Aflatoxin B1 Contamination of Dairy Feeds after Storage in Farm Practice in Tropical Environment

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Received 26 November, 2015/Accepted 9 June, 2016

The objective of this study was to determine the contamination of aflatoxin B1 (AF-B1) when keeping various dairy feeds in a farm environment. The study was carried out from March to May 2011 and involved 63 small holder dairy farms belonging to a single dairy cooperative in Chiang Mai province, Thailand. All feed samples used for milking cows including 4 commercial concentrates (CC1 to CC4), by-products from local corn processing factories fermented in plastic bags (SIL), and corn and cob meal or corn dust (CCD). Feed samples were collected 2 times at before and after storage. Farmers were requested to store CC1 to CC4 and CCD for a month and SIL for a week using their routine on-farm storage arrangements. All samples were measured for their AF-B1 concentrations by ELISA. Results showed that AF-B1 concentrations of CC1 to CC4, SIL and CCD before storage were 5.1, 4.1, 4.0, 4.2, 5.5 and 5.5 µg/kg, respectively, and after storage the concentration of AF-B1 were 9.7, 6.5, 9.8, 12.3, 11.4 and 20.0 µg/kg, respectively. CCD at after storage was the only feed that had mean level more than 20 µg/kg. Concentrations of AF-B1 at before storage in all feeds were significantly lower than after storage (P<0.01), and the increased ratio of AF-B1 levels was approximately 2 to 3 times. The study concluded that increased AF-B1 levels are related to feed types and farm conditions.

Key words : Dairy feed / Aflatoxin B1 / Commercial concentrate / Storage.

INTRODUCTION

Aflatoxins are toxic compounds that are mostly produced by Aspergillus flavus and A. parasiticus. Among aflatoxins, aflatoxin B1 (AF-B1) is highly toxic and carcinogenic to many animal species including human (Rao et al., 1977). For dairy cattle, toxicity of AF-B1 in feeds manifests as decreases in feed intake and efficacy, growth rate, milk production, health performance and reproductive performance, and increases in stress susceptibility, weight loss and liver damage. These symptoms incur economic losses in animal husbandry.

Production of AF-B1 is influenced by the type of substrate, fungal species, moisture content of the substrate, presence of minerals, and relative humidity of the surroundings, temperature and physical damage of the substrate (Alonso et al., 2011; Viquez et al., 1996). In a tropical country, Uganda, the extent of AF-B1 contamination is strongly influenced by differences in climatic conditions and storage time (Kaaya and Kyamuhangire, 2006). Maize samples stored for more than six months were found to contain AF-B1 greater than the 20 µg/kg which is the FDA/WHO regulatory limit as mean level (Kaaya and Kyamuhangire, 2006). In India, it was observed that AF contamination in storage was dependent on the storage system (Ahmad, 1993).

In Thailand, the storage times of energy-based feeds used in dairy farms vary from a few days to a few months depending on feed types. Dry feeds such as commercial concentrates with 2-level of plastic bag package
and by-products from local feed processing factories, --the so-called commodities, —are kept for at least one month. In contrast, silages, for example corn silage, are kept for at least a week. Despite the aflatoxin contamination of dairy feeds has been reported, little information is so far available regarding the aflatoxin contamination after storage in naturally farm practice. Therefore, the objective of this study was to investigate the AF-B1 concentrations when storing various feeds in a farm environment.

**MATERIALS AND METHODS**

**Farm and feeds management**

The study was carried out from March to May 2011 and involved 63 small holder dairy farms belonging to a single dairy cooperative in Chiang Mai province, Thailand. For feeding management, all farms had 2 sources of feeds, that is, energy sources as roughages, and protein sources as commercial concentrates and commodities. Commercial concentrates from 4 feed factories (CC1 to CC4) were available from local markets including a dairy cooperative shop, and were usually purchased once a month. During the study period (late summer in Thailand) the energy sources were mostly straw, but some farms could purchase by-products from local corn processing factories composed of corn husks and straws, for example corn silage, are kept for at least one month. In contrast, silages, for example corn silage, are kept for at least a week. Despite the aflatoxin contamination of dairy feeds has been reported, little information is so far available regarding the aflatoxin contamination after storage in naturally farm practice. Therefore, the objective of this study was to investigate the AF-B1 concentrations when storing various feeds in a farm environment.

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**Study design and feed sample collection**

An observational cohort study was designed to differentiate concentrations of AF-B1 in feeds between before and after storing in the farm environment. Feed samples were collected twice before and after storage. The intervals between before and after storage depended on the farm routine storage times for feeds. All feeds used for milking cows, except fresh roughages and straws, were collected before storage. After first collection, all feed packages were opened as they were ready to use, and stored in accordance with routine farm storage practice. The collections of after storage were performed either a week later for SIL or a month later for CC1 to CC4 and CCD. The feed samples were collected according to the technique prescribed by the Department of Livestock Development, Thailand (Department of Livestock Development, 2009). These samples were placed in plastic bags, transported to the laboratory in styrofoam boxes with recyclable ice and stored at -20°C until AF-B1 analysis.

