Prevalence and analysis of aflatoxin M_1 in milk and milk products of Bhagalpur, Bihar, India

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Abstract

Aspergillus flavus is ubiquitous saprophytic mould which produced aflatoxins in any of the food and feed items under congenial environmental conditions. Aflatoxin B_1 (AFB_1) is highly carcinogenic compound categorized as class I carcinogen. Aflatoxin B_1 in feed can be transformed into the milk as its hydroxylated derivative, aflatoxin M_1 (AFM_1). In the present investigation, the aflatoxin M_1 contamination in cattle milk/ milk products of Bhagalpur and surrounding districts was analysed and also the influence of various factors (types of cattle, regions and seasons) on the prevalence of aflatoxin M_1 contamination was monitored. Milk samples of cattle (cow, buffalo and goat) and milk products (curd, paneer, khoya, cheese, ice-cream, milk-shake and milk powder) collected in different seasons of various localities (urban, semi-urban and rural areas) were analysed for aflatoxin M_1 contamination.

Means of variable ± SD were recorded and data of regional variations was subjected to statistical analysis by one way analysis of variance using SPSS 16. Among the 182 milk samples analysed, maximum aflatoxin M_1 contamination occurred in the milk samples of cow (58%) followed by buffalo (52%), however, there was least (25%) contamination in Sudha dairy milk samples. Out of 75 aflatoxin positive samples, 29 contained milk toxin in the range of 0.05µg/kg to 0.5µg/kg, however, in remaining it was less than 0.05µg/kg. Only one sample (cow milk) aflatoxin M_1 was exceeding 0.5µg/kg. Eleven samples of milk products (khoya, curd, rabri and paneer) contained AFM_1 exceeding 0.05µg/kg. In most of the samples, aflatoxin was within the regulatory limits (USFDA, EU and CAC). Seasons and regions had pronounced influence on the prevalence of aflatoxin in milk and milk products. Periodical monitoring and evaluation of factors conducive for aflatoxin synthesis will help us to develop strategies for prevention and control.

Keywords: Aflatoxin B_1 (AFB_1), Aflatoxin M_1 (AFM_1), Cattle feed, Seasonal variation, ANOVA test, Bihar.

Introduction

Aflatoxins are the toxic metabolites of certain saprophytic moulds *Aspergillus flavus*, *A. parasiticus* and *A. nomius*[4] which are distributed worldwide in air, soil, food and feed[12,18]. International Agency for Research on Cancer (IARC 2002) has classified aflatoxin B_1 (AFB_1) as class I carcinogen. It has been evident that if AFB_1 is present in the diet of lactating animals, then aflatoxin M_1 (AFM_1) would occur in milk[2,9,10,19]. The contamination of aflatoxin M_1 also occurs when the moulds synthesizing aflatoxin pass to the milk and produce aflatoxin during transport, process and storage phases after milking[7].

AFM_1 is classified in group 2B carcinogen (possibilities of developing cancer among humans). Aflatoxin M_1 is the monohydroxy derivative of AFB_1 produced after metabolization in liver following consumption of contaminated feed by lactating cows/ cattle. After ingestion of contaminated feed, AFM_1 can be detected in cow's milk (with the percentage of 1-6% of AFB_1) from few hours to two days[15].

AFM_1 could be found in dairy products manufactured from contaminated milk, therefore, the only practical way to ensure the safety of milk and dairy products for human consumption is avoiding its contamination[27]. Dairy products are the one of the most important exposure factors through diet for AFM_1. High AFM_1 incidence in milk and milk products can create an important public health risk due to the fact that milk and milk products are consumed widely by all age groups and especially infants, children and old aged people[20]. The occurrence and incidence of AFM_1 contamination in milk and dairy products may vary according to individual, localities and also on agricultural practices[23]. Consumption of high amounts of concentrated feeds by high-yielding cows might result in high level of aflatoxin M_1 contamination in milk[6]. Geographical variation in the concentration of AFM_1 has also been reported[1,3].

Since AFM_1 has carcinogenic potency as high as that of aflatoxin B_1[16], thus many countries have set maximum acceptable levels for AFM_1 in milk and dairy products. US Food and Drug Administration (USFDA) has set a maximum permissible level of 0.5 µg/Kg of AFM_1 while in some European countries (EC), the maximum acceptable level is 0.05 µg/kg[29]. In order to maintain this limit, aflatoxin B_1 level in feeds for dairy animals must be kept limited[22].

