

Correlation Between Aflatoxin M1 in Milk and Milk Products in Dairy Animals Fed on Aflatoxin B1 Contaminated Ration

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Abstract

Aflatoxins are cancerogenic compounds produced predominantly by certain strains of the *Aspergillus* spp., Aflatoxin M1 (AFM1) is the major hydroxylation of aflatoxin B1 (AFB1), which is formed when animals ingest feed contaminated with aflatoxin B1. Contamination of milk and dairy products with aflatoxin M1 is a risk for human health. Aflatoxin M1 is relatively stable during milk pasteurization and storage as well as during the preparation of various dairy products. In this study, 80 samples represented by 20 samples of animal feed, 20 of raw milk, 20 of cream and 20 of butter were obtained from suppliers of the dairy company under study. The concentration of aflatoxin B1 and M1 were determined by high pressure liquid chromatography (HPLC).

Presence of AFB1 was detected in animal feed by a ratio of 55% in concentrations ranged between 7.16-69.81ppb with a mean value of 32.06 ± 1.47 ppb. While mean value of AFM1 in samples of raw milk, cream and butter (20 of each), were 30%, 25% and 25% with a mean value of 0.429 ± 0.031 , 0.272 ± 0.015 and 0.053 ± 0.003 ppb., respectively, while the acceptability of positive samples were 70%,75%, and 90%, respectively according to EOS, EC and Codex Regulation.

Key words: - Aflatoxin M1, B1, HPLC, milk and milk product.

Introduction

Milk and dairy products play a significant role in human diet since they are rich sources of bioavailable calcium and protein. However, many of the previous studies indicated the presence of AFM1 at high concentrations in dairy products (**Oruc *et al.*, 2006 and Aydemir *et al.*, 2010**).

Polluted milk and dairy products with aflatoxin M1 varies between 40% and 60% and is given by the presence of aflatoxin B1 in feed and raw materials used for the manufacture of feed which are intended for consumption by cattle, what has become an issue with sufficient global significance. There is a lot of evidences

indicating that chronic exposure to these toxins induces the production of cancer cells and subsequently public health complications, especially when it says that 20 to 50% of all cancers are related to factors diet. This toxin along with hepatitis B is considered as risk factors in China and North Africa, an estimated 250,000 deaths annually. According to FAO (**Food and Agriculture Organization, 2003**), over 25% of world production of cereals and raw materials for human and animal consumption, are contaminated with some kinds of toxin of fungal origin. In the United States, it has been estimated that economic losses caused by mycotoxins such as aflatoxin and fumonisin, are approximately 932 million dollars each year (**Van Egmond, 1989**).

A. parasiticus produces four major aflatoxins: B1, B2, G1 and G2, while AFB1 is the most toxic in the group and the toxicity is in the order of B1 > G1 > B2 > G2 (**Galvano et al., 1998**). Aflatoxin M1 is a major metabolite of aflatoxin B1 (AFB1), which is formed when animals ingest feed contaminated with aflatoxin B1. These metabolites are not destroyed during the pasteurization and heating process (**Brackett and Marth 1982 and Oruc et al., 2006**). The amount of AFM1 in milk depends on several factors, such as animal breed, lactation period and mammary infections. AFM1 could be detected in milk 12-24 hours after the AFB1 ingestion, reaching a high level after a few days. When AFB1 intake is stopped, AFM1 concentration in milk decreases to an undetectable level after 72 h (**Pittet, 1999 and Creppy, 2002**).

Evidence exists for the role of AFs in causing genetic mutations and primary liver cancer in human populations by modifying the structure of liver cells DNA. Aflatoxins are activated by oxidation into the epoxide form that reacts with DNA to form an N7 guanine adduct that produce chronic effects from ingestion of contaminated food (**Günsen and Büyükyörük, 2002**).

Temperature and moisture contents also affect the presence of aflatoxin B1 in feeds, *A. flavus* and *A. parasiticus* can easily grow in feeds having moisture between 13% and 18% and environmental moisture between 50% and 60%, furthermore, they can produce toxin (**Jay, 1992**) and the fungal toxins are very resistant, supporting more than 200°C of temperature, so sometimes it is commonly found when the fungus is destroyed (**Gingins, 2006**).

