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RESEARCH ARTICLE

Determination of aflatoxin B_1 in finished poultry feed samples collected from different poultry farms and markets of Lahore, Pakistan

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ABSTRACT

The present study was designed to determine the levels of aflatoxin B_1 (AFB_1) in the poultry finished feed samples collected from different poultry farms and local markets of Lahore, Pakistan. This study was conducted from July 2009 to June 2012 with each year divided into three periods i.e. July-October (hot and humid), November-February (winter) and March-June (moderate). During each period 80 samples were analyzed by competitive direct-Enzyme Linked Immuno-sorbent assay (CD-ELISA) constituting a total of 720 samples throughout the study. The levels of AFB_1 in poultry feed samples were highest during rainy seasons (48.2±20.0, 51.6±22.6 and 46.0±19.8 µg/kg) followed by Mar-Jun (29.9±10.4, 27.2±9.72 and 28.8±13.1 µg/kg) and Nov-Feb (19.7±6.30, 16.3±6.76 and 17.1±6.20 µg/kg). The levels were below maximum tolerable levels (MTL) for poultry as recommended by US-Food and Drug Administration (FDA) i.e. 20µg/kg during winter seasons only. The highest level during this study was 119.2µg/kg in Jul-Oct (2010-11). Percentage of samples below MTL was minimum during rainy season and at the peak during winter season confirming a high production of AFB_1 in stored feed during rainy season compared to other seasons. Poultry feed becomes highly contaminated with AFB_1 during rainy season due to high humidity and hot atmosphere which gives best favorable conditions for the growth of different storage fungi. This is the first most extensive study of levels of AFB_1 from poultry finished feed samples collected from different areas of Lahore (Pakistan).

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INTRODUCTION

Poultry industry plays an important role in the economy of Pakistan (Anonymous 2012). Although the demand of poultry meat is increasing day and day still this industry is facing some of the major problems like bacterial, viral, metabolic disorders and mycotoxicosis. Mycotoxicosis is considered to be the second most alarming issue in the poultry industry after increased poultry feed prices (Abidin et al 2011). Up till now, more than 300 chemically different mycotoxins have been identified but ochratoxins and aflatoxins are considered to be the most important ones (Perrone et al 2007, Abidin et al 2011).

Discussing about aflatoxins, many of toxigenic fungal species are being involved in the production of aflatoxins but the most important producers are *Aspergillus flavus* and *A. parasiticus* (Abidin et al 2011). 20 different types of aflatoxins have been identified like AFB_1, AFB_2, AFG_1, AFG_2 etc but among all these, AFB_1 is most toxic and important (Shareef et al 2008, Shi et al 2009, Mushtaq et al 2012). Generally, it causes hepatotoxicity, carcinogenicity, neurotoxicity, reproductive and developmental toxicity and coagulation abnormalities (Carolynoles et al 2006). It causes deleterious effects in poultry in the form of immunosuppression which might lead to the death also (Sravanan et al 2006). It also leads to poor feed conversion ratio (FCR), reduced body weight gain, listlessness and anorexia in broilers (Leeson et al 1995).

Aflatoxins present in the feed have a tendency to infiltrate most of the body tissue, muscles and fat depots

as residues and when these edible tissues/muscles are consumed by humans being become a potent source of contamination in human population. According to the available literature, a very little data is available regarding the determination of AFB_1 in poultry finished feed samples in Lahore Pakistan and in this data, study was conducted on country level collecting samples from all over the country and very few samples were collected from Lahore Pakistan (Hanif et al 2005, Khan et al 2011).

Lahore, being 25th largest urban population area of the world and presence of AFB_1 in poultry feed might be a great risk to a large number of population consuming poultry products fed on such contaminated feed. So the present study was designed to determine the levels of AFB_1 in poultry finished feed collected from poultry farms and markets of Lahore Pakistan and to investigate the percentage of samples above the maximum tolerable limits during different seasons.

MATERIALS AND METHODS

Study area

Lahore is the 2nd largest city of Pakistan with latitude 31°15′-31°45′ North, longitude 74°01′-74°39′ East and altitude of 217 meters. According to 2010 estimate made by government the population of Lahore is approximately 10 million. The weather condition of Lahore becomes very hot during summer which starts from May and ends at September. This weather becomes hot and humid during rainy season starting from mid July and ending at mid October while winter season starts after mid October and ends at mid February.

Samples collection

The study was carried out over a period of 3 years from July 2009 to June 2012. Samples were collected from different poultry farms and local markets of Lahore. The whole year was divided in to 3 periods i.e. July-October (hot and humid), November-February (winter) and March-June (moderate) and in each period 80 samples were collected constituting a total of 720 samples. From each feed lot 100 grams of finished poultry feed sample was collected in a sterile plastic bag, labeled and placed at 4°C till further use.

