



Observation of aflatoxins and substantial metals in some poultry feed

Arthur Sovi and Virgile Gnanguenon

Babol Noshirvani University of Technology

Abstract

Aflatoxins and heavy metals were investigated in some poultry feed samples used as starter, grower, developer, layer, rabbit feed and bran which obtained from the local market at Al-Qassim region, Saudi Arabia. The results indicated that different amounts of aflatoxins were found in the analyzed samples. They reached peak values of 70.6, 46.38 and 50.88 $\mu\text{g}/\text{kg}$ sample for aflatoxin B1, G1, and G2, respectively, however aflatoxin B2 was generally less than 2.0 $\mu\text{g}/\text{kg}$. The study showed that the levels of aflatoxins were generally below the permissible levels (100 - 200 $\mu\text{g}/\text{kg}$). The results, also indicated that different levels of lead, cadmium, chromium, cobalt, nickel, zinc, manganese, iron and copper were detected in all samples. They ranged from 0.10 - 3.21, 0.004 - 0.249, 0.14 - 1.82, 4.57 - 37.6, 9.77 - 42.93, 0.51 - 55.38, 0.43 - 10.20, 3.78 - 5.18, and 0.45 - 3.26 mg/kg for lead, cadmium, cobalt, zinc, manganese, iron, copper, chromium and nickel, respectively. The high levels of zinc, copper, manganese and iron may reflecting the deliberate addition of these metals to meet animal nutrient requirements. Cadmium levels were less than the permissible limit of 0.5 mg/kg in US feeds. Lead levels in most feed sample exceeded the permissible limit of < 1 mg/ kg in UK. However, they lower than the allowed lead content in feed ingredients according to the current official regulations (10 mg/ kg).

Keywords: Aflatoxin, heavy metals, feeds, poultry, monitoring.

INTRODUCTION

Aflatoxins and heavy metals are potential environmental contaminants with the capability of causing human health problems (Das, 1990; Bennett and Klich, 2003). Tracing of these contaminants in feeds are significant in poultry nutrition. During the last decades, the increasing demand of food and feed safety has stimulated research regarding the risk associated with consumption of food and feed contaminated by aflatoxins and/ or heavy metals. Aflatoxins are the most frequently found mycotoxins produced by fungi *Aspergillus flavus*, *Aspergillus parasiticus*, that cause liver damage in poultry and livestock. They lower the profitability of poultry production by decreased growth, feed conversion efficiency, egg production and break in immunity leading to heavy economic losses.

The four major types of the aflatoxins are called B1, B2, G1, and G2 based on their fluorescence under UV light (blue or green). Aflatoxin B1 is by far the most prevalent and the most potent natural carcinogen and is usually the major aflatoxin produced by toxigenic strains (Squire, 1981; Reddy and Waliyar, 2000). Survey of mycotoxins in different feedstuffs of plant origin were done all over the world by many investigators (Mirocha and Christensen, 1974; Ueno, 1977; Siame and Lovelace, 1989; Abdelhamid, 1990; Schollenberger et al., 2006; Wagacha and Muthomi, 2008). The fungal attack and production of aflatoxins may occur during pre-harvest or post-harvest, during storage and transportation of feed and at farm level itself like in feed troughs. High temperature and humidity are contribution factors that encourage fungal growth and aflatoxin production. A positive correlation

($r = 0.814$) was found to exist between the moisture and aflatoxin contents of the feed (Khan et al., 2005).

Heavy metals are among the major contaminants of food supply and may be considered the most important problem to our environment (Zaidi et al., 2005). Such a problem is getting more serious all over the world especially in developing countries. Heavy metals, in general, are not biodegradable, have long biological half-lives and have the potential for accumulation in the different body organs leading to unwanted side effects (Jarup, 2003; Sathawara et al., 2004). The extensive content of heavy metals such as lead and cadmium in food is associated with the etiology of a number of diseases, especially with cardiovascular, kidney, nervous as well as bone diseases (WHO, 1992, 1995; Steenland and Boffetta, 2000; Jarup, 2003). In addition, they are also implicated in causing carcinogenesis, mutagenesis and teratogenesis (IARC, 1993; Pitot and Dragan, 1996).

