# Prevalence of Fumonisin Contamination in Corn and Corn-based Feeds in Taiwan

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**ABSTRACT :** The purpose of this study was to investigate the prevalence of fumonisin contamination in corn and corn-based feeds in Taiwan. A total of 233 samples was collected from 8 feed mill factories located in four different regions in Taiwan. The presence of fumonisin $B_1$ ($FB_1$) and $B_2$ ($FB_2$) was determined by thin layer chromatograph, while the total fumonisin content was determined using immuno-affinity column cleanup and fluorometer quantitation. Our results showed that 55 samples of swine feeds had the highest percentage of incidence of $FB_1$ and $FB_2$ (41.8% and 41.8%, respectively), followed by 66 samples of duck feeds (40.9% and 37.8%). However, the percentage of incidence of $FB_1$ and $FB_2$ was much lower in 43 samples of broiler feeds (23.2% and 13.9%) and 69 samples of corn (17.3% and 10.1%). Corn and duck feeds were found to have a significant higher level of means of total fumonisins (5.4±1.5 and 5.8±0.6 ppm, respectively) than swine feeds (2.9±0.4 ppm) and broiler feeds (3.0±0.5 ppm). Comparing fumonisins distribution in different regions, the highest percentage of $FB_1$ incidence (39.2%) was found in the eastern region of Taiwan, and total fumonisins level (4.5±0.7 ppm) was significantly higher than other regions. However, the highest percentage of $FB_2$ incidence (32.0%) was found in the central region of Taiwan. Trimonthly analysis of data showed that both high percentage of $FB_1$ and $FB_2$ incidence (39.3% and 37.7%) and total concentration of fumonisin (5.7±0.4 ppm) were found in the period of Jan. to Mar., The incidence and concentration were significantly higher than other trimothly periods. These results indicate that fumonisin B mycotoxins are both widespread and persistent in feed-grade corn and corn-based feeds in Taiwan. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 4 : 610-614)***

**Key Words :** Fumonisins, Corn, Corn-Based Feeds, Taiwan

## INTRODUCTION

*Fusarium moniliforme* and *F. proliferatum* fungi are ubiquitous in white and yellow corn worldwide (Bezuidenhout et al., 1988; Gelderblom et al., 1988; Steyn, 1995; Shephard et al., 1996). Recently, these fungi have been identified as the source of fumonisin mycotoxins. They are known as secondary metabolites produced by *Fusarium moniliforme* on corn, in culture, and under natural conditions (Bezuidenhout et al., 1988; Alberts et al., 1990). Fumonisins commonly present in corn before and after harvest are fumonisin $B_1$ ($FB_1$), fumonisin $B_2$ ($FB_2$), and fumonisin $B_3$ ($FB_3$). They were isolated and chemically identified in South Africa (Bezuidenhout et al., 1988). They are water-soluble, heat-stable and alkaline-resistant aliphatic hydrocarbons with a terminal amine group and two tricarboxylic acid side chains (Steyn, 1995). The number and position of hydroxyl groups on the aliphatic hydrocarbon determine the structure as $FB_1$, $FB_2$, or $FB_3$; the first two are of approximately equal toxicity, while $FB_3$ is much less toxic. These mycotoxins are known to be responsible for the major toxicological effects of this fungus in animals including leukoencephalomalacia in horses (Kellerman et al., 1990), pulmonary edema syndrome in pigs (Harrison et al., 1990), and hepatic cancer in rats (Gelderblom et al., 1991). They may be involved in the epidemiology of esophageal carcinoma in humans in the Transkei region of South Africa and parts of China (Yoshizawa et al., 1994).

There is clear evidence that fumonisins in feed and food caused animal (Berry, 1988) and human (Smith et al., 1995) mycotoxicosis. Furthermore, Tseng and Liu (1997) pointed out that commercially available corn-based foodstuffs for human consumption in Taiwan are frequently contaminated with $FB_1$ and $FB_2$. They also assumed that fumonisins producing strains of *F. moniliforme* are widely distributed among economic crops such as corn, rice, sugarcane, and sorghum throughout the island (Tseng et al., 1995). However, there is no information available concerning the various degree of fumonisin present in animal feeds in Taiwan. The purpose of this study was to investigate the prevalence and concentration of $FB_1$ and $FB_2$ in corn and corn-based feeds in Taiwan.

