




# Prevalence of mycotoxins in feed and feed ingredients between 2015 and 2017 in Taiwan

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## Abstract

Contamination of feed by mycotoxins is a global epidemic that has a sizeable impact on animal health and causes economic losses. Mycotoxins, including aflatoxins (AFs), zearalenone (ZEN), fumonisins (FUMs), deoxynivalenol (DON), and ochratoxin A (OTA), lead to acute and chronic adverse effects in pigs. Animal feed and feed ingredients are commonly contaminated by one or more mycotoxins worldwide; however, the prevalence of mycotoxin contamination in feed and feed ingredients in Taiwan remains unclear. A total of 820 cornmeal and corn-based swine feed (pregnancy and nursery diets) samples provided by feed and animal producers were analyzed using the enzyme-linked immunosorbent assay method between January 2015 and December 2017 to determine the presence of mycotoxins. The results revealed that the most prevalent mycotoxin in Taiwan was DON, with 91.4% of positive samples between 2015 and 2017, followed by ZEN, AFs, and FUMs, with 70.2%, 58.0%, and 50.4% of positive samples, respectively. A similar prevalence of mycotoxins was observed in cornmeal and corn-based swine feed. Furthermore, 7.7% of the analyzed feed samples contained one mycotoxin, and 91.3% contained multiple mycotoxins. DON was the most prevalent mycotoxin in cornmeal and corn-based swine feed in Taiwan. Moreover, a high incidence of contamination by multiple mycotoxins was observed in swine feed. Awareness of mycotoxin presence in feed and development of mycotoxin detoxification strategies are unmet needs.

**Keywords** Cornmeal · Deoxynivalenol · Feed · Mycotoxin · Pig · Taiwan

## Introduction

Mycotoxins, including aflatoxins (AFs), zearalenone (ZEN), fumonisins (FUMs), deoxynivalenol (DON), and ochratoxin A (OTA), are secondary metabolites of fungi that have adverse effects on humans and animals (Hussein and Brasel 2001). Contamination of food and animal feed with mycotoxins is a global epidemic that has a sizeable impact on health and causes economic losses (Wu 2007; Bryden 2012). Mycotoxins exert

various acute and chronic effects on farm animals depending on species and susceptibility (Zain 2011). Deleterious effects of mycotoxins on animals include chronic growth impairment and acute mortality (Grenier and Applegate 2013). In addition, cocontamination of mycotoxins in feed has severe effects on animals because of additive and synergistic interactions between mycotoxins (Speijers and Speijers 2004).

Mycotoxins are prevalent in grains such as corn, soybean, wheat, and barley and their byproducts (Rodrigues and Naehrer 2012a; Rodrigues and Naehrer 2012b; Pinotti et al. 2016; Abudabos et al. 2017). Moisture and temperature are critical factors for mold growth and mycotoxin production (Bryden 2012). In addition, the prevalence of mycotoxins in feed and feed ingredients may vary by location and season (Rodrigues and Naehrer 2012a; Rodrigues and Naehrer 2012b; Pinotti et al. 2016).

Taiwan belongs to both tropical and subtropical climate zones and has hot and rainy seasons. More specifically, the weather in Taiwan is hot with high humidity in summer. Therefore, mold thrives on surfaces holding animal feed because of abundant

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moisture in the highly humid environment. A survey conducted in North Asia between January 2009 and December 2010 analyzed feed samples for the presence of AFs, DON, ZEN, FUMs, and OTA (Rodrigues and Naehrer 2012a). The results revealed that the most prevalent of these mycotoxins in the region were DON, ZEN, and FUMs (Rodrigues and Naehrer 2012a). DON was present in 70% of the tested samples with an average of 691 ppb (Rodrigues and Naehrer 2012a). However, awareness of the prevalence of mycotoxins remains limited and comprehensive surveys are rare in Taiwan. Only the prevalence of FUMs in corn and animal feed has been analyzed (Tseng and Liu 1999, 2001; Cheng et al. 2002). Contamination of feed with mycotoxins is expected to become more prevalent because of global warming and the increase in extreme weather events. Changes in environmental stress also have a considerable impact on mycotoxin production in mold (Magan et al. 2011). Thus, annual surveying of mycotoxins in feed is crucial for enabling feed and animal producers to assess the risk of and prevent mycotoxin contamination of feed.