Heavy metal contamination may occur due to irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, harvesting process, storage and/or sale. On the other hand, zinc and copper are essential trace minerals required for many biological processes and they have a positive influence on live-stock growth and reproduction. Due to the low zinc and copper content in some homegrown feeds compared with recommendations and varying bioavailability, supplementation of these metals is necessary for most live-stock species, and they are commonly added as mineral supplements (NRC, 1980; EC, 2003a, b). When these nutrients are added above requirements, however, the animal may restrict undesired accumulation of zinc and copper in tissues by adaptation of absorption and excretion leading to an increase in the metals content of manure (Nicholson et al., 1999; McBride and Spiers, 2001).

As far as the feed ingredients and the compound feed for poultry are an integral part of the consumer's food chain, they need to be assessed as potential sources of heavy metal contamination. As quality of feed is the main determinant factor in successful poultry farming in Saudi Arabia, therefore, the present study is dealing with tracing of aflatoxins and heavy metals in some poultry feeds obtained from the local market at Al-Qassim region, Saudi Arabia.

MATERIALS AND METHODS

Chemicals

Analytical grade standards for aflatoxins B1, B2, G1, and G2, 99% purity, were purchased from WinLab Limited, UK. Methanol (HPLC grades) and the other solvents were purchased from BDH. Ultra-pure deionized water of 15 M Ω .cm resistivity was obtained from a water purification system (PURELAB Option-R, ELGA, UK). Heavy metals standard samples of lead, cadmium, chromium, cobalt, nickel, zinc, manganese, iron, and copper were obtained from J. B. Baker Inc. (Phillipsburg, NJ, USA), while solid phase extraction column (Waters SPETM, C₁₈, 500 mg per column) was purchased

from Waters, USA.

Poultry feeds

A total of 72 of poultry feed samples namely, Starter (for chicken starting growth stage, samples 1 - 4), Grower (for chicken growing stage, samples 5 - 8), Developer (for chicken developing stage, samples 9 - 12), Layer (for egg layering stage, samples 13 - 16), Rabbit Feed (samples 17 - 20) and Bran (samples 21 - 24), each 10 kg were obtained from the local market of Al-Qassim region. Four replicates for each sample were used.

Sample preparation

Sampling plan was carried out according to FAO (1993) by taking ten 1-Kg samples from the same lot of feeds, incorporated together and then divided into composite samples (1 kg, each). Sub samples (200 g, each) were taken at random from the composite samples and divided into two groups (100 g, each). The first group was used for the determination of aflatoxins and the second group was employed for the assay of heavy metals. Aflatoxins were analyzed in starter, grower, developer layer, rabbit feed and bran, while heavy metal contents were measured in starter, grower, developer layer and rabbit feed.

Chromatographic analysis of aflatoxins

Extraction procedure

Aflatoxins were analyzed according to the procedure of AOAC (2002) with slight modifications. Feed samples (4 × 25 g, each) were taken and shaken with 50 ml of methanol: water (80:20, v/v) plus 5 gram of sodium chloride for 24 h at room temperature. The mixture was filtered under vacuum through porcelain funnel, evaporated to 1 ml by gentle stream of nitrogen and then subjected to solid phase extraction (SPE). The cartridges were preconditioned with 2 × 3 ml of methanol: water (80:20, v/v), and slowly aspirated.

Extracts were loaded onto the Sep-Pak Vac 6cc (500 mg) C₁₈ cartridges and eluted with methanol (2 × 3 ml) under vacuum using a 20-port vacuum manifold at rate of 5 ml/ min into glass vials (10 ml). After elution, solvent had passed through the extraction column and the residue was forcibly removed from the column by vacuum aspiration under increased vacuum. The eluate was evaporated to dryness under gentle stream of nitrogen and then re-dissolved in 1 ml of methanol and subjected for HPLC analysis. Aliquots (3 × 10 g, each) of the tested samples were fortified with 50 ng/ μ l of aflatoxins B1, B2, G1, and G2 to determine the percentages of recovery. Fortified samples were extracted as previously described.

