Jundishapur Journal of Natural Pharmaceutical Products 2011; 6(1): 42-50

Jundishapur Journal of Natural Pharmaceutical Products

EVALUATION OF AFLATOXINS CONTAMINATION IN BABY FOOD SUPPLEMENTS (MAMANA & GHONCHEH)

Kalantari H[1], Kalantari GH[1], Nazari Khorasgani Z[1*]

[1]*Department of Pharmacology & Toxicology, School of Pharmacy, Ahvaz Jundishapour University of Medical Sciences, Ahvaz, Iran*

Received: February 2011 ***Accepted: May 2011***

Abstract

One of the most important sources of human nutrition is cereals such as rice, wheat, corn and barley. Baby food supplements mostly consist of cereals. If these products are improperly prepared, they may get contaminated with various aflatoxins such as AFB_1, AFB_2, AFG_1 and AFG_2. The purpose of this investigation was to determine the aflatoxins contamination of baby food supplements such as Mamana and Ghoncheh, which are widely used in Iranian markets. In this study 14 samples of Mamana and 15 samples of Ghoncheh were investigated for aflatoxin B_1, B2, and G1. According to the CB method, extraction was carried out and then qualitatively and quantitatively by TLC-Scanner aflatoxins B_1, B2 and G_1 were identified and measured. The results of this study showed that 2 of 15 samples of Mamana and 2 of 14 samples of Ghoncheh were contaminated with aflatoxin B_1 and B_2. (< 2ppb).

Key words:

Aflatoxins, Baby food suplment, Health.

Introduction

Most food borne illnesses are largely attributed to microbiological contamination (1-5). Not surprisingly, there has been a sharp increase in national public interest about microbial and chemical food safety recently. Mycotoxins have been implicated as causative agents of adverse health effects in humans and animals that have consumed fungus-infected agricultural products (4, 6-9). Aflatoxins are toxic bis furanocoumarin fungal metabolites produced primarily by some strains of Aspergillus (A.) Flavus and most strains of Aspergillus parasiticus, plus related species, A. nomius and A. Niger in/on foods and feed (10, 11). There are four major aflatoxins B_1, B_2, G_1, G_2 plus two additional metabolic products, M_1 and M_2 that are of significance as direct contaminants of food and feed(12). The aflatoxins M_1 and M_2 were first isolated from milk of lactating animals fed aflatoxins preparations; hence, the M designation(13). Aflatoxins B_2 and G_2 were established as the dihydroxy derivatives of B_1 and G_1. Whereas, M_1 is 4-hydroxy aflatoxin B_1 and aflatoxin M_2 is 4 - dihydroxy aflatoxin B_2. The B series contain a cyclopentenone ring, instead of a D lactone ring in the G series, this difference being responsible for higher toxicity of the B series. Fungal toxins found in certain agricultural crops have been causing adverse health effects in humans and animals since ancient times (14). Today, They are estimated to occur at significant levels in around a quarter of world's food supply (15-20).

*Email: Znazarikh@yahoo.com

The occurrence of aflatoxins is influenced by certain environmental factors; hence the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during preharvest, storage and/or processing periods (21-23). Since crops are not yet grown under sterile conditions, the probability of mold infection and mycotoxin contamination is ever present and the carcinogenic effect of aflatoxin is well recognized(5, 24). One of the most vulnerable part of population are children, due to, their physiology, lack of food diversity, and a higher consumption relative to their body. Therefore, the significance and potential health risk of any contaminant in foods consumed by infants is increased and special attention must be paid to this problem. So the levels of aflatoxins contamination in Mamana and Ghoncheh baby food supplements were investigated.

Materials and Methods

All solvents and chemicals were ACS grade and purchased from Merck and Sigma company agents in Iran. All glassware in this study, before use , were soaked in dilute sulfuric acid (2M) for several hours, then rinsed extensively with distilled water to remove all traces of acid (checked by using pH paper), because acid may cause loss of aflatoxins.

