Aflatoxin M₁ Contamination in Raw Bulk Milk and the Presence of Aflatoxin B₁ in Corn Supplied to Dairy Cattle in Japan

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Kei-ichi Sugiyama¹, Hisaaki Hiraoka² and Yoshiko Sugita-Konishi¹,*

¹Division of Microbiology, National Institute of Health Sciences: 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan; ²Division of Fertilizer and Feed Inspection, Food and Agricultural Materials Inspection Center, Sendai Center: 1–3–15 Gorin Miyagino, Sendai 983–0842, Japan; *Corresponding author

Aflatoxin M₁ (AFM₁) is a hydroxylated metabolite of aflatoxin B₁ (AFB₁), which has been found in the milk of dairy cattle fed AFB₁-contaminated feeds. Since AFM₁ has been evaluated as a possible human carcinogen, the cancer risk arising from AFM₁ contamination in milk is a serious problem in food safety. To evaluate the risk of AFM₁ contamination in milk, it is necessary to analyze the risk factors of AFB₁ contamination in corn provided for concentrated feed in Japan. The AFM₁ level in domestic raw bulk milk was measured at three sampling times, January, February and June in 2004. The AFB₁ contamination in corn supplied to cows was determined at the same time as the sampling of raw milk. The AFM₁ contamination levels in milk in January, February and June 2004 were 0.011, 0.007 and 0.005 ng/g, respectively. The AFB₁ contamination level in the corn of the concentrated feed was higher from October of 2003 to February of 2004 than from April to June in 2004. This study provides evidence that AFM₁ contamination level in milk is parallel to that of AFB₁ in corn of concentrated feed, so monitoring of the AFB₁ level in corn is important to prevent the risk of AFM₁ contamination in milk in Japan.

Key words: aflatoxin M₁; aflatoxin B₁; raw milk; corn

Introduction

Mycotoxins, secondary metabolites of fungi, are toxic to humans and animals. Among them, aflatoxins are produced by Aspergillus flavus and A. paratisicus, and aflatoxin B₁ (AFB₁) has potent carcinogenic and mutagenic effects in humans¹. In addition, cows that consume feed mixtures containing corn polluted with AFB₁ can then excrete aflatoxin M₁ (AFM₁) as a hydroxylated metabolite of AFB₁ in milk²–⁵. AFM₁ has been categorized by the International Agency for Research on Cancer as a class 2B carcinogen and its carcinogenicity was estimated to be one-tenth of that of AFB₁ by the Joint Expert Committee on Contamination and Food Additives in 2001. In particular, infants and young children potentially at risk from AFM₁ contamination because they are major consumers of milk. Therefore, CODEX recommends the establishment of maximum residue limit for AFM₁ of 0.5 μg/kg. The ingestion level of AFB₁ by animals influences the amount of AFM₁ in secreted milk in a dose-dependent manner⁶. The AFM₁ levels in Japanese commercial milk have been previously investigated, but there was no information about the level of AFB₁ contamination in feed in that study⁷. For risk analysis of AFM₁ in milk in Japan, it is necessary to evaluate the levels of AFM₁ in raw bulk milk and AFB₁ in corn, which is the largest contributor to AFB₁ exposure in feed.

This study surveyed AFM₁ contamination levels in raw milk samples collected on three occasions through the winter and summer. The AFB₁ level in corn supplied to dairy cattle was also evaluated at the same sampling times.

Materials and Methods

Sampling

Raw bulk milk samples were obtained from 11 districts in Japan, i.e., Hokkaido, Tohoku, Kanto, Sinetsu, Tokai, Hokuriku, Kinki, Tyugoku, Shikoku, Kyushu and Okinawa region. They were collected in sterile bottles and immediately frozen. Sampling of raw milk samples was performed in January, February, and June in 2004. The frozen raw milk samples were sent to the National Institute of Health Sciences and maintained at −80°C until processing and analysis. The numbers of individual samples were 101 in January, 97 in February and 101 in June.