HPLC analysis

HPLC analysis was carried out in a Perkin Elmer-200 High Performance Chromatograph equipped with a degasser, quaternary LC pump model 2000Q/ 410, 20 μ l loop, with a Spheri-10 RP-18 column (25 cm × 4.6 mm i.d., 10 μ m) using a mobile phase of methanol : water (80:20, V/V) at a flow rate of 1.0 ml/ min and a LC200 UV detector. The ultraviolet detector was set at 360 nm. The Turbochrom Workstation Software package was used for instrument control, data acquisition, and data analysis.

The HPLC system was standardized on the same day as the samples were analyzed by injecting 20 μ l of standard solutions of freshly prepared aflatoxins B1, B2, G1 and G2 in methanol with concentrations ranging from 0.0 - 1.0 ng/ μ l. Areas under the peak versus concentrations were plotted and fit by simple linear

Table 1. Retention time (t_R), limit of detection (LOD) and limit of quantification (LOQ) for aflatoxins.

Compound	t_R , min	LOD $\mu\text{g/g}$	LOQ $\mu\text{g/g}$
Aflatoxin B1	2.27	0.010	0.030
Aflatoxin B2	2.53	0.002	0.007
Aflatoxin G1	4.02	0.003	0.011
Aflatoxin G2	3.01	0.010	0.033

HPLC analysis was carried out according to AOAC, 2002. LOD and LOQ are calculated according to Keith et al, 1983.

Table 2. Percent recovery of extractable aflatoxins from samples fortified with 50 ng μl .

Compound	% Recovery	RSD
Aflatoxin B1	68.20 \pm 2.30	3.37
Aflatoxin B2	95.80 \pm 5.10	5.32
Aflatoxin G1	89.24 \pm 1.40	1.57
Aflatoxin G2	70.28 \pm 1.40	1.99

Each value represents the mean for four replicates \pm S.D.

regression to obtain an equation for the standard curve. The amount of aflatoxins in each sample was thus calculated based on the slope of the standard curves.

Retention time (t_R), limits of detection (LOD) and quantification (LOQ) are presented in Table 1. The retention times for aflatoxins B1, B2, G1 and G2 were found to be 2.27, 2.53, 4.02 and 3.01 min, respectively.

Fortification study

The recovery experiments for aflatoxins were carried out at the level of 50 ng μl . The data indicated that the recovery percentages were ranged from 68.20 -95.80% with relative standard deviation (RSD) ranged from 1.57 - 5.32 as presented in Table 2.

Heavy metals determination

Metals were measured by using atomic absorption spectrometer (AAS, Shimadzu Model AA-6200, Kyoto, Japan), equipped with a hollow cathode lamp, a 10 cm long slot-burner head and air/acetylene flame. The operating conditions adjusted in the spectrometer were carried out according to the standard guidelines of the manufacture. The emission wavelength used, slit width, the correct coefficient for the calibration straight line, the working linear range and detection limit found for each metal are presented in Table 4.

Samples were processed for the analysis by the dry-ashing method. Samples were first dried in oven at 105°C for 24 h and then ground. The ground samples (5.0 g each) were placed in crucibles and few drops of concentrated nitric acid were added as ashing aid. Dry-ashing process was carried out in a muffle furnace by stepwise increase of the temperature up to 550°C and then left to ash at this temperature for 4 h (Crosby, 1977). The ash was left to cool and then rinsed with 1 M nitric acid. The ash suspension was filtered and the filtrate made up to the volume of 25 ml with 1 M nitric acid. Blank solutions were prepared under identical conditions and the average signal was subtracted from analytical signals of

samples.

Standards solutions were prepared by adequate dilution of a multi-element standard (1000 mg/L) obtained from J. B. Baker Inc. (Phillipsburg, NJ, USA). All solutions and dilutions were prepared with ultra pure deionized water (pH 7.0) of 15 M cm resistivity obtained from a water purification system (PURELAB Option-R, ELGA, UK). Standard curves for heavy metals using atomic absorption spectroscopy (AAS) were carried out using the amounts of elements versus the corresponding absorbance.

