We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

An Assessment and Control of AFM₁ in Milk and Main Dairy Products in Lahore, Pakistan

*Umaar Afzal Gill, Aneela Zameer Durrani
and Muhammad Usman*

Abstract

The main objective of this study is to investigate the presence of Aflatoxin M₁ (AFM₁) in local and processed milk and main dairy products available in Lahore. Total 60 milk samples and 120 samples of dairy products including butter (n = 30), cheese (n = 30), cream (n = 30), and yogurt (n = 30) were collected. Milk samples were collected from 3 different sources i.e. unprocessed milk from local milk shop (n = 20) and a local dairy farm (n = 20), and processed milk sample from a commercial shop (n = 20) while samples of each dairy product were also different i.e. processed (n = 15) and unprocessed (n = 15). Milk samples were analyzed using kit method while dairy product samples were analyzed by high performance liquid chromatography (HPLC) technique equipped with fluorescence detector (HPLC-FLD) followed by immunoaffinity column clean up. In second phase of the study, efficacy of three different toxin binders was compared and analyzed. The results showed that AFM₁ was detected in 16.7% of processed butter samples, 33.3% of processed cheese samples, 13.3% of local cream samples and 26.6% of processed yogurt samples and these samples exceeds European Union (EU) permissible limits of 0.05 ppb with mean concentration $0.090 \pm 0.180 \mu\text{g/kg}$ and $0.000 \pm 0.000 \mu\text{g/kg}$ for processed and local butter samples, $0.350 \pm 0.606 \mu\text{g/kg}$ and $0.000 \pm 0.000 \mu\text{g/kg}$ for processed and local cheese samples, $0.000 \pm 0.000 \mu\text{g/kg}$ and $0.542 \pm 1.085 \mu\text{g/kg}$ for processed and local cream samples and $0.552 \pm 1.001 \mu\text{g/kg}$ and $0.000 \pm 0.000 \mu\text{g/kg}$ for processed and local yogurt samples, respectively. Moreover, milk samples showed highest AFM₁ (62%) in local unprocessed dairy farm followed by samples from local milk shop (51%) and commercial dairy farm (31%). In addition, therapeutic efficacy of three different types of toxin binders showed that the toxin binder which had yeast wall (75%) and algae (25%) is the best to control AFM₁ under field conditions. Overall, results of this study are valuable for dairy farmers on one hand and law enforcement authorities on the other to comprehend and control AFM₁ problem in milk and main dairy products.

Keywords: AFM₁, Milk, Dairy products, HPLC, Toxin binders

1. Introduction

Milk and dairy products both are vital part of human nutrition and ideal sources of nutritional components because of their biochemical complexity for supplying

essential mixture of proteins, vitamins, calcium, amino acids and antioxidants [1]. Pakistan is 4th largest milk producing country in the world and produces 45 billion liters per year [2]. The extensive and vast dairy industry of Pakistan faces a lot of problems including Aflatoxin M₁ (AFM₁). AFM₁ are playing negative impacts on animal production as well as dangerous for human health [3, 4].

AFM₁ is a monohydroxylated product of Aflatoxin B₁ (AFB₁). When lactating mammals consume AFB₁ contaminated feed then production of AFM₁ becomes enhanced. After ingestion of AFB₁, hydroxylation reaction is occurred on tertiary carbon of difuran ring system which yields AFM₁ [5–7]. The biotransformation frequency of AFB₁ to AFM₁ in excreted milk is different in all lactating animals. But AFM₁ start producing in milk within 12 – 24 hours after AFB₁ ingestion from feed [8].

80% people especially children consume dairy products as an important part of their diet [9]. But it is a dejected reality that dairy products are compromised badly because of mycotoxins. These fungal toxins not only destroy these dairy products but also produce dreadful disease which causes chronic diseases in the consumer. The World Health Organization (WHO) has endorsed the depletion of Aflatoxins in food by establishing tolerable limits for Aflatoxins to fulfill Farm-to-Fork principle.

