Research Article

Occurrence of Aflatoxin M1 in Milk at Sindh Province, Pakistan

Deepesh Kumar Bhuptani[1], Atta Hussain Shah[1*], Gul Bahar Khaskheli[1], Zubair Ahmed Laghari[2], Ghulam Shabir Bahram[1], Muneer Ahmed Jamali[1], Tarique Ahmed Khokhar[1]

[1]Department of Animal Products Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam; [2]Department of Veterinary Parasitology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam.

Abstract | Among various disease-causing agents, fungi has remained prominent making foods unsafe and insecure. Food and feed contamination by mycotoxins are among key factors creating food insecurity. There are various forms of aflatoxin (AF) occurring in foods of plant origin (B_1, B_2, G_1, and G_2) and animal origin (M_1 and M_2). Aflatoxin M_1 (AFM_1) is a geno-toxic carcinogen and is less lethal but it is still cytotoxic. The present study was conducted on prevalence and quantification of AFM_1 in raw buffalo milk in Sindh province. A total of three hundred and fifteen raw milk samples (n=315) from dairy farms of three regions in Sindh province i.e., southern (Karachi, Thatta, Hyderabad), central (Mirpurkhas, Umerkot, Shaheed Benazirabad) and northern (Naushahro Feroze, Sukkur and Larkana) were collected and analyzed using commercial ELISA kit. The results revealed that AFM_1 was statistically higher ($P<0.05$) in district Karachi (0.7223 µg/L) as compared to Thatta and Hyderabad (0.5897 µg/L and 0.5697 µg/L, respectively) than FDA limit (0.5 µg/L) however, it was found relatively similar ($P>0.05$) in Hyderabad and Thatta districts of Southern zone. In central zone, numerically AFM_1 load was high ($P>0.05$) in samples of district Mirpurkhas (0.5306 µg/L) followed by Umerkot (0.5271 µg/L) and Shaheed Benazirabad (0.4966 µg/L). In northern zone, Naushahro Feroze and Sukkur (0.5294 µg/L and 0.5257 µg/L, respectively) also exhibited numerically high ($P>0.05$) AFM_1 level than Larkana (0.4951 µg/L) district. Based on current results it was concluded that milk samples throughout the Sindh province were contaminated with AFM_1 and further Karachi province was on top in contamination.

Keywords | Aflatoxin; Contamination; Milk; Prevalence; Quantification; Sindh

Received | February 13, 2022; **Accepted** | March 25, 2022; **Published** | July 30, 2022
***Correspondence** | Atta Hussain Shah, Department of Animal Products Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam; **Email**: vetdr_atta@sau.edu.pk
Citation | Bhuptani DK, Shah AH, Khaskheli GB, Laghari ZA, Bahram GS, Jamali MA, Khokhar TA (2022). Occurrence of aflatoxin m1 in milk at sindh province, Pakistan. J. Anim. Health Prod. 10(3): 368-374.
DOI | http://dx.doi.org/10.17582/journal.jahp/2022/10.3.368.374
ISSN | 2308-2801

INTRODUCTION

Milk is known as a whole food since it contains energetic constituents such as proteins, lactose, fat, mineral and vitamins which make it highly perishable commodity (Dirmitrov et al., 2003; Saha et al., 2003). Because of perishable nature, milk serves the best microbial growth medium thus it gets easily contaminated (Neumann et al., 2002; Imran et al., 2008; Hanif et al., 2012). Milk is considered as sterile but by the time it secretes out from mammary glands it either gets contaminated by udder or unhygienic practices i.e., milking, processing, transportation, and handling of milk, prevailing at the farms (Hassan, 2005). Producing and supplying safe food of animal or plant origin to consumers has remained a major issue in current scenario of changing climatic conditions and increasing population density.