The recovery study of the analytical procedure was carried out by spiking and homogenizing several already analyzed samples with varied amounts of standard solutions of the metals. The spiked samples were processed for the analysis by the dry-ashing method and reanalyzed as described above. The recovery percentages for the tested metals were ranged from 68 - 100% with RSD of 0 - 6.03% (Table 5).

Statistical analysis

Data were calculated as mean \pm standard deviation (SD) analyzed using analysis of variance (ANOVA). Probability of 0.05 or less was considered significant. The statistical package of Costat Program (1986) was used for all chemometric calculations.

RESULTS

Aflatoxins level in poultry feeds

The amount of aflatoxin B1, B2, G1, and G2 observed in the present study were presented in Table 3. The results indicated that different amounts of the aflatoxin B1, G1, and G2 were found in samples. They reached peak values of 70.6, 46.38 and 50.88 $\mu\text{g/kg}$ sample for aflatoxins B1, G1, and G2, respectively. However, aflatoxin B2 was <2.0 $\mu\text{g/kg}$ in all analyzed samples.

Heavy metals in poultry feeds

The typical concentration levels of essential and contaminated heavy metals in different samples were determined using AAS and presented in Table 6. Zinc was found at higher levels in rabbit feed followed by developer, starter, grower and then layer. Layer was highly contaminated with Ni followed by rabbit feed, starter, developer and then grower. The highest levels of Cr was detected in rabbit feed (5.11 mg/kg) and the lowest levels was recorded with grower (3.92 mg/Kg). All samples were contaminated with lead with levels of 2.93, 3.05, 2.45, 0.80 and 0.23 mg/kg for starter, grower, developer, layer and rabbit feed, respectively. In case of Cd, the levels were ranged from 0.02 to 0.20 mg/Kg. Copper was found at higher levels rabbit feed, while the lowest level was obtained with layer. Also, the data illustrate that Co levels were ranged from 0.21 - 1.54 mg/Kg. Iron was found at high levels for all the tested samples (45.10 - 54 mg/Kg) except layer contained 0.83 mg/ Kg. Manganese was found at higher levels in developer (40.72 mg/Kg) followed by rabbit feed, starter,

Table 3. Concentration levels of aflatoxins in some poultry feeds.

Samples	Amounts of aflatoxin, µg/kg sample			
	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2
Starter				
1	< 10.0	< 2.0	22.58	< 10.0
2	< 10.0	< 2.0	26.30	< 10.0
3	< 10.0	< 2.0	44.90	< 10.0
4	< 10.0	< 2.0	< 3.0	< 10.0
Grower				
5	< 10.0	< 2.0	< 3.0	14.42
6	< 10.0	< 2.0	< 3.0	20.62
7	< 10.0	< 2.0	< 3.0	34.64
8	< 10.0	< 2.0	< 3.0	20.58
Developer				
9	< 10.0	< 2.0	3.24	< 10.0
10	< 10.0	< 2.0	19.42	< 10.0
11	< 10.0	< 2.0	11.72	< 10.0
12	< 10.0	< 2.0	26.88	< 10.0
Layer				
13	< 10.0	< 2.0	46.38	< 10.0
14	< 10.0	< 2.0	22.82	< 10.0
15	< 10.0	< 2.0	23.90	< 10.0
16	< 10.0	< 2.0	32.17	< 10.0
Rabbit feed				
17	61.48	< 2.0	< 3.0	24.76
18	< 10.0	< 2.0	< 3.0	50.88
19	70.60	< 2.0	< 3.0	49.06
20	< 10.0	< 2.0	< 3.0	36.84
Bran				
21	25.00	< 2.0	< 3.0	< 10.0
22	20.00	< 2.0	< 3.0	< 10.0
23	10.00	< 2.0	< 3.0	< 10.0
24	25.00	< 2.0	< 3.0	< 10.0

Each value represents the mean for four replicates.

grower and then layer.

