Aflatoxin B$_1$, zearalenone and deoxynivalenol contamination in feedstuffs in Thailand

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Summary

Mycotoxin contamination of feedstuffs used in Thailand was studied by analyzing aflatoxin B$_1$, zearalenone and deoxynivalenol in raw materials of feeds and complete feeds collected in 2000-2003 with TLC method. For raw material samples, 1,373 imported and domestic samples of corn, soybean mill, peanut product, cassava and rice bran were examined, and for complete feed, 915 domestic samples were examined. The samples were extracted with acetonitril-water (84:16, v/v), and the extracts were then cleaned up with Mycosep 226 cleanup columns and developed on TLC with toluene-acetone (1:1, v/v). The plate was visually examined under ultraviolet light using pure standard toxins as references. For raw materials, the contamination level of aflatoxin B$_1$ higher than 20 µg/kg was found in 24.02 %, 10.96 %, 5.68 % and 4.29 % of the samples collected in 2000, 2001, 2002 and 2003, respectively. The zearalenone level higher than 200 µg/kg was found in 8.11 %, 1.33 %, 6.18 % and 0.71 % in 2000, 2001, 2002 and 2003, respectively. The deoxynivalenol level higher than 1,000 µg/kg was found in 7.50 %, 1.33 %, 0.83 % and 0 % in 2000, 2001, 2002 and 2003, respectively. The contamination in complete feeds was observed to change roughly from year to year in parallel with that in raw materials. The aflatoxin B$_1$ level higher than 20 µg/kg was found in 14.29 %, 13.39 %, 7.53 % and 1.39 % in 2000, 2001, 2002 and 2003, respectively. The zearalenone level higher than 200 µg/kg was found in 11.70 %, 1.79 %, 6.63 % and 1.39 % in 2000, 2001, 2002 and 2003, respectively. The deoxynivalenol level higher than 1,000 µg/kg was found in 12.33 %, 1.79 %, 2.11 % and 0 % in 2000, 2001, 2002 and 2003, respectively.

Key words : aflatoxin B$_1$, zearalenone, deoxynivalenol, feed, Thailand
Introduction

The contamination of mycotoxins can cause a wide variety of negative impacts on animal health, depending on various factors on their nature and concentrations. Especially chronic exposure to mycotoxins leading to unspecific symptoms often entails serious economic losses in animal production.

Aflatoxin B$_1$ (AFB$_1$) is a mycotoxin produced by *Aspergillus flavus* and *A. parasiticus*. AFB$_1$ exerts toxic and carcinogenic effects and immuno-suppression. Zearalenone (ZEN), a mycotoxin produced by *Fusarium culmorum* and *F. graminearum*, has an estrogenic activity, and exerts adverse effects on reproduction system and fertilization. Deoxynivalenol (DON), a trichothecene mycotoxin produced by various species of *Fusarium*, poses adverse effects on digestive tract and immune system.

In order to gain insight in the situation of feed contamination with mycotoxins in Thailand, mycotoxin contamination of feedstuffs used in Thailand was studied by analyzing AFB$_1$, ZEN and DON in raw materials of feeds and complete feeds collected in 2000-2003.

Materials and Methods

Raw material samples (n=1,373) of imported and domestic corn, soybean mill, peanut product, cassava and rice bran, and domestic complete feed samples (n=915) were collected from various regions of Thailand. They were analyzed by the three-toxin TLC screen method for AFB$_1$, ZEN and DON. Briefly, the samples (25 g) were extracted with 100 ml acetonitril-water (84:16, v/v) by shaking for 1.5 hr. After being filtrated with Whatman #4 paper, the extracts were cleaned up with Mycosep 226 cleanup columns. Purified extracts are then evaporated to dryness under N$_2$ gas, dissolved in toluene-acetonitrile (9:1, v/v), and spotted on TLC plates, which were developed with toluene-acetone (1:1, v/v). The plate was visually examined under ultraviolet light using pure standard toxins as references.

Results and Discussion

For raw materials, the contamination levels of AFB$_1$ higher than 20 $\mu$g/kg were noted in 14.02 %, 10.96 %, 5.68 % and 4.29 % of the samples collected in 2000, 2001, 2002 and 2003, respectively. The ZEN level was higher than 100 ppb in 11.41%, 11.30%, 22.20% and 2.86 % in 2000, 2001, 2002 and 2003, respectively. The DON level was higher than 1,000 $\mu$g/kg in 7.51 %, 1.33 %, 0.83 % and 0 % in 2000, 2001, 2002 and 2003, respectively (Table 1).
Table 1. The percent raw material feeds and complete feeds that contamination level of aflatoxin B₁ higher than 20 μg/kg, zearalenone level higher than 100 μg/kg and deoxynivalenol level higher than 1,000 μg/kg were examined in 2000, 2001, 2002 and 2003.

