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Seasonal pattern of aflatoxin M1 contamination in buffalo milk

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Abstract

The purpose of this survey was to evaluate the natural occurrence and content of aflatoxin M1 (AFM1), in buffalo milk samples. During June to December 2012, 90 samples were collected from North West region of Iran. AFM1 contents were determined by the competitive enzyme-linked imunosorbent assay (ELISA) technique. AFM1 was found in 54.4% of the samples by average concentration of 38.5±5.12 ng/L. The concentration of AFM1 in all of the samples were lower than Iranian national standard and FDA limit (500 ng/L), but in 16.3% of milk samples was higher than maximum tolerance limit accepted by European Union/Codex Alimentarius Commission (50 ng/L). The level of contamination in winter milk samples was significantly ($P < 0.05$) higher than in summer milk samples. The results indicated that the contamination of the samples with AFM1 in such a level could be a serious public health problem at the moment.

Keywords: Water buffalo, Milk, Aflatoxin M1, ELYSA.

1. Introduction

Aflatoxins are mycotoxins of major concern to the dairy industry and AFM1 is a main metabolite of aflatoxin B1 (AFB1), which is formed when animals ingest feed contaminated with AFB1. These metabolites are not destroyed during the pasteurization and heating process, the amount of AFM1 which is found in milk depends on several factors, such as animal breed, lactation period, mammary infections etc [1,2]. Many researchers showed that the relationship between amount of AFB1 ingested and AFM1 excreted in milk varies between 1% and 2% and it can be as high as 6% in high milk-producing cows [3]. High levels of AFM1 in milk and other dairy products are considered undesirable because it has toxic, teratogenic and carcinogenic properties [4].

Many countries have established regulations to control the levels of AFB1 in feeds and to have maximum permissible levels of AFM1 in milk to reduce this hazard [20]. The European Community and Codex Alimentarius Commission prescribed that the maximum level of AFM1 in milk and milk products should not exceed 50 ng/L [5]. Since AFM1 is excreted in the milk it may subsequently contaminate other dairy products such as cheese and yoghurt. Note that the one of the major areas buffalo breeding in Asia is North West region of Iran and this area has a favorable climate for the growth of toxigenic fungi [6-8]. Information on the occurrence of AFM1 in buffalo milk in Iran is limited. Few studies [7] have been conducted to determine the contamination level of AFM1 in buffalo milk.

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The present study describes the AFM1 contamination in buffalo milk samples produced in North West region of Iran.

2. Materials and Methods

2.1. Sampling

A total 90 samples of raw buffalo milk was obtained from different areas in North West region – Iran. Samples were collected throughout 2012, during June –September (summer indicator) and November – Jan (winter indicator). Sampling scheme was based on the relative volumes of milk for better precision and containing 45 samples in each season and 15 samples for each month.

All of the samples were transported at 2–4 °C in the icebox to the laboratory.

2.2. Method for analysis of AFM1

The quantitative analysis of AFM1 in the milk samples was performed by competitive enzyme immunoassay using Euroclone_ Aflatoxin M1 Elisa kit (Quantative EuroClone Aflatoxin M1, Cod. EEM005096. LOT. AM11110V).

2.3. Preparation of milk samples

Preparation of milk samples was conducted according to the instructions of kit. Milk samples were chilled to 10 °C and then centrifuged at 2000g for 5 min. The upper creamy layer was completely removed by aspirating through a Pasteur pipette and from the lower phase (defatted supernatant) 200 µL was directly used per well in the test.

2.4. ELISA test procedure

ELISA test procedure was conducted according to the instructions of kit. 200µl of standard solutions (were provided in 0, 5, 10, 25, 50 and 100 ng/L concentrations) and prepared samples were added into separate microplate wells and incubated for 30 min at room temperature (20–25 °C) in the dark. The liquid was then poured out and the wells were washed with washing buffer (250 µL) thrice.