DISCUSSION

Our findings are in agreement with Reddy and Waliyar (2000) who found that aflatoxin B1 is widely distributed in feed stuff. A vast majority of outbreaks in farm animals have been caused by aflatoxin, fumonisins and zearalenone and to a lesser extent by ochratoxin and ergot alkaloids. Production losses can occur even at low levels of exposure to mycotoxins in feed. A combination

of mycotoxins may pose a greater production loss than each of these mycotoxins separately. The economic losses have been associated in terms of reduced productivity, such as lowered egg production, reproductive effects, susceptibility to infections resulting in increased morbidity and finally mortality. Exposure of farm animals to mycotoxins through animal feed have in the past resulted in field outbreaks. The most farm animals affected by mycotoxins are poultry, swine, dairy cattle and horses. The actual cost of these losses has been estimated only for some outbreaks. A case study in India of an outbreak of aflatoxicosis in 11 465 layers and

Table 4. Standard conditions used in determination of different elements and their detection limits using atomic absorption spectrometer.

Element	Wavelength	Slit width (nm)	Current (mA)	Flow (L/min)	Limit of detection (mg/kg)	Limit of quantification (mg/kg)
Lead	217.0	0.7	12	2.0	0.010	0.030
Cadmium	228.8	0.7	8	1.8	0.004	0.013
Chromium	357.9	0.7	10	2.8	0.050	0.170
Iron	248.3	0.2	12	2.2	0.030	0.100
Cobalt	240.7	0.2	12	2.2	0.050	0.160
Nickel	232.0	0.2	12	2.2	0.040	0.150
Manganese	279.5	0.2	10	2.0	0.010	0.033
Zinc	213.9	0.7	8	2.0	0.005	0.018
Copper	324.7	0.7	6	1.8	0.020	0.070

Table 5. Percent recovery of heavy metals from samples fortified with 10 mg/kg.

Element	% Recovery \pm S.D	RSD
Copper	84.60 \pm 2.30	2.72
Lead	68.00 \pm 4.10	6.03
Cadmium	69.00 \pm 2.00	2.90
Iron	100.0 \pm 0.00	0.00
Cobalt	70.10 \pm 3.50	4.99
Manganese	100.0 \pm 0.00	0.00
Zinc	99.00 \pm 0.50	0.51
Nickel	81.00 \pm 4.10	5.06
Chromium	79.30 \pm 3.20	4.04

Each value represents the mean for four replicates \pm S.D.

5,000 pullets in a poultry farm revealed that an 18 day exposure of poultry to the contaminated feed containing 600 $\mu\text{g}/\text{kg}$ aflatoxin B1, contributed mainly from groundnut cake, resulted in a loss of about 10% of the initial investment (Prathapkumar et al., 1997). The major loss was observed to be due to a drop in egg production followed by mortality in birds and additional expenditure on the protein source. The balance was accounted for by medical and other miscellaneous expenditures.

Increased awareness and monitoring have led to fewer market outlets for grains containing mycotoxins. There are no official FDA tolerances for any mycotoxins. This means a zero tolerance. However, FDA has established an action level which permits grains or feedstuffs to be marketed in interstate commerce with up to 20 $\mu\text{g}/\text{kg}$ aflatoxin (US-FDA, 2001; GMP, 2005). At the present time, the tolerance for feed destined for market hogs is 200 and 100 $\mu\text{g}/\text{kg}$ for the breeding herd. Even though a tolerance level has been established, no "safe" level has been established for any mycotoxin in any diet (Diekman and Long, 1984; GMP, 2005). The potential for mycotoxins is reduced by timely grain harvest, drying for

1 - 2% below maximum moisture for storage (grain 14 - 15%), removal of all foreign material, cracked kernels, routine aeration of stored grains to prevent moisture accumulation, as well as weevil and temperature control in the grain (less than 80°F).

The use of fungal inhibitors, such as propionic acetic acid (1 - 2%) will help prevent fungal growth in grain and finished feed. The present study showed that the levels of aflatoxins are generally below the permissible levels (100: 200 ng/g). However, some of the tested samples support the growth of microflora and aflatoxins production with different levels. These differences could be attributed to that these samples in our study collected from the local market might be stored under different conditions. It is suggested that care must be exercised to avoid the poor conditions during the storage of feedstuffs.