Fourteen and fifteen samples of Mamana and Ghoncheh baby food supplements were purchased form drug stores in the city of Shiraz in Iran, respectively during 2002-2003. In order to investigate the storage conditions and the effect of time after manufacturing, samples were collected from five different dates of production (3 sample of each of the 5 batch number). For preparation of stock standard solutions, to containers containing 1 mg of each dried Aflatoxin Standards (B1, B2 and G1) , 1ml of benzene – acetonitrile (98:2) solution was added (1µg/1µl). Working standard was prepared by dilution of 20 µl of each stock standards solution up to 20 ml into a 20 ml volumetric flask(1ng/1µl). 50 g of each prepared sample was weighed into 500 ml Erlenmeyer flask and mixed with 25g of diatomaceous earth, moistened with 25 ml of water, and thoroughly shaken to obtain a homogeneous blend. Then, 250 ml $CHCl_3$ was added to the whole and shaken vigorously for 30 min on a vibrating shaker. 50 ml of $CHCl_3$ extract was recovered by filtering through a folded filter paper and transferred into a chromatographic column packed with silica gel. The column was eluted at a maximum flow rate with 150 ml hexane followed by 150 ml anhydrous ether and discarded. Then, aflatoxins were separated by passing 150 ml methanol- chloroform (3:97). The elute concentrated to near dryness on a steam bath, quantitatively transferred to a vial with $CHCl_3$ and evaporated to dryness. Then 200 µl $CHCl_3$ was added to the vial. 40µl of dissolved residue in $CHCl_3$ and also 2 µl(2ppb) of mixed working standard solution of aflaoxins (B_1, B_2 and G_1) were spotted on a silical gel coated plate. On each T.L.C. plate, 6 spots of dissolved residue of investigated samples, 2 spots of working standard and one spot of recovery sample were placed and then developed with Choloroform- acetone (90:10). The T.L.C plate was removed, dried at room temperature and scanned with a T.L.C scanner equipped with a densitometer. Afloxins were identified by comparing their retention times obtained for the chromatograms of samples with those obtained for the chromatograms of standards (B1, B2 &G1) analyzed under identical conditions and quantified from fluorescent intensities on a thin layer of chromatograms. The recovery of method was 98%.

Results and Discussion

Results are shown in figures 1-7. Interest in mycotoxins is due primarily to (1) their adverse effects on human and animals, (2) their widespread and avoidable contamination of agricultural commodities, (3) their recalcitrance to degradation during milling or processing and heat resistance, and their economic losses associated with reducing efficiency of production and causing diseases in livestock, or jeopardizing the taste of cereal products due to contaminated mycotoxins in wheat or barley grains. Aspergillus flavus is common and widespread in nature and is most often found when certain grains are grown under stressful conditions such as drought(19).

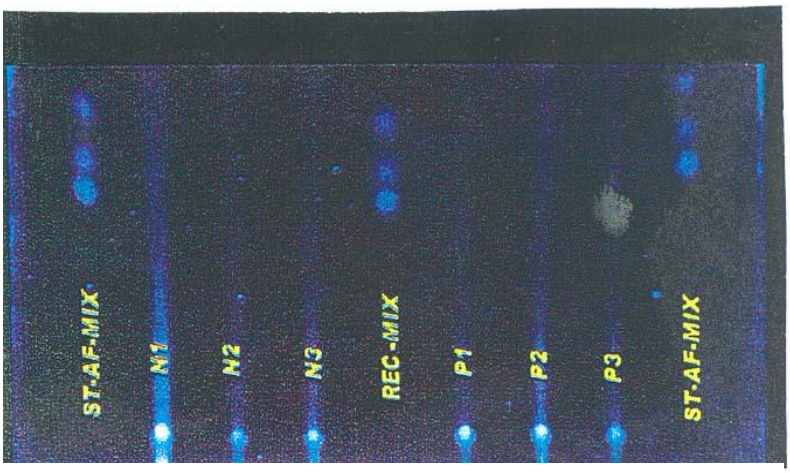

Fig. 1: florescent image of spotted samples on TLC no. 1
1: mixture std (B1,B2 and G1), 1' mixture standard for recovery, 2,3,4,5,6,7:spots of Ghoncheh baby food supplement samples.

