Postprint: Distribution Patterns of Mycotoxin Contamination in Meat-Type Poultry Compound Feed in Southwestern China

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Abstract

This study aims to understand the contamination distribution patterns of mycotoxins in meat poultry compound feed in southwestern China. A total of 100 meat poultry compound feed samples (early-stage meat duckling, early-stage broiler, and late-stage broiler) were randomly collected from feed mills of different scales in southwestern China (Sichuan, Chongqing, Guizhou, Guangxi, and Yunnan). The contents of aflatoxin B1 (AFB1), zearalenone (ZON), deoxynivalenol (DON), and fumonisin (FB) in the samples were determined using enzyme-linked immunosorbent assay (ELISA) kits for preliminary screening and high-performance liquid chromatography for confirmation. The results showed that the detection rates of AFB1, DON, ZON, and FB in meat poultry compound feed were 89%, 96%, 85%, and 94.74%, respectively; the non-compliance rates of AFB1 and ZON were 18% and 5%, respectively, while no samples exceeded the standard limits for DON and FB. The mean concentrations of AFB1, DON, and ZON in meat poultry compound feed differed among regions; specifically, Chongqing had the highest AFB1 content (11.76 g/kg), Sichuan had the highest DON content (1.23 mg/kg), and Yunnan had the highest ZON content (0.26 mg/kg). Among different types of meat poultry compound feed, the AFB1 content in descending order was early-stage meat duckling, late-stage broiler, and early-stage broiler feed; the highest DON and FB contents were found in early-stage broiler feed, while the highest ZON content was in late-stage broiler feed; there was no significant difference in the contents of the four mycotoxins among different types of meat poultry compound feed ($P>0.05$). In terms of feed mill scale, the contamination level of products from large-scale feed mills (annual output >100,000 tons) was lower than that of small-scale feed mills (annual output of 20,000-50,000 tons), but there was no significant difference in the contents of the four mycotoxins in meat poultry compound feed among different scales of feed mills ($P>0.05$). Thus, meat poultry compound

feed in southwestern China is widely contaminated by AFB1, DON, ZON, and FB, with AFB1 having a relatively high non-compliance rate, and the contents of the four mycotoxins vary among feed products from different regions, species, and feed mill scales.

Full Text

Mycotoxin Distribution of Meat-type Poultry Feeds in Southwest China

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Abstract

This study investigated the contamination and distribution patterns of mycotoxins in meat-type poultry feeds across Southwest China. A total of 100 compound feed samples for meat-type poultry (including duckling starter, broiler starter, and broiler grower feeds) were randomly collected from feed mills of varying scales in Southwest China (Sichuan, Chongqing, Guizhou, Guangxi, and Yunnan). The contents of aflatoxin B1 (AFB1), zearalenone (ZON), deoxynivalenol (DON), and fumonisin (FB) were determined using enzyme-linked immunosorbent assay (ELISA) kits for initial screening, followed by confirmatory analysis using high-performance liquid chromatography (HPLC). The results revealed detection rates of 89%, 96%, 85%, and 94.74% for AFB1, DON, ZON, and FB, respectively. The over-limit ratios were 18% for AFB1 and 5% for ZON, while no samples exceeded the limits for DON or FB. Significant regional variations were observed in the average contents of these mycotoxins: Chongqing exhibited the highest AFB1 level (11.76 g/kg), Sichuan showed the highest DON content (1.23 mg/kg), and Yunnan had the highest ZON concentration (0.26 mg/kg). Among different feed types, AFB1 content was highest in duckling starter feed, followed by broiler grower and broiler starter feeds. DON and FB contents were highest in broiler starter feed, while ZON was most concentrated in broiler grower feed. However, no statistically significant differences were found among feed types ($P>0.05$). Although large-scale feed mills (annual output >100,000 tons) demonstrated lower contamination levels compared to small-scale mills (20,000–50,000 tons annually), no significant differences in mycotoxin contents were observed across different mill scales ($P>0.05$). These findings indicate widespread contamination of meat-type poultry feeds in Southwest China with AFB1, DON, ZON, and FB, with AFB1 showing the highest over-limit ratio. The contamination levels vary by region, feed type, and feed mill scale.

Keywords: meat-type poultry; compound feed; mycotoxins; Southwest China; distribution patterns

Introduction

Mycotoxins are toxic secondary metabolites produced by molds in feed and grain. The most prevalent and hazardous mycotoxins in food and feed include aflatoxin B1 (AFB1), deoxynivalenol (DON), zearalenone (ZON), and fumonisin (FB). These compounds exhibit broad toxicological effects on humans and animals. Acute mycotoxicosis severely compromises health and can be fatal, subacute exposure impairs growth and immune function, while chronic exposure may induce tumorigenesis. Beyond impairing livestock performance, mycotoxins from moldy feed can accumulate in various tissues and organs, posing risks to food safety of animal origin.