Excessive application of low-quality fertilizers, pesticides, sewage sludge and other bio wastes has increased the concentrations of heavy metals in many agricultural soils worldwide above levels considered safe for food production (Fässler et al., 2010). The agricultural use of Hg-, As- and Pb-containing pesticides has been

Table 6. Concentration levels of heavy metals in some poultry feeds.

Samples	Concentrations of heavy metals (mg/kg)						
	Zn	Ni	Cr	Pb	Cd	Cu	Co
Starter							
1	29.65	2.44	4.07	3.11	0.147	8.55	1.14
2	31.08	2.88	4.18	2.50	0.221	7.75	0.99
3	31.14	3.03	4.39	3.18	0.249	8.54	1.32
4	30.26	3.26	4.33	ND	0.219	6.33	1.24
Mean ± SD	30.5 ± 0.71	2.90 ± 0.35	4.24 ± 0.15	2.93 ± 0.37	0.20 ± 0.04	7.79 ± 1.04	1.17 ± 0.14
Grower							
5	25.40	0.45	3.93	3.21	0.144	5.50	0.29
6	27.28	0.79	3.94	3.19	0.111	5.25	0.16
7	27.27	1.30	3.78	3.02	0.136	6.15	0.14
8	27.01	1.30	4.01	2.79	0.055	4.88	0.26
Mean ± SD	26.7 ± 0.90	0.96 ± 0.42	3.92 ± 0.10	3.05 ± 0.19	0.11 ± 0.04	5.45 ± 0.53	0.21 ± 0.07
Developer							
9	34.18	1.33	3.87	2.57	0.083	6.94	0.55
10	32.37	1.56	4.02	2.54	0.141	6.95	0.77
11	32.99	1.68	4.10	2.38	0.103	4.61	0.99
12	31.61	1.93	3.93	2.32	0.124	6.56	0.68
Mean ± SD	32.7 ± 1.09	1.63 ± 0.25	3.98 ± 0.10	2.45 ± 0.12	0.11 ± 0.03	6.27 ± 1.12	0.75 ± 0.19
Layer							
13	7.23	3.25	4.47	1.24	0.161	0.81	1.42
14	5.43	3.15	4.57	1.29	0.223	0.43	1.73
15	4.57	3.11	4.70	0.52	0.127	0.44	1.42
16	5.56	3.05	4.89	0.15	0.091	0.54	1.59
Mean ± SD	5.70 ± 1.11	3.14 ± 0.08	4.66 ± 0.18	0.80 ± 0.56	0.15 ± 0.06	0.56 ± 0.18	1.54 ± 0.15
Rabbit feed							
17	34.44	3.02	5.04	0.40	0.049	9.87	1.39
18	34.39	2.92	5.11	0.10	0.018	9.55	1.36
19	37.60	3.14	5.18	ND	ND	10.20	1.82
20	35.67	3.13	5.11	0.18	0.004	8.70	1.49
Mean ± SD	35.5 ± 1.50	3.05 ± 0.10	5.11 ± 0.06	0.23 ± 0.16	0.02 ± 0.02	9.58 ± 0.64	1.52 ± 0.21

ND: not determined. Each value represents the mean for four replicates.

totally prohibited in KSA and only a small number of approved pesticides contain other trace elements, of which Cu and Zn were the extensively used elements for pesticides in KSA. Copper is mainly used as CuSO₄ as a fungicide for fruits, while Zn is a minor constituent of some fungicides, such as mancozeb, that are applied to crops and vegetables. Heavy metals such as zinc, copper, iron, manganese, lead, chromium, cadmium, nickel and cobalt are potential bioaccumulative toxins as soils tend to act as long term sinks for these metals (Alloway, 1995). Although different heavy metals display a range of different properties and mobilities in the soil, losses are generally low and may occur through crop removal, leaching and soil erosion (Aldrich et al., 2002). The observed levels of zinc, copper, manganese and Iron may reflecting the deliberate addition of these metals to meet animal nutrient requirements.