Among various disease-causing agents, fungi has remained prominent making foods unsafe and insecure (Ahlberg et

al., 2019). Food and feed contamination by mycotoxins are among key factors creating food insecurity (Udomkun et al., 2017; Acaroz 2019). As per observations of food and agriculture organization, one-fourth (1/4th) of crop throughout the word is influenced by mycotoxins (Pankaj et al., 2018; Mahto et al., 2019). Mycotoxins are produced by three major genera i.e. *Aspergillus*, *Fusarium* and *Penicillium* (Reddy et al., 2009). Aflatoxins (a type of mycotoxin) are fungal metabolites produced by certain fungi (molds) causing contamination of food crops which ultimately pose serious threat to livestock and humans. There are various forms of aflatoxin occurring in foods of plant origin (B_1, B_2, G_1, and G_2) and animal origin (M_1 and M_2). Aflatoxin B_1 is the most harmful form due to its direct link with liver cancer in humans (Dirheimer 2000; Fink-Gremmels, 2008; Perdoncini et al., 2019). Aflatoxin M_1 is a geno-toxic carcinogen and is less lethal than aflatoxin B_1 (Creppy, 2002) but it is still cytotoxic (Dirheimer 2000; Caloni et al., 2006). In dairy animals, the main signs are liver and kidney impairment, weight loss and reduction in milk yield. Aflatoxins are metabolized by different types of enzymes e.g. cytochrome P450s and glutathione S-transferases (WHO, 2002; WHO, 2017).

The aflatoxin was first discovered in Brazil during 1960 where more than 0.1 million turkeys and many other farm animals expired due to intake of *Aspergillus flavus*-contaminated peanuts (Davis & Deiner, 1979). Aflatoxins have been isolated from variety of plant foods such as groundnuts, figs, maize, dry fruit, and animal origin food such as milk, yoghurt, meat, eggs, meat products (Mutegi et al., 2009; Perrone et al., 2014; Iqbal et al., 2015; Martinez-Miranda et al., 2019; Sumon et al., 2021). Two species of fungi i.e., *Apergillus flavus* and *Aspergillus parasiticus* are mainly responsible for public health impact (WHO, 2018). Food and feed at both levels (before and after harvest) stage may get contaminated (Kumar et al., 2017; Perdoncini et al., 2019).

Humans are exposed to aflatoxins when they eat food contaminated with fungal growth products. Evidence of acute aflatoxicosis in humans has been reported in many parts of the world, particularly third world countries. Conditions that increase the likelihood of acute aflatoxicosis in humans include limited food availability, environmental conditions that favour fungal growth in crops and the lack of regulatory systems to monitor and control aflatoxins. The extent of aflatoxin-related diseases in humans can be influenced by factors such as age, sex, nutritional status and / or simultaneous exposure to other pathogens such as viral hepatitis (HBV) or parasite infestation (Prandini et al., 2009). It is estimated that more than 5 billion people in developing countries around the world are at risk of contracting chronic aflatoxins from contaminated food (Guo et al., 2009). Animals and humans are exposed to aflatoxins by consuming contaminated products such as dairy products (milk, cheese and yoghurt) (Ogodo & Ugbogu, 2006). Aflatoxin has a food safety and public health problem due to its toxicity. When consumed, it can be toxic by altering intestinal integrity or modulating cytokine expression, which can cause growth retardation in children and / or immunosuppression. In the liver, aflatoxin may be converted by certain p450 enzymes into its DNA-reactive form aflatoxin B1 (AFB1)-8-9-epoxide, which binds to liver proteins and causes its failure, leading to acute aflatoxin poisoning or may bind to DNA, resulting in aflatoxin induction of hepatocellular carcinoma (liver cancer) (De La Campa et al., 2006).

In this context, present study was designed to determine the prevalence and quantify the level of aflatoxin M_1 in milk produced at different zones of Sindh Province, Pakistan.

MATERIALS AND METHODS

Collection of samples

Study was carried out to determine the prevalence of aflatoxin M_1 and to quantify its level in buffalo raw milk at farm level. A total of three hundred and fifteen (n=315) raw buffalo milk samples (composite sample) were collected from dairy farms from three geographical zones of Sindh province (105 samples from each zone) i.e., Southern zone (Karachi, Thatta, Hyderabad) Central zone (Mirpur Khas, Umerkot and Shaheed Benazirabad) and Northern zone (Naushahero Freoze, Sukkur, Larkana). The selection area from each zone was based on density of commercial dairy farming. All samples were transported (under chilled conditions i.e., 4°C) to the laboratory of Animal Products Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam for further analysis.

