In Vivo Kinetics and Biotransformation of Aflatoxin B₁ in Dairy Cows Based on the Establishment of a Reliable UHPLC-MS/MS Method

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The in vivo kinetics of aflatoxin B₁ (AFB₁) and its carry-over as aflatoxin M₁ (AFM₁) in milk as well as the toxin loads in the tissue of dairy cows were assessed through a repetitive feeding trial of an AFB₁-contaminated diet of 4 μg kg⁻¹ body weight (b.w.) for 13 days. This was followed by a clearance period that ended with a single dose trial of an AFB₁-contaminated diet of 40 μg kg⁻¹ b.w. An ultra-high performance liquid chromatography tandem mass spectrometry method was developed and successfully validated by the determination of linearity (R² ≥ 0.990), sensitivity (lower limit of quantification, 0.1–0.2 ng ml⁻¹), recovery (79.5–111.2%), and precision relative standard deviation (RSD) ≤14.7%) in plasma, milk, and various tissues. The repetitive ingestion of AFB₁ indicated that the biotransformation of AFB₁ to AFM₁ occurred within 48 h, and the clearance period of AFM₁ in milk was not more than 2 days. The carry-over rate of AFM₁ in milk during the continuous ingestion experiment was in the range of 1.15–2.30% at a steady state. The in vivo kinetic results indicated that AFB₁ reached a maximum concentration of 3.8 ± 0.9 ng ml⁻¹ within 35.0 ± 10.2 min and was slowly eliminated from the plasma, with a half-life time (T₁/₂) of 350.1 ± 10.2 min. Meanwhile, AFM₁ reached a plateau in plasma (0.5 ± 0.1 ng ml⁻¹) at 4 h after the ingestion. AFB₁ was found in the heart, spleen, lungs, and kidneys at concentrations of 1.6 ± 0.3, 4.1 ± 1.2, 3.3 ± 0.9 and 5.6 ± 1.4 μg kg⁻¹, respectively. AFM₁ was observed in the spleen and kidneys at concentrations of only 0.7 ± 0.2 and 0.8 ± 0.1 μg kg⁻¹, respectively. In conclusion, the in vivo kinetics and biotransformation of AFB₁ in dairy cows were determined using the developed UHPLC-MS/MS method, and the present findings could be helpful in assessing the health risks to consumers.

Keywords: aflatoxins, in vivo kinetics, biotransformation, dairy cow, UHPLC-MS/MS
INTRODUCTION

Aflatoxin B₁ (AFB₁), primarily produced by Aspergillus flavus, Aspergillus parasiticus, and Aspergillus nomius, is frequently found in different feeds and their raw materials (Kumar et al., 2016; Frazzoli et al., 2017). AFB₁ has been classified as a group 1 Carcinogen by the International Agency for Research on Cancer (IARC) (Global Health, 2012) because of its hepatic, carcinogenic, teratogenic, mutagenic, immunosuppressive, and reproductive toxicities to livestock and poultry (Gross-Steinmeyer and Eaton, 2012; Iqbal et al., 2019). Aflatoxin M₁ (AFM₁), derived from the 4-hydroxylated metabolite of AFB₁ (chemical structures shown in Supplementary Figure S1), is also a potential human carcinogen classified as group 1 by IARC (Ostry et al., 2017), and it is usually secreted into milk after the ingestion of AFB₁-contaminated diets.

In recent decades, there have been many reports on the natural occurrence of AFB₁ in feeds and AFM₁ in milk and milk products (Natour et al., 1991; Han et al., 2013; Canestrari et al., 2016). The amount of AFM₁ in milk and AFB₁ in feeds consumed by animals could lead to health risks to consumers (Gonçalves et al., 2017). Therefore, the maximum AFB₁ limits have been set as 5 μg kg⁻¹ for compound feeds and 20 μg kg⁻¹ for all feed materials in EU (European Commission (EC), 2003), 20 μg kg⁻¹ for different feeds in the United States (Food and Agriculture Organization (FAO), 2004), and 10 μg kg⁻¹ in concentrate supplementary feeds and 50 μg kg⁻¹ in feed materials in China (State Administration for Market Regulation, 2017). For AFM₁, China, several other Asian countries, and the United States have set a maximum level of 0.5 μg kg⁻¹ in raw milk and dairy products (Food and Drug Administration (FDA), 1996; ASEAN, 2015; National Health Commission of the people’s Republic of China, 2017), while a considerably lower level (0.05 μg kg⁻¹) is stipulated in the EU (European Commission (EC), 2006).

