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Potential natural exposure of endangered red-crowned crane (*Grus japonensis*) to mycotoxins aflatoxin B_1, deoxynivalenol, zearalenone, T-2 toxin, and ochratoxin A*

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Abstract: A survey was conducted to determine whether mycotoxins were present in the foods consumed by red-crowned cranes (*Grus japonensis*) in the Yancheng Biosphere Reserve, China. Collected in the reserve's core, buffer, and experimental zones during overwintering periods of 2013 to 2015, a total of 113 food samples were analyzed for aflatoxin B_1, deoxynivalenol, zearalenone, T-2 toxin, and ochratoxin A using high performance liquid chromatography (HPLC). The contamination incidences vary among different zones and the mycotoxins levels of different food samples also presented disparity. Average mycotoxin concentration from rice grain was greater than that from other food types. Among mycotoxin-positive samples, 59.3% were simultaneously contaminated with more than one toxin. This study demonstrated for the first time that red-crowned cranes were exposed to mycotoxins in the Yancheng Biosphere Reserve and suggested that artificial wetlands could not be considered good habitats for the birds in this reserve, especially rice fields.

Key words: Food, Mycotoxin, Red-crowned crane, Yancheng Biosphere Reserve
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1 Introduction

Mycotoxins are toxic substances produced as secondary metabolites mainly by filamentous fungi, such as *Aspergillus*, *Penicillium*, and *Fusarium* species (Nielsen *et al.*, 2009; Marin *et al.*, 2013). These metabolites have frequently been found worldwide in foods and feeds, and are known to cause acute and/or chronic toxicity in humans and animals, depending on the amount consumed (Scudamore *et al.*, 1997; Placinta *et al.*, 1999; Lee *et al.*, 2010; Li *et al.*, 2014). Fink-Gremmels (1999) has estimated that approximately 25% of crops worldwide are contaminated with mycotoxins.

To date, more than 400 mycotoxins have been identified as part of a growing interest in this field of research and availability of modern laboratory methods (Sulyok *et al.*, 2010). The most important

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mycotoxin members are aflatoxins (AFs), deoxynivalenol (DON), zearalenone (ZEN), T-2 toxin (T-2), and ochratoxin A (OTA), because of their ubiquitous nature, causing disease in humans and other domestic animals following ingestion of contaminated foods (Santos *et al.*, 2009; Zain, 2011). The International Agency for Research on Cancer (IARC) has classified AFs and OTA as Groups 1 and 2B carcinogens, respectively (IARC, 1993). AFs are fungal metabolites primarily from two species of *Aspergillus*, *A. flavus* and *A. parasitius*. AFs are extremely potent carcinogens in all animal species tested, including rats, mice, fish, hamsters, ducks, and humans. Among AFs, aflatoxin B_1 (AFB_1) is the most common and toxic form (Marin *et al.*, 2013). OTA, produced by many species of *Aspergillus* and *Penicillium*, is carcinogenic, nephrotoxic, teratogenic, immunotoxic, and hepatotoxic (Hussein and Brasel, 2001). DON, ZEN, and T-2 are mainly produced by the mold genus *Fusarium* (Zinedine *et al.*, 2007; Kolf-Clauw *et al.*, 2008). Long-term exposure of animals to feeds contaminated with DON causes reduced live weight gain, decreased nutrient efficiency, and increased susceptibility to infectious diseases (Swamy *et al.*, 2004). ZEN, also known as F-2 toxin, is of relatively low toxicity. However, a ZEN property that causes estrogenic syndromes in reproductive tracts of animals, such as pigs, cattle, and poultry, has been recognized (Minervini and Dell'Aquila, 2008). T-2 toxic effects include immunomodulation, cytotoxicity, inhibition of protein, DNA, and RNA syntheses, cell lesions in digestive tract, organs, and skin, and neural disturbances (Stafford and McLaughlin, 1973; Sklan *et al.*, 2003; Parent-Massin, 2004; Meissonnier *et al.*, 2008).

