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Research Article

Determination of Mycotoxins and Veterinary Medicines in Duck Flesh and Viscera and Assessment of Their Exposure

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Mycotoxins can accumulate in various feeds and thus may get in duck meat, which may have severe food safety and public health implications. This study examined mycotoxins and veterinary medications in duck meat marketed in eight marketplaces around China. For the determination of mycotoxins, including the mycotoxins aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin M1 (AFM1), T-2 toxin, zearalenone (ZEN), and ochratoxin A (OTA), a liquid-chromatography tandem mass spectrometry (LC-MS/MS) method was validated. Overall, 13 out of 48 samples (27%) presented AFB1, and AFB2 was present in 14 out of 48 samples with positive levels ranging from 0.5 μg/kg (gizzard) to 4.1 μg/kg (lung). Eleven samples were contaminated with AFM1. T-2 was also found in three parts of duck samples (duck gizzard, neck, and lung), and the 5th and 48th samples were contaminated with T-2. ZEN was found in 5 of 48 analyzed samples (10%), and OTA was present in 21 out of 48 samples. The maximum kinds of mycotoxins found simultaneously in duck samples were six in duck lungs. High co-occurrence of mycotoxins was verified in several samples. The detection rate of various veterinary drugs was 0–12.5% in duck meat samples, and the over standard rate was 2.1%. Co-occurrence of veterinary drugs was verified in several samples.

1. Introduction

Mycotoxins are, in general, stable molecules, which are challenging to remove from foods once they have been produced [1, 2]. Aflatoxins, metabolites of *Aspergillus flavus* and *Aspergillus parasiticus*, are highly toxic substances [3, 4]. Its harmfulness lies in its damaging effect on human and animal liver tissues, and it can cause liver cancer or even death in severe cases [5, 6]. They are present in soil and plants and various nuts, especially grain and oil products such as peanuts, corn, rice, soybeans, and wheat. [7–10]. Ochratoxin A (OTA) is another mycotoxin [7], and it mainly contaminates agricultural grain products such as oats, barley, wheat, corn, feed, and animal food (such as pig kidney and liver) [10, 11]. A variety of other mycotoxins (including various aflatoxins, deoxynivalenol, and zearalenone) also may contaminate foods [12–14].

Ducks are one of the most sensitive animals to mycotoxin contamination. In recent years, the global production amount of duck meat and duck eggs has been increasing year by year, so it is necessary to study the harmful effects of mycotoxins on ducks, especially ducklings [15]. The feed contaminated with multiple mycotoxins is particularly harmful to ducks [16]. Certain mycotoxins frequently cause harm to the muscle, stomach, and intestines of broilers, while such damage is uncommon in meat ducks. The

immune system, liver, and heart are the essential target organs of mycotoxin in ducks. After ducks eat mouldy feed, mycotoxins may damage the liver and kidneys of the animals. These mycotoxins may remain in the liver, lungs, heart, and other parts of animals [17]. Therefore, this study aims to investigate the residual mycotoxins in various tissues and organs of ducks purchased from the market, providing a reference for the occurrence of mycotoxins in various tissues of duck meat.

Veterinary drugs play an essential role in ensuring animal health and improving the quality of animal products, but they also have many adverse effects, such as the problem of veterinary drug residues in animal products. Although the maximum limits of veterinary drug residues in food and the management regulations for veterinary drugs have been issued successively, the abuse of veterinary drugs is still severe. Therefore, we studied the veterinary drug residues in duck meat samples.

2. Materials and Methods

2.1. Reagents and Standard Solutions. The standards of aflatoxin-B1 (AFB1), aflatoxin-B2 (AFB2), aflatoxin-M1(AFM1), T-2 toxin (T-2), ochratoxin A (OTA), and zearalenone (ZEN) all with purity >98% were purchased from Yuanye (Yuanye, Shanghai, China). EURO-DIAGNOSTICA and BIOO SCIENTIFIC CO provided the enzyme-linked immunosorbent assay kits. The standard veterinary medicines were all purchased from Dr. Ehrenstorfer (Augsburg, Germany). Each mycotoxin's standard stock solution at 10 mg/L was prepared in MeOH. From this, a mix of working solutions at 100 μg/L each was prepared in MeOH. All solutions and standards were stored at −18°C.

2.2. Sampling. Forty-eight duck meat samples were purchased from 8 different local markets in Taizhou. The samples were vacuum-packed (portions of 50 g) and stored at −18°C until the analysis.