International Agency for Research on Cancer (IARC) which is specialized cancer agency of World Health Organization (WHO) has categorized aflatoxins B₁ and M₁ into group 1 carcinogens [10, 11]. Therefore, different international organizations and countries have established standards for AFM₁. The European Commission Regulation 1831/2003 set permissible limits for AFM₁ in milk and dairy products of 0.050 µg/kg [12, 13]. According to Codex Alimentarius Commission permissible level of AFM₁ in butter is 50 µg/kg and in cheese is 250 µg/kg [14]. So, permissible limits of AFM₁ vary in milk and dairy products in different countries. But many countries including Pakistan also have no proper safety and regulatory limits and levels for AFM₁ in milk and dairy products [15] which may be due to oversight of policy makers with negligible research on aflatoxins.

Hence, this study was designed to gauge AFM₁ problem in milk and dairy products in and around Lahore from different sources. In the second phase of the study, efficacy of three different toxin binders were compared in a local dairy farm. The outcomes of the study will help dairy farmers on one hand and law enforcement agencies on the other to understand the gravity of AFM₁ problem in milk and main dairy products, and formulate strategies to control it.

2. Material and methods

2.1 Sample collection

Sampling strategy is comprehensively discussed in **Table 1**. After collection, samples were transported in icebox to University of Veterinary and Animal Sciences, Lahore where these were stored at –4°C till further processing.

2.2 Sample processing

2.2.1 Processing of milk samples through rapid test kit

Rapid test kit detects AFM₁ to the limit of 0.5 ng/ml-ppb. The kit was used according to manufacturer instructions. Briefly, 200 µL milk samples were pipetted into reagent microwell and after mixing, these samples were incubated at room

Sample	Sample category
Milk (n = 60)	10 ml unprocessed milk from local milk shop (n = 20)
	10 ml unprocessed milk local dairy farm (n = 20)
	10 ml processed milk sample from a commercial shop (n = 20)
Butter (n = 30)	50 g Processed butter samples (n = 15)
	50 g unprocessed butter samples (n = 15)
Cream (n = 30)	50 g processed cream samples (n = 15)
	50 g unprocessed cream samples (n = 15)
Cheese (n = 30)	50 g processed cheese samples (n = 15)
	50 g unprocessed cheese samples (n = 15)
Yogurt (n = 30)	50 g processed yogurt samples (n = 15)
	50 g unprocessed yogurt samples (n = 15)

Table 1.
Sampling strategy.

temperature for 2 minutes. Then dipstick was inserted in each sample and incubated for another 5 minutes at the same temperature. After that dipstick was taken out from each sample and samples were interpreted. There would be two lines i.e. test line (T) and control line (C) on dipstick. If $T \geq C$ then sample was considered negative while if $T < C$ or there was no test line then it was considered as positive.

2.2.2 Processing of samples of dairy products through HPLC

a. Chemicals and Reagents:

AFM₁ standards (10 µg/l in acetonitrile), Celite and HPLC grade acetonitrile of Sigma–Aldrich, Steinheim, Germany and Immunoaffinity column AflaTM of VICAM, USA were used.

AFM₁ standard curve or linearity curve was prepared by diluting the standards with acetonitrile at 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 µg/ml concentrations and stored in capped vials in refrigerator at – 4 C.

b. Samples Extraction:

5 g sample of each products and 5 g Celite mixed with 40 ml of dichloromethane in a 50 ml falcon tube. Then centrifuged at 21,000 rpm for 5 mins. After centrifugation the supernatant was separated and evaporated in water bath at 80 C. After evaporation the beaker was shifted in ultrasonic clean-up for 5 min. Then residues in beaker were dissolved in 10 ml mixture of methanol, water and n-hexane with the ratio of 3:5:2. Then 15 ml of this solution mixed by vortex mixture and again centrifuged at 21,000 rpm for 5 mins. After this, aqueous filtrate was passed through immunoaffinity column. The column was washed with 10 ml of water to remove toxins. After this column again washed with 2.5 ml of acetonitrile to get the final extract. This extract then dry under nitrogen steam at 40 C. After evaporation residues were dissolved with 2 ml mobile phase and mix by using vortex mixture. Finally, 20 µl sample was injected in HPLC for the analysis.

