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Mycotoxicological Study of Maize Feed Grain (1998–2018)

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Abstract—For maize grain, which has been important for the forage production of the country over the past two decades, persistent contamination with toxins of fusarium fungi, more often T-2/HT-2 toxins, fumonisins, and more rarely with deoxynivalenol and zearalenone in quantities that pose risks for animal intoxication was showed by enzyme immunoassay. The cases of superintensive accumulation of cyclopiazonic acid, citrinin, mycophenolic acid, and ochratoxin A were revealed. In 2016–2018, for maize grain from four subjects of the Central Federal District of the Russian Federation, a significant prevalence of T-2/HT-2 toxins, deoxynivalenol, and fumonisins with fluctuations in the frequency of occurrence of zearalenone over the years was found; diacetoxyscirpenol was detected in several samples from the Kursk and Voronezh oblasts. Contamination of grain with alternariol in 2016 and 2017 was mild both by frequency (5.3%) and accumulation levels (20–85 μg/kg), but the proportion of samples containing this toxin was 40.7% with a content range of 25–295 μg/kg in the yield of 2018. The influence of soil and climatic factors on the nature of mycotoxin contamination of the maize grain yield, the contribution of fungi belonging to dematiaceous hyphomycetes, and the prevailing variants of combined contamination with fusariotoxins are discussed.

Keywords: grain, maize, mycotoxins, diacetoxyscirpenol, alternariol, enzyme immunoassay

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INTRODUCTION

Maize grain, the global large-scale production of which exists in both southern and temperate latitudes, currently retains its importance in the nutrition of populations in Central, South America, and Africa, while it is used mainly for animal feed in other regions [1]. The high risk of contamination with mycotoxins associated with the susceptibility of this crop to pests and fungal diseases attracts the attention of specialists dealing with the problem of agrarian safety [2–4].

For maize plants, a variety of toxigenic phytopathogens belonging to the genera *Fusarium*, *Penicillium*, *Aspergillus*, *Alternaria*, *Stenocarpella*, and a particular susceptibility to infection due to slight damage of the cobs by insects and the possibility of direct transfer of spores are known, while the peculiarity of grain contamination with toxins is largely determined by soil-climatic factors [5–8]. Recently, the peculiarities of maize grain's contamination in the Czech Republic, Spain, and Portugal have been discovered [9].

In our country, according to the assessment of 1997–2001, 68.2% of grain samples traded in the field of fodder production contained mycotoxins, among which mainly fusariotoxins were present, and toxic metabolites characteristic for "storage molds" were found in only 8% of samples [10, 11]. This situation is confirmed by mycological analysis data: *Aspergillus* and *Penicillium* fungi were rarely found in the mycobiota grain, with *Fusarium* fungi dominating, along with dematiaceous hyphomycetes, which include representatives of the genera *Alternaria*, *Cladosporium*, *Drechslera*, *Myrothecium* etc. [10]. Consequently, selective grain studies were regularly continued according to an extended list of indices for both fusariotoxins and toxins, the range of expected producers of which is quite wide; however, the sum of the obtained data and their proper discussion did not take place. A regional survey of maize grain performed on 125 samples of yields from 2002–2005 in the Southern Federal District (Krasnodar krai, Stavropol krai, Rostov oblast) allowed establishing the peculiarities of its contamination with mycotoxins, but the study was only preliminary and limited to 14 samples in the Central District [12].

The goals of this study were a generalized assessment of maize grain's contamination with mycotoxins in 1998–2018 and a survey of the state of maize grain from the Central Federal District in 2016–2018 based on representative samples of material.

Table 1. Contamination of maize feed grain with mycotoxins (summarized data from 1998–2018)

Toxin	Occurrence		Content, μg/kg				
	n	n^+, %	diapason		average value	median value	90% percentile
			min	max			
T-2	331	197 (59.5)	4	2000	179	62	495.8
DON	270	93 (34.4)	40	3550	681	268	1780
ZEN	331	37 (11.2)	5	3000	246	54	480
FUM	331	208 (62.8)	20	38070	2645	689.5	7920
DAS	108	1 (0.9)	112		–	112	112
AOL	60	5 (8.3)	11	140	65	63	119.6
OA	331	25 (7.6)	5	390	76	20	233.2
CIT	230	8 (3.5)	20	953	273	57.5	866.9
AB_1	331	7 (2.1)	2	70	36	42	70
STE	314	3 (1.0)	5	15	8	5	13
MPA	73	9 (12.3)	25	629	197	100	443.4
CPA	108	2 (1.9)	126	1990	1058	1058	1803.6
EA	73	2 (2.7)	6	35	20.5	20.5	32.1
EMO	108	5 (4.6)	25	200	77	50	145.2

* n is the number of investigated grain samples; n^+ is the number of samples containing mycotoxin.

