AFLATOXIN RESIDUES IN FLUID MILK

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ABSTRACT

The content limit for aflatoxin B_1 in feedstuffs of the European Common Market Commission can result in the presence of aflatoxin M_1 in milk. Traces of aflatoxin M_1 have been found in milk, depending on the feedstuff used, feeding conditions and the season of the year. These results were confirmed by thin-layer chromatography, by chemical reactions and by fluorescence spectra.

Metabolic investigations show that the feeding of aflatoxin B_1 to cows results in a lower amount of aflatoxin M_1 in the milk. The amounts of M_1 (and M_2) to be found are low (*Table 1*); aflatoxin B_1 can also be found, but in still lower amounts. For estimating the excretion of aflatoxin M_1 in milk, roughly 1.5 per cent of the aflatoxin feed intake can be expected. However, it

Table 1. Excretion of aflatoxin M in milk during metabolic investigations

Excretion of aflatoxin M_1 related to aflatoxin feed intake (%)	Reference
1	1
0.35	2
0.7–0.2*	3
2–3	4
1.4	5

* Calculated from the results of Keyl and Booth

has to be taken into consideration that aflatoxin M is present in the milk 12 to 24 hours after feed intake and that it cannot be detected 3–5 days later. During continuous feeding of aflatoxins, as soon as an aflatoxin-free ration is given, the values decrease to zero. Therefore it is clear that aflatoxin M can only seldom be detected in milk samples taken on the market. Investigations in France, United States and England showed negative results; in South Africa 5 milk samples out of 21 showed an aflatoxin content of $\leqslant 0.16\ \mu g\ l^{-1}$. Neumann-Kleinpaul and Terplan[5] recently found in 8 dried milk products out of 166 samples aflatoxin M_1 in amounts which varied between 0.7 and 2.0 $\mu g\ l^{-1}$; this corresponds to 0.08 and 0.26 $\mu g\ l^{-1}$ respectively in fresh milk.

These results are only apparently favourable, because on the one hand the number of investigations is too low and, on the other hand, the sensitivity of the applied methods may not always have been adequate. Because of the numerous reports on aflatoxins in feedstuffs, aflatoxin M should be found more frequently. According to Lafont[6], 167 out of 380 coarse grain samples contained aflatoxin, i.e. 44 per cent; Jaquet and Boutibonnes[7] found aflatoxins in 9 out of 29 feedstuffs of different composition, i.e. 31 per cent. The presence of aflatoxins in feeds is not only limited to grains, peanut and copra meals; all the feedstuffs used for dairy cows, such as green meal, cottonseed meal, leguminosae, manioc, fishmeal and draff can be contaminated. As Shank and co-workers[8] demonstrated in Thailand, regional differences play an important role, as do rain seasons and hot periods. In addition, the harvest conditions in parts of the country can be responsible for the presence of aflatoxin M. This is in accordance with model experiments demonstrating that temperatures of between 20 and 30°C and a relative humidity of about 90 per cent, favour the development of aflatoxin moulds. Therefore one has to consider the presence of aflatoxins in milk under unsuitable harvesting conditions, especially if the feeding plants were contaminated with soil moulds. However, technical factors might also have an influence. Thus aflatoxins can grow on feedstuffs, such as green meals with insufficient dehydration, or in grain silos with insufficient ventilation which favour the growth of mould-nests. Therefore aflatoxin M should be detected more frequently in milk, at least at special periods, if a sufficiently sensitive method is applied. The method of Jacobsen and co-workers[9], which has a detection limit for aflatoxin M of 0.1 μg l^{-1} milk, makes this possible. Our method, developed in co-operation with Mücke[10], allows the determination of 0.04 μg aflatoxin per litre of milk (*Table 2*). Using this method we repeatedly found aflatoxin M in raw milk.

Table 2. Aflatoxins in raw milk

Origin of samples	Time of sampling	Number of samples	Number of positive samples	Amount of aflatoxin M_1 (μg l^{-1})
Single farms	February–April	36	12	0.04–0.25
Tanker (6–9000 l)	May 5	12	9	0.04–0.08
Bulk milk	February–March	7	7	0.05–0.13
	June	6	0	0

These results were obtained by the following methods:

(*i*) Two dimensional thin-layer chromatography.

(*ii*) Monoacylation of suspect spots, whereupon the R_f value changed from 0.2 to 0.7 as with the control spot.

(*iii*) Spray reagents, especially Fast blue salt B, developed by us.

(*iv*) Fluorimetric determination by means of excitation and emission spectra.

(*v*) Chick-embryo test in one special serious case.

From the results of *Table 2* it can be concluded that:

(*a*) The presence of aflatoxin M in fluid milk is possible. Therefore aflatoxin M will also be present in milk powder without any mould growth, because pasteurization and drying as applied in the dairy industry does not influence the aflatoxin content materially.

(*b*) There is a seasonal influence, because during pasture no aflatoxins could be detected in bulk milk.

In addition we were able to demonstrate that milk producers feeding larger amounts of concentrates, supplied milk with an especially high content of aflatoxin M. Therefore the question arises, how much aflatoxin in milk has to be expected. If one takes the standards for aflatoxin content in feedstuffs as tolerated by the Common Market Commission, an aflatoxin content of 0.05 mg kg^{-1} is tolerated for milk production rations. At a daily intake of 5 kg concentrates this means a daily digestion of 0.25 mg aflatoxin per cow. If we consider, as mentioned above, that 1.5 per cent passes into milk as aflatoxin M, one has on an average to tolerate roughly 0.4 μg aflatoxin M_1 per litre of milk. If, however, considerably higher amounts of aflatoxins are present in feedstuffs, as reported by Krogh[11] (up to 4 mg kg^{-1}), by Jacket and Boutibonnes[7] (up to 2 mg) and by our own investigations (up to 10 mg), amounts of aflatoxin M_1 in milk could be found which would be ten times higher than the tolerated amounts. From all this we can conclude that there is a certain carelessness in using feedstuffs. We also have to get rid of the notion that grain which is no longer suitable for human consumption should be usable as feedstuffs. It should be prohibited at least for lactating cows. Moreover the EEC tolerated limit for aflatoxins in milk production rations should be close to zero.

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