Historically, a number of mycotoxin studies have been performed because of the economic implications of these compounds on the animal husbandry industry or public health risks. However, in recent years, studies have focused on mycotoxin impacts on wildlife populations. Lawson *et al.* (2006) have identified hepatic AF residues in house sparrow (*Passer domesticus*) and greenfinch (*Carduelis chloris*) in Britain, and reported that the residues resulted from high dietary AF exposure, although the source(s) of dietary AFs was unknown. Some clusters of mycotoxicosis, with associated morbidity and mortality, also have occurred in cranes. About 9500 sandhill cranes (*Grus canadensis*) died in Gaines County, Texas and Roosevelt County, New Mexico, USA, between 1982 and 1987. The most probable cause of this mortality was mycotoxins produced by *Fusarium* spp. that grew on peanuts left in fields after harvest and found to be the predominant food of these dead cranes (Windingstad *et al.*, 1989). Pelleted feed contaminated by T-2 and DON sickened 80 of 300 captive whooping cranes (*G. americana*) and sandhill cranes, and caused the death of 15 of these cranes at the Patuxent Wildlife Research Center, Laurel, Maryland, USA, in 1987 (Olsen *et al.*, 1995).

The red-crowned crane (*G. japonensis*) is one of the rarest crane species and has been classified as "endangered" on the International Union for Conservation of Nature (IUCN) Red List (IUCN, 2014). The current population is estimated to be 2750 individuals (BirdLife International, 2015). A large proportion of the migratory population migrates from northeastern China to Yancheng in late October and overwinters in the Yancheng Biosphere Reserve, China, itself the largest wintering area for the migratory population, until early March (Ma *et al.*, 1999; Wang, 2008). Red-crowned cranes are omnivorous, whose diet includes rice, grain, wheat seedlings, shellfish, fish, shrimp, crabs, snails, seepweed seed and leaf, and reed roots (Ma *et al.*, 2000; Ma and Li, 2002; Dong *et al.*, 2005). As Yancheng is located in a climatic zone with temperature and humidity suitable for mold growth and mycotoxin production (Fan, 2008), it is likely that some of these foods are contaminated by mycotoxins. Such a situation might have a negative impact on the health and reproduction of these cranes, and thus potentially threatens the population. The objectives of this study are to confirm whether these five mycotoxins were present in the cranes' food and to discuss the potential for mycotoxin impact on the health of red-crowned cranes in the Yancheng Biosphere Reserve, China.

2 Materials and methods

2.1 Chemicals and instruments

Mycotoxin standards for AFB_1, DON, ZEN, T-2, and OTA were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA) and stored at −20 °C until use. Immunoaffinity columns (IACs) were obtained from R-Biopharm AG (Darmstadt, Germany). High

performance liquid chromatography (HPLC)-grade acetonitrile, methanol, and acetic acid were obtained from Merck KGaA (Darmstadt, Germany). Ultrapure water was produced by a Millipore Milli-Q system (Millipore, Billerica, MA, USA). All other chemicals and reagents used were of analytical grade.

2.2 Study area

This study was conducted in the Yancheng Biosphere Reserve (32°34′–34°28′N, 119°48′–120°56′E), which is located in Yancheng City, Jiangsu Province, China. The reserve was established for red-crowned cranes, other waterbirds, and coastal wetlands in 1983, and in 1992 the United Nations Educational, Scientific, and Cultural Organization (UNESCO) recognized it as an international biosphere reserve. The reserve was divided into three zones, the core, buffer and experimental zones, to integrate biodiversity conservation and economic development of local communities (Fig. 1). Red-crowned cranes in the reserve are the principal users of five major foraging habitats, including the common seepweed community (*Suaeda glauca*), a reed pond (*Phragmites communis*), farmland (wheat and rice fields), a salt pan, and an aquaculture pond. The seepweed community and reed pond were mainly located in the core zone with little human disturbance, as a type of natural wetlands, providing cranes with crabs, snails, seepweed seed and leaf, and reed root. The farmland, salt pan, and aquaculture pond comprised entirely artificial wetlands and occurred in the buffer and experimental zones, supplying cranes with rice grain, wheat seedings, shellfish, fish, and shrimp (Ma *et al.*, 2000; Liu *et al.*, 2013).

