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Determination of Aflatoxin B_1 Levels of the Feedstuffs in Traditional and Semi-industrial Cattle Farms in Amol, Northern Iran

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ABSTRACT

The study was conducted in order to measure the levels of Aflatoxin B_1 (AFB_1) in feedstuff by ELISA used by some of cattle farms in Amol (northern Iran). Out of 96 samples at 45 traditional and 51 semi-industrial cattle farms ones (concentrated feed, beet pulp and cotton meal) were selected in random basis during 3 different seasons of winter, spring and summer 2009. Out of 45 traditional samples, 20 samples (44.4%) and out of 51 semi-industrial samples, 22 samples (43.1%) showed AFB_1 contamination more than the limit accepted by EU (5 μg kg^{-1}). The contamination levels in concentrated feed, beet pulp and cotton seed were 58.8, 43.7 and 26.7%, respectively. The contamination level in winter, spring and summer were 56.4, 38.7 and 34.4%, respectively. Feedstuffs should be tested regularly in terms of AFB_1 and if they were contaminated, it would be better not to use them.

Key words: Aflatoxin B_1, feedstuff, traditional cattle farm, semi-industrial cattle farm

INTRODUCTION

Aflatoxins (Afs) are a group of secondary metabolites which are produced by *Aspergillus flavus, A. parasiticus* and *A. nomius* fungi on different kinds of food and feed (Richard, 2007; D'Mello and MacDonald, 1997). Human and animals contact with these toxins via oral, inhaling and direct contact and these toxins have various disorders. The molecular weight of this toxins are as low as other mycotoxin and for this reason, they are non-immunogenic. The main kinds of Aflatoxin are B_1, B_2, G_1 and G_2 which Aflatoxin B_1 (AFB_1) is the most important and toxic. These toxins have toxic effects such as acute and chronic toxicity, teratogenicity, mutagenicity, immunosuppression and carcinogenicity in mammals (Binder *et al*., 2007). If the feedstuff is being contaminated with AFB_1, the toxin is metabolized inside livestock body and Aflatoxin M_1 (AFM_1) will enter into the milk (Iheshiulor *et al*., 2011; Deshpande, 2002). The European Union (EU) determined the permissible limit of AFB_1 in feedstuff 5 μg kg^{-1}. Iran also follows this limit (Richard, 2007; European Commission Directive, 2003).

Numerous countries were successful to achieve toxin levels control in food and feed by suitable methods but many countries such as Iran have not yet reached to the standard limits. Prabakaran and Dhanapal (2009) detected Aflatoxins in some poultry feed that was in Kampu (220 ppb for AFB_1 and 45 ppb for AFB_2) and in Saamai (15 ppb for AFB_1). In Serbia toxigenic fungi and mycotoxins were isolated from animal feed. The frequent incidence of animal feed contamination were *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp. Natural occurrence of ochratoxin A is more frequent than incidence of aflatoxins (Krnjaja *et al.*, 2009). In Ankara, Turkey the levels of AFB_1 were reported between 0.03-1.61 μg kg^{-1} in foodstuff and feedstuff (Baydar *et al.*, 2005). In South Africa, aflatoxins were detected in animal feeds in 17 samples with levels ranged between 0.8±0.2 and 156±8 μg kg^{-1} (ppb) (Mngadi *et al.*, 2008). In India 23 different species of *Penicillium* in poultry feeds and cattle feeds were isolated (Rao *et al.*, 2011). The AFB_1 in 19 animal maize samples were measured with contamination ranging from 0.90 to 32.30 μg kg^{-1} (Oruc *et al.*, 2006). The feedstuff samples were analyzed at European, Mediterranean and Asian supermarkets for mycotoxins. 33% of Asian feedstuffs were positive which mostly contained deoxynivalenol, zearalenone, fumonisin and aflatoxin (Binder *et al.*, 2007). During 2003 to 2004 fungi were isolated in cereal types, legumes, fatty seeds and animal feeds in Libya. The contaminated animal feeds (35%) were over than the diet (30.63%) (Attitalla *et al.*, 2010). In a study by El-Maraghy fungi were found in 25 samples of sheep, cattle and camel feedstuffs collected in Libya. *Aspergillus, Penicillium* and *Fusarium* were the most common genera in the three substrates tested (El-Maraghy, 1996). In Belgium AFB_1 was detected in cattle farms feed with contamination levels 1.2-3.6 μg kg^{-1} that was less than the limit accepted by EU (5 μg kg^{-1}) (Stroka *et al.*, 2003). In brewers grain used to feed dairy cattle *Aspergillus* was reported with the most frequently (42.5%), followed by *Penicillium, Mucor, Rhizopus* and *Fusarium* (Simas *et al.*, 2006). In some studies co-occurring mycotoxins and fungal contamination in animal feeds were found (Mngadi *et al.*, 2008).

