AGRICULTURAL BIOLOGY,
2015, V. 50, № 4, pp. 503-512
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Sanitary quality of fodder, fodder supplements

UDC 636.085.19:633.2.03:632.4(470)

doi: 10.15389/agrobiology.2015.4.503rus
doi: 10.15389/agrobiology.2015.4.503eng

MYCOTOXIN CONTAMINATION OF MEADOW GRASSES IN EUROPEAN RUSSIA

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Acknowledgements:
The authors thank the Head of Central Veterinary Office for Moscow Province and the staff of Territorial Offices for Animal Health Control for assistance in sampling hay
Received May 19, 2015

A b s t r a c t

A toxicity of plants eaten by animals at grazing land is shown to be complicated with various causes and clinical manifestations. In addition to phytotoxins and «infection» factors carried onto the plants by the insects (e.g., bacterial corynetoxins), the toxic metabolites of endophytic and epiphytic fungi are considered to play the significant role. Based on early understanding, the local risks for cattle, sheep and horses during grazing and stable periods were caused mainly by ergotism, myrothecio- and fusariotoxicoses. Then, for a long time a mycotoxicological evaluation of local grass feeds was not carried out. To date, there is the only one study indicating differences between contaminations of the wild-growing gramineous plants and cultivated cereal crops (A.A. Burkin et al., 2010). The aim of the paper was to summarize our data of assaing 517 meadow grass samples from natural pastures and haying places in European Russia undertaken for the first time to determine contamination with mycotoxins. The spikes of fescue *Festuca* sp., couch grass *Elytrigia repens* (L.), timothy *Phleum* sp., and other locally occurring gramineous plants were selected in North Karelia, Tverskaya, Leningradskaya and Astrakhanskaya regions for July-October 2011. For summer and autumn 2014 collections the aboveground parts of gramineous plants and legumes were taken from Moskovskaya, Tverskaya, Astrakhanskaya regions and North Karelia. The average samples of the field sets of hay were obtained from the animal farms of Moskovskaya region from December 2013 up to April 2014. A multiple combined contamination of grassland gramineous plants and legumes by the mycotoxins was detected, particularly we have found the *Fusarium* fungi metabolites T-2 toxin (T-2), diacetoxyscirpenol (DAS), deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FUM), the *Alternaria* metabolite alternariol (AOL), the *Myrothecium* metabolite roridin A (RoA), the «storage fungi» metabolites aflatoxin B_1 (AB_1), sterigmatocystin (STE), cyclopiazonic acid (CPA), emodin (EMO), ochratoxin A (OA), citrinin (CIT), mycophenolic acid (MPA), PR-toxin (PR), and also the ergot alkaloids (EA). The common trend to changing the component composition and content of mycotoxins was revealed for collected samples of gramineous plants from Moskovskaya and Tverskaya regions (June-September) such as reed grass *Calamagrostis* sp., crested dog's tail *Cynosurus cristatus* L., sweet vernal grass *Anthoxanthum odoratum* L., cock's foot *Dactylis glomerata* L., bromegrass *Bromus* sp., bluegrass *Poa* sp., oats *Avena sativa* L., fescue *Festuca* sp., bentgrass *Agrostis* sp., couch grass *Elytrigia repens* (L.), timothy *Phleum* sp., bristly foxtail grass *Setaria* sp. The complex of all examined mycotoxins was found to be formed in plants during early growing period (June). Moreover, AOL, STE, CPA and EMO were detected almost in all locations (80-98 % of samples), whereas DAS, EA, AB_1, OA, STE and MPA occurred rarely (50-70 %). This period, in contrast to subsequent ones, was characterized by low level of T-2 (≤ 40 μg/kg), ZEN (≤ 56 μg/kg), EA (≤ 64 μg/kg), AOL (≤ 200 μg/kg) and EMO (≤ 315 μg/kg) and its negligible (no more than 10-fold) variations in all mycotoxins with the exception of RoA. In the second collection of samples (July) AOL and EMO remained the significant contaminants (89 and 100 %) with an increased incidence of T-2, DON, ZEN and a wider range of the fusariotoxins, CPA and EA amounts. During continued vegetation (August-September) there were stable high indices of prevalence and accumulation of T-2 (up to 795 μg/kg), AOL (up to 10000 μg/kg), EMO (up to 5620 μg/kg), a decreasing incidence of AB_1, CPA, OA, CIT, MPA, PR, DAS, DON, FUM, ZEN fusariotoxins, and super high level of ZEN (up to 5750 μg/kg) occurred occasionally. The peculiarities of contamination of the legumes, such as meadow clover *Trifolium pratense* L., white clover *Trifolium repens* L., narrow-leaved vetch *Vicia* sp., wood vetch *Vicia sylvatica* L., meadow peavine *Lathyrus pratensis* L., the meadow grasses and the hay of various botanic compositions are discussed. For the first time a contamination of herbage with STE has been shown. The data obtained on RoA are especially important due to limited information of its prevalence.

