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Determining the kinetic behaviour for the secretion of milk toxin as related to dosage level of aflatoxin B_1

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Aflatoxins are a group of extremely toxic and hepatocarcinogenic compounds that may pose a threat to animal and human health when present in feed and food sources. Maryamma and Sivadas (1975) showed that continuous feeding of diet containing 0.7 ppm aflatoxin B_1 produced hyperplasia of mammary epithelium in adult goats.

Aflatoxin M_1, a metabolite, appears to be present in the liver, kidney and urine as well as in the milk of animals if ingested food containing aflatoxins (Allcroft and Carnaghan, 1963; Allcroft et al., 1966, and de Iongh et al., 1964). Kiermeier (1973) demonstrated that the amount of such toxins is directly proportional to the amount of ingested aflatoxin B_1. Edds (1973) stated that the milk toxin secreted by cows ingested aflatoxin B_1 causes hepatotoxicity similar to that induced by aflatoxin B_1 in young ducks. Grant and Carlson (1971) showed that aflatoxin M_1 has an affinity for the casein fraction of milk. For this reason, the accumulation of milk toxin in fluid milk and milk products may pose serious hazards to young infants consuming such products.

Moreover, aside from the potential hazards to human population, the economic importance of low-level aflatoxin consumption on livestock production appeared to be considerable through lowered production, reduced weight gains, impaired resistance to infections (Pier, 1973) and decreased milk production (Newberne, 1973).

Our objectives were to isolate and identify fungi associated with market milk; screening test for determining the aflatoxin-producing isolates and to detect the effect of toxic isolates on the kinetic behaviour of milk toxin when animals received contaminated ration.

Materials and methods

a) Isolation, identification and screening for aflatoxin-synthesizing fungi

A total of 110 milk samples were collected from different street vendors at Assiut City. Samples were transferred aseptically to the laboratory in sterile 1-oz MacCartney bottles. Milk samples were cultured onto Littman-oxygal-agar plates (Moss and McQuown, 1969).

For identification of fungal species, the following references were used: Raper and Fennell, 1965; Raper and Thom, 1949; Brown and Smith, 1957; Gilman, 1957, and Booth, 1971. The fluorescence-agar-plate method of Hara et al. (1974) was used for detecting aflatoxin-synthesizing isolates.

b) Preparation of toxic feeds

The screened isolate with high toxicity was grown on polished rice medium, according to Shotwell et al. (1966). Measurable amounts of the toxic rice were incorporated into a balanced diet for feeding programme.

For an assay of the level of aflatoxin B_1 in fermented rice, the crude extract of 40 g was defatted with n-hexane. The coloured impurities were eliminated by using neutral alumina column chromatography, as previously described by Ling (1976). Fractionation and quantitation of aflatoxin B_1 was based essentially on the method of Nabney and Nesbitt (1965).

c) Feeding test

A cow of 5 years age and 200 kg L.B.W., and a goat of 3 years age and 20 kg L.B.W. were housed individually under sound conditions in Veterinary Hospital, Assiut University. The healthy animals were maintained on a balanced diet free from toxic rice before starting the experiment. The lactating animals were subjected to a toxic feeding programme for ten days as shown in table 2, then returned to uncontaminated diet.

Milk was collected from animals under investigation five days before and continued for five successive days after treatment.

d) Extraction and assay of milk toxin (M_1)

The fluid milk samples were assayed for milk toxin by the Official First Action Method of the Association of Official Analytical Chemist (1975b).

Results and discussion

39 species which belong to 14 genera were identified during this investigation.

The results revealed that *Aspergillus* was the most frequently encountered genus. *Cladosporium* and *penicillium* were the second most frequent. They were isolated from 44 samples out of 110. *Curvularia* was of low frequency in occurrence. 10 genera were of rare occurrence, namely, *Mucor, Alternaria, Cephalosporium, Fusarium, Geolegnia, Epicocum, Nigrospora, Phoma, Scopulariopsis,* and *Trimmatostroma.*

Aspergillus was represented by 10 species. *A. niger* and *A. flavus* were of moderate occurrence (isolated 40 and 28 times). The remaining 8 species were of rare occurrence as listed in table 1.

