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PREVALENCE OF AFLATOXIN B_1 IN FINISHED COMMERCIAL BROILER FEED FROM WEST CENTRAL PAKISTAN

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ABSTRACT

Aflatoxin contamination of poultry feed results in heavy economic loss and a potential risk for human being. Finished commercial broiler feed (n=96) samples collected from different broiler farms for the period June 2009 to May 2010 from south west Pakistan were analyzed for aflatoxin B_1(AfB_1) by using thin layer chromatography with a detection limit of 3µg/kg. Analysis revealed that 91.66% samples were positive with a range 10 to 166µg/kg and mean 47.64±2.55µg/kg. Highest level of AfB_1 was found in the month of September ranged from 42 to 166µg/kg with mean of 83.25±16.11µg/kg. Categorically 82.30% samples were above the permissible limit recommenced by United States Food and Drug Administration. A significant ($P<0.05$) effect of season on aflatoxin production was noted. The prevalence of AfB_1 may be attributed to poor harvesting technique, insect infestation, storage or environmental conditions.

Key words: aflatoxin, broiler, poultry feed, prevalence.

INTRODUCTION

Aflatoxins are a group of structurally related toxic compounds produced predominantly by certain strains of *Aspergillus flavus*, and *Aspergillus parasiticus* (Diaz *et al*., 2001). These molds are ubiquitous in nature and can grow and produce aflatoxins on any feed or grain used for poultry under favourable environmental conditions (Qazi and Fayyaz, 2006). United States (US) Food and Drug Administration (FDA) consider Aflatoxins to be an unavoidable contaminant of foods and have set regulatory levels. The permissible level for poultry is 20 ppb (Aravind *et al*., 2003; Azab *et al*., 2005). Aflatoxins are well intended the most prevalent, dangerous and studied mycotoxins (Krnjaja *et al*., 2008). Ingestion of feed contaminated with aflatoxins causes high morbidity and mortality resulting severe economic losses (Oguz *et al*., 2003; Diaz *et al*., 2008). Aflatoxins not only impairs weight gain, feed intake, feed conversion efficiency, egg production (Ortatatli *et al*., 2005), but also increases the susceptibility to environmental stress (Abdolamir *et al*., 2005) and severity of diseases like crop mycosis, salmonellosis, coccidiosis, aspergillosis, and Marek's disease (Ibrahim *et al*., 2000).

Although 18 different aflatoxins have been identified, only aflatoxins B_1, B_2, G_1, and G_2 are significant contaminant of agricultural commodities and pose a potential risk to poultry (Shareef *et al*., 2008). Among these, aflatoxin B_1 (AfB_1) is of great concern due to its carcinogenic, mutagenic, teratogenic (Shi *et al*., 2009), immunosuppressive (Hussain *et al*., 2010) and growth inhibitory effects (Khan *et al*., 2010). AfB_1 is usually the major aflatoxin produced by toxigenic strains (Qazi and Fyyaz, 2006).

The incidence and level of Aflatoxin contamination in food and feeds is continuously monitored and many surveys on natural occurrence are conducted every year in industrialized countries (Diaz *et al*., 2001). However, very little information is generated in developing countries. The inadequate technical, human and economic resources may be a reason for this lack of information (Cespedes and Diaz, 1997).

Keeping in view the importance of aflatoxins present study was planned to find out prevalence of aflatoxin B_1 in finished commercial broiler feed in and around Quetta district of Pakistan where no documented data is available. In addition to ascertain the prevalence of aflatoxin B_1, the effect of season on its concentration and categorization of the level of contamination following the general guidelines of FDA in finished commercial broiler feed was also studied.

MATERIALS AND METHODS

A base line study was conducted from June 2009 to May 2010 in and around Quetta district, situated between 30°15 North latitude and 66° 55 East longitudes, at an altitude of 1,675 meters in the North West part of Balochistan and at the West Central Pakistan. Quetta, is the capital city and the most populated district of Balochistan which is the largest province comprising the 45% of land mass of Pakistan. There are approximately 150 broiler farms with a flock rearing capacity of 1000 to 6000 birds.