2.3 Samples

A total of 113 samples were collected at 15–20 d intervals from November through to March 2013–2014 and 2014–2015 (Table 1). The corresponding foods were collected when cranes were observed feeding in foraging habitats. For each kind of food three sampling stations were chosen for each sampling date. The sample sites were more than 3 km from each other within the same wetland. In each site, the first available 50 g of each food was obtained at random. Then the same kind of food was combined to form a single sample. The samples were stored in an ice box in the field and transported to the laboratory within 8 h. All samples were ground using a grinder. The grinder was cleaned between samples thoroughly to avoid cross-contamination of samples. After grinding, samples were homogenized and stored in sealed plastic bags at −80 °C until subsequent HPLC analysis.

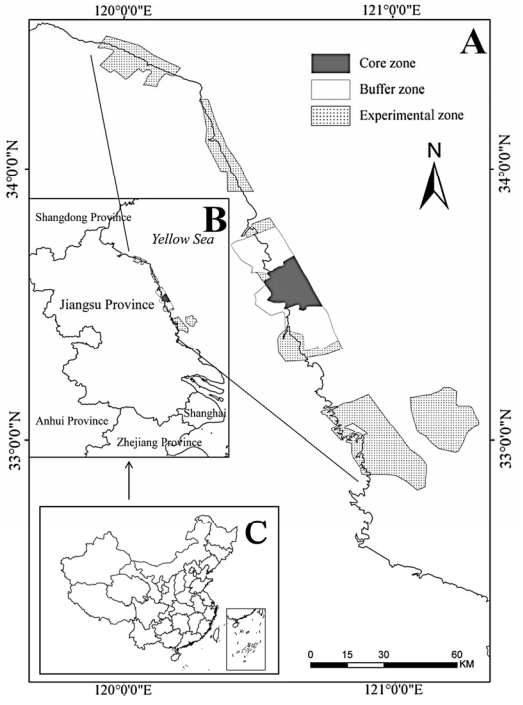

Fig. 1 Maps showing the Yancheng Biosphere Reserve functional zones (A), its location in Jiangsu Province (B), and its location in China (C)

Table 1 Sample information from the Yancheng Biosphere Reserve, China

Zone	Foraging habitat	Food sample	Number of samples	
			Nov.–Mar. 2013–2014	Nov.–Mar. 2014–2015
Core	Reed pond	Crabs	7	8
		Reed root	9	9
	Common seepweed community	Seepweed seed and leaf	9	9
		Crabs and snails	6	7
Buffer and experimental	Rice field	Rice grain	8	7
	Wheat field	Wheat seedlings	9	9
	Aquaculture pond	Fish and shrimp	7	9
	Salt pan	Shellfish and fish	NA[a]	NA

[a] NA: not available. According to the 2010–2015 winter survey, there were no cranes in salt pan, and no crane feeding was detected during the present sampling in this foraging habitat

2.4 AFB_1 analysis

A 25-g sample, prepared as above, was homogenized with 125 ml of methanol/water solution (70/30, v/v) and 5 g of NaCl for 30 min. The mixture was filtered through Whatman No. 4 paper, 15 ml of the filtrate diluted with 30 ml of ultrapure water, and then 15 ml of the resulting solution applied to an AFB_1 IAC. The column was then washed with 20 ml of ultrapure water and dried by blowing air through the column, and the target analyte was eluted three times with 1.0 ml of methanol. The eluate was evaporated to dryness at 40 °C under a stream of nitrogen. The residue was derivatized with trifluoroacetic acid according to the method of Khayoon *et al.* (2010). Then, 1 ml of acetonitrile/water (30/70, v/v) was added. A 20-μl sample was suction-filtered through a 0.45-μm nylon membrane and injected into the HPLC system.