Considering the widespread occurrence and intense toxicity, the in vivo kinetics of AFB₁ have been attracting more increasing attention. A few of studies have demonstrated that dietary AFB₁ is rapidly absorbed into the gastrointestinal tract of different animals and partially transformed to AFM₁ in milk for ruminant animals, such as cows and sheep, which are the primary source of AFM₁ in milk (Battacone et al., 2003; Zaghini et al., 2005; Corcuer et al., 2012). The rate of dietary AFB₁ carry-over as AFM₁ in milk ranged from 0.3 to 6.2% for cows (Applebaum et al., 1982; Frobish et al., 1986) and from 1.3 to 2.9% for sheep (Battacone et al., 2005; Battacone et al., 2009). Similarly, the in vivo toxicokinetics of AFB₁ have also been studied in different model animals, including rats, mice, and monkeys (Wong and Hsieh, 1980; Bastaki et al., 2010; Corcuer et al., 2012) but not in dairy cow. To date, there is no literature on the distribution of AFB₁ in different tissues and organs of dairy cows, which poses potential health risks to consumers. Notably, distinct differences in previous reports about the carry-over rate and in vivo kinetics of AFB₁ in various animal species were due to differences in AFB₁-delivery types, metabolic pathways, and animal susceptibility. Moreover, outdated detection methods, such as thin-layer chromatography (Stubblefield, 1986) and enzyme-linked immunosorbent assay (Diaz et al., 2004) have occasionally resulted in discrepant and contradictory results in earlier studies owing to complex sample pretreatment, lower sensitivity, and incomplete methodology.

The main objective of this study was to develop and validate an accurate and sensitive ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method to analyze the in vivo kinetics and biotransformation of AFB₁ in AFB₁-contaminated diet. Based on the model of dairy cows, the results will contribute to the understanding of the effects of dietary AFB₁ loads on its carry-over in milk, such as AFM₁, as well as distribution, and elimination of AFB₁ in vivo. The illustration of the kinetics and biotransformation of AFB₁ is shown in Supplementary Figure S2.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol, acetonitrile, and acetonitrile (all HPLC grade) were purchased from Merck (Darmstadt, Germany). Ammonium acetate (HPLC grade) was obtained from Sigma-Aldrich (St. Louis, MO, United States). Water was filtered using a Millipore system (Millipore, Billerica, MA, United States). AFB₁ (2.03 μg ml⁻¹), and AFM₁ (0.5 μg ml⁻¹) of analytical standard were purchased from Romer Labs (Union, MO, United States).

Preparation of Contaminated Diets

To produce AFB₁-contaminated maize, an AFB₁-producing strain (Aspergillus flavus 01) was isolated and identified at the mycotoxin research laboratory of Shanghai Academy of Agricultural Sciences, followed by cultivation on maize grains at 28°C for 28 days. The maize culture was then sterilized at 121°C, dried at 40°C for 60 h, and ground into powder. The concentrations of AFB₁ in contaminated maize flour and total mixed rations (TMR) feed were accurately determined according to the previously developed UHPLC-MS/MS method (Guo et al., 2017). Subsequently, 8.3 and 83 g of the obtained maize culture containing 240 mg kg⁻¹ AFB₁ were blended with 200 g of AFB₁-free TMR feed to develop two AFB₁ contaminated diets: Diet A, 4 μg kg⁻¹ body weight (b.w.) and Diet B, 40 μg kg⁻¹ b.w. for the animal trials, respectively. After finishing the diets, the animals were fed AFB₁-free TMR feed. The control group was directly fed AFB₁-free TMR feed.

