Postprint: Distribution Patterns of Mycotoxin Contamination in Layer Poultry Compound Feed in Southwest China

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Abstract

This study aimed to investigate the distribution patterns of mycotoxin contamination in Southwest China. Ninety-five compound feed samples for laying poultry (laying hens, young laying hens, and laying ducks) from feed mills of different scales in Southwest China (Sichuan, Chongqing, Guizhou, Guangxi, Yunnan) were analyzed for mycotoxin content [aflatoxin B1 (AFB1), zearalenone (ZON), deoxynivalenol (DON), and fumonisin (FB)]. The detection methodology involved initial screening by enzyme-linked immunosorbent assay (ELISA), with samples exceeding 80% of the national maximum limits re-analyzed using high-performance liquid chromatography. The results demonstrated: 1) Detection rates for AFB1, DON, ZON, and FB were 83.16%, 93.68%, 94.74%, and 100%, respectively. The exceedance rates for AFB1 and ZON were 4.21% and 3.16%, respectively, while no exceedances were observed for DON or FB. The average contents of AFB1, DON, and ZON in laying poultry feed products differed extremely significantly among different provinces and municipalities ($P<0.01$). The highest AFB1 content was detected in Chongqing at 10.9 g/kg, while the highest DON content was found in Sichuan at 1.07 mg/kg. 2) No significant differences ($P>0.05$) were found in the contents of the four mycotoxins among different laying poultry feed varieties. 3) The AFB1 content in laying poultry feed samples from feed mills of different scales differed significantly ($P<0.05$), with the highest AFB1 content observed in feed from small-scale feed mills (annual production <50,000 t) at 6.36 g/kg. These findings indicate that contamination by AFB1, DON, ZON, and FB is widespread in compound feed for laying poultry in Southwest China, with variations in mycotoxin content existing among different regions and feed mills of different scales.

Full Text

Mycotoxin Distribution in Compound Feed for Layer Poultry in Southwestern China

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Abstract

This study investigated the contamination patterns of mycotoxins in layer feed across southwestern China. A total of 95 compound feed samples for layer poultry (laying hens, pullets, and laying ducks) were collected from feed mills of varying scales in Sichuan, Chongqing, Guizhou, Guangxi, and Yunnan provinces. The samples were analyzed for aflatoxin B1 (AFB1), zearalenone (ZON), deoxynivalenol (DON), and fumonisin (FB) using enzyme-linked immunosorbent assay (ELISA) for initial screening. Samples exceeding 80% of the national maximum limit were re-analyzed using high-performance liquid chromatography (HPLC). The results showed: (1) Detection rates for AFB1, DON, ZON, and FB were 83.16%, 93.68%, 94.74%, and 100%, respectively. The exceedance rates were 4.21% for AFB1 and 3.16% for ZON, while no samples exceeded limits for DON or FB. Average contents of AFB1, DON, and ZON differed significantly among provinces ($P<0.01$), with the highest AFB1 content in Chongqing (10.9 g/kg) and the highest DON content in Sichuan (1.07 mg/kg). (2) No significant differences in the four mycotoxins were observed among different layer feed types ($P>0.05$). (3) AFB1 content varied significantly by feed mill scale ($P<0.05$), with the highest level in small-scale mills (<50,000 t annual production) at 6.36 g/kg. These findings demonstrate widespread contamination of AFB1, DON, ZON, and FB in layer compound feed in southwestern China, with variations in mycotoxin levels across regions and feed mill scales.

Keywords: layer poultry; compound feed; mycotoxins; southwestern China; distribution patterns

Introduction

Mycotoxins are diverse compounds produced by toxigenic fungi during growth [1] and are characterized by their stability and resistance to degradation. Approximately 25% of global crops are contaminated by mycotoxins annually,

causing numerous human and livestock poisoning incidents and economic losses amounting to hundreds of billions of dollars [2]. Common mycotoxins in feed include aflatoxin B1 (AFB1), zearalenone (ZON), deoxynivalenol (DON), and fumonisin (FB). Recent surveys have reported detection rates of 100% for mycotoxins in feed ingredients and products in some Chinese regions, with 96% of feed and raw materials contaminated by two or more mycotoxins simultaneously [3]. However, no studies have reported on the distribution patterns of mycotoxins in different types of layer compound feed in southwestern China. This research aims to characterize mycotoxin distribution patterns in layer compound feed across different provinces and cities in southwestern China, across different layer feed types, and across different feed mill scales, providing a scientific basis for mycotoxin prevention and control strategies to ensure animal and product safety.

Materials and Methods

1.1 Sample Collection

From August to October 2014, a total of 95 compound feed samples were collected from feed mills of different scales in Sichuan, Chongqing, Yunnan, Guizhou, and Guangxi. The samples included feed for pullets, peak-laying hens, and peak-laying ducks. Feed mills were categorized as small-scale (<50,000 t annual production), medium-scale (50,000–100,000 t), or large-scale (>100,000 t). Sampling followed the protocol specified in GB/T 14699.1-2005 (Feed Sampling). Each sample weighed at least 1,000 g, was ground to pass through a 20-mesh sieve, and stored at -20°C until analysis.