It is estimated that approximately 1 to 3% of the AFB1 initially present in animal feedstuff appears as AFM1 in milk (**Elgerbi et al., 2004**); cheese usually contains a higher concentration of AFM1 than the milk from which it is made. Association of AFM1 with casein seems to be the cause of this behavior. Currently available research results further show that the concentration of AFM1 is 2.5 to 3.3 fold higher in cheeses than that in milk from which these cheeses were made (**Kamkar et al., 2008**).

The residues of AFM1 remain soluble when milk is processed by heat or is fermented, during butter processing, protein membrane around fat globules is broken down and serum phase is separated. Due to the chemical structure of AFM1 and its affinity to casein, it was adsorbed on this fraction of protein (**Yousef and Marth, 1989**). Therefore, cream contained less AFM1 than milk, and butter contained less amount of AFM1 than cream. As a result of the associate effects of these factors, AFM1 concentration occurs in lipid phase (like butter and cream) was less than serum phase and protein fraction (**Blanco *et al.*, 1998 and Mohammadi, 2011**).

The parameters affecting levels of AFM1 contamination in milk are highly variable depending on the source of animal feeds, ecologic and economic factors in the farm, and also farm management. It seems that the kind of the animal feed and the harvesting time and temperature could be effective parameters in this regard (**Kuiper, 1999 and Tajkarimi *et al.*, 2007**).

As both aflatoxins B1 and M1 may cause cancer in humans, the risk posed by aflatoxins has been faced in different ways in different countries; several countries have established legislation to regulate the levels of mycotoxins to protect consumers (**Rastogi *et al.*, 2004 and Sarimehmetoglu *et al.*, 2004**). Codex Alimentarius prescribe that the maximum level of AFM1 in liquid milk and dried or processed milk products should not exceed 50 ng/kg (0.05ppb) (**Codex Alimentarius Commissions, 2001**).

In Europe, the maximum tolerated levels of AFM1 in milk and dairy products were regulated firstly by Reg. CE 2174/2003 (**EC, 2003**) that modified Reg.CE 466/2001 (**EC, 2001**) and then by Reg. 1881/2006 (**EC, 2006**) In accordance with these norms, the product to be screened is milk, in which the AFM1 concentration must not exceed 0.05 µg/kg (= 0.05ppb = 50 ppt), while dairy products must be obtained using milk conforming to the above AFM1 limits and standardized the maximum level of AFM1 in infant milk to be under 25 ng/l (**EC, 2006**).

However, there are thus differences in maximum permissible limit of AFM1 in various countries, It is much higher than those adopted in other states: 0.25 µg/kg in Switzerland and Iran and 0.20 µg/kg in Holland 0.020 µg/kg for butter in Netherlands (**Cavaliere *et al.*, 2006**), and in Turkey 0.50 µg/kg for milk and 0.25 µg/kg for cheese (**TFC, 2008**) while in France 0.03 for children's milk and 0.05 µg/kg for adult's milk (**Creppy 2002; Tekinsen and Eken, 2008 and Kamkar *et al.*, 2008**), according to US regulations the level of AFM1 in milk should not be higher than 500 ng/kg (**Stoloff *et al.*, 1991**).

The aim of the present work was to investigate the presence of AFB1 in animal feed and AFM1 in raw cow milk, cow cream and cow butter samples obtained from suppliers of the dairy company under study by using HPLC.

Material and Methods

This research is descriptive. The sampling was carried out for detection of the concentration of aflatoxin B1 and M1 in raw milk and feed for livestock consumption. Stage data were organized in the forms of acceptance (forms filled out by the owners of herds that agreed to participate in the study) creates subsequently conducted an inspection of the storage in herds for which they filled out the health inspection form. In the second phase proceeded with the test sample of 250g of the food field .

The samples were transported to the laboratory (Fac.Vet.Med., Benha University) . Then processed the samples obtained, 500ml of each (as required by the toxicology laboratory for HPLC analysis) where they were processed by HPLC technique for determination of aflatoxin M1. The limit of quantification LOQ of the technique was from 5ng/L-1 and the limit of detection (LOD) was less than 5ng/-1 concentration.