Animals and Diet Management

Five Holstein dairy lactating cows (b.w. = 500 ± 10 kg, 30–32 weeks of calving) were purchased from Zhaxueping Dairy Farm (Nanjing, China). Before the experiments began, the dairy cows were given feed and water daily for a week for acclimatization. The dairy cows were randomly divided into an experimental group (three cows) and a control group (two cow). TMR feed (20 kg) per cow per day was administered in equal doses at 0,700 h and 1700 h according to the methods of the National Research Council to ensure milk production of ≥10 kg. The feed was divided into small portions and given to the cows several times to ensure that all feed was consumed. The health of
all the dairy cows was monitored continuously during the experimental period. This experiment was approved by the Animal Ethics Committee of Shanghai Academy of Agricultural Science (Shanghai, China) (SYXK (Hu) 2015-0007) and Zhuozhou Jierong Bio-Technology Co., Ltd. (Zhuozhou City, Hebei, China) (SYXK (Ji) 2018-003).

Experiment Design and Sample Collection
During the carry-over trial, dairy cows were repeatedly fed with AFB1− contaminated TMR feed (4 µg kg−1 b.w.) or AFB1−free TMR feed for 13 days. All the dairy cows were milked at 0, 730 h and 1730 h, and the milk yield was recorded. Milk samples (10 ml) were collected twice daily according to the volume of daily milk production. All milk samples were stored at −20°C until analysis. After a 30-days clearance period, a higher single dose of AFB1 in contaminated TMR feed (40 µg kg−1 b.w.) was administered to the experimental cows. Successive milk samples (10 ml) were collected at 0.5, 1, 3, 6, 9, 24, 36, 48, 72, and 96 h after the administration of AFB1−contaminated diet for further carry-over analysis of AFB1. Simultaneously, 5 ml of blood from each cow was drawn from the caudal vein at 10, 35, 45, 60, 120, 180, 240, 360, 540, 720, 1,440, 2,160, and 2,880 min for the in vivo kinetic study of AFB1. Each blood sample was immediately collected in a heparinized tube and centrifuged at 2,739 g for 15 min. Subsequently, aliquots of plasma were transferred into clean tubes and stored at −20°C until use. After another 30-days clearance period, all the cows were sacrificed 6 h after the oral administration of AFB1 (40 µg kg−1 b.w.). Tissue samples from cows, including heart, liver, spleen, lung, and kidney, were collected and stored in liquid nitrogen until analysis. Blank milk, blood, and tissue samples from the control group were collected to establish the analytical method.

UHPLC-MS/MS Analysis
After thawing at room temperature, 200 µL of milk, plasma, and tissue homogenates, which were homogenized with normal saline (1/3, m/v), were separately transferred into a 2.5-mL centrifuge tube. Acetone (1.4 mL) of was added for protein precipitation and target extraction. The mixtures were blended by vortexing for 30 s and centrifuged at 16,099 g for 5 min. Subsequently, 1 ml of the supernatant was evaporated under a soft stream of nitrogen gas at 30°C for 10 min. The residues were then filtered through a 0.22 µm membrane filter for UHPLC-MS/MS analysis.

UHPLC-MS/MS analysis was performed on a Waters ACQUITY UPLC system coupled with an AB SCIEX Triple Quad TM 5500 mass spectrometer. LC separation was achieved on a Poroshell EC18 column (2.1 × 100 mm, 2.7 µm, Agilent, United States) with methanol (A) and 5 mmol L−1 ammonium acetate (B) as the mobile phase. The flow rate was 0.4 mL min−1 and a total of 8 min of gradient elution procedure was as follows: initial 10% A; 0.5 min, 10% A; 1.5 min, 50% A; 5.0 min, 90% A; 6.0 min, 90% A; 6.2 min, 10% A; and 8.0 min, 10% A. The injection volume was 3 µL, and the column temperature was 40°C.

Electrospray ionization was used in positive (ESI+ ) mode with the following parameters: ion spray voltage, 5500 V; source temperature, 500°C; ion source gas 1 (GS1), 50 psi; ion source gas 2 (GS2), 50 psi; and collision gas (CAD), 8 psi. The multiple reaction monitoring (MRM) mode was used for the quantification and confirmation of AFB1 and AFM1 with the parameters listed in Supplementary Table S1.

Carry-Over Analysis
The carry-over rate of AFB1 to AFM1 was calculated according to the following formula:

\[
\text{Carry – over rate (％) } = \frac{m_{\text{milk}} \times C_{\text{AFM1}}}{m_{\text{TMR}} \times C_{\text{AFB1}}} \times 100\%
\]

The \( m_{\text{milk}} \) and \( m_{\text{TMR}} \) are the milk yield (kg) and quantity of AFB1− contaminated TMR feed (kg) daily, respectively. C_{\text{AFM1}} and C_{\text{AFB1}} are the concentrations of AFM1 in milk (µg kg−1) and AFB1 in the diet (µg kg−1), respectively.

