Assessment of the level of contamination of feed with micromycetes and mycotoxins in the cattle industry of Ukraine in recent years

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Goal. To study the degree of contamination, the species of micromycetes and the content of mycotoxins (aflatoxin B1, sterigmatocystin, zearalenone, patulin) in feed for cattle for 2018 and the first half of 2019 and to carry out a retrospective analysis of contamination of feed with micromycetes over the last 5 years. Methods. Mycological and micotoxical study was conducted in accordance with generally accepted and developed in the laboratory of Toxicological monitoring methods of determination. Results. Level of contamination with micromycetes in 87 samples of feed for cattle in 2018–2019 exceeded the maximum permissible level (MPL) in 73.6% of samples. The major ones were: feed — 23.4%, mono feeds and silage — 18.7% each, straw — 10.9%, and hay — 7.8%. The main pollutants of the feed were micromycetes of the family Mucoraceae — 25.6%, and of species: Aspergillus Mich. — 24.7, Penicillium Linc. 9.5, Fusarium Linc. — 3.3, other — 36.9%. 35 samples of feed were examined on the presence of mycotoxins. Only one sample of feed has zearalenone (0,88 mg/kg, which is 76% higher than MPL). Aflatoxin B1, sterigmatocystin, patulin and zearalenone were outside the method definition, and did not exceed MPL. Conclusions. The level of contamination with micromycetes of feed for cattle in 2018 and the first half of 2019 remained high, including poor quality (higher than MPL), and was 73.3% and 73.8% respectively. The main pollutants of the feed were mold saprophytes of family Mucoraceae — 25.6%, and of species: Aspergillus Mich. — 24.7%, Penicillium Linc. — 9.5%, Fusarium Linc. — 3.3, other — 36.9%. The presence of aflatoxin B1, sterigmatocystin, patulin and zearalenone in quantities higher than MPL in 34 samples of feed was fixed. Only in the sample of feed zearalenone was determined — 0,88 mg/kg of feed, which is 76% higher than MPL.

Key words: biotic contaminants, toxin-forming mold saprophytes, microscopic fungi strains.
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One of the many negative environmental factors that affect the safety of feed materials and feeds are micromycetes and their secondary metabolites - mycotoxins. Their special danger lies in the latent form of impact on animal health. The presence of microscopic fungi in feed leads to a decrease in their consumption due to the deterioration of organoleptic qualities and causes a decrease in the absorption of nutrients and metabolic disorders in the body.

As a result, the costs of treatment of animals, as well as due to shortages and reduced quality of livestock products cause great economic losses [1-7]. According to the Food and Agriculture Organization of the United Nations (FAO), due to the high prevalence of microscopic fungi in almost all habitats and their high adaptive properties, mold saprophytes affect 25 - 40% of feeds each year [2]. Therefore, we believe that the systematic control of micromycetes and their secondary metabolites in feed at all stages of their manufacture and during storage is one of the main measures to prevent their negative impact on animal health and ensure the production of safe and quality products [4, 6, 7].

The purpose of the work is to investigate the degree of contamination, species affiliation of micromycetes and the content of mycotoxins (aflatoxin B1, sterigmatocystin, zearalenone, patulin) in the feeds used in the livestock industry of Ukraine in 2018 – the first half of 2019 and to compare the results with previous years.

Materials and methods. The study was conducted on the basis of the laboratory for toxicological monitoring of the NSC “IECVM” in the period 2018 – I half of 2019. We analyzed 87 samples of feed for dairy cattle from different regions of Ukraine: Kharkiv, Sumy, Odesa, Donetsk, Kyiv, Poltava, Cherkasy, Kirovograd, Ivano-Frankivsk and Ternopil regions.

Mycological studies of the samples were performed according to generally accepted methods, in particular: the degree of contamination of feed with microscopic fungi was determined by the number of colony-forming units (CFU) per 1 g of feed under primary seeding in nutrient medium (agar wort and Chapek) [7, 8]; species affiliation of isolates of microscopic fungi was determined by comparing cultural and morphological characteristics of the isolated mycobionta (features of crop growth on different nutrient media, their size, shape, width, structure of edges and center of colonies, growth intensity, surface characteristics, colony color, their reverse, mycelium, etc.) with the descriptions given in the determinants of micromycetes, and with museum strains of test cultures (property of NSC "Institute of Experimental and Clinical Veterinary Medicine") [9-12].
Mycotoxicological studies were performed following the guidelines "Simultaneous determination of mycotoxins (aflatoxin B1, zearalenone, patulin, sterigmatocystin) in feed and cereals by thin-layer or liquid chromatography", which were developed in the laboratory and approved by the State Committee of Veterinary Medicine of Ukraine (protocol №1, December 24, 2009).

**Research results.** During mycological monitoring in 2018–2019, 87 samples of feed and feed materials for cattle were studied, in particular: roughage and succulent feed (silage, haylage, hay, straw, mono-feed, beet pulp, beer pellets) – 56 samples, compound feed – 17, cereals (grain mixture, corn, barley, wheat) – 8 and other types of feed – 6 (Fig. 1). From the data shown in Fig. 1, it is seen that mostly coarse and succulent feeds, as well as compound feeds were subjected to mycological analysis.