Groups	Type of toxin binders	Dose rate
A (n = 10)	Clay based toxin binder	100 g/40 Kg feed
B (n = 10)	Whole yeast-based toxin binder	1 g/40 Kg feed
C (n = 10)	Yeast (75%) + Algae (25%)	10 g/40 Kg feed

Table 2.
Therapeutic trials in different groups.

c. HPLC Conditions:

The HPLC used for the analysis was a Shimadzu LC-10A series (Japan) with the fluorescence detector (HPLC-FLD) having excitation wavelength of 365 nm and emission wavelength of 435 nm.

2.3 Therapeutic trial

Three different toxin binders were used in respective groups A, B, and C each having 10 AFM₁ positive animals. The **Table 2** showed types of toxin binder and their dose rates. Each toxin binder was used on the daily basis for 7 days.

2.4 Sample collection and processing after therapeutics

At days 2nd, 3rd, 4th, and 7th 10 ml milk samples from each animal were collected in plain vacutainers and serum was extracted through centrifugation. After that milk samples were checked by using AFM₁ rapid test kit.

2.5 Statistical analysis

Collected data will be statistically scrutinized by SPSS 20.0 software and t-test as well as Chi square test was used to analyze the results.

3. Results and discussion

The results of this study were comprehensively described in **Tables 3** and **4**. **Figure 1** showed the linearity curve of AFM₁ standard concentrations of 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 µg/ml. The method showed linear response $R^2 = 1$.

Table 3 shows the level of AFM₁ of all dairy products that exceeds the tolerable limits (0.050 µg/kg) established by European Commission Regulation.

Our results concluded that 33.3% of processed butter sample showed positive recovery of AFM₁ with range concentration above EU limits (0.050 µg/kg) while no local butter sample with AFM₁ toxicity was found (**Table 3**). These results are only in agreement with a study conducted by Fallah et al. [16] who analyzed 31 butter samples and got 25.8% AFM₁-positive ones with range above permissible limits established by EU.

The similar trend was found in case of processed cheese samples which were found contaminated (33.3%) with AFM₁ (**Table 3**) whereas in another study the AFM₁ concentration was found much higher i.e., 78% [15]. There are many other such studies having positive percentages of AFM₁ higher than our results [16–18].

Similarly, analysis of processed and unprocessed yogurt samples showed that 26.6% of former were contaminated with AFM₁ above EU permissible limit whereas no sample in latter was found affected with these aflatoxins. Our results showed low positive percentage than documented by Iqbal and Asi [15] who found

Type of Sample	Group	Mean ± S.D	Range of Conc. of Aflatoxin M1 in ppb	Samples Exceeding EC limits (0.05 ppb)
Butter	Processed	.0900 ± .18000	.36	(5/30) 16.7%
	Local	.0000 ± .00000	.00	
	Total	.0450 ± .12728	.36	
Cheese	Processed	.3500 ± .60622	1.05	(10/30) 33.3%
	Local	.0000 ± .00000	.00	
	Total	.1750 ± .42866	1.05	
Cream	Processed	.0000 ± .00000	.00	(4/30) 13.3%
	Local	.5425 ± 1.08500	2.17	
	Total	.2713 ± .76721	2.17	
Yogurt	Processed	.5525 ± 1.00118	2.05	(8/30) 26.6%
	Local	.0000 ± .00000	.00	
	Total	.2763 ± .71889	2.05	

Table 3.
HPLC results of aflatoxin M₁ in dairy products.

Time of sampling		After 2 days		
Groups	Positive	Negative	Percentage	<i>p</i> -value (<i>p</i> < 0.05)
A	10	0	100%	0.000
B	9	1	90%	
C	3	7	30%	
Time of sampling		After 3 days		
Groups	Positive	Negative	Percentage	<i>p</i> -value (<i>p</i> < 0.05)
A	9	1	90%	0.000
B	7	3	70%	
C	0	10	00%	
Time of sampling		After 4 days		
Groups	Positive	Negative	Percentage	<i>p</i> -value (<i>p</i> < 0.05)
A	9	1	90%	0.000
B	6	4	60%	
C	0	10	00%	
Time of sampling		After 7 days		
Groups	Positive	Negative	Percentage	<i>p</i> -value (<i>p</i> < 0.05)
A	8	2	80%	0.000
B	6	40	60%	
C	0	100	00%	

Table 4.
Efficacy of different toxin binders in groups A, B, and C.