This study surveyed the prevalence of mycotoxins in cornmeal and corn-based swine feed (pregnancy and nursery diets) in Taiwan over a 3-year period. In total, 820 samples were analyzed for mycotoxin presence between January 2015 and December 2017.

## Materials and methods

### Sample collection and preparation

A total of 820 feed samples were provided by various feed and animal producers in Taiwan between 2015 and 2017. Criteria for sampling and sample preparation for mycotoxin analysis described in previous studies were strictly followed (Richard 2006; Whitaker 2006). Corn-based swine feed (pregnancy and nursery diets) and cornmeal (mainly imported from the USA and Brazil) samples were completely ground by using commercial blenders (Waring Products, Torrington, CT, USA). Homogenized feed subsamples of 20 g were weighed and mixed with 100 mL of 70% methanol at 25 °C for 3 min for AF, ZEN, and FUM extraction. For DON extraction, 20 g of homogenized feed samples were mixed with 100 mL of distilled water at 25 °C for 3 min. After extraction, the extracts were filtered using a Whatman No. 1 filter. The filtrates could be used for AF measurement directly without dilution. For ZEN and FUM quantification, filtrates were diluted 1:4 with 70% methanol and 1:19 with distilled water, respectively. For DON quantification, filtrates were diluted 1:4 with distilled water.

### Mycotoxin analysis

The filtrates and diluted filtrates were tested for mycotoxin content by using the enzyme-linked immunosorbent assay

(ELISA) method. AFs, including AFs B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>, were examined using AgraQuant Total Aflatoxin Assay 1/20 (Romer Labs, Newark, DE, USA). FUMs, including FUMs B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>, were examined using AgraQuant Total Fumonisin Assay 0.25/5.0 (Romer Labs, Newark, DE, USA). AgraQuant Zearalenone Plus Assay 25/1000 and AgraQuant Deoxynivalenol Assay 0.25/5.0 were used for ZEN and DON examination, respectively. ELISA was performed in accordance with the manufacturer's instructions, and signals were measured using a microplate reader with absorbance of 450 nm filter and a differential filter of 630 nm (SH-1000Lab, Corona, Japan). The concentration of each mycotoxin was determined on the basis of the slope of the standard curve. The method's limits of detection for AFs, ZEN, FUMs, and DON were 1, 20, 200, and 200 ppb, respectively, and their ranges of quantification were 1–20, 25–1000, 250–5000, and 250–5000 ppb, respectively. On the basis of the feed and animal producers' choices, the number of tests for mycotoxins may have varied each year.

## Results

The results revealed that 91.4% of the feed samples were contaminated by DON between 2015 and 2017 (Table 1). The second most prevalent mycotoxin was ZEN with 70.2% of positive samples between 2015 and 2017, followed by AFs and FUMs with 58.0% and 50.4%, respectively (Table 1). The average concentrations of mycotoxins in all tested feed samples were 1.2, 51.9, 592.9, and 782.6 ppb for AFs, ZEN, FUMs, and DON, respectively (Table 1). The average and maximum concentrations of all mycotoxins in the tested feed samples exceeded the quantification limit in the survey results (Table 1). The most annually prevalent mycotoxin in the tested feed samples was DON (Table 1), which was also the most prevalent mycotoxin in cornmeal, with 88.8% in positive samples at an average of 854.6 ppb between 2015 and 2017 (Table 2). The prevalences of ZEN and FUMs in cornmeal were comparable, with 56.1% and 55.8% in positive samples at averages of 68.2 and 696.7 ppb, respectively (Table 2). AFs were present in 35.2% of the positive samples, with an average of 0.8 ppb in cornmeal between 2015 and 2017 (Table 2). The average and maximum concentrations of ZEN, FUMs, and DON in cornmeal exceeded the quantification limit, whereas those of AFs did not (Table 2). In the swine feed samples, DON was the most prevalent mycotoxin in the nursery and pregnancy diets between 2015 and 2017, with 91.1% and 90.5% in positive samples, respectively (Tables 3 and 4). The second most prevalent mycotoxin in the nursery and pregnancy diets was ZEN, with 75.0% and 73.3% in positive samples, with averages of 43.5 and 49.8 ppb, respectively (Tables 3 and 4). AFs were present in 59.6% and 68.0% of the positive samples, with averages of 1.1 and 1.3 ppb in the