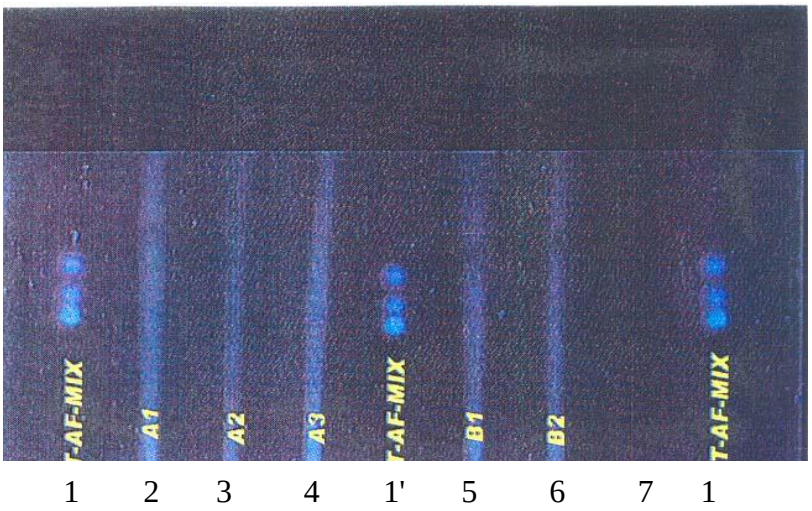

Fig. 2: florescent image of spotted samples on TLC no.2
1: mixture std (B1,B2 and G1), 1' mixture standard for recovery, 2,3,4,5,6:spots of Mamana baby food. supplement samples.

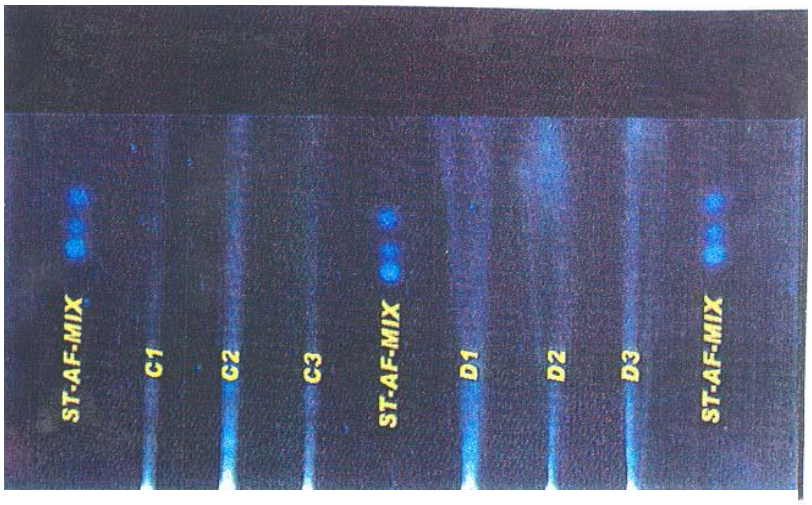

Fig. 3 : florescent image of spotted samples on TLC no.3
1: mixture std (B1,B2 and G1), 1' mixture standard for recovery, 2,3,4,5,6,7:spots of Mamana baby food supplement samples.

The mold occurs in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration and invades all types of organic substrates whenever and wherever the conditions are favorable for its growth. Favorable conditions include high moisture content and high temperature. Aflatoxins are potent, carcinogenic, mutagenic, teratogenic and immunosuppressive agents(25-27). The problem was first recognize following outbreak of Turkey " X" disease in the United Kingdom in 1960 (14).

Humans are exposed to aflatoxins by consuming foods contaminated with products of fungal growth. Such exposure is difficult to avoid because fungal growth in food is not easy to prevent and there is interest in the possible adverse effects resulting from long–term exposure to low levels of aflatoxins in food supply. In the USA, in 1964 an analysis was carried out on 78 samples of peanut farmers' stock from nine different regions (28). Aflatoxins levels varied form 0-91 µg/kg, but only two samples contained more than 50 µg/kg.

