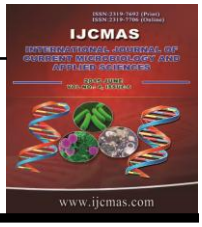
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 6 (2015) pp. 1139-1146**
http://www.ijcmas.com



Original Research Article

Evaluation of some dairy and beef cattle feed samples for fungal contamination in Markazi Province of Iran

Mohammad Rezaei[1], Issa Mohammad Pourfard[1], Mohammad Yahyaei[2], Mostafa Gholamrezaei[3], Reza Ghasemikhah[4] and Mehdi Kazemi-Bonchenari[5]*

[1]Department of Food Safety and Hygiene, school of public health, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Bioengineering, School of New Science and Technologies, University of Tehran, Tehran, Iran
[3]Department of Parasitology and Mycology, School of health, Tehran University of Medical Sciences, Iran
[4]Departmentof Parasitology and Mycology, School of Medicine, Arak University of Medical Sciences, Arak, Iran
[5]Department of Animal Science, Faculty of Agriculture and Natural Resources, Arak University, 38156-8-8349, Arak, Iran

**Corresponding author*

Keywords

Animal feeds, Fungal contamination, Toxins, Feed safety

A B S T R A C T

Mycotoxins are one the most important issues of human health. One of the most important ways to transfer of these toxins in to human diet is through animal product. Because of this, investigation on the fungal contamination of animals feed which are the main sources of mycotoxins has been taken special importance. The present study evaluated 40 dairy cattle and 35 beef cattle feed samples for fungal contamination in central province of Iran, Arak. The samples were taken from five commercial distributors of animal feed. Five feed samples of beef cattle (35 samples totally) and eight feed samples of dairy (40 samples totally) were selected from these distributes. Dichloran-Glycerol (DG18) selective medium was used in the present study. The results showed the fungal contamination of feeds in dairy feeds samples was 37.5% (15/40) and it was 37.1% (13/35) in beef cattle feeds. The average values for fungal contamination for dairy feeds and beef feeds were 3.63 and 3.52 CFU/g respectively. The results clear that the high rates of fungal contamination of dairy and beef cattle feeds might be as important health concerning issues in the research area and more studies are granted to minimize this contamination in animal nutrition and consequently in human health.

Introduction

Microscopic fungi and their metabolites, mycotoxins, are often found as contaminants in agricultural products before or after harvest as well as during transportation or storage. Animal feeds are routinely subject to contamination from diverse sources, including environmental pollution and activities of insects and microbes. Feed toxins include compounds of both plant and microbial origin. Among microbes, fungal contamination is a concerning issue in animal nutrition because of toxins which might be poisonous in animal products for human. There are consistent reports of worldwide contamination of feeds with fungi and their spores. In the tropics, Aspergillus is the predominant genus in dairy and other feeds(Dhand et al., 1998). Other species include Penicillium, Fusarium and Alternaria, which are also important contaminants of cereal grains(D'mello and Devendra, 1995, D'Mello, 2000). When cereal grains and animal feed are colonised by moulds there is a significant risk of contamination with the secondary metabolites of these fungi (Placinta et al., 1999). Mycotoxins are secondary metabolites that have adverse effects on human, animals and crops, resulting in illness and economic losses (Hussein and Brasel, 2001). These fungi contaminate wide range of agricultural products mainly cereal grains, during pre- and post-harvest stages (El Khoury et al., 2011, Guan et al., 2011) and factors such as season, humidity, temperature and drought in field as well as storage conditions (i.e. temperature, relative humidity and duration) have critical roles in production of aflatoxins (Kang'ethe and Lang'a, 2010, Dashti et al., 2009, EFSA, 2004). Regarding the toxins the human health concerns have been emphasis on mycotoxins which might be presented by animal products. Among four common aflatoxins available (aflatoxin B1, B2, G1 and G2), aflatoxin B1 (Af-B1) is the most prevalent and poisonous molecules and categorized as group 1 human carcinogen (Fallah, 2010, Guan et al., 2011, El Khoury et al., 2011). When lactating mammals ingest feeds containing aflatoxin B1, it is converted to Af-M1 by hydroxylation (Ruangwises and Ruangwises, 2010). Af-M1 is secreted to milk and subsequently entered individuals through consumption of milk and other dairy products (Prandini et al., 2009). Many studies showed that Af-M1 have teratogenic and mutagenic implication and recently, likewise Af-B1, classified as first group human carcinogen (Sassahara et al., 2005, Almeida Picinin et al., 2012). Fungal contamination should be examined individually for feeds of local areas in different countries and hence the results could be practical for the studied regions(Čonková et al., 2006). Because of this critical role of aflatoxinis, controlling the fungal contamination should be considered in animal nutrition to decrease aflatoxins concentration in human nutrition. In the present study, the feed samples prepared from some commercial dairy and beef feeds are evaluated for fungal contamination in central province of Iran, Arak.