**Table 1** Survey results of mycotoxins in feed and feed ingredients in Taiwan

	AF <sup>1</sup>	ZEN	FUM	DON
2015				
Number of tests <sup>2</sup>	223	224	224	224
% of positive samples <sup>3</sup>	56.1	65.6	61.2	85.7
Average concentration (ppb)	1.0	31.9	705.5	534.2
Average concentration in positive samples (ppb)	1.9	48.5	1188.0	623.0
Median concentration in positive samples (ppb)	1.6	39.0	1054.0	521.5
Maximum concentration in positive samples(ppb)	10.1	362.0	> 5000	4208.0
2016				
Number of tests	268	270	270	270
% of positive samples	77.6	85.2	51.1	92.6
Average concentration (ppb)	1.6	51.9	414.9	879.9
Average concentration in positive samples (ppb)	2.1	61.0	809.0	950.0
Median concentration in positive samples (ppb)	1.9	48.0	429.5	680.5
Maximum concentration in positive samples (ppb)	8.5	406.0	> 5000	> 5000
2017				
Number of tests	329	329	329	329
% of positive samples	43.5	61.1	57.8	94.2
Average concentration (ppb)	0.8	65.4	661.7	872.3
Average concentration in positive samples (ppb)	1.9	107.1	1146.0	923.0
Median concentration in positive samples (ppb)	1.48	60.5	958.5	712.0
Maximum concentration in positive samples (ppb)	> 20	> 1000	4824.0	> 5000
2015–2017				
Number of tests	820	823	823	823
% of positive samples	58.0	70.2	50.4	91.4
Average concentration (ppb)	1.2	51.9	592.9	782.6
Average concentration in positive samples (ppb)	2.0	73.8	1058.2	855.5
Median concentration in positive samples (ppb)	1.6	46.0	876.0	638.0
Maximum concentration in positive samples (ppb)	> 20	> 1000	> 5000	> 5000

<sup>1</sup> *AFs*, aflatoxins; *ZEN*, zearalenone; *FUMs*, fumonisins, *DON*, deoxynivalenol

<sup>2</sup> The tests were conducted on cornmeal and corn-meal-based swine feed (nursery and pregnancy diets)

<sup>3</sup> Positive results were determined when the analyzed values were greater than the detection limit of the method. The detection limits for aflatoxins, zearalenone, fumonisins, and deoxynivalenol were 1, 20, 200, and 200 ppb, respectively

nursery and pregnancy diets, respectively (Tables 3 and 4). FUMs were the least prevalent mycotoxin in the nursery and pregnancy diets, with 55.4% and 55.8% in positive samples and averages of 523.1 and 638.5 ppb, respectively (Tables 3 and 4). The average and maximum concentrations of all analyzed mycotoxins in the nursery and pregnancy diets exceeded the quantification limit in the survey results (Tables 3 and 4). The yearly trend analysis revealed that the percentage of samples containing DON in cornmeal and corn-meal-based swine feed was stably increased from 2015 to 2017 (Fig. 1a–d). A peak in the percentage of positive AF and ZEN samples were observed in 2016 (Fig. 1a–d). The lowest percentage of positive FUM samples were found in the nursery and pregnancy diets in 2016 (Fig. 1c, d). However, the percentage of samples containing FUM in cornmeal was slightly increased from 2015 to 2017 (Fig. 1b). Furthermore, the co-occurrence of