Baydar et al measured Aflatoxin B1, M1 and Ochratoxin A levels in infant formulae and baby foods marketed in Ankara,Turkey by enzyme-linked immunosorbent assay (ELISA) kits. For this purpose they collected 63 infant formulae, and baby foods randomly from pharmacies and supermarkets in the years of 2003 and 2004 in Ankara. According to results of their study the incidence of AFB1 contamination in these infant formulae and baby foods was very high, since 55 (87%) samples contained AFB1 above the detection limit, 0.025 ppb and were considered positive contamination. However, eight samples with AFB1 over the permissible level of 1 ppb were accepted for infant formulae and baby foods in Turkey. The average AFB1 level was found as 0.89 ± 1.10 ppb in 55 samples (29).

Paula. et al investigated the occurrence of Aflatoxins and Ochratoxin A in the 27 processed cereal-based foods (flours) and infant formulae (milk powder) available in the Portuguese market. 1 of 27 studied samples was contaminated with aflatoxin B1, contained a value of 0.009 µg AFB1/kg(30).

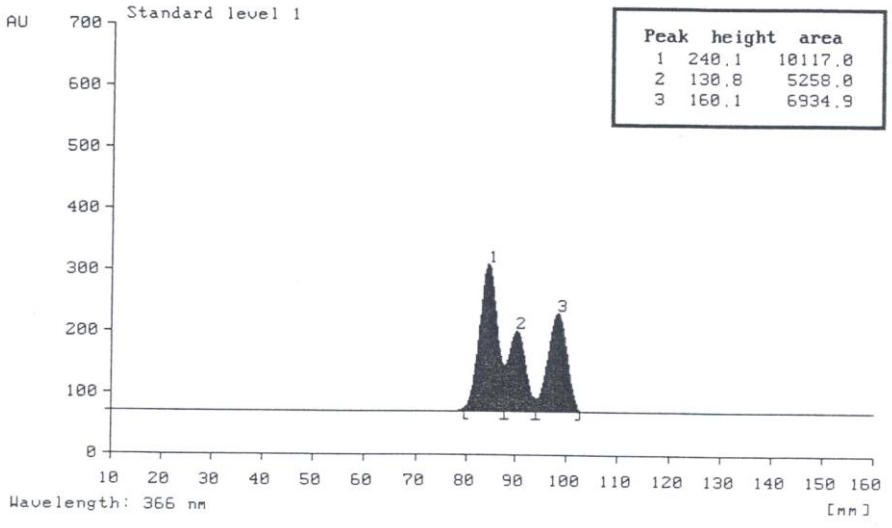

Fig. 4: data about mixture of Standards B_1, B_2 and G_1
1= aflatoxinB_1 (2ppb), 2 = aflatoxin B_2 (2pbb), and 3= aflatoxin G_1(2ppb).

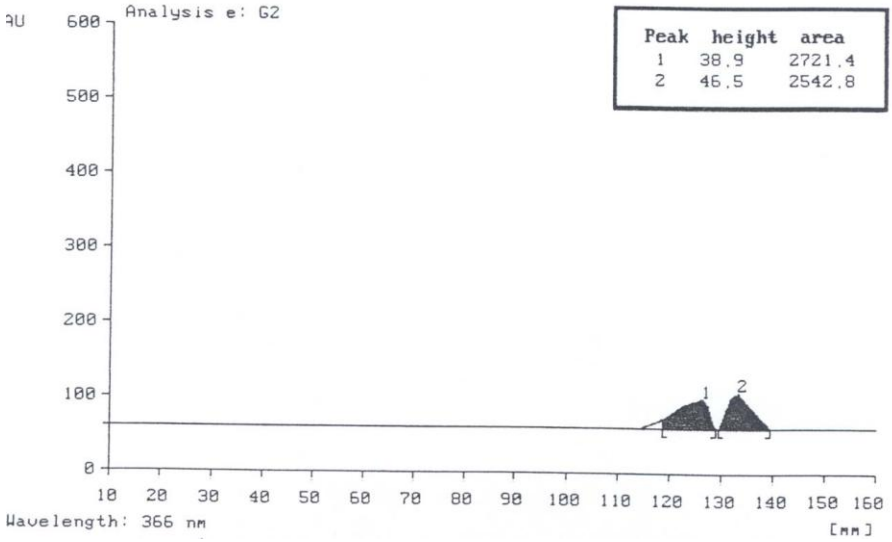

Fig. 5: data about contaminated Ghoncheh baby supplement with $B_1$and B_2
1= aflatoxinB_1 2 = aflatoxin B_2.