In the next stage, 200 µLof the diluted enzyme conjugate was added to the wells, mixed gently by shaking the plate manually and incubated for 15 min at room temperature in the dark. Again, the wells were washed thrice with washing buffer. Afterwards, 200 µL of substrate/chromogen was added, mixed gently and incubated in the dark at room temperature for 15 min. Finally, 50 µL of the stop reagent was added into the wells and the absorbance was measured at k = 450 nm in ELISA plate reader against air blank within 15 min. According to the Euroclone AFM1kit guidelines, the lower detection limit is 5ng/L for milk.

3. Statistical Analysis

The statistical methods used in this study were based on normal confidence intervals and analysis of variance (ANOVA). The levels were considered significantly different at $P<0.05$.

4. Results

The data obtained from chemical analysis of buffalo milk is given in Table 1. The mean of fat (7.15%) and protein (4.77%) milk samples during winter season was significantly higher than of mean fat (6.25%) and protein (4.04%) during summer season ($P<0.05$).

The results of the analyses of AFM1 level (ng/L) in water buffalo raw milk are shown in Table1. The presence of AFM1 was observed in 54.4% of all samples. The overall mean level of AFM1 in the samples was 38.5±5.12 ng/L (Table2). However, none of the samples was higher than the maximum tolerance level of AFM1 in liquid milk regarding Iranian national standard, and FDA standard (500 ng/L), [9]. But 8 (16.3%) of samples was higher than maximum tolerance limit accepted by European Union (EU) and Codex Alimentations Commission (50 ng/L), (European Commission [10].

Table 1. Chemical composition of buffalo milk

Parameter		Mean±SD			
		pH	Fat (%)	Protein (%)	Lactose (%)
Seasons	Summer	6.53±0.12^a	6.25±0.29^a	4.04±0.10^a	4.80±0.10^a
	Winter	6.79±0.22^a	7.15±0.18^b	4.77±0.13^b	4.83±0.16^a

Means ± SD in the same column with different letters are significantly different ($P<0.05$).

Table 2. Occurrence of aflatoxin M1 level in water buffalo milk collected in North West Region, Iran

Sample size (n)	Positive samples* n (%)	Min–Max	Concentration (mean ±SD) (ng/l)	Exceed legal limit n (%)	
				ISIRI and US FDA**	EC***
90	49 (54.4%)	8.4-90.3	38.5±5.12	0	8 (16.3)

* >5 ng/LAFM1; ** Institute of Standards and Industrial Research of Iran (ISIRI) and US Food and Drug Administration (US FDA) limits for AFM1 in milk are 500 ng/L.; *** European Commission (EC) limit for AFM1 in milk is 50 ng/L.

Table 3. Comparison between samples obtained in winter and summer seasons

	Winter	Summer
Sample size	45	45
AFM1 Concentration (mean ±SD) (ng/l)	45.79±1.2[a]	29.67±2.1[b]
Positive samples n (%)	30 (66.7%)[a]	19 (42.2%)[b]
Exceed legal limit n* (%)	6 (20%)	2 (10.5%)

* European Commission (EC) limit for AFM1 in milk is 50 ng/l.
Means ± SD in the same row with different letters are significantly different (P<0.05).

Statistical analyses of the data show that the percentage of AFM1 contamination in winter water buffalo milk (66.7%) is more than the summer milk samples (42.2%). Also the mean concentration of AFM1 in winter milk samples (45.79±1.2 ng/L) was significantly (P <0.05) higher than those obtained in summer (29.67±2.1 ng/L) (Table3).

5. Discussion

There is abundant production of milk in North West region of Iran and one of the major sources for milk production in this area is water buffalo. Therefore, the purpose of this study was to determine the AFM1 contamination in the milk of water buffalo in North West region of Iran.

