A preliminary survey of aflatoxin M₁ in dairy cattle products in Bida, Niger State, Nigeria

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This study investigated the aflatoxin M₁ 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 AFM₁ concentrations using High pressure liquid chromatography (HPLC). The results obtained showed 100% prevalence of AFM₁ 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 AFM₁ contents above the United States' tolerable limit of 0.5µg/kg. This high AFM₁ 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 M₁, HPLC, dairy products, Nigeria.

INTRODUCTION

Animals fed aflatoxin B₁(AFBI) and B₂(AF B₂) contaminated feeds excrete into their milk and urine the less toxic AFM₁ and M₂, respectively. AFM₁ is of particular interest being the hydroxylated metabolite of AFBI 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 AFBI 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 AFM₁ 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 AFM₁ into the human dietary system in Africa.

Aflatoxin M₁ (AFM₁) 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), AFM₁ 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 AFM₁, to the best of our knowledge, there are only four works on the toxin in Nigeria. Opadokun et al. (1978) analyzed but did not...
find AFM₃ 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 AFM₁ 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 AFM₁ 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 M₃ extraction

Aflatoxins were extracted and analyzed using AOAC official method 980.21 without modifications as described by Elzupir and Elhussein, (2010). About 40ml of chloroform and 3ml salt solution (10g NaCl in 50ml H₂O) was added into a separatory funnel containing 15ml 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

AFM₁ 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 AFM₁ 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 M₁ standard. The detection limit of the machine with regards to the toxins was 0.001µg/ml.

RESULT AND DISCUSSION

AFM₁ 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 AFM₁ is partitioned in the skim portion milk because the toxin binds to casein (Hamid, 2011), lower concentrations of AFM₁ is expected in skimmed milk than in fresh milk. This explains the lower AFM₁ content found in the partially skimmed milk (kindirmo) than the fresh milk in this investigation. The exception to this trend is the higher AFM₁ 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 B₁ 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 AFM₁ concentrations range from 2.04-4.00µg/l. However the AFM₁ 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 AFM₁ 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 AFM₁ 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 those 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,
concentrations of between 0.003 and 0.5µg/l, Elzupir and (2010) in 103 out of 123 samples from Thailand at 95.5% of fresh milk in Sudan and Elgerbi et al., (2004) in Elhussein, (2010) at concentrations of up to 6.90µg/l in 0.0 and 0.7µg/l in Pakistan, Ruangwises and Ruangwise, (2008) in 100 of 168 samples analyzed at levels between higher than AFM exceeding 3.13µg/l. The herein reported levels were 35 out of 49 Libyan milk and milk products at levels not exceeding EU limits. 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 AFB1 in feeds by good agricultural and manufacturing practices, and strict enforcement of mycotoxin regulatory legislation.

Table 1. Aflatoxin M1 concentration in milk and milk products from Bida, Nigeria

<table>
<thead>
<tr>
<th>Dairy products</th>
<th>Freshmilk</th>
<th>Nono</th>
<th>Kindirimo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total samples analysed</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Contaminated samples</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>AFM1 concentration %</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Range (µg/l)</td>
<td>0.407-0.952</td>
<td>0.248-2.510</td>
<td>0.139-1.238</td>
</tr>
<tr>
<td>Mean ±SD (µg/l)</td>
<td>0.665±0.190</td>
<td>0.924±0.626</td>
<td>0.575±0.341</td>
</tr>
<tr>
<td>Number of samples with AFM1</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Exceeding EU limits

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 AFM1 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 AFM1 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 AFM1 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 AFM1 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 M1 content of milk and milk products of this study is not surprising as it is consistent with the high AFB1 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 AFB1 in concentrations of up to 8000µg/kg. At AFB1 to AFM1 transfer rate of 0.05-10% (Smith and Moss, 1985), the daily intake of AFB1 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.

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