The Food and Agriculture Organization (FAO) estimates that 25% of global grain production is contaminated by mycotoxins annually. Ao Zhigang et al. reported that over 90% of feed ingredients in China test positive for mycotoxins, with more than 60% exceeding permissible limits. However, no previous studies have documented the distribution patterns of mycotoxins in meat-type poultry compound feeds specifically in Southwest China. Therefore, this research aimed to characterize mycotoxin distribution patterns across different provinces and municipalities in Southwest China, among various poultry feed types, and in relation to feed mill production scales. These findings provide scientific evidence to support mycotoxin prevention and control efforts, thereby safeguarding animal health and product safety.

1.1 Sample Collection

A total of 100 meat-type poultry compound feed samples (including duckling starter, broiler starter, and broiler grower feeds) were collected from feed mills of different production scales (20,000–50,000 tons, 50,000–100,000 tons, and >100,000 tons annually) across Southwest China (Sichuan, Chongqing, Yunnan, Guizhou, and Guangxi). Sampling was conducted from August to October 2014 following the procedures outlined in "Feed Sampling" (GB/T 14699.1-2005). Disposable gloves were worn during sampling, with multiple sampling points used for each batch. Gloves were changed between samples, and sampling tools were cleaned regularly. Each sample weighed at least 1,000 g, was ground to pass through a 20-mesh sieve, and stored at -20°C in sealed containers until analysis.

1.2 Sample Analysis

All samples were initially screened using ROMER ELISA kits and a microplate reader (DNM 9602). AFB1, ZON, and DON were analyzed in all samples, while FB was measured in 19 selected samples. High-performance liquid chromatography (HPLC) offers high sensitivity, excellent recovery, and good reproducibility.

Therefore, all samples with initial screening results exceeding 80% of the limit values were re-analyzed using HPLC (Agilent 1100, USA) with immunoaffinity column cleanup.

1.3 Data Statistics and Processing

Data were analyzed using the General Linear Model (GLM) procedure in SPSS 17.0. Results are expressed as "mean ± standard deviation." Duncan' s multiple range test was used for post-hoc comparisons. Differences were considered significant at $P<0.05$ and highly significant at $P<0.01$.

1.4 Limit Standards and Detection Methods

The limit standards and detection methods are detailed in Table 1.

Results and Analysis

Overall, the detection rates of AFB1 and ZON in meat-type poultry feeds exceeded 80%, while DON and FB detection rates were above 90%. AFB1 showed the highest over-limit ratio at 18%, with the other three toxins below 10%. The average values for positive samples were 28.73 g/kg for AFB1 and 0.61 mg/kg for ZON.

2.1 Mycotoxin Detection in Different Meat-type Poultry Feeds

As shown in Table 2, AFB1 detection rates in the three feed types were approximately 88%. The highest AFB1 over-limit ratio occurred in broiler grower feed (26.92%), compared to 18.42% in duckling starter feed and 11.11% in broiler starter feed. Duckling starter feed contained the highest average AFB1 concentration (8.14 g/kg), while broiler starter feed had the lowest (3.86 g/kg), though differences among feed types were not significant ($P>0.05$).

Broiler starter feed exhibited the highest DON detection rate at 100.00%, with duckling starter and broiler grower feeds both exceeding 90%. No DON samples exceeded regulatory limits. The average DON content was highest in broiler starter feed (0.87 mg/kg), with duckling starter (0.65 mg/kg) and broiler grower (0.66 mg/kg) feeds showing similar levels. No significant differences were observed among the three feed types ($P>0.05$).

ZON detection was highest in broiler grower feed (92.31%), with over-limit ratios ranging from 2.63% to 7.69% across feed types. Duckling starter feed had the lowest average ZON content (0.13 mg/kg), while broiler grower and starter feeds averaged 0.19 mg/kg and 0.18 mg/kg, respectively. No significant differences in ZON content were found among feed types ($P>0.05$).

FB detection was lowest in duckling starter feed (83.33%), with other feed types at 100.00%. No samples exceeded FB limits. No significant differences in FB content were observed among feed types ($P>0.05$), though broiler starter feed

showed higher average ZON content (0.70 mg/kg) than broiler grower feed (0.39 mg/kg).

2.2 Mycotoxin Contamination in Different Regions

As presented in Table 3, AFB1 detection rate in Southwest China was 89%, with an 18% over-limit ratio and average content of 6.5 g/kg. Yunnan showed the lowest detection rate (76.92%), while other provinces exceeded 90%, with Guangxi reaching 100.00%. Over-limit ratios ranked as: Chongqing (40%) > Yunnan (15.38%) > Sichuan (14.29%) > Guangxi (5.88%); Guizhou had no over-limit samples. Chongqing' s average AFB1 content (11.76 g/kg) was significantly higher than Guizhou's (1.11 g/kg) ($P<0.05$), with no other significant differences among provinces.