In Northern Iran AFB_1 was found in animal food from 10.4 to 68.8% (Moalem *et al.*, 2010). Also, in the same area Aflatoxin M_1 (AFM_1) was found in 100% of the pasteurized yogurt and local yogurt samples in autumn 2009 with concentrations of 2.1-61.7 ng L^{-1} and 7-53 ng L^{-1}, respectively (Barjesteh *et al.*, 2010).

In Uganda was reported maize aflatoxin levels mean 13-0.7 ppb (Bigirwa *et al.*, 2006). Aflatoxins were detected in food in Jordan by ELISA with a contamination of 3% (Salem and Ahmad, 2010). *Fusarium* and *Aspergillus* were isolated at high levels in maize grains produced in Karnataka (India) for the post harvest (Sreenivasa *et al.*, 2011). The AFB_1 was detected in 45.3% of the maize samples that 12.3% of them exceeding 5 μg kg^{-1} (Matumba *et al.*, 2009). Lutfullah and Hussain (2012) analyzed total aflatoxins in rice (25%), broken rice (15%), wheat (20%), maize (40%) barley (20%) and sorghum (30%), while in red kidney beans they were (20%), split peas (27%), chick pea (10%), cow pea (20%) and soybean (15%). Soleimany reported the occurrence of mycotoxins in cereal samples in morocco; 70, 40, 25, 36, 19, 13, 16 and 16% for Aflatoxins, OTA, ZEA, DON, FB_1, FB_2, T2 and HT2-toxin, respectively (Soleimany *et al.*, 2012). In India AFB_1 in wheat was determined = 5 ppb in 40.3% of samples (Toteja *et al.*, 2006).

The study was performed in order to measure the levels of AFB_1 in feedstuff used by some of traditional and semi-industrial cattle farms in Amol (Northern Iran) and the results were compared with permitted limits.

MATERIALS AND METHODS

Samples: Ninety six samples were selected from 3 different feedstuff ingredients (34 concentrated feed, 32 beet pulp and 30 cotton meal) at 2 kinds of cattle farms (45 traditional and 51 semi-industrial) in random basis in Amol during 3 different seasons of winter, spring and summer 2009. Twenty to forty grams of each sample were taken and immediately entered bags.

Materials: For determining AFB_1 levels ELISA kit was used (RIDASCREEN AFB_1 30/15, R-Biopharm AG, Darmstadt, Germany).

Sample preparation: The samples after transferring to laboratory firstly were grinded then 5 g of grinded sample mixed with 25 cc of 70% methanol then were shaken for 3 min with hand or shaker. Obtained extracts were refined with filter paper Whatman No. 1 and 1 cc of refined solution was used to evaluate toxin levels (Aksoy *et al.*, 2009).

AFB_1 analysis by ELISA: Fifty microliter of this solution was poured into every micropipette ELISA well. At first, with pipettes, standard samples and conjugated solutions and anti-Aflatoxin antibodies, were added to each well. After the first incubation, using substrate in wells, its red color was converted into blue and by using the stop solution its blue color was changed into yellow. AFB_1 concentration and absorbance was read by ELISA Reader at 450 nm. Data were analyzed by ANOVA utilizing SPSS software package. The results were analyzed by comparing them with standard limits (Moalem *et al.*, 2010; Ozaslan *et al.*, 2011; Salem and Ahmad, 2010; Jayeola *et al.*, 2011).

RESULTS

Out of 96 samples, 42 samples (43.7%) showed contamination more than the limit accepted by EU (5 $\mu g\ kg^{-1}$). Three different kinds of feedstuffs concentrated feed, beet pulp and cotton seed revealed 58.8, 43.7 and 26.7% contamination rate, respectively (Table 1). The differences among concentrated feed and beet pulp ($p = 0.012$) and concentrated feed and cotton seed ($p = 0.01$) contamination were statistically significant (t-test) but beet pulp and cotton seed ($p>0.05$) contamination revealed no statistically significant difference. The contamination level between concentrates in winter, spring and summer were found 56.7, 54.4 and 54.4%, respectively (Fig. 1).

