A practical Italian approach to controlling aflatoxins in milk

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Aflatoxins, the most widespread mycotoxins, are common in warm and humid climatic conditions like those existing in Latin American, Asian and African countries, southern regions of the USA and certain parts of Australia.

However, in some European countries imported feedstuffs, such as maize or oilseed meals, grown in warmer southern climates are used. Therefore aflatoxins can also be considered a significant problem in European dairy rations.

Ruminants are not efficiently protected against aflatoxin toxicity, since ruminal degradability of aflatoxin B1 is minor, although they are less susceptible to aflatoxicosis than swine and poultry. In adult ruminants, exposure to aflatoxins can depress feed efficiency, immunocompetence, damage the liver, decrease milk production and reduce reproductive efficiency.

The effects on feed efficiency presumably arise from impaired ruminal function, including reduced cellulose digestion, volatile fatty acid formation, proteolysis, and motility.

The amount of aflatoxin required to affect performance and impair health is much greater than the dietary amounts related to milk residues.

Aflatoxin B1 is transformed into aflatoxin M1 in the liver. Aflatoxin M1 is considered a hepatotoxic and carcinogenic compound both for humans and animals and found predominantly in milk.

The quick transfer of aflatoxin M1 to milk is explained by fast absorption of aflatoxin B1 in the rumen. The peak aflatoxin M1 concentration in milk occurs 24 hours after feeding the contaminated feed to the cow.

The relationship between the amount of aflatoxin B1 ingested and quantity of aflatoxin M1 in milk is quite variable. Most authors consider the transfer of aflatoxins to milk is between 1 and 3%, with a mean transfer of ≈ 1.7%.

However, transfer factors up to 5-6% occur in reality, which then would result in milk with aflatoxin M1 levels above the legal limit of 50ppt. A transfer over 6% has been measured by Veldman et al. (1992) in high producing cows at lactation peak.

**Regulatory limits**

More than 100 countries in the world have specific mycotoxin control legislation. More than half of these countries have aflatoxin legislation for food and feeds.

Commission Regulation (EC) No. 1881/2006 sets the level for aflatoxin M1 in milk at a maximum of 50ppt. A concentration of 50ppt of aflatoxin M1 can occur when total mixed ration contains in excess of 5ppb/kg aflatoxin B1 (recalculated on 12% of moisture content).

The low European regulatory limit of aflatoxin M1 in milk makes compliance difficult for the European countries, where feeding-stuffs are imported or can naturally contain aflatoxins.

In recent years European milk producers have been observing increasing levels of aflatoxin M1 in milk.

Numerous milk batches have been rejected repeatedly by milk processing plants because the aflatoxin M1 level exceeded 50ppt. Each kilogram of rejected milk, for example in Italy, cost a dairy farmer in 2008 about €0.30 for production plus €0.25 for disposal.

**Case study**

The objective of this study was to set up the most practical approach for controlling aflatoxin M1 in milk under the farm conditions of central Italy.

Data on aflatoxin M1 in milk was obtained from two dairy farms with 160 (Farm A) and 130 (Farm B) dairy cows in central Italy.

In addition to the milk contamination, the aflatoxin B1 in the feed was also analysed. All cows received the same feed based on ground maize, cotton seeds, maize silage.

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**Fig. 1. Contamination of milk with aflatoxin M1 at farm A (ppt).**

**Fig. 2. Contamination of milk with aflatoxin M1 at farm B (ppt).**
alfalfa and grass hay and protein concentrate during the period of the study (Table 1). Concentrate included a non-digestible activated clay-based mycotoxin adsorbent (Toxfin, Kemin AgriFoods Europe) at the dose of 28g/cow/day.

When the aflatoxin M1 level surpassed 35ppt, 30g/cow/day were added to the TMR through the mixer wagon. Average milk performance was 28-29kg/cow/day, with an average milk composition of 3.7% fat and 3.4% protein. Cows had a good health status and no obvious problems with fertility were recorded.

Milk was routinely tested for aflatoxin M1 contamination in a local laboratory.

Periodically, feed and feed ingredients were also checked for aflatoxin B1 in the same laboratory.

### Results of farm trials

Data collected at both farms confirmed the continuous presence of aflatoxin M1 in milk at levels of 12.4ppt and higher (Figs. 1 and 2).