The presence of AFM_1 needs to be considered as great human health risk and never it should be underestimated or neglected due to the unpredictability of climatic and environmental conditions and the unavailability of certain agricultural systems (characterized by poor economic conditions and/or lack of knowledge) to face and manage aflatoxin prevention/ contamination. The occurrence of AFM_1 in milk and dairy product especially cow's milk makes it a particular risk for humans because of its importance as a foodstuff for adults and especially children.

As a result of this worldwide situation, the objective of our work was to access the levels of aflatoxin AFM_1 contamination in the cattle milk/ milk products of Bhagalpur, Bihar and also to evaluate the influence of factors such as type of cattle, seasons and regions on the prevalence of aflatoxin M_1 contamination in milk in order to develop future strategies for prevention/ control.

Material and Methods

Milk sample collection: Raw milk of cow, buffalo and goat milk was collected from different regions (urban, semi-urban and rural area) in sterile plastic bottles while pasteurized milk samples were purchased from Sudha dairy of Bhagapur district. Samples were collected directly from dairy farms, milkman and farmers after morning milking. Different milk products (curd, paneer, khoya, cheese, ice-cream, milk shake and milk powder) were purchased from Bhagalpur market.

Extraction: AFM_1 was extracted following the AOAC (2000) method. 50 ml of different milk samples were shaken first and then 10 ml of saturated salt solution (40 gm NaCl / 100 ml water) and 120 ml chloroform (at 30ºC) were added in a 250 ml separating funnel and subsequently allowed to separate for 2 minutes. For milk powder, reconstitute 5 gm with 50 ml of water, however, for cheese, paneer, curd and Khoya 15 gm samples were blended with 1 ml saturated salt solution, 5 gm diatomaceous earth or celite and 100 ml chloroform for 60 seconds in a blender jar. Then lower $CHCl_3$ layer was drained into 125 ml Erlenmeyer flask.

In case of milk product, the chloroform solution was centrifuged (15 minutes at 2000 rpm) and then lower chloroform layer was drained into flask. After separating chloroform layer, 10 gm anhydrous sodium sulphate was added to $CHCl_3$ with stirring. It was further filtered into 100 ml graduated cylinder. Then filtrates were used for column chromatography.

Column chromatography: Column was half filled with $CHCl_3$ and then 2 gm silica gel slurry (made with $CHCl_3$) was added. 2 gms sodium sulphate was added above silica gel. Excess $CHCl_3$ was drained off and silica column sides were rinsed with $CHCl_3$. Sample extract was added in column and entire solution drained through column by gravity, simultaneously stirred with sodium sulphate gently. Graduated cylinder was rinsed with $CHCl_3$.

Then wash column with 25 ml of toluene – acetic acid (9 + 1) in order to remove coloured compounds. Further, it was washed with 25 ml of hexane – ether – acetonitrile (5 + 3 + 2) to remove fat. Aflatoxin M_1 was eluted with 40 ml $CHCl_3$ – acetone (4 + 1) which was then evaporated to dryness and subsequently it was used for TLC/ HPTLC.

Thin Layer chromatography: Samples residues were dissolved in 100 µl of benzene – actonitrile (9 + 1) and spotted on HPTLC plate. 20µl of test solutions and 2, 4, 6, 8 and 10µl of AFM_1 standard (0.25µg /ml) were spotted on HPTLC plate. The plate was developed in chloroform - actone – isopropanol (87 + 10 + 3) and aflatoxin M_1 was calculated in µg/ kg or ppb through fluorescence intensity and also through CAMAG TLC Scanner (Camag, Muttenz, Switzerland), using a D-2 lamp and K-400 filter along with a Sklar integrator.

Statistical analysis: The means of variable ± SD were recorded and regional variation was subjected to statistical analysis by One-way Analysis of Variance using SPSS 16.

Results

The results obtained by the qualitative and quantitative analysis of aflatoxin M_1 from different milk and milk products are shown in table 1. The study revealed that the per cent of contamination of aflatoxin M_1 depends upon the type of milk and milk products. We examined a total of 182 samples of milk and milk products among which 75(41.1%) were found contaminated with aflatoxin M_1.