## MATERIALS AND METHODS

### Sample collection

A total of 233 samples including 69 corn, 55 swine feeds, 66 duck feeds, and 43 broiler feeds was collected from 8 feed mill factories located in four regions (North, Central, South, and East) in Taiwan. The collection period was from July 1999 to June 2000, and all samples of corn

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grain were imported from USA, and transferred directly to mill factories from Kaoshiung and Keelung harbors in Taiwan. Samples (150 to 200 gm) were taken to a laboratory and immediately analyzed for fumonisins. Fumonisin analysis followed as the method of Shepard et al. (1996) with some modification. Standard $FB_1$ and $FB_2$ (Sigma, St. Louis, MO, USA) were dissolved in methanol (1 mg/ml) in brown vials, dried under nitrogen air, and stored (-20°C) for further assay.

**Extraction and clarification of samples**

Samples were finely ground by a Warner blender (model 34B697, CT, USA), and aliquots of 25 gm (dry matter basis) ground feed were extracted by blending with methanol:water (3:1, 125 ml) in a polytron mixer for 3 min. After centrifugation (500×g), the supernatant was filtrated through Whatman No. 4 filter paper, and 5 ml of crude filtrate was passed through an anion exchange column (Lichrolut 500, SAX 500 mg, Merck, Germany), which had been rinsed with 8 ml methanol. The column was then flushed successively with 8 ml methanol:water (3:1) and 3 ml of methanol. Fumonisins were then eluted with 14 ml of 0.5% acetic acid/methanol, and 1 ml of eluate was taken and dried under nitrogen air.

**Fumonisin analysis by thin layer chromatography (TLC)**

The extract was applied on a TLC plate (Silica Gel 60, Merck, Germany) with 20 μg FB1 and FB2 standards as controls. The TLC plate was dried with a blowing heater to evaporate solvent from spotted dot and developed with ethyl acetate/acetic acid/water (6:3:1; v/v/v). The plate was then air-dried, sprayed with p-anisaldehyde [0.5% in methanol/sulfuric acid/acetic acid, (90/5/5/, v/v/v)] and heated on a hot plate (about 100°C for 5 min).

**Quantification of fumonisins**

An immuno-affinity column was used for quantification (VICAM, FumoniTest, MA, USA). Samples (50 gm), sodium chloride (5 gm), and methanol/water (v/v, 80/20; 100 ml) were mixed in a beaker by polytron for 3 minutes. The crude filtrate was collected on Whatman No. 4 filter paper. An aliquot of 10 ml crude filtrate plus 40 ml PBS/0.1% Tween-20 buffered solution was mixed, and the fine filtrate was obtained after running through 1.0 μm microfibre fiber. Fine filtrate (10 ml) was loaded in to the column, washed with PBS/0.1% Tween-20, and eluted with 1 ml methanol (HPLC grade) successively. The eluate was collected in a glass tube. After mixing with developer, the glass tube was scanned in a fluorometer (VICAM, Series-4). The results were expressed in ppm (μg/g).

**Statistical analysis**

All data were subjected to statistical analysis according to feed types, geographic regions and trimonthly periods. Data were analyzed using the General Linear Models procedure (SAS, 1989). Differences between individual means were determined by Duncan's new multiple range test.

## RESULTS AND DISCUSSION

**Qualitative and quantitative comparison of fumonisins in corn and corn-based feeds**

The fumonisin spots on TLC plate are shown in figure 1. The spots occurred as reddish purple, and the retention factors (Rf) for $FB_1$ and $FB_2$ were 0.61 and 0.76, respectively. The color of spots increased deeply as concentrations of fumonisin increased (10, 20 and 30 μg), and samples with suspicious fumonisins showed similar retention factors as that in figure 1, only standards were presented for quick reference before quantitative assays.

The prevalence and concentration of fumonisins in corn and corn-based feeds are summaried in table 1. With the total of 233 samples collected, 55 samples of swine feeds had the highest percentage of incidence of $FB_1$ and $FB_2$

