



Original Article

Impact of Seasonal Variations (Aflatoxin M1 & Heavy metals) on Quality of Marketed Milk

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ABSTRACT

This study thoroughly examined the safety and quality of commercially accessible milk in light of the growing concerns about food safety and its effects on public health. **Objective:** To monitor the amount of aflatoxin and heavy metals found in commercially available milk from different cities in central Punjab, Pakistan. **Method:** The collection of milk samples was done from three major cities in central Punjab, including Lahore, Faisalabad, and Jhang, to measure the contamination of Aflatoxin M1 and heavy metals from 2018-2019. **Results:** The maximum concentration of AFM1 (0.38-1.65µg/L) was observed in the semi-flush season from Lahore, followed by Faisalabad (0.37-1.63 µg/L) and Jhang (0.35-1.62µg/L) whereas, the minimum concentration of AFM1(0.15-0.46µg/L) was observed during the lean season in the milk samples procured from Jhang, followed by Faisalabad (0.17-0.47µg/L) and Lahore (0.18-0.49µg/L). The levels of lead and cadmium in milk samples fluctuated seasonally, with Lahore having the highest contamination (Pb: 0.062ng/L, cadmium: 0.037ng/L), followed by Faisalabad and Jhang. Milk sample %ages exceeded the national and international aflatoxin M1 (AFM1) maximum residual limits (MRL). **Conclusion:** All milk samples from different milking seasons were above the EU's maximum residual level of 0.05µg/L. They were still within the Pakistan Standard and Quality Control Authority's(10µg/L) recommended range.

INTRODUCTION

Aflatoxins are mycotoxins that have been extensively researched. The *Aspergillus* genus of fungi, particularly *A. flavus*, *A. parasiticus*, and *A. nomius*, produce aflatoxins [1], when make colonies in food crops, including maize, cottonseed, sunflower seeds, tree nuts, and peanuts [2]. They can infect a variety of food goods both before and after harvest [3]. Four main types of aflatoxins, i.e., B1, B2, G1, and G2, can contaminate food and nutrition and pose substantial health risks to humans and animals [4] [5]. *Aspergillus parasiticus* and *Aspergillus flavus* are the main aflatoxins-producing fungi [6]. Due to their cancerous, metabolic, mutagenic, immunosuppressive, and

teratogenic effects, aflatoxin-contaminated food, feed, and agricultural products as a significant concern for the world [7]. Aflatoxin M1 (AFM1), a monohydroxylated precursor of aflatoxin B1 (AFB1), is produced during metabolism inside the liver of breastfeeding animals and then secreted into the raw milk of cattle that frequently fed feed containing aflatoxin B1. AFM1 has been categorized as a 2B carcinogenic group by the International Agency for Research on Cancer (IARC) because of its potential to affect the DNA molecule, resulting in various malignancies [8]. Since humans consume a substantial portion of milk, the AFM1 level in milk is regularly

maintained globally [9]. Milk is an excellent source of natural diet, used as nutrition worldwide because it contains fat, protein, and minerals [10]. Consumers are facing major health risks due to the presence of toxic aflatoxins along with certain heavy metals. Various studies have shown that AFM1 is reduced in raw milk by pasteurization, heat processing, and a few other methods that are still unsuccessful [3, 11]. It is becoming harder to totally purify raw milk after aflatoxins have entered the milk supply chain [12]. Health effects from consuming dairy products contaminated with AFM1 are likely [11, 13]. Recent research has emphasized the grave health risks connected to consuming milk infected with aflatoxins. Due to this, identifying aflatoxins in agricultural products and the mitigation techniques are essential areas of global research [14]. Pakistan was ranked fourth in the world for the production of dairy items and is behind India, China, and USA (GOP, 2016-17). On the other hand, Pakistan is at second rank regarding the production of dairy milk from buffalo [15]. The Pakistan Economic Survey was conducted from 2016 to 2017 and published the current statistics on the production of milk in Pakistan, which showed an augmentation of gross milk production. This revealed that there is an addition of 56,080,000 tons of milk in 2016-17.

By monitoring the milk throughout the year, this study sought to determine AFM1 common in commercially available milk supply chains in selected cities of Punjab, Pakistan. There have been several researches to look at the AFM1 contamination in Punjab, Pakistan's milk. Still, each of this research had limitations because of the sample size, location, point of sample collection, season, and milking time [15].