Analysis of samples

Milk samples were screened and quantified through Bio-Shield M_1 Ultra-Fast for Milk (ProGnosis Biotech, Greece) ELISA kit. In brief, 200 µl of the matrix diluent with 50 µl of the samples and standards in the dilution microwells was added than transferred 100 µl from each dilution microwells into the antibody coated microwells and incubated for 10 min at room temperature after that washed for four times and 100 µl of detection solution was added and again incubated for 5 min at room temperature. After that 100 µl of TMB substrate was added and left for 5 min in the dark at room temperature for the development of color and then 100 µl of stop solution was added and in the last absorbance was noted at 450 nm within 60 min.

Table 1: Overall prevalence of aflatoxin M_1 in Sindh province, Pakistan

Type of Aflatoxin	Extent of aflatoxin M_1; No. of samples (%)	
	Exceed tolerable limit	Within tolerable limit
Aflatoxin M_1 in Milk	164 (52.06)	151 (47.93)

*The maximum AFM_1 tolerable limit for liquid milk is 0.5 µg/L (FDA, 2019)

Table 2: Zone-wise prevalence of aflatoxin M_1 in Sindh province, Pakistan

Type of Aflatoxin	Prevalence; Number of positive samples (%)		
	Southern Zone	Central Zone	Northern Zone
Aflatoxin M_1 in Milk (n=105)	60 (57.14)	51 (48.57)	53 (50.47)

Table 3: District-wise prevalence and quantification of aflatoxin M_1 in Sindh province, Pakistan

Type of aflatoxin (No. of samples)	District-wise prevalence and quantification of AFM_1 Number of samples positive (%)								
	Southern Zone (n=105)			Central Zone (n=105)			Northern Zone (n=105)		
	KHI	THT	HYD	MPK	UMK	SBA	NFZ	SKR	LRK
AFM_1 n=35 (%)	26 (74.29)	19 (54.29)	15 (42.86)	18 (51.43)	16 (45.71)	17 (48.57)	17 (48.57)	18 (51.43)	18 (51.43)
AFM_1 load; µg/L (Mean)	0.7223	0.5897	0.5697	0.5306	0.5271	0.4966	0.5294	0.5257	0.4951

KHI: Karachi, THT: Thatta, HYD: Hyderabad, MPK: Mirpurkhas, UMK: Umerkot, SBA: Shaheed Benazirabad, NFZ: Naushahro Feroze, SKR: Sukkur, LRK: Larkana

Statistical Analysis

The data of the current research was statistically evaluated by computer-based program, Student Edition of Statistix (SXW), Version 8.1 (Copyright 2005, Analytical software-USA). Descriptive statistics i.e. percentages and inferential statistics i.e. analysis of variance (ANOVA) was used during the results analysis. Moreover, if significant difference existed among the average means of groups i.e., Least Significant difference (LSD) was applied to identify the distinct groups.

RESULTS

Prevalence of aflatoxin M_1 in Sindh province, Pakistan

Raw milk samples collected from all three zones were found contaminated with afltatoxin M_1. It was found that 52.06% samples were contained higher level of aflatoxin M_1 while 47.93% samples were within normal range (Table-1) compared to standard (The maximum AFM_1 tolerable limit for liquid milk is 0.5 µg/L; FDA, 2019).

Zone-wise AFM_1 prevalence results showed that milk samples from southern zone were more contaminated (57.14%) compared to that of northern and central zones having prevalence of 50.47% and 48.57%, respectively (Table-2). Moreover, nine districts from three zones of Sindh province were examined for the prevalence of AFM_1 in milk samples and the prevalence prevailed at each district is depicted in Table 3.

A total of n=26 (74.29%) milk samples from Karachi were positive for AFM_1 which was significantly high compared to those from Thatta (54.29%; n=19), Mirpurkhas, Sukkur and Larkana (51.43%; n=18), Naushahro Feroze and Shaheed Benazirabad (48.57%; n=17), Umerkot (45.71%; n=16) and Hyderabad (42.86%; n=15).

