

Aflatoxin M₁ in Milk of Cow in Relation to Lactation Period and Yields

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Abstract: Aflatoxin produced from few molds as *Aspergillus flavus*, *A. parasiticus* and *A. nomius* is highly carcinogenic, mutagenic to the animals and humans. These molds are present in the dietary intake of cattle feed. Present investigation was performed to observe the carryover of AFB₁ to the milk toxin of lactating cows. Cows during early lactation stage yields more milk compared to late lactating stage, hence, the same cows were used to keep the variable of physiology of cows as nearly constant. AFM₁ concentration was found independent to the intake of AFB₁ with the compound foods ingested by cows. Carryover of aflatoxin was observed comparatively less in case of cows of late lactation stage. With the yield of milk per day (milking twice), the carryover rate also varied. AFM₁ concentration was observed within the limit as set by EU. Frequent evaluation and testing of milk is necessary.

Keywords: Aflatoxin, AFB₁, AFM₁, Bhagalpur, Milk toxin.

1. Introduction

Mycotoxins are secondary metabolites produced by some toxigenic fungi under conducive conditions of temperature and humidity [1]. Broad range of food and feeds are contaminated by this toxin. Aflatoxins produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius* is highly toxic and hazardous to animals and human beings [2]. Aflatoxin M₁, also known as milk toxin, is a hydroxyl metabolite of aflatoxin B₁, metabolized in the liver of cattle and is being secreted in bile, urine, dung and also in milk of lactating cattle [3], [4]. These toxins can cause carcinogenic, mutagenic, teratogenic and immunosuppressive effect on many animal species including human. Humans may be exposed to aflatoxin M₁ through the consumption of contaminated milk. The toxin might cause unfavourable effects in the human body [1], [3]. However, aflatoxin M₁ is less poisonous than aflatoxin B₁, and according to the International Agency for Research on Cancer (IARC) classification, aflatoxin B₁ and M₁ were classified as group 1 and 2 of the human carcinogens, respectively [2], [4]. Milk in almost every part of world is the main dietary intake of infants, old and weak/ sick people. Hence, consumption of AFM₁ contaminated milk (exceeding the regulatory limit of USFDA, EU and CAC) can adversely affect the health of children, old and weak and susceptible people.

The complications related to aflatoxin M₁ contaminated milk and the significance of healthy milk and its dairy products have rendered various researchers to monitor the factors affecting the level of AFM₁ contamination in milk and its products [5]-[7]. Impact of various factors such as geographical regions, climatic factors, types of feeds etc. have been evidenced to influence the incidence and prevalence of aflatoxin M₁ in milk [1], [4], [5], [6]. However, in this specified geographical region, there is no previous recorded study with regard to aflatoxin M₁ level in milk in relation to the milk yields and lactation period of cows. The aim of this study was to determine the carryover rate of aflatoxin M₁ in the milk of early lactating period (3-4 weeks after calving) and late lactating period (34-36 weeks after calving) of cows.

The present study was undertaken in specified geographical regions of Bhagalpur and its nearby district. This maintained the type and quality feed, the hygienic practices performed by the breeders and the similar breed of cows for the comparative experimental study. During the early lactation stage, cows produce comparatively higher amount of milk (kg/day) than those of late lactation, however, the diet intake remains the same on an average. This point towards the scenario of metabolism of production of milk and level of aflatoxin M₁ in milk yield though with the same dietary intake. The study was done with the same cow in early lactation stage (2 – 4 weeks) and late lactation stage (34 – 36 weeks) after calving.

2. Materials and Methods

A. AFB₁ analysis in feed of cows

The qualitative and quantitative test for AFB₁ was done employing the methods previously discussed [7].

B. Milk sample collection

Raw milk was collected twice (morning and evening) from each cow and was stored in sterile plastic container. The samples of milk obtained during morning and evening time from each cow were mixed in the ratio of the quantity it was produced. The resultant quantity was labelled as Day 1 and sample from the mixture was considered for AFM₁ testing.

C. Extraction

For the determination of AFM₁, the method used was the AOAC, 2000. 50 ml of different milk samples was added to the 10 ml saturated salt solution (40gm NaCl in 100ml of water) and was shaken gently. Final preparation was further added to chloroform (120 ml) at 30 °C in a 250 ml separating funnel and allowed filtrate to settle for 2-3 minutes. Filtrate was then taken for column chromatography.

D. Column chromatography

Silica gel slurry made with 2gm silica gel and CHCl₃ was added to the column containing half-filled CHCl₃. Sodium sulphate (2 gm) was further added in the column. Excess CHCl₃ was drained out and sides of column was rinsed with CHCl₃. The milk sample was added to the column and drained entire solution through column simultaneously Sodium sulphate was stirred gently. The cylinder was rinsed with CHCl₃ and it was again added to the column. Column was then washed off with 25 ml toluene and acetic acid (9+1). This removed any coloured compounds in the column. Further, column was washed with 25 ml of hexane, ether and acetonitrile (5+3+2) for fat removal. Finally, aflatoxin M₁ eluted with 40 ml CHCl₃ and acetone (4+1). The elute was evaporated to dry and was used for TLC technique.