The graphs of concentration–time curves were prepared using Origin 9.0, (La Jolla, CA, United States), which were then used to illustrate the carryover of AFB1 and AFM1 in milk. All data are presented as mean ± standard deviation (SD).

In Vivo Kinetics and Tissue Distribution
After oral administration, in vivo kinetics of AFB1 was performed with DAS 2.0 (Shanghai, China) using non-compartmental analysis. AUC(0−t) and AUC (0−∞) are the areas under the plasma concentration–time curve from time 0−2,160 min and infinity, respectively. MRT(0−t) and MRT(0−∞) are the mean residence times from time 0−2,160 min and infinity, respectively, where \( T_{1/2} \) is the terminal elimination half-life. \( C_0 \) and \( C_{\text{max}} \) are the initial and maximal plasma concentrations, respectively. \( T_{\text{max}} \) is the time to maximal plasma concentration. All data are presented as mean ± SD.

The concentrations of AFB1 and AFM1 in different tissues from individual dairy cows, including the heart, liver, spleen, lungs, and kidneys were determined.

Method Validation
The analytical method for detecting AFB1 and AFM1 in plasma, milk, and various tissues, such as the heart, liver, spleen, lungs, and kidneys, was validated according to the guidelines on bioanalytical method validation provided by the European Medicines Agency (Blume et al., 2011). Linearity was evaluated in neat solvent and in plasma, milk, and various tissues spiked with AFB1 and AFM1 at concentrations of 0.1−200 ng mL−1. The calibration curves were drawn by plotting responses versus analyte concentrations, and the acceptable criterion of \( R^2 \) was ≥0.99. The lower limit of quantification (LLOQ) was the lowest concentration point of the calibration curves, which is typically defined as a theoretical signal-to-noise (S/N) ratio of 10. The lower limit of detection (LLD) was the lowest concentration that could be determined and defined as a theoretical S/N ration of 3. Note that S/N=SD/k, where SD is the standard deviation of the blank (n = 6) and k is the slope of the matrix-matched calibration curve. The recovery and precision were evaluated in blank samples spiked with LLOQ, low, intermediate, and high levels (LLOQ, 1, 50, and 200 ng mL−1 for plasma and milk; LLOQ, 1, 50, and 200 µg kg−1 for various tissues, respectively) of AFB1 and AFM1 in six replicates. RSD values on the same day and on five successive days were used to evaluate the
intra- and inter-day precision, respectively. The short-term (room temperature for 8 h) and long-term (-20°C for 20 days) stability of spiked plasma and tissue samples (1 and 50 ng ml⁻¹ for plasma and milk, 1 and 50 μg kg⁻¹ for various tissues), as well as the stability after three freeze-thaw cycles, were evaluated to ensure that the concentrations of AFB₁ and AFM₁ were not affected. In addition,
blank, spiked, and real plasma, milk, and spleen collected after AFB1 oral administration were individually analyzed and evaluated for specificity.

RESULTS AND DISCUSSION

Optimization of Extraction Solvent
In the current study, three different solvents (methanol, acetonitrile and acetone) at different extraction volumes (0.6, 1.0, 1.4, and 1.8 ml, respectively) were compared for the spiked milk samples (50 ng ml\(^{-1}\)). The extraction efficiency was evaluated according to the following formula:

\[
\text{Extraction efficiency} = \text{extraction recovery} \times \text{matrix effect} \times 100\%
\]

As shown in Supplementary Table S2, the highest extraction efficiency of 77.5 and 89.4% was achieved for AFB1 and AFM1, respectively, when 1.4 ml of acetone was selected. Similar trends were observed for AFB1 and AFM1 in plasma and different tissue samples. Therefore, 1.4 ml of acetone was selected as the extraction solvent for protein precipitation and target extraction.