10 species of *Penicillium* were recovered during this investigation. All the isolated *Penicillium* species were of rare occurrence. One species (other than *Aspergillus* and *Penicillium*) was of moderate occurrence, namely: *Cladosporium cladosporioides* (30 times). Also, another species was of low occurrence as *Curvularia spicifera* (14 times). 16 species were of rare frequency as listed in table 1.

It is evident by using the fluorescence-agar-plate method that only two strains of the fungal isolates, namely *A. flavus,* produced aflatoxin, while the other were not toxinogenic.

Table 1. List of fungi isolated from fluid milk samples and screened for their ability to produce aflatoxin.

Species		No. of cases of isolation
Aspergillus niger	Van Tiegh.	40
* *A. flavus*	Link	28
A. fumigatus	Fres.	6
A. amestelodami	(Mang.) Thom & Church	6
A. terreus	Thom	6
A. sydowii	(Bain. & Sart.) Thom & Church	4
A. ochraceus	Wilhelm	4
A. ustus	(Bain.) Thom & Church	2
A. versicolor	(Vuill.) Tirab.	2
A. nidulans	(Eidam) Wint.	2
Penicillium corylophilum	Dierckx	18
P. funiculosum	Thom	8
P. oxalicum	Curr. & Thom	6
P. janthinellum	Biourge	4
P. duclauxi	Delacroix	4
P. notatum	Westl.	4
P. cyclopium	Westl.	2
P. chrysogenum	Thom	2
P. jensenii	Zaleski	2
Penicillium species		2
Cladosporium cladosporioides	(Fres.) de Vries	30
C. herbarium	Link ex Fr.	10
C. sphaerospermum	Penz.	6
Curvularia spicifera	(Bain.) Boedijn	24
C. pallescens	Boed.	10
C. lunata	(Wakker) Boedijn	2
Mucor racemosus	Fres.	8
M. circinelloides	Van Tiegh.	2
Epicocum purpurascens	Ehrenb. ex Schlecht.	5
Alternaria alternata	(Fr.) Keissler	4
Fusarium solani	(Mart.) Sacc.	4
F. oxysporum	Schlecht. ex Fr.	2
Cephalosporium acremonium	Corda	4
C. roseum	Oud.	2
Geolegnia species		4
Phoma herbarum	Westend.	2
Nigrospora sphaerica	(Sacc.) Mason	2
Scopulariopsis brevicaulis	(Sacc.) Bainier	2
Trimmatostroma species		2

* Two isolates of *A. flavus* produced aflatoxin.
High occurrence, between 55 and 110 cases.
Moderate occurrence, between 27 and 54 cases.
Low occurrence, between 13 and 26 cases.
Rare occurrence, less than 13 cases.

Adequate identification of aflatoxin for these two isolates was obtained by thin-layer chromatography using chloroform : methanol (97 : 3 v/v) as a developer and co-chromatography with authentic sample in the same solvent system. A similar pattern has also been observed by many workers as Conder et al. (1963) and Wilson et al. (1968) investigated the production of aflatoxin by *A. flavus* isolated from certain common food sources including milk and its products. On the other hand, Van Walbeck et al. (1968) were able to detect aflatoxins not only by *A. flavus* but also by *Rhizopus* species and *A. ochraceus.*

The data given in table 2 revealed that the M_1toxin appears in the cow's milk in the next day after the animal had received a diet containing 3.245 mg B_1/kg ration. The initial level of the (M_1)toxin in the cow's milk on the first day was 0.07 μg/ml. The milk had gradually decreased to 0.056, 0.049 and 0.047 μg/ml milk, respectively.

After the animal received the second dose 2.272 mg B_1/kg ration at the end of the 4th day, toxin concentration increased to 0.056 μg/ml milk on the 5th day. This was followed by a second drop in its concentration to 0.042 μg/ml milk on the 6th day. The milk toxin concentration showed a similar pattern of fluctuation upon supplying the animal with the other doses. However, when the animal was supplied with a ration containing the same concentration at both the 6th and 7th days, the M_1 toxin concentration in milk was maintained at the same level (0.06 μg/ml milk) on the 7th and 8th days of the experiment. By increasing the dose to 4.868 mg/kg ration at the end of the 8th day, the M_1 toxin content consequently raised to its highest level of 0.088 μg/ml milk on the 9th day. This was followed

Table 2. Concentration of milk toxin (M_1) in cow's and goat's milk during the course of feeding animals of aflatoxin-B_1-containing ration.