mycotoxins and mycotoxin combinations in cornmeal and corn-meal-based swine feed between 2015 and 2017 was analyzed. The 3-year survey results revealed that 1.0% of the tested samples had mycotoxin levels below the detection limits, 7.7% of the tested samples were contaminated by one mycotoxin, and 91.3% of the tested samples were contaminated by multiple mycotoxins (Table 5). The main mixtures in cornmeal and corn-meal-based swine feed between 2015 and 2017 were AF + ZEN + FUM + DON, AF + ZEN + DON, ZEN + DON and, ZEN + FUM + DON, found in 23.54%, 16.16%, 13.87%, and 12.47%, respectively. Furthermore, 38.17% corresponded to ternary mixtures, 28.25% to binary mixtures, and 23.54% were quaternary mixtures (Table 6). Taken together, these results demonstrated that between 2015 and 2017 in Taiwan, DON was the most prevalent mycotoxin in all types of feed samples, followed by ZEN. In

**Table 2** Survey results of mycotoxins in cornmeal in Taiwan

	AF <sup>1</sup>	ZEN	FUM	DON
2015				
Number of tests	34	34	33	34
% of positive samples <sup>2</sup>	38.2	64.7	45.5	79.4
Average concentration (ppb)	0.7	40.0	816.4	625.6
Average concentration in positive samples (ppb)	1.8	56.9	1851	788.0
Median concentration in positive samples (ppb)	1.42	51.5	1781	712.0
Maximum concentration in positive samples (ppb)	4.34	116.0	>5000	2421.0
2016				
Number of tests	57	59	59	58
% of positive samples	61.4	72.9	59.3	87.9
Average concentration (ppb)	1.2	48.9	564.9	785.4
Average concentration in positive samples (ppb)	2.0	67.0	952.0	893.0
Median concentration in positive samples (ppb)	1.56	51.0	419.0	658.0
Maximum concentration in positive samples (ppb)	8.53	330.0	> 5000	2690.0
2017				
Number of tests	105	105	105	105
% of positive samples	20.0	49.5	57.1	92.4
Average concentration (ppb)	0.7	88.2	732.0	967.9
Average concentration in positive samples (ppb)	3.4	178.0	1281.0	1038.0
Median concentration in positive samples (ppb)	1.53	89.0	980.0	663.0
Maximum concentration in positive samples (ppb)	> 20	> 1000	4824.0	> 5000
2015–2017				
Number of tests	196	198	197	197
% of positive samples	35.2	56.1	55.8	88.8
Average concentration (ppb)	0.8	68.2	696.7	854.6
Average concentration in positive samples (ppb)	2.4	114.5	1133.7	928.7
Median concentration in positive samples (ppb)	1.55	56	807.5	663.0
Maximum concentration in positive samples (ppb)	> 20	> 1000	> 5000	> 5000

<sup>1</sup> *AFs*, aflatoxins; *ZEN*, zearalenone; *FUMs*, fumonisins; *DON*, deoxynivalenol

<sup>2</sup> Positive results were determined when the analyzed values were greater than the detection limit of the method. The detection limits for aflatoxins, zearalenone, fumonisins, and deoxynivalenol were 1, 20, 200, and 200 ppb, respectively

addition, cornmeal and corn-meal-based swine feed were commonly contaminated by multiple mycotoxins and AF + ZEN + FUM + DON was the most observed mixture.

## Discussion

In the present survey, we found that AFs, ZEN, FUMs, and DON were respectively present in 58.0%, 72.2%, 50.4%, and 91.4% of the analyzed samples between 2015 and 2017 in Taiwan. DON was the most prevalent mycotoxin in all types of feed samples, including cornmeal and the pregnancy and nursery diets. Furthermore, only 1% of the tested samples were below the detection limits, 7.7% of the feed samples tested positive for the presence of one mycotoxin, and 91.3% were contaminated by multiple mycotoxins.