AFB_1 analysis was performed on an HPLC system equipped with a fluorescence detector (HPLC-FLD) and separation achieved using a C_{18} column (150 mm×4.6 mm, 5 μm particle size; Shimadzu Corp., Kyoto, Japan). The mobile phase was acetonitrile/water (30/70, v/v) with a flow rate of 0.8 ml/min. Fluorescence was detected at 365 and 418 nm for excitation and emission, respectively. The column temperature was 30 °C and the limit of detection and limit of quantification (LOD and LOQ, respectively) of the method were 0.03 and 0.09 μg/kg, respectively. The mean recovery was 90% with a relative standard deviation (RSD) of 4%.

2.5 DON analysis

A 25-g sample was homogenized in 200 ml of ultrapure water for 30 min. The mixture was sequentially filtered through Whatman No. 4 and glass fiber filter papers, and then 2 ml of this extract was applied to a DON IAC. The column was then washed with 5 ml of ultrapure water and flushed with air to remove remaining water. DON bound to the column was eluted using three 1.5-ml volumes of methanol and the eluate evaporated to dryness at 40 °C under a steam of nitrogen. The residue was dissolved with 1 ml of acetonitrile/water (16/84, v/v) and a 20-μl sample was suction-filtered through a 0.45-μm nylon membrane and injected into the HPLC system.

DON analysis was carried out on an HPLC system equipped with an ultraviolet detector and a C_{18} column (150 mm×4.6 mm, 5 μm particle size). The mobile phase was acetonitrile/water (16/84, v/v), flow rate 0.8 ml/min, column temperature 30 °C, and detection at 218 nm. LOD and LOQ for this method were 4.2 and 13.44 μg/kg, respectively, and the mean recovery was 105% with an RSD of 5%.

2.6 ZEN analysis

A 25-g sample was homogenized with 125 ml of acetonitrile/water (75/25, v/v) for 30 min and filtered through Whatman No. 4 paper. A 10-ml volume of the extract was then diluted with 40 ml of phosphate buffered saline (PBS) and then 25 ml of the diluted sample applied to a ZEN IAC. The column was then washed with 20 ml of PBS and dried by blowing air through the column. ZEN was finally eluted using three 1.5-ml volumes of acetonitrile. A 20-μl sample of the extract was finally suction-filtered through a 0.45-μm nylon membrane and injected into the HPLC system.

Chromatographic separation was performed on a C_{18} column (150 mm×4.6 mm, 5 μm particle size), with a methanol/acetonitrile/water (10/44/46, v/v/v) mobile phase and 0.8 ml/min flow rate. The excitation and emission wavelengths were 274 and 440 nm, respectively, and the column temperature was 25 °C. LOD and LOQ for this method were 2.0 and 6.2 μg/kg, respectively, and the mean recovery was 83% with an RSD of 5%.

2.7 T-2 analysis

A 25-g sample was homogenized with 125 ml of methanol/water (90/10, v/v) for 30 min and filtered through Whatman No. 4 paper. A 7-ml volume of the extract was mixed with 28 ml of ultrapure water, passed through a glass fiber filter paper, and then 25 ml of the resulting solution was applied to a T-2 IAC. The column was washed with 20 ml of ultrapure water and T-2 eluted using three 1.5-ml volumes of methanol. The eluate was evaporated to dryness under a steam of nitrogen at 50 °C.

T-2 samples were derivatized with 4-dimethylaminopyridine (4-DMAP) and 2-naphthoyl chloride (2-NC) reagents according to a previously published method (Wang *et al.*, 2013). The sample was then reconstituted with 1 ml of acetonitrile/water (75/25, v/v) and suction-filtered through a 0.45-μm nylon membrane, and a 20-μl volume was analyzed on the HPLC system.

Chromatographic separation was performed on a C_{18} column (150 mm×4.6 mm, 5 μm particle size), with an acetonitrile/water (75/25, v/v) mobile phase and 0.6 ml/min flow rate. The excitation and emission wavelengths were 381 and 470 nm, respectively, and the column temperature was 30 °C. LOD and LOQ for this method were 1.8 and 5.4 μg/kg, respectively, and the mean recovery was 96% with an RSD of 6%.