2.3. Mycotoxin Analysis. Instrument and analytical conditions: The assays were performed using a Waters HPLC (Waters, Milford) with a QqQ mass spectrometer (Waters, Manchester, U.K.). A C18 column (150 × 4.6 mm) maintained at 30°C was used for analytical separation. The method was similar to those previously applied in food by Peromingo et. al [18]. The mobile phase A was water/acetic acid 99 : 1 (v/v). The mobile phase B was methanol/acetic acid 99 : 1 (v/v). The solvent gradient in volume ratios was as follows: 0–0.5 min, 95% A; 0.5–7 min, 95% A-35% A; 7–11 min, 35% A-25% A; 11–13 min, 25% A-0% A; 13–20 min, 0% A. The flow rate was 0.8 mL/min.

Mass spectrometry conditions were as follows: positive ion scanning, the capillary voltage of 3.0 KV; cone hole gas flow rate of 50 L/h; ion source temperature of 100°C; the dissolvent temperature of 400°C; collision energy of 20 eV; and dissolvent gas flow rate of 700 L/h.

Sample preparation: The extraction of mycotoxins in meat samples was performed according to the method optimized by Zou et al. [19] and Sulyok et al. [4]. Firstly, all samples were thawed. Briefly, 20 mL extraction solvent acetonitrile/water/acetic acid (79/20/1, v/v/v) were added with 2 g of each sample and the samples were extracted for 90 min at room temperature (200 rpm). After extraction, it was centrifuged. The supernatant was taken, and then the same volume of the same solvent was added and diluted to half of the original concentration. Five microliter was used for LC-MS/MS analysis.

2.4. Method Validation and Quality Control. The chromatographic peak area of mycotoxins was taken as the ordinate (Y), the corresponding content was taken as the abscissa (X), and a linear regression equation was obtained as a standard curve [20]. The limit of detection (LOD) and the limit of quantification (LOQ) of each mycotoxin was taken as the corresponding concentrations when the signal-to-noise ratio was 3 (signal-to-noise ratio = 3), and the signal-to-noise ratio was 10, respectively. Precision for inter- and intraday was expressed as relative standard deviation (% RSD). These parameters were determined by analysis of triplicate spiked samples on the same day (intraday) and three subsequent days (interday) at three concentration levels: 5 μg/kg, 40 μg/kg, and 100 μg/kg for mycotoxins (AFB1, AFB2, AFM1, OTA, ZEN, and T-2).

2.5. Veterinary Drug Analysis. The veterinary drugs in the samples were extracted according to the recommended method of each kit. Then, each veterinary drug in samples was determined according to the recommended procedure of the respective kit.

2.6. Statistical Analyses. Calibration curves were submitted to regression analysis by the least square method and analysis of variance (ANOVA), where the lack of fit was assessed. It was found that all curves were statistically significant ($p < 0.05$), and no lack of fit was found ($p < 0.05$). All statistical analyses were performed in Statistica 10.0 (StatSoft®).

3. Results and Discussion

3.1. Method Validation for the Determination of Mycotoxins. As shown in Table 1, good linearity was observed for six mycotoxins, with determination coefficients (R^2) higher than 0.979. LODs ranged from 0.01 to 0.02 μg/kg. LOQs ranged from 0.1 to 0.2 μg/kg. The results here obtained are similar to several other chromatographic methods reported in a literature review conducted by Zhang [21]. For multi-mycotoxin analysis, it is difficult to obtain identical optimization conditions because of the differences in physicochemical properties of these mycotoxins.

The percentage of recovery (%) was higher than 87% (Table 2). The relative standard deviation (% RSD) values for interday and intraday precision were lower than 11%. The results obtained are within the control limits recommended by Chinese Regulation (GB 5009.22–2016).

Table 1: Performance parameters: limit of detection (LOD), linear range, and coefficient of determination (R^2) obtained with developed LC-MS/MS method.

Mycotoxin	LOD (μg/kg)	Linear range (μg/kg)	Linearity (R^2)
AFB1	0.01	0.1–100	0.998
AFB2	0.01	0.1–100	0.997
AFM1	0.01	0.1–100	0.983
T-2	0.01	0.1–100	0.979
OTA	0.02	0.2–100	0.989
ZEN	0.01	0.1–200	0.996

Table 2: Results for the recovery and precision arising from the validation of the analytical method.