Qualitative and quantitative estimation of aflatoxins

1- Chemicals:

Standard and Blank aflatoxins B1 and M1 were purchased from Sigma Chemicals Co. St. Louis, MO. It was diluted in benzene:acetonitrile, chromatographic grade. Benzene came from Tedia (Fairfield, OH, USA) and acetonitrile from Merck (Darmstadt, Germany). Acetone, HPLC grade, employed for the extraction of aflatoxin M1 came from Merck (Darmstadt, Germany). The methanol, HPLC grade, used for the preparation of the mobile phase and elution of aflatoxin M1 in the immunoaffinity column came from Carlo Erba (Rodano, Milan, Italy). The water used in the analytical process was obtained through a Milli-Q purification and filtration system with an 18 M cm-1 resistivity (Millipore, Bedford, MA, USA). The present study used Easi-Extract Aflatoxin immunoaffinity columns, Product code RP71/70N, R-Biopharm Rhône, and Glasgow, Scotland. Column storage took place at a temperature ranging from 2 and 8° C and they were used at room temperature. The entire glassware used for aflatoxin determination was decontaminated by Alkaline Extran MA 01, 7555 (Merck, Darmstadt, Germany) at 20%, (pH > 12), remaining in contact for 24 hours and further washing with distilled water.

2- Standard Aflatoxins M1 (AFM1) solutions

The stock standard solutions of AFB1 and AFM1 were prepared by dissolving the solid standard in benzene:acetonitrile (98:2, v/v). The precise concentration was measured in Shimadzu UV-1601 PC spectrophotometer, as described by AOAC (2000). An intermediate standard solution from the stock solution was prepared in benzene: acetonitrile (98: 2, v/v) in a concentration of 9.855

ng ml⁻¹. This solution was utilized for the elaboration of a calibration curve in the range 0.1–9.8 ng/ml. All the solutions were packed in amber vials at -18°C.

3-Extraction and clean-up procedures for high-performance liquid chromatography (HPLC) analysis:-

The prepared samples were analyzed using a validated method by reversed-phase HPLC separation and fluorescence detection after post-column derivatization (Shundo and Sabino, 2006).

4- Determination of AFM1 and AFB1 by HPLC method:-

The presence of aflatoxins B1 and M1 detected by HPLC after post-column derivatization with the electrochemical generation of bromine (KOBRA cell – Rhone diagnostic technologies, UK) with a current of 100 µA and a fluorescence detector (Shimadzu LC-10 AD Model; 360 nm excitation wavelength; 435 nm emission wavelength; with Shim-Pack CLC – ODS column, 5 µm, 4.6 × 250 mm, preceded by a guard column Shim – Pack G – ODS, 5 µm, 4 × 10 mm). The mobile phase was deionized water-acetonitrile-methanol (60:20:20, v/v/v) with the addition of 350 µL of 4M HNO₃ and 120 mg of KBr at a flow rate of 1 ml/min. The injection volume was 50 µl. The quantification of aflatoxin was performed by measuring its peak areas at each retention time (23.4 min.) and comparing it with the calibration curve (Galvano *et al.*, 2001). The performance of the method, aflatoxin recovery and effectiveness of the cleanup procedure, was evaluated by the samples spiked with this aflatoxin, in duplicate, at level of 2.96 µg/kg.

Results and Discussion

Aflatoxins are toxic metabolites, generally produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. They can have immunosuppressive, mutagenic, teratogenic and carcinogenic effects, especially on the liver (Peraica *et al.*, 1999; Creppy, 2002; and Baskaya *et al.*, 2006). The most common are B1, B2, G1, G2 which are ingested by animals in contaminated pellets and forage; aflatoxin B1 (AFB1) is notoriously the most toxic of these metabolites. When ingested by dairy animals, the metabolite is biotransformed at the hepatic level by microsomal cytochrome P450 into AFM1 (Battacone *et al.*, 2005). Then it is excreted in this form in milk used for human consumption and, thanks to its affinity for casein, is also present in dairy products (Galvano *et al.*, 2001 and Lopez *et al.*, 2001), technology used in the production process and the water content in the final product also affect the presence of aflatoxin in milk product (Blanco *et al.*, 1998 ; Pittet, 1999 ; Lopez *et al.*, 2001 and Baskaya *et al.*, 2006).

In the present study recorded in **Table (1)** aflatoxin B1 (AFB1) was detected by 55.0% in the examined animal feed samples which ranged from 7.17 to 69.81 ppb with a mean value of 32.06 ± 1.47ppb. The European Community has reduced

the maximum admissible level of AFB1 in complete feeds for dairy cattle to 10 µg/kg ,as Lactating cows that eat feed containing 20 ppb or more aflatoxins may produce milk that exceeds the tolerance level for aflatoxins in milk (**EC,2003**).