Globally, mycotoxin contamination in feed has an impact on animal health and the economy (Wu 2007; Bryden 2012). A worldwide survey of mycotoxin prevalence revealed that 25%, 27%, 36%, 54%, and 55% of feed samples tested positive for contamination with OTA, AFs, ZEN, FUMs, and DON from 2004 to 2011 (Streit et al. 2013a). In North Asia and Taiwan, the most prevalent mycotoxin in feed samples was DON, with 70.0% in positive samples, followed by ZEN, FUMs, OTA, and AFs, with 57.0%, 43.0%, 20.0%, and 12.0%, respectively (Rodrigues and Naehrer 2012a). Similarly, the present survey found that DON was highly prevalent in feed and feed ingredients, with 91.4% in positive samples in Taiwan between 2015 and 2017; DON was followed in terms of prevalence by ZEN, AFs, and FUMs. Furthermore, cornmeal and finished feed were commonly contaminated by DON in Taiwan. These results were consistent with those of Rodrigues and Naehrer (2012a and b).

**Table 3** Survey results of mycotoxins in the nursery diet in Taiwan

	AF <sup>1</sup>	ZEN	FUM	DON
2015				
Number of tests	93	93	91	93
% of positive samples <sup>2</sup>	55.9	69.9	64.8	86.0
Average concentration (ppb)	1.0	30.0	615.5	455.0
Average concentration in positive samples (ppb)	1.7	43.0	970.0	529.0
Median concentration in positive samples (ppb)	1.6	36.6	918.0	497.5
Maximum concentration in positive samples (ppb)	5.9	111.0	> 5000	1370.0
2016				
Number of tests	76	76	76	76
% of positive samples	80.3	93.4	50.0	92.1
Average concentration (ppb)	1.6	52.8	340.0	634.0
Average concentration in positive samples (ppb)	2.1	57.0	679.0	688.0
Median concentration in positive samples (ppb)	1.9	45.0	495.0	596.0
Maximum concentration in positive samples (ppb)	4.8	326.0	1785.0	2652.0
2017				
Number of tests	91	91	91	91
% of positive samples	46.2	68.1	56.0	94.5
Average concentration (ppb)	0.8	49.6	582.0	774.9
Average concentration in positive samples (ppb)	1.6	73.0	1039.0	820.0
Median concentration in positive samples (ppb)	1.5	62.5	986.0	719.0
Maximum concentration in positive samples (ppb)	3.6	210.0	2705.0	2595.0
2015–2017				
Number of tests	260	260	260	260
% of positive samples	59.6	75.0	55.4	91.1
Average concentration (ppb)	1.1	43.5	523.1	619.3
Average concentration in positive samples (ppb)	1.8	57.1	919.0	682.3
Median concentration in positive samples (ppb)	1.6	44.5	818.5	589.5
Maximum concentration in positive samples (ppb)	5.9	326.0	>5000	2652.0

<sup>1</sup> *AFs*, aflatoxins; *ZEN*, zearalenone; *FUMs*, fumonisins; *DON*, deoxynivalenol

<sup>2</sup> Positive results were determined when the analyzed values were greater than the detection limit of the method. The detection limits for aflatoxins, zearalenone, fumonisins, and deoxynivalenol were 1, 20, 200, and 200 ppb, respectively

However, the demands on feed and animal producers for OTA analysis are low in Taiwan compared with those for analysis of other mycotoxins. The prevalence of OTA in cornmeal and corn-meal-based swine feed was not reported in the current survey. Therefore, the observed annual ranking of mycotoxin prevalence in feed samples may differ from those reported elsewhere. In summary, feed and feed ingredients are commonly contaminated by DON worldwide. The feed samples collected in Taiwan had the highest incidence of DON contamination compared with other locations.