Many studies suggest that A. flavus may infect cereals in a particular maize, and produce aflatoxins(15, 16, 31, 32). Also, there is abundant evidence from a number of sources which suggest that seeds of wheat, barley, oats, maize, and rice are not invaded to any serious extent by storage molds before harvest [20]. Very little information is available regarding contamination of food and feed with aflatoxins in Iran. To determine whether Mamana and Ghoncheh baby food supplements were contaminated with aflatoxins, samples were collected from drug stores in the city of Shiraz in Iran. The results of this study showed that 2 of the 15 (13.33%) samples of Mamana and 2 of the 14(14.28%) samples of Ghoncheh were contaminated with aflatoxin B_1 and B_2. (< 2ppb). With regards to W.H.O reports, these amounts of contamination are below the maximum level permitted (4 µg/kg) and could be considered safe, but as they are used in baby food supplements, it is worthy to consider their safety. If these products are not properly prepared, transported or stored they may get contaminated which will be dangerous.

In developed countries, the risks to consumers are much lower than those in developing countries. This is partly due to the high standards of safety and quality adopted by the major food suppliers and retailers and partly because of relatively tight controls on these contaminants in these countries. Since there are certain chemical substances and biological agents incorporated into food and feeds at any stage of production up to the point of feeding, there is a need for collaboration between all parties involved in feed and animal production, to establish the linkage between any identified or potential hazard and the level of risk. Effective control of food and feedstuffs requires multidisciplinary input. Good agricultural practices and good storage practices can minimize the problem, but regular monitoring is necessary to check that consumers are not being exposed to unacceptable levels of these natural toxins.

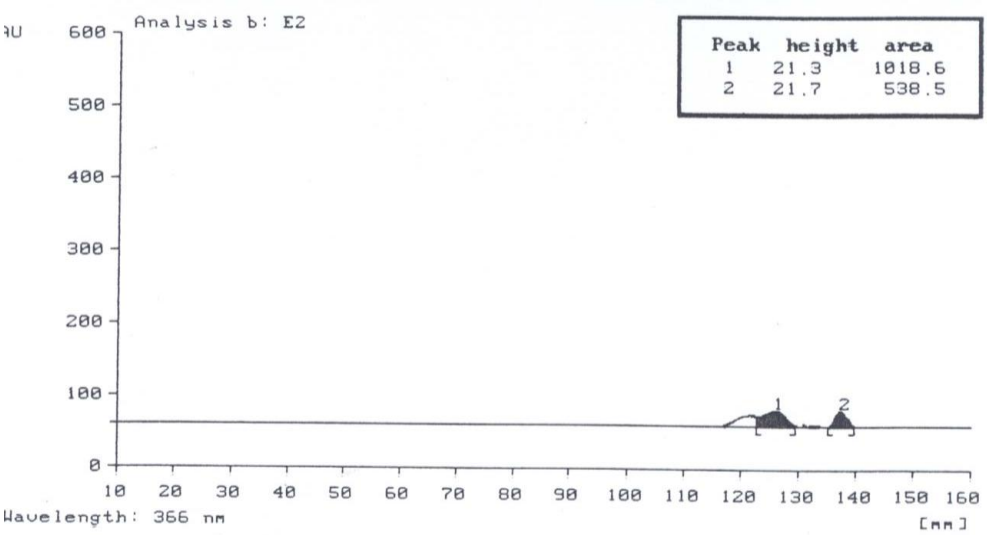

Fig. 6: data about contaminated Mamana baby supplement with $B_1$and B_2
1= aflatoxinB_1, 2 = aflatoxin B_2