E. Thin Layer chromatography

The residue of sample was dissolved in 100 µl of benzene and acetonitrile (9+1) solution by mixing them and further, spots on TLC plate were marked. Spot 20µl of test solutions and

2, 4, 6, 8 and 10µl aflatoxin M₁ standard (0.25 µg/ml). The plate was developed in chloroform - acetone – isopropanol (87+10+3) and Aflatoxin M₁ was calculated in µg/ kg or ppb.

3. Results and Discussion

Carry-over rate of low milking cows (<20 kg milk yield/ day) was observed (milked twice daily) as 1-2% of AFB₁ and high milking cows showed upto 6%. Multiple factors affecting the carry over rates were differences in species/ breed, physiology/ health of the cattle, biotransformation capacity of liver, types of feed etc. [8], [9]. C1, C2.....C10 indicate the cow numbered for experimental purposes.

Table 1 shows the summarized data of milk yield during early lactation stage of ten cows and their respective carryover rates of AFB₁. During early lactation, the cows were in 2-4 weeks after calving and same cows while in late lactation period (34 to 36 weeks after calving) were employed for our present investigation. Consumption of AFB₁ cattle feed was analysed for each cow and accordingly it was found that the mean intake of aflatoxin B₁ during early lactation period was 40.7 ± 3.6µg/ day. The mean concentration of AFM₁ toxin during early lactating cows was 0.05 ± 0.01µg/kg, which was 2.1± 0.027µg/day. The average carry over rate was 0.050. The carryover rate varied from 0.041 ± 0.007 to 0.058 ± 0.011. Various researchers have studied the metabolism of AFB₁ in cattle feed to AFM₁ in the milk yield [10]-[13].

Table 2 shows the AFB₁ intake and AFM₁ excretion by the selected cows during late lactation (34.36 weeks after calving).

Table 1
AFB₁ intake and AFM₁ excretion in milk of individual cow during early lactation (two to four weeks after calving)

Lactation period	Cows	AFB ₁ intake (µg/ day)	Milk yield (kg/ day)	AFM ₁ concentration in milk (µg/kg)	AFM ₁ excretion in milk (µg/day)	Carry-over rate
Early lactation (2 - 4 weeks after calving)	C1	42.8 ± 0.46	18.7 ± 1.24	0.06 ± 0.02	2.3 ± 0.46	0.051 ± 0.009
	C2	46.9 ± 1.02	18.3 ± 1.16	0.05 ± 0.01	2.4 ± 0.47	0.043 ± 0.008
	C3	35.8 ± 1.13	20.4 ± 0.72	0.05 ± 0.01	2.2 ± 0.42	0.058 ± 0.011
	C4	42.8 ± 1.03	18.6 ± 1.21	0.04 ± 0.01	1.8 ± 0.31	0.041 ± 0.007
	C5	41.7 ± 0.73	19.3 ± 1.1	0.05 ± 0.02	2.1 ± 0.44	0.048 ± 0.009
	C6	34.6 ± 1.21	17.8 ± 1.21	0.06 ± 0.01	2.1 ± 0.39	0.056 ± 0.011
	C7	40.5 ± 0.76	18.4 ± 1.17	0.05 ± 0.01	2.2 ± 0.51	0.057 ± 0.009
	C8	39.7 ± 1.44	20.7 ± 0.85	0.04 ± 0.01	1.7 ± 0.35	0.041 ± 0.008
	C9	40.5 ± 0.84	17.6 ± 1.19	0.05 ± 0.01	2.2 ± 0.53	0.053 ± 0.010
	C10	42.3 ± 1.12	19.6 ± 1.17	0.06 ± 0.02	2.5 ± 0.61	0.054 ± 0.011
Mean		40.7 ± 3.6	19.1 ± 1.06	0.05 ± 0.01	2.1 ± 0.27	0.050 ± 0.007

Table 2
Intake of aflatoxin and its excretion in milk of individual cows during late lactating period (34 to 36 weeks after calving)