Several mycotoxins are found simultaneously in feed and feed ingredients and exert adverse effects on animals because of combined mycotoxicosis (Speijers and Speijers 2004; Pierron et al. 2016). A global survey conducted between 2005 and 2011 reported that mycotoxins were undetectable in 16% of feed samples, whereas 26% of the samples were contaminated by one mycotoxin and 58% contained multiple mycotoxins

(Streit et al. 2013a). Between 2009 and 2011, 6% of a selection of feed samples in Asia tested negative for mycotoxins, whereas 12% were contaminated by one mycotoxin and 82% tested positive for multiple mycotoxins (Rodrigues and Naehr 2012b). In the present study, we ascertained that between 2015 and 2017 in Taiwan, 1.0% of the collected feed samples had mycotoxin levels below the detection limits, 7.7% were contaminated by one mycotoxin, and 91.3% were contaminated by multiple mycotoxins. Weather may be a critical factor contributing to the high incidence of mycotoxin co-occurrence in feed in Taiwan. Because the climate in Taiwan is relatively hot in summer and humid in winter compared with other Asian countries, mycotoxin-producing molds can thrive on feed and feed ingredients because of abundant moisture in the environment. Furthermore, abnormal weather conditions such as drought and excessive rain markedly increase mycotoxin contamination levels (Magan et al. 2011). These phenomena



**Table 4** Survey results for mycotoxins in the pregnancy diet in Taiwan

	AF <sup>1</sup>	ZEN	FUM	DON
2015				
Number of tests	93	94	93	94
% of positive samples <sup>2</sup>	61.3	67.0	62.4	87.2
Average concentration (ppb)	1.3	29.4	774.6	500.2
Average concentration in positive samples (ppb)	2.1	44.0	1255.0	573.0
Median concentration in positive samples (ppb)	1.6	38.0	1270.5	499.0
Maximum concentration in positive samples (ppb)	10.1	147.0	3171.0	1349.0
2016				
Number of tests	108	108	108	108
% of positive samples	83.3	86.1	47.2	93.5
Average concentration (ppb)	1.8	54.2	447.3	726.4
Average concentration in positive samples (ppb)	2.2	63.0	947.0	777.0
Median concentration in positive samples (ppb)	2.0	48.0	964.0	656.0
Maximum concentration in positive samples (ppb)	4.8	406.0	3752.0	2956.0
2017				
Number of tests	125	125	125	125
% of positive samples	60.0	68.0	60.8	95.2
Average concentration (ppb)	1.0	61.5	696.3	842.5
Average concentration in positive samples (ppb)	1.7	90.0	1145.0	885.0
Median concentration in positive samples (ppb)	1.5	55.0	994.5	709.0
Maximum concentration in positive samples (ppb)	9.5	742.0	3613.0	3591.0
2015–2017				
Number of tests	326	327	326	327
% of positive samples	68.0	73.3	55.8	90.5
Average concentration (ppb)	1.3	49.8	638.5	705.8
Average concentration in positive samples (ppb)	2.0	67.6	1125.2	764.2
Median concentration in positive samples (ppb)	1.6	46.0	1100.0	615.5
Maximum concentration in positive samples (ppb)	10.1	742.0	3613.0	3591.0

<sup>1</sup> *AFs*, aflatoxins; *ZEN*, zearalenone; *FUMs*, fumonisins; *DON*, deoxynivalenol

<sup>2</sup> Positive results were determined when the analyzed values were greater than the detection limit of the method. The detection limits for aflatoxins, zearalenone, fumonisins, and deoxynivalenol were 1, 20, 200, and 200 ppb, respectively