In case of milk samples, maximum contamination (58%) occurred in cow milk samples followed by buffalo milk, (52%) however, minimum contamination (25%) of aflatoxin M_1 was observed in Sudha milk (25%). Among the milk products, maximum (53.8%) contamination was observed in local made product khoya followed by curd (45%). Cheese and milkshakes were less contaminated by aflatoxin M_1, 25% and 28.5%, respectively.

The quantitative analysis of aflatoxin M_1 revealed that range of aflatoxin was high in buffalo milk (0.020-0.22µg/kg) followed by cow milk samples (0.011-0.156 µg/kg). Out of 182 samples tested, 28 samples contained toxin in the range of 0.05µg/kg to 0.5µg/kg. However, one sample (cow milk) was highly contaminated with aflatoxin, the level exceeding 0.5µg/kg. The remaining 46 samples were contaminated by aflatoxin M_1 but the quantity was less than 0.05µg/kg. USFDA has set a maximum permissible level for aflatoxin M_1 of 0.5 µg/Kg (in milk), however, in Europe and in some African and Asian countries, the maximum acceptable level of aflatoxin M_1 in milk is 0.05 µg/kg[17]. In order to maintain this level, it is desirable that the aflatoxin B_1 level in feeds of dairy animals must be limited to keep the levels of AFM_1 in milk < 0.05 µg/Kg[22].

Mean concentrations of aflatoxin M_1 in different milk and milk products were also determined. In our studies, cow (0.076µg/kg) and buffalo milk (0.073µg/kg) samples contained comparatively high AFM_1 concentration.

Regional variation of aflatoxin M_1 from different species: Regional variations in aflatoxin M_1 contamination of milk of different cattle (cows, buffaloes and goats) were also analysed. Aflatoxin M_1 contamination in milk of different cattle of urban, semi-urban and rural areas was monitored (Table 2). Considering all types of cattle, maximum contamination (58%) of aflatoxin M_1 occurred in milk of rural areas cow milk followed by buffalo (52%) and goat milk 48%. In semi-urban areas, the contamination of aflatoxin M_1 was comparatively less than rural areas where 48% of buffalo milk samples, 46% of cow milk samples and 36% of goat milk samples were positive to aflatoxin contamination.

However, in the milk samples of urban areas, per cent contamination was comparatively less than semi urban and rural area where 36% samples in milk from buffaloes were positive to aflatoxin AFM_1 followed by 32% from cow and goat. However, maximum concentration of aflatoxin M_1 was obtained in buffalo milk of rural areas (mean =0.126 µg/kg) followed by cow milk of rural areas (0.102µg/kg).

Seasonal variation: Table 3 shows the seasonal variation of aflatoxin contamination in milk of different cattle (cow, buffalo and goat). The observation revealed that the aflatoxin M_1 contamination was significantly high in monsoons and spring seasons compared to winter season. In wet seasons, out of 70 samples of cow milk, 32 (45.7%) were contaminated with aflatoxin M_1 followed by 28 (42.4%) of buffalo milk samples, however, in goat milk the per cent of contamination (35.2%) was less than cow and buffalo. In spring, out of 65 samples of cow milk, 22 samples (33.8%) contained aflatoxin M_1 which, however, was higher than buffalo milk in which out of 54 samples, 17 (31.4%) contained aflatoxin M_1.

Table 1
Aflatoxin M_1 contamination in different milk and milk products

Different milk and milk product	No. of samples	No. of positive samples	>0.5µg/kg	>0.05ug/kg	Range of aflatoxin M_1 (µg/kg)	mean±SD
Cow milk	25	14(58)	1	9	0.011- 0.156	0.076±0.046
Buffalo milk	23	12 (52.1)	0	6	0.020 - 0.22	0.073±0.062
Goat milk	9	3(33.3)	0	2	0.023- 0.072	0.045±0.025
Sudha dairy milk	8	2 (25)	0	0	0.010-0.016	0.013±0.004
milk powder	8	3 (37.5)	0	0	0.009 - 0.024	0.013±0.012
curd	20	9 (45)	0	4	0.016- 0.102	0.046± 0.029
khoya	13	7 (53.8)	0	4	0.025- 0.118	0.060± 0.035
paneer	23	8 (34.7)	0	1	0.013- 0.054	0.032±0.015
cheese	12	3 (25)	0	0	0.00- 0.022	0.013±0.012
rabri	24	8 (33.3)	0	2	0.017- 0.92	0.043±0.024
ice-cream	10	4 (40)	0	0	0.007- 0.031	0.018±0.010
Milk shake	7	2 (28.5)	0	0	0.005- 0.023	0.014±0.013
Total	**182**	**75 (41.1%)**	**1**	**28**	**0.00 - 0.156**	**0.037 ± 0.023**