Keywords: meadow grasses, gramineous plants, pod-bearing plants, hay, mycotoxins.

The toxicity of plants eaten by animals in pastures has been shown to be a complicated problem with various causes and clinical manifestations. In addition to phytotoxins and «infectious» factors carried onto the plants by the insects (e.g., bacterial corynetoxins), the toxic metabolites of endophytic and epiphytic fungi are considered to play a significant role [1]. Endophytes of meadow grasses that produce ergot alkaloids caused considerable damage to livestock in the United States and other countries [2, 3]. This problem was managed in recent years only due to the successful introduction of variety substitution technology [4-7]. Toxins of epiphytic fungi that infect vegetative forage plants are quite diverse and include phomopsines (*Phomopsis leptostromiformis*), diplodiatoxines (*Diplodia maydis*), slaframine (*Rhizoctonia liguminicola*), paspalines (*Claviceps paspali)*, ergot alkaloids (*Claviceps purpurea*), trichothecenes, and zearalenone [8-10].

The studies of etiology of mycetogenetic animal intoxication started in our country in the 1930s revealing the general risks for cattle, sheep and horses during grazing and stable periods caused mainly by ergotism, myrothecio- and fusariotoxicoses. At the first signs of poisoning, inspection of pastures for ergot and *Myrothecium* infection was recommended, as well as avoiding grazing on stubble in the fall and early spring in the meadows with the remains of overwintered crops and the young grass damaged with ground frosts. At the same time, experts noted drastic changes in the accumulation of mycotoxins due to unknown causes. Often, extensive fungal infections did not signify hazard, and, on the contrary, in the absence of visible signs plant infection, acute toxicity could arise [11, 12]. These observations are still the most convincing argument for the need for toxicological monitoring of grass fodder.

We performed the first study of meadow grass in August 2009 in North Karelia at the Arctic zone border along the White Sea coast within an area of about 150 km in length located between the biological stations of Saint-Petersburg State University and M.V. Lomonosov Moscow State University. Tops of sand lyme-grass, couch grass and timothy grass collected from several closely spaced plants were free from ochratoxin A, citrinin and T-2 fuzariotoxin, deoxynivalenol, zearalenone, but contained alternariol that is rare for grain (one of the toxic metabolites of *Alternaria* fungi) and diacetoxyscirpenol (trichothecene fuzariotoxin) [13]. In this way, the first confirmation of differences in contamination of wild grasses and cultivated crops has been obtained earlier.

Continuing our research, we were the first in Russia to perform the random mycotoxicological estimation of meadow grass stands the results of which are summarized in this article. At the same time, the data obtained on red clover (*Trifolium pratense* L.) fundamentally changed the conception of local risks associated with it as a common pasture culture. Different contamination of herbage and hay with sterigmatocystin was first described. The data on roridin A are of particular interest as the information on its prevalence is very limited.

The purpose of this study was to investigate vegetating meadow grass samples from natural pastures and hayfields in European Russia to determine contamination with mycotoxins.

