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Determination of fungal genera composition and total toxicity of feed produced in the Republic of Crimea

S. S. Ibragimova[1], O. V. Pruntova[2], S. I. Danilchenko[3], Ye. S. Yerofeeva[4]

[1,3,4] Branch of FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH") in the Republic of Crimea, Simferopol city, Russia

[2] FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia

[1] *ORCID 0000-0003-3886-7702, e-mail: ibragimova@arriah.ru*

[2] *ORCID 0000-0003-3143-7339, e-mail: pruntova@arriah.ru*

[3] *ORCID 0000-0001-7796-7349, e-mail: danylchenko@arriah.ru*

[4] *ORCID 0000-0001-5060-6105, e-mail: erofeeva@arriah.ru*

SUMMARY

Animal mycotoxicoses caused by ingestion of toxicogenic micromycete-contaminated feed are of major concern for agricultural industry and of great importance for production of agricultural products. In 2017–2019, feed and raw feed materials produced in the Republic of Crimea were tested for mold fungi and genera composition thereof; the feedstuffs were tested for total toxicity by bioassay in rabbits. A total of 252 samples including 124 grain feed samples, 70 forage samples, 58 mixed feed samples were selected for testing. Tests showed that the major detected contaminants were members of the following genera: *Mucor* (67.9%), *Penicillium* (26.6%), *Aspergillus* (13.1%), *Fusarium* (9.1%), *Alternaria* (8.7%), *Stachybotrys* (3.6%) and *Rhizopus* (2.0%). It was revealed that feed were exposed to mold fungi contamination during vegetation and harvesting as well as during transportation and storage. Thus, in spring the feed were more often contaminated with micromycetes of *Penicillium* genus (37.8%) and *Stachybotrys* genus (6.7%); feed collected and tested in autumn were more often contaminated with toxicogenic mold fungi of *Fusarium* genus (14.9%), *Alternaria* genus (13.9%) and *Rhizopus* genus (3.0%); in winter members of *Mucor* genus (78.0%) and *Aspergillus* genus (22.0%) were most often detected in feed. Tests for determination of total toxicity showed that 9 (7.3%) and 10 (8.1%) samples out of 124 tested grain feed samples were low toxic and evidently toxic, respectively. Tests of mixed feed samples for toxicity showed that 5 samples (8.6%) and 2 (3.4%) samples out of 58 mixed feed samples were low toxic and evidently toxic, respectively. It was shown that the proportion of contaminated feed was the highest in spring (25.0%) as compared to proportion of the contaminated feed in winter (18.2%), in autumn (13.7%) and in summer (4.5%).

Key words: fungi, mycological analysis, micromycetes, contamination, total toxicity.

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For correspondence: Selime S. Ibragimova, Post-Graduate Student, Head of Sector, FGBI "ARRIAH" Branch in the Republic of Crimea, 295494, Russia, Simferopol city, set. of Komsomolskoye, str. Shosseinaya, 21A, *e-mail: ibragimova@arriah.ru.*

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Определение родового состава грибов и общей токсичности кормов, произведенных на территории Республики Крым

С. С. Ибрагимова[1], О. В. Прунтова[2], С. И. Данильченко[3], Е. С. Ерофеева[4]

[1,3,4] Филиал ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ») в Республике Крым, г. Симферополь, Россия