**Sample preparation**

All feed samples were carefully mixed and finely ground, and then extracted by putting 20 g of ground samples into an Erlenmeyer flask. Then 100 ml of 70% methyl alcohol was added and the flask was revolved at 200 rpm for 30 min, after which the mixtures were filtered by Whatman no.4.

**AF-B1 analysis**

AF-B1 was determined by a direct competitive Enzyme-Linked Immunosorbent Assay using DOA-Aflatoxin F ELISA Test Kit (Chinaphuti et al., 2002). Briefly, 50 µl of either AF-B1 standards or the diluted samples were added into the antibody coated wells, 50 µl of AF-B1-HRP conjugate was added to each well, slightly shaken, and then incubated at room temperature for 30 min. The contents of the well were dumped into the appropriate waste container and the plate was washed 3-5 times by 0.01M phosphate buffer saline + 0.5% Tween 20 (PBS-T). After 100 µl of tetramethyl-benzidine substrate was added to the well and incubated 10 min at room temperature, then 100 µl of stopping solution (0.3M phosphoric acid) was added. The absorbance was measured at 450 nm using an automated MicroELISA reader (Biotex Instrument, 1996). The average absorbance was calculated and the results were expressed as maximal percentage of binding:

\[
\% \text{ maximal binding} = \frac{B}{B_0} \times 100
\]

Where B is the mean absorbance of aflatoxin standards or feed extract samples and B0 is the mean absorbance of aflatoxin standard at concentration 0 µg/kg.

**Statistical analysis**

AF-B1 concentration was calculated by maximal binding percentages. The natural logarithms were applied to adjust the AF-B1 concentrated data to the normal distributed data for further statistical analysis. Correlation of AF-B1 concentration between before and after storage was performed by Pearson’s correlation coefficient. Comparisons of AF-B1 concentrations between before and after storage were separately performed for each feed using repeated mixed model analysis; Proc mixed (SAS, 1997). The significance level was defined at P<0.05.

**RESULTS AND DISCUSSION**

The standard curve of the ELISA test kit is shown in Fig.1. The LOD of this AF-B1 test kit of commercial
AF-B1 IN DAIRY FEEDS AFTER STORAGE

23.2% (n=29) in after storage sample showed higher than 20 µg/kg. The percentage of samples exceeding 20 µg/kg during storage was 22.5% (27/ 120) (Fig.2). Viewed individually, 19.5% (17/87) of CC1-CC4, 33.3% (6/18) of SILEAGE, and 26.6% (4/15) of CCD, exhibited the percentage of aflatoxin contamination after storage over FDA regulatory levels limit.

Concentrations of AF-B1 before and after storage for concentrate was 1.04 µg/kg. The average recovery percentages of 50 µg/kg spiked AF-B1 was 88.4% (n=5). The average recovery percentage and limit of quantification (LOQ) in each feed type in this study when added 50 µg/kg of AF-B1 standard. (N=5)

<table>
<thead>
<tr>
<th>Feed type</th>
<th>Average Recovery Percentage (%)</th>
<th>LOQ (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial concentrate (CC1, 2, 3, 4)</td>
<td>88.4</td>
<td>3.43</td>
</tr>
<tr>
<td>Corn dust (CCD)</td>
<td>86.6</td>
<td>3.12</td>
</tr>
<tr>
<td>Silage (SIL)</td>
<td>90.7</td>
<td>6.93</td>
</tr>
</tbody>
</table>

Numbers of samples for CC1 to CC4, CCD and SIL were 32, 14, 14, 29, 20 and 16, respectively. The storage conditions for feeds in the farms which supplied the feed in this study are described in Table 2. Even though feed samples were collected from farms located in the same area, feed storage practice and storage conditions were different in each farm. Ranges of AF-B1 at before and after storage were 3.95-39.9 µg/kg and 4.11-114.9 µg/kg, respectively. Relationships of concentrations of AF-B1 of feeds between before and after storage in logarithm base 2 scales are shown in Fig.2. Based on FDA regulatory levels for AF-B1 (20 µg/kg), 3.15% (n=5) in before storage sample and

FIG. 1. Standard curve of AF-B1 analysis by ELISA (DOA-Aflatoxin ELISA Test Kit) at AF-B1 standard concentration 0, 4, 10, 20 and 40 µg/kg. (R² = 0.992)

TABLE 2. The condition of storage for feeds in farm which supplied the feed in this study. (N=125)