METHODS

The milk samples were collected from selected areas of these three major cities in central Punjab, including Lahore, Faisalabad, and Jhang, measuring Aflatoxin M1 and heavy metals. Samples are collected by using a simple random sampling technique. The study was completed in two years. For the statistical treatment of AFM1 in the milk samples, the Ridascreen® Fast Aflatoxin M1, R5812 competitive enzyme immunoassay kit from R-Biopharm AG in Darmstadt, Germany, was employed. Samples were prepared according to instructions given by the RIDASCREEN FAST test kit manual. Then centrifugation was done for 10 minutes at 3500 g at 10°C to separate the fat from the milk. The layer of fat was pipetted using Pasteur pipette and liquid milk was used directly for the further analysis technique.



Figure 1: Geographical overview of the cities (Milk Samples Collected Areas) (adapted from Akbar N., 2019) [16]

Milk samples were subjected to wet digestion by following the method determined by [17]. In 100 mL digestion flask, 1 mL of milk was mixed with 10 mL of concentrated nitric acid (Riedel-de Haen) [16]. Heating was done for the time period of 20 minutes. Afterward, cooling was done at room temperature. After 20 minutes of heating, chill. Adding 5 mL perchloric acid and heating until white vapours develop, then lowering sample to 2-3 mL. Heavy metal's concentration was determined by atomic absorption spectrophotometric through graphite furnace atomic absorption spectrometry method as described by [17]. The concentration of heavy metals was calculated by using a graphite atomic absorption spectrophotometer with an Analyst 800 (Perkin Elmer, USA). Measurements like integrated absorbance peak areas were calculated by using single-element hollow lamps. Cadmium at 228.8 nm, and Lead at 283.3 nm. The results were expressed in ng/L. In the 2nd year all of the above-mentioned materials and methods were repeated to get the data of two years. Standard Error of Mean, Mean, percentages and frequency were calculated through the statistical software Statistix to meet the requirements. ANOVA was applied for the determination of seasonal variation, followed by LSD.

RESULTS

In the study period of 2018-2019, 360 milk samples were collected. The maximum concentration of AFM1 (0.38-1.65 µg/L) was found in the semi-flush season (end of December), from Lahore, followed by Faisalabad (0.37-1.63 µg/L) and Jhang (0.35-1.62 µg/L) whereas, the minimum concentration of AFM1 (0.15-0.46 µg/L) was recorded during the lean season (June) in the milk samples procured from Jhang, followed by Faisalabad (0.17-0.47 µg/L) and Lahore 0.18-0.49 µg/L during the year 2018 (Table 1).

Table 1: AFM1 (Aflatoxin M1) range µg/L in selected cities of central Punjab recorded during the year 2018

Cities	Flush milking season				Lean milking season			Semi flush Milking season				Av. AFM1 ±SD	
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov		Dec
Aflatoxin M1 range (µg/L)													
Lahore N=60	0.65 -1.5	0.66 -1.5	0.48 -0.9	0.37 -1.3	0.18 -0.4	0.14 -0.6	0.18 -0.7	0.06 -0.9	0.36 -1.6	0.66 -1.2	0.67 -1.5	0.38 -1.65	0.639± 0.251 ^{ab}
Faisal- abad N=60	0.56 -1.4	0.55 -1.4	0.47 -0.8	0.36 -1.2	0.17 -0.5	0.13 -0.7	0.17 -0.6	0.05 -0.9	0.35 -1.6	0.65 -1.1	0.66 -1.8	0.37 -1.63	0.638± 0.248 ^{ab}
Jhang N=60	0.54 -1.4	0.55 -1.4	0.45 -0.8	0.34 -1.2	0.15 -0.4	0.11 -0.6	0.15 -0.6	0.03 -0.8	0.33 -1.5	0.63 -1.0	0.64 -1.5	0.35 -1.62	0.36± 0.247 ^{ab}

The study showed (Table 2) that 100 percent milk samples collected from selected cities in different milking seasons exceeded EU MRL (0.05 µg/L). In contrast, all the collected samples were within the prescribed limit (10 µg/L) set by Pakistan Standard and Quality Control Authority (PSQCA).

