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NATURAL OCCURRENCE OF *FUSARIUM* MYCOTOXINS IN GRAINS AND FEEDS IN TAIWAN[1]

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Abstract

The occurrence of ***Fusarium*** mycotoxins in grains and feeds was surveyed from 1978 to 1981. No ***Fusarium*** mycotoxins were detected, using thin-layer chromatography, gas-liquid chromatography and spectrodensitometry, in 428 polished rice samples from various districts of Taiwan intended for human consumption. Maize imported from USA was contaminated with zearalenone, T-2 toxin, and deoxynivalenol. For instance, 10.9% of maize samples were contaminated with zearalenone at the levels of 49 to 303 μg/kg; 7.6% contained T-2 toxin at the levels of 78 to 650 μg/kg and 7% were contaminated with deoxynivalenol from 95 to 312 μg/kg. One of the 8 maize samples from South Africa was contaminated with zearalenone at 303 μg/kg and another contained deoxynivalenol at 140 μg/kg. Altogether 160 feed samples were examined for ***Fusarium*** mycotoxins, of which 6.9% swine feeds were contaminated with zearalenone at the levels of 162 to 1,203 μg/kg and 8.3% chicken feeds at levels between 203 to 1,973 μg/kg, and 11.1% feedstuffs at the levels of 126-152 μg/kg.

Key words: T-2 toxin; zearalenone; deoxynivalenol; diacetoxyscirpenol; grains; feeds.

Introduction

Species of *Fusarium* are widespread in nature as saprophytes in many plant materials and as pathogens of various field crops. The major mycotoxins produced by these fungi are known as zearalenones and trichothecenes.

Mirocha *et al.* (1976) have reported the natural occurrence of zearalenone in

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cereal grains and feeds involved in mycotoxicoses. Usually its estrogenic properties are evident and it causes infertility in cattle and swine. Other surveys have shown its presence in maize, barley, grain sorghum and sesame meal (Eriksen, 1968; Shotwell, 1977; Bennett and Shotwell, 1979; Shotwell *et al.*, 1980; Thiel *et al.*, 1982).

Trichothecenes have also been found in maize, associated with death of cows in a Wisconsin (U.S.A.) dairy herd (Hsu *et al.*, 1972). Morooka *et al.* (1972) isolated two toxic substances and identified as deoxynivalenol and nivalenol from barley and wheat infected with *Fusarium* in the region of Kagawaken, Japan. Eppley *et al.* (1974) found that 54% of 173 maize samples from various grain elevators in the midwest United States contained a skin irritating factor, presumably T-2 toxin. Deoxynivalenol has been identified as emetic principal in field maize infected with *Fusarium* (1976). Maize and barley have frequently been found to contain deoxynivalenol, sometimes in large amounts (Yashizawa and Morooka, 1977; Vesonder *et al.*, 1978; Ishii *et al.*, 1975; Jemmali *et al.*, 1978) and a toxic metabolite has been isolated from moldy sorghum infected with *F. incarnatum* (Rukmini and Bhat, 1978).

Recently, Ueno (1983) reviewed the natural occurrence of trichothecenes in worldwide, noting that surveys of the natural occurrence of the trichothecenes in agricultural commodities and feeds are still few. Most analyses have come from isolated outbreak on farms or from selected samples. The only general survey was for deoxynivalenol in wheat in Canada in 1980 and 1981 (Tremholm *et al.*, 1983) and for the same toxin in maize in the midwest United States (Vesonder and Ciegler, 1979). The occurrence of *Fusarium* mycotoxins, toxicoses and of toxin-producing strains in Taiwan is not well known. Since the hazards to animal health posed by *Fusarium* mycotoxins have only recently become known, this report deals with the occurrence of some *Fusarium* mycotoxins (zearalenone, T-2 toxin, diacetoxyscirpenol and deoxynivalenol) in imported maize, domestic polished rice and in feeds in Taiwan.

Materials and Methods

Sample Collection and Preparation

Imported maize (total=311) samples from Thailand, South Africa and the United States were supplied by Agricultural Product, Utilization Department, Union Industrial Research Laboratory after collection by Kaushung and Taichung Bureau of Commodity Inspection and Quarantine during 1978 and 1981. Polished rice samples (total=428) intended for human consumption and mixed feeds (total=160) for poultry and livestock were purchased from various districts of Taiwan (Fig. 1).

