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DETERMINATION OF AFLATOXIN M1 CONCENTRATIONS IN FULL-FAT COW'S UHT MILK SOLD FOR CONSUMPTION IN NAJRAN-SAUDI REGARDING ITS PUBLIC HEALTH SIGNIFICANCE*

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ABSTRACT

This study aimed to evaluate the concentrations of aflatoxin M1 in full fat, cow's UHT milk solid in Najran–Saudi Arabia with regard to its public heath significance. 96 samples of different brands full fat, cow's UHT milk were randomly punched from different supermarkets at Najran city during the period of September 2011 to January 2012. The samples were examined for AFM1 using the competitive enzyme-linked immunosorbent assay (ELISA), AFM1 residues were detected in 79 samples (82.30% of total). The minimum concentration was 0.01, the maximum concentration was 0.19 and the mean value was 0.058±0.0053 $\mu g/L^{-1}$. Data also indicated that AFM1 residues concentrations detected in all the positive samples were below the tolerated level of AFM1. So it could be concluded that contamination of AFM1 in dairy products marketed in Najran city does not appear to be serious public health problem at the moment.

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INTRODUCTION

Good health starts with good nutrition and good nutrition can protect against diseases later in life. Liquid milk is a common health drink consumed by people of all age groups especially children. A large population in our countries depends on milk from local suppliers. Milk is a product of biological evolution, its role in human nutrition is well known and its biochemical complex which appears to be the only material to function solely as a source of food. The complements of proteins in milk are ideal in quality and balance to satisfy human amino acid requirements. Confirmation of this nutritive image is the widespread use of milk and milk products as a part of the daily diet of peoples in the highly developed countries. As a consequence, such societies enjoy almost complete freedom from nutritional disease among infants, children, young and adults. In contrast, the underdeveloped areas of the world have a primitive or nonexistent milk supply and have numerous inhabitants suffering from nutrient deficiencies, especially infants and children (**Cashman 2002** and **Hoppe et al. 2006**).

Human health is highly attractive world, so food safety remains a major challenge to food producers and to legislators endeavoring to adequate consumer protection. Both man and animals live under a certain degree of "biological hazard" from natural toxicants that occur in food and foodstuffs **(Allam et al. 1999-a**; **1999-b** & **2002** and **Abdelhamid et al. 2002**).

Naturally occurring toxins such as mycotoxins pose intense challenges to food safety widespread in many countries, especially in tropical and subtropical regions where temperature and humidity conditions are optimum for growth of moulds and toxins production, so they are found in a wide variety of agricultural products (such as corn, wheat, soybean, barley and rice) (**FAO** (**1991**), and animal foods as well as meat products, milk products including ultra-high treated (UHT) milk and as a result of carry-over from contaminated animal feed (**Blunden et al. 1991**; **Moustafa 1994**; **Adams** & **Moss 1995**; **Orriss 1997**; **Sayed et al. 2000**; **Takahashi-Ando et al. 2004**; **Cavaliere et al. 2006** and **Trucksess et al. 2006**). Mycotoxins contamination of food and feeds remains a worldwide problem, the United Nation Food and Agriculture Organization (FAO) has estimated that up to 25% of the world's food crops are significantly contaminated with mycotoxins (**Jelinek et al. 1989**; **Smith et al. 1994** and **WHO 1999**). Mycotoxins are unavoidable food contaminants even when good agricultural practices are applied.

Crop transfers through international trade have made aflatoxins contaminated food a worldwide problem (**Sherif 2003**).

