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Mycotoxilogical monitoring. Part 2. Wheat, barley, oat and maize grain*

G. P. Kononenko [1], A. A. Burkin [2], Ye. V. Zotova [3]

All-Russia Research Institute of Veterinary Sanitation, Hygiene and Ecology – Branch of the Federal State-Financed Scientific Institution "Federal Scientific Centre – All-Russian Research Institute of Experimental Veterinary Medicine named after K.I. Scryabin and Ya.R. Kovalenko of the Russian Academy of Sciences, Moscow, Russia (ARRIVSHE – Branch of the FSFSI FSC ARRIEVM RAS), Moscow, Russia

[1] *ORCID 0000-0002-9144-615X, e-mail: kononenkogp@mail.ru*

[2] *ORCID 0000-0002-5674-2818, e-mail: aaburkin@mail.ru*

[3] *ORCID 0000-0002-1479-8602, e-mail: ezotova63@gmail.com*

SUMMARY

Results of mycotoxicological survey of representative samples of feed and procured wheat, barley, oat and maize grain batches are demonstrated. The samples were submitted by the Veterinary Service officials, livestock farmers and feed mill operators, agricultural producers, specialized commercial business operators and farm owners in seven Federal Districts of the Russian Federation in 2009-2019. Similar amounts of wheat and barley grain samples were received from the Central, Volga, Ural and Siberian Federal Districts. The amount of wheat samples delivered from the Southern Federal District prevailed over the number of barley samples, and the maize samples were mostly delivered from the regions of the Central Federal District. *Fusarium* toxins including T-2 toxin, diacetoxyscirpenol, deoxynivalenol, zearalenone and fumonisins of group B as well as alternariol, ochratoxin A, citrinin, aflatoxin B1, sterigmatocystin, cyclopiazonic acid, mycophenolic acid, ergot alkaloids and emodin were detected and measured according to the validated competitive ELISA procedure. Generalization of the results demonstrated domination of *Fusarium* toxins and active involvement of alternariol in the contamination of all types of feed grains as well as high occurrence of emodin in ear cereals and increased occurrence of T-2 toxin and ochratoxin A in barley. Shift of medians and 90%-percentile of the basic contaminants to lower values as compared to mean and maximal ones was reported thus being indicative of their possible accumulation at the levels outside the typical range. The highest levels of T-2 toxin, deoxynivalenol and ochratoxin A as well as 90%-percentile values exceeded the acceptable levels. The maize grains demonstrated the whole complex of the tested *Fusarium* toxins with the prevalence of T-2 toxin, deoxynivalenol, zearalenone and fumonisins; and the maximal amounts of these mycotoxins by several times exceeded the accepted regulatory levels. Diacetoxyscirpenol, aflatoxin B1, sterigmatocystin, cyclopiazonic acid and ergot alkaloids are classified as rare feed grain contaminants. High prevalence of alternariol and emodin known as "diarrhea factor" as well as maize grain contamination with mycophenolic acid (mycotoxin having an immunosuppressive effect) are for the first time reported in this paper. These data support the need of their introduction in the group of regulated substances significant for public health. Original monitoring data systematized and summarized in the paper are given in electronic format in section Additional materials.

Key words: wheat, barley, oat and maize grain, mycotoxins, monitoring, enzyme-linked immunosorbent assay (ELISA).

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For correspondence: Galina P. Kononenko, Doctor of Science (Biology), Professor, Head of Laboratory for Mycotoxicology and Feed Hygiene, ARRIVSHE – Branch of the FSFSI FSC ARRIEVM RAS, 123022, Russia, Moscow, Zvenigorodskoe shosse, 5; *e-mail: kononenkogp@mail.ru*.

