Aflatoxin Contamination in Shrimp Feed and Effects of Aflatoxin Addition to Feed on Shrimp Production

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ABSTRACT

One hundred fifty samples of shrimp feed were collected from the eastern and southern regions of Thailand, and aflatoxins B1, B2, G1, and G2 (AFB1, AFB2, AFG1, and AFG2) in them were analyzed. AFB1 contamination ranged from a non-detectable level (<0.003 ppb) to 0.651 ppb. Metabolites of AFB1 were less abundant than AFB1. To study the effects of aflatoxin in feed on shrimp production, black tiger shrimp were divided into four groups of 30 shrimp per group, tested in triplicate, and fed diets containing 0 (control), 5, 10, or 20 ppb of AFB1 for 10 consecutive days. After 7 or 10 days of consumption on each diet, the shrimp were weighed and sacrificed for laboratory examination. AFB1 and its metabolites were not detected in shrimp muscle. The mortality rate was slightly higher in the AFB1-treated groups than in the control group. The body weight of the surviving shrimp was decreased to 46 to 59% of the initial body weight in the AFB1-treated groups but not in the control group. Histopathological findings indicated hepatopancreatic damage by AFB1 with biochemical changes of the hemolymph. These results show that aflatoxin contamination in shrimp feed may cause economic losses by lowering the production of shrimp. Feed contaminated at a level of 20 ppb or lower (i.e., at the observed natural contamination level) may pose a very low risk, if any, to human health.

Aflatoxins B1, B2, G1, and G2 (AFB1, AFB2, AFG1, and AFG2) are secondary metabolites of Aspergillus flavus and Aspergillus parasiticus and have been observed to contaminate many kinds of foodstuffs such as fish meal, soybean, rice bran, peanut, corn, and other cereals (3, 14, 23). Among these metabolites, AFB1 is most toxic to humans and animals (7, 12, 16, 18). Feed contaminated with aflatoxins tends to cause growth retardation and an increase in the mortality rate of farm animals and a decrease in the production of animal-derived foods. In addition, feed contamination indirectly causes a risk to human health through the ingestion of aflatoxin residue in animal products.

Conditions favoring the growth of the fungus and aflatoxin production are high humidity and high temperature (4, 11); therefore, Thailand is one of the high-risk areas for aflatoxin contamination. Natural contamination in feed has been studied extensively for cattle, swine, shrimp, fish, and poultry feed (8, 15, 20, 24–26, 29). Significant amounts of AFB1 and its metabolites formed in animal bodies have been detected in the muscle, liver, and eggs of farm animals given AFB1-contaminated feed (1, 2, 7, 9, 10, 19, 21). However, aflatoxin contamination in feed and its effects on the production of shrimp, which is widely cultured in Thailand, have not been reported.

To estimate the risk of aflatoxin contamination in shrimp feed, the natural contamination of aflatoxin in shrimp feed was surveyed. The toxic effects and tissue residue of aflatoxin in shrimp fed a diet artificially contaminated with AFB1 were also studied.

MATERIALS AND METHODS

Sampling of shrimp feed. One hundred fifty samples (50 each) of commercial shrimp feed, which was mainly composed of fish meal, soybean, and corn, were collected by means of multi-stage random sampling from 10 provinces in the eastern and southern regions of Thailand, where there are many shrimp farms. We collected five samples from each province for a total of 50 samples from 10 provinces in each of the following seasons: summer (March to June 1997), rainy (July to October 1997), and winter (November 1997 to February 1998). The recovery rate of AFB1 spiked to the aflatoxin-free feed at 5, 10, and 20 ppb was 80.0 to 85.5%, 82.5 to 90.0%, and 85.0 to 98.0%, respectively.

Animals and feeding. Three hundred sixty black tiger shrimp, 3.5 months old, were acclimated for 10 days and then entered into an experiment with a completely randomized design. They were divided by weight into four groups of 30 shrimp per group and tested in triplicate. One of the four groups was fed commercial conventional feed, which was confirmed to be free from aflatoxins through analysis by high-pressure liquid chromatography (HPLC), as a control. The other groups were fed conventional feed supplemented with 5, 10, or 20 ppb of AFB1 for 10 consecutive days. The feed was composed of 25% fish meal,
### Table 1. Detection of aflatoxin contamination in shrimp feed in Thailand

<table>
<thead>
<tr>
<th>Season</th>
<th>Eastern region</th>
<th>Southern region</th>
<th>Total</th>
<th>Range of aflatoxin levela (ppb)</th>
<th>Eastern region</th>
<th>Southern region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>7/25 (28)</td>
<td>1/25 (4)</td>
<td>8/50 (16)</td>
<td>0.003–0.012</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Rainy</td>
<td>22/25 (88)</td>
<td>7/25 (28)</td>
<td>29/50 (58)b</td>
<td>0.003–0.651</td>
<td>0.003–0.058</td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>14/25 (56)</td>
<td>6/25 (24)</td>
<td>20/50 (40)b</td>
<td>0.003–0.314</td>
<td>0.003–0.022</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43/75 (57)</td>
<td>14/75 (19)</td>
<td>57/150 (38)</td>
<td>0.003–0.651</td>
<td>0.003–0.058</td>
<td></td>
</tr>
</tbody>
</table>

a Range of the AFB1, AFB2, AFG1, or AFG2 level.

b Significantly different ($P < 0.01$) from the value in the summer season.