Table 2: Milk samples %ages exceeding National and International aflatoxin M1 (AFM1) MRL in selected cities of central Punjab in 2018

Cities	International/ National Standards	Flush Milking Season				Lean milking season			Semi flush Milking season				
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Lahore N=60	% samples exceeding EU MRL (0.05 µg/L)	100	100	100	100	100	100	100	100	100	100	100	100
	% samples exceeding PSQCA MRL (10 µg/L)	0	0	0	0	0	0	0	0	0	0	0	0
Faisalabad N=60	% samples exceeding EU MRL (0.05 µg/L)	100	100	100	100	100	100	100	91	100	100	100	100
	% samples exceeding PSQCA MRL (10 µg/L)	0	0	0	0	0	0	0	0	0	0	0	0
Jhang N=60	% samples exceeding EU MRL (0.05 µg/L)	100	100	100	100	100	100	100	85	100	100	100	100
	% samples exceeding PSQCA MRL (10 µg/L)	0	0	0	0	0	0	0	0	0	0	0	0

During the year 2019, a maximum concentration of AFM1 (0.34-1.65µg/L) was observed in the semi-flush season from Lahore, followed by Faisalabad (0.33-1.62µg/L) and Jhang (0.33-1.57µg/L) whereas, the minimum concentration of AFM1(0.11-0.62µg/L) was noted during the lean season (June) in the milk samples procured from Jhang, followed by Faisalabad (0.14-0.64µg/L) and Lahore (0.16-0.68µg/L)(Table.3).

Table 3: AFM1 (Aflatoxin M1) range ug/L in selected cities of central Punjab recorded during the year 2019

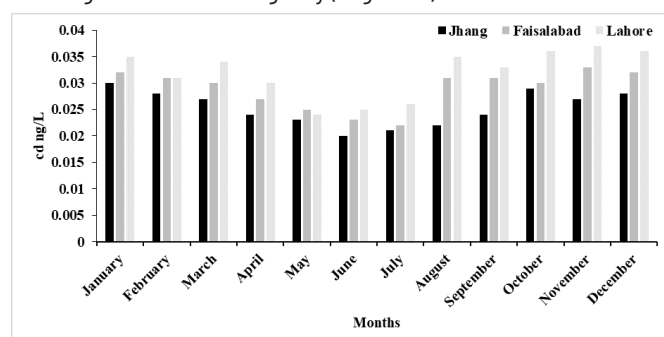
Cities	Flush milking season				Lean milking season			Semi flush Milking season				Av. AFM1 ±SD	
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov		Dec
Aflatoxin M1 range (µg/L)													
Lahore N=60	0.64-1.5	0.67-1.5	0.44-0.8	0.39-1.2	0.17-0.4	0.16-0.7	0.19-0.7	0.08-0.9	0.34-1.6	0.64-1.2	0.62-1.5	0.37-1.7	0.639±0.251 ^{ab}
Faisalabad N=60	0.55-1.4	0.58-1.4	0.43-0.8	0.35-1.2	0.16-0.5	0.14-0.6	0.18-0.6	0.07-0.9	0.33-1.6	0.66-1.2	0.61-1.5	0.35-1.6	0.638±0.248 ^{ab}
Jhang N=60	0.51-1.4	0.52-1.4	0.40-0.7	0.34-1.1	0.15-0.4	0.11-0.6	0.15-0.7	0.04-0.8	0.33-1.5	0.61-0.9	0.60-1.4	0.31-1.5	0.36±0.247 ^{ab}

Milk samples %ages exceeding National and International aflatoxin M1 (AFM1) MRL in selected cities of central Punjab during 2019, collected from Lahore and Faisalabad, whereas Jhang results showed that 92% of samples exceeding the EU, MRL as presented in Table 4. The overall number of samples and fractions per month in the different milking seasons in selected cities of central Punjab, higher than the PSQCA MRL, are shown in Table 4.

Table 4: Milk samples %ages exceeding National and International aflatoxin M1 (AFM1) MRL in selected cities of central Punjab in 2019

Cities	International/ National Standards	Flush Milking Season				Lean milking season			Semi flush Milking season				
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Lahore N=60	% samples exceeding EU MRL (0.05 µg/L)	100	100	100	100	100	100	100	100	100	100	100	100
	% samples exceeding PSQCA MRL (10 µg/L)	0	0	0	0	0	0	0	0	0	0	0	0
Faisalabad N=60	% samples exceeding EU MRL (0.05 µg/L)	100	100	100	100	100	100	100	100	100	100	100	100
	% samples exceeding PSQCA MRL (10 µg/L)	0	0	0	0	0	0	0	0	0	0	0	0
Jhang N=60	% samples exceeding EU MRL (0.05 µg/L)	100	100	100	100	100	100	100	92	100	100	100	100
	% samples exceeding PSQCA MRL (10 µg/L)	0	0	0	0	0	0	0	0	0	0	0	0

Seasonal variation of cadmium in milk samples collected in different milking seasons from selected cities of central Punjab was observed during 2018-2019. In the present study, 360 milk samples were collected. The highest contamination (0.037ng/L) was observed in Lahore, followed by Faisalabad (0.034ng/L) and Jhang City (0.031ng/L). The maximum concentration(0.037ng/L) of Cd was recorded during the flush milking, and the minimum concentration (0.020ng/L) was observed during the lean milking season in Jhang City(Figure 2).