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Микотоксикологический мониторинг. Сообщение 2. Зерно пшеницы, ячменя, овса, кукурузы*

Г. П. Кононенко[1], А. А. Буркин[2], Е. В. Зотова[3]

Всероссийский научно-исследовательский институт ветеринарной санитарии, гигиены и экологии – филиал федерального государственного бюджетного научного учреждения «Федеральный научный центр – Всероссийский научно-исследовательский институт экспериментальной ветеринарии имени К. И. Скрябина и Я. Р. Коваленко Российской академии наук» (ВНИИВСГЭ – филиал ФГБНУ ФНЦ ВИЭВ РАН), г. Москва, Россия

[1] *ORCID 0000-0002-9144-615X, e-mail: kononenkogp@mail.ru*

[2] *ORCID 0000-0002-5674-2818, e-mail: aaburkin@mail.ru*

[3] *ORCID 0000-0002-1479-8602, e-mail: ezotova63@gmail.com*

РЕЗЮМЕ

Представлены результаты микотоксикологического обследования средних образцов от партий фуражного и заготовляемого зерна пшеницы, ячменя, овса и кукурузы, предоставленных специалистами ветеринарных служб, животноводческих и комбикормовых предприятий, компаний-

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сельхозпроизводителей, специализированных коммерческих организаций и владельцами крестьянских фермерских хозяйств из 7 федеральных округов Российской Федерации в период с 2009 по 2019 г. Сравнимые по объему выборки зерна пшеницы и ячменя были получены из Центрального, Приволжского, Уральского и Сибирского федеральных округов. Из Южного федерального округа число образцов пшеницы было больше, чем ячменя, а зерно кукурузы поступало в основном из субъектов Центрального федерального округа. 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 проводили по аттестованной процедуре с использованием конкурентного иммуноферментного анализа. В ходе обобщения результатов установлена доминирующая роль фузариотоксинов и активное участие альтернариола в контаминации всех видов зернофуража, а также частая встречаемость эмодина в зерне колосовых культур и повышенная распространенность Т-2 токсина и охратоксина А в зерне ячменя. 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 их накопления за пределами типичного диапазона. Наибольшие уровни Т-2 токсина, дезоксиниваленола и охратоксина А, а также показатели 90%-го процентиля превышали допустимые нормы содержания. В зерне кукурузы комплекс анализированных фузариотоксинов представлен полностью с наибольшей встречаемостью Т-2 токсина, дезоксиниваленола, зеараленона и фумонизинов, и максимальные количества этих микотоксинов в несколько раз превышали принятые уровни нормирования. Диацетоксисцирпенол, афлатоксин B_1, стеригматоцистин, циклопиазоновая кислота и эргоалкалоиды отнесены к редким контаминантам кормового зерна. Факт обширной распространенности в зерне альтернариола и эмодина, известного как «диарейный фактор», а также контаминации зерна кукурузы микофеноловой кислотой – микотоксином с иммунодепрессивным действием, приведены в данной работе впервые. Эта информация подтверждает необходимость их введения в группу нормируемых санитарно-значимых показателей. Исходные данные мониторинга, систематизированные и обобщенные в данной работе, представлены в электронном виде в разделе «Дополнительные материалы».

Ключевые слова: зерно пшеницы, ячменя, овса, кукурузы, микотоксины, мониторинг, иммуноферментный анализ.

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Для корреспонденции: Кононенко Галина Пантелеевна, доктор биологических наук, профессор, заведующий лабораторией микотоксикологии и санитарии кормов ВНИИВСГЭ – филиал ФГБНУ ФНЦ ВИЭВ РАН, 123022, Россия, г. Москва, Звенигородское шоссе, д. 5, *e-mail: kononenkogp@mail.ru.*

INTRODUCTION

Grain contamination with toxic metabolites of microscopic fungi (mycotoxins) has been and remains the focus of attention of the global agricultural science being the key issue of the veterinary welfare [1, 2]. In our country the hygiene of the feed grain is of practical importance for all livestock sectors not only due to significant scope of its use in the compound feed but also due to still growing interest to hydroponic fodder and new technologies of its preservation under high humidity conditions. The feed rations of the majority of food-producing animals and poultry are based on grain ingredients – wheat, barley and maize grain with oat grain being of traditional interest for horse breeding sector.

For many years, the wide spread of *Fusarium* spp. contamination of grain, often growing into long-lasting epiphytoties, has been gaining increased attention due to the risk of toxicoses in animals [3]. In 1995–2005, the targeted governmental program was implemented in order to assess the consequences of the grain contamination with toxigenic *Fusarium* fungi and to forecast the development of the situation in the cultivation areas [4]. That was when the data on *Fusarium* toxins' spread pattern were obtained for the first time [5, 6]. Hereafter, regional surveys were initiated as for other mycotoxins significant for public health [7]. Enforcement of the Technical Regulation of the Customs Union on Safety of Grain (TR CU 015/2011) prescribing their admissible levels in feed was an important outcome of these efforts [8].

