African Journal of Food Science and Technology (ISSN: 2141-5455) Vol. 3(10) pp. 273-276, December, 2012
Available Online http://www.interesjournals.org/AJFST

Full Length Research Paper

A preliminary survey of aflatoxin M_1 in dairy cattle products in Bida, Niger State, Nigeria

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Accepted 4 December, 2012

This study investigated the aflatoxin M $_1$ contents of fresh milk, fermented defatted skimmed milk (*nono)* and full fat or partially skimmed milk (*kindirmo)* from two dairy farm settlements in Bida Local Government Area of Niger State, Nigeria. Thirty samples (10 of each product) were collected from the sampling sites and evaluated for their AFM_1 concentrations using High pressure liquid chromatography (HPLC). The results obtained showed 100% prevalence of AFM_1 in the three products at mean concentrations of 0.665, 0.924,0.575µg/l respectively. All the samples had toxin levels above the European Union action level of 0.05µg/l for milk and milk products while 5 out of the 30 samples had AFM_1 contents above the United States' tolerable limit of 0.5µg/kg. This high AFM_1 level in Nigerian milk and its product elicits public health concern which necessitates regulation of mycotoxins in foods and feeds in the country

Keywords: Aflatoxin $M_{1,}$ HPLC, dairy products, Nigeria.

INTRODUCTION

Animals fed aflatoxin $B_1(AFB_1)$ and $B_2(AFB_2)$ contaminated feeds excrete into their milk and urine the less toxic AFM_1 and M_2, respectively. AFM_1 is of particular interest being the hydroxylated metabolite of AFB_1 and is known to have 2-10% of the carcinogenic potency of the parent compound (Zinedine et al., 2007). The carryover of this carcinogen in cow at a transfer ratio (consumed AFB_1 to excreted AFM1) of 200:1 (Smith and Moss, 1985) which could be as high as 40:0.05 (JECFA, 2001) into human and animal milk that are the main sources of nutrition for infants (European Commission, 2002) whose vulnerability due to undeveloped immune system is obvious, poses serious health concern. Its stability to heat, cold storage, freezing and drying (Yousef and Marth, 1989) during processing makes dairy products another important route of AFM_1 exposure. Milk and milk products are traditional staple food commodities for the nomadic population of Northern Nigeria and many other parts of Africa. They are recognized by the elites as natural balanced diet and so are increasingly consumed by the urban populace in the continent. Therefore, they can no longer be ignored as they are among the main entry routes of AFM_1 into the human dietary system in Africa.

Aflatoxin M_1 (AFM_1) was initially classified by the International Agency for Research on Cancer (IARC) as a group 2B agent carcinogenic to humans (IARC, 1993) due to lack of data. However, when further studies demonstrated its *in vivo* genotoxicity and cytotoxicity effects (Caloni et al., 2006), AFM_1 was reclassified as a group 1 human carcinogen (IARC, 2002). Thus, its potential risk to human health makes its presence in milk and milk products undesirable and necessitates regular monitoring and control of the toxin in dairy products particularly in Nigeria and indeed Africa which has the favourable warm and humid climate, compelling environmental (drought) and socio-economic (ignorance of the toxin and poor infrastructure to manage mycotoxin prevention strategies) factors that enhance aflatoxins production on foods and feeds (Wagacha and Muthomi, 2008) and their subsequent carry over to dairy products.

In spite of this need for monitoring of AFM_1, to the best of our knowledge, there are only four works on the toxin in Nigeria. Opadokun et al. (1978) analyzed but did not

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find AFM_1 in cow milk samples in farms in Kano State while Ogunbanwo et al. (2005) worked only on imported powdered milk and milk products marketed nationwide. On the other hand Atanda et al. (2007) investigated the toxin in human and cow milk, yogurt, ice cream and cheese from the South western region of Nigeria. Makun et al. (2010) examined AFM_1 in only imported powdered milk marketed in Lagos. All these studies were done using the not very sensitive thin layer chromatographic methods and in locations that did not cover Bida, the area under current investigation. In order to fill these gaps, this pilot study was undertaken to determine the prevalence and levels of AFM_1 in fresh cow milk and locally produced skimmed (defatted) milk ("nono") and full fat or partially skimmed milk ("kindirmo") in Bida local government area of Niger State, Nigeria using the more sensitive high pressure liquid chromatographic (HPLC) method.