The excreted amount of aflatoxin M1 in milk of dairy cows was estimated to represent 1-2% of ingested aflatoxin B1 (**Van Egmond 1989**). The extent of transfer from feed to milk (carry –over) is influenced by various nutritional and physiological factors including animal health, hepatic biotransformation capacity and for individual animals, from day to day, and from one milking to the next. In high-yielding cows, the consumption of significantly higher amounts of concentrated feeds might result in carry-over percentages as high as 6.2% (**Veldman et al. 1992**).

Several authors have tried to determine whether the current legislation on aflatoxin B1 in feed (2002/32/EC (OJL 140, 30.05.2002)) for lactating animals is sufficient to keep aflatoxin M1 levels in milk below the threshold of 0.05 µgKg⁻¹. **Pettersson(1998)** has established a model calculation to determine the carry-over of ingested aflatoxin B1 to aflatoxin M1 in milk and expressed as Aflatoxin M1(ng kg⁻¹ milk) = 10:95 0:787 × (µg aflatoxinB1 intake day⁻¹)

While grain with high levels of aflatoxin can be used for ethanol production and it is illegal to sell grain with levels greater than 20 ppb aflatoxin for lactating dairy cows, and the seller of the grain is responsible for damage resulting from the sale of grain. As a general rule, maintain grain in storage at low humidity and low temperatures, usually below 40 degrees F if weather permits and the grain or related feed consumed by cows should be subjected for scanning (**Harper, 2003**).

While aflatoxin M1 (AFM1) in raw cow milk (**table 1**) was detected by 30% and ranged from 0.176 to 0.593 with mean value of 0.429 ± 0.031 ppb, the incidence was 70% of less than 0.050 ppb and was accepted according to maximum permissible limit (MPL) stipulated by (**EC, 2003 ; CAC, 2001 and EOS, 2010**) , while 30% of +ve samples were unaccepted and ranged from 0.05 to 0.200 ppb by 5%, 0.200 to 0.35 by 5% , 0.35 to 0.5 by 5% and 15% of more than 0.50ppb (**Table 2, 3**).

The incidence of AFM1 in milk and AFB1 in animal feed give a significant positive correlation represented by (0.68) .This was agreed with **Heshmati and Milani (2010)** They showed that the rate between the amount AFB1 ingested by cattle and the quantity of AFM1 excreted in milk is usually 0.32- 6.2%, while estimated by **Elgerbi et al., (2004)** by 1 to 3%, while in this study it was 1.34%.

According to **table (2)**, 15% of +ve examined milk samples was over above 0.5 ppb aflatoxin, the grain should be replaced; if AFM is below 0.5 ppb aflatoxin or has declined markedly. Records should be maintained for all feeds, feeding practices, milk contamination and animal health and performance for all cases of aflatoxin contamination of milk (**Pietri et al., 1997 and Shundo and Sabino, 2006**).

In cow cream **table (1)** AFM1 was detected by 25% and ranged from 0.121 to 0.481 ppb with a mean value of 0.272 ± 0.015 ppb and acceptability by 75% according to (CAC, 2001 ; EC, 2003 and EOS, 2010) who stipulated 0.05% ppb for milk product as MPL, in which the frequency distribution of AFM1 in cow cream was 75% less than 0.050 ppb ,10% from 0.050 to 0.200ppb ,10% from 0.200 to 0.350 ppb while 5% was from 0.350 to 0.500 ppb (**Table 2**) , the ratio of AFM1 in the positive cream samples made from contaminated milk was 63.4% which was agreed with **Bakirci (2001)** who investigated the levels of AFM1 in the products made from contaminated milk namely butter, butter milk, cream, skim milk. The mean AFM1 level found in cream samples was 64.4% of AFM1 concentration of bulk-tank milk. Whereas, mean AFM1 level of skim milks was 3% higher than those of bulk-tank milk. Levels of AFM1 in butter samples in his study were less, and they were as 33.80% of AFM1 amounts of bulk-tank milk.