Quantification of aflatoxin B_1 from poultry feed samples

Competitive direct Enzyme Linked Immuno-sorbent Assay (CD-ELISA) was used for the quantitative analysis of aflatoxin B_1 according to the method described by Niaz et al (2012) with a few modifications. Briefly described, sample was ground to the particle size of fine instant coffee. 100 mL of extraction solvent (70% methanol) was added in 20 grams ground feed sample (sample to extraction solvent ration was 1:5). The mixture was blended for a minimum of 2 minutes and filtered through a Whatman # 1 filter paper and filtrate was used for further analysis. CD-ELISA was performed using commercially available aflatoxin kit (Unitox®, Affinitech Ltd., Product code AFL-T-0050, Suite 2, Bentonville, AR, 72712). Tetramethylbenzidine (TMB) was used as substrate for color development (blue color). In final step, reaction was stopped changing the color from blue to yellow by stopping solution and the intensity of color was measured by ELISA reader (Bio-Tek ELX-800TM). The intensity of color was inversely proportional to aflatoxin concentration in feed samples.

Data analysis

Mean along with standard deviation (Mean±SD) was calculated during each period (n=80) along with median and maximum levels of AFB1 (µg/kg).

RESULTS

Levels of AFB_1 during each seasonal period

Mean levels along with standard deviation (Mean±SD), median and maximum levels of AFB_1 quantified during each period have been shown in Table 1. The limit of detection (LOD) in this method was 1 µg/kg and all samples (n=720) of poultry finished feed were found positive for AFB_1.

Table 1: Mean levels of AFB_1 (Mean±SD) along with median and maximum levels of AFB_1 detected from poultry finished feed samples during each period.

Year	Period	AFB1 µg/kg	Median–Maximum (µg/kg)
2009-10	Jul-Oct	48.2±20.0	42.2-101
	Nov-Feb	19.7±6.30	19.5-31.1
	Mar-Jun	29.9±10.4	29.6-58.9
2010-11	Jul-Oct	51.6±22.6	44.8-119
	Nov-Feb	16.3±6.76	15.1-41.2
	Mar-Jun	27.2±9.72	26.5-47.1
2011-12	Jul-Oct	46.0±19.8	42.5-109
	Nov-Feb	17.1±6.20	16.4-39.7
	Mar-Jun	28.8±13.1	26.6-84.2

During 2009-10; Mean±SD, median and maximum levels of AFB_1 during Jul-Oct were 48.2±20.0, 42.2 and 101µg/kg respectively; while these levels were 19.7±6.30, 19.5 and 31.1µg/kg respectively during Nov-Feb. During Mar-Jun, these levels were in between those detected in other two periods of the year (29.9±10.4, 29.6 and 58.9µg/kg respectively) in all 80 samples analyzed during each seasonal period.

During 2010-11; Mean±SD, median and maximum levels of AFB_1 during Jul-Oct were 51.6±22.6, 44.8 and 119µg/kg respectively. These levels were 16.3±6.76, 15.1 and 41.2µg/kg respectively during Nov-Feb while during Mar-Jun, these levels were in between those detected in other two periods of the year (27.2±9.72, 26.5 and 47.1µg/kg respectively) in all 80 samples analyzed during each time period.

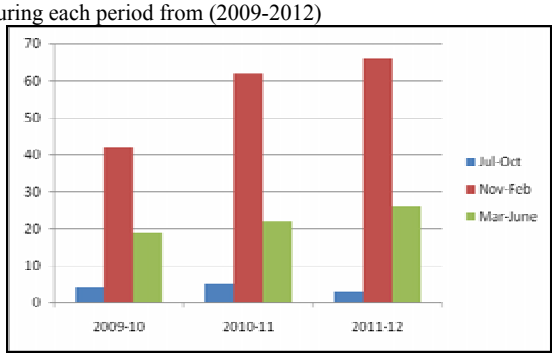
During 2011-12; Mean±SD, median and maximum levels of AFB_1 during Jul-Oct were 46.0±19.8, 42.5 and 109µg/kg respectively however; these levels were 17.1±6.20, 16.4 and 39.7µg/kg respectively during Nov-Feb. On the other hand these levels were in between those detected in other two periods of the year (28.8±13.1, 26.6 and 84.2µg/kg respectively) in Mar-Jun from all 80 samples analyzed during each seasonal period.

Number of samples below maximum tolerable levels

Graph 1 presents the number of samples below MTL during each period of the study. The number of samples below MTL for chicks (20 ppb as recommended by FDA) was highest during Nov-Feb in all three years when the

environmental temperature was low in Lahore Pakistan. During this period, the number of samples below MTL was highest (n=80) during 2011-12 (66) followed by 2010-11 (62) and 2009-10 (42). On the other hand minimum number of samples was below MTL i.e. 5 during 2010-11 followed by 4 during 2009-10 and 3 during 2011-12. However; in moderate season (Mar-Jun), this number was moderate i.e. 26 during 2011-12 followed by 22 during 2010-11 and 19 during 2009-10.

