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Mycotoxicological monitoring. Part 3. Feedstuffs from raw grain processing*

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SUMMARY

The paper presents the results of mycotoxicological testing of the production batches of sunflower cake and meal, feedstuffs of soybean and wheat bran processing received from domestic processing establishments and livestock farms from 2009 to 2019. Detection and measurement of the content of fusariotoxins, including T-2 toxin, diacetoxyscirpenol, deoxynivalenol, zearalenone and fumonisins of B group, as well as alternariol, ochratoxin A, citrinin, aflatoxin B_1, sterigmatocystin, cyclopiazonic acid, mycophenolic acid, ergot alkaloids and emodin was carried out by a competitive ELISA in accordance with certified procedure. The summarized results demonstrate the predominant role of alternariol in the contamination of sunflower cake and meal, as well as the frequent occurrence of T-2 toxin, ochratoxin A, citrinin, cyclopiazonic acid, sterigmatocystin, mycophenolic acid and emodin. For the main contaminants, a shift in the medians and 90% percentile towards the lower values of the average and maximum contents was observed, which indicates the possibility of their accumulation beyond the typical range. The summary and results of mycotoxicological study of wheat bran and feedstuffs of soybean processing for a complete list of 14 parameters are presented in this paper for the first time. It was found that the range of mycotoxins that can contaminate soybean meal, cake and full-fat soybean is quite wide, which is consistent with the results of the study of soybean seed mycobiota composition. It was demonstrated that soybean meal can accumulate high concentrations of mycophenolic acid – up to 1,255 µg/kg. As for the wheat bran batches, cases of contamination with diacetoxyscirpenol and the frequent occurrence of T-2 toxin, emodin and ergot alkaloids were detected. The initial monitoring data, systematized and summarized in this paper, are presented in electronic form in the section "Additional materials". The prospects of testing of feedstuffs from processing other oilseeds, as well as from wheat and corn grain processing are discussed.

Key words: sunflower meal/cake, soybean meal/cake, full-fat soybean, wheat bran, mycotoxins, monitoring, enzyme-linked immunosorbent assay.

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Микотоксикологический мониторинг. Сообщение 3. Кормовая продукция от переработки зернового сырья*

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РЕЗЮМЕ

Представлены результаты микотоксикологического обследования производственных партий подсолнечного жмыха и шрота, кормовой продукции от переработки сои и пшеничных отрубей, полученных из перерабатывающих предприятий и животноводческих хозяйств страны за период с 2009 по 2019 г. Детектирование и измерение содержания фузариотоксинов, включающих Т-2 токсин, диацетоксисцирпенол, дезоксиниваленол, зеараленон и фумонизины группы В, а также альтернариола, охратоксина А, цитринина, афлатоксина B_1, стеригматоцистина, циклопиазоновой кислоты, микофеноловой кислоты, эргоалкалоидов и эмодина проведено по аттестованной процедуре с использованием конкурентного иммуноферментного анализа. В ходе обобщения результатов установлена доминирующая роль альтернариола в контаминации подсолнечного жмыха и шрота, а также частая встречаемость Т-2 токсина, охратоксина А, цитринина, циклопиазоновой кислоты, стеригматоцистина, микофеноловой кислоты и эмодина. Для основных контаминантов отмечено смещение медиан и 90%-го процентиля в сторону меньших значений по отношению к средним и максимальным содержаниям, что указывало на возможность случаев их накопления за пределами типичного диапазона. Обобщение и результаты микотоксикологического исследования пшеничных отрубей и кормовой продукции от переработки соевых бобов по полному перечню из 14 показателей приводятся в этой работе впервые. Установлено, что спектр микотоксинов, способных участвовать в контаминации соевого шрота, жмыха и сои полножирной, достаточно широк, что согласуется с результатами изучения состава микобиоты семян этой культуры. В соевом шроте показана возможность накопления высоких концентраций микофеноловой кислоты – до уровня 1255 мкг/кг. В партиях пшеничных отрубей выявлены случаи загрязненности диацетоксисцирпенолом и частая встречаемость Т-2 токсина, эмодина и эргоалкалоидов. Исходные данные мониторинга, систематизированные и обобщенные в данной работе, представлены в электронном виде в разделе «Дополнительные материалы». Обсуждаются перспективы обследования кормовой продукции от переработки семян других масличных культур, а также зерна пшеницы и кукурузы.

Ключевые слова: подсолнечный шрот/жмых, соевый шрот/жмых, соя полножирная, пшеничные отруби, микотоксины, мониторинг, иммуноферментный анализ.

