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Short Communication

Mycotoxin Contamination in Japanese Domestic Feed

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This study summarizes the levels of the mycotoxins aflatoxin B_1, deoxynivalenol, zearalenone, ochratoxin A, and fumonisin B_1 in domestic feed produced in Japan. We collected samples of Japanese domestic feed used in livestock farming establishments or by small farmers from April 2012 to March 2014, and measured mycotoxin concentrations in each sample. Regarding corn, deoxynivalenol had the highest detection rate (84%), maximum value (2370 µg/kg), and mean value (400 µg/kg). These results suggest that deoxynivalenol is a major mycotoxin contaminant in Japanese-produced domestic feed. Zearalenone and fumonisin B_1 presented the second highest detection rates. The maximum concentration of zearalenone was 1200 µg/kg in grass samples, but its median was under 25 µg/kg, and, overall, it occurred at low concentrations. The detection rate of fumonisin B_1 was about 30%, but its maximum concentration in corn was 2400 µg/kg, slightly higher than deoxynivalenol. Overall, mycotoxin concentrations were lower in grass than in corn. Although deoxynivalenol was detected in samples collected from all regions, concentrations in samples from Hokkaido were particularly high. Fumonisin B_1 was mainly distributed in Kanto and the southern regions. Concentrations of ochratoxin A and aflatoxin B_1 were low; however, the maximum concentration of aflatoxin B_1 was 22 µg/kg in corn. Although aflatoxin B_1 and ochratoxin A were rarely detected, they occasionally presented high levels, therefore requiring attention. Overall, mycotoxins produced by *Fusarium* sp. fungi require attention in Japanese-produced domestic feed.

Key words: aflatoxin, deoxynivalenol, fumonisin, Japanese domestic feed, mycotoxin, zearalenone

Introduction

Mycotoxin contamination of foods, especially cereals and animal feeds, is a serious problem worldwide. To avoid feed mycotoxin contamination, it is necessary to accurately assess the most likely conditions of such contamination. The worldwide surveys of feed mycotoxin contamination[1,2] did not include samples of forage crops produced in Japan. However, some mycotoxins have been reported from Japanese forage corn and rice[3,4]. To ensure the safety of feed crops produced in Japan, it is necessary to accurately assess the concentration of mycotoxins in such crops. Therefore, the present study measured mycotoxin concentrations in domestic feed (forage crops) produced by livestock farming establishments or by small farmers in Japan. The data will contribute for developing measures against mycotoxin contamination of Japanese-produced feed.

Materials and Methods

Samples of Japanese domestic feed used in livestock farming establishments or by small farmers were collected from

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Abbreviations: AFB_1: aflatoxin B_1, DON: deoxynivalenol, FB_1: fumonisin B_1, OTA: ochratoxin A, ZEA: zearalenone

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Fig. 1. Regions of Japan surveyed in this research.

April 2012 to March 2014. Based on Japanese regions' classification, Japan was divided into the following six blocks (**Fig. 1**): Hokkaido, Tohoku, Kanto, Hokuriku-Tokai-Kinki, Chugoku-Shikoku, and Kyushu-Okinawa. However, no samples were collected from Tohoku and Okinawa. Samples were randomly collected from small sites in multiple plots in each region.