Table 1: The contamination levels of feedstuffs to AFB_1 according to the type of cattle farm

	Concentrated feed			Beet pulp			Cotton meal			Sum		
Feedstuffs cattle farm	N	P	%	N	P	%	N	P	%	N	P	%
Traditional	16	10	62.5	15	6	40.0	14	4	28.6	45	20	44.4
Semi-industrial	18	10	55.6	17	8	47.1	16	4	25.0	51	22	43.1
Sum	34	20	58.8	32	14	43.7	30	8	26.7	96	42	43.7

N: Number of samples, P: Positive (over than 5 $\mu g\ kg^{-1}$)

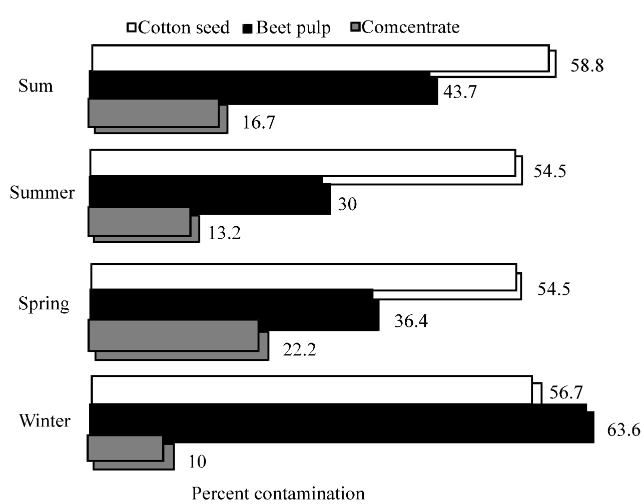

Fig. 1: The contamination levels of samples to AFB_1 according to season

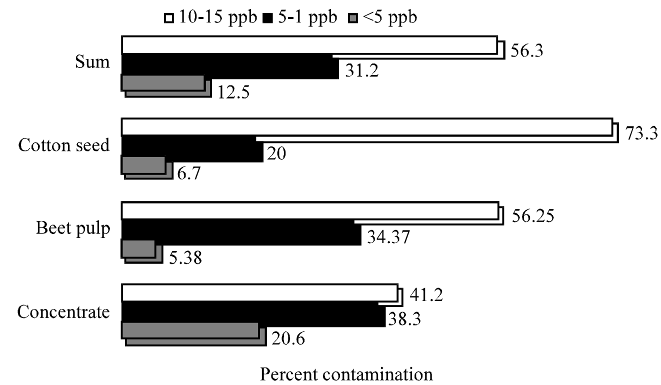

Fig. 2: The distribution of AFB_1 level in feedstuff (ppb = μg kg^{-1})

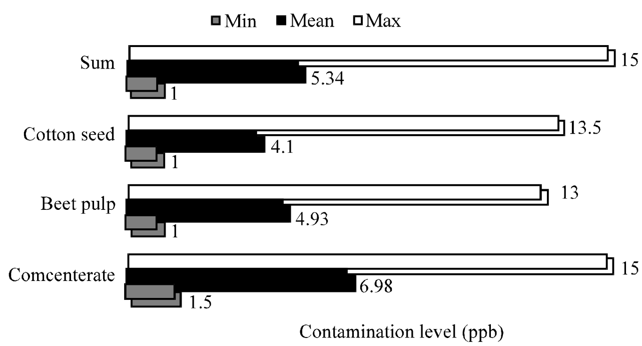

Fig. 3: The contamination levels of samples with AFB_1 according to the kinds of samples

The contamination between 10-15 ppb was 12.3% (Fig. 2). In general, max, min and mean of the concentration were 1, 15 and 5.3 μg kg^{-1}, respectively (Fig. 3). The difference for contamination level in winter and summer was significant ($p = 0.001$) but between spring and summer and winter and spring was not significant ($p>0.05$), AFB_1 contamination level in traditional, semi-industrial cattle farms were 44.4 and 43.1%, respectively and no significant difference ($p>0.05$) was found between them (Table 1).

