

Survey on Contaminated Raw Milks with Aflatoxin M₁ in the Sarab Region, Iran

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Abstract: Mycotoxins are those groups of fungus metabolites which as the result of the consumption of contaminated food causes human or animal illness or death and the resulted illness from this is called mycotoxicosis. In this study 100 samples from milk were analyzed for the presence of aflatoxin M₁ by thin layer chromatography. This study showed that from 100 samples were analyzed a total of 84 samples (84%) were positive for aflatoxin M₁ and 16 samples (16%) were negative. The range of aflatoxin M₁ content was 30-630 ng/lit. Contamination rate of aflatoxin M₁ in tanks milk in around (84%) with amounts ranging 30 to 630 ng/lit. All contaminated samples had a level of aflatoxin M₁ above the European countries standard (50ng/lit). Therefore, the following suggestions are made. Determined standard complications for aflatoxin limit value in feed and aflatoxin M₁ in milk and dairy products- use of effective methods for treatment of contaminated feed, milk and dairy products.

Key words: Raw milk, aflatoxin M₁, sarab, Iran.

INTRODUCTION

According to the increasingly population growth need to diet also increased. In this mean, dairy products such as milk play an important role in relieving food needs specially from providing of proteins, minerals, vitamins and etc. aspect. Because of this reasons nurturing of dairy livestock industry have been expanded and daily requirement to dairy products has been increased. On the other hands, in unsafe order, milk as a risk agent can endanger the human life and children and neonates expose on a risk. Understanding of toxicities subsequent fungi toxins has a long history so that disease which called aspergillosis yields to wasting of one Hundred thousand turkeys. More studies about exploring of the causative agent of death yields to this finding that toxins were produced by two groups of fungus which are called aspergillus flavus and aspergillus parasiticus (18,19). After this time more studies were done on aflatoxins and their carcinogenesis effect and their toxicity effects caused that countries legislated such laws that by this means can calculate this toxins in foods so that nowadays in 56 world countries, inspection of foods from existence of this toxins apply. Entrance of aflatoxins to milk products occurred by two way:

1- Indirect contamination; to wit consumption of contaminated products by animals and entrance of these toxins to milk that about 1-2% of ingested B₁ aflatoxin expelled as M₁ aflatoxin. (18,19,20)

2- Direct contamination; to wit contamination of milk and its products by fungus and then production of mycotoxins. (18,19)

According to this point that M₁ aflatoxin is very similar with B₁ from terms of chemical structure and in fact is of 4-hydroxy aflatoxin B₁ derivatives and on the other hand because of carcinogenesis, mutation and teratogenic effects of aflatoxins (14,17,21) and resistance of several aflatoxins including M₁ and M₂ against common physical agents (3,6,23), there are many studies about detection of contaminated milks from one hand and routes to sanitation of contaminated milks from other hand. The objective of this study was to determination of the raw milks contamination to aflatoxin M₁.

MATERIALS AND METHODS

Chemical Material Used:

Fixed chloroform with 5% ethanol 96°, toluene, glacial acetic acid 99.6%, acetonitrile, diethyl ether without peroxide which its ethanol is lower than 5%, N-hexane, acetone, methanol, dried sodium sulfate, NaCl, sodium dodecyl sulfate, silica gel 60 solely to column and standard M₁ chromatography, filter paper, fiberglass. It must be remember that all material used were specialized to chromatography.

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Laboratory Equipment Used:

TLC-scanner modeled camac Swiss made, condenser evaporator Swiss made, chromatography glass plates 20*20cm modeled Whatman USA made, Syringe 10 and 100 microliter Hamilton Co. made, glass column for columnar chromatography longed 30cm and 1cm in diameter and 15ml volume that lower terminal of the column were striped and makes 4mm in diameter a small circle that equipped to an appropriate plug, mixer, regulatory ultraviolet lamp on 360nm wavelength, appropriate equipments to evaporation of the chloroform in conical tubes in azotes gas flow and at 40-50 degree centigrade temperature, regulatory drier at 70-105 degree centigrade, micropipette 100 and 1000 microliter modeled Eppendorf, regulatory bain marie at 0-100 degree centigrade made by Swiss gerber Co., shaker mixer and other common laboratory materials.