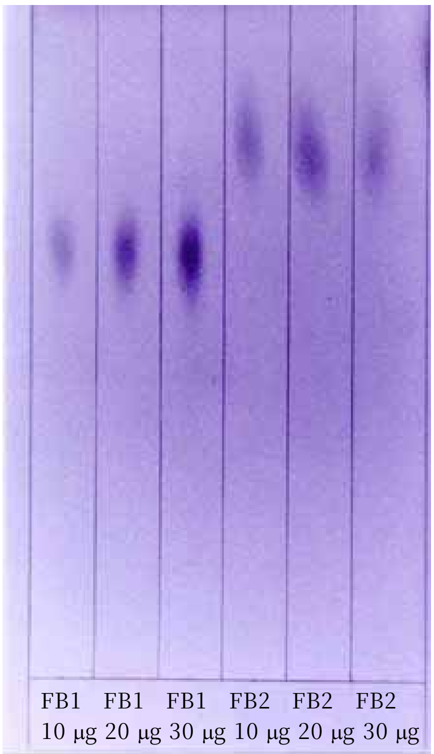


**Figure 1.** Chromatograph of fumonisins by thin layer chromatography (TLC). The TLC plate was developed after loading different concentrations of $FB_1$ and $FB_2$ standards (10 μg, 20 μg and 30 μg) onto the silicate plate.

**Table 1.** Prevalence and concentration of fumonisins ($FB_1$, $FB_2$) in corn and corn-based feeds in Taiwan

| Feed types | Toxins | Percentage of incidence[a] (positive/total samples), % | Mean of total fumonisins, (μg/g)±SD[b] |
|---|---|---|---|
| Swine feeds | $FB_1$ | 41.8% (23/55) | 2.9±0.4[d] |
| | $FB_2$ | 41.8% (23/55) | |
| Broiler feeds | $FB_1$ | 23.2% (10/43) | 3.0±0.5[d] |
| | $FB_2$ | 13.9% (6/43) | |
| Duck feeds | $FB_1$ | 40.9% (27/66) | 5.8±0.6[c] |
| | $FB_2$ | 37.8% (25/66) | |
| Corn | $FB_1$ | 17.3% (12/69) | 5.4±1.5[c] |
| | $FB_2$ | 10.1% (7/69) | |

[a] Percentage of positive samples from different samples detected by thin layer chromatograph.
[b] Means of concentration fumonisins detected by affinity chromatograph method based on positive samples only.
[c,d] Means in the same column with different superscripts differ significantly (p<0.05).

(41.8% and 41.8%) respectively, followed by 66 samples of duck feeds (40.9% and 37.8%). However, the percentage of incidence was much lower in 43 samples of broiler feeds (23.2% and 13.9%) and 69 samples of corn (17.3% and 10.1%). The percentage of incidence of $FB_1$ detected in corn and different kinds of feed samples was higher than for the corresponding $FB_2$ in this survey. Similar observations have been reported by Chu and Li (1994), Doko and Visconti (1994), and Tseng and Liu (1997). Means of fumonisins concentration in corn and duck feeds (5.4±1.5 ppm and 5.8±0.6 ppm) were significantly higher (p<0.05) than that of broiler feeds (3.0±0.5 ppm), and swine feeds (2.9±0.4 ppm). Most of the corn for animal feeds in Taiwan was originally imported from the United States of America. Fumonisins could be produced during shipping and storage before use. Moreover, feed mills use a poor quality of corn whose contents are high in broken corn and foreign materials for duck feed, and, therefore, a high incidence of fumonisin contamination could be expected. Osweiler et al. (1992) reported that corn screenings contaminated with fumonisin mycotoxins are the most likely source of fumonisin toxicosis. The concentration of contamination and prevalence of fumonisin observed in this study were lower than those found in over 50% of corn samples in Southeast Asia (Yamashita et al., 1995) and Italy (Doko and Visconti, 1994). In addition, our results of fumonisin concentrations were lower than those reported in China with a mean of 35.3 ppm contamination in samples collected from households (Yoshizawa et al., 1994).

**Quantification of fumonisins by regions**

Distribution of fumonisins in corn and corn-based feeds in different geographic regions of Taiwan is shown in table 2. The percentage of $FB_1$ incidence and means of total fumonisins contamination levels (39.2%, 4.5±0.7 ppm) in the eastern region of Taiwan were significantly higher (p<0.05) than that of other geographic regions. Although, the highest percentage of $FB_2$ incidence (32.0%) was found in the central region of Taiwan. However, mean of total fumonisins was low in this region. The climate of the eastern region of Taiwan is much more humid and cold during winter season, providing an optimal growth condition for *Fusarium* spp. Tseng et al. (1995) found that *F. moniliforme* was widely distributed in economic crops including corn, sugarcane, and sorghum in eastern Taiwan. Therefore, our results confirmed that fumonisins are produced more in the eastern regions than in the other regions of Taiwan in corn and corn-based feeds. Fuminisins were also found as natural contaminants of mid-western United States corn in over 60% of samples collected from 1988-1991 crops, and up to 10% of the samples had levels of toxin in the 10-59 ppm level (Murphy et al., 1993).