At p value < 0.05, for the quantitative incidence of AFM_1 in the milk and milk products, the mean data showed significant difference between raw milk of cows, buffaloes and goats to that of Sudha dairy milk, milk powder and milk shake for all three districts under observation. For statistical analysis of one way variance of milk and milk products, CD (at 5%) which clearly show remarkable difference of AFM1 incidence in raw milk to that of processed milk products

Table 2
Regional variation of aflatoxin M_1 from different species

Species	Region	Positive sample (%)	Mean ± SD
Cow	Rural	58	0.102± 0.192
	Semi-urban	46	0.074± 0.112
	Urban	32	0.038±0.036
Buffalo	Rural	52	0.126±0.261
	Semi-urban	48	0.105±0.222
	Urban	36	0.031± 0.028
Goat	Rural	48	0.033±0.021
	Semi-urban	36	0.035±0.022
	Urban	32	0.032±0.017

Table 3
Seasonal variation of aflatoxin M_1 from different season

Season	Species	Total no. of sample	Positive sample (%)	Range of aflatoxin M_1			Mean
				≥0.5µg/kg	0.05 to 0.5µg/kg	<0.05µg/kg	
Spring	Cow	65	22 (33.8)	2	7	13	0.137±0 .193
	Buffalo	54	17 (31.4)	3	8	6	0.177± 0.227
	Goat	29	6 (20.6)	0	2	4	0.038± 0.027
Summer	Cow	104	32 (30.7)	3	12	17	0.128±0.175
	Buffalo	96	24 (25)	2	13	9	0.099±0.146
	Goat	40	7 (17.5)	0	2	5	0.036± 0.027
Monsoon	Cow	70	32 (45.7)	4	18	10	0.143± 0.217
	Buffalo	66	28 (42.4)	2	9	17	0.100±0.198
	Goat	17	6 (35.2)	0	1	5	0.041±0.020
Winter	Cow	92	25 (27.1)	0	6	19	0.034± 0.021
	Buffalo	84	18 (21.4)	0	7	11	0.039± 0.025
	Goat	41	15 (26.5)	0	0	15	0.015± 0.012

At p value < 0.05, for the quantitative incidence of AFM_1 in the milk of cattle, the mean data showed significant difference between raw milk obtained during winter season to any other seasons (summer, spring, monsoons). For statistical analysis of Season X milk, CD is 0.0343 (at 5%) which clearly show that milk obtained during spring, summer and monsoon, the mean value of aflatoxin is significantly higher compared to winter.

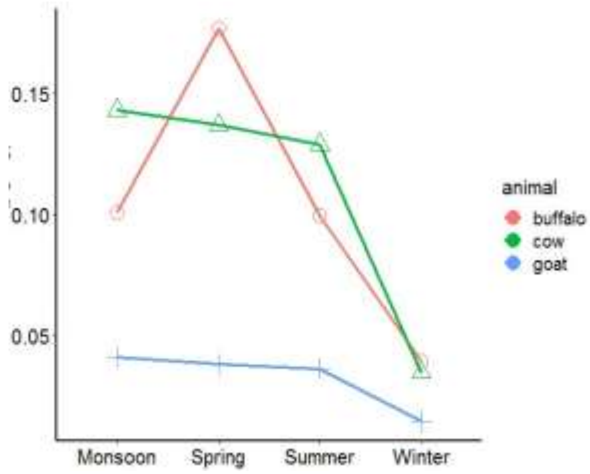

Fig. 1: Mean aflatoxin M_1 contamination among milk samples of different cattle and season
At p<0.05, the mean value of AFM_1 in milk obtained from three cattle generates a CD value of 0.0524. The critical difference clearly shows that the mean value AFM_1 incidence in milk obtained from cows and buffaloes are highly significant compared to goat milk

Among different seasons, winter milk samples of different cattle milk showed less contamination than spring, summer and monsoon. During those seasons, cow milk contamination rate was higher than buffalo and goat milk. 21.4% and 25% of buffalo milk were contaminated in winter and summer respectively. Per cent of aflatoxin positive sample was significantly low in goat milk than cow and buffalo.