According to **table (1)** the incidence of AFM1 in cow butter was detected by 25% of examined samples and was ranged from 0.027 to 0.075 ppb with a mean value of 0.053 ± 0.003 ppb, this examined +ve samples was accepted by 90% **Table (3)** according to (CAC, 2001 ; EC, 2003 and EOS, 2010) as MPL not exceeded 0.05% in which , 90% less than 0.050 ppb and 10% from 0.050 to 0.20 ppb, **table (2)**, and the ratio of AFM1 in cow butter made from contaminated milk was 12.3% in this study and was less than **Bakirci, (2001)** which was 33.80% , and this could be attributed to the butter processing in which protein membrane around fat globules is broken down and serum phase is separated and due to the chemical structure of AFM1 and its affinity to casein, it was adsorbed to this fraction of protein. Therefore, cream contained less AFM1 than milk, and butter contained less amount of AFM1 than cream. As a result of the associate effects of these factors, AFM1 concentration occurs in lipid phase (like butter and cream) less than serum phase and protein fraction, (**Mohammadi, 2011**).

In many countries of Europe, the low level of aflatoxin M1 have been found may be due to stringent regulation of aflatoxin B1 in complementary feedstuffs for dairy cattle. But almost 99% of contaminated samples exceeded the European communities and Codex Alimentarius recommended limits (**EC, 2003**). Some surveys conducted on the occurrence of AFM1 in milk and its products as reported by **Hussain and Anwar (2008)** revealed that all of the raw milk samples examined from 14 districts of Punjab, Pakistan the contamination with aflatoxin M1 reached 99.4% more than European Union limit (0.05 mg/l). **Dashti, et al. (2009)** showed that all fresh milk samples except one from Kuwaiti markets were contaminated with aflatoxin M1 ranging from 4.9 to 68.7 ng/L, but 8 samples exceed maximum tolerant limit. **Elzupir and Elhussein (2010)** analyzed 44 bulk milk samples in Khartoum state. The percentage of AfM1 contamination has been found in these samples were 42/44 (95.45%) with contamination level ranging between 0.22 and

2.07mg/L-1. The contamination of AfM1 in these samples was expected as it is consistent with the presence of AFB1 in feed. Therefore, dairy cattle AFB1 exposure must be reduced by (GMP) good manufacturing practices (**Elzupir and Elhussein, 2010**).

On the other hand **Abdallah, et al., (2012)** 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 health significance. 96 samples of different brands were randomly punched from different supermarkets, AFM1 residues were detected in 79 samples (82.30% of total). Data also indicated that AFM1 residues concentrations detected in all the positive samples were below the tolerated level of AFM1. This finding agreed with **Mahdavi, et al. (2010)** in Iran who established the local milk as a main source of AFM1 exposure for lactating women. Whereas in Egypt, raw milk was recurrently a cause of many public health problems due to the lack of the hygienic measures and investigations. The consumers are depending only on heat treatment of this milk; however AFM1 is resistant to thermal inactivation (**Park, 2002**). Therefore, raw milk may be regarded as a serious risk factor for AFM1 exposure. In spite of the significance of promoting the sanitary measures of raw milk, the animal feed should be free of fungal growth especially in current screened area which has high temperature and humidity conditions.

In Italy, after the outbreak of maize contamination by AFB1 that occurred in 2003, and the subsequent finding of AFM1 in milk (**Giorni et al., 2007**), the Ministry of Health established a maximum permissible value of 0.45 µg/kg (= 0.45 ppb=450ppt) of mycotoxin in hard, long term ripened cheeses. This value, although considered at the time as an interim measure to deal with the crisis, has not since been updated and some authors have used it as the threshold value for AFM1 contamination in cheeses (**Oruc, et al., 2006**).

It is too difficult to compare the data from the literature due to wide differences between and within the countries related to animal feeding and environmental factors, extraction and analysis procedures, and regulatory limits for aflatoxins in feeds and milk.

Many authors showed that seasonal effect influences concentration of aflatoxin M1. They reported higher concentration of AFM1 in cold seasons as compared to hot seasons the reason being in winters mostly milking animals are fed with compound feeds and thus concentration of aflatoxin B1 increases which in turn enhances AFM1 concentration in milk. Moreover, temperature and moisture contents also affect the presence of aflatoxin B1 in feeds. *A. flavus* and *A. parasiticus* can easily grow in feeds having moisture between 13% and 18% and environmental moisture between 50% and 60% (**Giorni, et al., 2007** and **Fallah, 2010**).

Shortly, the results of this study showed that there was a risk from milk, cream and butter from animals fed with AFB1 contaminated feed, since all the age

groups including infants and children consume milk and dairy products daily. Therefore the prevention of aflatoxin formation in feeds is very important. Avoiding contamination appears to be the only practical way to ensure the safety of milk and milk products for human consumption. For this reason, it is considered that food substances should be produced from healthy raw material and kept in convenient conditions to prevent aflatoxin formation.