**Trimonthly results of fumonisin survey**

Temperature and humidity might fluctuate differently in different regions of Taiwan. Data analyzed trimonthly to detect any seasonal effect on incidence and total means of fumonisins. Trimonthly results of prevalence and concentration of fumonisins in corn and corn-based feeds in Taiwan are shown in table 3. The percentage of incidence of $FB_1$ and $FB_2$ (39.3% and 37.7%) and means of total fumonisins contamination levels (5.7±0.4 ppm) in the period of Jan.-Mar. were significantly higher (p<0.05) than that of the other periods. However, mean of total fumonisins in Oct.-Dec. period was significantly higher

**Table 2.** Distribution and concentration of fumonisins ($FB_1$, $FB_2$) in corn and corn-based feeds in different geographic regions of Taiwan

| Geographic regions | Toxins | Percentage of incidence[a] (positive/ total samples), % | Mean of total fumonisins, (μg/g)±SD[b] |
|---|---|---|---|
| North | $FB_1$ | 14.7% (5/34) | 3.5±0.7[d] |
| | $FB_2$ | 20.5% (7/34) | |
| Central | $FB_1$ | 36.8% (28/78) | 2.9±0.4[d] |
| | $FB_2$ | 32.0% (25/78) | |
| South | $FB_1$ | 30.1% (28/93) | 2.9±0.5[d] |
| | $FB_2$ | 22.5% (21/93) | |
| East | $FB_1$ | 39.2% (11/28) | 4.5±0.7[c] |
| | $FB_2$ | 28.5% (8/28) | |

[a] Percentage of positive samples from different samples detected by thin layer chromatograph.
[b] Means of concentration fumonisins detected by affinity chromatograph method based on positive samples only.
[c,d] Means in the same column with different superscripts differ significantly (p<0.05).

**Table 3.** Trimonthly results of prevalence and concentration of fumonisins ($FB_1$, $FB_2$) in corn and corn-based feeds in Taiwan

| Periods | Toxins | Percentage of incidence[a] (positive/total samples), % | Mean of total fumonisins, (μg/g)±SD[b] |
|---|---|---|---|
| Jan.- Mar. | $FB_1$ | 39.3% (24/61) | 5.7±0.4[c] |
| | $FB_2$ | 37.7% (23/61) | |
| Apr.- Jun. | $FB_1$ | 37.5% (15/40) | 3.2±0.4[e] |
| | $FB_2$ | 32.5% (13/40) | |
| Jul.- Sep. | $FB_1$ | 19.1% (13/68) | 3.3±1.0[e] |
| | $FB_2$ | 10.2% (7/68) | |
| Oct.- Dec. | $FB_1$ | 31.2% (20/64) | 4.5±0.8[d] |
| | $FB_2$ | 28.1% (18/64) | |

[a] Percentage of positive samples from different samples detected by thin layer chromatograph.

[b] Means of concentration fumonisins detected by affinity chromatograph method based on positive samples only.

[c,d,e] Means in the same column with different superscripts differ significantly ($p<0.05$).

than that of in Apr.-Jun. period. Although, there was the second high percentage of incidence of $FB_1$ and $FB_2$ was noticed in Apr.-Jun. period (37.5% and 32.5%). Mould and mycotoxins contamination of raw materials occur during the pre-harvest and /or the post-harvest periods. During this periods, temperature and humidity play an important role in the growth of fungi and fumonisins contamination. During the period of Jan.-Mar., the climate of Taiwan with its uniform low temperature (mean, 22°C, min. 20°C, max. 26°C) and high relative humidity (65-90%), this condition is suitable to growth of fumonisin-producing fungi (Jackson and Bennett, 1990).

## CONCLUSIONS

In conclusion, the results of this survey clearly show that there were various fumonisins and contamination levels in corn and corn-based feeds in Taiwan. Because corn is the major energy resource in compound feed formula and is present in at least 60% or more in compound feeds in Taiwan, it is necessary to regulate fumonisin contamination in feed industries by intensive monitoring of feed-grade corn and related compound feeds regularly before use. Due to the fact that in Taiwan there is little information about this topic, this work has shown the need for targeted studies to determine the sources of fumonisin contamination entering the Taiwan feed industry and to establish strategies for control.

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