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Assessment of Aflatoxin M1 in Milk from Chakwal City

Uzair Ahmad*

Data Scientist and AI/ML Academician, Northeastern University, Pakisthan

Abstract

Background and Objective: Contamination with aflatoxins in raw milk is a burning issue in the World regarding human health. Aflatoxins are secondary metabolites of toxigenic fungi, and are potential mutagens, carcinogens, teratogens, genotoxins and other health hazards to human. These toxins are produced by *fusarium*, *penicillium* and *aspergillus Spp*. Aflatoxin M1, hydroxylated forms of Aflatoxin B1 (AFB1), predominantly present in milk.

Materials and methods: ELISA technique was us to check the quantity of aflatoxin M1 in milk samples. Aflatoxin M1 test kit was used for the detection of AFM1 in milk by carrying out the procedure of Competitive Enzyme Linked Immunosorbent Assay (ELIZA). Competitive Enzyme Linked Immunosorbent Assay (ELIZA) is a cost effective, quick and reliable technique for the quantitative analysis of AFM1. German Federal board of Health (Kaniou-Grigoriadou, Eleftheriadou, Mouratidou, & Katikou, 2005) also included this process in their official collection of test procedure.

Results and discussion: The results showed that every sample was contaminated with AFM1. The results from the test shows that every sample was contaminated with AFM1. According to the findings of different farms all samples were found positive with Aflatoxin M1 but only 15% were found higher value than European Commission while all 100% samples were below value according to the Pakistan Standard and Quality Control Authority acceptable limits Maximum concentration was shown in Seth Shahmeer Dairy Farm and Zumurd Dairy Farm 0.585 μg/l and minimum in Al Hadi Dairy Farm with value 0.252 μg/l.

Keywords: Aflatoxin • AFM1 • Milk • Elisa kit • Chakwal

Introduction

Milk is an important part of our life. It is used worldwide and it is most drinking item in world, not includes water, it provides all nutrients for normal growth and development of children and young ones [1]. Milk is a major component in daily diet of growing children. It provides all the important nutrients which are necessary for body development that's why it is known as perfect food [2]. In world, milk has been utilized widely to obtain the nutrients and energy from it [3]. It is being utilized by human being in all phases of his life from infant to adolescent and from adolescent to adult phase. It is equally important for humans of all age groups and specially given to the newly born babies who cannot ingest the solid food to acquire the all essential nutrients. The babies take milk for essential nutrients as the milk is said to be complete diet. Therefore, it is of extreme apprehension to consider the contaminants of milk as it may be contaminated with different contaminants [4]. Pakistan is on 4th number in the world for the dairy production and is behind USA, China and India [5]. Pakistan's production of dairy milk from buffalo is being ranked on second in the world's production of milk from buffalo [6]. Aflatoxin M1 is a known carcinogen of the aflatoxin class, a class of mycotoxins belonging to three species of Aspergillus which contaminate plant and plant products: *Aspergillus flavus*, *Aspergillus parasiticus* and rare *Aspergillus nomius*. *Aspergillus flavus* still generates aflatoxins of the B type. The hydroxylated metabolite of aflatoxin B1 is aflatoxin M1 and can be present in milk or dairy products collected from animals which have consumed toxic feed. In susceptible animals, the highly toxic potential of aflatoxin M1 is approximately one order of magnitude lower than that of aflatoxin B1 [7]. The inactivation by-product of aflatoxin B1 is generally known to be aflatoxin M1. Peanuts, meal, maize, and cottonseed meal are the primary fact of aflatoxins in feed. Metabolic by-products aflatoxins are produced principally by the molds *Aspergillus parasiticus* and *Aspergillus favus* are the most generally examined mycotoxins. Positive conditions for the improvement of mycotoxins might be available during all periods production of food, i.e., during plant development in the field, during harvesting, stockpiling and transport and during the innovative creation of food [8].