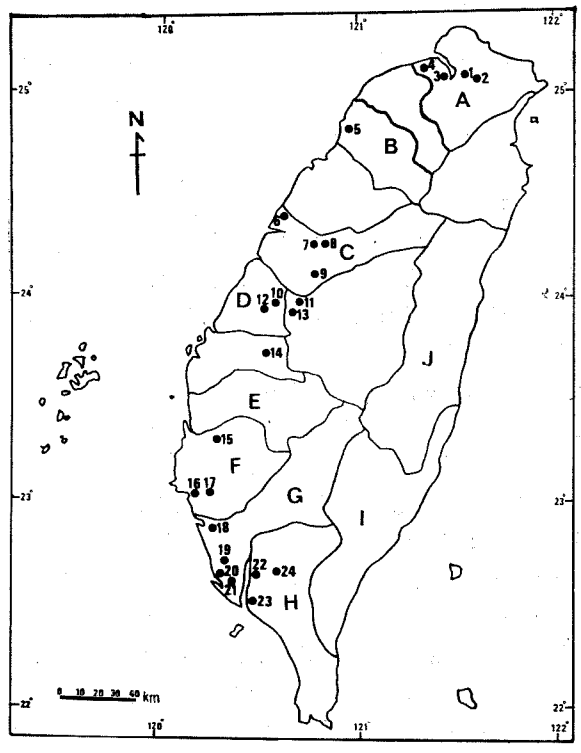

Fig. 1. Sampling locations of polished rice and feeds in various districts of Taiwan.

Rice:	A. Taipei;	B. Hsinchu;	C. Taichung;	D. Chunghua;
	E. Chiayi;	F. Tainan;	G. Kaohsiung;	H. Pington;
	I. Taitung;	J. Hualien.		
Feed:	1. Taipei;	2. Nankang;	3. Sahchung;	4. Linkou;
	5. Hsinchu;	6. Tachia;	7. Shinkang;	8. Tungshin;
	9. Taiping;	10. Yuanlin;	11. Tsaotung;	12. Yungching;
	13. Nantou;	14. Touliu;	15. Hsinying;	16. Tainan;
	17. Yungkang;	18. Luchu;	19. Tsoying;	20. Kaohsiung;
	21. Fengshan;	22. Pingtung;	23. Hsinyuan;	24. Neipu

The samples (3 kg) were collected and immediately stored at 4°C before analysis. All samples were ground in a Krups 75 mill (Ribert Krups 5650 Solinger 19, Germany), passed through a 2-mm sieve, and mixed thoroughly. The representative samples (100g) were separately assayed for zearalenone, T-2 toxin, diacetoxyscirpenol and deoxynivalenol.

Chemical Analyses

Solvents for extraction and clean-up were purchased from Wako Chemical Industries, Ltd, Japan (First class reagent grade) and ALPS Chemical Co., Ltd, Taiwan (Ultra pure). Those for HPLC analysis were from E. Merck, Germany (LC

grade). Authentic toxins of zearalenone, T-2 toxin, diacetoxyscirpenol and deoxynivalenol were prepared by C. J. Mirocha at the University of Minnesota, USA.

Fusarium mycotoxins from maize kernels, polished rice and feeds were analyzed following the methods of Mirocha *et al.* (1976; 1977) with the modifications described below:

A. Zearalenone analysis:

a. Extraction and clean up: Except ground maize 25 g, polished rice or feed were extracted as described by Mirocha *et al.* (1977).

b. Thin-layer chromatography: Pre-coated E. Merck silica gel TLC plates (20× 20 cm; 0.25 mm thickness, without fluorescence indicator) previously scribed into 18 lines were spotted with 30 μl, 50 μl and 100 μl of test extracts and 5 μg of standard zearalenone. The plates were then developed with chloroform-ethanol (97:3, v:v). Zearalenone fluoresces blue-green under a 356 nm UV lamp. Identification was confirmed as previous described (Mirocha *et al.*, 1977).