	Medium			High			Low		
Analyte	Recovery (%) ($n=6$)	Intraday precision (%) ($n=6$)	Interday precision (%) ($n=6$)	Recovery (%) ($n=6$)	Intraday precision (%) ($n=6$)	Interday precision (%) ($n=6$)	Recovery (%) ($n=6$)	Intraday precision (%) ($n=6$)	Interday precision (%) ($n=6$)
AFB1	91.3	5.82	6.38	105.9	8.90	7.83	95.6	3.61	8.91
AFB2	87.2	8.61	9.60	103.8	6.37	8.67	90.8	5.87	7.34
AFM1	103.7	7.31	6.83	93.7	6.96	9.18	87.3	9.18	9.66
T-2	87.6	5.86	10.61	92.6	7.82	9.60	87.5	10.60	5.87
OTA	90.5	7.90	9.61	89.1	9.10	8.17	91.3	9.24	8.20
ZEN	106.9	9.66	7.90	88.6	7.91	6.15	106.1	9.61	8.93

Levels used for precisions and recovery assays were level 1 = 5 μg/kg, level 2 = 40 μg/kg, and level 3 = 100 μg/kg for AFB1, AFB2, AFM1, OTA, T-2, and ZEN.

3.2. Mycotoxins in Real Samples. Overall, 13 out of 48 samples (27%) presented AFB1, as shown in Table 3. The duck heart is the part where AFB1 appears most, and 50% of heart samples contained AFB1, followed by the neck and lung. AFB1 was not detected (<LOD) in any sample. Thirteen samples were contaminated with AFB1 with levels ranging from 0.1 to 1.3 μg/kg.

Among the emerging mycotoxins studied, AFB2 was one of the most usual mycotoxins, present in 15 out of 48 samples with positive levels ranging from 0.5 μg/kg (gizzard) to 4.1 μg/kg (lung). AFB2 was present in five parts of duck samples, from which the duck neck showed the highest level, followed by the duck heart (Table 4). For duck leg samples, AFB2 was not detected in any sample. Duck lung is the part where AFB2 appears most, present in 63% of samples.

AFM1 was also found in six parts of duck samples (Duck gizzard, breast, neck, heart, leg, and lung). Overall, 11 samples were contaminated with AFM1 (Table 5). The positive samples contaminated with AFM1 ranged from 0.1 to 4.0 μg/kg. Several positive samples showed total AFM1 higher than those laid down for milk and other products by the Chinese State standard (0.5 μg/kg, GB2761-2011). There is a specific correlation between AFM1 and AFB1 because the primary phase biotransformation of AFB1 is AFM1.

Not detected. T-2 was also found in three parts of duck samples (Duck gizzard, neck, and lung), with levels ranging from 0.2 to 1.1 μg/kg (Table 6). Overall, five samples were contaminated with T-2. Duck lung is the part where T-2 appears most, present in 25% of samples, followed by neck and gizzard. In duck heart, leg, and breast samples, T-2 was not detected in any sample.

Regarding ZEN, it was found in 5 of 48 analyzed samples (10%), with the positive levels ranging from 12.8 to 17.2 μg/kg (Table 7). ZEN was present in three parts of duck samples, from which, duck lung showed the highest level (17.2 μg/kg), followed by duck breast and gizzard. For duck neck, heart, and leg samples, ZEN was not detected in any sample. Duck gizzard and breast are the parts where ZEN appears most, present in 25 percent of total samples.

In general, the results obtained for the AFB1 regulated are lower than those reported in the literature: around 7 μg/kg for AFB1 in fresh and processed meat in Egypt [1]. In another report [22], an analysis of 22 retail products showed one Parma meat with a very high level of OTA contamination (56–158 μg/kg) that exceeded the Italian regulatory limit of 1 μg/kg, and their results were higher than the OTA contents of our study. In some other studies [23, 24], lower contents of mycotoxin were detected in meat samples.

3.3. Veterinary Drug Residue in Actual Samples. As shown in Table 9, in the duck meat samples collected, the detection rate of various veterinary drugs was 0–12.5%, and veterinary drug residues were detected in various parts of duck meat and various duck viscera, and the overall detection rate was not high. There was only 1 sample with veterinary drug residue exceeding regulatory limits. Among the 48 samples of duck meat and duck viscera, three veterinary drugs, oxytetracycline, clenbuterol hydrochloride, and streptomycin, were all detected in 4 groups of duck meat or viscera samples, and the detection rates of other veterinary drugs were lower. Five veterinary drugs were detected simultaneously in the Duck gizzard and duck neck samples. Few kinds of veterinary drugs were detected in the duck meat and duck viscera samples from other parts. Chloramphenicol, which the state prohibits from being used in animal food, is detected in the samples. Once detected, it will be judged to exceed the standard. For chloramphenicol residues, the total detection rate was 2.1%.