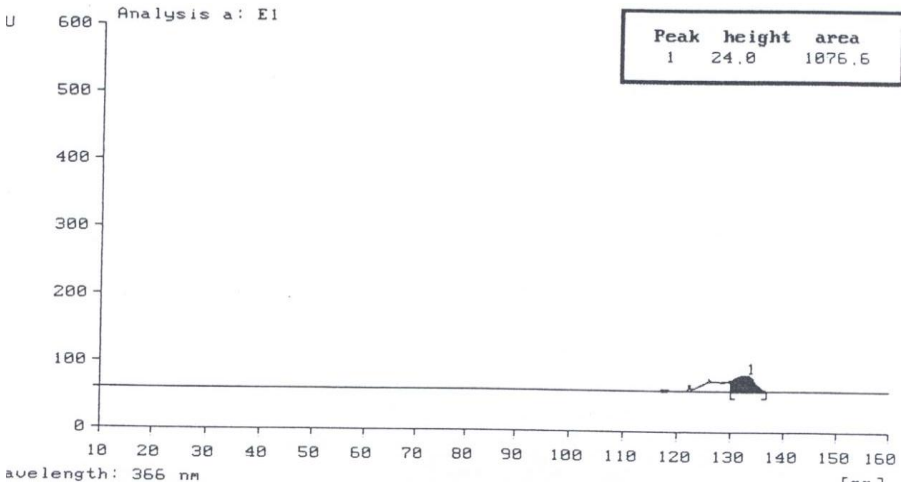

Fig. 7: data about contaminated Mamana baby supplement with B_1
1= aflatoxinB_1.

Acknowledgment

This work is Pharm. D thesis of Mis. GH. Kalantari which was supported by a grant from vice chancellor of research, Ahvaz Jundishapur University of Medical Sciences.

References

1. Hawkes C, Ruel M. The links between agriculture and health: an intersectoral opportunity to improve the health and livelihoods of the poor. Bull. World Health Organ. 2006; 84(12): 984-90.
2. Hussein HS, Brasel JM. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology 2001; 167(2): 101-34.
3. Park DL. Surveillance programmes for managing risks from naturally occurring toxicants. Food Addit. Contam. 1995; 12(3): 361-71.
4. Blunden G, Roch OG, Rogers DJ, Coker RD, Bradburn N, John AE. Mycotoxins in food. Med. Lab. Sci. 1991; 48(4): 271-82.
5. Hussain AM. Mycotoxins as carcinogens. Basic Life Sci. 1985; 34: 87-96.
6. Nizami HM, Zuberi SJ. Aflatoxin and liver cancer in Karachi, a preliminary survey. J. Pak. Med. Assoc. 1977; 27(6): 351-2.
7. Zhang MD. Aflatoxins and primary liver cancer--a population based case-control study. Zhonghua Yu Fang Yi Xue Za Zhi. 1992; 26(6): 331-3.
8. Chu FS. Mycotoxins: food contamination, mechanism, carcinogenic potential and preventive measures. Mutat. Res. 1991; 259(3-4): 291-306.
9. Nepote MC, Piontelli E, Saubois A. Occurrence of Aspergillus flavus strains and aflatoxins in corn from Santa Fe, Argentina. Arch. Latinoam. Nutr. 1997; 47(3): 262-4.
10. Abbas HK, Zablotowicz RM, Weaver MA, Horn BW, Xie W, Shier WT. Comparison of cultural and analytical methods for determination of aflatoxin production by Mississippi Delta Aspergillus isolates. Can. J. Microbiol. 2004; 50(3): 193-9.