c. Spectrodensitometer: For quantitative analysis in maize and polished rice, the zearalenone positive samples were re-spotted on TLC plates, developed as described above, air dried and scanned using a SD-300 Spectrodensitometer (Schoeffel Instrument Corporation, New Jersey, U.S.A.), equipped with a scanning thin-layer plate stage with a search unit (Mode OPM 30 quartz prism monochromator), light source (200–700 nm, set at 313 nm) and interference wedge monochromators (400–700 nm, set at 443 nm). Zearalenone was quantified by peak height measurement against standards, giving a limit of detection of 50 ng (Fig. 2).

d. High pressure liquid chromatography: Quantitative analysis of feeds was performed with a Waters Associates Model ALC/GPC-204 instrument equipped with M-6000A pump, U6K universal injector, μ-Bondapak C_{18} (4 mm id×30 cm) and Model 440 UV detector (236 nm filter) set at sensitivity of 0.01 absorption unit full scale (AUFS).

Each standard was injected into the column eluted at 2 ml/min using a CH_3CN: H_2O (1:1, v:v) solvent system. Zearalenone in feed samples was quantified by comparing peak heights against a calibration curve prepared using serial dilutions of a standard dissolved in methanol. The limit of detection was 1 ng (Fig. 3 and Fig. 4).

e. Recovery: Quadruplicate 25 g samples of toxin-free ground maize, polished rice and feed were individually spiked with 40 μg of zearalenone in 1 ml methanol in sealed containers and analyzed. Average recoveries were 53% for maize and 71% for polished rice, 79% for pig feed, 75.5% for chicken feed and 75% for feedstuff.

B. T-2 toxin and diacetoxyscirpenol analyses:

a. Extraction and clean up: Except ground maize 25 g, polished rice or feed were extracted as described by Mirocha *et al.* (1976).

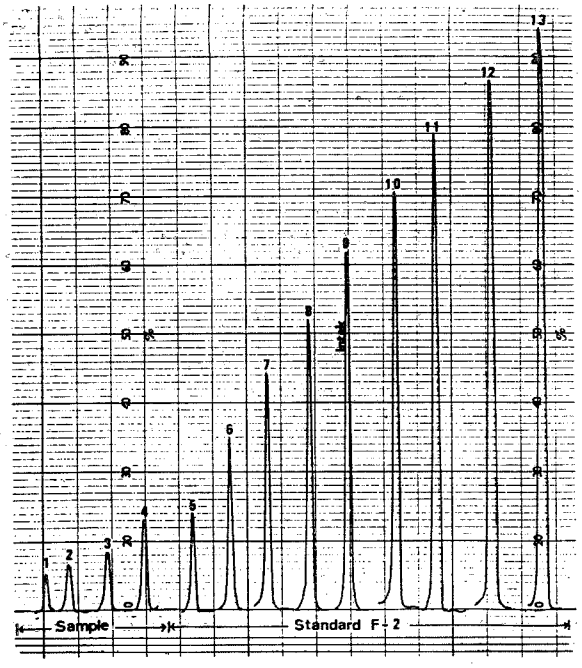

Fig. 2. Typical spectrodensitometric recording of authentic zearalenones (F-2) and some corn samples contaminated with zearalenone.

Peak numbers: 1 CI-69, 2 CI-74, 3 CI-76, 4 CI-80, 5=200 ng, 6=300 ng, 7=400 ng, 8=500 ng, 9=600 ng, 10=700 ng, 11=800 ng, 12=900 ng, and 13=1,000 ng.