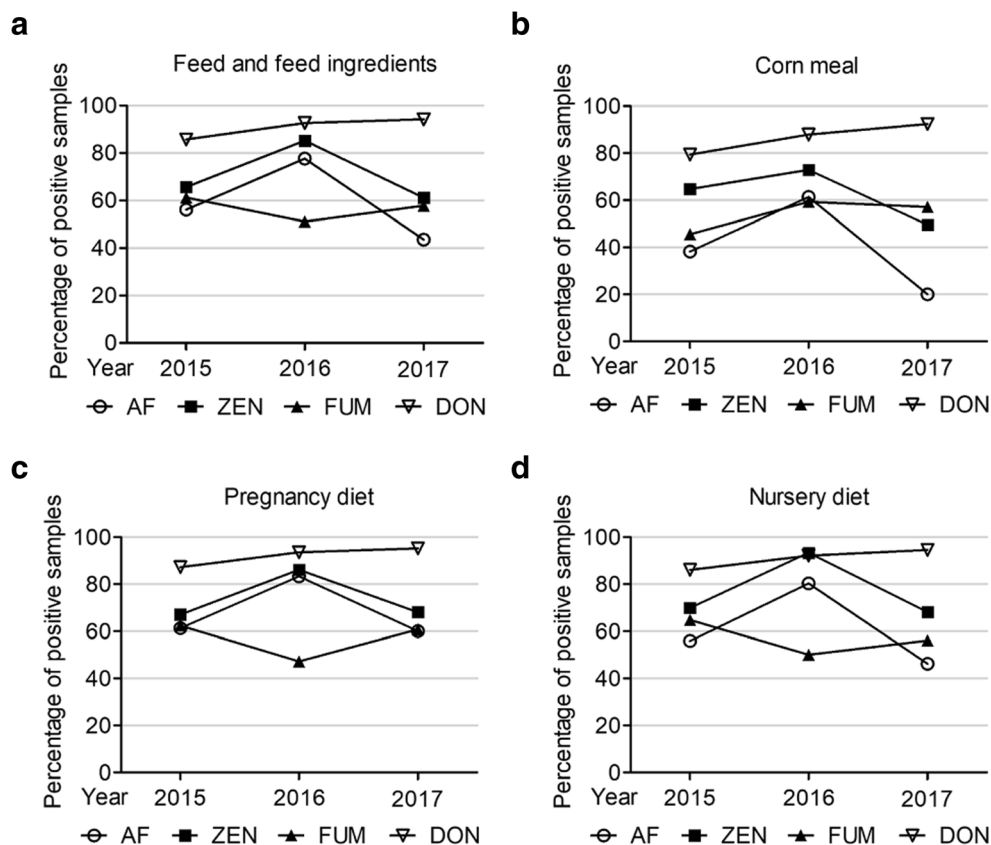
seemingly increase the prevalence of multimycotoxin contamination in Asia. However, safe tolerance levels in animals may differ according to interactions between mycotoxins. Thus, the additive and synergistic toxic effects caused by multimycotoxin contamination in feed on animals must be considered in future studies.

In Taiwan, the percentage of addition of mycotoxin binders in swine feed is 70% in nursery diet and 95% in pregnancy diet, respectively. It is reported that the adsorption efficacy of binders or adsorbents is limited to only a few mycotoxins. Further, based on the current survey of mycotoxin prevalence, an alternative strategy for efficient detoxification of mycotoxins is required in Taiwan. Mycotoxin binders in combination with enzymatic or microbial detoxification might be a feasible approach. In addition, boosting the immune system by natural immunomodulating agent is also an alternative strategy for reduction of adverse effects caused by mycotoxins

on animals. Further, the permissible limit of AFs in swine feed is 50 ppb in Taiwan. However, no maximum admitted levels for other mycotoxins are established in Taiwan. In the present study, the AF concentrations in feed and feed ingredients comply with the maximum admitted levels in Taiwan. However, it is still needed to establish the permissible limit of other mycotoxins, such as ZEN, FUM, and DON, in feed and feed ingredients in the future.

Several mycotoxins and their metabolites were identified in fungus and plants by using chromatographic and immunochemical methods (Berthiller et al. 2013). However, only OTA, AFs, ZEN, FUMs, and DON have been broadly examined in animal feed and feed ingredients (Streit et al. 2013a; Murugesan et al. 2015). Some modified mycotoxins—so-called masked mycotoxins—are metabolites that normally remain inactive and undetectable during analysis for parent mycotoxins (Freire and Sant’Ana 2018). These modified

**Fig. 1** Percentage of samples testing positive for aflatoxins (AF), zearalenone (ZEN), fumonisins (FUM), or deoxynivalenol (DON) each year



mycotoxins can transform into active parent mycotoxins in the gastrointestinal tracts of animals (Freire and Sant'Ana 2018). The global prevalence of mycotoxins in feed and feed ingredients has been surveyed annually by various research groups (Cheng et al. 2002; Streit et al. 2013a; Pinotti et al. 2016; Abudabos et al. 2017); however, the prevalence of masked mycotoxins in feed and feed ingredients remains unclear. In addition, the toxicities of individual and combined masked mycotoxins in animals remain to be investigated in future studies.