2.8 OTA analysis

A 25-g sample was homogenized with 100 ml of acetonitrile/water (60/40, v/v) for 30 min and filtered through Whatman No. 4 paper. A 2-ml volume of the extract was diluted with 22 ml of PBS and then applied to an OTA IAC. The column was washed with 20 ml of PBS and dried by flushing the column with air. OTA was eluted using three 1.5-ml volumes of methanol and 20 μl of the eluate was suction-filtered through a 0.4-μm nylon membrane and injected into the HPLC system.

Chromatographic separation was achieved on a C_{18} column (150 mm×4.6 mm, 5 μm particle size), with an acetonitrile/acetic acid/water (51/2/47, v/v/v) mobile phase and 1 ml/min flow rate. Fluorescence was detected at 333 and 443 nm for excitation and emission, respectively, and the column temperature was 40 °C. LOD and LOQ were 0.09 and 0.28 μg/kg, respectively, and the mean recovery was 84% with an RSD of 3%.

2.9 Statistical analysis

Levels of mycotoxins were analyzed with one-way analysis of variance (ANOVA). The comparisons using the chi-square test were carried out for the contamination incidence of mycotoxins in separate zones. In all the tests, the differences were considered significant at $P<0.05$. Data analyses were performed using the software package SPSS 16.0.

3 Results

In the core zone, only seepweed seed and leaf were contaminated with AFB_1. The incidence and levels in the samples from buffer and experimental zones were significantly higher than those from core zone (Table 2). In the buffer and experimental zones, levels of AFB_1 and OTA were significantly higher in rice grain than in fish and shrimp, and levels of DON and ZEN were higher in rice grain than in wheat seedings (Table 3).

The co-occurrence of mycotoxins in mycotoxin-positive samples is shown in Table 4. Among the samples, 59.3% (16/27) were found to be co-contaminated with multiple mycotoxins. Among samples collected in the buffer and experimental zones, co-occurrence was 66.7% (16/24), while in the core zone, no samples were contaminated by multiple mycotoxins. In individual food-types and taking into account mycotoxin-positive samples, co-occurrence was the most frequent in rice grain (100.0%), which also showed the most frequent combination of 3 mycotoxins (40.0%), followed by 4 mycotoxins (33.3%) and 2 or 5 mycotoxins (13.3%). In fish and shrimp, the mycotoxin co-occurrence appeared in one only positive sample (6.3%). Wheat seedling samples were only contaminated with DON or ZEN.

Table 2 Occurrences of AFB_1, DON, ZEN, T-2, and OTA in red-crowned crane's food in the core, buffer, and experimental zones

Mycotoxin	Zone	Positive samples*	Contamination incidence (%)	Concentration (μg/kg)	
				Mean±SD	Range
AFB_1	Core	6 (64)	9.4[a]	1.90±0.62[a]	LOD–2.43
	Buffer and experimental	18 (49)	36.7[b]	39.92±25.10[b]	0.09–80.30
DON	Core	0 (64)	0[a]		
	Buffer and experimental	18 (49)	36.7[b]	778.63±400.23	LOD–1587.30
ZEN	Core	0 (64)	0[a]		
	Buffer and experimental	24 (49)	49.0[b]	201.01±140.97	LOD–447.30
T-2	Core	0 (64)	0[a]		
	Buffer and experimental	11 (49)	22.4[b]	38.08±28.31	LOD–100.20
OTA	Core	0 (64)	0[a]		
	Buffer and experimental	18 (49)	36.7[b]	5.14±3.76	LOD–10.70

LOD: limit of detection. * The data in parentheses represent the total sample numbers. [a, b] Values with different superscripts are significantly different ($P<0.05$) between zones within the same mycotoxin

Table 3 Concentrations of AFB_1, DON, ZEN, T-2, and OTA in red-crowned crane's food in the core, buffer, and experimental zones