DISCUSSION

Contamination to aflatoxins in different kinds of food and feed is inevitable. This toxin has various impacts on human and animals specialist carcinogenicity. Contaminated feedstuffs is AFB_1 too metabolized inside livestock body and AFM_1 will enter into the milk. With due attention to milk and other dairy products used by human vastly, it is necessary to monitor consumer, health especially sensitive persons such as children and the old. Therefore, much research is done for determining this toxin in food and feed (Hell and Mutegi, 2011; Farombi, 2006).

In Kenya detected 53.2% of Aflatoxin contamination at feedstuff over than 20 ppb (Lanyasunya *et al*., 2005). Reddy and Salleh in Malaysia collected animal feeds (corn grain) in order to determine AFB_1 by ELISA. AFB_1 were detected in 81.2% samples ranging from 1.0-135 μg kg^{-1} (Reddy and Salleh, 2011). Whereas our study 43.7% of samples was contaminated over than the limit accepted by EU (5 μg kg^{-1}) and 56.3% were under 5 μg kg^{-1}. In Sudan, 64.29% of the samples of animal feed were contaminated with Aflatoxins at average concentration of

130.63 μg kg^{-1} (Elzupir *et al*., 2009). Whereas, in our study were found max, min and mean of the concentration 1, 15 and 5.3 μg kg^{-1}, respectively. In Turkey AFB_1 and total aflatoxin contamination of the crop were reported 43 and 32%, respectively (Alptekin *et al*., 2009). The occurrence of AFB_1 at animal feed in Turkey by ELISA method was found to be 95% (Aksoy *et al*., 2009). In Southern Vietnam AFB_1 levels in feeds were 7.5 μg kg^{-1} (Thieu *et al*., 2008).

Charoenpornsook and Kavisarasai in Thailand found out 25 samples of feedstuff, 23 samples (92%) contained AFB_1 with mean concentrations 7.56 μg kg^{-1} (Charoenpornsook and Kavisarasai, 2006). In Kenya AFB_1 was isolated in 98.6% of animal feeds (Kangethe *et al*., 2007). In north Italy feedstuffs such as corn and concentrated feed were to have by ELISA. Out of 541 samples in 2004, 8.1% were contaminated with AFB_1 and in 2005 no sample was contaminated (Decastelh *et al*., 2007). In Bangladesh poultry feed samples were reported to have from 0 to 98 ppb AFB_1. The maximum of incidence was at June, July and August, while from November to February it was not detected in any feed. Similar to our study higher amount was observed in rainy season (Khan *et al*., 2005). In Iran AFB_1 was detected in 43.6% maize samples (Karami-Osboo *et al*., 2012). In this research unfortunately due to humidity in north of Iran, 43.7% of samples were over than standard limit. Due to harmful effects of this toxin on human body especially its carcinogenicity and immunosuppression in children; it is very hazardous for dairy products users, therefore it requires much attention (Shrif *et al*., 2008; Iheshiulor *et al*., 2011; Richard, 2007).

To decrease and reach to permissible limit of AFB_1 levels in human used livestock products and with due attention to this point that prevention is always better than remedy, only effective method is controlling contamination of feedstuffs and use of healthy feedstuffs. To minimize AFM_1 in milk and other dairy products and in order to ensure consumer health feedstuffs ingredients should be tested regularly in terms of AFB_1 and fungi growth should be prevented in order to have healthy feedstuffs for animal feeding.

CONCLUSION

Considering various effects of AFB_1 in human and livestock and the fact that a lot of livestock products such as milk and other dairy products used as main food for human, it is necessary to check AFB_1 levels in food and feed.

REFERENCES

Aksoy, A., D. Guvenc, O.H. Muglali, O. Yavuz and Y.K. Das, 2009. Occurrence of aflatoxin B1, T-2 toxin and zearalenone in compound animal feed. J. Anim. Vet. Adv., 8: 403-407.

Alptekin, Y., A.D. Duman and M.R. Akkaya, 2009. Identification of fungal genus and detection of aflatoxin level in second crop corn grain. J. Anim. Vet. Adv., 8: 1777-1779.