Materials and Methods

Study area

This study was performed in Markazi province, central Iran. The area has a population of approximately 600000 and is located around 49° east longitude and 34° north latitude with an average height of 1750 meters above sea levels and a mean annual precipitation of about 300 mm and the annual relative humidity about 50%. The maximum temperature may rise up to 35°C in summer and may fall to minus 25 °C in winter (Fig.1).

Feed sample preparation

The five commercial animal feed distributers for dairy and also five distributers for beef cattle feeds are selected to collect the samples. Eight feed samples of dairy cattle nutrition (totally 40 samples) and seven feed samples of beef cattle nutrition (totally 35 samples) are subjected to analysis for fungal contamination.

Fungal culture

Yeast isolation was performed according to ISO21527-2 (ISO, 2008). 20 g of each sample were homogenized for 5 min in 180 ml peptone-water. Decimal dilutions in 9% (w/v) NaCl solution were prepared and inoculated in to Dichloran 18% mass fraction glycerol agar (DG18) medium. Plates were incubated for 3–5 days at 30°C, after which yeast colonies were counted.

Composition of DG18

The composition of the DG18 was yeast extract (5 g), Glucose (10 g), chloramphenicol (0.1 g), Agar (12- 15 g), PO_4H_2K (1 g), $MgSO_4.H_2O$ (0.5 g), dichloran (2, 6-dichloro-4-nitroaniline) (0.002 g), glycerol (220 g), enzymatic digestion of casein (5 g), distilled Water (1 liter).

Statistical analysis

Statistical analyses were done by using SAS software(SAS, 1999-2000). The results showing $p< 0.05$ were considered as significant.

Results and Discussion

The data for fungal contamination analysis for different feeds in five factories of dairy cattle feeds and beef cattle feeds are presented in Table 1. Moreover a discriminate analysis on the tested samples was carried out and results in each source of animal feeds are shown in Fig.1 (dairy cattle feeds) and Fig.2 (beef cattle feeds).

The counts of yeasts in dairy cattle feed are ranged from 6×10^1 to 1.6×10^5 CFU/gr and in beef cattle feed was 3×10^1 to 5.6×10^6 CFU/gr. This result clarifies the greater range of fungal contamination of beef cattle feeds in the research area. In this study the mean content of fungal contamination in dairy cattle feed was 16436.75 ± 6.32 CFU/gr and this value for beef cattle feeds was 291839.6 ± 5.91 CFU/gr. The significant difference was observed for different factories supplied animal feeds. The greatest fungal contamination for both dairy cattle feeds and beef cattle feeds was found for factory E (4.63 and 5.22 CFU/gr, for dairy feeds and beef feeds respectively). The lowest values for fungal contamination were 2.52 CFU/gr for dairy feeds and were 2.13 CFU/gr for beef feeds. The average values for fungal contamination for dairy feeds and beef feeds were 3.63 and 3.52 CFU/gr respectively.

Moulds are common contaminants of animal feeding products and cause their spoilage. However, for some products, certain mould species have to be present if the desired appearance and rheological and sensory characteristics are to develop (Belén Flórez et al., 2007).

Contamination of feeds takes effect from different sources, such as failure to comply with health and contamination of product line. In feed mills fungal contamination may be retained at various points along the production line, contaminating subsequent batches of meal as they are processed. Therefore in addition to the feed per se, the processing pathway also may include in increasing the fungal pollution.

Table.1 Averages logarithmic comparisons of fungal contamination (CFU/gr) among five different factories feeds

Factory	Dairy cattle feeds	Beef cattle feeds
A	2.52[a]	2.13[a]
B	3.28[b]	2.66[ab]
C	3.8[bc]	3.54[bc]
D	3.92[c]	4.05[c]
E	4.63[d]	5.22[d]
SD	0.1568	0.23

[a,b,c] Least squares means within the same row without a common superscript differ (P < 0.05).