1.2 Sample Analysis

All samples were analyzed for AFB1, ZON, and DON content, with FB measured in a randomly selected subset of 21 samples. Initial screening employed ELISA kits from ROMER for all toxins. Since ELISA cannot provide accurate quantification [4], samples with results exceeding 80% of the maximum limit were re-analyzed using HPLC (Agilent, USA) with immunoaffinity column cleanup.

1.3 Evaluation Criteria

Maximum limits and detection methods are detailed in Table 1 . Samples exceeding these limits were classified as non-compliant, while those below the detection limit were considered not detected.

1.4 Statistical Analysis

Data were analyzed using SPSS 17.0 GLM procedure for analysis of variance across all feeds, by feed type, province, and feed mill scale. Results are presented as mean ± standard deviation. Duncan' s multiple range test was used for

post-hoc comparisons. $P<0.05$ was considered significant and $P<0.01$ highly significant.

Results and Analysis

Mycotoxin detection results in layer feed are summarized in Table 2 . All four mycotoxins were detected, with AFB1 showing the lowest detection rate (83.16%), while DON, ZON, and FB exceeded 90%. AFB1 and ZON had the highest exceedance rates (3.16% each), whereas no samples exceeded limits for DON or FB. AFB1 exhibited the greatest variation range, followed by ZON.

2.1 Mycotoxin Contamination in Different Layer Feed Types

As shown in Table 3 , AFB1 detection rates were 77.05% in laying hen feed, 85.71% in pullet feed, and 100% in laying duck feed. The highest AFB1 exceedance rate occurred in duck feed (10%), with no exceedances in hen feed. Duck feed contained the highest AFB1 level (6.43 g/kg), while pullet feed had the lowest (3.17 g/kg), though differences among the three feed types were not significant ($P>0.05$). DON detection rates exceeded 90% across all feed types, reaching 100% in pullet feed, yet no samples exceeded limits. No significant differences in DON content were observed among feed types ($P>0.05$). ZON detection rates ranged from 92% to 95% across feed types, with exceedance rates of 5% in duck feed and 3.28% in hen feed, but none in pullet feed. ZON content did not differ significantly among feed types ($P>0.05$). FB detection reached 100% in hen and duck feeds (excluding pullet feed with only one sample), with no exceedances and no significant differences in content ($P>0.05$).

2.2 Mycotoxin Contamination in Different Provinces of Southwestern China

Table 4 shows that Chongqing had the highest AFB1 detection rate (100%) and exceedance rate (15.38%) in layer feed, while Yunnan, Guangxi, and Guizhou had higher detection rates than Sichuan but no exceedances. Sichuan showed 75% detection and 2.08% exceedance rates. Chongqing' s average AFB1 content was significantly higher than other regions ($P<0.01$). For DON, Sichuan and Guizhou achieved 100% detection without exceedances. Sichuan feed contained the highest DON level (1.07 mg/kg), significantly exceeding Guangxi ($P<0.01$), while other regions showed no significant differences ($P>0.05$).

As presented in Table 5 , Guizhou and Yunnan achieved 100% ZON detection, with other regions exceeding 80%. Yunnan had the highest exceedance rate (5.88%), followed by Sichuan (4.17%). Guizhou feed contained the highest ZON level (0.20 mg/kg), while Guangxi had the lowest, significantly lower than Sichuan, Guizhou, and Yunnan ($P<0.05$). For FB, detection rates reached 100% across all regions except Guizhou, with no exceedances. Sichuan had the highest FB content (1.11 mg/kg) and Guangxi the lowest (0.4 mg/kg), though regional differences were not significant ($P>0.05$).

2.3 Mycotoxin Contamination by Feed Mill Scale

Table 6 indicates that AFB1 detection rates exceeded 80% across all mill scales, with large-scale mills showing the lowest rate and no exceedances. Medium-scale mills had the highest detection rate but lower exceedance than small-scale mills. Small-scale mills showed the highest exceedance rate (7.14%). AFB1 content followed the trend: small-scale > medium-scale > large-scale, with significant differences between small- and large-scale mills ($P<0.05$). Duck feed contained the highest AFB1 levels across all scales, particularly in medium-scale mills.

As shown in Table 7 , DON detection exceeded 90% across all mill scales without exceedances, and content differences were not significant ($P>0.05$). Pullet feed contained the highest DON levels in both small- and large-scale mills.

Table 8 reveals that medium-scale mills achieved 100% ZON detection, compared to 89.29% and 95.65% in small- and large-scale mills, respectively. Medium-scale mills had the highest exceedance rate (4.76%) and large-scale the lowest (2.17%). ZON content did not differ significantly among mill scales ($P>0.05$). Duck feed from small-scale mills contained the highest ZON level (0.24 mg/kg).

Table 9 shows 100% FB detection across all mill scales without exceedances. Medium-scale mills had the highest FB content (1.03 mg/kg) and small-scale the lowest (0.6 mg/kg), though differences were not significant ($P>0.05$).