Analyses of mycotoxins were carried out as previously reported[1]. In brief, for determining deoxynivalenol (DON) contamination, a 25-g portion of ground (Romer® Series II Mill; Romer Labs. Inc., MO, USA) feed sample was thoroughly mixed with 100 mL of acetonitrile:water (84:16). After filtration (filter paper #1; Whatman, UK), the filtrate was purified using a multifunction column (MultiSep 227 Trich+ and Multisep® #216, Romer Labs Inc.). After bringing the filtrate to an appropriate concentration, DON was measured using an ultraviolet detector for high performance liquid chromatography (HPLC-UV Agilent 1100 Series; Hypersil® ODS column, 2.1 × 100 mm, 5-μm; 30°C, 220 nm; Agilent® Technologies, Germany). For aflatoxin B_1 (AFB_1), the extract solution was obtained as described for DON, and 4 mL of the filtrate was then slowly passed through a Mycosep #226 (Romer Labs Inc.) column; 100 μL of the eluent was diluted with 440 μL of water, and AFB_1 was measured by HPLC-fluorescence (Agilent 1100 Series, Zorbax SB-Aq column 4.6 mm × 150 mm, 5-μm; 30°C, excitation: 360 nm, emission: 440 nm; Agilent® Technologies). For zearalenone (ZEA), the extraction and purification method followed that of AFB_1, and ZEA was measured by HPLC-fluorescence (Agilent 1100 Series, Hypersil® ODS column 2.1 mm × 100 mm, 5 μm; 30°C, excitation: 235 nm, emission: 460 nm; Agilent® Technologies). For fumonisin B_1 (FB_1) determination, a 25-g portion of ground feed sample was thoroughly mixed with 100 mL of acetonitrile:water (50:50). After pH adjustment (6–9), 3 mL of sample extract was diluted with 8 mL of methanol:water (3:1), and this 11-mL sample was passed through a preconditioned Multisep® #211 column (Romer® Labs. Inc.). After rinsing the purified sample with 8 mL of methanol/water (3:1), FB_1 was eluted with 10 mL of methanol:acetic acid (99:1) and the solvent was evaporated at 60°C. The sample was then dissolved in 1 mL of methanol before derivatization with naphthalene 2,3-dicarboxaldehyde (NDA). The methanol-dissolved sample was supplemented with 5 mL of 0.05 M sodium borate buffer (pH 9.5), 0.5 mL of sodium cyanide reagent, and 0.5 mL of NDA reagent (25 mg NDA in 100 mL of methanol). This mixture was vortexed and heated for 20 min at 60°C. After cooling to room temperature, the reaction was stopped with 7 mL of 0.05 M phosphate buffer:acetonitrile (40:60), and FB_1 was finally measured by HPLC-fluorescence (Agilent 1100 Series, Hypersil® ODS column 2.1 mm × 200 mm, 5 μm; 40°C, excitation: 420 nm, emission: 500 nm; Agilent® Technologies). For ochratoxin A (OTA) determination, 25 g of ground sample was extracted with 100 mL of acetonitrile:water (60:40). The sample extract (4 mL) was diluted with 28 mL of phosphate buffered saline (PBS, pH 7.4) and applied to an OchraStar® immuno-affinity column (Romer® Labs Diagnostic, Austria). After washing the purified sample with 2 × 10 mL of 0.2 M phosphate buffered saline, OTA was eluted with 3 mL of methanol:acetic acid (98:2) and measured by HPLC-fluorescence (Agilent 1100 Series, Hypersil® ODS column 2.1 mm × 200 mm, 5 μm; 40°C, excitation: 330 nm, emission: 460 nm; Agilent® Technologies). The lower limits of quantitation (LOQ) for mycotoxin concentrations were as follows: AFB_1, 1 μg/kg; DON, 50 μg/kg; ZEA, 10 μg/kg; OTA, 0.5 μg/kg; and FB_1, 100 μg/kg. Concentrations below the respective LOQ were considered "not detected" (ND) and used as zero.

Results and Discussion

Mycotoxin concentrations in corn and grass samples over the 2-year period are summarized in **Table 1**. Considering corn samples, DON had the highest detection rate (84%), maximum value (2370 μg/kg), and average value (400 μg/kg). This maximum value exceeded the Japanese regulation

Table 1. Mycotoxin concentaration in Japanese domestically produced feed: shown in crop types.