Fig.1 Location of Markazi provinces

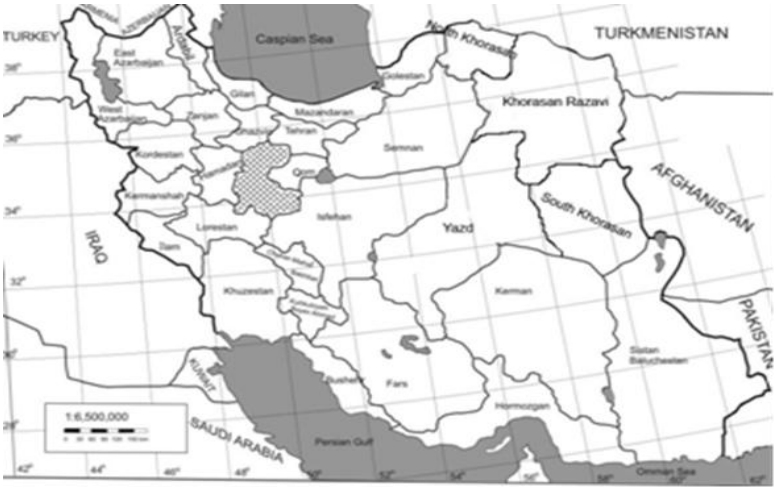


Fig.2 Fungal contamination in dairy cattle feed

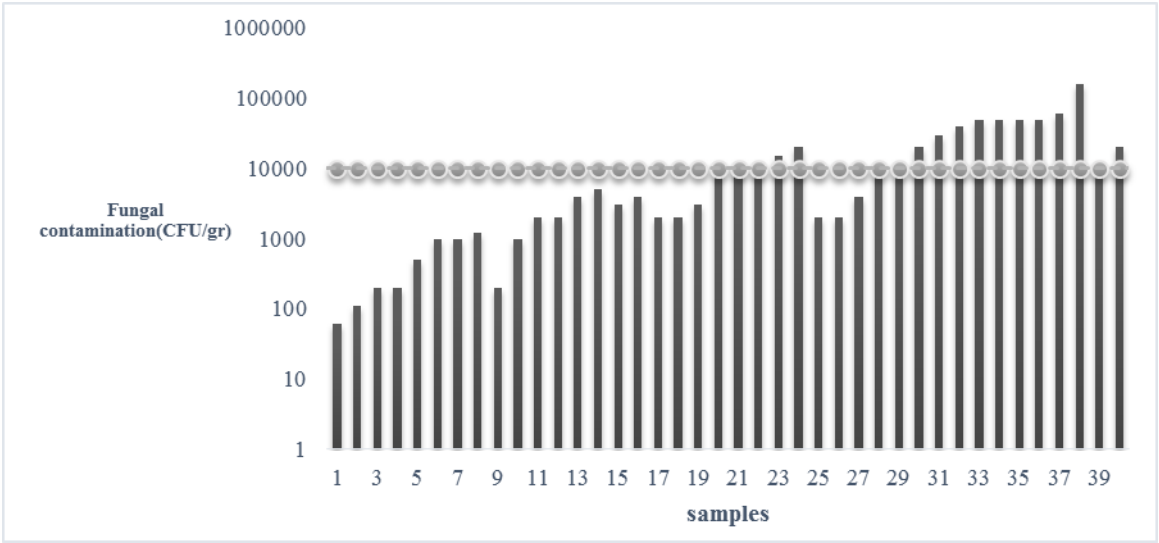
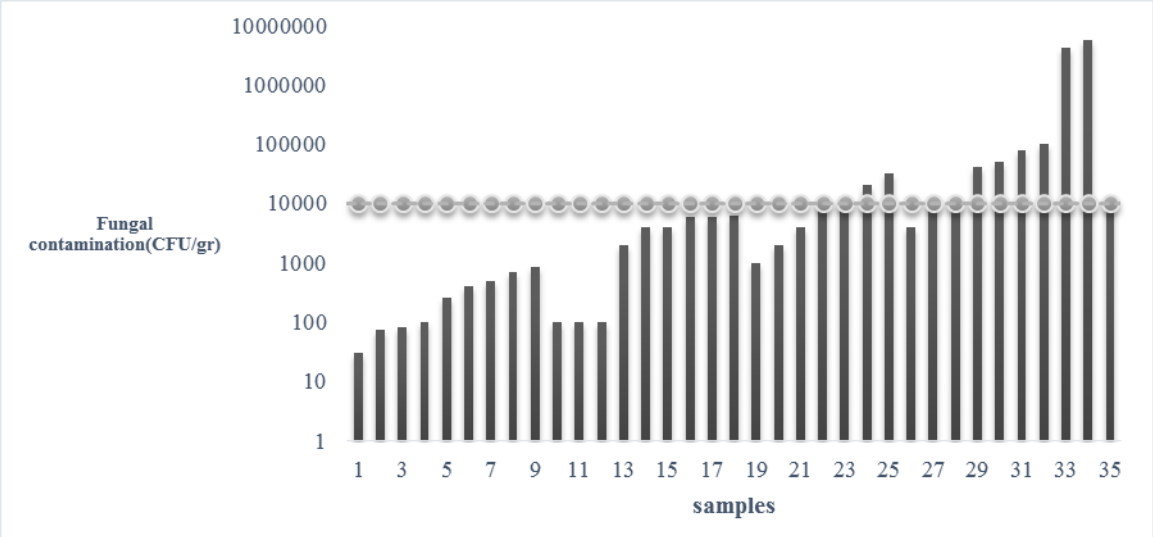