<table>
<thead>
<tr>
<th>Farm environment/characteristics</th>
<th>Number of feed sample (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Store in feed storage room</td>
<td>114 (91.2)</td>
</tr>
<tr>
<td>Higher temperature at feed storage area</td>
<td>82 (65.6)</td>
</tr>
<tr>
<td>Higher humidity at feed storage area</td>
<td>95 (76)</td>
</tr>
<tr>
<td>Regular clean feed storage area</td>
<td>28 (22.4)</td>
</tr>
<tr>
<td>Once a week</td>
<td>7 (5.6)</td>
</tr>
<tr>
<td>Twice a month</td>
<td>21 (16.8)</td>
</tr>
<tr>
<td>Method of cleaning</td>
<td></td>
</tr>
<tr>
<td>Sweep</td>
<td>24 (19.2)</td>
</tr>
<tr>
<td>Wash with detergent and water</td>
<td>4 (3.2)</td>
</tr>
<tr>
<td>Sunlight pass through feed</td>
<td>65 (52)</td>
</tr>
<tr>
<td>Store in feed container</td>
<td>111 (88.8)</td>
</tr>
<tr>
<td>Regular clean feed containers (once a month)</td>
<td>111 (88.8)</td>
</tr>
<tr>
<td>Feed screening test before purchase</td>
<td>22 (17.6)</td>
</tr>
<tr>
<td>Feed source</td>
<td></td>
</tr>
<tr>
<td>Co-operative shop</td>
<td>106 (84.8)</td>
</tr>
<tr>
<td>Local shop/market</td>
<td>22 (17.6)</td>
</tr>
<tr>
<td>Other region</td>
<td>14 (11.2)</td>
</tr>
</tbody>
</table>

FIG. 2. Concentration of AF-B1 of feeds at before and after storage in logarithm base 2 scales. The vertical and horizontal solid lines indicate AF-B1 concentration at 20 µg/kg, respectively.

TABLE 1. Average recovery percentage and limit of quantification (LOQ) of ELISA AF-B1 analysis in each feed type in this study when added 50 µg/kg of AF-B1 standard. (N=5)
In comparison among feed types, no significant difference was observed in AF-B1 concentrations of feeds at before storage, but CCD at 1 month storage increased AF-B1 after storage more than that of other feeds (P<0.05). Compared to commercial concentrates stored for a month, a concentration of AF-B1 for SIL after 1 week storage was not significantly different from that of CC1, CC3 and CC4, but was significantly higher than that of CC2. Differences after storage for any feeds might be related to storage conditions of feeds and feed mechanical and chemical composition such as moisture content in grain and corn seed (Jayaraman and Kyamuhangire, 2006; Oberheu and Dabbert, 2001). For storage condition, the higher increases of AF-B1 concentrations in both SIL and CCD might be due to the greater quantity of air transferred into the feed, as compared with commercial concentrates (CC1 to CC4) that were contained in 2 levels of bag: inner and outer plastic bags. In this study, SIL had a high moisture content and was mostly placed outside the storage room in high temperatures; the changed conditions might be appropriate for aflatoxin-producing fungi.

It is generally accepted that AF-B1 in feed transfers to milk as aflatoxin M1 (van Egmond, 1989; Veldman, 1992). The mean percent of daily AF-B1 intake that was transferred to aflatoxin M1 was 1.74 (Frobish et al., 1986). In this area, average day milk yield for crossbred Holstein-Friesians was 11.8±0.5 kg/day (Pongpiachan et al., 2003) and the average weight of used concentrates was 5 kg/cow/day (data not shown). The calculated AF-B1 concentration in concentrates must be less than approximately 7 µg/kg to prevent aflatoxin M1 levels in milk exceeding the EU limit of 0.05 µg/l. In our study, only 30% of samples before storage had AF-B1 above 7 µg/kg, and this might indicate that predicted aflatoxin M1 concentration in milk must be lower than 0.05 µg/l. This was in agreement that aflatoxin M1 in milk in this area was mostly less than 0.05 µg/l (Mongkon et al., 2014; Suriyasathaporn and Nakprasert, 2012).

In this study, AF-B1 concentrations of dairy feeds in Thailand at before storage were acceptable for the Thai limit, as FDA limitation, of AF-B1 at 20 µg/kg, but were relatively high for the EU limit of 5 µg/kg. However, 1-month storage at farm condition of commercial concentrates and corn dust increased AF-B1 concentration to approximately 2 to 3 times of that before storage, and corn silage stored for a week at farm condition increased AF-B1 at about 2 times. These indicate that increased AF-B1 levels are related to feed types and farm condition. Farmers should carefully store all feeds and use them as soon as possible to avoid the increase of AF-B1 in animal feeds.
ACKNOWLEDGMENTS

The authors acknowledge the Royal Golden Jubilee Ph.D. Program, the Thailand Research Fund, and the National Research Council for financial support. The authors also thank the officers and farmers of Patang Dairy Cooperative for data and sample collection.

REFERENCES