Zone	Sample type	Concentration (μg/kg)				
		AFB_1	DON	ZEN	T-2	OTA
Core	Reed root					
	Seepweed seed and leaf	1.90±0.69[a]				
	Crabs[c] and snails					
Buffer and experimental	Rice grain	47.60±19.67[b]	1015.55±319.38[a]	221.84±135.81[a]	38.08±36.28	6.50±2.44[a]
	Wheat seedlings		146.87±120.12[b]	11.00±1.52[b]		
	Fish and shrimp	1.57±0.99[b]				1.37±1.17[b]

Data are expressed as mean±SD. [a, b] Values with different superscripts are significantly different ($P<0.05$) between food samples. [c] Crabs collected from reed pond and common seepweed community

Table 4 Frequencies of co-occurrences of AFB_1, DON, ZEN, T-2, and OTA in red-crowned crane's food

Zone	Sample type	Co-occurrence (%)			
		2[b]	3[b]	4[b]	5[b]
Core	Reed root	0	0	0	0
	Seepweed seed and leaf	0	0	0	0
	Crabs[a] and snails	0	0	0	0
Buffer and experimental	Rice grain	13.3	40.0	33.3	13.3
	Wheat seedlings	0	0	0	0
	Fish and shrimp	6.3	0	0	0

[a] Crabs collected from reed pond and common seepweed community. [b] Number of co-occurring mycotoxins

4 Discussion

Mycotoxins were detected in foods consumed by red-crowned cranes while overwintering in the Yancheng Biosphere Reserve. To our knowledge, this study is the first to assess mycotoxin contamination of the red-crowned crane's food. The present results indicated that these cranes were potentially exposed to mycotoxins during the entire wintering period. Seasonal weather patterns, preharvest, harvest, and storage conditions can place agricultural crops at risk for mycotoxin contamination (Schrödter, 2004; Georgiadou *et al.*, 2012; Torres *et al.*, 2014). Some studies have shown that rice not only contains considerable amounts of fungi but is also contaminated with mycotoxins (Park *et al.*, 2005; Nguyen *et al.*, 2007; Bansal *et al.*, 2011; Qiu and Shi, 2014). The high rice contamination observed in this study probably resulted from environmental conditions, especially moisture and temperature, which are conducive to fungal growth and toxin bioproduction. Some fungi, such as *Fusarium*, can infect wheat at some or any stage of growth, leading to fungal diseases (Xu, 2003; Lu *et al.*, 2008), and under favorable conditions, molds can produce mycotoxins (Krnjaja *et al.*, 2015). Fungicides are widely used to control these diseases (D'Mello *et al.*, 1998). However, pesticides, putting birds at risk, are not used in the reserve because of the active education program for local farmers. Therefore, pesticides are not implicated in mycotoxin production. It is most likely that time, temperature, humidity, and physical damage were primary factors interacting in complex ways to induce the toxins observed in wheat seedings. At present, all of the aquaculture production is based on the use of commercial foodstuffs. Many of the raw materials used in these feeds, including peanuts, wheat, soybean, maize, and fishmeal, have been shown to be contaminated with mycotoxin (Binder *et al.*, 2007; Rodrigues and Naehrer, 2012; Streit *et al.*, 2012). In addition, feed production, transport, and storage are other factors that can contribute to the presence of mycotoxins in animal feed (Jouany, 2007; Zinedine *et al.*, 2007). Mycotoxins in feed could carry over into animal organs or tissues (Marin *et al.*, 2013). AFB_1 contamination in fish and shrimp might be explained by the fact that the aquafeeds were

contaminated. Among the foods collected in the core zone, only seepweed seed and leaf were contaminated by AFB_1, and at very low concentration. To our knowledge, no study regarding mycotoxin occurrence in common seepweed has been reported. It is possible that this plant is susceptible to infection by AF-producing fungi, as are wheat, barley, and maize.