[2] ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия

[1] *ORCID 0000-0003-3886-7702, e-mail: ibragimova@arriah.ru*

[2] *ORCID 0000-0003-3143-7339, e-mail: pruntova@arriah.ru*

[3] *ORCID 0000-0001-7796-7349, e-mail: danylchenko@arriah.ru*

[4] *ORCID 0000-0001-5060-6105, e-mail: erofeeva@arriah.ru*

РЕЗЮМЕ

Микотоксикозы животных, причиной которых является употребление контаминированных токсинообразующими микромицетами кормов, представляют серьезную проблему для сельского хозяйства и имеют большое значение при производстве сельскохозяйственной продукции. В период с 2017 по 2019 г. проведен анализ кормов и кормового сырья, произведенных на территории Республики Крым, на содержание и родовой состав плесневых грибов, изучена общая токсичность кормовой продукции методом постановки биопробы на кроликах. Для проведения исследований было отобрано 252 образца, из которых бóльшую часть составили зерновые корма – 124 пробы; 70 проб были представлены грубыми кормами, 58 – комбикормами. В результате проведенных исследований установлено, что основными контаминантами были представители таких родов, как: *Mucor* (67,9%), *Penicillium* (26,6%), *Aspergillus* (13,1%), *Fusarium* (9,1%), *Alternaria* (8,7%), *Stachybotrys* (3,6%) и *Rhizopus* (2,0%). Выявили, что корма подвержены контаминации плесневыми грибами как в период вегетации и уборки, так и во время перевозки и хранения. Так, микромицетами родов *Penicillium* (37,8%) и *Stachybotrys* (6,7%) чаще были контаминированы корма весной; токсинообразующими плесневыми грибами родов *Fusarium* (14,9%), *Alternaria* (13,9%) и *Rhizopus* (3,0%) – корма, отобранные и исследованные осенью; представителей родов *Mucor* (78,0%) и *Aspergillus* (22,0%) наиболее часто выявляли в зимний период. При определении общей токсичности кормов и кормовой продукции установили, что из 124 исследуемых образцов зерновых кормов слаботоксичными были 9 (7,3%), токсичными – 10 проб (8,1%). Из 58 испытуемых образцов комбикорма слабую токсичность проявили 5 проб (8,6%), а выраженную токсичность – 2 образца (3,4%). Показано, что самый высокий процент токсичного корма выявляли в весенний период (25,0%), зимой данный показатель составил 18,2%, осенью – 13,7%, летом – 4,5%.

Ключевые слова: плесневые грибы, микологический анализ, микромицеты, контаминация, общая токсичность.

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Для корреспонденции: Ибрагимова Селиме Серверовна, аспирант, руководитель сектора Филиала ФГБУ «ВНИИЗЖ» в Республике Крым, 295494, Россия, г. Симферополь, пос. Комсомольское, ул. Шоссейная, 21А, *e-mail: ibragimova@arriah.ru.*

INTRODUCTION

Quality of feed becomes very important in the context of animal and poultry farming intensification. Contamination of feed with mold fungi being producers of mycotoxins, low-molecular-weight secondary metabolites of micromycetes, are of particular concern [1, 2]. Mycotoxin diversity, high toxicity and various mycotoxicosis manifestations make the said issue highly significant.

There are currently more than 100,000 various fungi species including more than 250 toxicogenic fungi species [2–4]. There are following mycotoxins: aflatoxins, trichothecene mycotoxins, ochratoxins, fumonisin, zearalenone and its derivatives, ergot alkaloids, cyclopiazonic acid, patulin, citrinine, etc. Ochratoxin A, deoxynivalenol, T-2 toxin, zearalenone and aflatoxin are the most widespread and dangerous [5, 6].

Contamination of feed and feed products with mycotoxins reduces feed nutritional value and contributes to development of various non-infectious diseases – foodborne mycotoxicoses that can be acute or chronic. Disease clinical signs and course depend on the level of feed contamination, animal physiological status and pathogenic characteristics of the fungi contaminating feed products. Acute mycotoxicosis is characterized with neurotoxic symptoms: agitation or depression, fatigue, incoordination, gastrointestinal and cardiovascular disorders, hyporeflexia, convulsions. Chronic mycotoxicosis is characterized with depression, anemia, decrease in weight and reduced performance, abortions [7, 8].

Contamination of feed with mycotoxins at low concentrations also poses a serious threat: after the ingestion by farmed animals they can partially accumulate in tissues and organs and after being metabolized they can enter the products derived from these animals (meat, milk, eggs). Thus, aflatoxin B_1, ingested with the feed transforms in aflatoxin M_1 and is excreted with milk. Therefore, products derived from the animals that have been fed with mycotoxin-contaminated feed are potentially toxic for humans [1, 9].

It is important to note that mycotoxins are characterized by uneven distribution - their concentration in different points of the same batch of feed varies significantly that affects the test results [1].

Failure to observe the requirements for raw feed materials and finished feed harvesting and storage is one of the main causes of the mold fungi growth and, therefore, results in changes in the fungal microflora species and quantitative composition. The mycotoxin contamination level in feed depends on the level of its exposure to toxicogenic mold fungi [10].

According to the Food and Agricultural Organization of the United Nations (FAO), annually, mycotoxins contaminate about 25% of the world grain crop. The majority of countries in the world regulates mycotoxin content in feed and food. In the Russian Federation, maximum allowable levels for mycotoxins are laid down by Technical Regulations of the Customs Union 021/2011 on food safety and 015/2011 on grain safety, GOSTs and TUs for different types of products and feed. According to the literature data, the following mycotoxins are the most often detected in the Russian Federation: deoxynivalenol, T-2 toxin, zearalenone and aflatoxin. There are no documents regulating allowable levels of mold fundi species contamination [11].