Technique. In July and October 2011 spikes were selected of fescue *Festuca* sp., couch grass *Elytrigia repens* (L.), timothy grass *Phleum* sp. and other locally occurring grasses, particularly, reed canary grass *Phalaroides arundinacea* (L.) Rauschert, sand lyme-grass *Leymus arenarius* (L.) Hochst. in North Karelia (Loukhskii District); reed grass *Calamagrostis* sp., cock's foot *Dactylis* *glomerata* L., bromegrass *Bromus* sp., bluegrass *Poa* sp., common reed *Phragmites australis* (Cav.) Trin. ex Steud. in Tver' Province (Vyshnevolotskii Region); reed grass, canary reed grass, cock'sfoot, bromegrass, bluegrass, rye *Secale* sp. in Leningrad Province (Luzhskii, Priozerskii, Pushkinskii regions), and bromegrass, bluegrass, pampas grass *Cortaderia* sp., and common reed in Astrakhan' Province (Enotaevskii Region). In summer and fall of 2014, aerial parts of gramineous (reed grass, crested dog's tail *Cynosurus cristatus* L., sweet vernal grass *Anthoxanthum odoratum* L., canary reed grass, cock's foot, bromegrass, lyme-grass, foxtail *Alopecurus* sp., bluegrass, oats *Avena sativa* L., fescue, bentgrass *Agrostis* sp., couch grass, ryegrass *Lolium* sp., rye, timothy grass, bristly foxtail grass *Setaria* sp.) and leguminous plants (narrow-leaved vetch *Vicia* sp., wood vetch *Vicia sylvatica* L., red clover *Trifolium pratense* L., white clover *Trifolium repens* L., meadow peavine *Lathyrus pratensis* L.) were conducted in Moscow Province (Kashirskii, Noginski, Ruzskii regions in June to September), Tver' Ptovince (Vyshnevolotskii Region, in July and September), Astrakhan' Province (Enotaevski Region, bank of the Volga river in October) and in North Karelia (Loukhski District, the White Sea coast in August). The plants were cut at a height of 3-5 cm from the soil surface and dried at 50 °C. Average samples from production batches of hay were provided by agricultural enterprises of Moscow region in the period from December 2013 to April 2014.

Ground air-dried plant material was extracted with a mixture of acetonitrile and water in the volume ratio 86:14; extraction agent consumption of 10 ml per 1 g of sample weight. Extracts 10-fold diluted with a buffer solution were used for indirect competitive enzyme-linked immunosorbent assay (ELISA). Content of T-2 toxin (T-2), diacetoxyscirpenol (DAS), deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FUM), ergot alkaloids (EA), alternariol (AOL), roridin A (RoA), aflatoxin B_1 (AB_1), sterigmatocystin (STE), cyclopiazonic acid (CPA), emodin (EMO), ochratoxin A (OA), citrinin (CIT), mycophenolic acid (MPA), and PR-toxin (PR) was evaluated using certified enzyme immunoassay [14]. The lower limit of quantification was determined by the 85 % level of antibody binding.

Results. AOL was found in all the spikes collected in July-October 2011. DAS was found in one sample from Tver' region only (Table 1).

1. Contamination of spikes with fusariotoxins, alternariol, and ergot alkaloids in wild grass in different regions of the European part of Russia (collected in 2011)

Mycotoxin	n^+/minimal-maximal content, rg/kg			
	Tver' Province, July (n = 29)	North Karelia, August (n = 19)	Leningrad Province, September (n = 65)	Astrakhan Province, October (n = 48)
T-2	23/14-450	1/10	19/8-125	5/8-60
DAS	1/100	–	–	–
DON	2/126, 225	–	1/150	–
ZEN	–	–	–	–
EA	19/10-69,000	9/19-16,980	27/17-5,250	2/10, 80
AOL	11/40-560	5/343-8,310	52/40-1,320	39/45-1,260

N o t e. T-2 — T-2 toxin, DAS — diacetoxyscirpenol, DON — deoxynivalenol, ZEN — zearalenone, EA — ergot alkaloids, AOL — alternariol; n — number of samples studied, n^+ — number of positive samples. Dash means that no positive samples were found.

In two areas (North Karelia, Astrakhan' Province), grass rarely contained small amounts of T-2, in other areas (Tver' and Leningrad provinces) T-2 in the amount of more than 100 μg/kg was common, DON was rare. In Tver' and Leningrad Provine and in North Karelia, within the range of ergot, extensive contamination of spikes with ergot alkaloids (EA) was observed, and ultra-high accumulation of EA could be the consequence of sclerotia maturation. Further extended analysis of 29 samples from Tver' Province (16 mycotoxins) showed extensive contamination of spikes with EMO (24 samples, 80-4,680 μg/kg) and less frequent contamination with OA, STE (7 samples, 8-25 mcg/kg), and CPA

(3, 125-160 μg/kg). This meant that not only toxigenic fungal species of *Fusarium* and *Alternaria* and producers of ergot alkaloids, but also the representatives of other micromycetes allegedly belonging to the *Aspergillus* and *Penicillium* genera or perhaps a number of other genera played their role in the infection of growing plants.