11. Takahashi H, Kamimura H, Ichinoe M. Distribution of aflatoxin-producing Aspergillus flavus and Aspergillus parasiticus in sugarcane fields in the southernmost islands of Japan. J. Food Prot. 2004; 67(1): 90-5.
12. Carvajal M, Bolanos A, Rojo F, Mendez I. Aflatoxin M1 in pasteurized and ultrapasteurized milk with different fat content in Mexico. J. Food Prot. 2003; 66(10): 1885-92.
13. Martins ML, Martins HM. Aflatoxin M1 in yoghurts in Portugal. Int. J. Food Microbiol. 2004; 91(3): 315-7.
14. Asao T, Buchi G, Abdel-Kader MM, Chang SB, Wick EL, Wogan GN. Aflatoxins B and G. J. Am. Chem. Soc. 1963; 85(11): 1706-7.
15. Charmley LL, Rosenburg A, Trenholm HL. Factors responsible for economic losses due to Fusarium mycotoxin contamination of grains, foods, and feedstuffs. In: Miller JD, Trenholm HL. (eds.) Mycotoxins in Grain: Compounds other than Aflatoxin. St. Paul MN, Eagan Press, 1994: 487-540.
16. Bankole SA, Mabekoje OO. Occurrence of aflatoxins and fumonisins in preharvest maize from south-western Nigeria. Food Addit. Contam. 2004; 21(3): 251-5.
17. Mendez-Albores JA, Arambula-Villa G, Preciado-Ortiz RE, Moreno-Martinez E. Aflatoxins in pozol, a nixtamalized, maize-based food. Int. J. Food Microbiol. 2004; 94(2): 211-5.
18. Bueno DJ, Oliver G. Determination of aflatoxins and zearalenone in different culture media. Methods Mol. Biol. 2004; 268: 133-7.
19. Ciegler A, Bennett JW. Mycotoxins and mycotoxicoses. BioScience 1980; 30: 512-5.
20. Magnoli AP, Monge MP, Miazzo RD, Cavaglieri LR, Magnoli CE, Merkis CI, Cristofolini AL, Dalcero AM, Chiacchiera SM. Effect of low levels of aflatoxin B on performance, biochemical parameters, and aflatoxin B in broiler liver tissues in the presence of monensin and sodium bentonite. Poultry Sci. 2011; 90(1): 48-58.
21. Krishnamachari KA, Bhat VR, Nagarajan V, Tilak TB, Tulpule PG. The problem of aflatoxic human disease in parts of India-epidemiological and ecological aspects. Ann. Nutr. Aliment. 1977; 31(4-6): 991-6.
22. Muriuki GK, Siboe GM. Maize flour contaminated with toxigenic fungi and mycotoxins in Kenya. Afr. J. Health Sci. 1995; 2(1): 236-41.
23. Outbreak of aflatoxin poisoning--eastern and central provinces, Kenya, January-July 2004. MMWR Morb. Mortal. Wkly. Rep. 2004; 53(34): 790-3.
24. Klich MA. Aspergillus flavus: the major producer of aflatoxin. Mol. Plant Pathol. 2007; 8(6): 713-22.
25. Sharma RP. Immunotoxicity of mycotoxins. J. Dairy Sci. 1993; 76: 892.
26. Dvorackova I, Pichova V. Pulmonary interstitial fibrosis with evidence of aflatoxin B1 in lung tissue. Toxicol. Envir. Hlth. 1986; 18: 153-7.
27. KPimpukdee, Kubena L, Bailey C, Huebner H, Afriyie-Gyawu E, Phillips T. Aflatoxin-induced toxicity and depletion of hepatic vitamin A in young broiler chicks: Protection of chicks in the presence of low levels of Novasil Plus(TM) in the diet. Poultry Sci. 2004; 83(5): 737-44.
28. Taber HH, Schroeder HW. Aflatoxin – producing potential of isolates of the Aspergillus flavus – oryzae group from peanuts (Arachis hypogaea). Appl. Microbiol. 1967; 15: 140-4.
29. Baydae T, Erkekoglu P, Sipahi S, Sahin G. Aflatoxin B1, M1 and Ochratoxin A levels in Infant

formulae and baby foods marketed in Ankara, Turkey. J. Food Drug Anal. 2007; 15(1): 89-92.
30. Paula CA, Eric AS, Cristina MMA, Hans PvE. Occurrence of Aflatoxins and Ochratoxin A in Baby Foods in Portugal. Food Anal. Methods 2010; 3(1): 22-30.
31. Anderson HW, Nehring EW, Wichser WR, Chem JAF. Aflatoxin contamination of corn in the field. J. Agr. Food Chem. 1975; 23: 775-82.
32. Rambo GW, Tuite J, Caldwell RW. Incidence of aflatoxin in preharvest corn from Indiana in 1971 and 1972. Cereal Chem. 1974; 51: 848-53.