Conditions: Single beam was used, excitation wavelength 313nm, emission wavelength 443 nm, gain set at 7, balance 18, density computer O.D. at 2.0 and chart speed 1 cm/min.

b. Thin-layer chromatography: The concentrated extracts were spotted onto TLC plates together with authentic standards and developed with ethyl acetate-toluene (3:1, v:v). T-2 toxin and diacetoxyscirpenol fluoresced under a 365 nm UV lamp. Identification was confirmed as previous report (Mirocha *et al.*, 1976).

c. Gas liquid chromatography: A Hitachi Model 163 gas chromatograph equipped with FID was used for quantitative analysis. Nitrogen was used as carrier gas at 30 ml/min. The column (6′×1/8′) consisted of 10% SE-30 adsorbed on chromosorb w (100/120 mesh). Temperatures of both the injector and detector were set at 310°C, with a temperature program of 200°C to 280°C at 7.5°C/min. The toxins were analyzed as their trimethylsilyl ester derivatives by using Tri-Sil-TBT as silylation reagents (Pierce Chemical Co. Box 117, Rockford, Illinois 61105, U.S.A.). Each twenty microliter of concentrated extracts was placed in 2.5 ml vials, and 50 μl of a standard solution of T-2 toxin (0.5 μg/μl) or diacetoxyscirpenol in acetone was put in another vial. After evaporating to dryness under nitrogen, 20 μl of Tri-Sil-TBT were added to each vial and left for 20 min at room temperature. The peak

enhancement technique was used to confirm the identity of T-2 or diacetoxyscirpenol. Sensitivity of GLC method for identification of T-2 toxin and diacetoxyscirpenol was lower than 40 ng (Fig. 5).

d. Recovery: Twenty five grams of uncontaminated maize or polished rice were spiked with 50 μg of T-2 toxin or diacetoxyscirpenol. The average recoveries from these tests for T-2 toxin in maize, polished rice and feed were 64%, 70% and 71% and with diacetoxyscirpenol 55%, 61% and 60%, respectively.

C. Deoxynivalenol analysis:

a. Extraction and clean up: Ground maize, polished rice and feed (50 g) were extracted with 200 ml of methanol containing 1% NaCl (55:45, v:v) and 100 ml petroleum ether (b.p. 60–70°C) in a blender and filtered through Whatman #1 filter paper. Residues were rinsed with 10 ml of the methanolic mixture and washes were

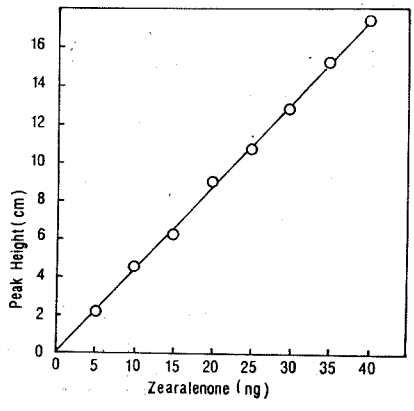

Fig. 3. Calibration curve of zearalenone estimated by high pressure liquid chromatography.

Conditions: 1. Column: μ-Bondarpark C_{18}
2. Excitation wavelength: 236 nm
3. Solvent system: CH_3CN: H_2O (60:40, v:v)
4. Flow rate: 2 ml/min

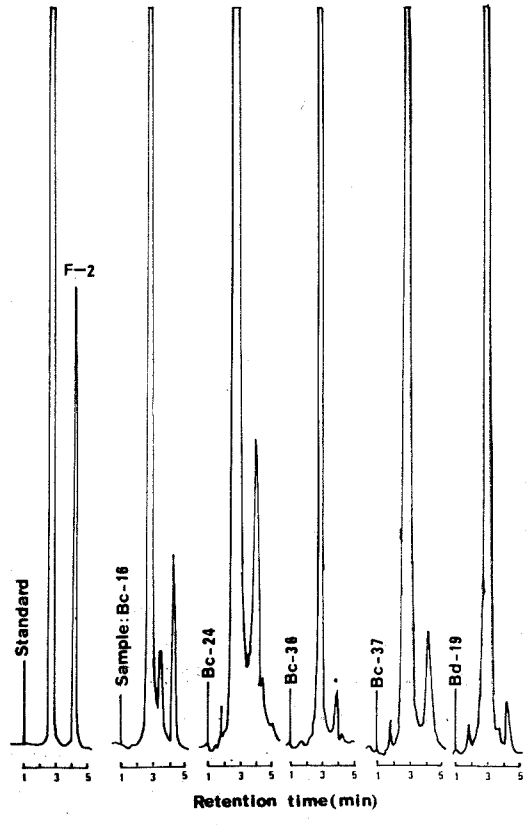

Fig. 4. High pressure liquid chromatograms of authentic zearalenone (F-2) and five chicken feeds contaminated with zearalenone. Experimental conditions are the same as in the legend of Fig. 3.