Fig.3 Fungal contamination in beef cattle feed



In this study DG18 culture are used and according to ISO 21527-2 (ISO, 2008) and based on this method total fungal count should be under 10^4 CFU/gr. By using this method our results revealed the presence of fungal contamination in 13 samples of beef cattle feed and 15 samples of dairy cattle feeds. Worldwide, fungal contamination of foods and feeds, with the consequent mycotoxin production, is a considerable problem. Placinta et al. 1999 in a review reported that global contamination of animal feed with trichothecenes, zearalenone (ZEA) and fumonisins (Placinta et al., 1999). Jaimez et al., (2004) showed the fungal contamination status of feed ingredients in Spain. They found that 91 of samples positively showed to contain fungal contamination. One sample of corn and another of cotton seed were Ochratoxin A (OTA) contaminated exceeded limit of European countries, whereas none of the samples contaminated with ZEA (Jaimez et al., 2004).

In a study, Mycotoxins were found in 18% of the Feed samples from western Canada (Abramson et al., 1997). Rezaei et al, about aflatoxin contamination of feed material produced in Qom province, reported that all samples demonstrated total aflatoxin levels lower than European Union standard and National Standard of Iran recommended limits(Rezaei et al., 2014b).

The effects of moulds and their metabolites are investigated from the point of the relationship to human and animal health(Hintz, 1990). Many mycotoxins in food and feed are nephrotoxic, hepatotoxic, immunosuppressive, or carcinogenic(Ostrý and Ruprich, 1998). That is why many countries accept the recommended limits for fungal contamination and mycotoxin levels in food and feed. Mycotoxins have adverse effects on human, animals and crops, resulting in illness and economic losses (Rosa et al., 2006). Milk and meat are essential for human and have been and still are important parts of the diets of people. Meat consumption is globally increasing every year: the growth rate of livestock and meat production has increased annually by 2.2% during 2002 to 2010 (FAO, 2002 , FAO, 2009, FAO, 2012). Accompanying with this increase in animal products using, public interest is increasing in food quality and food safety due to concerns about

contamination by microorganisms such as bacteria and fungi (Dorn-In et al., 2013).

According to previous studies in the different food and feeds in Markazi province (Rezaei et al., Rezaei et al., 2013, Fani et al., 2013, Rezaei et al., 2014a)and also, looking to the results of the present study clear that controlling fungal contamination of animal feeds in the research area needs more attention and practical decisions to do preventing transfer of these toxins in to animal body or in to animal products are required. More studies also granted to be done for evaluating the kind of fungal contamination, identification of special toxins in feeds, suggesting the methods to decrease high concentration of fungal in animal feeds.

The average values for fungal contamination for dairy feeds and beef feeds were 3.63 and 3.52 CFU/gr respectively. The results of the present study showed that fungal contamination of both dairy and beef cattle feeds are considerable and preventing or decreasing of contamination is necessary for future programming. Future studies are required to present practical ways to control these high amounts of fungal contamination in both beef and dairy cattle feeds.

