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Peculiarities of feed contamination with citrinin and ochratoxin A

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ABSTRACT

Occurrence of citrinin and ochratoxin A in different feed ingredients and compound feeds was screened by accredited methods based on the indirect competitive enzyme-linked immunosorbent assay. High frequency co-occurrence of both toxins was found in wheat grain and processed sunflower seeds. Citrinin levels exceeded those of ochratoxin A in the majority of co-contaminated feed samples, and the ratio of (1.1 - 10):1 proved to be the most frequent. A possible role of *Aspergillus* and *Penicillium* fungi in separate and simultaneous OTA and CIT occurrence in feeds is also discussed.

Keywords: Ochratoxin A; Citrinin; Immunoassay; Feeds; Cereals

1. INTRODUCTION

Ochratoxin A (OTA) and citrinin (CIT) have long been known as factors of mycotoxic animal nephropathy. Both toxins were detected in barley grain by Krough and Hasselager [1]. The contaminated barley was able to induce experimental nephropathy in rats and pigs. Later OTA and CIT were found in mouldy barley and rye the consumption of which caused swine nephropathy in natural conditions [2]. In 1970s the incidence rate of these nephrotoxins was first determined for barley and oat grain received from Danish pig farms [3] as well as for hay and feed wheat, oats, barley and rye in Canada [4-6]. After that various feeds were investigated for OTA and CIT occurrence in India [7].

In recent years it has been established that OTA has properties of genotoxic carcinogen while CIT acts as non-enzymatic bioactivator of OTA, that is, CIT promotes OTA-DNA adduct formation thus increasing the negative effect of OTA [8]. In this connection the investtigation of the distribution of both toxins in agricultural commodities is of particular relevance. Therefore, in this study we made a large-scale assessment of the contamination of compound feeds and several types of feed ingredients (cereal grain, processed grain and oil seeds) with OTA and CIT. The obtained data were presented as separate and simultaneous OTA and CIT occurrence values and as ratios of their levels.

2. MATERIALS AND METHODS

2.1. Sample Collection

A total of 2380 samples of 10 different feed products (wheat, barley and maize grain, wheat bran, soy-bean and sunflower seed meal and cakes, maize "gluten", compound feeds) were submitted to our Institute in 2003-2009 from poultry and pig farms in Russia either in connection with complaints about livestock performance or for a regular feed quality control as part of veterinary sanitation program. Wheat and barley grain originated from the central part of European Russia. Sunflower oil-seed meal and cakes as well as maize "gluten" were home-manufactured while most samples of maize grain and soy-bean oil-seed meal and cakes were imported.

2.2. Mycotoxin Analyses

The determination of CIT and OTA was performed by the accredited methods based on indirect enzyme-linked immunosorbent assay (ELISA) [9,10]. First, finely ground feed samples were shaken with the mixture of acetonitrile and water (84:16, v/v) in an orbital shaker for 1 - 2 s, then incubated for 14 - 16 h at room temperature and shaken again. Filtrates were diluted with buffer and subjected to indirect ELISA. The microtiter plate wells were each coated with 200 µl solid phase antigen solutions. After overnight incubation at 4˚C, the plates were washed four or five times by filling each well with 300 µl 0.05 M phosphate-buffered saline containing 0.05% Tween 20. Then, 100 µl of the solutions of either toxin standard or sample extracts and 100 µl of specific antibody solution were added together into each well and incubated for 1h at room temperature. The plates were

washed again four or five times, as already described, and 200 µl of horseradish peroxidase conjugate was added. After incubation for 1h at room temperature, the plates were washed again four times and 200 µl of o-phenylenediamine substrate solution was added. Then, incubation at room temperature in the dark for 30 min followed, and the reaction was terminated by adding 50 µl of 4 M sulfuric acid with 0.1 M sodium sulfite to each well. Absorbency was determined at 490 nm using Dynatech MR-250 reader (Germany). Sample results were calculated from the standard curves. The limits of detection of OTA and CIT were 4 and 10 ppb respectively. Intercomponent cross of test systems for OTA and CIT were about 0.1%. Sample extracts and standards were run in duplicate. Variation coefficient did not exceed 15%. Data was processed with the Minitab Statistical Software (version 15).

3. RESULTS AND DISCUSSION

Total values of toxin occurrence in compound feeds were calculated for the set of 1231 specimens and amounted to 29.0% for OTA and 12.9% for CIT. Feed ingredients which usually make the basis of such ration compositions differed in frequency of toxic contamination with OTA and CIT. These toxins were seldom detected in soy-bean oil-seed meal and cake—only in 2 and 3 specimens out of 183 examined. The number of OTA-positive samples was rather similar in different kinds of grain (30 out of 318 samples of wheat, 15/190 barley and 17/187 maize), appeared to be higher for wheat bran (10/48) and was the highest for maize "gluten" (27/55) and sunflower oil-seed meal and cake (79/168). The incidence rate of CIT in the same feed ingredients (with the exception of sunflower oil-seed meal and cake) was two and more times less than that of OTA. The drop in the number of CIT-positive sunflower oil-seed meal and cake samples was not so sharp (51/168). The results corresponded to the ones obtained earlier [9,11].