Method Validation
Good linear relationships were obtained with correlation coefficients \((R^2) > 0.99\) in neat solvent and blank plasma, milk, and tissues (Supplementary Table S3). The LLODs and LLOQs for AFB1 and AFM1 in different matrices were in the range of 0.03–0.2 ng ml\(^{-1}\) (μg kg\(^{-1}\)) and 0.1–0.5 ng ml\(^{-1}\) (μg kg\(^{-1}\)), respectively. Satisfactory recoveries and precisions for AFB1 and AFM1 at various spiking levels are listed in Table 1. The recoveries ranged from 79.5 to 102.3% for milk; 82.8–107.9% for plasma; 88.7–111.2% for heart; 83.3–109.6% for liver; 86.8–104.8% for spleen; 85.8–103.0% for lung; and 85.1–106.6% for kidney. The intra- and inter-day RSDs were in the range of 3.0–12.3% and 6.8–14.7%, respectively, for various matrices, indicating the acceptable reproducibility of the
proposed method. The concentration at each spiking level of all samples after the short-term, long-term, and three freeze–thaw cycle stability tests were in the range of 82.2–102.0% (Supplementary Table S4), which indicated that AFB1 and AFM1 in all the biological matrices were stable. Moreover, no endogenous interference was observed at the respective retention times of AFB1 (5.0 min) and AFM1 (5.5 min) in plasma, milk, and spleen matrices (Figure 1), verifying the good selectivity of this method.

**Carry-Over Rate of AFB1 to AFM1 in Milk**

The repetitive ingestion of 4 μg kg⁻¹ b.w. of AFB1 for 13 days (intoxication period) demonstrated that the concentrations of AFM1 in the milk increased rapidly from the first day, with concentrations remaining in the range of 2.6–3.8 μg kg⁻¹ till day 13 (Figure 2A). As presented in Supplementary Table S5, this result was similar to that previously reported in cows that were fed a diet containing ~ 86 μg AFB1 daily for 7 days (Britzi et al., 2013). After the intoxication period (13 days), the cows were fed AFB1-free feeds, and the milk was collected for 7 days (clearance period). The concentration of AFM1 in milk decreased gradually and could not be detected after 2 days. These results corresponded with those of previous studies that reported the clearance period typically lasted less than 3 days for AFB1 (Diaz et al., 2004). As depicted in Figure 2B, the carry-over rate of AFM1 in milk during the continuous ingestion experiment was in the range of 1.15–2.30% at a steady state, which was consistent with the range of 1–3% that has been reported in previous studies (Diaz et al., 2004; Van Eijkeren et al., 2006; Masoero et al., 2007) (Supplementary Table S5).

Furthermore, a high single dose (40 μg kg⁻¹ b.w.) of feed artificially contaminated with AFB1 showed that AFM1 in milk increased rapidly and the highest concentration of AFM1 was observed at 24 h (21.3 ± 2.9 μg kg⁻¹) (Figure 3A). After its plateau, AFM1 concentration decreased rapidly and could not be detected after 96 h. The disappearance pattern of AFM1 in milk is depicted in Figure 3B, and the disappearance of AFM1 in milk can be expressed as: y = 117.95e⁻₀.₀⁰₉₅ₙ R² = 0.9569.

Overall, no significant differences were observed in the carry-over of AFB1 to AFM1 with different administration approaches and concentrations, similar to the results of previous studies on cows and sheep. However, the observed plateaus and clearance periods of AFM1 in milk were partially variable (Supplementary Table S5). These variations may be related to the different dietary sources of AFB1, for example, pure AFB1 or naturally

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**TABLE 2 | Primary toxicokinetic parameters of AFB1 after a single oral administration (40 μg kg⁻¹ b.w.) to dairy cows (n = 3).**

| Toxicokinetic parameter | Unit | Mean ± SD |
|-------------------------|------|-----------|
| AUC (0–1) | ng min mL⁻¹ | 1763.3 ± 132.5 |
| AUC (0–∞) | ng min mL⁻¹ | 2162.7 ± 359.6 |
| MRT (0–1) | min | 703.5 ± 56.6 |
| MRT (0–∞) | min | 1220.7 ± 94.1 |
| T₁/₂ | min | 931.1 ± 30.8 |
| C₀ | ng mL⁻¹ | 0.0 ± 0.0 |
| Cₘ₉₉ | ng mL⁻¹ | 3.8 ± 0.9 |
| Tₘ₉₉ | min | 35.0 ± 10.2 |

AUC₀–ₙ = area under the plasma concentration-time curve from time 0–2,160 min, AUC₀–∞ = area under the plasma concentration-time curve from time 0 to infinity, MRT (0–1) = mean residence time from time 0–2,160 min, MRT (0–∞) = mean residence time from time 0 to infinity, T₁/₂ = terminal elimination half-life, C₀ = plasma concentration at time 0; Cₘ₉₉ = maximal plasma concentration; Tₘ₉₉ = time to maximal plasma concentration; SD, standard deviation.