<table>
<thead>
<tr>
<th>Years</th>
<th>Raw Material (1,373 samples)</th>
<th>Complete Feed (915 samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AFB₁ &gt; 20 ppb (%)</td>
<td>ZON &gt; 100 ppb (%)</td>
</tr>
<tr>
<td>2000</td>
<td>24.02</td>
<td>11.41</td>
</tr>
<tr>
<td>2001</td>
<td>10.96</td>
<td>11.30</td>
</tr>
<tr>
<td>2002</td>
<td>5.68</td>
<td>22.20</td>
</tr>
<tr>
<td>2003</td>
<td>4.29</td>
<td>2.86</td>
</tr>
</tbody>
</table>

Note: AFB₁ = Aflatoxin B₁
ZON = Zearalenone
DON = Deoxynivalenol

Mycotoxins contamination in incomplete feeds was observed to change from year to year roughly in parallel with that in raw materials. The AFB₁ level higher than 20 ppb was found in 14.29 %, 13.39 %, 7.53 % and 1.39 % in 2000, 2001, 2002 and 2003, respectively. The ZEN level was higher than 100 μg/kg in 16.15 %, 4.29 %, 15.36 % and 1.39 % in 2000, 2001, 2002 and 2003, respectively. The DON level was higher than 1,000 μg/kg in 12.33 %, 1.79 %, 2.11 % and 0 % in 2000, 2001, 2002 and 2003, respectively (Table 1).

Regarding each item of raw materials and mycotoxins, 2.47 %, 0.87 %, 2.41 % and 0 % of soybean meal samples in 2000, 2001, 2002 and 2003, respectively, were contaminated with AFB₁ higher than 20 μg/kg. The contamination at the level higher than 20 μg/kg was also observed in 31.18 %, 31.71 %, 21.67 % and 19.23 % of corn samples, and in 64.91 %, 51.85 %, 20.69 % and 9.09 % of cassava samples, in 2000, 2001, 2002 and 2003, respectively. The peanut product and rice bran were contaminated with AFB₁ higher than 20 μg/kg only in 2002 (3.28 %) and 2000 (13.33 %), respectively. The contamination at this level was not noted in broken rice in either year. For the other materials, contamination was observed in 22.22 %, 13.89 %, 14.71 % and 0 % in 2000, 2001, 2002 and 2003, respectively.

Contamination with ZEN at the level higher than 100 μg/kg was observed in 14.81 %, 15.53 %, 28.92 % and 0 % of soybean meal samples, in 4.30 %, 12.20 %, 16.67 % and 0 % of corn samples, in 22.22 %, 11.32 %, 27.05 % and 16.67 % of peanut product samples, 16.67 %, 11.54 %, 15.28 % and 0 % of rice bran samples, in 19.30 %, 3.70 %, 10.34 % and 0 % of cassava samples, in 4.76 %, 0 %, 6.67 % and 0 % of fish meal samples, in 2000, 2001, 2002 and 2003, respectively. Broken rice was contaminated at
the level higher than 100 µg/kg only in 2002. For the other materials, contamination at the level higher than 100 µg/kg was observed in 5.55 %, 8.33 %, 5.88 % and 0 % in 2000, 2001, 2002 and 2003, respectively.

Contamination with DON at the level higher than 1,000 µg/kg was observed in 9.88 %, 0.97 %, 0.40 % and 0 % of soybean meal samples, in 16.67 %, 0 %, 0.82 % and 0 % of peanut product samples, in 3.33 %, 3.85 %, 0 % and 0 % of rice bran samples, and in 15.79 %, 3.70 %, 10.34 % and 0 %, in 2000, 2001, 2002 and 2003, respectively. None of broken rice and fish meal samples were contaminated with DON higher than 1,000 ppb in either year. Contamination at this level was found in 11.11 %, 2.78 %, 0 % and 0 % of the other materials.

All over the results show current status of mycotoxin contamination in complete feeds and raw materials used for domestic animals in Thailand. Heavy contamination with AFB1 was noted in corn and cassava, while that with ZEN was noted in soybean meal, corn, peanut products, rice bran and cassava. Heavy contamination with DON was noted only in cassava. The study to elucidate the relationship between mycotoxin contamination in feeds and animal diseases in Thailand is now in progress.