According to the global scientific data the list of mycotoxins capable of inducing chronic intoxication and highly dangerous remote effects in animals is far from being exhausted, and improvement of the system of grain safety control remains highly relevant. An increasing number of countries realize the need for regular monitoring researches and continuous operation of specialized structures for coordination of actions and rapid expert evaluation of the received data. In our country, the long-run objective of the establishment of the national information resource aimed at the mitigation of mycotoxicosis risk in farm animals involves systematization of analytical investigations and data reporting under the unified approach, which combines their overall assessment and accessibility for updating and use by the experts.

The work was aimed at compilation of the results of the survey of mycotoxin contamination of feed and procured wheat, barley, oat and maize grain delivered from seven Federal Districts of the Russian Federation in 2009–2019 with the source data being reported to the electronic database.

MATERIALS AND METHODS

The test objects included representative samples of feed and procured grain batches submitted by the Veterinary Service officials, livestock farmers and feed mill operators, agricultural producers, specialized commercial business operators and small-scale farm owners in 2009–2019. Harvesting areas were documented and officially confirmed for 623 wheat, oat, barley and maize samples, 30 samples were delivered without data on their origin, and four samples were collected from the imported consignments (Table 1). Twenty-seven oat grain samples were submitted for testing mostly from the Tula, Moscow Oblasts and Krasnodar Krai as well as from the Bryansk, Kursk, Tyumen Oblasts and Sakha (Yakutia) Republic.

The tested mycotoxins included T-2 toxin (T-2), diacetoxyscirpenol (DAS), deoxynivalenol (DON), zearalenone (ZEA) and fumonisins of group B (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). The samples were prepared using standardized procedure based on liquid-liquid extraction and indirect competitive ELISA [9]. 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 record form in the database: type of grain (WH, BAR, OA, MAI), region (FD and REGION), reporting year (1–11), tested mycotoxins. Microsoft Excel 2016 and Statistica (Version 6) software were used for statistical data processing including calculation of percentage of occurrence based on n^+/n ratio and the following three values for positive samples – the arithmetical mean, the median and the 90% percentile.

RESULTS AND DISCUSSION

The data in Table 1 demonstrate that the coverage of the grain sampling areas was the highest in the Central and Volga Federal Districts (FD). In the Southern, Ural and Siberian FD this parameter was significantly lower and as for the Northwestern and Far Eastern FD the samples were delivered only from two regions of each. The total numbers of tested samples of wheat, barley and maize were quite comparable, but their regional distribution was uneven. Comparable sizes of wheat and barley grain samples were delivered only from the Central, Volga, Ural and Siberian FD, but samples delivered from the Southern FD considerably varied in size. Maize grain was mostly submitted from the regions of the Central FD.

All tested mycotoxins except from DAS and AB_1 were detected in wheat grain (Table 2). T-2 and DON as well as AOH and EMO were the most frequently detected ones. The occurrence rate of other mycotoxins amounted to 1.0%–4.9%. The quantities of toxins generally varied within two orders of magnitude; only in case of ZEA the variation range did not exceed one order of magnitude. Shift of medians to lower values as compared to mean ones was reported for all contaminants being indicative of unified dissymmetric distribution of contents where half of the data was less than other ones. The highest quantities exceeded the threshold concentrations calculated for 90% of values (90% percentile) being the most pronounced in case of T-2, DON, AOH and EMO that suggested their possible abnormal accumulation against the typical contamination. Moreover, in case of DON the 90% percentile value several times exceeded its admissible level of 1,000 μg/kg [8]. It should be emphasized that against the relatively rare occurrence both maximum levels of OA and CIT detected in grain and their threshold concentrations calculated for 90% of values were significantly higher than the recommended standards [8, 10].