The corn used as feed for dairy the cattle was monitored by the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan. The data regarding AFB₁ contamination levels from July 2003 to February 2005 are taken from the routine monitoring data. The number of samples monitored in each month ranged from 7 to 24.
Analytic methodology

AFM1 in raw bulk milk samples were analyzed according to the method of Nakajima et al. Briefly, for AFM1, raw milk was homogenized with a vortex mixer and diluted with phosphate-buffered saline (pH 7.4). The diluted milk was passed through an immunofinity column of AFM1 (Vicam, Watertown, MA, USA), and the concentration of AFM1 was determined by HPLC with a Lichrospher 100 column, RR-18, 5 μm, 250 by 4 mm. The mobile phase was isocratic, water–acetonitrile (25:75, v/v), and all separations were performed at a flow rate of 1.0 mL/min. Then, aliquots of 100 μL were injected into the HPLC unit. The column temperature was maintained 40°C. The excitation and emission wavelengths were set at 365 nm and 435 nm, respectively. A recovery test of AFM1 in milk was performed in samples spiked with 0.05 and 0.10 ng/mL of AFM1. Determination of AFB1 in corn samples was performed according to the HPLC-florescence detector method validated previously. Briefly, 50 g of corn was extracted with acetonitrile-water (9:1, v/v). A portion of the extract was purified with a multifunctional column (Multisep #226, Romer Lab, USA). The HPLC column for AFB1 was Inertsil ODS-3, 5 μm, 250 by 4 mm (GL Science, Co., Tokyo). The mobile phase was acetonitrile–methanol–water (2:3:5, v/v/v) and the flow rate was 1.0 mL/min. The wavelengths for excitation and emission were 365 and 450 nm, respectively.

Statistical analyses

Data were expressed as the mean±standard deviation and analyzed by ANOVA.

Results and Discussion

The validation of the AFM1 analytical method revealed that the recovery was 99.60±3.52% in samples spiked with 0.5 ng/g and 86.73±17.32% in samples spiked with 0.05 ng/g. The detection limit (LOD) and the quantitation limit (LOQ) for AFM1 were 0.005 ng/mL and 0.01 ng/mL, respectively. The LOD and LOQ corresponded to signal levels of three times and ten times the background noise on the chromatogram, respectively. For the AFB1 analytical method, the recovery rates were 84.50±8.2% and 90.7±5.3% from samples spiked with 1 ng/g and 10 ng/g, respectively. The LOD was 1 ng/g and LOQ was 2 ng/g.

The AFM1 level in each sampling district at the indicated periods is shown in Fig. 1. Even though there was little difference in the AFM1 levels among districts, the AFM1 level in January 2004 tended to be higher than that in June 2004. The AFM1 level in February 2004 ranged between that in January 2004 and June 2004. The mean and standard deviations of the AFM1 levels are shown in Table 1. The samples collected in January 2004 contained 0.011±0.0035 ng/mL, while the samples obtained in June 2004 contained 0.005±0.0016 ng/mL AFM1. This result seems to be consistent with the report of Dragacci and Frémy, who found that the contamination level of AFM1 in milk was higher in winter than summer. They indicated that seasonal factors such as lactation yield, feed consumption and the dosage of concentrated feed contributed to the seasonal difference of AFM1 level in milk. However, our investigation indicated that the value of AFM1 concentration in February 2004 was lower than in January 2004, which suggests that other factors have a major influence on the AFM1 level in milk, at least in Japan. The Joint FAO/WHO Expert Committee on Food Additives has determined that the AFB1 concentration in feed is closely related to the AFM1 concentration in milk. Recently, Prandini et al. reported the most important risk factor for the AFM1 level in milk to be the AFB1 concentration in feed components, such as meal, groundnuts, cottonseed and maize. Therefore, the risk factors for AFM1 in milk were investigated in Japan.