MATERIALS AND METHODS

The objects of study were representative samples of maize grain (1998–2018) obtained from feed-milling establishments and livestock farms in Moscow, Kaluga, Ryazan, Kursk, Voronezh, Oryol, Rostov oblasts, Krasnodar krai, Stavropol krai, Primorsky krai, and the Republic of Mordovia as well as 144 samples (2016–2018) from the parties with documentary evidence of the harvest of grain within the boundaries of the Central Federal District subjects (Kursk, Belgorod, Voronezh, and Lipetsk oblasts) and its use for feeding purposes. The procedure for sample preparation and quantitative determination of mycotoxins was carried out in accordance with a certified standardized procedure, including liquid extraction and indirect competitive enzyme immunoassay [13]. For the determination of T-2/HT-2 toxins (T-2), 8-oxotrichothecenes of the 4-deoxynivalenol group (DON), zearalenone (ZEN), group B fumonisins (FUM), alternariol (AOL), ochratoxin A (OA), aflatoxin B_1 (AB_1), sterigmatocystin (STE), roridin A (ROA), citrinin (CIT), and mycophenolic acid (MPA) commercial enzyme immunoassay reagent kits (All-Russia Research Institute of Veterinary Sanitation, Hygiene, and Ecology, Russia) were used. Cyclopiazonic acid (CPA), ergot alkaloids (EA), emodin (EMO), and PR-toxin (PR) were determined using enzyme immunoassay test systems developed and metrologically certified in the laboratory. For statistical data processing, Microsoft Excel 2016 was used with the calculation of parameters of positive samples: arithmetic average, median, and 90% percentile.

RESULTS AND DISCUSSION

According to the data presented in the Table 1, in the maize feed grain, the group of contaminants had a broad composition and included 14 of the 16 studied mycotoxins. In terms of occurrence, fusariotoxins with T-2 and FUM domination prevailed followed by DON and ZEN. Only one sample of 108 was positive for DAS, a highly toxic trichothecene, similar to T-2 in terms of toxicity, and it was associated with T-2 in an amount of 112 μg/kg. The decisive role of T-2, DON, ZEN, and FUM in contamination is consistent with the previously obtained data [11], and this confirms the validity of these mycotoxins' introduction into the list of controlled indices for maize grain supplied for feeding purposes [14].

MPA, AOL, and OA were found out of the toxins of fungi of other taxonomic groups with a frequency of more than 5%, and CIT, EA, EMO, AB_1, CPA, and STE were detected less frequently. Most of these mycotoxins were produced by fungi prone to vigorous growth on harvested crops with abrupt changes in humidity and temperature. Cases of intensive accumulation of CPA, CIT, MPA, and OA (Table 1) indicate the possibility of enhancing the toxicity of feed grain when postharvest storage conditions were impaired. Similar situations with stored maize grain were described in other countries [15, 16]. Toxins of

Table 2. Contamination of maize grain by fusariotoxins and alternariol (Kursk, Voronezh, Belgorod, Lipetsk oblasts, 2016–2018)

Year (*n*)	n^+, amount of toxin (min–max), μg/kg					
	T-2	DAS	DON	ZEN	FUM	AOL
2016 (26)	26	0	26	12	25	4
	15–210		50–3300	20–650	50–1580	20–85
2017 (91)	91	0	76	10	76	2
	5–998		50–2500	20–420	50–9976	24.44
2018 (27)	27	7	23	19	20	10
	12–560	50–180	50–3620	20–3970	655–5000	25–295

* *n* is the number of investigated grain samples; n^+ is the number of samples containing mycotoxin.

dematiaceous hyphomycetes were rare, for example AOL, the known metabolite of *Alternaria* spp. [17], found in 6.7% of samples, and anthraquinone EMO, which can be synthesized by *Drechslera catenaria* [18] and *Cladosporium fulvum* Cooke [19], which was found in only 2.7% of samples. Some of these toxins were not detected at all, such as macrocyclic trichothecene ROA, the metabolite of fungi of the genus *Myrothecium*.