Further examination of meadow grass was performed using a panel of 16 analytical test systems, and, in most cases, legume samples were collected along with grass. At the beginning of the growing season (June), multiple concomitant contaminations of grass with the all analyzed mycotoxins were observed in Moscow Province (Table 2). The amount of EA was not great (beyond 64 μg/kg). AOL, STE, CPA, and EMO were widely distributed, the rate of DAS, OA, CIT, MPA, AB_1 and EA was around 50 %, and this value was 35 and 23 % for RoA and PR, respectively. The frequency of T-2, ZEN, and DON detection was inferior to DAS, and FUM was found in 2 % of samples only.

2. Mycotoxin incidence and content in meadow grasses in different regions of the European part of Russia, depending on the timing of the growing season (collected in 2014)

Mycotoxin	Incidence,%/minimal-maximal content, rg/kg							
	Moscow Region				Tver' Province		North Karelia	Astrakhan Province
	June (*n* = 92)	July (*n* = 55)	August (*n* = 46)	September (*n* = 17)	July (*n* = 21)	September (*n* = 16)	August (*n* = 22)	October (*n* = 18)
T-2	32/3-40	65/4-760	70/4-795	65/4-630	86/4-2,510	69/5-540	23/3-630	33/3-220
DAS	52/104-500	22/89-500	2/125	–	29/117-400	6/220	–	6/160
DON	15/126-315	31/117-930	2/125	12/78.400	24/79-400	6/120	5/570	6/500
ZEN	17/27-56	27/52-145	11/25-5,750	12/79, 1,520	29/49-100	–	–	–
FUM	2/66, 85	4/126, 215	–	–	–	–	–	–
EA	70/2-64	53/2-52,200	35/3-2,000	24/2-870	67/4-33,000	31/2-125	100/2-10,000	-
AOL	98/19-200	89/28-830	100/28-2,240	100/31-10,000	86/25-135	94/24-9,770	100/40-4,680	83/40-2,510
RoA	35/8-265	20/2-80	–	–	24/21-50	–	5/7	–
AB_1	51/2-16	15/4-10	2/5	–	10/6, 8	–	23/4-10	6/3
STE	80/10-53	33/9-95	59/8-190	71/13-80	29/32-70	75/13-135	68/12-45	44/12-40
CPA	95/151-660	49/115-1,380	11/166-330	–	76/200-515	–	18/132-280	11/155, 158
EMO	87/33-315	100/73-3,890	100/56-5,620	100/72-5,130	86/59-330	100/59-5,370	95/63-870	94/51-2,500
OA	55/8-28	36/8-21	13/7-9	–	57/6-20	–	14/9-13	17/10-370
CIT	50/33-160	27/66-295	9/42-70	–	19/91-175	–	–	–
MPA	60/14-84	40/14-80	15/10-150	24/12-25	67/18-45	–	41/20-170	28/23-35
PR	23/105-400	24/120-430	2/165	–	19/200-250	–	–	–

N o t e. T-2 — T-2 toxin, DAS — diacetoxyscirpenol, DON — deoxynivalenol, ZEN — zearalenone, FUM — fumonisins, EA — ergot alkaloids, AOL — alternariol, PoA — roridin A; AB_1 — aflatoxin B_1, STE — sterigmatocystin, CPA — cyclopiazonic acid, EMO — emodin, OA — ochratoxin A, CIT — citrinin, MPA — mycophenolic acid, PR — PR-toxin; *n* — number of samples studied. Dash means that no positive samples were found.

The amount of most mycotoxins varied insignificantly: it was within the same range in DAS, DON, ZEN, FUM, STE, CPA, MPA, and PR, or it was in the same range in all the others, except RoA. Presumably, different grasses respond to infection-producing fungi similarly in the initial stage of plant development. The same feature was observed in Leningrad Region in the seeded grass herbage prior to the first cut [15].