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**FIGURE 3 | Time-concentration profiles of AFM1 in milk after a single oral administration of AFB1 (40 μg kg⁻¹ b.w.) (A) and the disappearance pattern of AFM1 in the milk (B).** Values are presented as mean ± standard deviation, n = 3.

**FIGURE 4 | Time-concentration profile of AFB and AFM1 in the plasma after a single oral administration of AFB1 (40 μg kg⁻¹ b.w.) (n = 3). Values are presented as mean ± standard deviation.
AFB1-contaminated corn, cottonseed, and ground-peanut meal, varying levels of AFB1 dose, as well as the differences between individual animals (Battacone et al., 2003; Battacone et al., 2012; Sumantri et al., 2012).

In Vivo Kinetics
The concentration–time profiles of AFB1 and AFM1, as well as the toxicokinetic parameters in plasma after a single dose (40 μg kg⁻¹ b.w.) of AFB1 are presented in Figure 4 and Table 2. The results indicated that AFB1 was rapidly absorbed in all studied cows with the highest concentrations (Cmax = 3.8 ± 0.9 ng ml⁻¹) approximately 35.0 ± 10.2 min after oral administration. Meanwhile, AFB1 was rapidly eliminated in cows (T1/2 = 931.1 ± 30.8 min) and transformed into AFM1, which plateaued in the plasma (0.5 ± 0.1 ng ml⁻¹) at 4 h after ingestion. As presented in Supplementary Table S5, the values of the primary kinetic parameters in this study were significantly different from those of other animals, such as rats, mice, monkeys, and broiler chickens (Bastaki et al., 2010; Cui et al., 2017). This can be attributed to many factors, including the differences in AFB1 intake, gastrointestinal absorption, animal health, and particularly in the activity of cytochrome P450 (CYP450) enzymes, which play an important role in the transformation of AFB1 to AFM1 in the liver (Applebaum et al., 1982; Gross-Steinmeyer and Eaton, 2012).

Tissue Distribution
After a single oral dose of AFB1 (40 μg kg⁻¹ b.w.), all tissues were analyzed via the validated UHPLC-MS/MS method. The concentrations of AFB1 in the heart, spleen, lungs and kidneys were 1.6 ± 0.3, 4.1 ± 1.2, 3.3 ± 0.9 and 5.6 ± 1.4 μg kg⁻¹, respectively. Although the liver is typically considered the most susceptible organ for AFB1, neither aflatoxin was detected in all the live samples. It is likely that AFB1 in the liver was completely cleared because of the time taken between last feed and sacrifice (Corcuera et al., 2012; Cui et al., 2017). Moreover, AFM1 was observed in the spleen and kidneys at concentrations of 0.7 ± 0.2 and 0.8 ± 0.1 μg kg⁻¹, respectively. In summary, these results verified the effects of AFB1 and AFM1 accumulation in different tissues, particularly in the spleen and kidneys, which could pose health risks for both dairy cows and consumers.

CONCLUSION
An accurate and reliable UHPLC-MS/MS method was established and validated for the simultaneous determination of AFB1 and AFM1 in the plasma, milk, and tissues of dairy cows. And the method was applied to investigate in vivo kinetics and biotransformation of AFB1 in dairy cows. A rapid absorption, distribution, and excretion of AFB1 was observed in dietary cows with relatively high residues detected in kidneys, lungs, heart, and spleen. A certain amount of AFB1 (1.15–2.30%) could also be transformed to AFM1, as another important risk factors and then excreted into milk. This comprehensive study will be of great value in the evaluation and control of AFB1 contamination in feeds to reduce the health risks posed to both humans and animals.

DATA AVAILABILITY STATEMENT
The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT
The animal study was reviewed and approved by the Animal Ethics Committee of Shanghai Academy of Agricultural Science (Shanghai, China) (SYXK (Hu) 2015-0007) and Zhuozhou Jierong Bio-Technology Co., Ltd. (Zhuozhou City, Hebei, China) (SYXK (Ji) 2018-003).