Table 3 also shows the variation in the level of aflatoxin concentration in milk of different cattle (cow, buffalo and goat). It was observed that the concentration of aflatoxin M_1 was high during the spring season (figs. 1 and 2). In this season, mean value of M_1 concentration was maximum in buffalo (0.177µg/kg) followed by cow milk samples (0.137µg/kg), however, the mean value was the lowest in goat milk samples (0.038µg/kg). In this season two cow milk samples (out of 22 samples) were grouped in the category in which the concentration exceeded 0.5µg/kg, however, 7 samples came under the range between the 0.5µg/kg to 0.05µg/kg and rest 13 samples concentration was less than 0.05µg/kg.

In case of buffalo milk, out of 17 samples, concentration of aflatoxin M_1 in 3 samples was more than 0.5µg/kg, 8 in the range of 0.5µg/kg to 0.05µg/kg, 6 samples contained concentration less than 0.05µg/kg. However, out of 6 positive samples of goat, 2 in between the range of 0.5µg/kg to 0.05µg/kg and in 4 samples aflatoxin M_1 contamination was less than 0.05µg/kg.

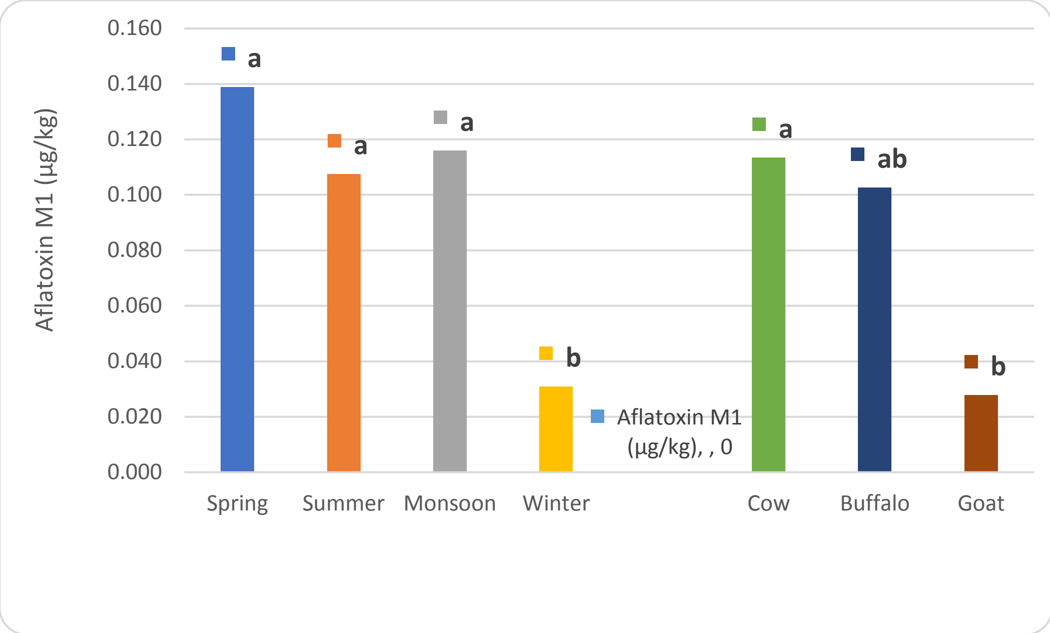

Fig. 2: Variations of aflatoxin M_1 contamination in the milk of different cattle (cow, buffalo, goat) in different seasons.