### Table 2. Aflatoxin levels in contaminated samples of shrimp feed in Thailand

<table>
<thead>
<tr>
<th>AF</th>
<th>Eastern region</th>
<th>Southern region</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB1</td>
<td>0.605 ± 0.099a</td>
<td>0.044 ± 0.012</td>
</tr>
<tr>
<td>AFB2</td>
<td>0.060 ± 0.034</td>
<td>0.014 ± 0.011</td>
</tr>
<tr>
<td>AFG1</td>
<td>0.013 ± 0.003</td>
<td>0.005 ± 0.001</td>
</tr>
<tr>
<td>AFG2</td>
<td>0.003 ± 0.001</td>
<td>0.003 ± 0.000</td>
</tr>
<tr>
<td>Total</td>
<td>0.170 ± 0.019a</td>
<td>0.016 ± 0.004</td>
</tr>
</tbody>
</table>

a Significantly different ($P < 0.01$) from the value in the southern region.

The seasonal and geographical patterns of aflatoxin contamination depend on the production of aflatoxins by fungi. A high incidence of aflatoxin-producing molds occurs in both the rainy and winter seasons. Levels of AFB1 contamination of >10 ppb in animal feed and ca. 70 ppb in shrimp feed have been observed in humid tropical and savanna regions. The aflatoxin contamination in shrimp feed was more frequent in the eastern region than in the southern region (Table 1). AFB1 was prepared from moldy corn and then extracted and mixed in shrimp feed. Hemolymph was collected, and serum separated from it was kept at −20°C until biochemical testing by spectrophotometry by Dry Chem (EKTACHEM DT60II and DTSCII, Kodak, Rochester, N.Y.). For histopathology, the hepatopancreas was removed, fixed in Davidson’s fixative, and processed by conventional methods for paraffin sections and staining with hematoxylin and eosin.

Analysis of aflatoxin in feed and shrimp muscle. AFB1, AFB2, AFG1, and AFG2 in shrimp feed were extracted and partially purified according to the methods of Sugano et al. (25). Briefly, 50 g of feed was extracted with 70 ml of 0.1 N hydrochloric acid and 250 ml of chloroform. The extract was filtered through Celite and sodium anhydrous sulfate. The filtrate was applied to a Sep-pak florisor cartridge (Waters Co., Tokyo, Japan) and eluted sequentially with chloroform–methanol (9:1 [vol/vol] 10 ml) and acetone–distilled water (99:1 [vol/vol] 50 ml) to purify aflatoxins. AFB1, AFB2, AFG1, AFG2, AFM1, and aflatoxicol in shrimp muscle were extracted according to the method of the AOAC International (6) and purified using a Sep-pak florisor cartridge as described above. The acetone–distilled water fraction was evaporated to dryness, dissolved in benzene–acetone (98:2 [vol/vol] 1 ml), and loaded on an HPLC column (Shimadzu Co., Tokyo, Japan). AFB1, AFB2, AFG1, and AFG2 were determined by HPLC using a fluorescence monitor, with excitation at 365 nm and emission at 425 nm, and a normal-phase column (Nucleosil 50-10, Techlab, Blacksburg, Va.), with 10% water-saturated chloroform–cyclohexane–acetone–ethanol (50:15:2:1 [vol/vol/vol/vol]) as the mobile phase at a flow rate of 1 ml/min. For the determination of AFM1 and aflatoxicol, HPLC was performed with a reverse-phase column (µ-Bondapak C18, Waters Co.) using 45% methanol as the mobile phase at a flow rate of 1 ml/min. Aflatoxin levels were calculated by measuring the areas under the chromatogram and standard curves with a C-R3A chromatopac (Shimadzu).

Statistics. Statistical significance ($P < 0.01$) was computed by analysis of variance, the Student Newman-Keuls test, or Duncan’s multiple range test.

### RESULTS

Aflatoxin contamination in feed. Feed was contaminated more frequently in the eastern region than in the southern region (Table 1). Contamination occurred most frequently during the rainy season and next most frequently during the winter and summer, in that order (Table 1). Consistent with the higher frequency of contamination in eastern regions, the residual levels of AFB1 detected in samples were higher in the eastern region than in the southern region (Table 2). The same tendency was noted for AFB2 and AFG1, whereas the residual levels of AFG2 were similar in the two regions (Table 2). The highest level of AFB1 was 0.651 ppb, which was found in a sample from the eastern region (Table 1).

Effects of aflatoxin on shrimp. On day 8 of the experiment, the body weight of the shrimp was slightly lower in the 5-ppb group (9.80 g/shrimp) than in the other groups, while the mortality rate was significantly higher in the 10- and 20-ppb groups than in the 5-ppb and control groups (Fig. 1). On day 11 of the experiment, the body weight was significantly lower and the mortality rate significantly higher in the 20-, 10-, and 5-ppb groups than in the control group (Fig. 1).