DON detection rate reached 96% regionally, with no over-limit samples and an average content of 0.73 mg/kg. Sichuan, Guizhou, and Yunnan achieved 100.00% detection rates. Sichuan exhibited the highest average DON content (1.23 mg/kg), which was highly significantly greater than other regions ($P<0.01$), while no significant differences were found among the remaining regions ($P>0.05$).

As shown in Table 4, ZON detection rate was 85% regionally, with a 5% over-limit ratio and average content of 0.16 mg/kg. Guangxi had the lowest detection rate (58.82%), while Guizhou and Yunnan reached 100.00%. Over-limit ratios were 9.09% in Guizhou, 9.52% in Sichuan, and 7.69% in Yunnan; Chongqing and Guangxi had no over-limit samples. Average ZON contents in Guizhou, Sichuan, and Yunnan were highly significantly higher than in Chongqing and Guangxi ($P<0.01$), with no significant differences within these groupings ($P>0.05$).

FB analysis included partial samples from Sichuan, Chongqing, Guangxi, and Yunnan, showing a 94.74% detection rate, 0% over-limit ratio, and average content of 0.61 mg/kg. Yunnan had the lowest FB detection rate (83.33%), while Sichuan, Chongqing, and Guangxi reached 100.00%. No significant differences in average FB content were observed among provinces ($P>0.05$).

2.3 Mycotoxin Contamination in Different Scale Feed Mills

As indicated in Table 5, AFB1 detection rates exceeded 80% across all mill scales. Small-scale mills showed higher AFB1 over-limit ratios and average contents compared to medium- and large-scale mills, though differences were not significant ($P>0.05$). Notably, duckling starter feed from small-scale mills contained 13.08 g/kg AFB1 on average, exceeding the limit value (10 g/kg).

DON detection rates exceeded 90% across all mill scales, with large-scale mills showing the lowest detection rate (91.11%) and average content (0.6 mg/kg). Small- and medium-scale mills had similar average DON contents, with no significant differences among scales ($P>0.05$). The highest average DON contents were observed in broiler starter feed from small- and medium-scale mills.

As shown in Table 6, medium-scale mills exhibited the highest ZON detection and over-limit rates. Average ZON contents were similar between small- and medium-scale mills, while large-scale mills showed the lowest content (0.13 mg/kg). No significant differences were found among the three scales ($P>0.05$). The highest average ZON content occurred in broiler starter feed from medium-scale mills (0.24 mg/kg), while broiler grower feed from large-scale mills averaged 0.22 mg/kg.

Discussion

This survey analyzed 100 meat-type poultry feed samples from Southwest China, revealing widespread contamination by four mycotoxins. DON and FB detection rates exceeded 90%, while AFB1 and ZON rates approached 90%. AFB1 showed the highest over-limit ratio (18%), followed by ZON (5%), with no DON or FB samples exceeding limits. Mycotoxin contents varied with feed type, region, and mill scale.

All three poultry feed types were extensively contaminated with AFB1. Broiler grower feed exhibited a significantly higher AFB1 over-limit ratio compared to duckling starter and broiler starter feeds, suggesting feed type influences AFB1 contamination levels. Although duckling starter and broiler grower feeds showed similar average AFB1 contents, duckling starter feed represents a more serious contamination concern due to lower AFB1 tolerance in ducklings. Zhang Ziqiang et al. reported national poultry feed AFB1 detection rates of 100.00% with average content of 9.49 g/kg in 2009, higher than our findings, likely reflecting improved AFB1 control as enterprises have become more aware of its hazards. Cheng Chuanmin et al. found a 3.61% AFB1 over-limit ratio in corn during the second half of 2013, lower than our 18% result, possibly because compound feeds contain multiple ingredients and are more susceptible to contamination.

Chinese dietary habits include consumption of animal organs, which are primary sites for AFB1 accumulation in broilers. Research indicates that when broilers were fed diets containing 24.71 g/kg AFB1, liver residues reached 20.52%. Epidemiological and experimental studies suggest that human AFB1 intake should not exceed 3 ng/kg body weight daily to avoid liver cancer risk. Based on our average feed AFB1 contents, consumption of 50–100 g liver daily by a 50 kg person would result in intakes of 1.67–3.34 ng/kg BW (duckling starter feed), 1.58–3.17 ng/kg BW (broiler grower feed), and 0.79–1.58 ng/kg BW (broiler starter feed). While broiler starter feed falls within the safe range, duckling starter and broiler grower feeds exceed safety thresholds. Moreover, maximum AFB1 contents in all three feed types exceeded 24.71 g/kg. Therefore, despite reduced detection rates and average contents, AFB1 contamination remains a serious concern requiring enhanced monitoring.