Acknowledgments

This article was financially supported by authors. The authors thank the all people who contributed to this study.

References

Abramson, D., Mills, J., Marquardt, R. & Frohlich, A. 1997. Mycotoxins In Fungal Contaminated Samples Of Animal Feed From Western Canada, 1982-1994. *Canadian Journal Of Veterinary Research,* 61**,** 49.

Almeida Picinin, Cristina, L., Oliveira Pinho Cerqueira, Mônica Maria Vargas, E. A. Q. L., Ângela Maria Toaldo, I. M. B. L. & Bordignon-Luiza, M. T. 2012. Influence Of Climate Conditions On Aflatoxin M1 Contamination In Raw Milk From Minas Gerais State, Brazil. *Food Control,* 31**,** 419–424.

Belén Flórez, A., Álvarez-Martín, P., López-Díaz, T. M. & Mayo, B. 2007. Morphotypic And Molecular Identification Of Filamentous Fungi From Spanish Blue-Veined Cabrales Cheese, And Typing Of Penicillium Roqueforti And Geotrichum Candidum Isolates. *International Dairy Journal,* 17**,** 350-357.

Čonková, E., Laciaková, A., Štyriak, I., Czerwiecki, L. & Wilczyńska, G. 2006. Fungal Contamination And The Levels Of Mycotoxins (Don And Ota) In Cereal Samples From Poland And East Slovakia. *Czech J. Food Sci. Vol,* 24**,** 33-40.

D'mello, J. 2000. Anti-Nutritional Factors And Mycotoxins. *Farm Animal Metabolism And Nutrition.***,** 383-403.

D'mello, J. & Devendra, C. 1995. Anti-Nutritional Substances In Legume Seeds. *Tropical Legumes In Animal Nutrition.***,** 135-172.

Dashti, B., Al-Hamli, S., Alomirah, H., Al-Zenki, S., Abbas, A. B. & Sawaya, W. 2009. Levels Of Aflatoxin M1 In Milk, Cheese Consumed In Kuwait And Occurrence Of Total Aflatoxin In Local And Imported Animal Feed. *Food Control,* 20**,** 686-690.

Dhand, N. K., Joshi, D. V. & Jand, S. K. 1998. Fungal Contaminants Of Dairy Feed And Their Toxigenicity. *Indian Journal Of Animal Sciences,* 68**,** 1095-1096.

Dorn-In, S., Hölzel, C. S., Janke, T., Schwaiger, K., Balsliemke, J. & Bauer, J. 2013. Pcr-Sscp-Based Reconstruction

Of The Original Fungal Flora Of Heat-Processed Meat Products. *International Journal Of Food Microbiology,* 162**,** 71-81.

Efsa 2004. Opinion Of The Scientific Panel On Contaminants In The Food Chain On A Request From The Commission Related To Aflatoxin B1 As Undesirable Substance In Animal Feed. *The European Food Standard Agency Journal,* 39**,** 1-27.

El Khoury, A., Atoui, A. & Yaghi, J. 2011. Analysis Of Aflatoxin M1 In Milk And Yogurt And Afm1 Reduction By Lactic Acid Bacteria Used In Lebanese Industry. *Food Control,* 22**,** 1695-1699.

Fallah, A. A. 2010. Aflatoxin M1 Contamination In Dairy Products Marketed In Iran During Winter And Summer. *Food Control,* 21**,** 1478-1481.

Fani, A., Rezaei, M., Moini, A., Fani, P., Mirzajani, P., Malekirad, A. A., Rafeie, M. & Atabak, N. 2013. Aflatoxin Occurrence In Nuts Consumed In Arak, Iran. *Int. J. Food Nutr. Saf,* 4**,** 91-97.

Fao 2002 Report Of The 19th Session Of The Intergovernmental Group On Meat And Dairy Products, Medium Term Projections For Meat And Dairyproducts To 2010.

Fao 2009. How To Feed The World In 2050.

Fao 2012. Global And Regional Food Consumption Patterns And Trends.

Guan, D., Li, P., Cui, Y., Zhang, Q. & Zhang, W. 2011. A Competitive Immunoassay With A Surrogate Calibrator Curve For Aflatoxin M1 In Milk. *Analytica Chimica Acta,* 703**,** 64-69.