In Table 2, it is shown that the standard diet composition for dairy cattle contained various feed components, but over 44% was accounted for by corn and corn silage in Japan. The corn silage is from domestic sources and contains no AFB1 (personal communica-

![Fig. 1. AFM1 concentrations in raw bulk milk in several regions of Japan](image-url)

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Number of samples</th>
<th>Mean (ng/mL)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan. 2004</td>
<td>101</td>
<td>0.011</td>
<td>0.0035</td>
</tr>
<tr>
<td>Feb. 2004</td>
<td>97</td>
<td>0.007</td>
<td>0.0021</td>
</tr>
<tr>
<td>Jun. 2004</td>
<td>101</td>
<td>0.005</td>
<td>0.0016</td>
</tr>
</tbody>
</table>

The mean values are significantly different from each other (Scheffe’s post-hoc test, p<0.01).
tion). However, about 95% of corn in concentrated feed is imported from the USA. This is generally accepted to be contaminated with AFB$_1$. Therefore, AFB$_1$ from imported corn may be a major determinant of milk AFM$_1$ concentration in Japan. In fact, MAFF has established the regulatory level at 20 mg/kg for AFB$_1$ in feed, and performs regular monitoring for AFB$_1$ contamination in imported corn and feed.

Since the concentrated feed is prepared with corn imported about 1–2 months previously in Japan, the feed supplied in January 2004 would have been made from corn imported during October to December 2003. Similarly, the corn imported between April and May 2004 was used for dairy cow feed around June 2004.

Figure 2 shows the monitoring results from July 2003 to February 2005. As can be seen, more contaminated corn was used to prepare the concentrated feed which was supplied to cattle in January 2004, when the first sampling was carried out. The average contamination levels of AFB$_1$ from December 2003 to January 2004 were lower than from November to December 2003. It is thought that this result corresponds to the reduction in the AFM$_1$ concentration in milk from January to February 2004, as shown in Table 1. The tendency that AFB$_1$ contamination in 2003 is higher than in 2004 probably reflects the difference in the periods when the corn was supplied.

These results suggest that the contamination levels of AFM$_1$ in raw milk in Japan are dependent on the amount of AFB$_1$ contained in imported corn which is supplied to dairy cattle. Moreover, it is considered that AFB$_1$ contamination level does not depend on season factors, because higher levels of AFB$_1$ in imported corn were not always observed in winter (Fig. 2). These findings are the first to demonstrate a parallel relationship between the AFM$_1$ concentration in raw milk and the AFB$_1$ concentration in imported corn in Japan. The monitoring of AFB$_1$ contamination levels in imported corn over the long term will be required to protect the Japanese population against AFM$_1$-induced health risks, because the AFB$_1$ level in imported corn varies from year to year (Fig. 2). In addition, the monitoring of contamination levels of AFB$_1$ in feeds other than imported corn may also be necessary.

In summary, our results indicate that AFB$_1$ in imported corn for dairy cattle is a major contributor to AFM$_1$ content in raw milk. To ensure the safety of milk for human health it is extremely important to avoid providing feed contaminated with AFB$_1$ to cows. Hence, regular monitoring of not only the AFM$_1$ level in milk, but also the AFB$_1$ level in feed, will be required to protect the public, especially infants and young children, against AFM$_1$ toxicity.

Acknowledgements

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References

5) Chopra, R. C., Chhabra, A., Prasad, K. S. N., Dudhe, A., Murthy, T. N., Prasad, T. Carry-over of aflatoxin M$_1$ in

Table 2. Standard diet composition for dairy cattle

<table>
<thead>
<tr>
<th>Feed</th>
<th>% of contained dry basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>22.94</td>
</tr>
<tr>
<td>Corn</td>
<td>21.72</td>
</tr>
<tr>
<td>Rice hay</td>
<td>17.99</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>11.94</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>7.92</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>6.42</td>
</tr>
<tr>
<td>Cotton seed</td>
<td>4.95</td>
</tr>
<tr>
<td>Beet pulp</td>
<td>4.66</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.54</td>
</tr>
<tr>
<td>Tricalcium phosphate</td>
<td>0.54</td>
</tr>
<tr>
<td>Salt</td>
<td>0.43</td>
</tr>
<tr>
<td>Vitamin supplement</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Fig. 2. AFB$_1$ concentrations in imported corn supplied to dairy cattle in Japan

A bar represents the average during each month in 2003, 2004 and 2005. Contamination levels above the averages are indicated by points. The arrows represent the sampling points of raw milk, as shown in Table 1.