In a month, in July, the incidence index decreased in DAS, RoA, AB_1, STE, CPA, OA, CIT, and MPA. At that, there was an increase in both fusariotoxins (most dramatic in T-2) and EA, AOL, STE, CPA, and EMO content range. It was during this period that the ultrahigh amount of EA was observed in plants, up to 52,200 μg/kg.

In August and September, the T-2 contamination in plants remained the same, while DAS was detected even rarer. It is essential to note the cases of ZEN quantities accumulation greater than 1,000 μg/kg, as this conceivably could be due to *Fusarium* species that tend to change their metabolic response under the influence of environmental factors [16]. Prevalence of AOL and EMI remained widespread, the intensity of AOL accumulation progressed smoothly, and the amount of this toxin could reach 10,000 μg/kg in September. EMO content was stable with the limit value of about 5,000 μg/kg. The incidence of many other toxins decreased. Thus, RoA was not found in grasses in August, and AB_1, OA, CIT, CPA, MPA, and PR were rarer compared to in July. AB_1, OA, CIT, CPA, and PR were never found.

The dynamics of DON and STE incidence and content were unstable. The maximum DON amount was 930 μg/kg. Content lower than 10 μg/kg was typical of STE, and this value was rarely exceeded only in the later stages the growing season (August and September).

Some of the trends described for grasses in Moscow Region, were the same in Tver' Province (see Table 2). Thus, the incidence of most mycotoxins decreased, the range of AOL and EMO increased, and ultra-high amounts of EA and ZEN were not observed in September compared to in July. In North Karelia (August) and Astrakhan Province (October), grasses were poor in mycotoxins with a dominant spread of AOL and EMO, but in smaller quantities compared to that in September in Moscow and Tver' provinces. A low infectious load in the place of growth could be the reason for this.

Seasonal changes in contamination of grasses, various in different mycotoxin groups, were likely the result of processes in the composition of microbiota. It could be of a regular character, but the effect of climatic or environmental factors could not be excluded. The most important future purpose for researchers is obtaining statistically significant information on the nature of meadow and pasture grass contamination with micromycetes and mycotoxins within the entire period of their economic use from the extended database. The results presented in this study is the first attempt to perform a mycotoxicological estimate of natural herbage grasses which will later make it possible to move on to exploration of some of the most common plant species.

3. Contamination of samples of meadow leguminous plants with mycotoxins in Moscow and Tver' provinces, and North Karelia (2014)

Mycotoxin	n^+/minimal-maximal content per sample, μg/kg				
	red clover (n = 35)	white clover (n = 5)	narrow-leaved vetch (n = 11)	wood vetch (n = 12)	meadow peavine (n = 6)
T-2	29/3-108	4/3-4	8/3-50	3/5-6	1/6
DAS	19/122-550	–	2/132, 133	–	–
DON	11/89-405	–	–	3/126-140	1/67
ZEN	15/31-190	1/90	–	–	–
FUM	18/42-300	3/56-245	–	2/56, 56	1/100
EA	35/2-490	5/4-9	5/3-62	12/2-15	3/3-8
AOL	35/30-830	5/129-400	11/19-310	12/23-100	6/20-540
RoA	17/7-185	1/100	3/35-125	–	1/4
AB_1	29/3-22	4/5-15	3/5	4/2-6	5/2-6
STE	28/18-200	4/42-115	10/25-2,320	11/15-45	6/10-70
CPA	35/190-2,455	5/176-540	6/158-525	9/107-560	2/155, 165
EMO	35/260-27,540	5/155-5,500	11/63-1,585	11/51-1,000	6/50-150
OA	35/7-105	4/10, 11	5/11-15	4/8-16	1/10
CIT	30/42-340	3/72-90	3/91-125	–	–
MPA	33/14-130	5/22-40	3/28-40	2/16, 16	–
PR	35/148-910	3/158-315	3/176-400	2/133, 150	–

N o t e. T-2 — T-2 toxin, DAS — diacetoxyscirpenol, DON — deoxynivalenol, ZEN — zearalenone, FUM — fumonisins, EA — ergot alkaloids, AOL — alternariol, PoA — roridin A; AB_1 — aflatoxin B_1, STE — sterigmatocystin, CPA — cyclopiazonic acid, EMO — emodin, OA — ochratoxin A, CIT — citrinin, MPA — mycophenolic acid, PR — PR-toxin; n — number of samples studied, n^+ — number of positive samples. Dash means that no positive samples were found.