Quantification of aflatoxin M_1 in Sindh province, Pakistan

Quantification of milk samples collected from three districts of Southern zone namely, Karachi, Hyderabad and Thatta was carried out for AFM_1 analysis (Table 3) and the results revealed that AFM_1 in milk samples from Karachi was statistically higher (0.7223 µg/L) than Thatta and Hyderabad (0.5897 µg/L and 0.5697 µg/L, respectively). In the central zone of Sindh province three main districts i.e. Mirpurkhas, Umerkot and Shaheed Benazirabad were examined for the AFM_1 (Table 3). The mean AFM_1 limit was noted numerically high in district Mirpurkhas (0.5306 µg/L) followed by Umerkot (0.5271 µg/L) and Shaheed Benazirabad (0.4966 µg/L), however the difference among means of all districts was non-significant (P>0.05). Similarly, the average AFM_1 quantification in Northern zone (Naushahro Feroze, Sukkur and Larkana districts) was recorded and results showed that mean concentration of aflatoxin M_1 in the milk samples collected from Naushahro Feroze, Sukkur and Larkana, districts were 0.5294 µg/L, 0.5257 µg/L and 0.4951 µg/L, respectively which was sta-

tistically similar to each other (P>0.05).

DISCUSSION

Milk is well thought-out to be the nature's perfect diet containing all essential elements thus extensively used throughout the world. Due to its high nutrient contents, and perishable nature it serves as an excellent medium for the microbial growth which ultimately contaminate the milk quality (Singh et al., 2015). Among other feed stuffs for dairy cattle, cottonseed cake – a meal remains with high proteineous, energy and fibre source for ruminants. The extremely multipurpose seed provide one of the utmost human attire fabric and is also a vital food for dairy animals feed. Aflatoxin can be arisen because of storage proteins and carbohydrates in growing cottonseed (Khilosia, 2011). When mold contaminated feed (aflatoxin B_1) is eaten by the ruminant animals for example dairy animals, this contaminant is transformed to another type of toxin i.e. aflatoxin M_1 underneath the impact of cytochrome P_{450} oxidase system which carried over to milk and ultimately infects consumers. Hence, the amount of aflatoxin M_1 secreted in milk can be equal to the 3% of aflatoxin B_1 ingested through contaminated feed (Diaz et al., 2004). Aflatoxins are acutely and chronically toxic to human and animals, leading to the acute damage to liver, liver cirrhosis, carcinomas induction and teratogenic effects (WHO, 2018).

The results of the present study showed all milk samples carried aflatoxin with more than 50% milk samples with carriage of above maximum residual level (MRL) recommended by FDA (2019) i.e. 0.5 µg/L. Jawaid et al. (2015) who reported that 96.43% (81 out of 83) milk samples were contaminated with the AFM_1 in the range of 0.01 to 0.76 µg/L. Similarly, Raza (2006) found 33.33% AFM_1 in fresh milk samples procured from Karachi, Sindh. Additionally, current findings are in line with the results of Akbar et al. (2019) who also found that 70% (672 out of 960) raw milk samples surpassed the United States permitted maximum residue limits (MRL 0.50 µg/L), with a complete AFM_1 contamination limit that ranged in between 0.3 to 1.0 µg/L during the winter season in Punjab province. One of the reports from Bangladesh revealed 33.33% of the raw milk, pasteurized milk and ultra-high temperature treated milk samples crossed the border line set by the European Union (50 ng/L) for AFM_1 in milk however, the levels of afltatoxin M_1 in yoghurt and milk powder were within limits (Sumon et al., 2021). Abyaneh et al. (2019) reported prevalence of aflatoxin M_1 from raw, pasteurized and UHT milk samples collected from 117 cities throughout the Iran during winter using HPLC-FLD technique. The results revealed that 54.7% samples of milk were positive containing load of <10 to 150 ng/L. Only three milk samples (0.6%) crossed the border line of aflatoxin M_1 as described by the Iranian regulations (100 ng/L). The aflatoxin content was higher in samples obtained from humid areas. AFM_1 Levels were the highest in winter (48.70 ng/Kg). The level of AFM_1 in raw, UHT treated and pasteurized milk were 55.08, 94.81 and 49.76 ng/kg, respectively (Abyaneh et al., 2019).