	DON	ZEA	OTA	FB_1	AFB_1
Corn					
Mean (μg/kg)	400	46	<0.5	140	<1
Maximun (μg/kg)	2370	570	3	2400	22
Median (μg/kg)	250	<10	<0.5	<100	<1
Detection ratio (%)	84	50	5	29	1
Number of samples	124	124	124	124	124
Grass					
Mean (μg/kg)	210	110	<0.5	28	<1
Maximun (μg/kg)	3010	1200	18	850	<1
Median (μg/kg)	<50	<10	<0.5	<100	<1
Detection ratio (%)	44	48	32	10	<1
Number of samples	90	90	90	90	90

DON: deoxynivalenol, ZEA: zearalenone, OTA: ochratoxin A, FB_1: fumonisin B_1, AFB_1: aflatoxin B_1
Detaction ratio: calculated following formula {(Number of over limit of qualification value/Number of samples) *100}

value (1 or 4 mg/kg)[5]. In fact, 11 corn samples exceeded 1000 μg DON per kg in the present research. These data suggest that DON is a mycotoxin that requires attention in Japanese-produced feed. The next highest detection rates were for ZEA and FB_1. The maximum concentration of ZEA was 570 μg/kg, but its median value was under 10 μg/kg, and it generally occurred at low concentrations. Moreover, no samples presented ZEA values exceeding that regulated for Japan (1 mg/kg)[5]. The detection rate of FB_1 was 29%, which was not high. However, the maximum concentration of FB_1 was 2400 μg/kg, equivalent to that of DON. The detection rate of OTA was 5%, and its maximum value was 3 μg/kg. Both detection rate and maximum concentration were low, indicating that OTA is not a matter of frequent concern in Japanese-produced feed. Although AFB_1 was detected in only one sample, its concentration was 22 μg/kg, therefore exceeding the Japanese regulation value of 20 μg/kg[5] for corn.

Detection rates of mycotoxins were generally lower in grass (grass silage and hay) than in corn, except for OTA. Average values were also lower in grass than in corn, except for ZEA. The maximum concentration of ZEA (1200 μg/kg) was also higher in grass than in corn, exceeding the regulation value for Japanese feed (1 mg/kg)[5], and two samples presented values over 1000 μg/kg. However, the median values of OTA and ZEA were bellow 2 μg/kg.

Table 2 shows the results for corn in each region. In Hokkaido, the maximum concentration of DON was 2370 μg/kg, the mean was 450 μg/kg, the median was 300 μg/kg, and the detection rate was over 90%. All these values exceeded those measured in the other regions (excluding Kyushu where there was only one sample). In Kanto, the maximum concentration of FB_1 was 2400 μg/kg, the mean was 410 μg/kg, and the median was 190 μg/kg. These values were higher than those measured in other regions. In Hokuriku-Tokai-Kinki, and Chugoku-Shikoku, FB_1 had high detection rates and means, although the number of samples was small. The detection ratio of FB_1 was much lower in Hokkaido than in Kanto region, suggesting this mycotoxin is more largely distributed in Kanto than in Hokkaido. However, there were no samples from the Tohoku region, which is located between Hokkaido and Kanto. Uozumi et al.[3] reported high concentrations of DON (maximum 16 mg/kg) and FB_1 (maximum 5.5 mg/kg) in forage corn grown in the Tohoku region. Because these data were collected during a cultivation experiment, rather than from domestic feed, they cannot be directly compared with our results. However, it is interesting that the Tohoku region seems to have intermediate DON and FB_1 concentrations between Hokkaido and Kanto. These regional differences might be strongly related to climate[6], but also to human social activities and/or transportation of crops. However, detailed research is necessary to find the influence of such factors in mycotoxin contamination concentrations.

In summary, mycotoxin concentrations in Japanese domestic feed crops varied according to crop type and to the region where the feed was produced. In general, the results

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Table 2. Mycotoxin concentaration in Japanese domestically produced feed (corn): shown in production eareas.