Moreover, mycological control for mold fungi exposure allows for detection of toxicogenic micromycete contamination of feed and feed products at early stages. Toxicogenic micromycetes include fungi of the following genera: *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Fusarium*, *Alternaria*, *Helminthosporium*, *Ustilago*, *Tilletia*, *Nigrospora* etc., that can produce mycotoxins under favorable conditions (relative humidity of 85–95%, temperature of 4–30 °C) [3]. Therewith, the mycological analysis is an important step of the feed quality control.

The work was aimed at determination of generic composition of mold fungi contaminating the feed produced in the territory of the Republic of Crimea, examination of dependence of mold fungi exposure level on the season of the year and environmental conditions (temperature, humidity), and determination of total toxicity of feed by bioassay.

MATERIALS AND METHODS

Test materials: samples of feed and mixed feed taken on farms and backyards located in the Republic of Crimea. Samples were collected in accordance with GOST ISO 6497-2014 "Feeding stuffs. Sampling", GOST 13586.3-2015 "Grain. Acceptance rules and sampling methods", GOST 13496.0-80 "Mixed feeding-stuffs. Rules of sampling of average sample".

Reagents and nutrient media: distilled water (GOST 6709-72 "Distilled water. Specifications"), Czapek-Dox and Sabouraud media (OOO "SPC Biokompas-S", Russia), filter paper, formaldehyde, OP-7 surfactant, ammonia, pure acetone for analysis (OOO "KhlorenKhima", Russia), refined sunflower oil (GOST 1129-2013 "Sunflower oil. Specifications").

Laboratory animals: rabbits, live weight: 2.0 to 2.5 kg, having integral non-pigmented skin.

All experiments in animals were carried out in strict accordance with the international standards on laboratory animal keeping and handling laid down in GOST 33216-2014 "Guidelines for accommodation and care of animals. Species-specific provisions for laboratory rodents and rabbits" and GOST 33215-2014 "Guidelines for accommodation and care of animals. Environment, housing and management", adopted by the Interstate Council for Standardization, Metrology and Certification as well as in accordance with Directive of the European Parliament and the Council 2010/63/EU of 22 September 2010 on protection of animals used for scientific purposes.

Sample preparation methods: mycological analysis was performed in accordance with GOST 13496.6-71 "Mixed feed. Method of detachment of fungi" and in accordance with the "Methodical guidelines for sanitary and mycological evaluation of feed and for feed quality improvement" (approved by the USSR Ministry of Agriculture on February 25, 1985). The test materials were seeded in agar media-containing Petri dishes for mold fungi cultivation. Tests were carried out in quintuplicate. The seeds in Petri dishes were kept in thermostat at 25 °C for 10 days. The Petri dishes were examined at day 3, 5, 7 and 10 of incubation [12, 13].

The thermostat and isolation room were tested for cleaning quality and sterility, respectively, in accordance with the "Methodical guidelines for disinfection, pre-sterilization cleaning and sterilization of medical devices" [14]. Before test material seeding, two Petri dishes with the agar medium were placed on the laboratory bench and two Petri dishes with the agar medium were placed in thermostat. The said Petri dishes were opened and left for 15 minutes, then, they were placed in thermostat and kept similarly to the seeded material.

Grain feed was tested for external and internal spore contamination. To test grains for external spore contamination, the test materials were seeded onto Sabouraud medium in Petri dishes, 10 grain kernels per dish, so as the kernels did not touch each other.

To test grain for internal spore contamination the grain kernels were treated with 3% formaldehyde solution (treatment period – 2 minutes) and washed once with 0.2% ammonium solution and then with distilled water after their treatment; the treated kernels were placed onto Sabouraud agar in Petri dishes, 10 grain kernels per dish. Five Petri dishes were used for each test.

Forage feed was chopped into 2 cm-long pieces in a sterile Petri dish. Then, the pieces were transferred with a sterile forceps onto Czapek-Dox agar in Petri dishes (10 forage pieces per dish, 5 dishes) [13].