Attitalla, I.H., L.K.T. AL-Ani, M.A. Nasib., I.A.A. Balal, M. Zakaria, S.S.M. El-Maraghy and S.M.R. Karim, 2010. Screening of Fungi associated with commercial grains and animal feeds in Al-Bayda Governorate, Libya. World Applied Sci. J., 9: 746-756.

Barjesteh, M.H., I.G. Azizi and E. Noshfar, 2010. Occurrence of aflatoxin M1 in pasteurized and local yogurt in Mazandaran province (Northern Iran) using ELISA. Global Veterinaria, 4: 459-496.

Baydar, T., A.B. Engin, G. Girgin, S. Aydin and G. Sahin, 2005. Aflatoxin and Ochratoxin in several of commonly consumed retail ground samples in Ankara, Turkey. Ann. Agric. Environ. Med., 12: 193-197.

Bigirwa, G., G. Sseruwu, A.N. Kaaya, E. Adipala and S. Okanya, 2006. Fungal microflora causing maize ear rots in Uganda and associated aflatoxins. J. Boil. Sci., 6: 540-546.

Binder, E.M., L.M. Tan, L.J. Chin, J. Handl and J. Richard, 2007. Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. Anim. Feed Sci. Technol., 137: 265-282.

Charoenpornsook, K. and P. Kavisarasai, 2006. Mycotoxins in animal feedstuffs of Thailand. KMITL Sci. Technol. J., 6: 25-28.

D'Mello, J.P.F. and A.M.C. Macdonald, 1997. Mycotoxins. Anim. Feed Sci. Technol., 69: 155-166.

Decastelh, D., J. Lai, M. Gramaglia, A. Monaco and C. Nachtmann *et al*., 2007. Aflatoxins occurrence in milk and feed in Northern Italy during 2004-2005. Food Control, 18: 1263-1266.

Deshpande, S.S., 2002. Fungal Toxins. In: Handbook of Food Toxicology, Deshpande, S.S. (Ed.). Marcel Decker, New York, USA., pp: 387-456.

El-Maraghy, S.S., 1996. Fungal flora and aflatoxin contamination of feedstuff samples in Beida Governorate, Libya. Folia Microbiol. (Praha), 41: 53-60.

Elzupir, A.O., Y.M.H. Younis, M.H. Fadul and A.M. Elhussein, 2009. Determination of aflatoxins in animal feed in Khartoum State, Sudan. J. Anim. Vet. Adv., 8: 1000-1003.

European Commission Directive, 2003. Commission directive 2003/100/EC of 31 October 2003 amending annex 1 to directive 2002/32/EC of the European parliament and of the council on undesirable substances in animal feed. Off. J. Eur. Union, L285: 33-37.

Farombi, E.O., 2006. Aflatoxin contamination of foods in developing countries: Implications for hepatocellular carcinoma and chemo preventive strategies. Afr. J. Biotechnol., 5: 1-14.

Hell, K. and C. Mutegi, 2011. Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. Afr. J. Microbiol. Res., 5: 459-466.

Iheshiulor, O.O.M., B.O. Esonu, O.K. Chuwuka, A.A. Omede, I.C. Okoli and I.P. Ogbuewu, 2011. Effects of mycotoxins in animal nutrition: A review. Asian J. Animal Sci., 5: 19-33.

Jayeola, C.O., A. Oluwadun, L.E. Yahaya, L.N. Dongo, A.A. Ajao and F.C. Mokwunye, 2011. Comparative analysis of detecting ochratoxin A in cocoa powder samples using high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). Afr. J. Food Sci., 5: 513 -521.

Kangethe, E.K., G.M. Mibui, T.F. Randolph and. A.K. Langat, 2007. Prevalence of Aflatoxin M1 and B1 in milk and animal feeds from urban smallholder dairy production in Dagoretii division Nairobi Kenya. Est. Afr. Med. J., 84: 83-86.

Karami-Osboo, R., M. Mirabolfathy, R. Kamran, M. Shetab-Boushehri and S. Sarkari, 2012. Aflatoxin B1 in maize harvested over 3 years in Iran. Food Control., 23: 271-274.

Khan, M.M.H., B. Chowdhury, M.R.H. Bhuiya and M. Rahim, 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.

Krnjaja, V., J. Levic and S. Stankovic, 2009. Ubiquity of toxigenic fungi and mycotoxins in animal feeds in republic of Serbia. Biotechnol. Anim. Hus., 25: 477-491.