All feedingstuffs analysed in the trial period were positive for aflatoxin B1 with the highest concentrations in cotton seeds and ground maize.

It was observed that the aflatoxin B1 level in ground maize and cotton seeds was relatively low, but aflatoxin M1 in milk was unexpectedly high when both ingredients and especially cotton seeds were present in the cow diet. About 20-50% variation in analysis results of aflatoxin B1 is common. An error in aflatoxin B1 analysis in cotton seeds is even higher due to presence of linters, where the great amount of mycotoxin can accumulate and which can be occasionally excluded from the analysis during sample grinding.

Therefore, strict control of the experimental error is crucial for a good forecast of aflatoxin M1.

The first peak of aflatoxin M1 contamination was observed at the end of November/beginning of December 2007 at both farms.

Consequently, feed materials and TMR were tested for aflatoxin B1 level and relatively high contamination was found in ground maize (6.3ppb and 3.4ppb for Farm A and B respectively) although it was far below 20ppb — the EU limit for feed grade maize. The contamination of other feedingstuffs was low.

Another increase of aflatoxin M1 level at farm A started in January/February 2008. Since maize and cotton seeds were the main concern, both feed materials were again checked for the presence of aflatoxin B1. Results showed a slight increase of maize contamination that can explain higher levels of aflatoxin M1 in the milk.

In the middle of March 2008 there was a new arrival of cotton seeds. There were no other changes in the diet and new cotton seeds apparently caused the incidence of high aflatoxin M1 levels on both farms. Cotton seeds were analysed and levels of 8.5ppb (Farm A) and 11.0ppb (Farm B) were found to be well below the EU maximum limit of 20ppb for cotton seeds.

The final level of aflatoxin B1 in the TMR was 1.6 and 1.37ppb for farm A and B respectively and remained below the legal limit of 3ppb in feed.

The risk of having unacceptable level of aflatoxin M1 in milk in April 2008 was so high, that the cotton seeds were completely excluded from the diet on both farms.

The drop of milk contamination after exclusion of cotton seeds was fast and sharp, as also described by Jouany and Diaz, 2005.

In order to reduce the aflatoxin M1 level, cows occasionally received 30g/cow/day more mycotoxin binder.

### Table 1. The TMR composition used in the study.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Volume (kg/cow/day)</th>
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<tbody>
<tr>
<td>Maize silage</td>
<td>16</td>
</tr>
<tr>
<td>Ground maize</td>
<td>5.7</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>3.7</td>
</tr>
<tr>
<td>Hay</td>
<td>2.7</td>
</tr>
<tr>
<td>Cotton seeds</td>
<td>1.3</td>
</tr>
<tr>
<td>Protein concentrate</td>
<td>8.0</td>
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</tbody>
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This additional inclusion of the mycotoxin binder resulted in significant drops of aflatoxin M1 levels.
Taking into consideration the accuracy of analyses, sampling procedure and that the major part of aflatoxin B1 was bound by mycotoxin binder, it was suggested that the carry-over of contaminant seemed to exceed 6% in high producing cows.

Conclusion

There are many factors, which influence aflatoxin M1 levels in milk. Among them are diet composition, accuracy of analyses and sampling procedures, cow performance and health and use of mycotoxin binder. According to the results obtained the actual transfer factor of aflatoxin B1 from feed to aflatoxin M1 in milk was higher than considered as a basis for the EU legislation.

Consequently, the legal maximum level of 50ppt of aflatoxin M1 in milk could be exceeded, even when the feed contains less than 5ppb of aflatoxin B1.

In the dairy industry, the estimation of aflatoxin B1 carry over from feed to milk greater than 5-6% can be used by feed-processor and farmers to avoid contamination in milk exceeding the legally permitted limits.

The management practices implemented in two dairy farms in central Italy (Fig. 3) maintained safe aflatoxin M1 levels in milk as well as good cow health.

The most effective ways to reduce milk contamination were the use of a suitable non-digestible mycotoxin binder and the exclusion of suspicious raw materials, such as cottonseeds, from the diet. It was also practical to pay more attention to preventative actions when level of aflatoxin B1 in maize exceeded 2ppb and the level of aflatoxin M1 in milk went above 35ppt.

![Fig. 3. Programme of aflatoxin M1 control at farm level.](image-url)