Glutamic oxaloacetic transaminase was decreased in the 20-ppb–treated group, whereas glutamic pyruvic transaminase was increased in all treated groups (Table 3). There were no significant changes in the creatinine, glucose, urea nitrogen, total protein, or phosphorus levels.

A histopathological study demonstrated that consumption of the AFB1 diet caused hepatopancreatic damage, the degree of which was slightly higher in the 20- and 10-ppb groups than in the 5-ppb group (Table 4).

Aflatoxin residues in shrimp muscle. AFB1 and its metabolites were not detected in shrimp muscles.

### DISCUSSION

The seasonal and geographical patterns of aflatoxin contamination depend on the production of aflatoxins by fungi. A high incidence of aflatoxin-producing molds occurs in both the rainy and winter seasons. Levels of AFB1 contamination of >10 ppb in animal feed and ca. 70 ppb in shrimp feed have been observed in humid tropical and savanna regions.
semitropical regions (22, 28). AFB1 residues in shrimp feed in the eastern region of Thailand were higher than those in the southern region. Furthermore, the levels of AFB1 contamination in shrimp feed in both regions ranged from a nondetectable level (<0.003) to 0.651 ppb (Table 2), which is lower than the legal levels (10 to 1,000 ppb) for animal foodstuffs in Asian and African countries (13, 27). The result of the feeding experiment in shrimp demonstrates that the level of aflatoxin contamination in shrimp muscle remains lower than 0.003 ppb after the consumption of a diet containing 20 ppb of AFB1 for 10 days. It has been estimated regarding the potency of aflatoxin that 0.013 cancers per year per 100,000 people are generated by consuming 1 ng of aflatoxins per kg of body weight per day for hepatitis B virus carriers (5). The consumption of this level of aflatoxin corresponds to the consumption of 10 g of food containing 5 ppb of aflatoxin by a person with a body weight of 50 kg. Thus, taken together with the observed natural contamination levels in shrimp feeds, the feeding experiment appears to indicate that shrimp produced with feed in Thailand pose a very low risk to human health.

The 50% lethal dose of AFB1 in Penaeid shrimp has been reported to be 100.5 ppm (24). In the present study, however, the consumption of an AFB1 (5, 10, or 20 ppb)-containing diet retarded the growth of black tiger shrimp and increased their mortality rate (Fig. 1), which could cause economic losses. Biochemical studies demonstrated a significant increase in the serum glutamic pyruvic transaminase activity in AFB1-treated animals (Table 3), indicating that AFB1 damages the hepatopancreas. Moreover, a histopathological study showed that the degree of hepato-pancreatic damage was slightly higher in the 20- and 10-ppb groups than in the 5-ppb group (Table 4). These findings clearly show that the hepatopancreas is a target of the toxic effects of AFB1, in accord with the fact that the liver is a major target of AFB1 in mammals and birds. Consistent with this notion, necrosis and atrophy of the hepatopancreas have been observed in aflatoxin-treated shrimp (17, 22).

In conclusion, this study demonstrates that aflatoxin contamination in shrimp feed may cause economic losses by lowering the production of shrimp. The risk of aflatoxins in shrimp as a food appears to remain very low, al-

**TABLE 3.** *Biochemical changes in shrimp fed an AFB1 diet*

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 8</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GOT</td>
<td>GPT</td>
</tr>
<tr>
<td>Control</td>
<td>261.7 ± 1.5 (unit)</td>
<td>21.0 ± 2.0 (unit)</td>
</tr>
<tr>
<td>5 ppb</td>
<td>244.5 ± 1.4</td>
<td>47.5 ± 1.6</td>
</tr>
<tr>
<td>10 ppb</td>
<td>228.2 ± 2.1</td>
<td>41.1 ± 1.4</td>
</tr>
<tr>
<td>20 ppb</td>
<td>187.7 ± 2.6</td>
<td>37.5 ± 2.5</td>
</tr>
</tbody>
</table>

*GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase.*
TABLE 4. Histopathological changes in shrimps fed the AFBI diet

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 8</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.15 ± 0.02$^a$</td>
<td>0</td>
</tr>
<tr>
<td>5 ppb</td>
<td>1.67 ± 0.04</td>
<td>1.17 ± 0.02</td>
</tr>
<tr>
<td>10 ppb</td>
<td>1.75 ± 0.03</td>
<td>1.58 ± 0.03</td>
</tr>
<tr>
<td>20 ppb</td>
<td>1.80 ± 0.01</td>
<td>1.67 ± 0.01</td>
</tr>
</tbody>
</table>

$^a$ Degree of hepatopancreatic damage: necrosis of tubule epithelium and vacuolation. Grading index from none (0) to severe (4).

though the levels of aflatoxins in human foods should be kept as low as possible to reduce the incidence of hepatic cancer.

ACKNOWLEDGMENT

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REFERENCES