Heavy metals with different levels were observed poultry feed in our study with lead being in higher concentration compared to cadmium. This is in accordance to Ona et al. (2006) where plants were found to be capable of absorbing extra lead from soil and that some plants naturally absorbed far more lead than others. Cadmium levels were found in all samples to be less than the permissible limit of 0.5 mg/kg in US feeds (NRC, 1980). Lead levels in most sample exceed the permissible limit of < 1 mg/kg in the United Kingdom (Nicholson, 1999), however, they are lower than the allowed lead content in feed ingredients according to the current official regulations (10 mg/kg) (Alexieva et al., 2007). Chromium was detected in all feed samples and was generally found in higher concentration than other contaminated heavy metals (that is, Ni, Co, Cd and Pb). In the current regulations (Act No 2001/2006) there are no maximum allowed concentrations of chromium or nickel for feed ingredients and compound feed. The maximum allowed limits for chromium concentrations in human foods that range between 0.1 and 0.5 mg/kg. However, the limits for the allowed nickel content in different human foods range between 0.1 - 8 mg/kg.

Conclusion

It is concluded that the present study showed that the levels of aflatoxins are generally below the permissible levels (100:200 ng/g). Thus, the consumption of average amounts of these feeds does not pose a health risk for the consumer. The potential for mycotoxins could be reduced by avoiding the poor conditions during the storage of feedstuffs. The present study provides additional data on heavy metals pollution in Saudi Arabia and also can help in risk assessment of consumer exposure to the expected heavy metals. It is therefore suggested that regular survey of aflatoxins and heavy metals should be done to evaluate whether any health risks from toxins exposure do exist and to protect the end user from food that might injure their health.

REFERENCES

- Abdelhamid AM (1990). Occurrence of some mycotoxins (aflatoxin, ochratoxin A, citrinin, zearalenone and vomitoxin) in various Egyptian feeds. *Arch. Tierernahr.* 40: 647-664.
- Alexieva D, Chobanova S, Ilchev A (2007). Study on the level of heavy metal contamination in feed materials and compound feed for pigs and poultry in Bulgaria. *Trakia J. Sci.* 5: 61-66.
- Aldrich AP, Kistler D, Sigg L. (2002). Speciation of Cu and Zn in drainage water from agricultural soils. *Environ. Sci. Technol.* 36: 4824-4830.
- Alloway BJ (1995). Soil processes and the behavior of metals. In: *Heavy metals in soils.*, 2nd Ed, B.J. Alloway (Ed), Kluwer Academic Publ., Dordrecht, The Netherlands pp. 11-37.
- AOAC International (2002). Natural toxins. In: P. Cunniff, *Official methods of analysis of AOAC international.* 17th Ed., Association of Official Analytical Chemists Gaithersburg, Maryland, USA.
- Bennett JW, Klich M (2003). Mycotoxins. *Clin. Microbiol. Rev.* 16: 497-516.
- Costat Program. Version 2, Cohort Software, Minneapolis, MN, USA, 1986.
- Crosby NT (1977). Determination of metals in foods. A review. *The Analyst* 102: 223-268.
- Das A (1990). Metal ion induced toxicity and detoxification by chelation therapy. In: *A Text Book on Medical Aspects of Bio-inorganic Chemistry*, 1st ed, CBS, Delhi pp. 17-58.
- Diekman MA, Long GG (1984). Mycotoxins and reproduction in swine. *Animal Nutr. Health* pp. 22-28.
- EC (European Commission) (2003). Opinion of the scientific committee on animal nutrition on the use of zinc in feedstuffs. European Commission, Health and Consumer Protection Directorate, Brussels, Belgium.
- FAO (Food and Agriculture Organization) (1993). Sampling Plans for Aflatoxin Analysis in Peanut and Corn. *FAO Food Nutr. Paper* 55: 75.
- Fässler E, Robinson BH, Stauffer W, Gupta SK, Papritz A, Schulin R (2010). Phytomanagement of metal-contaminated agricultural land using sunflower, maize and tobacco. *Agric. Ecosys. Environ.* 136: 49-58.
- GMP (2005). Regulations on Product Standards in the Animal Feed Sector, GMP14.
- IARC (1993). Cadmium and cadmium compounds. In: *Beryllium, Cadmium, Mercury and Exposure in the Glass Manufacturing Industry*, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. *Int. Agency for Res. on Cancer* 58: 119-237.
- Jarup L (2003). Hazards of heavy metal contamination. *Br. Med. Bull.* 68: 167-182.
- Khan MMH, Chowdhury B, Bhuiya MR, Rahim M (2005). Variation of aflatoxin level in different poultry feeds used in different poultry farms of Bangladesh round the year. *Int. J. Poult. Sci.* 4: 382-387.
- Keith LH, Crummett WB, Deegan J, Libby RA, Taylor JY, Wentler G (1983). Principles of environmental analysis. *Anal. Chem.* 55: 2210-2218.
- McBride M, Dpiers G (2001). Trace element contents of selected fertilizers and dairy manures as determined by ICP-MS. *Commun. Soil Sci. Plant Anal.* 32: 139-156.
- Mirocha CJ, Christensen CM (1974). Mycotoxins, *Purchase I.F.H.* (Ed), Elsevier pp. 129-148.
- Nicholson F, Chambers B, Williams J, Unwin R (1999). Heavy metal contents of livestock feeds and animal manures in England and Wales. *Bioresour. Technol.* 70: 23-31.
- NRC (1980). Mineral Tolerance of Domestic Animals. National Academy Press, Washington, DC.
- Ona LF, Alberto AM, Prudente JA, Sigua GC (2006). Levels of lead in urban soils from selected cities in a central region of the Philippines. *Environ. Sci. Pollut. Res.* 13(3): 177-183.