Prevalence of aflatoxin B_1

Sampling, Processing and Analysis: The finished commercial broiler feed samples (n=96, 1kg each) were randomly collected on weekly basis from two government and private poultry farms in and around Quetta District. Grab method (Richard, 2000) was used to collect a fairly representative sample. The samples were collected in sterilized polythene bags and shifted to the toxicology laboratory of Center for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan (CASVAB, UoB) within 24 hours for further processing and analysis.

The collected samples were dried at 80°C for 3 hours, grounded, passed from sieve No. 20 and stored in a double layer paper bag enveloped with polythene bag at -18°C until they were analyzed (within one week of collection).

Samples were analyzed quantitatively by Thin Layer Chromatography (TLC) to determine AfB_1 contents. Extraction and cleanup process were carried out by using the method no. 993.17, while quantification was done by using the method no. 998.03 of AOAC (2000).

Effect of Season on AfB_1 concentration: The effect of season on AfB_1 concentration was studied by dividing the whole year into four quarters viz., quarter 1 (June to August), quarter 2 (September to November), quarter 3 (December to February) and quarter 4 (March to May) as suggested by Anjum (1990).

Categorization of level of contamination of AfB_1 Collected samples were categorized into low (<20µg/kg), medium (20-50µg/kg) and high (>50µg/kg) level of contamination of AfB_1 following the general guidelines of FDA (Hanif *et al*., 2006).

Statistical Analysis: Data revealed was subjected to statistical analysis using the One-Way Analysis of Variance as a Complete Randomized Design (CRD) using the general linear models procedure of statistical analysis software (SPSS 16 for windows). The means showing significant differences (P<0.05) were compared using Duncan's Multiple Range Test (DMR).

RESULTS

Prevalence of AfB_1 in finished commercial broiler feed samples (n=96) is presented in Table 1. It was found that 91.6% of the examined samples were contaminated with AfB_1. In the present study detection limit was 3µg/kg and the concentration of AfB_1 in positive samples ranged from 10 to 166µg/kg (mean 47.64±2.55µg/kg). The level of contamination was highest during the month of September (mean 83.25±16.11µg/kg) followed by October and August (mean 62.75±7.04 and 55.25±4.48µg/kg, respectively). The lowest level of contamination was found in the month of January that ranged from 10 to 30µg/kg with mean of 18.80±3.81 µg/kg. A significant difference in the means of AfB_1 was observed during months (P<0.05) Table 1. The effect of season on aflatoxin is depicted in Figure 1. The results revealed that highest concentration of AfB_1 was found in the quarter September to November (mean 66.04±7.03µg/kg), while it was lowest in the quarter December to February (mean 34.42±4.63µg/Kg). Following the general guidelines of FDA about contamination level of aflatoxins in poultry feed the results of the study reveals that 17.71% samples were categorize in low, 51.04% medium and 31.25% in high level of contamination (Table 2).

Table 1. Spectrum of Aflatoxin B_1 in broiler feed with a detection limit of 3µg/kg

Month	Total Samples	% +ve	Mean ± SEM for +ve samples (µg/Kg)	Range for +ve samples (µg/Kg)
Jun. 2009	8	87.5	40.42±7.05^{bc}	12-58
Jul. 2009	8	100	48.12±2.52^{bc}	39-58
Aug. 2009	8	100	55.25±4.48^{bc}	39-70
Sep. 2009	8	100	83.25±16.11^{a}	42-166
Oct. 2009	8	100	62.75±7.04^{b}	39-100
Nov. 2009	8	87.5	50.14±9.12^{bc}	17-83
Dec. 2009	8	100	55.37±3.35^{bc}	42-70
Jan. 2010	8	62.5	18.80±3.81^{d}	10-30
Feb. 2010	8	75	19.50±3.45^{d}	10-30
Mar. 2010	8	100	36.50±1.62^{cd}	30-42
Apr. 2010	8	87.5	41.71±1.82^{bc}	36-50
May 2010	8	100	40.75±1.58^{bc}	36-50
Overall	**96**	**91.66**	**47.64±2.55**	**10-166**

^{abcd} Values within same column followed by different superscript differ significantly (P<0.05)