Objectives

Aim this research is that no research had been done in this area, so I focused on the adulteration, physiochemical, and aflatoxin detection of milk in this area. By performing these tests, I will be able to explain the exact condition of milk. Some other edges of this research have been listed below:

- Milk quality enhanced
- Target big market
- Higher yield
- Animal health
- Human health

Material and Methods

Collection of samples

90 loose milk samples were collected from different farms of chakwal. The sampling was done to describe the level of Aflatoxin M1 in the milk of these farms. Milk samples (n=100) were collected by simple random sampling technique from the farms. The samples were collected in sterilized sampling bottles and transported to the laboratory in an ice box. Afterwards these samples were kept frozen at -40°C prior to analysis at the laboratory. Following is the map of Chakwal city. 5 samples were collected from each farms of chakwal city (Figure 1 and Table 1).

Determination of aflatoxin M1

Aflatoxin M1 in milk samples was determined by using Competitive ELIZA

***Address for Correspondence:** Uzair Ahmad, Data Scientist & AI/ML Academician, Northeastern University, Pakisthan, E-mail: uzairahmedmalik@gmail.com*

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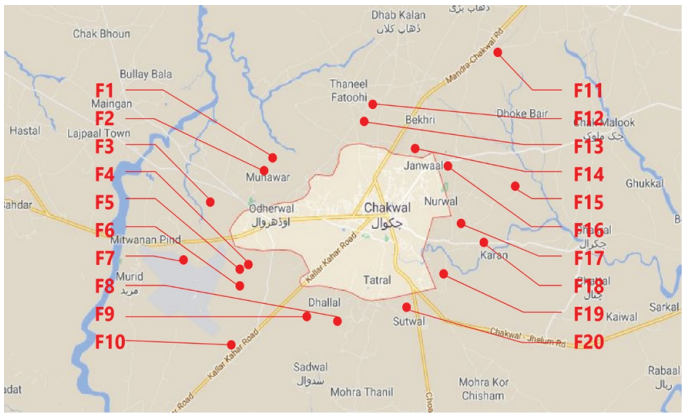

Figure 1. Geographical locations of raw milk samples from selected twenty farms of Chakwal City.

Table 1. Codes of selected farms.

Name	Abbreviations
Solid Not Fat	SNF
Total Solids	TS
Institute of Food Science and Technology	IFSN
Bismillah Dairy Farm	F1
Al Najaf Dairy Farm	F2
Al Hadi Dairy Farm	F3
Milk Lime Dairy Farm	F4
Anmol Dairy Farm	F5
Al Noor Dairy Farm	F6
Al Madina Dairy Farm	F7
Daud Suleman Dairy Farm	F8
Hussain Dairy Farm	F9
Anwar Pehlwan Dairy Farm	F10
Malik Dairy Farm	F11
Mian Sardar Dairy Farm	F12
Seth Shameer Dairy Farm	F13
Umar Raza Dairy Farm	F14
Al Awan Dairy Farm	F15
Kahoot Dairy Farm	F16
Zamurd Dairy Farm	F17
Afzal Dairy Farm	F18
Munawar Dairy Farm	F19
Karam Dairy Farm	F20

(RIDASCREEN FAST AFM1, R-Biopharm) procedure was used to detect the Aflatoxin M1 in milk samples as recommended by R-biopharma GmbH [9].

Reagents:

- 6 × standard solutions of 0, 5, 10, 20, 40 and 80 ppt
- 1 × conjugate- peroxidase
- 1 × anti-aflatoxin M1 antibody
- 1 × Red chromogen Pro- tetramethylbenzidine
- 1 × Stop solution-1N sulphuric acid
- 1 × Washing buffer- salt containing 0.05% Tween 2

Method: Aflatoxin M1 test kit was used for the detection of AFM1 in milk by carrying out the procedure of Competitive Enzyme Linked Immunosorbent Assay (ELIZA). Competitive Enzyme Linked Immunosorbent Assay (ELIZA) is a cost effective, quick and reliable technique for the quantitative analysis of AFM1. German Federal board of Health also included this process in their official collection of test procedure [10].

Sample preparation: Samples were prepared according to directions described by the RIDASCREEN FAST test kit. Centrifugation was the first step which was done for 10 minutes at 3500 g at 10°C for the separation of fat from the milk. After centrifugation, the layer of fat was separated by the Pasteur pipette and liquid at lower phase was used directly in the analysis [11].