Granulated mixed feed was ground with LZM-1 laboratory mill; 10 g of the feed were taken and poured with 100 ml of 0.1% aqueous OP-7 surfactant solution to prepare primary suspension. The following suspension dilutions were prepared using serial dilution method: 1:1 000 dilution – for good-quality feed and 1:10 000 dilution – for the feed with spoilage signs (macroscopic signs of mold fungi contamination, characteristic odour, etc.). The 1:1 000 dilution and 1:10 000 dilution were seeded onto 5 and 8 Petri dishes containing agar medium, respectively [12, 13].

Generic classification of fungi was performed based on morphological characteristics of the colonies, mycelia and sporangium structure [15].

Determination of total toxicity of feed. Test feed samples were ground and sifted through a 1 mm-pore-size sieve for tests for total toxicity. Ground feed sample (50 g) was transferred to 500 cm^3 conical flask with tight stopper and 150 cm^3 of acetone were added. The flask was placed on orbital shaker and the extraction was performed for 3 hours. Resulting extract was filtered through paper filter and transferred to the cup for evaporation. It was concentrated up to complete solvent odour removal and to oily residual mass. Vegetable oil was added to make the sample volume to 1 cm^3 when amount of the extract after evaporation was not sufficient for testing [13].

Bioassay was performed in rabbits that have integral non-pigmented non-scaly skin. Vegetable oil used for the extract dissolution was preliminary tested for its toxicity in rabbits by applying it twice on the sheared skin area at an 24-hour interval. Skin reactions were recorded on the next day after second application and then for 3 days. The vegetable oil that had not induce any redness of the rabbit epidermis was considered suitable for use.

Then, one half of the extract was applied to 6 × 6 cm preliminary sheared skin area of rabbit thigh or shoulder with glass spatula by gentle rubbing. The second half of the extract was applied on the next day. The oily extract of the test feed was kept in refrigerating chamber before the second application. The skin reactions were recorded on the next day after the second extract application and the rabbits were observed for the next 3 days.

One 6 × 6 cm sheared skin area was used as a control one. No extract was applied to this area. One rabbit was used for maximum four simultaneous bioassays.

Results of tests of feed for their toxicity were evaluated based on inflammatory reaction presence or absence. The feed sample was considered toxic when apparent hyperemia, soreness, swelling, manifested by a strong thickening of the skin as well as ulcers or solid scab were observed on the skin area in rabbits [13].

RESULTS AND DISCUSSION

At first stage, the level of feed contamination with micromycetes and generic composition of mold fungi contaminating feedstuffs harvested on the territory of the Republic of Crimea were determined.

Table 1
Characterization of tested feed samples

Таблица 1
Характеристика исследуемых образцов корма

Sampling period	Average weight of collected sample	Number of collected samples	Sample type
Winter	2.0 kg	23	grain feed (barley, wheat, oat)
		17	forage (hay, straw)
		10	mixed feed
Spring	2.0 kg	22	grain feed (barley, wheat, oat)
		13	forage (hay, straw)
		10	mixed feed
Summer	2.0 kg	30	grain feed (barley, wheat, oat)
		12	forage (hay, straw)
		14	mixed feed
Autumn	2.0 kg	49	grain feed (barley, wheat, oat)
		28	forage (hay, straw)
		24	mixed feed

Tests were performed in the FGBI "ARRIAH" Branch located in the Republic of Crimea in 2017–2019.

Feed and feed product samples were collected in backyards and on farms located in the Belogorsky, Krasnogvardeysky, Leninsky and Saksky Raions of the Republic of Crimea. Average sample weight was 2.0 kg (for each sample taken separately). Numbers of collected samples and sampling periods are given in Table 1.

A total of 252 feed samples including 124 grain feed samples, 70 forage samples and 58 mixed feed samples were collected for tests of the feedstuffs harvested in the Republic of Crimea for toxicogenic mold fungi and for fungal generic composition. Feed samples were collected and tested in different seasons of the year to determine dependence of the level of feed contamination with mold fungi on environmental conditions (air temperature, relative humidity).

Mold fungi were differentiated and classified to genera based on the grown colonies morphology; mycelium and sporangium structures were examined by light microscopy.

Results of tests of mold fungi contaminating the feedstuffs harvested in the territory of the Republic of Crimea are shown in Table 2.

Micromycetes of the following genera were detected during feed sample tests for determination of contaminating fungi generic composition: *Mucor* – in 171 samples (67.9%), *Penicillium* – in 67 samples (26.6%), *Aspergillus* – in 33 samples (13.1%), *Fusarium* – in 23 samples (9.1%), *Alternaria* – in 22 samples (8.7%), *Stachybotrys* – in 9 samples (3.6%), *Rhizopus* – in 5 samples (2.0%).