Table 2. Categorization of Aflatoxin B_1 contamination in poultry feed with respect to General guidelines of FDA

Month	Total samples	Level of Contamination (%)		
		Low contamination (<20 µg/Kg)	Medium contamination (20-50 µg/Kg)	High contamination (>50 µg/Kg)
Jun. 2009	8	37.5	37.5	25
Jul. 2009	8	0	75	25
Aug. 2009	8	0	37.5	62.5
Sep. 2009	8	0	25	75
Oct. 2009	8	0	25	75
Nov. 2009	8	25	25	50
Dec. 2009	8	0	37.5	62.5
Jan. 2010	8	75	25	0
Feb. 2010	8	75	25	0
Mar. 2010	8	0	100	0
Apr. 2010	8	12.5	87.5	0
May 2010	8	0	100	0
Overall	96	17.71	51.04	31.25

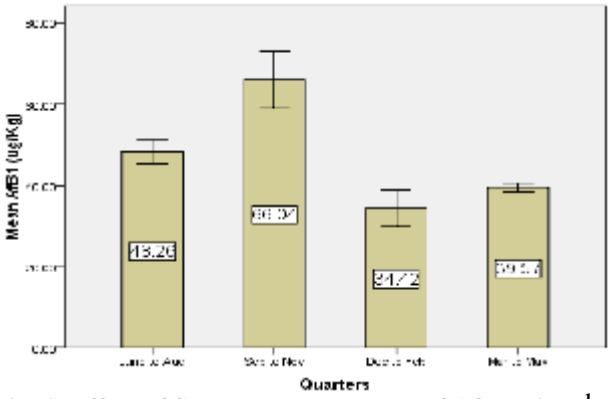

Fig. 1. Effect of Season on Prevalence of Aflatoxin B[1]

DISCUSSION

Contamination of commercial feed with aflatoxins is not an unusual phenomenon. Several workers (Cespedes and Diaz, 1997; Charoenpornsook and Kavisarasai, 2006; Shareef, 2010 and Moalem *et al*., 2010) from different parts of the world have reported incidence of aflatoxins form 40 to 92%. The maximum level of AfB_1 concentration observed in the current study was 166ug/kg. In other studies the highest values detected by Bhatti *et al*. (2001) Azab *et al*. (2005), Mngadi *et al*. (2008) and Alkhalaf *et al*. (2010) were 78, 120, 2000, and 70 µg/kg, respectively.

The differences in the prevalence and levels of contamination of aflatoxins in finished poultry feeds depend on number of factors such as the geographical area, climatic conditions (Dersjant-Li *et al*., 2003), type of raw material used, birds and insects damage to grains used in formula feed (Thompson and Henke, 2000), and storage conditions (Moss, 2002). Similarly sampling procedure and preparation of samples is an important tool that plays a vital role in the integrity of test result produced (Davis *et al*., 1980).

The season has a major impact on fungal growth and aflatoxin production. The aflatoxin production is influenced by environmental conditions like humidity and temperature (Hanif *et al.,* 2008, Sultana and Hanif, 2009). In the present study high levels of AfB_1 in the quarter September to November and low concentrations during December to February indicates the effect of season. In Pakistan, monsoon (hot season with heavy rain fall in which there is persistent high relative humidity and temperature) commences from July to September (Yunus *et al*., 2009) provide conditions ideal for the fungal growth ultimately resulting in increased production of aflatoxins.

According to the general guidelines of FDA about contamination level of aflatoxins in poultry feed the results of the present study are alarming that 82.30 % samples were above the permissible limits of 20ppb as recommended by USFDA (Ghahri, 2010). Such high level of aflatoxin in feed is not only hazardous to poultry because of direct exposure but also suggests a high risk to human health with the possibility of indirect exposure through contaminated meat, eggs and other poultry products and by products (Maqbool *et al*., 2004; Bintvihok and Kositcharoenkul, 2006).

The present study clearly describes the existing status of aflatoxin B_1 contamination of finished commercial broiler feed in Quetta. This situation needs to be considered with strict surveillance and quality control of poultry feed.

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