Acute toxicity of mycotoxins requires intake of relatively large quantities of contaminated foods over a short period. The mean weight of adult red-crowned cranes is 6–11 kg and the average daily food consumption is 324.3 g (Ma and Li, 2002; Dong *et al.*, 2005). Because of a lack of information regarding the relative sensitivity of red-crowned cranes to mycotoxins, the 50% lethal doses (LD_{50}) of other birds were referenced here to assess the possibility of acute toxicity in these cranes (Pain *et al.*, 2004). Toxin concentrations leading to acute toxicity are presented in Table 5. If the LD_{50} for cranes is similar to that for these other birds, all values of mycotoxin-positive samples were lower than these threshold concentrations and the possibility of acute toxicity in these cranes was ruled out (Tables 2 and 5). In spite of this, comparison of these concentrations to those used in laboratory experiments suggested that these cranes could have been subject to chronic mycotoxic effects. These negative effects include growth retardation, tumor development, and immunotoxicity, thus increasing crane susceptibility to predation and disease (Carnaghan, 1965; Choudhury, 1971; Huff *et al.*, 1986; Kamalavenkatesh *et al.*, 2005). Moreover, although low mycotoxin concentrations do not cause morbidity in controlled laboratory settings, it might reduce the survival probability of wild species that rely on highly efficient metabolic systems to cope with extreme environments, such as winter conditions (Oberheu and Dabbert, 2001). In addition, the simultaneous occurrence of more than one mycotoxin was common in analyzed mycotoxin-positive samples. The implications of the interaction between different mycotoxins have been investigated. For example, when ZEN and DON occur simultaneously in the same substrate at permitted feed concentrations, the combination causes serious physiological effects in weaning piglets (Chen *et al.*, 2001). Specifically, ZEN and DON decrease the levels of globulin, albumin, total protein, anticlassical swine fever antibody titers, and the mRNA expression of interferon (IFN)-α, tumor necrosis factor (TNF)-γ, and interleukin (IL)-2. The serum enzyme activities of γ-glutamyltransferase, aspartate aminotransferase, and alanine aminotransferase increase at the same time. Histopathologically, alterations of the kidney, spleen, uterus, lymph node, and liver were found. A combination of T-2 and ZEN decreases cultured renal cell viability and increases reactive oxygen species production and heat shock protein 70 expression, compared with their individual effects (Bouaziz *et al.*, 2013). Combined exposure to several classes of mycotoxins could lead to an additive effect, with a few minor exceptions, which indicated a synergistic interaction (Speijers and Speijers, 2004). Therefore, multiple mycotoxin contamination might pose a serious health hazard to the endangered cranes in the Yancheng Biosphere Reserve. Although artificial wetlands offer lower food availability and increased human disturbance, cranes fly to these wetlands to feed (Wang *et al.*, 2011; Li *et al.*, 2013). This might result from the limited carrying capacity of the natural wetlands, not the presence of food types in the artificial areas. The loss and degradation of wetlands worldwide has adversely affected waterbirds, which depend on wetland habitats (Ma *et al.*,

Table 5 Mycotoxin LD_{50} values for selected bird species and the concentrations leading to acute toxicity in red-crowned crane

Mycotoxin	Species	LD_{50} (mg/kg)	Concentration* (mg/kg)
AFB_1	Ducklings	0.335 (Lijinsky and Butler, 1966)	6.2–11.4
	Chickens	6.5–16.5 (Smith and Hamilton, 1970)	120.3–559.6
DON	Ducklings	27 (Yoshizawa and Morooka, 1974)	499.5–915.8
	Chickens	140 (Huff *et al*., 1981)	2590.0–4748.3
ZEN	Chickens	>15 000 (Chi *et al*., 1980)	>508 750.5
T-2	Chickens	4.97 (Chi *et al*., 1978)	91.9–168.6
OTA	Chickens	3.3 (Peckham *et al*., 1971)	6.1–111.9

* The concentrations leading to acute toxicity in red-crowned crane are calculated based on mycotoxin LD_{50} values for selected bird species, the mean weight of adult red-crowned cranes, and the average daily food consumption