Day	Cow		Goat	
	Dose of dietary aflatoxin (B_1) mg/kg ration	Concentration of milk toxin (M_1) (μg/ml milk)	Dose of dietary aflatoxin (B_1) mg/kg ration	Concentration of milk toxin (M_1) (μg/ml milk)
1	3.245	0.07	3.254	0.281
2	0.0	0.056	0.0	0.261
3	0.0	0.049	0.0	0.227
4	0.0	0.047	0.0	0.211
5	2.272	0.056	0.0	0.126
6	0.0	0.042	2.272	0.164
7	2.272	0.060	0.0	0.098
8	2.272	0.060	1.623	0.126
9	4.468	0.088	0.0	0.123
10	0.0	0.074	0.0	0.112
15	0.0	--	0.0	--

0.0 = Lactating animals received a diet free from mouldy rice.
-- = (M_1) toxin not detected in cow's and goat's milk.

again by a drop in the M_1 toxin content on the 10th day (0.074 µg/ml milk). It is particularly noteworthy that the M_1 toxin failed to be detected 5 days after the dose was discontinued (day 15).

Concerning the goat milk it was found that the M_1 toxin level appeared on the next day after aflatoxin-containing ration was fed. The relation between the toxin concentration and time showed a closely similar pattern of fluctuation as obtained in dairy cows. However, concentration of M_1 toxin was more secreted by the lactating goat than by the dairy cow.

The detection of M_1 toxin in the milk of lactating animals one day after the intake of aflatoxin-containing ration and the rapid drop of this toxin concentration after discontinued treatment agrees closely with the findings of Kely and Booth (1971) and Polan et al. (1974).

The fluorescence intensity of the total quantity of M_1 toxin excreted in milk was less than one per cent of the total amount of ingested aflatoxin B_1. These findings are generally in agreement with the observation of Linde et al. (1965) and Polan et al. (1974).

From the results achieved it is indicated that M_1 toxin was found to appear in the milk of lactating animals shortly after the aflatoxin-containing ration was fed. The kinetic behaviour for the secretion of such toxins is directly related to the aflatoxin in concentration in the ration. It is also clear from these findings that the initial level established at the beginning of the feeding period is inconstant and has a tendency to decrease. However, some of the aflatoxins consumed by the animal and not eliminated by the udder appear to be present in liver, kidney and urine (Allcroft et al., 1966).

As the aflatoxins and their metabolites are extremely toxic and hepatogenic compounds that may pose a threat to animal and human health, it will be necessary to evaluate and determine the role played by milk and its products in transmitting such toxins to man.

Summary

39 species which belong to 14 genera were isolated from 110 milk samples collected from different dairy sources at Assiut City. The most frequent fungi were *A. niger, A. flavus* and *Cladosporium cladosporioides,* followed by *Curvularia spicifera* and *Penicillium corylophilum.* The remaining species were of rare occurrence.

The fluorescence-method for detecting aflatoxin-producing strains demonstrated the presence of two isolates namely *A. flavus* possesses this property. One of these toxic isolates was proved to produce high level of aflatoxin B_1. It was used as a tool for determining the kinetic behaviour for the secretion of M_1 toxin in milk of lactating animals which had received a toxic diet. The results revealed the following:

1. The milk toxin was detected in the milk of lactating animals next day after the toxin-containing ration was fed.
2. The level of milk toxin is gradually decreased when the feeding programme was interrupted.
3. When the animals supplied a ration containing the same concentration of aflatoxin for successive days, the milk toxin concentration was maintained at the same level.
4. The total amount of milk toxin secreted was less than one per cent of the amount of aflatoxin B_1 received.

5. The concentration of milk toxin secreted by goats was higher than that of dairy cows.
6. Milk toxin failed to be detected five days after the feeding programme was discontinued.

Key words: milk toxin, toxic feeds, feeding test, aflatoxin-synthesis

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