In barley grain the highest occurrence rates were reported for T-2 and EMO, moderate ones – for DON, AOH and OA, the lowest ones – for ZEA, CIT, STG and MPA. DAS, FUM, AB_1 and CPA were absent (Table 3). Two- and even more-fold median displacement from the average values to the lower ones was reported for all contaminants excluding AOH, and the excess of the highest values over the threshold concentrations calculated for 90% of the values (90% percentile) was reported for all but EA. With high prevalence of T-2 contamination the 90% percentile value was consistent with the admissible levels and the highest amount exceeded it in over six times. As for less prevalent DON and OA, the comparison of the both parameters indicated real possibility of the excess contamination of this type of grain [8]. Higher T-2 occurrence in barley grain as

Table 1
Areas, from which representative samples of wheat, barley and maize grain were delivered (according to documented and official data, 2009–2019)

Таблица 1
Территории, с которых направлены средние образцы зерна пшеницы, ячменя, кукурузы (согласно документальным и ответственным подтверждениям, 2009–2019 гг.)

Federal District (Region) of the Russian Federation	Number of samples		
	wheat	barley	maize
Central (Belgorod, Bryansk, Voronezh, Kaluga, Kursk, Lipetsk, Moscow, Oryol, Ryazan, Tambov, Tula, Yaroslavl Oblasts)	65	58	190
Volga (Kirov, Nizhny Novgorod, Orenburg, Penza, Samara, Saratov, Ulyanovsk Oblasts, Republic of Bashkortostan, Republic of Mordovia, Republic of Tatarstan)	41	29	2
Southern (Volgograd, Rostov Oblasts, Krasnodar Krai, Stavropol Krai)	75	14	9
Urals (Kurgan, Sverdlovsk, Tyumen, Chelyabinsk Oblasts)	17	12	–
Siberian (Irkutsk, Novosibirsk, Omsk Oblasts, Altai Krai, Krasnoyarsk Krai)	44	26	–
Northwestern (Vologda, Pskov Oblasts)	1	1	–
Far Eastern (Amur Oblast, Primorsky Krai)	2	1	2
Origin is not specified	14	6	10
Imported grain	–	1	3
Total	259	148	216

Table 2
Mycotoxins in wheat grain (summary data, 2009–2019)

Таблица 2
Микотоксины в зерне пшеницы (обобщенные данные 2009–2019 гг.)

Toxin	Occurrence n^+/n (%)	Level, µg/kg				
		range		mean	median	90% percentile
		min	max			
T-2	83/259 (32.0)	2	225	26	12	48
DON	62/259 (23.9)	40	7,920	1,092	550	3,155
DAS	0/74	–	–	–	–	–
ZEA	11/259 (4.2)	10	215	68	40	205
FUM	6/165 (3.6)	75	1,990	478	150	1,200
AOH	52/240 (21.7)	11	675	60	32	116
OA	12/247 (4.9)	4	270	52	8	236
CIT	8/163 (4.9)	24	1,000	349	128	853
AB_1	0/101	–	–	–	–	–
STG	3/125 (2.4)	4	250	90	–	–
CPA	1/132 (1.0)	63	–	–	–	–
MPA	4/151 (2.6)	63	1,255	602	545	1,179
EA	4/121 (3.3)	6	144	43	10	105
EMO	56/126 (44.4)	5	706	118	60.5	301

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

compared to wheat grain as well as concordance of DON and ZEA occurrence parameters support the previously reported data [11]. More pronounced OA concentration in barley grain (20.4%) as compared to wheat grain (4.9%) was reported for the first time.

DAS, ZEA, FUM, AB_1 and CPA were not detected in 27 tested oat grain samples; OA, CIT, STG, MPA and EA were detected in singular samples; other mycotoxins were ranged as T-2 > EMO > DON > AOH according to their occurrence rate (see the Figure). Maximum concentrations of T-2 and DON detected in oat grain exceeded the current standards [8].

In spite of low amount of samples and irregular testing of this type of grain during the reported years, the obtained data can be useful for the complex research project targeted at the examination of oat contamination with toxicogenic fungi in the regions practicing intensive cultivation of this grain, especially in the northern part of the Non-Black-Earth region, Volga FD and other territories [12, 13]. Moreover, these data are of indisputable value for the horse breeding sector, where this grain is widely used due to its high nutrition and content of biologically active substances having a stimulating effect of the behavioral responses of horses. Previous pilot study of several samples submitted by the equestrian centers and horse breeding farms also demonstrated high T-2 contamination, moderate DON and AOH contamination and low ZEA contamination [12].