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INTRODUCTION

Feed base improvement is one of the most important tasks in animal farming in the Russian Federation. Animal health, productivity, immunobiological status, the quality and safety of animal products depend heavily on the sanitary state of feedstuffs and the balance of nutrients in them. In domestic compound feeds, macro-components supplementing the grain part are mainly represented by sunflower and soybean cake and meal, to a somewhat lesser extent – by-products of grain and starch processing. A wide network of fat-and-oil establishments and developed grain and starch processing industries completely cover the demand of domestic feed producers for sunflower cake and meal, grain bran, as well as for all types of by-products from complex corn grain processing. Supplies of soybean meal, cake and full-fat extruded soybean traditionally come from the regions specialized mainly in growing this crop (Southern and Far Eastern Federal Districts) and are supported by imported raw materials in order to meet the current market demands.

The first stage of testing of these types of feedstock for mycotoxin contamination was performed at the Laboratory for Mycotoxicology and Feed Hygiene, ARRIVSHE, in 2002–2009 [1, 2]. During this period frequent occurrence of ochratoxin A and citrinin at the level of 190 and 1,020 μg/kg was established in sunflower cake and meal; T-2 toxin, deoxynivalenol, sterigmatocystin, cyclopiazonic acid were less frequent; no zearalenone and diacetoxyscirpenol was detected. In addition, a weak contamination of soybean meal with T-2 toxin was reported. Deoxynivalenol and zearalenone were detected rarely and in small quantities, ochratoxin A and citrinin – in single samples, and fumonisins of B group, aflatoxin B_1, diacetoxiscirpenol, sterigmatocystin and cyclopiazonic acid could not be detected. However, in some imported product batches, the levels of deoxynivalenol exceeded 2,000 μg/kg, and zearalenone – 200 μg/kg. The situation with waste from flour mills received only a limited assessment. Recently, the peculiarities of contamination of sunflower seeds and feedstuffs from their processing have become the subject of special consideration [3–5].

The aim of this work is to sum up the results of testing of production batches of sunflower meal, cake, feedstuffs from soybean processing (meal, oil cake, full-fat soybean) and wheat bran for mycotoxins from 2009 to 2019 and to provide input data to the electronic registration database.

MATERIALS AND METHODS

Pooled samples from the production batches of sunflower meal and cake, soybean meal and cake, full-fat extruded soybean and wheat bran, provided by specialists of the veterinary services, livestock and feed producing establishments, agricultural companies, specialized commercial organizations and owners of backyard farms in 2009–2019 were used for the purpose of the study. As for sunflower cake and meal (121 samples), 107 samples were received from the processing establishments and holdings with documentarily confirmed addresses, located in Belgorod, Volgograd, Voronezh, Kursk, Oryol, Rostov, Saratov, Tambov oblasts, the Krasnodar Krai, the Primorsky Krai, the Republic of Tatarstan; 2 samples were received from the Ukraine, and 12 samples were provided with no data or its reliability was doubtful. Of the 80 samples of soybean meal, cake and processed soybean, 8 were imported, 6 were received from the Far Eastern Federal District (the Amur oblast, Primorsky Krai). Information about the origin of the rest of the feedstuffs from soybean processing, as well as of 20 samples of feed bran was not available.

The group of mycotoxins to be detected included T-2 toxin (T-2), diacetoxyscirpenol (DAS), deoxynivalenol (DON), zearalenone (ZEA), fumonisins of B group (FUM), alternariol (AOH), ochratoxin A (OA), citrinin (CIT), aflatoxin B_1 (AB_1), sterigmatocystin (STG), cyclopiazonic acid (CPA), mycophenolic acid (MPA), ergot alkaloids (EA), and emodin (EMO). Sample preparation was performed in accordance with the official harmonized methodology based on liquid extraction and indirect competitive enzyme immunoassay [6]. Detection limits determined based on 85% antibody binding were as follows: 2 µg/kg (AB_1), 3 µg/kg (EA), 4 µg/kg (T-2, OA, STG), 20 µg/kg (ZEA, AOH, CIT, MPA, EMO) and 50 µg/kg (DAS, DON, FUM, CPA). The following coding pattern was used for filling-in the record form in the database: tested mycotoxins, type of raw material, test year, and the location of the establishment or farm.

Microsoft Excel 2016 and Statistica (Version 6) programmes were used for statistical processing including calculation of percentage of occurrence based on n^+/n ratio and following three values for positive samples – the arithmetical mean, the median and the 90 percentile.