- Pitot CH, Dragan PY (1996). Chemical carcinogenesis, 5th ed. In: Casarett, Dolls (Eds.), Toxicology Int. Ed. McGraw Hill, New York pp. 201-260.
- Prathapkumar SH, Rao VS, Paramkishan RJ, Bhat RV (1997). Disease outbreak in laying hens arising from the consumption of fumonisin contaminated food. *Brit. Poult. Sci.* 38: 475-479.
- Reddy SV, Waliyar F (2000). Properties of Aflatoxin and It Producing Fungi. *Aflatoxin. Int. Crops Res. Inst. for the Semi-Arid Tropics.*
- Sathawara NG, Parikh DJ, Agarwal YK (2004). Essential heavy metals in environmental samples from western India. *Bull. Environ. Contam. Toxicol.* 73: 756-761.
- Schollenberger M, Muller HM, Ruffe M, Suchy S, Planck S, Drochner W (2006). Natural occurrence of 16 fusarium toxins in grains and feedstuffs of plant origin from Germany *Mycopathologia* 16: 43-52.
- Siame BA, Lovelace CEA (1989). Natural occurrence of zearalenone and trichothecenes toxins in maize-based animal feeds in Zambia. *J. Sci. Food Agric.* 49: 25-35.
- Steenland K, Boffetta P (2000). Lead and cancer in humans: Where are we now? *Am. J. Ind. Med.* 38: 295-299.
- Squire NP (1981). Ranking animal carcinogens: a proposed regulatory approach. *Sci.* 214: 877-880.
- Ueno Y (1977). Trichothecenes, overview address, In: *Mycotoxins in Human and Animal Health.* Rodricks JV, Hesselstine CW, Mehlman MA (Eds.), Pathotox Publishers. Part Forest South III, pp. 189-207.
- US-FDA, United States-Food and Drug Administration (2001). Guidance for Industry: Fumonisin levels in human foods and animal feeds. (www.cfsan.fda.gov/~dms/fumongu2.htm1); background paper in support of fumonisin levels in corn and corn products intended for human consumption (www.cfsan.fda.gov/~dms/fumonbg3.htm1); Washington, DC, Nov 9, 2001.
- Wagacha JM, Muthomi JW (2008). Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. *Int. J. Food Microbiol.* 124: 1-12.
- WHO (1992). Cadmium. *Environmental Health Criteria, Geneva.* Vol. 134.
- WHO (1995). Lead. *Environmental Health Criteria, Geneva.* Vol. 165.
- Zaidi MI, Asrar A, Mansoor A, Farooqui MA (2005). The heavy metal concentrations along roadside trees of Quetta and its effects on public health, *J. Appl. Sci.* 5: 708-711.