59 AFM₁-positive samples. Many other such studies also showed higher incidents of AFM₁ in yogurt samples than our study [19–21]. Lastly, results of unprocessed cream samples showed the highest concentration of AFM₁ whereas no aflatoxin

Figure 1.
Linearity curve of aflatoxin M₁ standard concentrations.

Figure 2.
Positive percentage in each group after offering toxin binders.

was detected in processed cream samples as shown in **Table 3**. These results have not only local impacts but high impact at global level too. Because at global level, particularly in underdeveloped and developing countries, the topic of aflatoxins in dairy sector impacting humans as well as animals is a neglected topic.

During the second phase of the study, milk samples collected on 2nd, 3rd, 4th, and 7th day showed significantly different efficacies of three toxin binders in groups A, B, and C. It was found that toxin binder used in group C had significantly higher (<0.05) efficacy as compared to those used in groups A and B. This toxin binder C had yeast wall (75%) in combination with algae (25%) so it showed best results and eradicated AFM₁ from all the animals after 48 h. The reason behind is the main role of yeast wall in the whole yeast which binds with mycotoxin and its binding ability is catalyzed by algae so the product having this combination provided the best results. Whereas, the clay-based toxin binder used in group A showed comparatively worst results in controlling AFM₁ due to their lack of binding with these mycotoxins as described by Chestnut et al. [22]. Other disadvantages associated with clay-based toxin binders are their probable interaction with the essential nutrients [23] and high inclusion rates [24]. On the other hand, whole yeast-based toxin binder showed significantly lower efficacy as compared to group C and higher

efficacy as compared to group A. The reason behind would be that whole yeast alone does not have good binding ability with mycotoxins so it also failed to control AFM₁, comparatively. The whole findings are summarized in **Figure 2**.

4. Conclusion and recommendations

The results of this study confirmed that processed and unprocessed milk and main dairy products i.e. butter, cheese, cream, and yogurt has significantly ($p < 0.05$) higher contamination of AFM₁ than EU permissible limit. Moreover, toxin binder having yeast wall (75%) and algae (25%) showed significantly ($p < 0.05$) higher efficacy to control AFM₁ in milk samples. The study depicts alarming situation of AFM₁ problem and stresses the need of establishing permissible limits of AFM₁ by the regulatory bodies to avoid their adverse effects on public health in Pakistan as well as in other developing countries. It is noted with grave concern that the issue of aflatoxins is one of the most discussed topic at global level but it neglected in underdeveloped countries. So, this study emphasizes the need of research on the impact and control of aflatoxins in milk, and other dairy products which are consumed by human beings.

Declaration

The authors declare no conflict of interest.

Author details

Umaar Afzal Gill¹, Aneela Zameer Durrani^{2*} and Muhammad Usman²

¹ Department of Food Sciences and Human Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan

² Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore, Pakistan

*Address all correspondence to: aneela@uvas.edu.pk

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Zulueta A, Maurizi A, Frígola A, Esteve M, Coli R, Burini G (2009). Antioxidant capacity of cow milk, whey and deproteinized milk. *International Dairy Journal*. 19(6-7): 380-385.
- [2] Iqbal S, Asi M, Ariño A (2011). Aflatoxin M1 contamination in cow and buffalo milk samples from the North West Frontier Province (NWFP) and Punjab provinces of Pakistan. *Food Additives and Contaminants: Part B*. 4(4): 282-288.
- [3] Jalil H, Rehman HU, Sial MH, Hussain SS (2009). Analysis of milk production system in peri-urban areas of Lahore (Pakistan): A case study. *Pakistan Economic and Social Review*. 229-242.
- [4] Shuib NS, Makahleh AS, Salizawati MS, Bahrudin. (2017). Determination of aflatoxin M1 in milk and dairy products using high performance liquid chromatography-fluorescence with post column photochemical derivatization. *Journal of Chromatography A*. 1510: 51-56.
- [5] Murphy PA, Hendrich S, Landgren C, Bryant CM (2006). Food mycotoxins: an update. *Journal of food science*. 71(5).
- [6] Ruangwises N, Ruangwises S (2010). Aflatoxin M1 contamination in raw milk within the central region of Thailand. *Bull Environ Contam Toxicol*. 85(2): 195-198.
- [7] Tsakiris IN, Tzatzarakis MN, Alegakis AK, Vlachou MI, Renieri EA, Tsatsakis AM (2013). Risk assessment scenarios of children's exposure to aflatoxin M1 residues in different milk types from the Greek market. *Food Chem Toxicol*. 56: 261-265.
- [8] Rahimi E, Bonyadian M, Rafei M, Kazemeini H (2010). Occurrence of aflatoxin M1 in raw milk of five dairy species in Ahvaz, Iran. *Food Chem Toxicol*. 48(1): 129-131.
- [9] Silva RAd, Chalfoun SM, Silva MAMdCUdL, Pereira MC (2007). Inquérito sobre o consumo de alimentos possíveis de contaminação por micotoxinas na ingesta alimentar de escolares da cidade de Lavras, MG. *Ciência e Agrotecnologia*.
- [10] IARC. 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. World Health Organization.
- [11] Iqbal SZ, Asi MR, Selamat J (2014). Aflatoxin M1 in milk from urban and rural farmhouses of Punjab, Pakistan. *Food Additives & Contaminants: Part B*. 7(1): 17-20.
- [12] EC (2006). Commission Regulation (EC) 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Off J Eur Union*. 50: 5-24.
- [13] Zachariasova M, Dzuman Z, Veprikova Z, Hajkova K, Jiru M, Vachavikova M, Zachariasova A, Pospichalova M, Florian M, Hajslova J (2014). Occurrence of multiple mycotoxins in European feedingstuffs, assessment of dietary intake by farm animals. *Animal Feed Science and Technology*. 193: 124-140.
- [14] Codex Alimentarius Commission (2001). Maximum level for aflatoxin M1 in milk. *Codex Stand*. 232.
- [15] Iqbal SZ, Asi MR (2013). Assessment of aflatoxin M1 in milk and milk products from Punjab, Pakistan. *Food Control*. 30(1): 235-239.
- [16] Fallah AA, Jafari T, Fallah A, Rahnema M (2009). Determination of aflatoxin M1 levels in Iranian white and cream cheese. *Food and chemical toxicology*. 47(8): 1872-1875.

- [17] Elkak A, El Atat O, Habib J, Abbas M (2012). Occurrence of aflatoxin M₁ in cheese processed and marketed in Lebanon. *Food Control*. 25(1): 140-143.
- [18] Oruc HH, Cibik R, Yilmaz E, Gunes E. Fate of aflatoxin M₁ in Kasha cheese. *Journal of Food Safety* [Internet]. Wiley; 2007 Feb;27(1). Available from: <http://dx.doi.org/10.1111/j.1745-4565.2007.00062.x>
- [19] Galvano F, Galofaro V, Ritieni A, Bognanno M, De Angelis A, Galvano G (2001). Survey of the occurrence of aflatoxin M₁ in dairy products marketed in Italy: second year of observation. *Food Additives & Contaminants*. 18(7): 644-646.
- [20] Hussain I, Anwar J, Munawar MA, Asi MR (2008). Variation of levels of aflatoxin M₁ in raw milk from different localities in the central areas of Punjab, Pakistan. *Food Control*. 19(12): 1126-1129.
- [21] Issazadeh K, Darsanaki R, Pahlaviani M (2012). Occurrence of aflatoxin M₁ levels in local yogurt samples in Gilan Province, Iran. *Ann Biol Res*. 3(8): 3853-3855.
- [22] Chestnut AB, Anderson PD, Cochran MA, Fribourg HA, Twinn KD (1992). Effects of Hydrated Sodium Calcium Aluminosilicate on Fescue Toxicosis and Mineral Absorption. *J. Anim. Sci*. 70:2838-2846.
- [23] Moshtaghian J, Parsons CM, Leeper RW, Harrison PC, Koelkebeck KW (1991). Effect of sodium aluminosilicate on phosphorus utilization by chicks and laying hens. *Poult. Sci*. 70:955-962.
- [24] Devegowda, G., M. V. L. N. Raju, N. Afzali and H. V. L. N. Swamy (1998b). Mycotoxin picture worldwide: Novel solutions for their counteraction. In: *Biotechnology in the Feed Industry: Proceedings of Alltech's 14th Annual Symposium* (Ed. T. P. Lyons and K. A. Jacques). Nottingham University Press, Nottingham. pp. 241-255.