RESULTS AND DISCUSSION

The predominant role in contamination of sunflower meal and cake belonged to AOH produced by fungi of the genus *Alternaria*, with the average values of 306 and 193 µg/kg with the possibility of accumulation up to 1,990 and 953 µg/kg. As for the group of fusariotoxins, only the T-2 detection rate was significant – 21.4 and 37.3% at low concentrations with the ranges of 4–16 and 5–25 µg/kg, only in specific cases it was possible to determine DON and ZEA; DAS and FUM were not detected (Tables 1, 2).

Toxins produced by fungi of other taxa, mainly the genera *Aspergillus* and *Penicillium*, except for AB_1, were detected in both types of products. EA were equally rare in small concentrations; OA, MPA and EMO were found with approximately equal frequency exceeding 50%, while the frequency of contamination with OA was higher than with CIT. For other toxins, these parameters varied by 1.5–2 times in meal and cake. The rows arranged according to the average content values coincided and were as following: CIT, CPA, MPA (75–97 µg/kg) > OA (14 and 16 mg/kg) > STG (7 µg/kg). Medians and threshold concentrations for 90% of the values (90 percentile) for all the contaminants were lower than the average and maximum values, which was indicative of a skewed distribution of the numbers of accumulation cases, i.e. the possibility of their accumulation beyond the typical range for each specific product sample.

Thus, according to the data obtained, sunflower meal and cake are characterized as the ones containing multiple similar contaminants, including AOH, T-2, OA, CIT, CPA, STG, MPA and EMO, and their occurrence frequency varies from 10.4 to 83.8%. This gives every reason to consider

Table 1
Mycotoxins in sunflower meal (summary data for 2009–2019)

Таблица 1
Микотоксины в подсолнечном шроте (обобщенные данные 2009–2019 гг.)

Toxin	Occurrence n^+/n (%)	Content, µg/kg				
		range		average	median	90 percentile
		min	max			
T-2	15/70 (21.4)	4	16	9	9	13
DON	1/70 (1.4)	375	–	–	–	–
DAS	0/34	–	–	–	–	–
ZEA	1/70 (1.4)	66	–	–	–	–
FUM	0/33	–	–	–	–	–
AOH	57/68 (83.8)	19	1,990	306	104	839.2
OA	48/70 (68.6)	4	93	14	10	25.6
CIT	29/67 (43.3)	20	1,020	87	44	106.2
AB_1	0/41	–	–	–	–	–
STG	5/48 (10.4)	4	12	7	6	11.2
CPA	11/58 (19.0)	50	123	77	72	109
MPA	32/61 (52.5)	24	379	75	44	179.1
EA	1/43 (2.3)	11	–	–	–	–
EMO	29/54 (53.7)	15	278	72	52	159.2

n – number of tested samples (число исследованных образцов);
n^+ – number of mycotoxin-containing samples (число образцов, содержащих микотоксин).

Table 2
Mycotoxins in sunflower meal (summary data for 2009–2019)

Таблица 2
Микотоксины в подсолнечном жмыхе (обобщенные данные 2009–2019 гг.)

Toxin	Occurrence n^+/n (%)	Content, µg/kg				
		range		average	median	90 percentile
		min	max			
T-2	19/51 (37.3)	5	25	12	10	18.4
DON	0/51	–	–	–	–	–
DAS	0/23	–	–	–	–	–
ZEA	0/51	–	–	–	–	–
FUM	0/16	–	–	–	–	–
AOH	41/50 (82.0)	20	953	193	79	536
OA	32/51 (62.7)	4	62	16	9.5	36.8
CIT	11/51 (21.6)	20	126	80	79	126
AB_1	0/29	–	–	–	–	–
STG	10/39 (25.6)	4	11	7	5.5	9.2
CPA	21/39	50	142	81	71	120
MPA	9/44 (53.8)	20	334	97	63	222.8
EA	3/33 (9.1)	5	40	19	–	–
EMO	17/30 (56.7)	10	5,000	369.5	59	229.4

n – number of tested samples (число исследованных образцов);
n^+ – number of mycotoxin-containing samples (число образцов, содержащих микотоксин).

both types of the above-mentioned products to be high-risk raw materials. It should be noted that the results of the tests carried out in 2008–2010 for a shorter list of parameters that included T-2, DON, ZEA, FUM, OA and AB_1 in regards to the relevant samples of sunflower meal and cake received from holdings and establishments of the European part of the country, revealed that mycotoxins, except for the missing FUM, were extremely rare (detection frequency of 1.9 to 2.7%) and the meal was much more contaminated with AB_1 in comparison to the cake (28.6%) [7]. It should be admitted that such discrepancies in assessment are very unexpected and difficult to explain.