In red clover samples selected in Moscow and Tver' provinces and in Northern Karelia, the complex of mycotoxins usually consisted of 12-15 ingredients (Table 3). AOL, EMO, OA, PR, CIT, and MPA were widespread or were of close prevalence, PoA was quite substantially sizeable (in half of samples). An ultra-high content of EMO (up to 27,540 μg/kg) was observed in this plant in different habitats in July, August and September.

Our findings fundamentally change the existing ideas about local risks associated with this major pastoral culture. Earlier, clover contamination with *Rhyzoctonia leguminicola* fungus observed in some regions of the world (particularly in the US) and «slobber» syndrome in cattle and horses caused by slaframine and swainsonine were considered of economic importance [17]. Extensive multiple mycotoxin contamination characteristic of a variety of fungi species is not in complete agreement with a developed system of biochemical protection of these plants against fungal infection [18].

Despite the smaller sample size of white clover, as a whole it has the same features of extensive contamination as red clover. Widespread or close incidence of the same group of mycotoxins was observed, but the mycotoxin limit accumulation did not reach the values typical of red clover.

The number of narrow-leaved vetch, meadow pea-vine, and wood vetch samples was also small (6 to 12). In these plants, AOL, STE, and EMO were found almost everywhere, and they were inferior to both clover species in the content of other mycotoxins. Similarities in T-2 common incidence and rare DAS and CIT were observed in narrow-leaved vetch and clovers. A possibility of intensive CTE contamination (up to 2,320 μg/kg) is one of the *Vicia* sp. features.

In general, the accumulation of the amounts of T-2 exceeding 100 μg/kg was not observed in the late stages of vegetation in leguminous plants, unlike grasses. Unfortunately, the small sample sizes of these plants provide just a general idea of mycotoxin contamination. To verify the data and evaluate the seasonal variation in legume contamination, it is necessary to have more extensive biomaterial. It is important to expand the species composition of the crops studied including economically valuable plants, such as alfalfa, sainfoin, bird's foot deer vetch, goatэs rue, sweet clover, and others.

4. Mycotoxin incidence and content in meadow grass and hay samples in Moscow Province (collected in 2014)

Mycotoxin	Incidence, %/minimal-maximal content, rg/kg	
	meadow grasses (n = 227)	hay (n = 120)
T-2	54/3-795	94/3-1,410
DAS	32/89-550	19/100-1,445
DON	19/78-930	28/87-1,620
ZEN	21/25-5,750	45/20-10,000
FUM	4/66-300	8/97-250
EA	57/2-52,200	83/2-3,160
AOL	96/19-0000	98/21-10,000
RoA	23/2-265	13/5-65
AB_1	30/2-6	25/2-25
STE	64/8-200	88/6-7,940
CPA	60/115-2,455	67/63-5,130
EMO	95/33-27,540	100/33-17,780
OA	41/7-105	14/5-30
CIT	35/33-340	33/28-515
MPA	46/10-150	88/15-10,000
PR	23/105-790	26/50-1,070

N o t e. T-2 — T-2 toxin, DAS — diacetoxyscirpenol, DON — deoxynivalenol, ZEN — zearalenone, FUM — fumonisins, EA — ergot alkaloids, AOL — alternariol, PoA — roridin A; AB_1 — aflatoxin B_1, STE — sterigmatocystin, CPA — cyclopiazonic acid, EMO — emodin, OA — ochratoxin A, CIT — citrinin, MPA — mycophenolic acid, PR — PR-toxin; n — number of samples studied.

Identifying distinct seasonal dynamics in cereal contamination and interspecific differences in mycotoxins content in leguminous plants, we felt it appropriate to generalize the results of Moscow Provicne obtained within the entire period of observation, and to compare them with the data on the dry grass fodder contamination harvested in the same area a year before. All 16 of mycotoxins participated in grass contamination in summer and fall providing multicomponent contamination with varying amounts of T-2, ZEN, EA, AOL, and EMO within two to three orders of magnitude (Table 4).