It is necessary to use potentially sensitive analysis (HPLC), which determined AFM1 concentration in raw milk from different herds suppliers of a dairy company in order to correlate the presence of the fungus *Aspergillus flavus* in dairy cattle feed, so that preventive measures are taken regarding the collection and storage conditions and at the same time warn companies of the responsibility that comes with free milk products that have not undergone a proper process of traceability. Controlled continuously for presence of AFM1 contamination (*Dashti, et al., 2009*).

Table (1): Incidence and concentrations of aflatoxins (ppb) in the examined samples of animal feed “AFB1”, cow milk and its products “AFM1” (n=20).

Aflatoxins	+ve samples		Min	Max	Mean± S.E*	Correlation coefficient
	No.	%				
<u>Aflatoxin B1:</u> Animal feed	11	55	7.16	69.81	32.06 ± 1.47	
<u>Aflatoxin M1:</u> Cow Milk	6	30	0.176	0.593	0.429 ±0.031++	+ 0.68**
Cow cream	5	25	0.121	0.481	0.272 ±0.015	+ 0.61
Cow butter	5	25	0.027	0.075	0.053 ±0.003	+ 0.56

S.E*= Standard error of mean

++ = High significant differences (P<0.01)

+ = Significant differences (P<0.05)

** = Significant positive correlation between AFB1 in animal feed and AFM1 in milk and its products

Table (2): Frequency distribution of aflatoxins AFM1 ppb in the +ve examined samples of milk, cream and butter.

Samples Range	Cow milk		Cow cream		Cow butter	
	No	%	No	%	No	%
≤ 0.050	14	70	15	75	18	90
0.050 > 0.200	1	5	2	10	2	10
0.200 > 0.350	1	5	2	10	0	0
0.350 > 0.500	1	5	1	5	0	0
> 0.500	3	15	0	0	0	0
Total	20	100	20	100	20	100

Table (3): Acceptability of the examined samples of cow milk and its products based on their levels of aflatoxin M1 (n=20).

Milk products	MPL (ppb)	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Cow milk	0.05*	14	70	6	30
Cow cream	0.05*	15	75	5	25
Cow butter	0.05*	18	90	2	10

*MPL: Maximum Permissible Limit stipulated by EOS (2010); CAC (2001) and EC (2003).