Soybean meal in the domestic feed production belongs to the main type of raw materials from soybean processing; cake and extruded full-fat soybean are used far less frequently. The summary and results of mycotoxicological testing of these products for a complete list of 14 parameters are presented in this paper for the first time (Fig. 1) for soybean meal (49 samples), the previously established fact that it is less contaminated with mycotoxins compared to the products from sunflower seed processing was fully confirmed [1, 2]. Only T-2, EA and EMO had 10% or higher occurrence frequency, and DAS, OA and CIT were not found, while other toxins were detected less frequently and with average concentrations of tens of µg/kg. Only for MPA in individual samples, concentrations exceeded this threshold and were 337 and 1,255 µg/kg (Fig. 1A). For soybean cake and full-fat soybean (31 samples), the situation was quite similar: the absence of DAS, the presence of T-2 and EMO with the occurrence frequency of more than 10%, and a lower incidence of ZEA and AOH; however, some clear differences were also observed (Fig. 1B). These include not only the increased frequency of contamination with T-2, DON, FUM, EMO and the intensity of accumulation of DON, FUM, but also detection of OA and CIT, although in small quantities close to the method detection limit, and the absence of a number of toxins – AB_1, STG, CPA, MPA and EA.

It can be assumed that the observed shifts in the nature of contamination of cake and full-fat soybean in comparison to meal are associated with different origins of the raw materials, and can also depend on transportation and storage conditions. Similarly noticeable fluctuations in the results are likely to occur for the meal, which also comes from geographically remote areas. Indeed, according to the work of N. Strashilina et al., for 166 samples of meal with non-specified origin, contamination with AB_1 was 100%, all the tested fusariotoxins (T-2, ZEA, DON, and FUM) occurred with a frequency of 20.9 to 28.6%, and OA was detected in 2.2% of the cases [7].

In general, the range of mycotoxins that can contaminate by-products of soybean processing is quite wide and is quite consistent with the results of studies of mycobiota associated with soybean seeds. According to the Slovak University of Agriculture in Nitra, fungi of the genus *Aspergillus* and *Penicillium* were widely present in the

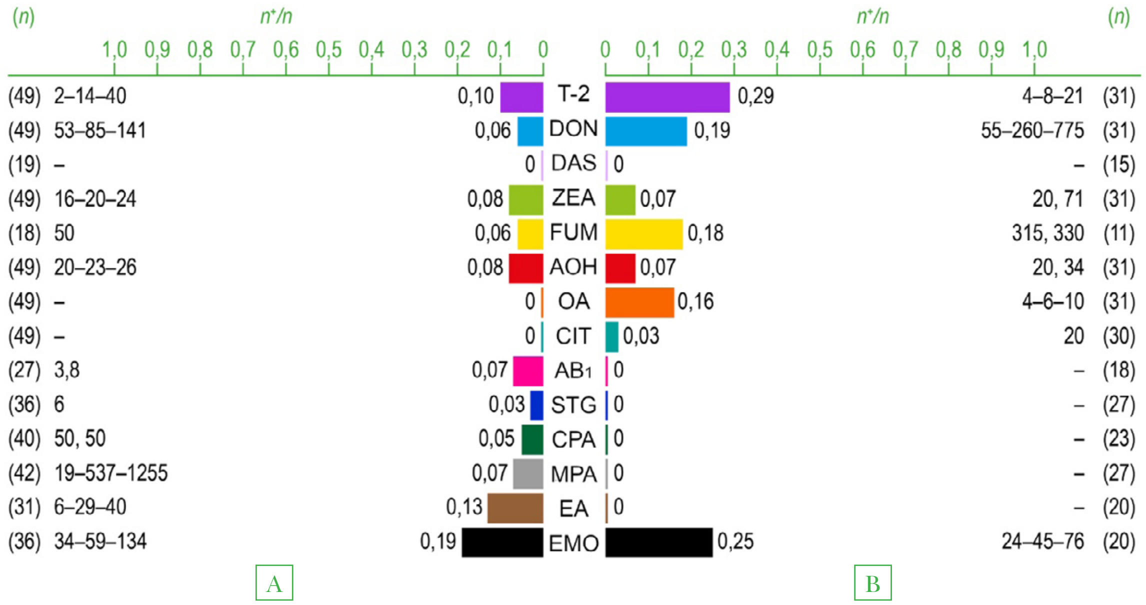

Fig. 1. Mycotoxin occurrence (n+/n) and content (µg/kg, min – average – max) in soybean meal (A), soybean cake and full-fat soybean (B)