DON was ubiquitous in meat-type poultry feeds, with broiler starter feed showing the highest detection rate (100.00%). Although duckling starter and broiler grower feeds had slightly lower detection rates, none exceeded regulatory limits.

Zhen Yangguang et al. and Wang Jinyong et al. investigated DON contamination in Chinese poultry feeds in 2008 and 2012, respectively, reporting detection rates consistent with our study but higher average contents. This may reflect improved DON prevention as enterprises increased awareness and detection capabilities. The high DON levels reported in 2012 may also be attributed to widespread wheat scab outbreaks that year.

ZON detection was lowest in duckling starter feed (76.32%) and highest in broiler grower feed (92.31%), possibly because ducklings are more sensitive to ZON, prompting stricter raw material screening. Cheng Chuanmin et al. and Lei Yuanpei et al. reported significant variations in ZON detection rates among different feed ingredients, consistent with our findings. While poultry are less sensitive to ZON than swine, ZON can accumulate in tissues with a half-life of approximately six months. Our study indicates that although ZON contamination levels in poultry feeds are relatively low, contamination is widespread.

Chongqing exhibited the highest average AFB1 content (11.76 g/kg), while Guizhou showed the lowest (1.11 g/kg), likely due to Chongqing's hot summers, rainy autumns, and high humidity favoring *Aspergillus flavus* growth. Regional differences in AFB1 contamination between China and Southeast Asia have been reported, consistent with our findings. High AFB1 over-limit ratios were observed in Chongqing, Sichuan, Guangxi, and Yunnan, with a maximum value of 104.1 g/kg in Guangxi. Wang Jinyong et al. reported an average AFB1 content of 16 g/kg in poultry feeds in 2013, lower than our positive sample average of 28.73 g/kg, indicating more severe AFB1 contamination in Southwest China.

Chen Xinyi reported 100.00% DON detection in compound feeds (pig and poultry) from 18 provinces in 2011, consistent with our results. However, our average DON content (0.73 mg/kg) was slightly lower than Chen' s (0.88 mg/kg), possibly due to improved prevention measures and different feed types. Sichuan showed higher average DON content than other regions. As a field toxin, DON production requires optimal temperature, humidity, oxygen, and energy. Grain produces substantial DON when moisture content is 22%, relative humidity approximately 85%, and temperature 20°C. Sichuan's climate during crop planting, growth, and harvest is most favorable for DON production, explaining the severe contamination. Zhen Yangguang et al. reported regional variations in DON content across 11 provinces in 2009, consistent with our findings.

Guangxi showed the lowest ZON content, possibly because sampling occurred in autumn when dry conditions in Guangxi are unfavorable for mold growth. Wang Ruojun et al. reported ZON content of 0.084 mg/kg in national complete feeds in 2003, lower than our 0.16 mg/kg average, likely due to different feed types and sampling times. Du Ni' s 2014 survey of national feed and ingredients found similar ZON averages, indicating that ZON contamination in Southwest China is consistent with national levels.

FB detection rates were very high across regions, though no samples exceeded

limits and average contents were far below regulatory thresholds. Regional differences in average FB content were observed, indicating widespread but low-level contamination in Southwest China.

All mill scales showed extensive AFB1 contamination. Small- and medium-scale mills exhibited more severe AFB1, ZON, and DON contamination than large-scale mills. Duckling starter feed from small-scale mills exceeded AFB1 limits, likely because large-scale mills have greater raw material selection capacity, adequate funding, and better laboratory facilities, enabling more effective mycotoxin control.

Conclusion

1. This study found detection rates of 89%, 96%, 85%, and 94.74% for AFB1, DON, ZON, and FB, respectively, in meat-type poultry feeds from Southwest China. Over-limit ratios were 18% for AFB1 and 5% for ZON, with no DON or FB samples exceeding limits. Average contents were 6.5 g/kg for AFB1, 0.73 mg/kg for DON, 0.16 mg/kg for ZON, and 0.61 mg/kg for FB.

2. Mycotoxin contents varied with feed type, region, and mill scale. Duckling starter feed showed the highest average AFB1 content; broiler starter feed had the highest average DON and FB contents; broiler grower feed contained the highest average ZON content. Regionally, Chongqing had the highest average AFB1 content, Sichuan showed the highest DON and ZON contents, and Guangxi had the highest FB content. Small-scale mills exhibited the highest average AFB1 content, while medium-scale mills showed the highest average DON and ZON contents.

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