Lactation period	Cows	AFB ₁ intake (µg/ day)	Milk yield (kg/ day)	AFM ₁ concentration in milk (µg/kg)	AFM ₁ excretion in milk (µg/kg)	Carry-over rate
Late lactation (34 - 36 weeks after calving)	C1	38.7 ± 1.59	13.4 ± 0.67	0.03 ± 0.02	0.9 ± 0.72	0.023 ± 0.009
	C2	36.9 ± 1.01	14.6 ± 1.14	0.04 ± 0.02	0.7 ± 0.34	0.019 ± 0.010
	C3	36.4 ± 1.24	12.1 ± 0.86	0.04 ± 0.01	0.7 ± 0.89	0.019 ± 0.006
	C4	35.8 ± 0.97	10.2 ± 1.34	0.03 ± 0.01	0.6 ± 0.17	0.017 ± 0.008
	C5	34.9 ± 1.41	11.8 ± 1.41	0.03 ± 0.02	0.4 ± 0.66	0.011 ± 0.006
	C6	37.2 ± 1.51	11.2 ± 1.23	0.05 ± 0.03	1.0 ± 0.53	0.027 ± 0.010
	C7	34.6 ± 1.34	10.9 ± 0.94	0.05 ± 0.02	0.5 ± 0.81	0.014 ± 0.013
	C8	36.8 ± 0.84	9.6 ± 1.21	0.03 ± 0.02	0.3 ± 0.59	0.008 ± 0.009
	C9	36.4 ± 1.17	11.4 ± 1.34	0.04 ± 0.01	0.4 ± 0.61	0.011 ± 0.010
	C10	36.9 ± 1.45	12.3 ± 1.53	0.05 ± 0.01	0.7 ± 0.29	0.019 ± 0.007
Mean		36.4 ± 1.08	11.6 ± 1.39	0.04 ± 0.02	0.6 ± 0.21	± 0.005

An average the daily intake of AFB₁ during late lactating period was $34.4 \pm 1.08 \mu\text{g/day}$. During late lactation period the yield of milk from same cow, however, decreased ($9.4 \pm 1.45 \text{ kg/day}$). Mean production of milk from the cows were $10.1 \pm 1.39 \text{ kg/day}$ during this period of milking. Mean AFM₁ concentration in milk was $0.04 \pm 0.02 \mu\text{g/kg}$, which was $0.5 \pm 0.21 \mu\text{g/day}$. The mean carry-over rate observed in this period was recorded to be 0.016 ± 0.005 (ranging from 0.009 ± 0.008 to 0.024 ± 0.009).

There was no significant correlation among AFM₁ concentration in milk, carryover rate by individual cow and the amount of AFB₁ consumed by respective cow. Our observations are in conformity with the previous work [14]. They fed the cattle with AFB₁ contaminated feed (57 to $311 \mu\text{g/day}$ per cow) and the resultant carry-over rate was found independent of AFB₁ intake ingested.

Remarkable differences were found in the carry over rate of aflatoxin between early lactation and late lactation period of milking. While comparing two different lactating periods, the difference of carryover rate was found in a factor of 3.1. Variation among the AFM₁ excretion has been observed among the cows of same stage fed with similar AFB₁ concentration. This has been supported by the fact that conversion of AFB₁ to AFM₁ in liver is partially excreted by the urine and by way of bile to faeces (apart from milk) [12]. The variation in the carry-over rate of each cow can be supported with the fact that the metabolism/ conversion of AFB₁ to AFM₁ can be due to variation in activities of enzyme of the MFO (mixed function oxidase) system. Moreover, the conversion of AFB₁ is not only converted to AFM₁ but also to AFP₁ and AFQ₁ [10].

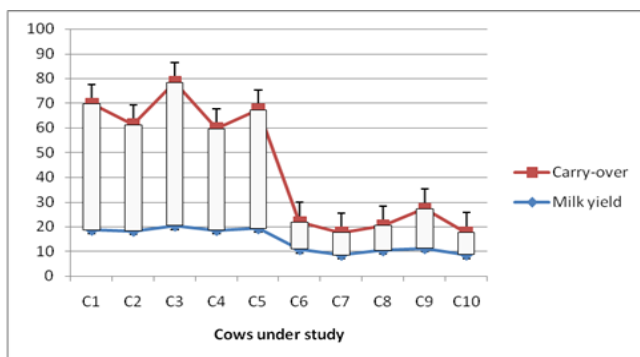


Fig. 1. Relationship between milk yield and carry-over rate of aflatoxin

Figure 1 shows relation between the milk yield and carry-over rate of aflatoxin from AFB₁ to AFM₁. The data reflects the facts that with the variation in the milk yield, the carry-over rate can be predicted (if other variables such as lactation period, age of the cows etc. remain same). This is in conformity with the results obtained by earlier researchers [15], [16]. The metabolism of AFB₁ to AFM₁ is performed in the liver of lactating cows and are disposed out of the body through bile, urine, faeces and milk [3], [4]. Partial conversion of AFB₁ occurs to AFM₁ occurs and rest to other forms of toxins. It is evident that more milk production by the cow, there are the

chances of more AFM₁ contamination in milk. Previous workers [11], [17] observed that high milk yield could intensify the effect on AFM₁.

4. Conclusion

The presence of AFM₁ in the milk of the cows is a result of carryover by the ingestion of AFB₁ contaminated feed. AFB₁ is metabolized and hydroxylated to form AFM₁ in the cows which are excreted through the milk. Mean percent AFM₁ contamination was comparatively high in the milk produced by early lactating cows than late lactating cows. The food intake by early lactating cows and late lactating cows are nearly similar. The intake of diet/feed were metabolized to excrete bile, urine, faeces and milk. Hence, the toxin gets excreted out through various discharges. Thus it relates to the finding that amount of toxins will vary with the amount of excretions. The observation leads to a finding that amount of milk yielded by the cows is in correlation with the AFM₁ contamination of the milk.

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