Continuous change in climatic conditions and agriculture practices patterns play vital role in occurrence of microbial contamination. Elevated temperature and humid conditions support fungal growth and mycotoxin production. In present study, a total of three zones i.e. Southern (Karachi, Thatta, Hyderabad), Central (Mirpurkhas, Umerkot, Shaheed Benazirabad) and Northern (Naushahro Feroze, Sukkur, Larkana) of Sindh province were examined for the prevalence of AFM_1 and found that southern zone had highest percent of AFM_1. Among other factors brings variation in occurrence percentage of aflatoxin, season is one of the major contributors. Seasonal effect influences concentration of aflatoxin M_1 concentrations which are the highest in winter and the lowest in summer (Hussain & Anwar, 2008; Rossi et al., 1996; Blanco et al., 1988). The reason behind this might be in winter commonly dairy animals are fed with compound feeds and therefore concentration of aflatoxin B_1 rises that in turn increases concentration of AFM_1 in milk. Furthermore, moisture and temperature also affect the occurrence of aflatoxin B_1 in feeds. *A. parasiticus* and *A. flavus* can definitely grow in feeds having moisture ranging 13-18% and environmental moisture ranging 50-60%. In the current study, variation in the prevalence of aflatoxins could also be due to high humidity in the southern zone due to costal (Karachi and Thatta) and highly irrigated areas (Hyderabad), followed by northern areas (upper Sindh) because of heavy low humidity. Southern region of Sindh province is somehow hot but mostly humid region due the nearby sea compared to that central and northern region which is to some extent hot and less humid.

Furthermore, prevalence of aflatoxin was high in Karachi, Thatta and Hyderabad for AFM_1. Reasons behind this high prevalence could be humidity and commercial dairy farming at large scale among these districts compared to rest of study areas. Due to urbanization especially in Karachi and Hyderabad and high salinity in the surrounding of Thatta, cultivation of forage is going to be reduced day by day. High demand of cottonseed cake to fulfill the requirements for commercial dairy farm particularly in urban areas causing increased transportation of grasses from anterior Sindh and storage for longer periods which provide favourable niches for growth of moulds. Lack of proper aeration/ventilation during the storage of cottonseed cake purchased in a lot and stocked due to price fluctuation

could favors occurrence of aflatoxins (Iqbal et al., 2010; Iqbal et al., 2015; Yunus et al., 2015; Yunus et al., 2019). Proper ventilation is very crucial during transportation of cottonseed cake to other districts. It has been argued that environmental factors and pre and post harvesting conditions (especially soil condition and type of seed planted, pest attack etc) of the crops are fundamental cause of variation in aflatoxins levels (Khilosia, 2011; Iqbal et al., 2014). In this regard, results of the present study are also in accordance with those of Yunus et al. (2020) who observed that reason behind 80% of total aflatoxin intake of dairy animals was the contaminated cottonseed cake. Akbar et al. (2019) observed that Eastern area in Punjab had higher than standard limits of AFM_1 contamination in milk (0.705 µg/L). Samples of milk collected from the Northern area were found to be broadly infected (86.9%) and surpassed the US MRL, followed by the Eastern area, with 72.3% milk samples being polluted with >0.5 µg/L AFM_1.

CONCLUSION

Based on current results it was concluded that milk samples throughout the Sindh Provinces were contaminated with AFM_1 and further Karachi province was on top in contamination. It is recommended that cottonseed cake (a prime source of aflatoxin in dairy animals) need to be properly analyzed before offering to animals.

ACKNOWLEDGEMENTS

Authors want to thank the Higher Education Commission of Pakistan for Indigenous Scholarship and staff of Department of Animal Products Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam.

CONFLICT OF INTEREST

There is no any conflict of interest.

NOVELTY STATEMENT

Aflatoxin is of great public health concern due to its immunosuppressive and carcinogenic effects on consumers. Its prevalence in various foods of animal origin such as milk and milk products may pose serious threat to public health. In this context, present study was designed to determine the prevalence and quantify the level of aflatoxin M1 in milk produced at different zones of Sindh Province, Pakistan.

AUTHORS CONTRIBUTION

AHS conceived and designed the study, DKB performed the experimental work, ZAL & TAK analysed and interpreted the data, GBK, MAJ & GSB revised the manuscript.