A total of 50 feed samples were collected in winter period. Mold fungi of the following genera were detected during mycological tests of the said samples: mold fungi of *Mucor* genus were detected in 39 samples (78.0%), *Aspergillus* – in 11 samples (22.0%), *Penicillium* – in 7 samples (14.0%), *Fusarium* – in 6 samples (12.0%), *Alternaria* – in 5 samples (10.0%), *Stachybotrys* – in 2 samples (4.0%), *Rhizopus* – in one sample (2.0%).

In spring 45 feed samples were collected and tested, mold fungi of the following genera were detected: mold fungi of *Mucor* genus were detected in 33 samples (73.3%), *Penicillium* – in 17 samples (37.8%), *Aspergillus* – in 9 samples (20.0%). Micromycetes of *Alternaria* genus and *Stachybotrys* genus were detected in 3 (6.7%) and 3 samples (6.7%), respectively. Mycromycetes of *Rhizopus* genus were detected in 1 sample (2.2%) out of the tested feed samples.

In summer, 56 feed samples were collected for testing. The feed samples were found to be contaminated with the mold fungi of the following genera: mold fungi of *Mucor* genus were detected in 43 samples (76.8%), *Penicillium* – in 20 samples (35.7%), *Aspergillus* and *Fusarium* genera – in 2 samples (3.6%) and 2 samples (3.6%), respectively. Mold fungi of *Stachybotrys* genus were detected in one sample (1.8%).

In autumn, 101 feed samples were collected and tested, 56 (55.4%) out of them were found to be contaminated

Table 2
Generic diversity of the fungi contaminating tested feed

Таблица 2
Родовое разнообразие грибов, контаминирующих исследуемые корма

Genus of detected micromycete	Sample type	Number of contaminated feed samples collected in different seasons of the year				
		winter	spring	summer	autumn	total
Mucor	grain feed	18	16	22	23	79
	forage	13	10	9	17	49
	mixed feed	8	7	12	16	43
Penicillium	grain feed	4	9	14	12	39
	forage	1	2	2	6	11
	mixed feed	2	6	4	5	17
Alternaria	grain feed	2	3	0	5	10
	forage	0	0	0	4	4
	mixed feed	3	0	0	5	8
Aspergillus	grain feed	4	6	2	5	17
	forage	4	3	0	3	10
	mixed feed	3	0	0	3	6
Stachybotrys	grain feed	1	1	1	0	3
	forage	1	2	0	3	6
	mixed feed	0	0	0	0	0
Fusarium	grain feed	6	0	2	10	18
	forage	0	0	0	2	2
	mixed feed	0	0	0	3	3
Rhizopus	grain feed	0	0	0	0	0
	forage	1	0	0	1	2
	mixed feed	0	1	0	2	3

with fungi of *Mucor* genus, 23 samples (22.8%) – with *Penicillium,* 15 samples (14.9%) – *Fusarium*. Fungi of *Alternaria* genus were detected in 14 samples (13.9%), *Aspergillus* genus – in 11 samples (10.9%); micromycetes of *Stachybotrys* genus and *Rhizopus* genus were detected in 3 (3.0%) and 3 (3.0%) samples, respectively.

According to the literature data, fungi of *Rhizopus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Aspergillus*, *Fusarium*, *Mucor*, *Trichoderma*, *Stachybotrys* genera and others also play dominating role in contamination of plant feed in the Russian Federation and in European countries; their genera and species diversity depend on the environmental climatic conditions [3, 16, 17].

The tests have shown that feedstuffs are exposed to mold fungi both in vegetation and harvesting periods as well as during their transportation and storage. Some toxicogenic micromycetes rapidly grow and multiply at temperatures below 20 °C, the other ones – at 30–50 °C. Favorable conditions for phytopathogenic micromycetes propagation at the stage of feed crop growing and failure

Table 3
Results of tests of feed for total toxicity

Таблица 3
Результаты исследований общей токсичности кормов

Season	Toxicity of feed samples					
	grain feed			mixed feed		
	non-toxic	low toxic	toxic	non-toxic	low toxic	toxic
Winter	18	2	3	9	1	0
Spring	16	2	4	8	1	1
Summer	29	1	0	13	1	0
Autumn	42	4	3	21	2	1

to comply with the feed harvesting technology can result in mycotoxin accumulation in the products. Degree of feed exposure to toxicogenic micromycetes directly influences the level of feed contamination with mycotoxins and, as a result, agricultural product safety and probability of mycotoxicoses in animals.