	DON	ZEA	OTA	FB_1	AFB_1
Hokkaido					
Mean (μg/kg)	450	52	<0.5	<100	<1
Maximun (μg/kg)	2370	570	3	1110	<1
Median (μg/kg)	300	29	<0.5	<100	<1
Detection ratio (%)	93	57	5.1	23	<1
Number of samples	98	98	98	98	98
Kanto					
Mean (μg/kg)	270	26	<0.5	410	<1
Maximun (μg/kg)	1430	310	<0.5	2400	<1
Median (μg/kg)	110	<10	<0.5	190	<1
Detection ratio (%)	56	25	0	56	0
Number of samples	16	16	16	16	16
Hokuriku-Tokai-Kinki					
Mean (μg/kg)	44	23	<0.5	210	<1
Maximun (μg/kg)	260	110	<0.5	590	<1
Median (μg/kg)	<50	<10	<0.5	170	<1
Detection ratio (%)	17	33	0	50	0
Number of samples	6	6	6	6	6
Chugoku-Shikoku					
Mean (μg/kg)	<50	<10	0.7	130	7
Maximun (μg/kg)	90	<10	2	200	22
Median (μg/kg)	50	<10	<0.5	<100	<1
Detection ratio (%)	66	0	33	33	33
Number of samples	3	3	3	3	3
Kyushu					
Mean (μg/kg)	581	<10	<0.5	<100	<1
Maximun (μg/kg)	581	<10	<0.5	<100	<1
Median (μg/kg)	581	<10	<0.5	<100	<1
Detection ratio (%)	100	0	0	0	0
Number of samples	1	1	1	1	1

DON: deoxynivalenol, ZEA: zearalenone, OTA: ochratoxin A, FB_1: fumonisin B_1, AFB_1: aflatoxin B_1
Detaction ratio: calculated following formula {(Number of over limit of qualification value/Number of samples) *100}

suggest that mycotoxins produced by *Fusarium* sp. fungi require attention in Japanese-produced feed. Contamination by DON was high in all regions, and contamination by FB_1 was observed in Kanto and southern regions. The degree of contamination by OTA and AFB_1 was low. Although these results are similar to those for East Asia (Korea, China, and Taiwan) reported in a global survey[1)], our data result from a fragmentary survey. Future research including detailed surveys (increasing the number of sampling points and sample size, for example) is therefore needed.

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Conflict of interest

The authors have no conflict of interest.

References

1. Binder EM, Tan LM, Chin LJ, Handl J, Richard J. Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. *Anim Feed Sci Technol*. 2007; **137**: 265–282. doi:10.1016/j.anifeedsci.2007.06.005
2. Hassan ZU, Al-Thani RF, Migheli Q, Jaoua S. Detection of toxigenic mycobiota and mycotoxins in cereal feed market. *Food Control*. 2018; **84**: 389–394. doi:10.1016/j.foodcont.2017.08.032
3. Uozumi S, Ashida N, Owari T, et al. Differences in resistance to corn ear rot and mycotoxin accumulation between cultivars of silage corn (*Zea mays* L.). *Jpn Soc Grassl Sci.* 2013; **59**: 98–104. (in Japanese with English abstract).
4. Uegaki R, Tohno M, Yamamura K, Tsukiboshi T. Changes in the concentration of fumonisins in forage rice during the growing period, differences among cultivars and sites, and identification of the causal fungus. *J Agric Food Chem*. 2014; **62**: 3356–3362. PMID:24628734, doi:10.1021/jf405358h
5. Food and Agricultural Materials Inspection Center Reference value of harmful substances in feed. http://www.famic.go.jp/ffis/feed/r_safety/r_feeds_safetyj22.html, Accessed February 28, 2018.
6. Van der Fels-Klerx HJ, Liu C, Battilani P. Modelling climate change impacts on mycotoxin contamination. *World Mycotoxin J*. 2016; **9**: 717–726. doi:10.3920/WMJ2016.2066