MATERIAL AND METHODS

Sampling

Thirty samples of fresh milk and its products (*nono* and *kindirmo*) produced by two local dairy farmers were collected in Bida and its environs. Immediately after ten of each of the product were purchased and were transported"to the laboratory in an ice packed box. The samples were stored at -20 °C deep-freezer until used for analysis. At the time of analysis samples were brought up to room temperature.

Aflatoxin M_1 extraction

Aflatoxins were extracted and analyzed using AOAC official method 980.21without modifications as described by Elzupir and Elhussein, (2010). About 40ml of chloroform and 3ml salt solution (10g NaCl in 50ml H_2O) was added into a separatory funnel containing15ml of the sample (fresh milk, *nono, kindirmo*) securely stoppered, shaken gently. The chloroform was eluted in 250ml beaker and then evaporated to dryness over a water bath at 50°C. The extract was dissolved in 10ml of acetonitrile and defatted twice with 15ml petroleum ether. The petroleum ether layer was discarded while the residue was transferred to an amber vial and then evaporated to dryness. This was stored at –20°C deep freezer until analysis. The dry film was redissolved with 200 µl mobile phase (Acetonitrile: water: acetic acid 10:50:40) for HPLC analysis.

High Pressure Liquid Chromatographic Technique

AFM_1 was analyzed on Agilent technologies 1200 series high performance liquid chromatography with UV detection as described by Cora et al., (2005) at wavelength of 365nm. The octadecylsilyl group (ODS) column, 4.6mm x 150mm x 5µm was used at ambient temperature of 25°C. Acetonitrile: water and acetic acid in ratio 10:50:40 v/v/v respectively was used as mobile phase at flow rate of 0.8ml/min. The injection volume was 20µL.The analyses were carried out with aflatoxin standards (Sigma Chemical Company, St. Louis, MO, USA) of known concentrations with AFM_1 eluting at retention time of 1.921. Calibration curve with correlation factor of 0.923 was obtained using series of dilutions in methanol containing $600pgm^{-1}$, 1200 pgm^1, and 2400 pgm^1 of aflatoxin M_l standard. The detection limit of the machine with regards to the toxins was 0.001µg/ml.

RESULT AND DISCUSSION

AFM_1 was detected in all the samples analyzed with contamination range between 0.139-2.510µg/l and average concentration of 0.665, 0.924 and 0.575 for fresh milk, *nono* and *kindirmo* (Table 1). Since 80% of AFM_1 is partitioned in the skim portion milk because the toxin binds to casein (Hamid, 2011), lower concentrations of AFM_1 is expected in skimmed milk than in fresh milk. This explains the lower AFM_1 content found in the partially skimmed milk (*kindirmo*) than the fresh milk in this investigation. The exception to this trend is the higher AFM_1 levels in skimmed milk (*nono*) than in fresh milk, and this could be because the sources of the two were different with the animals from which the former was obtained consumed more aflatoxin B_1 contaminated feeds than those that produced the fresh milk (Hamid, 2011).

The result obtained in this study could be compared with those reported by Atanda et al., (2007). These workers demonstrated that fresh milk produced in Ogun and Oyo States of Nigeria had AFM_1 concentrations range from 2.04-4.00g/l. However the AFM_1 of the content of this study was observed to be higher than those reported by Ogunbanwo et al, (2005) and Makun et al., (2010) who found AFM_1 concentrations in imported powdered milk at concentrations below 0.5ug/l. The low concentrations of the toxin in the two aforementioned studies might be because the marketed powdered milk were from developed countries and so compliance to international regulatory limits might have been achieved at the point of manufacture. Opadokun et al., (1978) analyzed 92 milk samples but did not find AFM_1 because the method used with a detection limit of 200µg/l was not sensitive enough to detect the toxin which is usually found in concentrations below 7.0µg/l.