REFERENCES

Abyaneh HK, Bahonar A, Noori N, Yazdanpanah H, Aliabadi MHS (2019). Aflatoxin M1 in raw, pasteurized and UHT milk marketed in Iran. Food Addit Contam Part B Surveill, 12(4):236-244. https://doi.org/10.1080/19393210.2019.1611666

Acaroz U (2019). Assessment of aflatoxin M1 levels in tulum and white cheeses in afyonkarahisar, turkey. Fresenius Environ. Bullet. 28 (11a): 8663-8668

Ahlberg SH, Randolph D, Okoth S, Lindahl J (2019). Aflatoxin Binders in Foods for Human Consumption—Can This be Promoted Safely and Ethically. Toxins., 11(7):410. https://doi.org/10.3390/toxins11070410

Akbar N, Nasir M, Naeem N, Ahmad M, Iqbal S, Rashid A, Imran M, Gondal TA, Atif M, Salehi B, Sharifi-Rad J, Martorell M, Cho WC (2019). Occurrence and Seasonal Variations of Aflatoxin M1 in Milk from Punjab, Pakistan. Toxins., 11: 574; http://doi..org/10.3390/toxins11100574.

Blanco JL, Dominguezs L, Gomez-Lucia E, Garayzabal JFF, Gracia JA, Suarez G (1988). Presence of aflatoxin M1 in commercial ultra-high temperature treated milk. Appl. Environ. Microbiol., 56; 1622–1623. https://doi.org/10.1128/aem.54.6.1622-1623.1988

Caloni F, Stammati A, Frigge G, De Angelis I (2006). Aflatoxin M1 adsorption and cytotoxicity on human intestinal in-vitro model. Toxicon, 47: 409-415. https://doi.org/10.1016/j.toxicon.2005.12.003

Creppy EE (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicol. Lett., 127 (1-3):19-28. http://doi..org/10.1016/ S0378-4274(01)00479-9.

Davis ND, Diener UL (1979). Mycotoxins. In: Food and Beverage Mycology. Edited by Beuchat, L. R. AVI Publishing Company West Port, CT: 397-444.

De La Campa R, Hooker DC, Miller ID, Schaafsma AW, Hammond BG (2006). Modeling effects of environment, insect damage and Bt genotypes on fumonisin accumulation in maize in Argentina and the Philippines. Mycopathologia., 159: 539–552. https://doi.org/10.1007/s11046-005-2150-3

Diaz DE, Hagler WMJr, Blackwelder JT, Eve JA, Hopkins BA, Anderson KL, Jones FT, Whitlow LW (2004). Aflatoxin binders ii: Reduction of aflatoxin M_1 in milk by sequestering agents of cows consuming aflatoxin in feed. Mycopathologia. 157: 233–241. https://doi.org/10.1023/B:MYCO.0000020587.93872.59

Dirheimer G (2000). A review of recent advances in the genotoxicity of carcinogenic mycotoxins. In carcinogenic and anticarcinogenic factors in food: Symposium., 18(11): 180. Vch Verlagsgesellschaft Mbh

Dirmitrov T, Jorbineva MT, Iliev, Mihailova G (2003). Effect of activated Lacto peroxidase system on fatty acid content of sheep milk and white brined cheese. Zhivotno"dni Nauki, 38 (12): 94-97.

FDA (Food and Drug Administration) (2019). Compliance

policy guide sec. 638.100. Action level for aflatoxin in animal food: 1-4. https://www.fda.gov/ICEI/Compliance manuals/ compliance policy guidance manual/ default.htm.

Fink-Gremmels J (2008). The role of mycotoxins in the health and performance of dairy cows. Vet. J., 176(1): 84-92. https://doi.org/10.1016/j.tvjl.2007.12.034.

Guo Y, Zhao L, Ma Q, Ji C (2021). Novel strategies for degradation of aflatoxins in food and feed: A review, Food Res. Int. 140: 109878. https://doi.org/10.1016/j.foodres.2020.109878.

Hanif M, Zahoor ST, Iqbal Z, Haq I, Atta MA (2012). Effect of storage on rheological and sensory characteristics of cow and buffalo milk yoghurt. Pakistan J. Sci., 22 (2):61-70.