Analysis of the test results showed that fungal generic composition in feed changes depending on the season of the year. Thus, in spring the feed was more often contaminated with micromycetes of *Penicillium* genus (37.8%) and *Stachybotrys* genus (6.7%); in autumn, the feed was more often contaminated with toxicogenic mold fungi of *Fusarium* genus (14.9%), *Alternaria* genus (13.9%) and *Rhizopus* genus (3.0%); members of *Mucor* genus (78.0%) and *Aspergillus* genus (22.0%) were the most often detected in winter.

Taking into account the direct influence of the degree of exposure of the feed to toxicogenic mold fungi on the level of contamination of feed with mycotoxins, the next stage of the work was to determine the total toxicity of samples of micromycete-contaminated feed and feed products.

A total of 124 grain feed samples and 58 mixed feed samples were tested for toxicity by bioassay in rabbits. Feed toxicity was assessed based on presence or absence of inflammatory reaction in rabbits and the tested feed samples were classified into three categories: toxic, low toxic and non-toxic [13]. The feed sample was considered toxic when apparent hyperemia, soreness, swelling manifested by a strong thickening of the skin as well as ulcers or solid scab were observed on the rabbit skin area pre-treated with the said feed extract. The extract of low toxic feed caused hyperemia that lasted for 2–3 days, skin exfoliation, soreness and swelling manifested by slight thickening of the skin followed by formation of discrete crusts. The feed sample was considered non-toxic when inflammatory reaction was absent or hyperemia was observed for maximum 2 days and no skin exfoliation was observed after treatment with the feed extract.

Results of tests of feed for total toxicity are given in Table 3.

Tests of 124 grain feed samples for their toxicity showed that 9 samples (7.3%) out of them were low toxic, 10 samples (8.1%) were toxic. Tests of 58 mixed feed samples showed that 5 samples (8.6%) out of them were low toxic, 2 samples (3.4%) were evidently toxic.

In winter 33 samples were tested: 3 grain feed samples (9.1%) were considered toxic and 2 grain feed samples (6.1%) and 1 mixed feed sample (3.0%) were considered low toxic.

In spring 32 samples were tested, 4 grain feed samples (12.5%) and 1 mixed feed sample (3.1%), were found toxic and 2 grain feed samples (6.3%) and 1 mixed feed sample (3.1%) were found low toxic.

In summer 44 samples were tested: 1 grain feed sample (2.3%) and 1 mixed feed sample (2.3%) were found low toxic.

In autumn 73 samples were tested: 3 grain feed samples (4.1%) and 1 mixed feed sample (1.4%) were considered toxic, 4 grain feed samples (5.5%) and 2 mixed feed samples (2.7%) were considered low toxic.

The figure shows that the proportion of the feed found contaminated was the highest in spring (15.6%).

Feed and feed products collected and tested in all seasons were found toxic to various degree. Thus, 4 samples (5.5%) were found toxic and 6 samples (8.2%) were found low toxic out of 73 samples collected and tested in autumn. Three samples (9.1%) were found toxic and 3 samples (9.1%) were found low toxic out of 33 samples collected and tested in winter. Five samples (15.6%) were found toxic and 3 samples (9.4%) were found low toxic out of 32 samples collected and tested in spring. No toxic samples were found and only 2 samples (4.5%) were found low toxic out of 44 samples collected and tested in summer.

Feed and feed products considered low toxic and toxic based on the laboratory test results are not allowed for feeding livestock animals [18].

CONCLUSION

Tests showed that fungal genera composition changes depending on the season of the year. Thus, feedstuffs were more often contaminated with micromycetes of *Penicillium* genus (37.8%) and *Stachybotrys* genus (6.7%) in spring; with toxicogenic mold fungi of *Fusarium* genus (14.9%), *Alternaria* genus (13.9%) and *Rhizopus* genus (3.0%)