In maize grain the group of contaminants was more diverse and only STG was not included in it. The complex of the tested *Fusarium* toxins was represented in full with high prevalence of T-2, DON and FUM and lower prevalence of ZEA and DAS (Table 4). The highest levels of T-2, DON, FUM and ZEA several times exceeded their regulatory standards. The data on the dominating role of these mycotoxins were consistent with the previous conclusion [14] and supported the relevance of their introduction into the list of regulated substances in the maize grain supplied for use in feed [8]. Among other tested metabolites the significant contribution of AOH and MPA should be noted with their occurrence rate 13.3% and 8.6% and maximum levels 295 µg/kg and 397 µg/kg, respectively. OA, CIT and EMO mycotoxins were rare to detect and their levels amounted to maximum 50 µg/kg and AB_1, CPA and EA were detected only sporadically.

Results of tests of wheat, barley, oat and maize grain contaminants relevant for public health carried out in 2009–2019 were for the first time consolidated and analyzed in detail in this paper. Dominant role of *Fusarium* toxins along with potential high-level accumulation as well as evidence of co-contamination with all components of the complex were confirmed for maize grain. Significant EMO occurrence in ear cereals was reported that is likely to be the basic route of its introduction into the compound feeds (see Part 1). The role of this secondary metabolite for plants is not quite clear for now and has been actively

Table 3
Mycotoxins in barley grain (summary data, 2009–2019)

Таблица 3
Микотоксины в зерне ячменя (обобщенные данные 2009–2019 гг.)

Toxin	Occurrence n^+/n (%)	Level, μg/kg				
		range		mean	median	90% percentile
		min	max			
T-2	105/148 (70.9)	4	660	48	25	100
DON	35/148 (23.6)	50	7,060	654	130	2,380
DAS	0/60	–	–	–	–	–
ZEA	2/148 (1.4)	100	1,250	675	–	–
FUM	0/67	–	–	–	–	–
AOH	29/137 (21.2)	5	397	85	71	141
OA	29/142 (20.4)	4	250	23	5	71
CIT	5/137 (3.6)	30	1,120	300	146	734
AB_1	0/69	–	–	–	–	–
STG	2/107 (1.9)	10	40	25	–	–
CPA	0/92	–	–	–	–	–
MPA	1/106 (1.0)	334	–	–	–	–
EA	4/94 (4.3)	5	164	49	14	120
EMO	68/94 (72.3)	13	1,400	205	101	495

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

discussed in the scientific publications [15]. Genotoxic AOH was classified as significant for public health and relevant for all types of feed grain as well as highly active immunosuppressive MPA that is relevant for maize grain.

To see the source data of the monitoring with the regions and sampling date specified please follow the link http://doi.org/10.29326/2304-196X-2020-2-33-139-145. In section Additional materials. The option of the database updating as well as its accessibility allow for any selection of data processing *inter alia* by type of grain, occurrence of contamination with an individual mycotoxin or co-contamination with mycotoxins as well as by region or territory. Over the recent years, DON and ZEA co-contamination has been frequently occurring in wheat [11], the same as T-2 and DAS co-contamination – in maize grain [14]. The data received as for individual territories allow for unique possibility to forecast risk of contamination in the specific area with due consideration of the soil-climatic and cultivation factors, which are able to influence the mycotoxicological situation due to their effect on the microscopic fungus growth conditions, competitive interactions and toxin production [16–18].