Frequent detection of fusariotoxins in herbs (20-96 %), as well as EA, AOL, and RoA can be attributed to the spread of «field» fungi. In contrast, high values of this parameter for all other mycotoxins (23-90 %) were quite unexpected, and a special study on the identification of micromycetes providing biosynthesis of these substances is required. So far, it is assumed that the probability of accumulation of mycotoxins typical of «storage fungi», OA in particular, is extremely low in vegetative plants [19].

The frequency of detection of the majority of mycotoxins in herbs and hay was quite comparable, but there was a significant (approximately 2-fold) increase in this parameter in T-2, ZEN, and MPA in hay, and the range of content clearly shifted towards higher values. We have already discussed possible sources and causes of accumulation of significant amounts of ZEN in vegetative grasses and hay [16]. In selective toxicological evaluation of hay from different regions of the European part of the country, the upper T-2 and MPA limits exceed 2,000 μg/kg [20]. An abnormally high level of STE (2,510, 3,550, and 7,940 μg/kg) was found in three samples of perennial hay grasses and motley grass. Differences in vegetative herbage and hay on the intensity of STE contamination were found for the time. Their reasonable interpretation can be started only after the completion of the search of micromycetes responsible for STE biosynthesis. It is obvious that these fungi not only continue to develop in the drying phase, but also intensify toxin formation under these conditions. In addition, the causes of significant STE accumulation may be associated with the peculiarities of the botanical composition of the forage.

Hay AOL contamination remained high; there were no significant changes in DON, FUM, AB_1, OA, and CIT contamination, the limit amounts of EA and RoA were lower compared to herbs. The situation of EA can be explained by ergot sclerotia shedding during tedding, stacking hay in bales or stacks, as well as during transportation. Reduced incidence of PoA (from 23 % in plants to 13 % in hay) was accompanied by a decrease in the average value of accumulation from 48 to 20 μg/kg. These results are of particular value, since the information on RoA prevalence is very limited. RoA incidence and content in cultivated herbs in multi-mowing fields was low [8]. Cases of accumulation of 100 μg/kg of RoA were rare in meadow herbs. Of all the samples containing this toxin (n^+ = 51), its content was 100 to 265 μg/kg in seven samples only. In this context, the possibility of myrotheciotoxicosis in ruminants in the territories studied raise doubts.

In contrast, a high-limit content of T-2, DAS, ZEN, and EA in meadow plants in a number of areas of European Russia, as well as in dry green fodder, undoubtedly represents a threat to animal health and, consequently, fusariotoxicosis and ergotism can be considered as a real problem. Indeed, in the late 1990s, when calves were pastured in Kursk Province with frostbitten corn herbage heavily contaminated with *F. sporotrichioides,* massive acute toxicosis with sores in mouth was diagnosed in some areas. Cases of ergotism, claviceps-toxicosis and zearalenone-toxicosis in cattle, sheep and horses have not been recorded in our country, but such examples have been described in other countries [21-24]. Unfortunately, the negative effects of the intake of AOL, EMO, STE, CPA, MPA, and PR at thousands or tens thousands micrograms per 1 kg have not been evaluated experimentally, but the threat may be very serious due to the combination of various forms of their direct and long-term toxic effects [25].

Thus, many grass and leguminous forage species are a source of complex mycotoxin combinations which is subject to changes in vegetation, and has its own features of composition and ratio of individual components in different crops. Herbage T-2, zearalenone, mycophenolic acid, and sterigmatocystin contamination prior to drying may result in extremely intensive contamination of hay. The selective evaluation of meadow herbage mycotoxin contamination performed in our country for the first time indicates the need for increased monitoring studies, studies of other plants, as well as for toxicological experiments to assess the risk of combined effects of mycotoxin intake with grass and leguminous fodder on animals. In the future, it is also very important to pay attention to mycological and mycotoxicological assessment of *Asteraceae* meadows of economic importance, sedge herbage that make up the bulk of hay in the northern regions of the forest zone (slender sedge, water sedge, etc.), as well as of the plants belonging to other botanical families widely represented at natural forage lands.

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