Hintz, H. 1990. Molds, Mycotoxins, And Mycotoxicosis. *The Veterinary Clinics Of North America. Equine Practice,* 6**,** 419-431.

Hussein, H. S. & Brasel, J. M. 2001. Toxicity, Metabolism, And Impact Of Mycotoxins On Humans And Animals. *Toxicology,* 167**,** 101-34.

Iso 2008. Microbiology Of Food And Animal Feeding Stuffs -Horizontal Method For The Enumeration Of Yeasts And Moulds - Part2 : Colony Count Technique In Products With Water Activity Less Than Or Equal To 0,95. *21527-2*

Jaimez, J., Fente, C. A., Franco, C. M., Cepeda, A. & Vázquez, B. I. 2004. A Survey Of The Fungal Contamination And Presence Of Ochratoxin A And Zearalenone On Spanish Feed And Raw Materials. *Journal Of The Science Of Food And Agriculture,* 84**,** 832-840.

Kang'ethe, E. & Lang'a, K. 2010. Aflatoxin B1 And M1 Contamination Of Animal Feeds And Milk From Urban Centers In Kenya. *African Health Sciences,* 9.

Ostrý, V. & Ruprich, J. 1998. Determination Of The Mycotoxin Fumonisins In Gluten-Free Diet (Corn-Based Commodities) In The Czech Republic. *Central European Journal Of Public Health,* 6**,** 57-60.

Placinta, C. M., D'mello, J. P. F. & Macdonald, A. M. C. 1999. A Review Of Worldwide Contamination Of Cereal Grains And Animal Feed With Fusarium Mycotoxins. *Animal Feed Science And Technology,* 78**,** 21-37.

Prandini, A., Tansini, G., Sigolo, S., Filippi, L., Laporta, M. & Piva, G. 2009. On The Occurrence Of Aflatoxin M< Sub> 1</Sub> In Milk And Dairy Products. *Food And Chemical Toxicology,* 47**,** 984-991.

Rezaei, M., Akbaridastjerdi, H., Jafari, H., Farahi, A., Shahabi, A., Javdani, H., Teimoory, H., Yahyaei, M. & Malekirad, A. A. 2014a. Assessment Of Dairy Products Consumed On The Arakmarket As Determined By Heavy Metal Residues. *Health (1949-4998),* 6.

Rezaei, M., Karimi, F., Yahyaei, M., Javdani, H., Shahabi, A. & Farahi, A. A Survey Of Microbial Total Count And Prevalence Of Escherichia Coli In Raw Milk In Markazi Province, Iran. *Research Opinions In Animal & Veterinary Sciences*, 12**,** 474-7.

Rezaei, M., Mohammadpourfard, I., Malekirad, A. A., Ghasemikhah, R. & Sayadi, M. 2014b. Aflatoxin Contamination Of Feed Materials In Qom Province, Iran. *Iranian Jornal Of Toxicology*, 8**,** 1025-1027.

Rezaei, M., Shariatifar, N., Jahed Khaniki, G. & Javadzadeh, M. 2013. Nitrite In Hamburgers In Arak, Iran. *Food Additives & Contaminants: Part B*, 6**,** 285-288.

Rosa, C. A., Ribeiro, J. M., Fraga, M. J., Gatti, M., Cavaglieri, L. R., Magnoli, C. E., Dalcero, A. M. & Lopes, C. W. 2006. Mycoflora Of Poultry Feeds And Ochratoxin-Producing Ability Of Isolated Aspergillus And Penicillium Species. *Vet Microbiol*, 113**,** 89-96.

Ruangwises, N. & Ruangwises, S. 2010. Aflatoxin M1 Contamination In Raw Milk Within The Central Region Of Thailand. *Bulletin Of Environmental Contamination And Toxicology*, 85**,** 195-198.

Sas, I. 1999-2000. Sas/Stat User's Guide. *Sas Institute Inc., Cary, Nc.,* Version 8.

Sassahara, M., Pontes, N. D. & Yanak, E. K. 2005. Aflatoxin Occurrence In Foodstuff Supplied To Dairy Cattle And Aflatoxin M1 In Raw Milk In The North Of Parana State. *Food And Chemical Toxicology*, 43**,** 981-984.