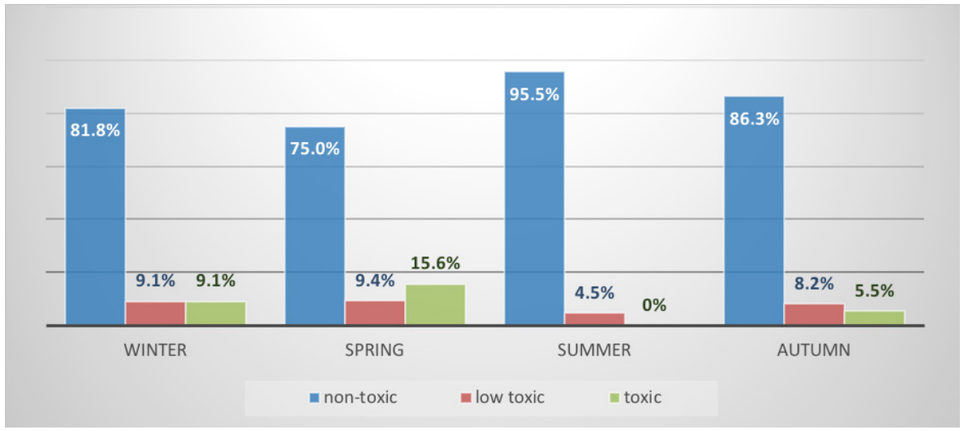

Fig. Total toxicity of feed and feed products in different seasons of the year

Рис. Общая токсичность кормов и кормовой продукции в зависимости от сезона года

in autumn; with fungi of *Mucor* genus (78.0%) and *Aspergillus* genus (22.0%) in winter.

Tests of feed for total toxicity showed that 9 samples (7.3%) were low toxic and 10 samples (8.1%) were low toxic out of tested 124 grain feed samples. Five samples (8.6%) were considered low toxic and 2 samples (3.4%) were considered toxic out of tested 58 mixed feed samples.

Low toxic feed samples were detected during all four seasons: in summer (4.5%), autumn (8.2%), winter (9.1%) and spring (9.4%). Toxic feed samples were detected in autumn (5.5%), winter (9.1%) and spring (15.6%). No toxic feed samples were detected in 44 feed samples collected and tested in summer.

REFERENCES

1. Ivanov A. V., Tremasov M. Ya., Papunidi K. Kh., Chulkov A. K. Animal mycotoxicoses (etiology, diagnosis, treatment, prevention) [Mikotoksikozy zhivotnyh (etiologiya, diagnostika, lechenie, profilaktika)]. M.: Kolos; 2008. 133 p. (in Russian)

2. Popova O. M. Immunological, biochemical and microbiological characteristics of cows with T2- and aspergillus-associated toxicosis and correction thereof [Immunobiologicheskie, biohimicheskie i mikrobiologicheskie pokazateli korov pri associirovannom T-2 i aspergillotoksikoze i ih korrekciya]: Author's Abstract of Thesis for Doctor of Biology Degree. Kazan; 2016. 40 p. (in Russian)

3. Yakimova E. A. Generic composition and quantityof microscopic fungi in feed and raw feed materials for animals. [Vidovoj sostav i kolichestvennoe soderzhanie mikroskopicheskih gribov v kormah i kormovom syr'e dlya zhivotnyh]. *Biotika*. 2015; 6 (7): 3–12. eLIBRARY ID: 26591267. (in Russian)

4. Alshannaq A., Yu J.-H. Occurrence, toxicity, and analysis of major mycotoxins in food. *Int. J. Environ. Res. Public Health*. 2017; 14: 632. DOI: 10.3390/ijerph14060632.

5. Tremasov M. Ya., Papunidi K. Kh., Semenov E. I., Tarasova E. Yu. Current problems of veterinary toxicology. *Vestnik veterinarii*. 2012; 4 (63): 16–18. eLIBRARY ID: 18258778. (in Russian)

6. Koshchaev A. G., Khmara I. V. Peculiarities of seasonal mycotoxin contamination of raw grain and mixed fodders in Krasnodar Region. *Veterinaria Kubani*. 2013; 2: 29–30. eLIBRARY ID: 19008503. (in Russian)

7. Ivanov I. I. Mycotoxin sources and distribution and mycotoxicosis prevention in animals in the Republic of Mariy-El [Istochniki, rasprostranenie mikotoksinov i profilaktika mikotoksikozov zhivotnyh v Respublike Marij El]: Author's Abstract of Thesis for Candidate of Science Degree (Biology). Kazan; 2002. 18 p. (in Russian)

8. Papunidi K. Kh., Tremasov M. Ya., Fisinin V. I., Nikitin A. I., Semenov E. I. Mycotoxins (in food chain) [Mikotoksiny (v pishchevoj cepochke)]: Monograph. 2nd edition, revised. Kazan: FGBSI "Federal Centre for Toxicological, Radiological and Biological Safety"; 2017. 158 p. (in Russian)