Hassan SS (2005). Quality Assurance of Various Dairy Products. MSc Thesis, Department of Chemistry, University of Peshawar, Pakistan.

Hussain I, Anwar J (2008). A study on contamination of aflatoxin M1 in raw milk in the Punjab province of Pakistan. Food Cont., 19: 393–395. https://doi.org/10.1016/j.foodcont.2007.04.019

Imran M, Khan H, Hassan SS, Khan R (2008). Physico - chemical characteristics of various milk samples available in Pakistan. J. Zhejiang University Sci., 9: 546-551. https://doi.org/10.1631/jzus.B0820052

Iqbal SZ, Asi MR, Jinap S (2014). A survey of aflatoxin M1 contamination in milk from urban and rural farmhouses of Punjab, Pakistan. Food Additives and Contaminants Part-B, 7(1): 17-20. https://doi.org/10.1080/19393210.2013.828322

Iqbal SZ, Jinap S, Pirouz AA, Faizal ARA (2015). Aflatoxin M1 in milk and dairy products, occurrence and recent challenges: A review. Trends Food Sci. Technol, 46: 110–119. http://doi..org/10.1016/j.tifs.2015.08.005. 58

Iqbal SZ, Paterson RRM, Bhatti IA, Asi MR (2010). Survey of aflatoxins in chilies from Pakistan produced in rural, semi-rural and urban environments. Food Addit. Contaminants. Part-B, 3(4): 268-274. https://doi.org/10.1080/19393210.2010.520341

Jawaid S, Talpur FN, Nizamani SM, Afridi HI (2015). Contamination profile of Aflatoxin M1 in milk supply chain of Sindh, Pakistan. 2015. Toxicol. Rep., 2: 1418 – 1422. https://doi.org/10.1016/j.toxrep.2015.10.011

Khilosia LD (2011). A Surveillance Study of Mycotoxins in the South African Industry with Specific Reference to Aflatoxin B1 in Feed and Aflatoxin M1 in Farm Gate and Selected Commercially Available Dairy Milk. University of Johannesburg. 001 – 430.

Kumar P., Mahato D. K., Kamle M., Mohanta T. K., Kang S. G. (2017). Aflatoxins: A global concern for food safety, human health and their management. Front. Microbiol., 7: 2170.

Mahato DK, Lee KE, Kamle M, Devi S, Dewangan KN, Kumar P, Kang SG (2019). Aflatoxins in Food and Feed: An Overview on Prevalence, Detection and Control Strategies. Front Microbiol., 10: 2266. https://doi.org/10.3389/fmicb.2019.02266.

Martinez-Miranda MM, Rosero-Moreano M, Taborda-Ocampo G (2019). Occurrence, dietary exposure and risk assessment of aflatoxins in arepa, bread and rice. Food Control., 98: 359–366. https://doi.org/10.1016/j.foodcont.2018.11.046.

Mutegi CK, Ngugi HK, Hendriks SL, Jones RB (2009). Prevalence and factors associated with aflatoxin contamination of peanuts from Western Kenya. Int. J. Food Microbiol., 130: 27–34. http://doi..org/10.1016/j.ijfoodmicro.2008.12.030

Neumann CG, Harris DM, Rogers LM (2002). Contribution of animal source foods in Improving diet quality and function in children in the developing world. J. Nutrit., Res. 22: 193-220. https://doi.org/10.1016/S0271-5317(01)00374-8

Ogodo A, Ugbogu O (2016). Public Health Significance of Aflatoxin in Food Industry – A Review. European J. Clin. Biomed. Sci., 2: 51-58. https://doi.org/10.11648/j.ejcbs.20160205.14.

Pankaj SK, Shi H, Keener KM (2017). A review of novel physical and chemical decontamination technologies for aflatoxin in food. Trends Food Sci. Technol., 71. http://doi..org/10.1016/j.tifs.2017.11.007.

Perdoncini MRFG, Sereia MJ, Scopel FHP, Formigoni M, Rigobello ES, Beneti SC, Marques LLM (2019). Growth of fungal cells and the production of mycotoxins. Cell Growth., 23.