Рис. 1. Встречаемость (n+/n) и содержание микотоксинов (мкг/кг, мин. – среднее – макс.) в соевом шроте (А), соевом жмыхе и сое полножирной (В)

mycobiota of feed soybean sampled at agricultural establishments of the country and were accompanied by representatives of the genera *Cladosporium*, *Alternaria* и *Fusarium* [8]. The possibility of asymptomatic colonization of soybean with the fungus *Fusarium verticillioides*, capable of FUM biosynthesis, has been recently shown by American researchers [9]. The predominance of the AOH-producing species *Alternaria alternata* and the presence of this toxin is shown in soybeans from Argentina [10, 11]. In soya bran, which is one of the most important components of feed in Brazil, the fungi *Aspergillus* (*A. flavus*, *A. fumigatus*, *A. niger*), *Penicillium* (14.93%), as well as *Fusarium* (3.25%) were frequently detected [12]. Several potentially toxigenic species of the genus *Aspergillus* were identified in soybean seeds and soybean flour imported to Armenia from the USA, Canada, Spain and Greece, with rare occurrence of *Penicillium cyclopium*, *P. lanosum* and *Fusarium moniliforme*, with AB_1 being found in 6 out of 17 samples (7–50 µg/kg),

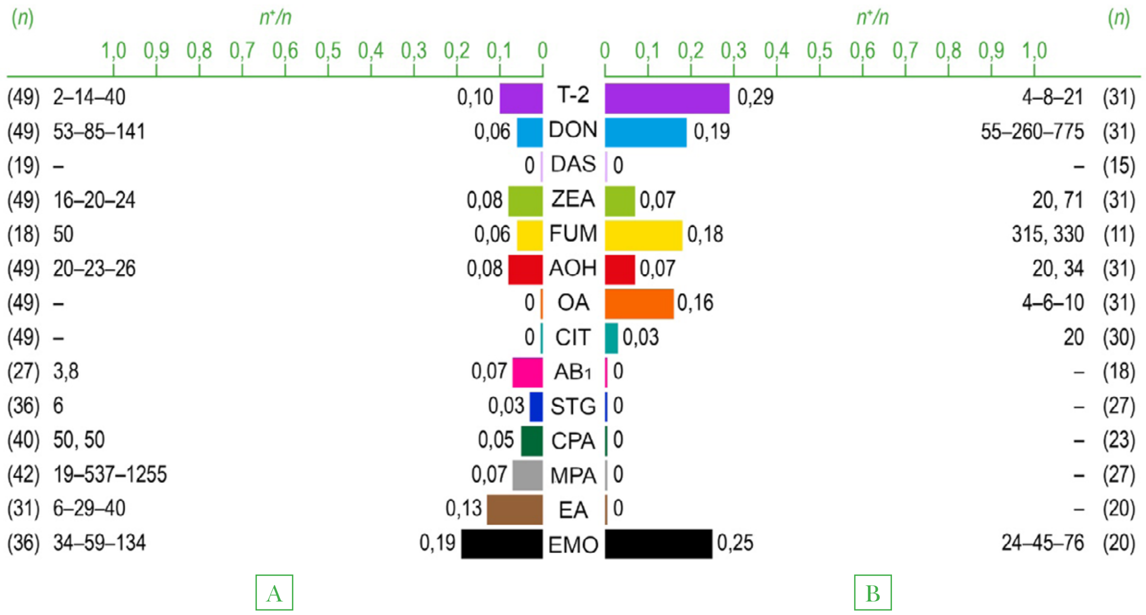

Fig. 2. Mycotoxin occurrence (n+/n) and content (µg/kg, min – average – max) in wheat bran (A) and wheat grain (B) (cit. for Part 2)

Рис. 2. Встречаемость (n+/n) и содержание микотоксинов (мкг/кг, мин. – среднее – макс.) в пшеничных отрубях (А) и в зерне пшеницы (В) (цит. по сообщению 2) and STG (150 µg/kg) and ZEA (2,000 µg/kg) in some single samples [13]. The nature and intensity of mycotoxin contamination of soybean products is undoubtedly conditioned by a combination of factors such as soil, climate and ecology.