Nowadays the main mycotoxins of interest are aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, ergot alkaloids and deoxynivalenol (**Hussein** & **Brasel 2001**; **Bhat** & **Vasanthi 2003** and **Cleveland et al. 2003**). Aflatoxins (AFs) are a group of polyketide-derived furanocoumarins, with at least 16 structurally related toxins that have been characterized, these toxins are produced by a number of different Aspergillus species (**CAST 1989**; **FAO 1991**; **Abdelhamid et al. 1993**; **Goto et al. 1996**; **Klich et al. 2000**; **Ito et al. 2001** and **Peterson et al. 2001**). However, in the agriculture commodities, they are primarily produced by *Aspergillus flavus*; *Aspergillus parasiticus* and *Aspergillus nomius* (**Moss 1998**; **Galvano et al. 2001**; **Creppy 2002** and **Oliveira** & **Ferraz2007**). There are four major categories of AFs (AFB1, AFB2, AFG1 & AFG2) all of which occur naturally (**Anonymous 1998**) and according to the research by joint (**FAO/WHO 1996**) AFB1 is usually predominant of mould producing compounds and the most toxic of the four categories (**Bhat** & **Miller 1991** and **Brera et al. 1998**). Meanwhile, AFB1 and AFB2 have assumed the biosynthetic precursor of the other significant members of aflatoxins family (**Dutton et al. 1985**), M1 and M2 are 4-hydroxy derivatives of AFB1 and AFB2 respectively (**Chifiriuc et al. 2010**). Several research workers reported that there is a linear relationship between the amount of AFM1 in milk and AFB1 in feed which is consumed by dairy cattle (**Bakirci 2001**). The half-time of AFMl in milk is relatively short, if the intake of AFBl ceases, AFMl levels in milk are reduced by about 50% within 25-36 hours (**FAO/WHO 1996**) while, decrease to an untraceable level within 72 hours after removal of contaminated source (**Rahimi** & **Karim 2008**). In fact, AFM1 has a high affinity for casein (**Brackett** & **Marth 1982**; **Galvano et al. 1998**; **Baskaya et al. 2006** and **Barbiroli et al. 2007**) and can be detected in milk through 12-24 hours as a direct result of ingestion of food contaminated with aflatoxin B1 by many mammals including dairy cattle and humans (**Stoloff 1977** and **Ghazani 2009**). The conversion rate of aflatoxin B1 to aflatoxin M1 present in milk ranges between 0.5% and 5%, yet values as high as 6% have been reported by **Pipet** (**1998**).

AFM1 recognized as milk toxin exhibits, carcinogenic **(IARC 2002**), genotoxic (**Lafont et al. 1989**) and cytotoxic effects (**Neal et al. 1998**). AFB1 and AFM1 have been classified by the International Agency for Research on Cancer as human carcinogens class 1A (carcinogen) and 2B (probable carcinogen) respectively (**IARC 2002**), because of their potential risks and also to minimize their hazard, the World Health Organization recommends the reduction in its consumption to a minimum, because there is still not enough information available to establish a tolerable exposure level (**WHO 2002**). Fortunately, AFM1 has been found to be about 10 times less carcinogenic than AFB1 (**Lafont et al. 1989**). Researchers from US Division of Epidemiology and Surveillance have articulated that the ingestion of mycotoxin-contaminated animal-based food products could pose a concern to public health (**Hollinger** & **Ekperigin 1999**).

AFM1 is relatively stable in raw and processed milk products, pasteurization, sterilization and ultra-high-temperature (UHT) treatment or processing result in negligible destruction of AFM1 (**Galvano et al. 1996**; **Yaroglu et al. 2005** and **Unusan 2006**). It has been reported that AFM1 was a resistant to thermal inactivation during food processing for procedures such as pasteurization and autoclaving (**Bakirci 2001**) and **Park** (**2002**). This toxin was found in foods in spite of disappearing fungal organisms **(Macdonald** & **Castle 1996**).

To secure safety of foodstuffs, regular monitoring of mycotoxins is necessary and for this purpose the following work is to provide a qualitative overview about aflatoxin M1 residues in marked UHT milk at Najran city, Saudi Arabia.

MATERIAL AND METHODS

Sampling:

A total of 96 samples of different brands full-fat cow's UHT milk, were randomly purchased from different supermarkets in various districts of Najran city in the period from September 2011 to January 2012. The samples were labeled, collected and transported to the laboratory in an insulated container at about 4°C and analyzed before their expiration dates exceeded.

Methods:

The samples were examined for AFM1 using the competitive enzyme-linked immunosorbent assay (ELISA), (RIDASCREEN Aflatoxin M1, R-Biopharm) test kits and the procedures were as described by **Biopharm and Gmb** (**1999**). This method is quick, reliable and cost effective for estimation of AFM1 and has been included in the official collection of test procedures by the German Federal Board of Health. All UHT milk samples were prepared and defattened using the

method outlined in the ELISA kits, as briefly described. The mean lower detection limit of RIDASCREEN® AFM1 test is 0.01 $\mu g/L^{-1}$ for milk.

Statistical analysis:

Analysis of data was performed according statistical programmed of **SPSS** (**1997**).