Table 3: Levels of AFB1 in duck meats (μg/kg).

Source of samples	Number of samples	Average (μg/kg)					
		Duck breast	Duck gizzard	Duck neck	Duck heart	Duck leg	Duck lung
Market 1	3	1.3	—	0.5	0.5	—	LOQ
Market 2	3	<LOQ	—	LOQ	—	—	—
Market 3	3	—	—	—	—	0.1	LOQ
Market 4	3	—	—	—	—	—	—
Market 5	3	—	—	LOQ	LOQ	—	—
Market 6	3	—	—	—	0.3	—	—
Market 7	3	—	—	—	LOQ	—	—
Market 8	3	—	—	—	—	—	0.1

Not detected. When the content of mycotoxin found in infusion was < LOQ, it was considered the arithmetic mean between the limit of detection (LOD) and the limit of quantification (LOQ).

Table 4: Levels of AFB2 in duck meats (μg/kg).

Source of samples	Number of samples	Average (μg kg^{-1})					
		Duck breast	Duck gizzard	Duck neck	Duck heart	Duck leg	Duck lung
Market 1	3	3.1	0.5	0.5	0.5	—	3.6
Market 2	3	—	—	4.1	3.9	—	3.1
Market 3	3	—	—	—	—	—	3.8
Market 4	3	—	—	1	—	—	—
Market 5	3	—	—	—	0.9	—	—
Market 6	3	—	<LOQ	—	—	—	—
Market 7	3	—	—	—	2	—	1.1
Market 8	3	—	—	—	—	—	<LOQ

Not detected. When the content of mycotoxin found in infusion was < LOQ, it was considered the arithmetic mean between the limit of detection (LOD) and the limit of quantification (LOQ).

Table 5: Levels of AFM1 in duck meats (μg/kg).

Source of samples	Number of samples	Average (μg/kg)					
		Duck breast	Duck gizzard	Duck neck	Duck heart	Duck leg	Duck lung
Market 1	3	—	2.3	—	—	—	1.5
Market 2	3	—	—	—	—	—	1.8
Market 3	3	—	—	—	—	2.1	4.0
Market 4	3	—	—	—	0.6	—	—
Market 5	3	—	—	—	0.5	—	—
Market 6	3	—	—	0.3	—	—	—
Market 7	3	—	—	—	—	—	0.1
Market 8	3	—	—	0.4	0.1	—	—

Table 6: Levels of T-2 in duck meats (μg/kg).

Source of samples	Number of samples	Average (μg/kg)					
		Duck breast	Duck gizzard	Duck neck	Duck heart	Duck leg	Duck lung
Market 1	3	—	0.2	<LOQ	—	—	—
Market 2	3	—	—	—	—	—	—
Market 3	3	—	—	—	—	—	1.1
Market 4	3	—	—	—	—	—	0.5
Market 5	3	—	—	—	—	—	—
Market 6	3	—	—	—	—	—	—
Market 7	3	—	—	<LOQ	—	—	—
Market 8	3	—	—	—	—	—	—

Not detected. When the content of mycotoxin found in infusion was < LOQ, it was considered the arithmetic mean between the limit of detection (LOD) and the limit of quantification (LOQ).

Table 7: Levels of ZEN in duck meats (μg/kg).

Source of samples	Number of samples	Average (μg/kg)					
		Duck breast	Duck gizzard	Duck neck	Duck heart	Duck leg	Duck lung
Market 1	3	—	14.4	—	—	—	o
Market 2	3	—	12.8	—	—	—	—
Market 3	3	—	—	—	—	—	17.2
Market 4	3	16.9	—	—	—	—	—
Market 5	3	13.6	—	—	—	—	—
Market 6	3	—	—	—	—	—	—
Market 7	3	—	—	—	—	—	—
Market 8	3	—	—	—	—	—	—

Not detected. Among the mycotoxins studied, OTA was most often found and was present in 21 out of 48 samples (Table 8), with positive levels ranging from 0.72 μg/kg (gizzard) to 1.02 μg/kg (lung). OTA was present in six parts of duck samples, from which duck lung showed the highest level, followed by duck breast, neck, heart, gizzard, and leg. OTA has been detected in all the duck parts studied. Duck gizzard and lung are the part where OTA appears most, present in 63% of samples.