Statistical processing of the results of grain analysis was performed in order to identify the extreme and central trends for each series of values (Table 1). The ranges of T-2, DON, ZEN, and FUM were extremely wide and were 3–4 orders of magnitude, while the ranges for other mycotoxins were 1–2 orders of magnitude. The median values for them differed noticeably from the average values, which was expected and indicated that the distribution was asymmetric, in which half of the values were significantly smaller than the rest. The threshold concentrations found at 90% of the values in the samples (90% percentile) for T-2, FUM, and DON exceeded the allowable values, especially for T-2, which exceeded the threshold by almost five times. The maximum levels of accumulation were extremely high for T-2 (2000 μg/kg) and FUM (38 070 μg/kg), and they exceeded the standards of the maximum content by three times or more for DON and ZEN. This indicates that the grain from the territories in which there was an intensive defeat of the maize cobs by *Fusarium* can pose a serious danger to animals. The reason for the sharp increase in the content of fusariotoxins could be the prolonged infestation of plants by highly toxigenic fungi produced under conditions promoting their active growth.

The development intensity of fusarium of the cob observed in all areas of maize cultivation, and, as a result, the contamination degree of the crop with mycotoxins is determined by a variable set of biotic, abiotic, and technological factors [20]. However, regular observations of the situation in the main grain-producing areas of our country were not performed. A regional survey of maize grain (125 crop samples from 2002–2005) in the Southern Federal District (Krasnodar krai, Stavropol krai, Rostov oblast) showed that contamination is characterized by a significant prevalence of fusariotoxins (92% of samples), especially FUM (89.6%), with a lower incidence of T-2, DON, and ZEN [12]. During the same years in the local grain harvest from the territories of the Central District, contamination of T-2 was found in all 14 studied samples, while FUM, DON, and ZEN were less frequent [12].

The results of the annual mycotoxicological examination of grain grown in Kursk, Voronezh, Belgorod, and Lipetsk oblasts in 2016–2018 are presented in Table. 2. All samples contained T-2, and DON and FUM were in second place by the frequency of detection. The incidence of ZEN was 28.5% on average in 144 samples and was regularly lower than that of DON. In general, the situation was similar to that described for feed grain of different territorial affiliation and harvest time (Table 1), although some features were detected.

In the grain harvested in 2018, DAS was identified quite often and always together with T-2 (Table 2). The biosynthesis of this group of toxins of the trichothecene series is known for several species of *Fusarium* fungi identified in the composition of the mycobiota of grain crop seeds, in particular, *F. sporotrichioides*, *F. poae*, and *F. langsethiae* [21]. Probably, under the ecological and climatic conditions of this year, an atypical representative of the toxin-forming complex of these fungi has gained an advantage or its regular participants realized the potential of toxin formation in a different way. AOL, one of the toxins characteristic of the fungus of the genus *Alternaria*, was annually found in samples from Central Russia, but such contamination had a mild frequency (5.3%) and level of accumulation in early 2016 and 2017. However, the proportion of positive samples reached 40.7% with the

Table 3. Contamination of maize grain by fusariotoxins (Kursk, Voronezh, Belgorod, Lipetsk oblasts, 2016–2018)

Year (n)	n^+							
	T	TD	TF	TDF	TDZ	TDZF	TDDsZ	TDZFDs
2016 (26)		–	–	14	1	11	–	–
2017 (91)	1	14	14	52	1	9	–	–
2018 (27)		2	3	4	2	9	3	4

* n^+ is the number of samples containing one and combinations of two, three, four, and five fusariotoxins.

highest content of 295 μg/kg in 2018 (Table 2). The situation could probably be aggravated under the influence of the prevailing weather factors.

It should also be noted that, MPA was found in this area in the amount of 125 μg/kg in only one grain sample during the entire study period, but none of the other seven toxins, for the accumulation of which fungi of the genera *Aspergillus*, *Penicillium*, etc. are considered responsible, were detected. In general, grain from the territories of the Central Federal District during these years was characterized by multiple combined contamination with fusariotoxins (Table 3). Contamination with T-2 was found only in a single sample, and combinations of 2–5 toxins with T-2 + DON + FUM and T-2 + DON + FUM + ZEN domination were presented in all the others.

Thus, for the last two decades, maize feed grain has been characterized by persistent contamination by toxins of fusarium fungi, more often T-2, FUM, and somewhat less often DON and ZEN with cases of superintensive accumulation representing a serious danger to animals. A high degree of risk has been confirmed in relation to other rarely detected toxins of microscopic fungi that are prone to saprophytic and saprotrophic habitats. The contamination of grain with mycotoxins from central Russia in recent years has been characterized by an intense combined contamination with fusariotoxins and an increase in the incidence of DAS and AOL. The previous regional survey was conducted in Krasnodar krai and Stavropol krai in 2002–2005 but it was not repeated later. Considering the large volume of maize grain production and the significant fluctuations in the mycotoxicological situation, it is necessary to introduce the repetition of such projects into routine practice.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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