Methods:

In this study of 246 Sarab milk collection centers by chance about 25 samples of 100 centers were collected in each season, in different process and same intervals and after transportation to lab, samples were evaluated by International dairy federation (IDF) method to existence probability of M₁ aflatoxin. In this study, prior 50ml milk transferred to a 250ml Separator funnel and 10ml chilled to 4 degree centigrade saturated NACL were added to it and then 10ml sodium dodecyl sulfate 5% in room temperature were added to it. Then 125 ml chilled to 4 degree centigrade chloroform were shaded on it and were shaking slowly for 1 minute so that two blue and chloroform phases were make. Chloroform layer were transferred to a conical Erlenmeyer and 5gr sulfate sodium were added to it and were fixed for 15minute and sometimes was shook. Finally, by a filter paper noted solution were filtered and 75ml of it were taken. Achieved extract were transferred to chromatography column and logged solution were washed with 25ml toluene+ glacial acid acetic (9+1) to the extent that solution receive to upper level of sodium sulfate. Then washing solution was out and column was washed with 25ml n-hexane and then was washed with 25ml mixed acetonitrile, diethyl ether and n-hexane (10+30+60). Then remnant aflatoxin m₁ was washed with 60ml mixed chloroform and acetone and washing result were collected in bottom round balloons and evaporated by rotary evaporator. It must be remember that all washing processes must be continue without interruption and in all stages chromatography column not be dried. Solution was evaporated at 50 degree centigrade under neutralized gas including nitrogen. After chilling of the tube, by a Hamilton syringe 100µl chloroform was added to it and mixed for 1 minute. After extraction, spots related to standard and analysis samples were located on a TLC plates. Then plates were transferred rapidly to chromatography tanks with 100ml mixed isopropanole, acetone and chloroform (5+10+85) and finally after preparation of the extraction, plates of chromatography were transferred to TLC scanner and based on under curve area and below formula, concentration of aflatoxin m₁ were calculated by ng.

$$\text{Aflatoxin } m_1 \text{ concentration (ppb)} = \frac{\text{sample under curve area}}{\text{standard under curve area}} \times \text{standard concentration} \times \frac{100}{\text{recycling percent}}$$

RESULTS AND DISCUSSION

According to the obtained results, among 100 understudying raw milk samples numbered 84 samples had aflatoxin M₁ in range of 30-630 ng/lit and 16 cases in terms of existence of aflatoxin were detected negative. On the other hand, based on data in table 1 maximum contamination rate is belonged to autumn and winter with 388 and 390 ng/lit respectively. Whereas, contamination rate in spring and summer was 372 and 320 ng/lit respectively. It has been revealed that contamination rate in summer was lower than other seasons. In fact, ANOVA test consider this amounts as significant. However, aflatoxin M₁ in Sarab area milks is higher than accepted permissible ranges in other countries such as European Union countries that in countries permissible range is approximately between 0.05-0.5 µg/Lit.

Table 1: contamination rate of raw milks in Sarab area to aflatoxin M₁ during 2009-2010.

Season	No. of samples	Contamination average (ng/Lit)	Minimum Contamination (ng/Lit)	Maximum Contamination(ng/Lit)
Spring	25	372	30	341
Summer	25	320	69	390
Autumn	25	388	50	592
Winter	25	390	91	630

Discussion:

Based on that contamination of milk and its products to aflatoxin M₁ is worldwide (1,2,3,4,5,8,10,11,12,13,15,16,18,21,22,24) our country also isn't exception so that done studies in our country about aflatoxin M₁ in contaminated milks indicates this fact that not only produced milks about contamination

to aflatoxin M₁ are in high frequency but contamination rate in these milks is higher than standard levels exist in European countries. It must be noted that until there isn't any complied standards in Iran thus European countries standards considered. In other study by Karim *et al.*, 1361 on 61 milk samples (52 samples was belonged to Tehran around cow keepers and 9 samples was belonged to pasteurized milks) revealed that 92.31 % of raw milks and all of the pasteurized milks were contaminated to aflatoxin M₁. In that study maximum contamination rate were determined 25 and 20.1 µg/Lit in raw and pasteurized milks respectively (1). In other study that carried out by Karim *et al.*, 1387 by ELISA method demonstrated that 82.2% of samples were contaminated to aflatoxin M₁ and contamination rate in referred milks to Tehran milk factory was 259.5 ng/Lit (2). In our research, of 100 raw milk samples numbered 84 samples were contaminated to aflatoxin M₁ and contamination rate was 30-630 ng/Lit. According to this and previous researches results revealed that contamination rate of aflatoxin M₁ in Iran produced milks is higher than other countries and standard limits. Also demonstrated that contamination rate in cold seasons is higher than hot seasons so this findings are consistent with other researches results (2,7) whereas this relationship is not referred in some others. Because of its worldwide until there isn't any sufficient assume to minimizing the aflatoxin M₁ from milks. In sum, appropriate management procedures in safe feeding and without contamination or minimizing the animals feed contamination to aflatoxin M₁ is one of the most important way to minimizing the milk contamination.

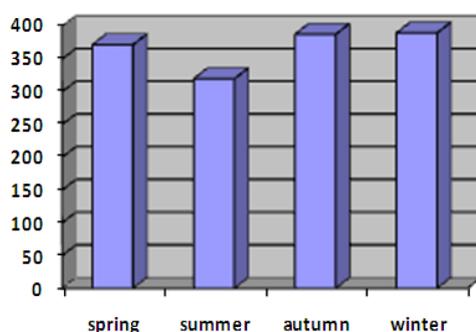


Diagram 1: comparative columnar diagram of contamination rate of raw milks at different season to aflatoxin M₁.

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