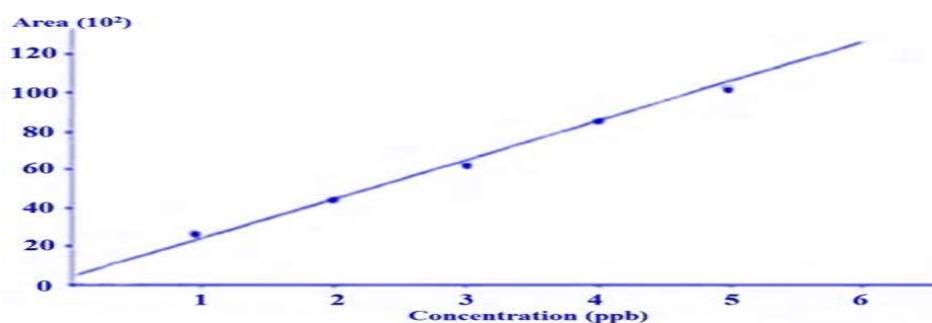


Figure (1): Calibration curve of aflatoxin M1 by using HPLC

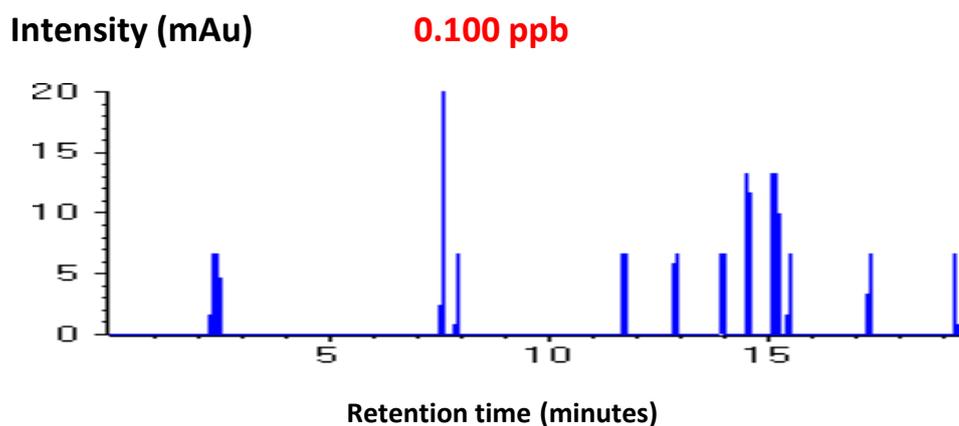


Figure (2): HPLC chromatogram of aflatoxin M1 in the examined cow milk sample (Standard)

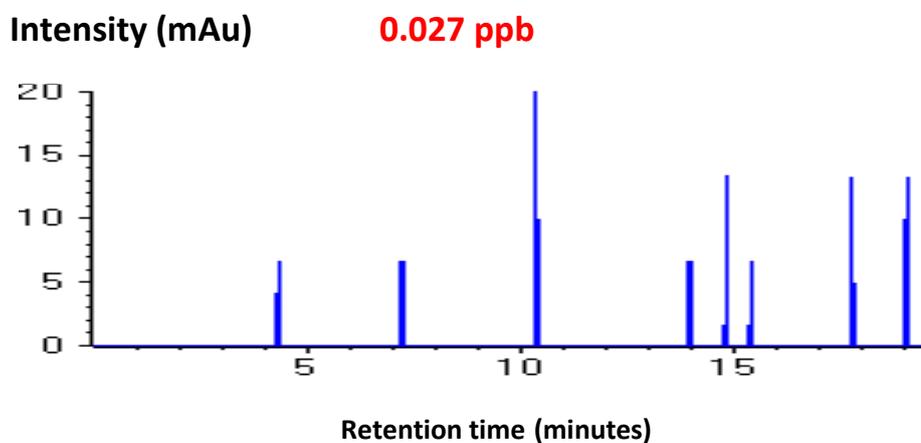


Figure (2): HPLC chromatogram of Aflatoxin M1 in the examined cow milk sample (Positive)

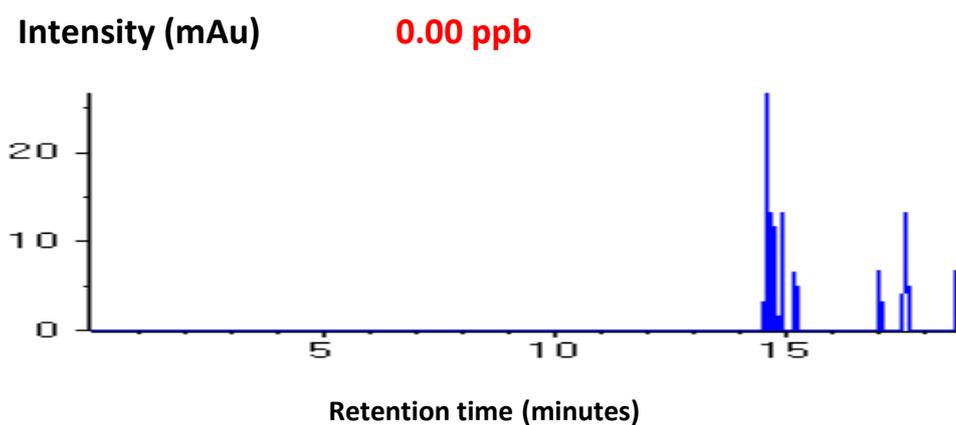


Figure (3): HPLC chromatogram of Aflatoxin M1 in the examined cow milk sample (negative)

Conclusion and recommendations

In response to the above, there is positive relationships between AFB1 in animals feed and AFM1 in milk, cream and butter, and has shown the serious risk for public health since all age groups, including infants and children, consume milk worldwide. So it is also extremely important to maintain low levels of AFB1 in the dairy animals feed. In order to achieve this, dairy cow feeds should be kept away from contamination as much as possible. However, frequent analytical surveillance by food control agencies is highly recommended to control the incidence of Mycotoxins contamination, especially in dairy products. Implementing a food control system, such as the Hazard Analysis and Critical Control Point (HACCP) system in the food industries. Control storage insects and check grain every 2 weeks in storage, also antifungal agents can be applied to grain.

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