When compared with values from other regions of the world, the absolute concentrations obtained in this investigation were similar to these observed in tropical developing countries, such as the values obtained by Kamkar, (2011) in 85 out of 111 milk samples from Iran at concentrations of up to 0.725µg/l, Hussain and Anwar,

Table 1. Aflatoxin M_1 concentration in milk and milk products from Bida, Nigeria

Dairy products	Freshmilk	*Nono*	*Kindirmo*
Total samples analysed	10	10	10
Contaminated samples	10	10	10
AFM_1 concentration %	100%	100%	100%
Range (µg/l)	0.407-0.952	0.248-2.510	0.139-1.238
Mean ±SD (µg/l)	0.665±0.190[a]	0.924±0.626[a]	0.575±0.341[a]
Number of samples with AFM1 Exceeding EU limits	10	10	10

Note: *1Each values is the mean ± standard deviation of 10 determinations*
2Similar superscript not significantly different P>0.05
3European Union action limit is 0.05 µg/l

(2008) in 100 of 168 samples analyzed at levels between 0.0 and 0.7µg/l in Pakistan, Ruangwises and Ruangwise, (2010) in 103 out of 123 samples from Thailand at concentrations of between 0.003 and 0.5µg/l, Elzupir and Elhussein, (2010) at concentrations of up to 6.90µg/l in 95.5% of fresh milk in Sudan and Elgerbi et al., (2004) in 35 out of 49 Libyan milk and milk products at levels not exceeding 3.13µg/l. The herein reported levels were higher than AFM_1 levels reported in milk and milk products from temperate, developed countries such as Argentina (0.012-0.014µg/l), Croatia (0.011-0.058µg/l), Italy (0.015-0.280µg/l) and Portugal (0.005-0.08µg/l) by Lopez et al. (2003), Bilandzic et al. (2010), Galvano et al. (1996) and Martins et al. (2005) respectively. The regional variation shown above with higher AFM_1 contents observed in developing countries in the tropics than levels in developed countries in temperate regions might be for two reasons. The hot and humid climatic conditions of the tropics favour growth of aflatoxigenic fungi and aflatoxin production (Klich, 2002) in animal feeds with consequent more AFM_1 contamination of milk products than in the less favourable climate of the temperate region. Secondly, the developed countries have imposed strict control of the quality of feeds provided to animals which reduces AFM_1 incidence (Martins et al., 2005).

A total of 19 out 30 (63.3%) contaminated samples showed action level above 0.5 (µg/l) permitted by US regulation, whereas all the contaminated samples exceeded the prescribed limit of 0.05µg/lby European Union. The high aflatoxin M_1 content of milk and milk products of this study is not surprising as it is consistent with the high AFB_1 concentrations in feeds and feeding stuffs in Nigeria. Animal feeds and, cereals and peanuts which are the usual components of animal feeds and feeding stuffs in Nigeria have been shown by various workers (Gbodi et al., 1984, Gbodi et al., 1986, Opadokun, 1992, Ezekiel et al., 2012) to contain AFB_1 in concentrations of up to 8000µg/kg. At AFB_1 to AFM_1 transfer rate of 0.05-10% (Smith and Moss, 1985), the daily intake of AFB_1 by lactating cows at such concentrations found in Nigerian feeding stuffs may lead to production of milk with toxin content above the EU legislated level of 0.05µg/l as observed in this work.

The present AFM_1 contamination of milk and milk products with 100% of the samples having unacceptable toxin levels above the safe limit set by EU raises public health concern because the host substrate is a major source of nutrition for the very vulnerable infants and children. Therefore the pastoralist who is the major producer of milk and its products in Bida should be enlightened by Government authorities through the Nomadic Education programme on the potential health consequence of aflatoxin and ensure the use of controlled animal feeds. This also calls for reduction of AFB_1 in feeds by good agricultural and manufacturing practices, and strict enforcement of mycotoxin regulatory legislation.

ACKNOWLEDGEMENT

The authors are grateful to Adenike Adebayo of Drug Quality Control Laboratory, College of Medicine, Lagos State University Teaching Hospital, Lagos, Nigeria of the for the HPLC analysis.

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