A swift decreasing trend in cadmium contamination (0.034-0.020 ng/L) was seen from March to July, it was observed that Cd contamination trends in all selected cities were found to be almost similar to each other for all milking seasons(Figure 3).

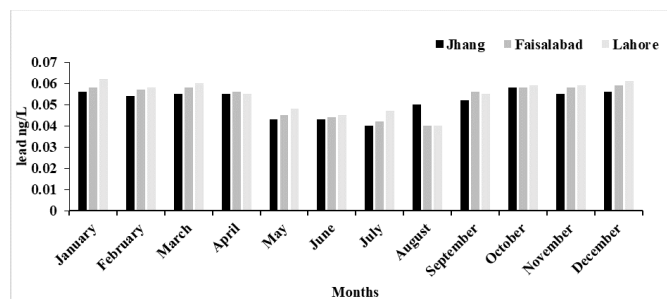


Figure 3: Seasonal variation of lead concentration in milk samples collected from central Punjab during 2018-19

DISCUSSION

In developing countries, food and feed aflatoxin contamination is a burning issue. The results showed that AFM1 toxicity in selected cities of Punjab was higher than the allowed EU MRL (0.05 g/L). These findings of the current studies agree with the results published by [18], who reported AFM1 toxicity in the milk from the Sindh province of Pakistan and concluded that 94 percent of samples contain contamination. Out of which, 70 percent of samples exceed the standard values of US regulations. Another study reported that 53.8% of raw milk, as well as milk products, were contaminated with perceptible concentrations of AFM1 (≤ 50 ng/L) while 24.2% of samples had concentrations of AFM1 more than the allowed limit standardized by the European Union i.e., EU; 50 ng/kg. According to current work, the maximum average of 82.4 ± 7.8 ng/kg of AFM1 was found in the raw milk samples. The findings of this study strengthen the presence of rigorous concentration for AFM1 in animal feed, particularly considering the elevated prevalence rate of hepatitis cases in the major cities of Punjab, Pakistan [19]. According to some studies, the food supply in Africa may contain up to one-third more aflatoxins than in the United States. According to the findings, AFM1 was found in all milk products [20, 21]. About 99% of the samples were reported with greater values of AFM1 contamination as the standards prescribed by EU, In comparison, only 9% of samples were able to pass the regulatory levels of United States, when 87 milk samples analyzed from Indian market [22]. It ended up with a higher concentration of AFB1. When livestock are fed AFB1-contaminated sources, the AFB1 is released as AFM1 in milk. In Pakistan, another study reported more elevated level of AFM1 during winter season [23], and compared to the other seasons. The current research exposed that the primary two reasons for milk contamination include animal feed and its handling. For instance, the handling of corn and cotton seed cake. When these ingredients are harvested and stored during humid weather leads to increased concentration of toxins that results in higher milk AFM1 level [16, 24]. Comparing the

heavy metal contents in milk samples with the maximum permissible limits (MPL) (2.6 $\mu\text{g}/\text{kg}$ for Cd and 20 $\mu\text{g}/\text{kg}$ for Pb, respectively.) established by International Dairy Federation, current study showed that 100 percent milk samples collected from selected cities in different milking seasons, exceeding maximum permissible limit of lead and cadmium. The higher heavy metals concentration in milk may be due to the utilization of sewerage water for the usage in agricultural fields. These metals may be increased in the drinking water being used for the animals.

CONCLUSIONS

The current study indicated that all milk samples alarmingly exceeded AFM1 (aflatoxin) maximum residual limits (MRL), with European MRL criteria. Results showed that the feed given to the dairy animals contained AFB1 and heavy metals, mainly responsible for milk contamination. Seasonal variations were also noted in the concentrations of heavy metals, including cadmium and lead, with Lahore showing the most contamination. Furthermore, this study also reported a greater AFM1 and heavy metals concentration in the winter season.

Authors Contribution

Conceptualization: FS

Methodology: MA

Formal analysis: FZ

Writing, review and editing: AI, TT, ZA, FS

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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