The distribution of toxin-positive feed samples with different types of contamination (two toxins or only one) is presented in **Table 1**. The frequency of separate CIT occurrence was about 10% in compound feeds and in all the ingredients with the exception of "gluten" where CIT did not occur separately. OTA as a single contaminant was found in more than half (60% - 80%) of the samples of barley grain, maize grain, wheat bran, "gluten" and compound feeds. Meanwhile, less than half (40%) of the samples of wheat grain and sunflower oil-seed meal and cakes were OTA-positive. These results are also confirmed by the works of other researchers. As limited surveys conducted in India indicated, about 44% and 51% samples of compound feeds and feed ingredients were CIT-positive singly and in co-occurrence with OTA [7], while OTA in different seasons was present either in 76% or in 48% of sunflower cake samples [12]. Ready-made poultry feeds in Pakistan provided about 50% of OTA-positive samples [13].

The percentage of simultaneous OTA + CIT occurrence in compound feeds was high enough (27%). OTA + CIT occurrence was also detected in half of the toxin-positive samples of sunflower oil-seed meal and cakes and in wheat grain. It could be explained by the fact that wheat grain and sunflower oil-seed meal and cakes have traditionally been used as invariable components in ration preparation in Russia. In France, too, a high percentage (33%) of wheat samples (collected from farms in various parts of the country) were co-contaminated with OTA + CIT [8].

Table 1. Occurrence of ochratoxin A and citrinin in different kinds of feedstuffs and in compound feeds.

Feeds	Analyzed samples, *n*	Positive samples, n^+		Positive samples, n^+ with a single toxin and with both		
		OTA	CIT	OTA	OTA + CIT	CIT
Wheat grain	318	30	19	14	16	3
Barley grain	190	15	6	10	5	1
Maize grain	187	17	7	12	5	2
Wheat bran	48	10	2	9	1	1
Maize "gluten"	55	27	10	17	10	0
Soy-bean oil-seed meal and cake	183	2	3	1	1	2
Sunflower oil-seed meal and cake	168	79	51	34	45	6
Compound feeds	1231	357	159	246	111	48
Total	2380	537	257	343	194	63

The data on the toxin levels in the samples with separate and simultaneous occurrence is summarized in **Table 2**. Amounts of the toxins were below 100 μg/kg in most of the samples with one toxin with the exception of a few samples of grain, "gluten" and compound feeds. In all the cases of joint toxin presence in grain and sunflower oil-seed meal and cakes the toxin accumulation was higher and in several cases reached 1000 μg/kg.

In samples with simultaneous toxin occurrence equal amounts of toxins (4/194, 2.1%) and predominance of OTA (17/194, 8.8%) were seldom found (**Table 3**). In wheat grain, sunflower oil-seed meal and cake, as well as in compound feeds this feature was quite distinct, possibly because on these types of feeds the fungi producing both toxins equally or with prevalence of OTA occurred very seldom. Also, it is possible that sometimes at the time of processing there can appear conditions promoting equilibrium biosynthesis of toxins or a shift towards the accumulation of OTA. Therefore, in OTA-positive grain products with a larger ratio of the outer layers of the grain kernel low concentrations of CIT are detected rather frequently [14].

In the absolute majority of samples (173/194, 89.1%) CIT amounts were higher than those of OTA. In two samples of this group the ratio of abundance (the ratio of CIT amount to OTA amount) was in marked contrast to the rest and amounted to 92.5 (compound feed) and 113.3 (sunflower meal). In all the other 171 samples CIT levels were 1.1 - 33.0 times higher than those of OTA with the predominance of samples with 1.1 - 10 times abundance (**Figure 1**). This range was typical of the most samples of wheat grain, sunflower oil-seed meal and cake as well as of compound feeds. In all the samples of barley, maize grain and "gluten" and wheat bran with simultaneous toxin occurrence and CIT prevalence the ratio of CIT/OTA levels did not exceed 10 times.

For comparison: the relation of OTA and CIT amounts in one geese feed sample equalled 4 [15], while CIT lev-

Table 2. Levels of OTA and CIT in different kinds of feedstuffs and compound feeds with separate and simultaneous toxin occurrence.

Feeds	Level of toxins, μg/kg, in the samples			
	With separate toxin occurrence		With simultaneous toxin occurrence	
	OTA	CIT	OTA	CIT
Wheat grain	4 - 55	79; 173; 175	6 - 270	20 - 1000
Barley grain	4 - 25	371	5 - 102	32 - 998
Maize grain	5 - 141	20; 22	29 - 390	25 - 953
Wheat bran	4 - 10	24	5.6	50
Maize "gluten"	4 - 126	0	12 - 100	16 - 62
Soy-bean oil-seed meal and cake	4	14; 30	21	20
Sunflower oil-seed meal and cake	4 - 100	16 - 91	4 - 186	14 - 1020
Compound feeds	4 - 250	12 - 182	4 - 141	16 - 740
Total	4 - 250	12 - 371	4 - 390	14 - 1020

Table 3. Relation of OTA and CIT levels in different kinds of feedstuffs and compound feeds with simultaneous toxin occurrence.