9. Medetkhanov F. A., Hairullin D. D., Mullakayeva L. A., Ovsyannikov A. P. Study guide for veterinary toxicology [Uchebno-metodicheskoe posobie po veterinarnoj toksikologii]. Kazan: FGBEI "Kazan State Academy of Veterinary Medicine"; 2017. 133 p. (in Russian)

10. Baybakova J. P., Husainov I. T., Kryuchkova M. A., Tremasov M. J., Ahmetov F. G. Sanitary-mycology evaluation of forage quality. *Veterinary Medicine*. 2010; 94: 296–297. (in Russian)

11. Abakin S. S. Mycotoxins: diagnosis and prevention [Mikotoksiny: diagnostika i profilaktika]. *In: Proceedings of the Stavropol Research Institute for Animal Farming and Feed Production*. 2006; 2: 89–97. eLIBRARY ID: 17244251. (in Russian)

12. GOST 13496.6-71. Mixed feed. Method of detachment of fungi (with amended No. 1, 2). M.: Standartinform; 2011. 4 p. Available at: http://docs.cntd.ru/document/1200024325. (in Russian)

13. Methodical guidelines for sanitary and mycological evaluation of feed and for feed quality improvement [Metodicheskie ukazaniya po sanitarno-mikologicheskoj ocenke i uluchsheniyu kachestva kormov]. M.: Main Veterinary Department of the USSR Ministry of Agriculture; 1985. 68 p. Available at: http://docs.cntd.ru/document/1200108240. (in Russian)

14. MU-287-113. Methodical guidelines for disinfection, pre-sterilization cleaning and sterilization of medical devices [Metodicheskie ukazaniya po dezinfekcii, predsterilizacionnoj ochistke i sterilizacii izdelij medicinskogo naznacheniya]. M.: Federal Centre for State Sanitary and Epidemiological Surveillance of the RF Ministry of Health; 1998. Available at: http://docs.cntd.ru/document/1200031410. (in Russian)

15. GOST 10444.12-2013. Microbiology of food and animal feeding stuffs. Methods for the detection and colony count of yeasts and moulds. M.: Standartinform; 2014. 7 p. Available at: http://docs.cntd.ru/document/1200107308. (in Russian)

16. Potekhina R. M., Ermolaeva O. K., Sagdeeva Z. Kh., Semenov E. I. Mycological assessment of feed in the Republic of Tatarstan. *Veterinarnyj Vrach*. 2019; 1: 19–23. DOI: 10.33632/1998-698X.2019-1-19-24. (in Russian)

17. Richard J. L. Some major mycotoxins and their mycotoxicoses – an overview. *Int. J. Food Microbiol*. 2007; 119 (1–2): 3–10. DOI: 10.1016/j.ijfoodmicro.2007.07.019.

18. GOST 31674-2012. Feeds, compound feeds, material for compound feeds. Methods for the determination of common toxicity. M.: Standartinform; 2014. 15 p. Available at: http://docs.cntd.ru/document/1200096705. (in Russian)

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INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Selime S. Ibragimova, Post-Graduate Student, Head of Sector, FGBI "ARRIAH" Branch in the Republic of Crimea, Simferopol city, Russia.

Olga V. Pruntova, Doctor of Science (Biology), Professor, Chief Expert, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia.

Sergey I. Danilchenko, Candidate of Science (Veterinary Medicine), Head of Laboratory and Diagnostic Centre, FGBI "ARRIAH" Branch in the Republic of Crimea, Simferopol city, Russia.

Yelena S. Yerofeeva, Post-Graduate Student, Head of Food Safety Laboratory, FGBI "ARRIAH" Branch in the Republic of Crimea, Simferopol city, Russia.

Ибрагимова Селиме Серверовна, аспирант, руководитель сектора Филиала ФГБУ «ВНИИЗЖ» в Республике Крым, г. Симферополь, Россия.

Прунтова Ольга Владиславовна, доктор биологических наук, профессор, главный эксперт информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Данильченко Сергей Иванович, кандидат ветеринарных наук, руководитель лабораторно-диагностического центра Филиала ФГБУ «ВНИИЗЖ» в Республике Крым, г. Симферополь, Россия.

Ерофеева Елена Сергеевна, аспирант, заведующий лабораторией безопасности пищевых продуктов Филиала ФГБУ «ВНИИЗЖ» в Республике Крым, г. Симферополь, Россия.