Recent decades have been marked by the expansion of the area of soybean cultivation in moderate latitudes, and the relevance of its contamination control remains very high. However, the number of works tackling this issue is small. Recently, the species *Alternaria alternata*, as well as individual representatives of the genera *Cladosporium* and *Fusarium*, have been identified in collection and selection planting soybean seeds in Belarus [14]. Therefore, there is need for mycotoxicological testing of soybean not only in the regions of its traditional cultivation, but also in the territories where lands are actively being exploited – the Belgorod, Rostov oblasts, Altai and Stavropol krais, the Republic of Adygea, and the Republic of Tatarstan. Of undoubted importance is gathering of information regarding other promising types of feedstuffs from oilseed processing, in particular cotton [15] and rapeseed. In 2018, a comprehensive analysis of rapeseed cake sample received from the Krasnodar Krai, revealed only CPA and EMO in small quantities – 50 and 32 µg/kg.

Analysis of 20 samples of wheat bran showed that the frequency of occurrence of T-2, EMO > DON, AOH, OA (Fig. 2A) corresponds to the one typical for wheat grain (Fig. 2B). DAS, ZEA, FUM, AB_1 and CPA were not detected, while OA, CIT, STG, MPA, and EA were identified in single samples. A significantly higher number of T-2, EA and DAS detection can be noticed.

Despite the small number of samples, the data obtained can serve a basis for future research projects aimed at studying the distribution of mycotoxins in fractions separated during the production of wheat flour and cereals. Initial monitoring data with the type of raw material, its origin and the year of sample receipt are provided in electronic form in the section "Additional materials" at: http://doi.org/10.29326/2304-196X-2020-3-34-213-219.

Unfortunately, the situation regarding mycotoxin contamination of distiller's dried grain, obtained by drying and granulating of distillery waste, which is widely used in feed production, is still unclear in our country. However, data from American researchers indicate multiple and intense contamination of the distiller's dried grains with solubles (DDGS) – DON, ZEA and FUM were detected in 70–90% of samples, with their concentrations reaching values of 13,920, 8,107 and 9,042 µg/kg; AB_1 and T-2 at levels up to 89 and 226 µg/kg, respectively, were also often detected [16]. During this period, we did not have an opportunity to continue mycotoxicological testing of gluten feeds, the by-products which are very popular among starch-processing plants. In 2015 and 2016, the laboratory received two samples of corn gluten from the People's Republic of China for testing, one of which demonstrated the presence of fusariotoxins T-2, DON, ZEA, FUM and STG in the amounts of 145, 1,860, 1,080, 1,260 and 11 µg/kg, respectively; the other contained DON and ZEA in the amount of 2,320 and 2,230 µg/kg. The obtained data and preliminary results [2, 7], indicative of the intensive multiple contamination of this product, as well as combined contamination of corn grain (see Part 2) testify to the necessity for mandatory testing of corn gluten for a complete list of parameters. Insufficient attention is paid to other types of products from the complex processing of corn grain, which are widely used in feed production, such as dry corn germ, as well as cake and corn germ meal. However, 16 samples of corn germ cake received in 2009–2010 from holdings in the European part of the country showed that contamination with T-2, DON, ZEA, FUM, OA and AB_1 was quite significant with a frequency of 43.8 to 75% [7].

CONCLUSION

During extensive monitoring, conducted as an annual data collection for the period from 2009 to 2019, multiple contamination of sunflower meal and sunflower oil cake with alternariol, T-2 toxin, ochratoxin A, citrinin, cyclopiazonic acid, sterigmatocystin, mycophenolic acid and emodin was confirmed with the frequency of occurrence from 10.4 to 83.8%, which entitles these products to be regarded as high-risk. For the safe use of raw materials based on soybean, appropriate regional monitoring projects should be considered in the territories of its intensive cultivation and industrial processing. Imported batches of cake and meal should be inspected on a regular basis due to their possible contamination with a wide range of mycotoxins. Cases of diacetoxyscirpenol detection and the frequent occurrence of T-2 toxin, emodin and ergot alkaloids described for wheat bran for the first time show the need for a mandatory incoming control of these products at feed producing establishments.

Additional materials to the paper (records forms with database) can be found at http://doi.org/10.29326/2304-196X-2020-3-34-213-219.

Дополнительные материалы к этой статье (учетные формы с базой данных) можно найти по адресу http://doi.org/10.29326/2304-196X-2020-3-34-213-219.

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