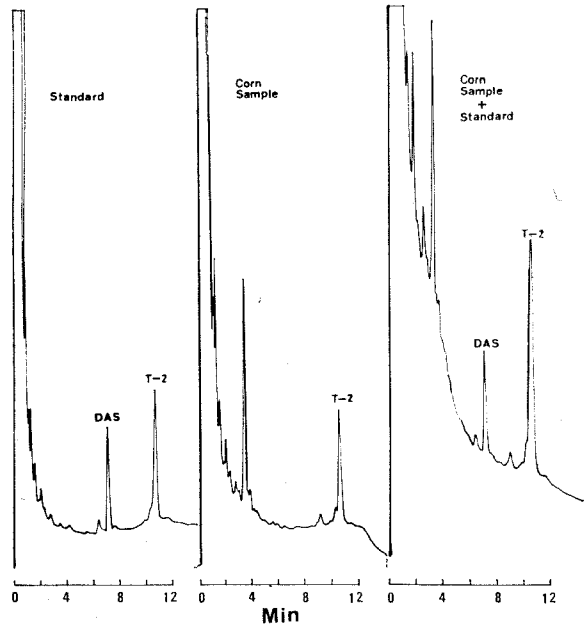

Fig. 5. Profiles of gas-liquid chromatograms of authentic T-2 toxin and diacetoxyscripenol as well as a corn sample contaminated with T-2 toxin. One microgram per microliter of standard TBT derivatives or 2 μl of sample was injected to the GLC equipped with 10% SE-30 column (6′×1/8′); N_2 flow rate 30 ml/min; temperature program 200–280°C at 7.5°C/min; Range set 10, attenuator 16; chart speed 5 mm/min.

combined. The filtrate was allowed to separate and after further partitioning with ether, the methanol-NaCl layer was extracted with chloroform 10 ml three times. The combined $CHCl_3$ extract was evaporated to dryness over a steam bath, redissolved in acetone and transferred to an 8-ml vial and again evaporated to dryness. The residue was reconstituted with 2 ml methanol-H_2O (2:3, v:v) and passed through a Sep-pak C_{18} cartridges into a 2-ml vial. Eluate was dried by flux of N_2 and redissolved in 1 ml acetone for TLC and GLC analyses.

b. Thin-layer chromatography: Concentrated extracts were applied to precoated TLC plates and developed in chloroform—acetone (90:10, v:v). Plates were air dried, sprayed with *p*-anisaldehyde solution (methanol: glacial acetic acid: concentrated sulfuric acid: *p*-anisaldehyde=7:1:0.5:0.15, v/v/v/v). Plates were heat-dried with a hot air blower and deoxynivalenol showed up immediately as a yellow spot.

For quantitative analysis of deoxynivalenol, gas liquid chromatography was used as for T-2 toxin and diacetoxyscirpenol. Average recoveries of toxin, from three tests in maize, polished rice and feed were 76%, 78% and 75%, respectively (Fig. 6).

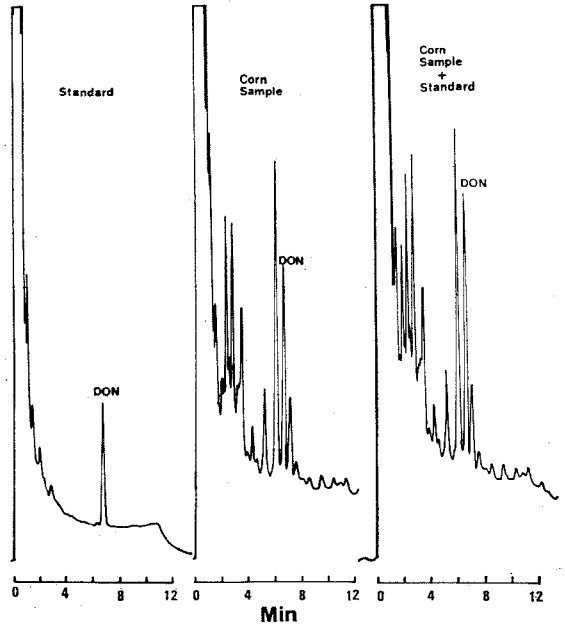

Fig. 6. Profiles of gas-liquid chromatograms of authentic deoxynivalenol (DON) and a corn sampl contaminated with deoxynivalenol. All of the conditions are the same as in Fig. 5.