Lanyasunya, T.P., L.W. Wamae, H.H. Musa, O. Olowofeso and I.K. Lokwaleput, 2005. The risk of mycotoxins contamination of dairy feed and milk on smallholder dairy farms in Kenya. Pak. J. Nutr., 4: 162-169.

Lutfullah, G. and A. Hussain, 2012. Studies on contamination level of aflatoxins in some cereals and beans of Pakistan. Food Control., 23: 32-36.

Matumba, L., M. Monjerezi, E. Chirwa, D. Lakudzala and P. Mumba, 2009. Natural occurrence of AFB_1 in maize and effect of traditional maize flour production on AFB_1 reduction in Malawi. Afr. J. Food Sci., 3: 413-425.

Mngadi, P.T., R. Govinden and B. Odhav, 2008. Co-occurring mycotoxins in animal feeds. Afr. J. Biotechnol., 7: 2239-2243.

Moalem, S.H.H., I.G. Azizi and M. Azarmi, 2010. Prevalence of aflatoxin B_1 in feedstuffs in Northern Iran. Global Veterinaria, 4: 144-148.

Oruc, H.H., M. Cengiz and O. Kalkanli, 2006. Comparison of aflatoxin and fumonisin levels in maize grown in Turkey and imported from the USA. Anim. Feed Sci. Technol., 128: 337-341.

Ozaslan, M., I. Caliskan, I.H. Kilic and I.D. Karagoz, 2011. Application of the ELISA and HPLC test for detection of aflatoxin in Pistachio. Sci. Res. Essays, 6: 2913-2917.

Prabakaran, J.J. and S. Dhanapal, 2009. Analysis of proximate composition and aflatoxins of some poultry feeds. Asian J. Biotechnol., 1: 104-110.

Rao, V.K., P. Shilpa, S. Girisham and S.M. Reddy, 2011. Incidence of mycotoxigenic penicillia in feeds of Andhra Pradesh, India. Int. J. Biotechnol. Mol. Biol. Res., 2: 46-50.

Reddy, K.R.N. and B. Salleh, 2011. Co-occurrence of moulds and mycotoxins in corn grains used for animal feeds in Malaysia. J. Anim. Vet. Adv., 10: 668-673.

Richard, J.L., 2007. Some major mycotoxins and their mycotoxicosis: An overview. Int. J. Food Microbiol., 119: 3-10.

Salem, N.M. and R. Ahmad, 2010. Mycotoxins in food from Jordan: Preliminary survey. Food Control, 21: 1099-1103.

Shrif, S.O., E.E. Salama and M.A. Abdel-Wahhab, 2008. Mycotoxins and child health: The need for health risk assessment. Int. J. Hyg. Environ. Health. 10.1016/j.ijheh.2008.08.002

Simas M.M.S., M.B. Botura, B. Correa, M. abino, C.A. Mallmann, T.C.B.S.C. Bitencourt and M.J.M. Batatinha, 2006. Determination of fungal microbiota and mycotoxins in brewers grain used in dairy cattle feeding in the State of Bahia. Brazil. Food Control.

Soleimany, F., S. Jinap and F. Abas, 2012. Determination of mycotoxins in cereals by liquid chromatography tandem mass spectrometry. Food Chem., 130: 1055-1060.

Sreenivasa, M.Y., R.S. Dass, A.P.C. Raj and G.R. Janardhana, 2011. Mycological evaluation of Maize grains produced in Karnataka (India) for the post harvest fungal contamination. World Applied Sci. J., 13: 688-692.

Stroka, J., C. Von-Holst, E. Anklam and M. Reutter, 2003. Immunoaffinity column cleanup with liquid chromatography using post-column bromination for determination of aflatoxin B1 in cattle feed: Collaborative study. AOAC official method 2000.02. J. AOAC Int., 86: 1179-1186.

Thieu, N.Q., B. Ogle and H. Pettersson, 2008. Screening of aflatoxins and zearalenone in feedstuffs and complete feeds for pigs in Southern Vietnam. Trop. Anim. Health Prod., 40: 77-83.

Toteja, G.S., A. Mukherjee, S. Diwakar, P. Singh and B.N. Saxena *et al*., 2006. Aflatoxin B1 contamination in wheat grain samples collected from different geographical regions of India: A multicenter study. J. Food Protect., 69: 1463-1467.