Test procedure: 50 µL of milk samples was added in each well. 50 µL of AFM1 standard solution was added in separate wells. 50 µL of enzyme conjugate was added to each well. 50 µL of anti-aflatoxin M1 antibody solution was added afterwards. It was mixed gently and incubated for 10 minutes at room temperature. This was incubated in dark for the time period of 1 hour. After this, the liquid inside the well was poured out on the absorbent paper to clean out properly. Washing buffer was used to wash the wells twice. The amount of washing buffer used was 250 µL. afterwards, 50 µL of stop solution was added to each well, mixed gently and absorbance was measured at 450 nm [12].

Evaluation of AFM1: The value of absorbance obtained of the samples was divided with the absorbance of zero standards. For the calculation of maximum absorbance, it was multiplied by the 100. Thus, the zero standards were made equivalent to 100% and the absorbance values were quoted in percentages [13].

Results and Discussion

All the collected samples from different farms were tested to detect the quantity of AFM1. The results from the test shows that every sample was contaminated

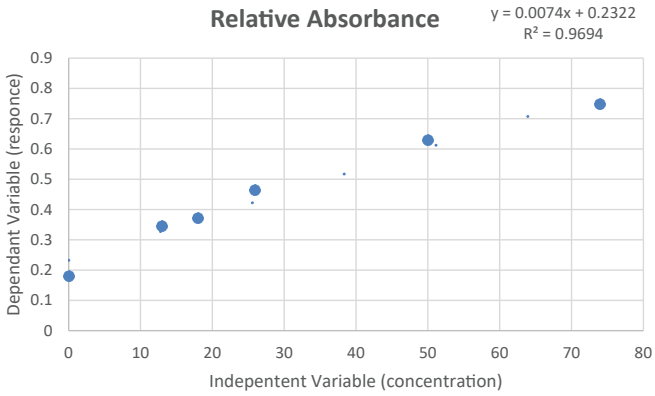

Figure 2. Relative absorbance curve.

Table 2. Ascertainment of AFM1 in raw milk samples from selected farms of Chakwal City.

Name of Farms	Mean % ± SEM
F1	0.432 ± 0.001
F2	0.467 ± 0.001
F3	0.252 ± 0.001
F4	0.324 ± 0.001
F5	0.475 ± 0.001
F6	0.481 ± 0.001
F7	0.274 ± 0.001
F8	0.447 ± 0.001
F9	0.448 ± 0.001
F10	0.456 ± 0.001
F11	0.464 ± 0.001
F12	0.422 ± 0.001
F13	0.585 ± 0.001
F14	0.480 ± 0.001
F15	0.523 ± 0.001
F16	0.391 ± 0.001
F17	0.585 ± 0.001
F18	0.444 ± 0.001
F19	0.456 ± 0.001
F20	0.454 ± 0.001

with AFM1. According to the findings of different farms all samples were found positive with Aflatoxin M1 but only 15% were found higher value than European Commission while all 100% samples were below value according to the Pakistan Standard and Quality Control Authority acceptable limits Maximum concentration was shown in Seth Shahmeer Dairy Farm and Zumurd Dairy Farm 0.585 µg/l and minimum in Al Hadi Dairy Farm with value 0.252 µg/l. In all raw milk samples of the different farms only 3 samples showed aflatoxin M1 contamination over acceptable limit 0.5 µg/l by European Commission. In contract to this fact, all the samples were within the prescribed limit by Pakistan Standards and Quality Control Authority (PSQCA) which is 10 µg/l. The standard solutions of concentration from 0 to 80 ppt AFM1 were used to find calibration/standard curve. The results showed the linearity of the standard curve over the range studied. Figure 2 gives the calibration curve of standard solutions of AFM1 with concentrations of 0, 5, 10, 20, 40, and 80 ppt by ELISA analysis (Table 2).

Conclusion

According to the findings of different farms all samples were found positive with Aflatoxin M1 but only 15% were found higher value than European Commission while all 100% samples were below value according to the Pakistan Standard and Quality Control Authority acceptable limits. Maximum concentration was shown in Seth Shahmeer Dairy Farm and Zumurd Dairy Farm 0.585 µg/l and minimum in Al Hadi Dairy Farm with value 0.252 µg/l shown in (Table 2). In all raw milk samples of the different farms only 3 samples showed aflatoxin M1 contamination over acceptable limit 0.5 µg/l by European Commission. In contract to this fact, all the samples were within the prescribed limit by Pakistan Standards and Quality Control Authority (PSQCA) which is 10 µg/l.

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