Results

Altogether 428 rice samples intended for human consumption were collected from various districts of Taiwan (Fig. 1) during 1978 and 1981 and were analyzed for zearalenone, T-2 toxin, diacetoxyscirpenol and deoxynivalenol. None of these *Fusarium* mycotoxins was found in any rice samples grown in Taiwan. The incidence and levels of zearalenone in imported maize from Thailand, South Africa and the United States are shown in Table 1. Of 82 imported samples, 8 contained zearalenone. Nearly, 11% of 73 samples of maize from the United States were zearalenoee-positive, containing 49 to 303 μg zearalenone per kg. Zearalenone was detected in one of the eight samples from Thailand.

Maize samples from South Africa and the United States were also examined for T-2 toxin and diacetoxyscirpenol (Table 1). Of the 124 samples tested, only 9 (7.6%) were definitely contaminated with T-2 toxin, all from the United States, with levels of 78 to 650 μg T-2 toxin per kg. No diacetoxyscirpenol was found in any sample.

Deoxynivalenol was found in maize imported from both South Africa and the United States. Seven of the 100 samples (7%) came from the United States with levels ranging between 95 and 312 μg deoxynivalenol per kg (Table 1). The only

positive sample from South Africa was contaminated with 140 μg/kg of deoxynivalenol.

No T-2 toxin, diacetoxyscirpenol or deoxynivalenol was found in any of 160 feed samples, which included swine feeds, chicken feeds and other feedstuffs. However, some were contaminated with zearalenone (Table 2). Four of 58 swine feeds (6.9%) were contaminated with 162 to 1,203 μg zearalenone per kg, the highest level was found in feed from Tachia county.

The occurrence of zearalenone in various kinds of chicken feeds is illustrated

Table 1. *Incidence and levels of zearalenone, T-2 toxin, and deoxynivalenol in imported maize for human consumption*

Source imported from	Zearalenone			T-2 toxin			Deoxynivalenol		
	Samples analyzed	Content (μg/kg)	No. of samples	Samples analyzed	Content (μg/kg)	No. of samples	Samples analyzed	Content (μg/kg)	No. of samples
U.S.A.	73	ND	65	118	ND	109	100	ND	93
		49	2		78-98	2		95	1
		106-170	4		134	1		124-185	3
		244	1		213-217	3		219-264	2
		303	1		317	1		312	1
					415	1			
					650	1			
South Africa	8	ND	7	6	ND	6	5	ND	4
		303	1					140	1
Thailand	1	ND	1						

ND: Not detected

Table 2. *Occurrence of zearalenone in domestic swine feeds prepared from maize imported from the United States and South Africa*

Kind of mixed feed	Location(1)	No. of samples		Level (μg/kg)
		Examined	Contaminated	
Growing pig	3, 6, 7, 10, 11, 24	6	0	
Developing pig	1, 3, 6, 9, 10, 18	7	1	1,203
Starter pig	1, 4, 6, 7, 10, 11 12, 14, 20	16	0	
Sow	5, 6, 7, 20	4	2	243-300
Broiler pig	3, 10, 15	4	0	
Miscellaneous	1, 3, 5, 7, 9, 10 11, 13, 14	21	1	162

(1): Figure indicates the location in Fig. 1.

Table 3. *Occurrence of zearalenone in domestic chicken feeds prepared from maize imported from the United States and South Africa*

Kind of mixed feed	Location(1)	No. of samples		Level (μg/kg)
		Examined	Contaminated	
Growing chicken	1, 2, 5, 8, 10,11,13 19,20,21,22,23,24	18	0	
Developing chicken	1, 2, 5, 7, 10,11,20 21, 22, 23	11	0	
Starter chicken	1, 2, 3, 5, 10,11,13 15,16,19,20,22,24	26	4	354-1,973
Broiler chicken	1, 6, 9, 10, 13, 15 17, 20, 21, 22	19	2	405-1,238
Laying hen	9, 10, 17	4	0	
Miscellaneous	1, 6, 10	6	1	203

(1): Footnotes as for Table 2.