Graph 1: Number of samples below maximum tolerable levels during each period from (2009-2012)



*****(Graph shows the number of samples below maximum tolerable levels as recommended US-FDA for whole world which 20 ppb (FAO, 2004)

DISCUSSION

Mycotoxins are small non-immunogenic molecules commonly known as heptans which mean that they cannot produce an immunogenic response alone. These specific heptans are being conjugated with different carrier proteins making them immunogenic and hence production of antibodies can be carried out against different regions of macromolecules including heptans. Such antibodies are used to produce simple sensitive and specific ELISA and radio-immuno assay (RIA) etc. These assays are less expensive than any other analytical method used for detection of mycotoxins and LOD also increases many times in these methods (Abidin and Khatoon 2012). LOD in our study was 1 ppb.

Mycotoxins pose greater economical damages in the poultry industry and chicks are highly susceptible to pathological alterations induced by aflatoxins (Anjum et al 2012). In this study, poultry finished feed samples collected from different poultry farms and markets of Lahore Pakistan were analyzed for the presence of AFB_1 during different seasons i.e. Jul-Oct (hot and humid), Nov-Feb (winter) and Mar-Jun (moderate). It was noticed that levels were far beyond the maximum tolerable limits for chicks during hot and humid season. It might be due to improper storage of the feed resulting into the growth of storage fungi and production of mycotoxins within the feed. Many toxigenic fungi infect the crops during pre-harvest and harvest stages. These fungi remain as such and when they get favorable conditions (hot and humid environment) they grow rapidly and produce different mycotoxins (Abidin and Khatoon 2012). Similar to our results, Rashid et al (2012) reported high levels of AFB_1 in poultry finished feed samples collected from Quetta Pakistan in hot and humid environment while Anjum et al (2012) reported high levels of AFB_1 in poultry feed ingredients collected from Rawalpindi Pakistan during July-August. Discussing about other countries, Okoli et al (2006) from Nigeria and Tangendjaja et al (2008) from Indonesia reported high levels of AFB_1 in poultry feed ingredients during rainy seasons. However; contrary to our study, Bhatti et al (2001) reported that there was not any correlation between the month of study and the levels found in poultry feed ingredients.

During the winter season (Nov-Feb), relatively lower levels of AFB_1 were detected in feed as compared to other periods. Interestingly, the mean levels during this period were lower than the maximum tolerable levels as recommended by FDA which is 20µg/kg. This might be due to improper growth of storage fungi present within the feed lot and in consequence low production of AFB_1 within feed leading to low detection levels in winter season. Similarly lower levels of AFB_1 in poultry feed were detected in Quetta Pakistan by Rashid et al (2012) during winter months. During moderate weather (Mar-Jun) the mean levels were in between those found in rainy and cold weathers. Though the mean levels were higher than maximum tolerable levels yet these were less than the mean levels detected during rainy season. During this study the maximum level found was 119.21 ppb throughout 3 years study. Similarly Hanif et al., (2005) reported a maximum level of 120 ppb in poultry finished feed from a total of 182 samples collected from all over Pakistan.

US- Food and Drug Administration (FDA) recommend 20µg/kg to be the worldwide range of maximum tolerable limits /permissible levels for poultry (Aravind et al 2003, FAO 2004, Azab et al 2005). During our study, minimum percentage of samples was below MTL during hot and humid environments whereas maximum percentage of samples was below MTL during winter. However; during Mar-Jun, the percentage of samples below MTL was in between that of what observed during rainy and winter seasons. This is due to the fact that minimum amount of AFB_1 was produced in the feed during winter season as this season does not positively support the growth of stored toxigenic fungi within feed. But as the weather became hot, the levels of AFB_1 in feed samples started increasing and these levels became maximum as soon as the weather became hot and humid.

Aflatoxin present in feed causes hepatomegaly, fatty change in liver, periportal fibrosis, hyperplasia of bile duct etc. Birds exposed to aflatoxins become immunosuppressive and prone to many other diseases (Espada et al 1992, Ibrahim et al 2000). Proper prevention strategies should be adapted to avoid the entry of mycotoxins in the feed. Once mycotoxins get entry into poultry food chain their 100% removal is impossible. In such case, proper control strategies should be adapted to avoid the adverse effects of mycotoxins in poultry (Abidin and Khatoon, 2012).

Conclusion

It can be concluded from this study that poultry feed becomes highly contaminated with AFB_1 during hot and humid environment. This data clearly shows the current status of poultry feed used at the farms of Lahore Pakistan. Strict prevention strategies should be adapted to

prevent the entry of toxigenic fungi in the poultry food chain. Harvesting of the crops should be done when the moisture level becomes lower than 14% and these crops should be stored as soon as possible at a cool and dry place. This study recommends further investigation of levels of AFB_1 in the meat and tissues (liver, kidney etc) of birds at farm and market level in Lahore Pakistan.

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