At $p<0.05$, the one way analysis of variance of AFM_1 incidence in season has a CD value of 0.0343 and that of AFM_1 milk has 0.0524. The CD value among seasons statistically supports the data obtained where the AFM1 incidence in spring, summer and monsoon are highly significant compared to that of winter. The CD value among the cattle further supports the data obtained where the mean value of AFM_1 incidence in cows and buffaloes are highly significant compared to that of goat milk

In the summer, mean value of M_1 concentration was maximum in cow milk (0.128µg/kg) which was more than buffalo milk samples (0.099µg/kg), however, the mean value was low in goat milk samples (0.036µg/kg). Out of 32 cow milk samples of this season, 3 samples contained the level exceeding 0.5µg/kg, however 12 samples had fallen in the range between 0.5µg/kg to 0.05µg/kg and rest 17 samples had less than 0.05µg/kg of aflatoxin M_1. In case of buffalo milk, out of 24 positive samples, concentration of 2 samples was more than 0.5µg/kg, 13 in between the range of 0.5µg/kg to 0.05µg/kg and 9 samples had the amount less than 0.05µg/kg. However, out of 7 positive samples of goat milk, 2 were in the range of 0.5µg/kg to 0.05µg/kg and 5 samples concentration of aflatoxin M_1 was less than 0.05µg/kg.

In the monsoon, mean value of M_1 concentration was maximum in cow milk (0.143µg/kg) which was followed by buffalo milk samples (0.100µg/kg) and the mean value was minimum in goat milk samples (0.041µg/kg). In this season, 4 cow milk samples (out of 32 positive samples) were grouped in the category of exceeding the regulation limit of 0.5µg/kg, however 18 samples were in the range of 0.5µg/kg to 0.05µg/kg and rest in 10 samples, the amount of toxin was less than 0.05µg/kg.

In case of buffalo milk, out of 28 positive samples, 2 contained more than 0.5µg/kg, 9 had fallen in the range of 0.5µg/kg to 0.05µg/kg, in 17 samples contamination level was less than 0.05µg/kg. However, out of the 6 positive samples of goat, only one sample could fall in the range of 0.5µg/kg to 0.05µg/kg and in 5 samples concentration of aflatoxin M_1 was less than 0.05µg/kg.

In winter, concentration of aflatoxin M_1 was lowest than other season. In this seasons mean value of M_1 concentration was maximum in buffalo milk (0.039µg/kg) where it was more than cow milk samples (0.034µg/kg) and mean value was minimum in goat milk samples (0.015µg/kg). In this season, six cow milk samples (out of 25 positive samples) were grouped in the category in which concentration was in between the 0.5µg/kg to 0.05µg/kg and rest in 19 samples concentration was less than 0.05µg/kg. In case of buffalo milk, out of 18 positive samples, 11 were in the range of 0.5µg/kg to 0.05µg/kg, in 7 samples, concentration was less than 0.05µg/kg. However, out of 15 positive samples of goat milk, concentrations of aflatoxin M_1 of all samples were less than 0.05µg/kg.

The mean value of aflatoxin M_1 was found maximum in spring which was highly significant as compared to winter but was at par in summer and monsoon (Fig. 2). However, during the spring, summer and monsoon, there were non-significant differences with respect to the levels of aflatoxins M_1. Mean value of aflatoxin M_1 in winter season was 0.030µg/ kg which was significantly different from other three seasons (spring, summer and monsoon).

Minimum value of aflatoxin M_1 was found in goat milk which was however, significantly low as compared to cow and buffalo. Maximum level of milk toxin was found in cow which was at par with buffalo milk samples.

The analysis of variance with respect to aflatoxin M_1 content in different cattle for different seasons differed significantly. F-value for season was found to be 4.702 whose probability value was 0.001. This reflects the facts that the levels of aflatoxin M_1 contamination of different cattle milk (cow, buffalo, goat) are influenced with the seasons. Mean values of aflatoxin M_1 level in cow and buffalo were at par which however, was significantly high from goat milk (Fig. 2).

Discussion

In case of milk samples, maximum aflatoxin M_1 contamination occurred in cow milk (58%) followed by buffalo milk (52%), however, the least per cent contamination was obtained in Sudha milk samples (25%). The result was in conformity with the previous work[17] in which they analyzed 55,40, 30, 24 and 20 milk samples of buffaloes, cows, goats, sheep and camel for AFM_1 and contamination level was 34.5%. 37.5%, 20% and 16.7% respectively.