Discussion

3.1 Impact of Feed Type on Mycotoxin Content

AFB1 contamination varied among feed types, with detection rates of 77.05%, 85.71%, and 100% in laying hen, pullet, and duck feeds, respectively. Previous studies reported AFB1 detection rates exceeding 90% in compound feed [5-7]. The highest exceedance rate occurred in duck feed, likely due to stricter limits for ducks ($\$ 15\mu g/kg) compared to hens ($20\mu g/kg$), while pullets ($\$10$ g/kg) have the lowest tolerance, prompting more stringent raw material selection. Zhang et al. [5] reported a 7.78% exceedance rate for AFB1 in poultry feed, consistent with our findings. The average AFB1 content in southwestern China (4.09 g/kg) was lower than previous reports (9.49 g/kg [5] and 8.27 g/kg [7]), possibly due to enhanced regulatory oversight.

DON detection was high across all feed types without exceedances, and contents did not differ significantly. The average DON content in this study was substantially lower than Wang et al.' s [8] 2013 survey, possibly because their samples were collected following the 2012 wheat scab outbreak.

Li et al. [9] reported 100% ZON detection in wheat and corn in 2004, while Cheng et al. [10] found varying detection rates across feed types in 2014, consistent with our results showing >90% detection in layer feeds with exceedances in duck and hen feeds. ZON contents (0.15–0.18 mg/kg) were lower than Wang et al.' s [11]

2013 findings but similar to Huang et al.' s [12] statistics for complete feeds (0.14–0.22 mg/kg). Jiang et al. [13] reported higher ZON levels (0.34 mg/kg) in Guangxi poultry feed using ELISA, likely due to method-related false positives.

FB detection reached 100% in hen and duck feeds without exceedances, with an average content of 0.75 mg/kg. This differs from Wang' s [14] 2012 global survey showing 64% detection and 0.86 mg/kg average, possibly due to different feed sources and types.

3.2 Impact of Province on Mycotoxin Content in Layer Feed

Climate variations among Sichuan, Chongqing, Guizhou, Yunnan, and Guangxi influence mycotoxin levels. Liu et al. [15] reported regional differences in aflatoxin-producing *Aspergillus* strains across 17 Chinese provinces, and Zhang et al. [5] documented geographical variation in AFB1 across 11 provinces, consistent with our findings. Chongqing showed the highest AFB1 detection, exceedance, and content, likely due to hot summers, rainy autumns, and high humidity favoring *Aspergillus flavus* growth and toxin production [16].

Zhen et al. [17] reported higher DON levels in northern China, while Cheng et al. [18] found regional variation in wheat feed ingredients. Our study showed significantly higher DON in Sichuan than Guangxi. As a field toxin, DON production requires specific temperature (20°C), humidity (~85%), and grain moisture (22%) [19]. Sichuan' s climate during crop growth and harvest appears most favorable for DON production.

Guangxi showed the lowest ZON detection and content, likely due to dry autumn conditions unfavorable for *Fusarium* growth, while Sichuan, Chongqing, Yunnan, and Guizhou receive abundant rainfall year-round, resulting in more severe ZON contamination [20]. Cheng et al. [10] reported ~10% exceedance rates nationwide except in northeast China. Southwestern layer feed showed only 3.16% ZON exceedance despite high detection rates, suggesting feed mills screen raw materials to ensure product quality.

FB is widespread in corn, wheat, and sorghum globally, with levels influenced by climate and storage [14]. He et al. [21] reported higher FB1 contamination in Sichuan corn, consistent with our finding of elevated FB in Sichuan layer feed, likely due to the region' s humid, temperate climate favoring FB production. Ogunlade et al. [22] demonstrated that FB1 levels below 15.2 mg/kg do not affect layer performance or egg quality. With an average FB content of 0.75 mg/kg, southwestern layer feed appears safe regarding FB contamination.

3.3 Analysis of Mycotoxin Differences Among Feed Mill Scales

Except for DON and FB, small- and medium-scale mills showed more severe AFB1 and ZON contamination than large-scale mills. This likely reflects greater financial resources in large-scale mills enabling stricter raw material selection

and monitoring, while small-scale mills face funding constraints and less rigorous quality control.

Conclusions

1. AFB1, ZON, DON, and FB were detected in southwestern Chinese layer compound feed at rates of 83.16%, 94.74%, 93.68%, and 100%, respectively. Exceedance rates were 3.16% for both AFB1 and ZON, with no exceedances for DON or FB. Average contents were 4.09 g/kg for AFB1, 0.17 mg/kg for ZON, 0.86 mg/kg for DON, and 0.75 mg/kg for FB.
2. Mycotoxin levels varied by feed type, region, and mill scale. Duck feed contained the highest AFB1, while pullet feed had the highest DON content. Chongqing feed showed the highest AFB1, Sichuan the highest DON, and Guizhou the highest ZON. Small-scale mills produced feed with the highest AFB1, medium-scale mills the highest FB, while DON and ZON contents were similar across mill scales.

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Note: Figure translations are in progress. See original paper for figures.