Milk composition is shown in Table 1 and the differences between two seasons was found statistically significant (P<0.05). It is important to realize milk composition depends on a variety of factors including species, genetic variation, lactation period, individual animal variability, animal nutrition, seasons and type of feed consumed [3]. Low amounts of fat and protein content of milk in summer could be due to greater secretion of prolactin during long periods of light in this season [11]. In addition, composition of diet may affect the different components amount of milk. The diet formula used during these two seasons were completely different, therefore feeding a diet based on low fiber and high grain in winter probably may have led to increased milk fat levels [12].

Screening survey to determine the occurrence of AFM1in row and pasteurized cow milk was done in many difference region of Iran [13,14], But few studies reported AFM1 contamination in water buffalo milk samples from Iran, in this field, Rahimi et al. (2010) [7] assessment the Occurrence of aflatoxin M1 in raw milk from cow, water buffalo, camel, sheep, and goat in Ahvaz (Iran) by competitive ELISA technique and showed that AFM1 was found in 42.1% of the samples by average concentration of 43.3 ± 0.80 ng/kg. The incidence rates of AFM1 in raw cow, water buffalo, camel, sheep, and goat milks were, 78.7%, 38.7%, 12.5%, 37.3%, and 27.1%, respectively. The concentration of AFM1 in all of the samples were lower than Iranian national standard and FDA limit (500 ng/l), but in 36% of raw cow milk, 8% water buffalo milk, 3.9% sheep milk, and 5.7% raw goat milk samples were higher than maximum tolerance limit accepted by European union/Codex Alimentarius Commission (50 ng/L) [7].

Our results indicate that AFM1 was found in 54.4% water buffalo milk samples by average concentration of 38.5 ng/L (Table 3).

The low presence of AFM1 in water buffalo milk is probably related to the fact that these species in Iran are mainly fed by grazing and they are fed on stored grains for three–four months only. Moreover, cotton-seed cake, corn, and concentrated feed are not used

for these species in this area and these kind of feed commodities are the major sources of AF contamination. Whereas, cows fed on manufactured feed stuffs made of various stored grain products, by products of agricultural industry. Also, use of dry bread for fed of cows in small and traditional herds is usual in this area. That is prone to fungal infection and to subsequent contamination with aflatoxins during storage. The lower presence of AFM1 in buffalo milk than in cow milk could be given by data in the literature. Some authors have shown that strains of *Lactobacilli* (present in higher concentrations in buffalo than in cow milk) are able to bind AFB1 and AFM1, thus decontaminating the milk secreted [3].

The incidence rates of AFM1 in raw milks was 66.7% and 42.2% in winter and summer season respectively, Also the mean concentration of AFM1 in winter milk samples was significantly ($P < 0.05$) higher than those obtained in summer (Table3). In view of the high levels of AFM1 in milk detected by us (especially in winter), and according to numerous authors, a seasonal effect influences AFM1 occurrence. Higher incidence of AFM1 contamination during cold seasons than hot ones has been expressed by many researchers [16-19]. Because in winter cows fed diets containing high levels of AFB1and a relationship between AFM1 occurrence level in milk and AFB1 content of feed was reported [15-17].

Out-pasturing of milking cows was the most important factor in the low levels of aflatoxin in milk in summer and spring seasons [15-17] and findings of these researchers also demonstrated low levels or absence of AFM1 in the summer season. Therefore it is possible to say that the results obtained in present study were parallel to the results of prior Studies.

6.Conclusion

High levels of AFM1 in milk and other dairy products are considered undesirable because it has toxic, teratogenic and carcinogenic properties.

Considering the results, this survey revealed that AFM1 contamination in buffalo milk samples from North West region – Iran. Therefore, consumption of this type milk could be a serious public health problem at the moment.

Reducing the levels of AFB1 in animal feedstuffs by improved processing and storage practices can be the initial approach to deal with this problem.Furthermore, it is important to inspect and control milk, dairy products and animal feed for presence of aflatoxins in a regular manner to evaluate the hygienic managements.

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Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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