In our investigations, out of 182 milk samples analyzed, only one sample of milk contained aflatoxin M_1 exceeding 0.5µg/kg. In 29 samples, the toxin range was in between 0.05 µg/kg, however, remaining samples contained less than 0.05 µg/kg. USFDA has set a maximum permissible level of 0.5µg/kg for aflatoxin M_1 in milk while in Europe and some African and Asian countries, the maximum acceptable level of aflatoxin M_1 in milk is 0.05 µg/kg[29]. To achieve this objective, aflatoxin B_1 level in feeds for dairy animals must be limited to keep levels of AFM_1 in milk <0.05 µg/kg[22]. In previous works, aflatoxin M_1 contamination was observed in camel and cow milk[5,13,15,24]. Occurrence of aflatoxin M_1 in goat milk has also been reported[30].

Many studies declared contamination of AFM_1 in sheep milk[2]. Comparatively high levels (Mean value 0.027ppm) of aflatoxin M_1 have been reported in earlier work while working on cow milk[1]. Concentrations of aflatoxin M_1 in Sudha dairy milk, milk powder, cheese and milk shake samples were less than 0.05 µg/kg, mean of 0.013 ± 0.004, 0.013 ± 0.012, 0.013 ± 0.011 and 0.014 µg/kg respectively.

In our study, Sudha dairy milk (which was pasteurized milk) showed less risk of AFM_1 toxin than fresh milk of cow, buffalo and goat. Our results are in conformity with the earlier work that raw milk contained more aflatoxin level than pasteurized and UHT (Ultra high temperature processed) milk[25,31]. The explanation to our findings is that the pasteurized milk is being stored in refrigerator, however, the raw milk is kept at room temperature. It is known that aflatoxin level does not increase at 4ºC or below temperatures but its level increases in optimum room temperatures due to secondary contamination[14]. AFM_1 in milk can be reduced by good manufacturing, transporting and storage practices. For food safety purposes, there is a need for quality control during processing and distribution of those products[6,11].

Regional variation of aflatoxin M_1 from different species: While considering the type of cattle and the regions, it was found that maximum contamination (58%) of milk was in cow milk of rural areas followed by buffalo (52%) and goat milk (48%). In semi-urban area, the contamination of aflatoxin M_1 was comparatively low than rural areas. However, in urban areas contamination percentage was lower than semi-urban and rural areas. Maximum concentration of aflatoxin M_1 was obtained from buffalo milk of rural area (mean = 0.126µg/kg) followed by cow milk of rural area (0.102µg/kg). In urban and semi-urban areas, cattle rearing is maintained in comparatively hygenic conditions. They feed their cattle with commercial and freshly harvested feeds where the chances of aflatoxin B_1 contamination are comparatively low[8].

However, during the spring, summer and monsoon, there were non-significant differences with respect to the levels of aflatoxins M_1. Mean value of aflatoxin M_1 in winter season was 0.030 µg/kg, which was significantly different from spring and monsoon seasons. Our results are in contrary to the previous works[21,27] who revealed that the levels of AFB_1 and AFM_1 in feedstuff and milk were maximum during winter followed by autumn, spring and summer. Seasonal and geographical effects are factors which have pronounced influence in the level of AFM_1[9,28].

High temperature and high humidity prevailing in this locality during spring, summer and monsoons provided ideal condition for *A. flavus* infestation and aflatoxin synthesis. Once the food/ feed ingredients get infested, aflatoxin enters into the food chain[9,12,26]. Aflatoxin M_1 can be minimized through good preharvest (crop rotation, irrigation, fungal resistance, biological control) during harvest (ripening, dry matter) and post harvest (storage, cooling, dryness etc.) management of feed crops.

Conclusion

Aflatoxin contamination can occur in milk and milk products either through carry-over of aflatoxin B_1 from feed to milk by cattle or by secondary contamination with *Aspergillus flavus*. Per cent contamination of aflatoxin was comparatively high in cow raw milk of rural areas followed by buffalo milk, however, the level of contamination in most of the samples was within the regulatory limits (USFDA, EU and CAC).

Congenial environment (condusive temperature coupled with high humidity) of spring and wet seasons of this locality favoured *A. flavus* growth and aflatoxin synthesis in cattle feeds and subsequently to milk and milk products.

Acknowledgement

Authors are thankful to UGC, Regional office, Kolkata for financial assistance (Sanction no. – F.PSB-34/13-14 ERO).

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(Received 20th October 2020, accepted 07th December 2020)