Table 8: Levels of OTA in duck meats (μg/kg).

Source of samples	Number of samples	Average (μg/kg)					
		Duck breast	Duck gizzard	Duck neck	Duck heart	Duck leg	Duck lung
Market 1	3	0.96	0.94	0.95	0.95	—	0.93
Market 2	3	0.93	0.94	—	0.92	0.92	0.94
Market 3	3	0.93	—	—	0.94	0.92	1.02
Market 4	3	—	—	—	—	—	0.94
Market 5	3	—	0.93	0.95	—	—	0.92
Market 6	3	—	0.93	—	—	—	—
Market 7	3	0.86	—	—	—	—	—
Market 8	3	—	0.72	—	—	—	—

Not detected. The maximum number of mycotoxins found simultaneously in duck samples was six in duck lung, followed by neck and gizzard with five mycotoxins, and heart and breast with four mycotoxins each. There were three types of mycotoxins detected in duck leg samples, which was the least. Thus, high co-occurrence of mycotoxins was verified in these samples.

Table 9: Detection of veterinary drug residues in duck meat and viscera samples.

Duck	Oxytetracycline	Chlortetracycline	Tetracycline	Clenbuterol hydrochloride	Chloramphenicol	Gentamicin	Streptomycin	Furans
Breast	Relevance amount (samples)	0	0	1	0	0	0	1
	Over standard rate (%)	0	0	0	0	0	0	0
Gizzard	Relevance amount (samples)	1	0	1	1	0	1	1
	Over standard rate (%)	0	0	0	0	0	0	0
Neck	Relevance amount (samples)	0	1	1	1	1	0	1
	Over standard rate (%)	0	0	0	0	12.5	0	0
Heart	Relevance amount (samples)	1	1	0	0	0	1	1
	Over standard rate (%)	0	0	0	0	0	0	0
Leg	Relevance amount (samples)	1	1	1	0	0	0	0
	Over standard rate (%)	0	0	0	0	0	0	0
Lung	Relevance amount (samples)	1	0	0	0	0	0	1
	Over standard rate (%)	0	0	0	0	0	0	0

In the test, it was found that some duck meat samples contained multiple veterinary drugs simultaneously. Although this can synergize the drugs and improve the drug effect, it also increases the veterinary drug residues. In the samples with veterinary drug residues detected, there were six samples with two or more veterinary drug residues. The potential harm to human health caused by veterinary drug residues in animal food has been recognized by more and more people. The detection rate of veterinary drug residues in duck meat samples was 0–12.5%, and the exceeding rate was 2.1%. Only one sample had chloramphenicol residues exceeding the standard. Chloramphenicol can cause bone marrow hematopoietic disorders, leading to severe aplastic anemia, agranulocytosis, and other diseases.

For this reason, countries around the world and China have banned its use in animal breeding. In addition, samples containing chloramphenicol, gentamicin, gentamicin, and streptomycin were also found simultaneously. The combined use of such a variety of veterinary drugs enhances disease resistance and increases the residues of veterinary drugs, which is worthy of attention.

4. Conclusions

Six mycotoxins were simultaneously determined in 48 duck meat samples using an LC-MS/MS method, and the analytical performance (such as linearity, reproducibility, and sensitivity) of the method showed that it was accurate and sensitive for all the mycotoxins analyzed. The analysis indicated the presence of mycotoxins in 43% of the samples. AFB1 was present in 27% of the samples, with levels ranging from 0.1 to 1.3 μg/kg. The maximum number of mycotoxins found simultaneously in duck samples was six in duck lung, followed by neck and gizzard with five mycotoxins, and heart and breast with four mycotoxins each. There were three types of mycotoxins detected in duck leg samples, which was the least. Thus, high co-occurrence of mycotoxins was verified in these samples. The detection rate of 8 veterinary drugs in duck meat and viscera samples was 0–12.5%, and the exceeding rate was 2.1%. Co-occurrence of several veterinary drug residues was found in some duck meat and viscera samples.

Data Availability

All the data used to support the findings of this study are included in the paper.

Conflicts of Interest

The authors declare no conflicts of interest.

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