RESULTS AND DISCUSSION

Data outlined in table, 1 and 2 showed the analysis of 96 samples of commercial UHT whole milk, AFM1 residues were detected in 79 samples (82.30%) of total in different levels as showed in table, (2) with the mean value 0.058±0.0053 $\mu g/L^{-1}$. Whereas, all the positive samples were below the limit permitted by **SASO** (**1996**). Moreover, 17 samples (17.70%) total of UHT whole milk samples were not contain AFM1as recorded in table, (1).

Table, (1): Occurrence and frequency distribution of examined "UHT milk" samples based on their AFM1 concentration (n=96):

Full-fat cow's UHT milk examined samples	Not detected samples	Positive samples	Frequency distribution of examined samples according to their AFM1 concentrations ($\mu g/L^{-1}$)*		Min.	Max.	Mean ± SE
			< 0.20	> 0.20			
Number	17.00	79.00	79.00	00.00	0.01	0.19	0.058±0.0053
Percentage	17.70%	82.30%	82.30%	00.00%			

*According to SASO (1996)

Table, (2): Occurrence and frequency distribution of positive examined "UHT milk" samples based on their AFM1 concentration (n=79):

Range of AFM1($\mu g/L^{-1}$)	No. of positive samples	Percentage*
0.01 - 0.04	24	30.38%
0.05 - 0.09	30	37.97%
0.10 - 0.14	19	24.05%
0.15 - 0.19	06	07.60%
Detected	79	100.00%

*According to 0.2 $\mu g/L^{-1}$ limit; N0. 79 x < 0.20 (100%)

A number of countries particularly developing ones, have established acceptance levels for AFM1, from 0.05 ppb in most European countries to 0.5 ppb in the United States, (**Van Egmond 1989**). (**Chen** & **Gao 1993** and **Van Egmond 1995**) have reported that in case of AFM1 in liquid milk, regulatory limits throughout the world are influenced by economic considerations, degree of development and may vary from one country to another, the Saudi Arabia Standard Organization has laid down the limit for total aflatoxins in liquid milk and its products (except dried milk) as 0.2 $\mu g/L^{-1}$, and for infants and children foods as 0.05 $\mu g/kg^{-1}$, (**SASO 1996** and **Gulf Standard 1997**).

As far as we knew, no available information dealing with the occurrence of AFM1 in UHT milk in Saudi Arabia. Elsewhere, nearly similar findings were reported by **Martins** & **Martins** (**2000**); **Roussi et al.** (**2002**) and **López et al.** (**2003**). While, higher prevalence was revealed by **Kamkar** (**2008**); **Tekinşen** & **Eken** (**2008**); **Gundinc** & **Filazi** (**2009**); **Atasever et al.** (**2010**) and **German et al.** (**2010**). Moreover, lower findings were reported by **Unusan** (**2006**) and **Fallah** (**2010**).

In the light of the above data, it could be noticed that the obtained positive results of this study were below the tolerated level of AFM1 in UHT milk collected from Najran city, Saudi Arabia, that is may be due to locality of Najran city in the south part of Saudi Arabia with a hot climatic to some extent, mostly all over the year. Factors include environmental temperature, humidity and moisture content of the feed as well as pH, affecting AFM1 production by the mold. Consequently, fungi present in haystacks may not easily produce high levels of toxins during storage conditions and following the consumption of contaminated feed with AFB1, conversion of AFB1 to AFM1 which takes place in the liver may not leads to elevated levels of AFM1 in milk **(Sassahara et al. 2005** and **Prandini et al. 2009**).

Regarding the public health significance, mycotoxins attract worldwide attention because of the significant associated with their impact on human health (**CTA 1997**). Mycotoxins in small amount contamination of feed and food is a current problem, however their continuous intake even in microdoses can result in their accumulation in the human bodies, during a long term consumption which can cause a variety of ill human effects ranging from allergic responses to immunosuppressive, mutagenic, teratogenic and carcinogenic effects especially on the liver, probably underestimates the effect of mycotoxins as a cause of human mortality (**Varman** & **Evans 1991**; **El-Shinawy et**