2010). From 1984 to 2008, the natural wetlands in the reserve's core zone decreased by up to 73% (Wang, 2012). From 1980 to 2008, the area of natural wetlands was in danger, with human activities as the main driving forces of reserve wetland degradation (Gu *et al.*, 2012). The deterioration of natural wetland quality has led to changes in feeding habitats in many waterbird species, increasing their reliance on artificial wetlands (Zuo *et al.*, 2004). Before 1996, there was no record of these cranes feeding on farmlands (Li *et al.*, 1997). Almost all cranes select the core zone as their roost habitat, but some of them move to artificial wetlands for daytime feeding because of the limited natural wetlands (Lv, 2007).

Artificial wetlands have been recognized as important areas for conservation of cranes and other waterbirds (Bellio *et al.*, 2009; Wang *et al.*, 2011; Dias *et al.*, 2014). These wetlands provide diurnal roosts and feeding grounds for birds. Lee *et al.* (2007) found that unplowed rice fields provide more food for red-crowned cranes and white-naped cranes (*G. vipio*) than plowed fields in the Cheolwon area of the Civilian Control Zone area of South Korea, and for conservation efforts, made recommendations that fields should remain unplowed until the foraging birds leave. However, from the present study, when compared with natural wetlands in the Yancheng Biosphere Reserve, artificial wetlands, especially rice fields, might not serve as good habitats for these birds.

Based on the present results, efficient and effective recommendations that conservationists could employ are needed to mitigate crane's food safety risks associated with these toxins. Thus, the following recommendations are provided for crane conservation. First, natural wetlands should be conserved and restored, providing birds with sufficient food resources, decreasing the use of artificial areas, and reducing mycotoxin exposure. Second, fish farmers in the buffer and experimental zones should be educated regarding the harm of contaminated feeds and encouraged to use toxin-free products. Third, monitoring of rice fields for mycotoxins should be done routinely and continuously. Once mycotoxins from waste rice grain are identified as a potential cause of bird mortality, local rice growers should be persuaded to till the waste grain. Fourth, local farmers should be encouraged to plant grains that are less susceptible to the growth of mycotoxin-producing mold to minimize the birds' exposure to toxins.

In conclusion, foods potentially utilized by red-crowned cranes were contaminated with mycotoxins in the Yancheng Biosphere Reserve. The mycotoxin incidences and concentrations in samples collected in the buffer and experimental zones were higher than those from the core zone. Mycotoxin-contaminated foods might represent a serious health threat to these birds. As cranes are migratory birds, future work will focus on collecting the cranes' foods from other overwintering areas, breeding areas, and stopover sites to determine whether these foods are also contaminated by mycotoxins.

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Compliance with ethics guidelines

Da-wei LIU, Hong-yi LIU, Hai-bin ZHANG, Ming-chang CAO, Yong SUN, Wen-da WU, and Chang-hu LU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：盐城生物圈保护区越冬丹顶鹤受到真菌毒素威胁的研究

目　的：调查在盐城生物圈保护区越冬的丹顶鹤食物是否受到真菌毒素污染以及评估丹顶鹤受到真菌毒素威胁的风险。

创新点：首次证明在盐城生物圈保护区越冬的丹顶鹤受到真菌毒素的威胁。

方　法：2013 年 11 月至 2015 年 3 月，两个越冬期内在盐城生物圈保护区采集丹顶鹤不同觅食生境内 113 份食物样品。使用高效液相色谱法对这些食物中毒素（黄曲霉毒素 B_1（AFB_1）、脱氧雪腐镰刀菌烯醇（DON）、玉米赤霉烯酮（ZEN）、T-2 毒素（T-2）和赭曲霉毒素 A（OTA））含量进行检测。

结　论：盐城生物圈保护区内丹顶鹤食物受到真菌毒素的污染，人工湿地尤其是稻田不适宜作为鸟类的觅食地。

关键词：食物；真菌毒素；丹顶鹤；盐城生物圈保护区