Feeds	Samples with simultaneous toxin occurrence, n^+	Including samples with different ratio of toxin levels		
		CIT > OTA	CIT = OTA	CIT < OTA
Wheat grain	16	15	0	1
Wheat bran	1	1	0	0
Barley grain	5	4	0	1
Maize grain	5	4	0	1
Maize "gluten"	10	5	0	5
Soy-bean oil-seed meal and cake	1	0	1	0
Sunflower oil-seed meal and cake	45	43	0	2
Compound feeds	111	101	3	7
Total	194	173	4	17

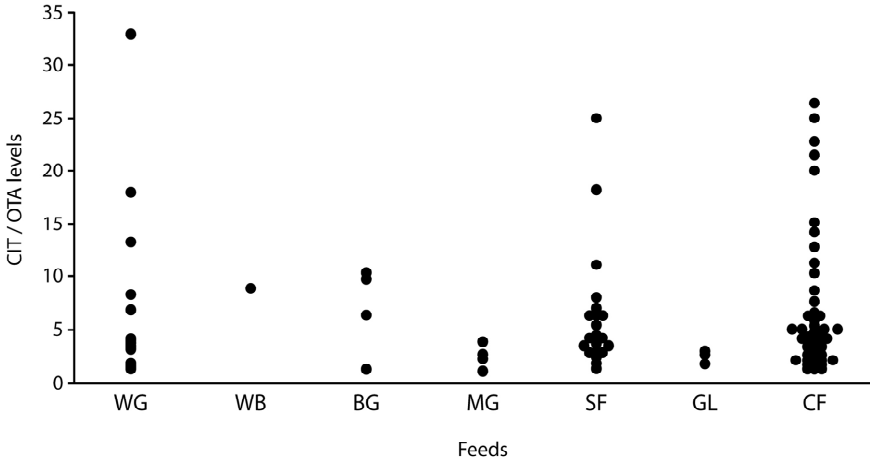

Figure 1. Ratio of CIT/OTA levels in samples of feedstuffs [WG = wheat grain; WB = wheat bran; BG = barley grain; MG = maize grain; SF = sunflower oil-seed meal and cake; GL = maize "gluten"] and compound feeds [CF] with simultaneous toxin occurrence when CIT levels prevail.

els in seven cereal samples with simultaneous toxin occurrence (the samples were collected in 1998 in Bulgarian villages with a history of Balkan endemic nephropathy) were 2 - 200 times higher than those of OTA [16].

Obviously the revealed differences in the character of OTA and CIT occurrence in feeds are connected with the peculiarities of feed infection with microscopic toxigenic fungi, but so far we have not been able to give any plausible interpretation of the results. A large number of fungi species belonging to the genera *Aspergillus* and *Penicillium* are able to produce OTA and CIT [17,18]. However, no special investigation and identification of CIT producers in feeds has been attempted in our country, and there is very limited information on taxonomy and incidence of fungal species able to produce OTA. Only 11 *Aspergillus ochraceus* Wilhelm isolates and 5 *Penicillium viridicatum* Westling isolates obtained from different kinds of feeds were reported as being capable of producing OTA [19]. Not long ago an active strain of *A. alliaceus* Thom. & Church No. 115 was isolated from barley grain [20]. It is noteworthy that namely this species is most often considered to be a possible source of OTA in agricultural commodities [21,22].

The simultaneous occurrence of OTA and CIT in feeds is not unexpected. According to numerous scientific reports, the microscopic fungus *P. viridicatum* [4,23-26] later re-identified as *P. verrucosum* [17,27,28] as well as separate representatives of the species *P. purpurrescens*, *P. palitans*, *P. cyclopium* [17] are able to produce both mycotoxins. The joint production of substantial amounts of CIT and smaller amounts of OTA has recently been confirmed in our laboratory for a few strains of *P. viridicatum* Westl. isolated from the contaminated samples of feed pea (Urals, near Tiumen), from wheat grain (Yakutia) and from oat grain (European Russia, Lipetsk region) (unpublished data). According to the published data, the type of substrate and the surrounding conditions have a marked effect on the final biosynthetic profile the fungi capable of producing both toxins [24]. This, possibly, can explain significant variations in toxin ratios in feeds with simultaneous occurrence of OTA and CIT.

4. CONCLUSION

The data obtained in the course of this research confirm the wide incidence of OTA and CIT in feeds and especially in wheat grain, by-products of the industrial processing of maize grain, sunflower seeds and compound feeds. In most samples with joint toxin occurrence the CIT amount is 1.1 - 10 times greater than the OTA amount. The conditions of the biochemical interaction between OTA and CIT in vivo which could increase the negative effect of OTA on the human organism as well as the role of the quantitative relation between these substances will have to be cleared up in future. If CIT is able to activate OTA at equal or ten times greater concentrations, it becomes essential that the regulation for OTA in feeds with extremely high incidence rate of CIT should be revised. We hope that our data about OTA and CIT distribution in feeds will be conducive to further improvement of feed safety standards which, in its turn, will diminish the danger of animal mycotoxicoses.

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