Currently, immunochemical and chromatographic methods are widely used for the detection of mycotoxins in feed (Murugesan et al. 2015). The immunochemical method, such as ELISA, is defined as routine screening which could be performed with commercially available test kits. The advantages of ELISA method are minimal sample preparation and

simple measurement procedure. However, the limitations of ELISA method are cross-reactivity to similar compounds and low sensitivity compared to chromatographic methods. The chromatographic methods, such as high-performance liquid chromatography (HPLC) and liquid chromatography coupled to mass spectrometry (LC-MS), are able to detect multiple mycotoxins simultaneously in a sample due to high specificity and sensitivity (Rodrigues and Naehrer 2012a; Streit et al. 2013b). In the future, the use of the latest analytical methods, such as LC-MS, will increase the precision on the determination of the concentrations of multiple mycotoxins present in swine feed of Taiwan.

In conclusion, 3289 mycotoxin analyses were performed on 820 samples between 2015 and 2017 in Taiwan. Among the tested samples, DON was the most prevalent mycotoxin in cornmeal and corn-meal-based swine feed. In addition, a high

**Table 5** Co-occurrence of mycotoxins in feed and feed ingredients in Taiwan

	Non-detected <sup>1</sup>	Single mycotoxin	More than one mycotoxin
2015 (%) <sup>2</sup>	2.2	8.5	89.3
2016 (%)	0	3.3	96.7
2017 (%)	0.9	10.6	88.5
2015–2017 (%)	1.0	7.7	91.3

<sup>1</sup> The analyzed values were below the detection limits of the method. The detection limits for aflatoxins, zearalenone, fumonisins, and deoxynivalenol were 1, 20, 200, and 200 ppb, respectively

<sup>2</sup> The tests were conducted on cornmeal and corn-meal-based swine feed (nursery and pregnancy diets)

**Table 6** Prevalence of mycotoxin combinations in feed and feed ingredients in Taiwan

	Non- detected <sup>1</sup>	AF	ZEN	FUM	DON	AF + ZEN	AF + FUM	AF + DON	ZEN + FUM	ZEN + DON	FUM + DON	AF + ZEN + FUM + DON	AF + ZEN + FUM + DON + FUM	AF + ZEN + FUM + DON + DON	AF + ZEN + FUM + DON + FUM + DON	AF + ZEN + FUM + DON + FUM + DON + DON	
2015 (%) <sup>2</sup>	2.71	2.71	0.45	1.81	7.24	0.91	2.72	5.43	0.91	11.77	6.34	2.26	2.26	9.5	14.02	5.88	25.34
2016 (%)	0	0	0	1.23	2.47	1.23	0.82	4.11	0.82	9.88	1.65	4.12	4.12	31.28	7.41	4.53	30.45
2017 (%)	0.93	0.31	0.62	2.17	7.45	0.31	0.31	4.66	0	18.32	12.11	0.93	0.93	9.32	15.22	10.25	17.09
2015–2017 (%)	1.14	0.89	0.38	1.78	5.85	0.76	1.15	4.71	0.51	13.87	7.25	2.29	2.29	16.16	12.47	7.25	23.54
	Non-detected	Unary mixture						Binary mixture		Ternary mixture			Quaternary mixture				
2015–2017 (%)	1.14	8.9						28.25		38.17			23.54				

<sup>1</sup> The analyzed values were below the detection limits of the method. The detection limits for aflatoxins, zearalenone, fumonisins, and deoxynivalenol were 1, 20, 200, and 200 ppb, respectively

<sup>2</sup> The tests were conducted on cornmeal and corn-meal-based swine feed (nursery and pregnancy diets)

incidence of contamination by multiple mycotoxins was observed in the tested samples. Sustainable surveying and understanding of the prevalence of mycotoxins could provide valuable information for feed and animal producers and improve animal health by implementing mycotoxin management strategies in livestock industries.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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