Perrone G, Haidukowski M, Stea G, Epifani F, Bandyopadhyay R, Leslie JF (2014). Population structure and Aflatoxin production by Aspergillus Sect. Flavi from maize in Nigeria and Ghana. Food Microbiol., 41: 52–59. http://doi..org/10.1016/j.fm.2013.12.005

Prandini A, Tansini G, Sigolo S, Filippi L, Laporta M, Piva G (2009). On the occurrence of aflatoxin M1 in milk and dairy products. Food Chem. Toxicol., 47(5): 984-91. http://doi..org/10.1016/j.fct.2007.10.005.

ProGnosis Biotech, SA (2015). Bio-Shield M1 Milk & Youghurt and Bio-Shield Aflatoxin Total Extra Sensitive, immunoassay method for determination of the Aflatoxin M1 in Milk & Yoghurt and total Aflatoxin in grains, nuts, spices, cereals and other commodities including animal feed, respectively. https//www.prognosis-biotech.com.

Raza R (2006). Occurrence of aflatoxin M1 in the milk marketed in the city of Karachi, Pakistan. Chemical Society of Pakistan, 28(2):155-157.

Reddy KRN, Abbas HL, Abel CA, Shier WT, Oliveira CAF, Raghavender CR (2009). Mycotoxin contamination of commercially important agricultural commodities. Toxin Rev., 28: 154–168. https://doi.org/10.1080/15569540903092050

Rossi J, L Cataluffi, M Gobbetti (1996). Presence of aflatoxin M1 in cow and ewe milk from some farms in Umbria. Industria del Latte, 32(1–2): 37–41.

Saha BK, Ali MY, Charaborty M, Islam Z, Hira AK (2003). Study on the preservation of raw milk with hydrogen peroxide for rural dairy farmers. Pakistan J. Nutrit., 2 (1): 36-42. https://doi.org/10.3923/pjn.2003.36.42

Singh S, Chandel V, Soni P (2015). Quality analysis of milk and milk products collected from Jalandhar, Punjab, India. Department of Biotechnology, Lovely 62.

Sumon AH, Islam F, Mohanto, Kathak RR, Molla NH, Rana S, Degen GH, Ali N (2021). The Presence of Aflatoxin M1 in Milk and Milk Products in Bangladesh. Toxins (Basel), 13(7): 440. https://doi.org/10.3390/toxins13070440

Udomkun P, Wiredu AN, Nagle M, Müller J, Vanlauwe B, Bandyopadhyay R (2017). Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application. A review. Food Cont., 76:127-138. https://doi.org/10.1016/j.foodcont.2017.01.008

WHO (World Health Organization) (2002). Evaluation of certain mycotoxins (Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 906.

WHO (World Health Organization) (2017). Evaluation of certain contaminants in food (Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives).

WHO Technical Report Series WHO Technical Report Series, No.1002.

WHO (World Health Organization) (2018). Food Safety Digest. Department of Food Safety and Zoonoses. Updated risk assessment on aflatoxins undertaken by the Joint FAO/ WHO Expert Committee on Food Additives (JECFA).

Yunus AW, Imtiaz N, Khan H, Ibrahim MNM, Zafar Y (2019). Aflatoxin contamination of milk marketed in Pakistan: A longitudinal study. Toxins. 11: 110; http://doi..org/10.3390/ toxins11020110. www.mdpi.com/journal/toxins.

Yunus AW, Sulyok M, Böhm J (2015). Mycotoxin cocktail in the samples of oilseed cake from early maturing cotton varieties associated with cattle feeding problems. Toxins, 7(6): 2188–2197. https://doi.org/10.3390/toxins7062188

Yunus AW, Ullah A, Lindahl JF, Anwar Z, Ullah A, Saif S, Ali M, Zahur AB, Irshad H, Javaid S, Imtiaz N, Farooq U, Ahsan A, Fatima Z, Hashmi AA, Abbasi BHA, Bari Z, Khan IU, Ibrahim MNM (2020). Aflatoxin contamination of milk produced in peri-urban farms of Pakistan: Prevalence and contributory factors. Front. Microbiol., 11:159. https://doi.org/10.3389/fmicb/2020/00159.