AUTHOR CONTRIBUTIONS
WG and ZF performed the experiments; KF and JM contributed to the sample preparation, and WG wrote the manuscript; DN performed the data processing; ET and ZZ reviewed the manuscript; ZL and ZH conceived and designed the experiments.

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SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2021.809480/full#supplementary-material

REFERENCES
Applebaum, R. S., Brackett, R. E., Wiseman, D. W., and Marth, E. H. (1982). Response of Dairy Cows to Dietary Aflatoxin: Feed Intake and Yield, Toxin Content, and Quality of Milk of Cows Treated With Pure and Impure Aflatoxin. J. Dairy Sci. 65, 1503–1508. doi:10.3168/jds.s0022-0302(82)82374-6

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Doses of Aflatoxin B1. J. Dairy Sci. 86, 2657–2675. doi:10.3168/jds.s0022-0302(03)73862-4

Battacone, G., Nudda, A., Palomba, M., Mazzette, A., and Pulina, G. (2009). The Transfer of Aflatoxin M1 in Milk of Ewes Fed Naturally Contaminated by Aflatoxins and Effect of Inclusion of Dried Yeast Culture in the Diet. J. Dairy Sci. 92, 4997–5004. doi:10.3168/jds.2008-1684

Battacone, G., Nudda, A., Palomba, M., Pascale, M., Nicolussi, P., and Pulina, G. (2005). Transfer of Aflatoxin B1 From Feed to Milk and From Milk to Curd and Whey in Dairy Sheep Fed Artificially Contaminated Concentrates. J. Dairy Sci. 88, 3063–3069. doi:10.3168/jds.2005-0230(05)72987-8

Battacone, G., Nudda, A., Rassu, S. P. G., Decandia, M., and Pulina, G. (2012). Aflatoxin B1 Risk Management in Farmigianno Reggiano Dairy Cow Feed. Ital. J. Food Saf. 157, 233–241. doi:10.1023/b:myco.0000020587.93872.59

Corcuera, L. A., Vettorazzi, A., Arbillaga, L., González-Peñas, E., and López de Diaz, D. E., Hagler Jr., W. M., Blackwelder, J. T., Eve, J. A., Hopkins, B. A., Gross-Steinmeyer, K., and Eaton, D. L. (2012). Dietary Modulation of the Excretion Pattern of Aflatoxin B1 and Ochratoxin A After Simultaneous Oral Administration to Fasted F344 Rats. Mycopathologia. 183, 271–277. doi:10.1007/s11046-011-9821-y

Ostry, V., Malir, F., Toman, J., and Grosse, Y. (2017). Mycotoxins as Human Carcinogens: The IARC Monographs Classification. Mycotoxin Res. 33, 65–73. doi:10.1007/s12550-016-0265-7

State Administration for Market Regulation (2017). Hygienical Standard for Feeds: GB 13078-2017. Beijing, China: Standards Press of China.

Stubblefield, R. D. (1986). Thin-layer and High Performance Liquid Chromatographic Methods for the Analysis of Aflatoxins in Animal Tissues and Fluids. Diagn. Mycotoxins. 33, 257–269. doi:10.1079/978-1-4615-2382-8.16

Sumantiri, I., Murti, T. W., Van, d. P., Boehm, J., and Agus, A. (2012). Carry-Over of Aflatoxin B1-Feed into Aflatoxin B1 in Corresponding Feeds. Toxins. 4, 294. doi:10.3390/toxins4060294

Wong, Z. A., and Hsieh, D. P. H. (1980). The Comparative Metabolism and Toxicokinetics of Aflatoxin B1 in the Monkey, Rat, and Mouse. Toxicol. Appl. Pharmacol. 55, 115–125. doi:10.1016/0041-0084(80)90227-6

Zaghini, A., Martelli, G., Roncada, P., Simioni, M., and Rizi, L. (2005). Polymannosilicic acid as aflatoxin B1 and feed for laying hens: Effects on Egg Quality, Aflatoxin B1 and M1 Residues in Eggs, and Aflatoxin B1 Levels in Liver. Poult. Sci. 84, 825–832. doi:10.1093/ps/84.6.825

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