Table 4. *Occurrence of zearalenone in domesitc feedstuffs prepared from various meals and maize imported from the United States and South Africa*

Kind of feedstuff	Location(1)	No. of samples		Level (μg/kg)
		Examined	Contaminated	
Rice meal	1, 7	2	0	
Maize meal	13	2	0	
Wheat meal	8	1	0	
Soybean meal	8, 13	2	0	
Fish meal	8	2	0	
Miscellaneous	1, 6, 14, 15	9	2	126-152

(1): Footnotes as for Table 2.

in Table 3. About 8.3% of samples were contaminated with zearalenone. Among the positive samples, 15.3% of 26 starter chicken feeds was contaminated with zearalenone between 354 to 1,973 μg/kg; 10.5% of 19 broiler chicken feeds contained 405 to 1,238 μg/kg; and one of the 6 miscellaneous feeds contaminated zearalenone at 203 μg/kg. The feedstuffs which included duck feed, maize meal, wheat meal, soybean meal and miscellaneous feedstuff were also examined for zearalenone, T-2 toxin, deoxynivalenol and diacetoxyscirpenol. Only two of the 16 feedstuffs were contaminated with zearalenone at the level of 126 to 152 μg/kg (Table 4).

Discussion

Survey of polished rice in Taiwan in 1978 and 1981 indicated that this staple

was not contaminated by zearalenone, T-2 toxin, deoxynivalenol and diacetoxyscirpenol. However, some maize imported from the United States sampled at that time was contaminated by the first three *Fusarium* toxins as shown in Table 1. Maize samples collected from South Africa and Thailand were too small so that it was hard to draw any conclusive result, thus a further study is required. Survey of feeds intended for animal consumption indicates contamination with zearalenone but no other *Fusarium* mycotoxins. Some implication of these mycotoxins in mycotoxicoses of farm animals is well established. Recently, there is an increasing concern of the hazards of *Fusarium* mycotoxins to animal and human health in many countries. We need to continuously inspect imported maize and domestic feeds for contamination with *Fusarium* and other mycotoxins.

Studies of the incidence of *Fusarium* mycotoxins, specially trichothecenes, in agricultural commodities in the past have been handicapped due to lack of suitable analytical methods. The methods we have used for the extraction, clean-up and quantitative analyses of *Fusarium* mycotoxins in this study were originally developed by Mirocha *et al.* (1976, 1977) with slight modification. Zearalenone was quantitatively analyzed by high pressure liquid chromatography and the spectrodensitometric methods. These methods we have developed for zearalenone are useful in future studies.

Acknowledgements

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泛存於臺灣穀粒和飼料 *Fusarinm* Mycotoxins 之研究

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從一九七八年至一九八一年間，全面探討本省穀粒和飼料汚染 *Fusarium* mycotoxins，利用化學分析法包括薄層色層分析法，氣相色層分析法及分光濃度計量法，定性定量結果，發現被試428件食米中，全部未被毒素汚染。自美國進口玉米部份被 zearalenone, T-2 toxin 和 deoxynivalenol 汚染，其百分比及含毒量如下：zearalenone 10.9%，含毒量介於 49–303 μg/kg；T-2 toxin 7.6%, 78–650 μg/kg；deoxynivalenol 7%, 95–312 μg/kg。從南非進口玉米共八件，其中一件被 zearalenone 汚染，含毒素量爲 140 μg/kg，另一件被 deoxynivalenol 汚染含 140 μg/kg。調查160件飼料，全部未被 T-2 toxin, deoxynivalenol 和 diacetoxyscirpenol 汚染，但有些飼料含 zearalenone 毒素如猪飼料佔 6.9%，含毒量介於 162–1,203 μg/kg；鷄飼料佔 8.3%介於 203–1,973 μg/kg，一般飼料佔11.1%，含毒量爲 126–152 μg/kg。