al. 1994; **Peraica et al. 1999**; **Pitt 2000**; **Kocabas** & **Sekerel 2003**; **Kovacs 2004**; **Baskaya et al. 2006** and **Chifiriuc et al. 2010**).Aflatoxin B_1 and aflatoxin M_1 are known as hepatoxins and hepatocarcinogens. Their harmful effects in human, especially infants are vital (**Qian et al. 1984** and **Chu 1991**). In developed countries, aflatoxin contamination rarely occurs at levels that cause notable aflatoxicosis in humans. Epidemiological studies done in Asia and Africa that have demonstrated a positive association between dietary aflatoxins and liver cell cancer (**FAO/WHO 1996**; **Haggag et al. 2001**; **Bhat** & **Vasanthi 2003** and **Sherif 2003**). Severe aflatoxicosis, a pathological condition caused by aflatoxin intake is produced when moderate to high levels of aflatoxins are consumed, acute episodes of disease ensue and may include hemorrhage, vomiting, abdominal pain, alteration in digestion, absorption and/or metabolism of nutrients, stunted growth, pulmonary edema, acute liver damage, convulsions, coma, and possibly death with cerebral edema and fatty involvement of the liver, kidneys and heart. Chronic aflatoxicosis can result from ingestion of low to moderate levels of aflatoxins (**USFDA 2000**). The importance of aflatoxin is emphasized by a recent outbreak of aflatoxicosis in Kenya in April 2004 in which 125 people died **(Lewis et al. 2005**).

Synergistic effects may observe as a result from interaction of aflatoxins with hepatitis B virus in the etiology of liver cancer and could interact with HIV/AIDS **(Montesano et al. 1997** and **FAO 1997-b** & **2001**).

CONCLUTION AND RECOMMENDATION

The results of this study indicate that occurrence of aflatoxin M1 concentrations in UHT milk samples sold in Najran region were slight, probably because of slight to moderate contamination feedstuff consumed by the milking cows. It can be concluded that contamination of aflatoxin M1 in dairy products marketed in Najran city does not appear to be a serious public health problem at the moment, although the presence of this toxin in most sample analyzed. However, frequent analytical surveillance by food control agencies is highly recommended to control the incidence of mycotoxin contamination in Saudi Arabia, especially in dairy products. Implementing a food control system, such as the Hazard Analysis and Critical Control Point (HACCP) system in the food industries **(FAO 2001**), suggest an efficient means for limiting mycotoxin contamination in the Saudi's food supply.

The most effective way of controlling aflatoxin M1 in food supply is to reduce contamination of raw material and supplementary feedstuffs for dairy cattle with aflatoxin B1. Specific regulation exist in many countries **(FAO/WHO 1996**), and practical programs are being developed as the Codex Committee on Food Additives and Contaminants has developed, a code of practice for reducing aflatoxin B1 in raw materials **(Van Egmond et al. 1997**). Reduction can be achieved by good manufacturing practices and good storage practices.

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تقدير تركيزات الأفلاتوكسين إم١ في الحليب البقري كامل الدسم المُعامل بالحرارة الفائقة المُباع للاستهلاك في نجران-السعودية مع بيان الأهمية الصحية

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أجريت هذه الدراسة للكشف عن تواجد مادة الأفلاتوكسين إم١ السامة في الحليب البقري كامل الدسم المُعقم بالحرارة الفائقة (UHT) المُسوق للاستهلاك في مدينة نجران بالسعودية، وذلك باستخدام تقنية الإليزا ELISA (فحص إنزيمات المناعة المرتبطة)، وقد تم إجراء الدراسة في الفترة من سبتمبر ٢٠١١م إلى يناير ٢٠١٢م على عدد ٩٦ عينة عشوائية من حليب الابقار كاملة الدسم المعامل بالحرارة الفائقة بـ UHT، حيث دلت النتائج على تواجد سُم AFM1 في ٧٩ عينة (٨٢,٣٠%) بتركيزات تتراوح بين 0.01-0.19 ميكروجرام لكل لتر، وبمتوسط قدرة 0.058±0.0053 ميكروجرام لكل لتر، ومن ناحية أخرى تبين أن مستويات التلوث بـ AFM1 في عينات الحليب المعقم (UHT) منخفضة ولا تتجاوز الحد الأقصى المسموح به من هيئة التقييس الموحد السعودية، وبالتالي لا تشكل خطورة على صحة المستهلك في مدينة نجران بالسعودية، هذا وقد تم مناقشة النتائج والأهمية الصحية ومدى خطورة وتأثير سُم الأفلاتوكسين إم١ على صحة الإنسان وما يجب أن يُتبع من إجراءات للمساهمة في إنتاج أقل تلوثاً بالسموم الفطرية.

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