493.0 ng/g AFB1 in 74.55% of 110 morbid liver samples of poultry birds. These values were too high and so far, no author has reported such a

high residue levels in the healthy or morbid liver tissues of poultry birds.

Experimental studies conducted by different workers revealed aflatoxin residues in liver of broiler birds following administration of different dietary AF levels for variable periods. Micco *et al.* (1988) fed 50 ng/kg AFB<sub>1</sub> for 36 and 87 days and found 0.02 and 0.01 ng/g AFB<sub>1</sub> residues in liver after 36 and 64 days, respectively. Chen *et al.* (1984) fed 2057 ng/g AFB<sub>1</sub> and 1323 ng/g AFB<sub>2</sub> for 35 days and observed that mean AF residue in liver was less than 3 ng/g. Feeding of 3000 ng/g AFB<sub>1</sub> to boiler chicks for 7 days resulted in 0.15±0.09 ng/g AFB<sub>1</sub> in liver tissue (Bintvihok *et al.*, 2002). Zaghini *et al.* (2005) reported 4.13±1.95 AFB<sub>1</sub> residues in liver after feeding the birds on diet contaminated with 2500 ng/g AFB<sub>1</sub> levels for 28 days. Hussain *et al.* (2010) in an experimental study reported that feeding of AFB<sub>1</sub> from 1600 to 6400 ng/g to the broiler birds for 7 days resulted a maximum level of 6.97ng/g AFB<sub>1</sub> in liver tissue. These authors also reported a dose related increase of AFB<sub>1</sub> residues in liver.

The maximum tolerance levels (MTL) of AFB<sub>1</sub> and total AF residues in human foods in European community (EC) and many other countries is 2.0 and 4.0 ng/g, respectively (Anonymous, 2004a & b). In present study among healthy birds, only one (0.50%) liver sample had 2 ng/g AFB<sub>1</sub>. None of the liver samples of healthy birds had AF levels at or above 4 ng/g whereas 16 (24.62%) liver samples of sick birds had 4 ng/g or higher AF levels. The differences between AFB<sub>1</sub> or AF residue levels of liver tissue from healthy and sick birds suggested that livers having morphological gross abnormalities should not be used for human consumption.

Begum *et al.* (2001) from Pakistan in tissues of broiler chicks at market age reported 19-26 ng/g AFB<sub>1</sub> residues in kidneys. These residue levels in kidney were too high as compared with the residues of AFB<sub>1</sub> in kidney in the present study as well as in experimental studies reported by many authors. Experimental studies of aflatoxicosis by different workers reported the presence of aflatoxin residues in kidney. Chen *et al.* (1984) fed 2057 ng/g AFB<sub>1</sub> and 1323 ng/g AFB<sub>2</sub> for 35 days and reported the detection of combined aflatoxin residues in kidneys. Micco *et al.* (1988) fed 50 ng/kg AFB<sub>1</sub> for 36 and 87 days and found 0.05 ng/g and 0.06 ng/g AFB<sub>1</sub> residues in kidneys after 36 and 64 days, respectively. Our finding of AFB<sub>1</sub> and AF residue levels in kidneys of healthy broiler birds were in comparison with these reports. Kidney tissues of healthy birds have AFB<sub>1</sub> and total AF levels below the MTL for EC, however, 7.69 and 6.15 percent of the sick birds had AFB<sub>1</sub> and AF residues above the specified MTL.

The presence of aflatoxin residues in muscles is most important from the human health perspective because muscles are the largest edible component of broiler birds. In the present study AFB<sub>1</sub> and AF residue levels in muscles of broiler birds were 0.02-1.99 and 0.02-2.99 ng/g, respectively. Eleftheriadou *et al.* (2004) from Greece reported that aflatoxin residues were detected in 40.8% samples and that the highest value was 7.6 ng/g which was higher than that observed in present study (1.85 ng/g). Bintvihok and Davitiananda (2002) determined aflatoxin residues in 450 samples of muscle

tissues of broiler birds in Thailand and stated that 96 (21.33 %) samples had mean value of 0.0451 ng/g AF. In present study, 84.21% muscle samples of healthy birds contained aflatoxin with a mean value of 0.15 ng/g which was higher than that reported by Bintvihok and Davitiananda (2002). Begum *et al.* (2001) reported that muscle samples collected from market age birds contained 19 ng/g AFB<sub>1</sub> residues. However, no other author has reported such a high values of AFB<sub>1</sub> residues in filed or experimental studies.

Many workers reported the presence of aflatoxin residues in the muscles tissues after feeding the diet having different AFB<sub>1</sub> levels for variable time periods (Chen *et al.*, 1984; Micco *et al.*, 1988; Bintvihok *et al.*, 2002; Zaghini *et al.*, 2005). Hussain *et al.* (2010) reported maximum residues in the muscles as 0.22-3.27 ng/g after feeding on diet containing up to 6400 ng/g AFB<sub>1</sub>. In present study highest residue levels of AFB<sub>1</sub> and AF were 1.85 and 2.196 ng/g, respectively suggesting that the muscle component of broiler meat may not pose aflatoxin associated health hazard to human population.

In conclusion the AFB<sub>1</sub> levels in the muscles and kidneys of healthy and sick broiler birds at market age was found below 2.0 ng/g, hence, safe for human consumption. The AFB<sub>1</sub> levels in liver tissue of 23.0% sick birds was above 2.0 ng/g hence liver showing gross morphological changes should not be allowed for human consumption.

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