CONCLUSION

During the large-scale monitoring involving annual data collection in 2009–2019 general characteristics and peculiarities of mycotoxin contamination of wheat, barley, oat and maize grain were determined. The findings supported the relevance of the regular assessment of the grain contamination with *Fusarium* toxins and ochratoxin A. The list of public health-significant substances regulated in grain is recommended to be extended by inclusion of emodin (anthraquinone toxin known as "diarrhea factor") as well as alternariol, citrinin and mycophenolic acid having especially hazardous forms of toxicity and negative remote effects. The obtained data can be relevant

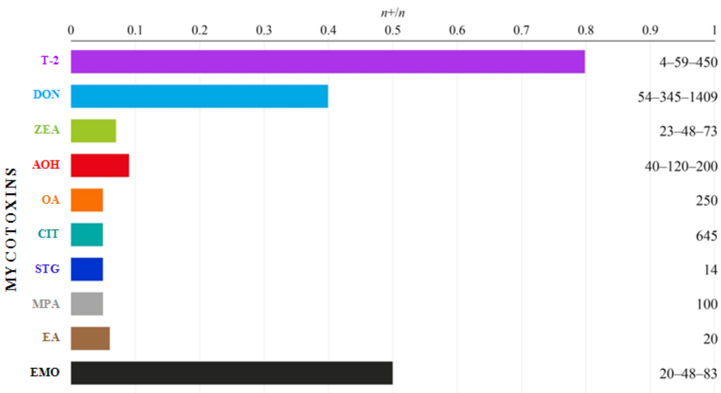

Fig. Mycotoxin occurrence (n^+/n) and content (μg/kg, min – mean - max) in oat grain (summarized data)

Рис. Встречаемость (n^+/n) и содержание микотоксинов (мкг/кг, мин. – среднее – макс.) в зерне овса (обобщенные данные)

Table 4
Mycotoxins in maize grain (summarized data, 2009–2019)

Таблица 4
Микотоксины в зерне кукурузы (обобщенные данные 2009–2019 гг.)

Toxin	Occurrence n^+/n (%)	Level, µg/kg				
		range		mean	median	90% percentile
		min	max			
T-2	203/216 (94.0)	2	1,040	151	120	310
DON	154/216 (71.3)	50	6,590	740	382.5	1,963
DAS	10/59 (17.0)	50	250	122	92.5	205
ZEA	50/216 (23.2)	20	4,455	345	55.5	674
FUM	167/216 (77.3)	50	15,800	1,334	500	3,152
AOH	21/158 (13.3)	20	295	71	57	140
OA	4/164 (2.4)	5	16	10	–	–
CIT	3/141 (2.1)	20	40	27	–	–
AB_1	1/113 (1.0)	16	–	–	–	–
STG	0/112	–	–	–	–	–
CPA	1/75 (1.3)	126	–	–	–	–
MPA	8/93 (8.6)	25	397	158	100	345
EA	1/67 (1.5)	6	–	–	–	–
EMO	2/74 (2.7)	25	45	35	–	–

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

for the assessment of the general situation in the agricultural production, verification of the criteria of the mycotoxin regulation in grain and for the improvement of the system of grain product control.

Additional materials to the paper (records forms with database) can be found at: http://doi.org/10.29326/2304-196X-2020-2-33-139-145.

Дополнительные материалы к этой статье (учетные формы с базой данных) можно найти по адресу: http://doi.org/10.29326/2304-196X-2020-2-33-139-145.

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INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Galina P. Kononenko, Doctor of Science (Biology), Professor, Head of Laboratory for Mycotoxicology and Feed Hygiene, ARRIVSHE – Branch of the FSFSI FSC ARRIEVM RAS, Moscow, Russia.

Alexey A. Burkin, Candidate of Science (Medicine), Leading Researcher, ARRIVSHE – Branch of the FSFSI FSC ARRIEVM RAS, Moscow, Russia.

Yelena V. Zotova, Candidate of Science (Veterinary Medicine), Senior Researcher, ARRIVSHE – Branch of the FSFSI FSC ARRIEVM RAS, Moscow, Russia.

Кононенко Галина Пантелеевна, доктор биологических наук, профессор, заведующий лабораторией микотоксикологии и санитарии кормов ВНИИВСГЭ – филиал ФГБНУ ФНЦ ВИЭВ РАН, г. Москва, Россия.

Буркин Алексей Анатольевич, кандидат медицинских наук, ведущий научный сотрудник ВНИИВСГЭ – филиал ФГБНУ ФНЦ ВИЭВ РАН, г. Москва, Россия.

Зотова Елена Владимировна, кандидат ветеринарных наук, старший научный сотрудник ВНИИВСГЭ – филиал ФГБНУ ФНЦ ВИЭВ РАН, г. Москва, Россия.