![Fig. 1. Types of feed for cattle, subjected to mycological research in 2018 and the first half of 2019: 1 – roughage and succulent feed; 2 – compound feeds; 3 – grain feed; 4 – other types of feed](image)

The degree of contamination with microscopic fungi of feed for cattle was determined. It was found that in 2018 and the first half of 2019, only 26.7 and 26.2% of feeds had a permissible degree of contamination (less than $5 \times 10^4$ spores per 1 g of feed), and 73.3 and 73.8% of feeds exceeded the maximum allowable level (MAL), respectively [8]. Compound feeds – 23.4% (15 samples), mono-feeds and silage 18.7% (12 samples each), roughage: straw – 10.9% (7 samples), hay – 7.8% (5 samples) were the most contaminated with micromycetes.

Among the studied, the smallest number of feed samples exceeding the MAL was found among cereals – 3.1% (2 samples). To determine the composition of mycobiota of feed and feed raw materials in 2018 and the first half of 2019, 360 isolates of mold and yeast-like fungi were isolated and identified (in 2018 – 163, in 2019 – 197 isolates).
Fig. 2. Generic composition of mycobiota of feed and feed materials for cattle in 2018 and the first half of 2019: ■ 2019; ■ 2018

In particular, the number of Aspergillus Mich. isolated during the study period was 89 isolates (24.7%), Penicillium Linc. – 34 (9.5%), Fusarium Link. – 12 (3.3%), family Mucoraceae – 92 isolates (25.6%).

Representatives of other genera – 133 isolates (36.9%). It should be noted that the number of isolates of the genus Aspergillus Mich. was higher in 2018. This is due to heat and dryness in early summer of that year (temperatures in June reached 30-35°C), and such climatic conditions are optimal for the growth of many species of micromycetes of the genus Aspergillus Mich.

The number of isolates of the Mucoraceae family was also identified in a clear dependence on weather conditions (temperature and humidity). In particular, due to the cool, rainy weather in July 2019, the number of isolates increased 1.5 times compared to the previous year (in 2018 – 37, in 2019 – 55 isolates). The taxonomic affiliation of isolates of potentially toxin-forming microscopic fungi was determined (table).

**Species taxonomy of toxin-forming micromycetes isolated from feed samples for cattle in 2018 and the first half of 2019**

| Type of toxin-forming micromycete, toxic metabolite | Number of isolates, pcs. | The total species number of isolates in the genus,% |
|---------------------------------------------------|--------------------------|-----------------------------------------------|
| **Genus Aspergillus Mich.**                       |                          |                                               |
| Asp. flavus — aflatoxins B1, B2, G1, G2, H1, H2, sterigmatocystin, penitrema, tremogens, ohalates, etc. | 35                       | 39.4                                          |
| Asp. amstelodami — aflatoxin, sterigmatocystin    | 5                        | 5.6                                           |
| Asp. niger — aflatoxin, ohalates                  | 11                       | 12.4                                          |
| Asp. sydowi — sterigmatocystin                    | 5                        | 5.6                                           |
| Asp. fumigatus — aflatoxin, fumigatin, fumitoxin A-D, fumitremorgin, etc. | 5                        | 5.6                                           |
| Asp. candidus — citrinin, tremogen                | 6                        | 6.7                                           |
| Asp. oryzae — aflatoxin, orysochlorin, maitoricin ochratoxins A, B, C, D, aflatoxin, patulin, etc. | 6                        | 6.7                                           |
| Other species                                     | 89                       | 100                                           |
| Total                                            |                          |                                               |
| **Genus Penicillium**                             |                          |                                               |
| Pen. lanosum — citrinin                           | 11                       | 32.4                                          |
**Table 1.** Toxigenic properties of selected molds isolated from feed samples

| Species                      | Total | Toxic properties |
|------------------------------|-------|------------------|
| Pen. commune                  | 8     | 23.5             |
| Pen. stoloniferum            | 5     | 14.7             |
| Pen. casei                   | 2     | 5.9              |
| Other species                | 34    | 100              |

| Total | 86 |

**Family Mucoraceae**

| Genus Mucor Mich. | — toxic properties | 34 | 37.0 |
|-------------------|--------------------|----|------|
| Genus Rhizopus Ehrenb. | — aflatoxin, toxic properties | 42 | 45.6 |
| Other species     | 92                 | 100 |

**Family Ascomycota**

| Genus Fusarium Linc. | — moniliformin, vomitoxin, T-2 toxin, etc. | 7 | 58.3 |
|----------------------|---------------------------------------------|---|------|
| Fusarium moniliforme, var. lactis | — fusaric acid, vomitoxin, moniliformin, T-2 toxin, etc. | 2 | 16.7 |
| Other species        | 12                             | 100 |

**Conclusions**

When determining the degree of cattle feed contamination with microscopic fungi in 2018 and the first half of 2019, it was found that 73.6% (73.3% in 2018, 73.8% in 2019) of feed was of poor quality. The main contaminants of feed were mold saprophytes of the family Mucoraceae – 25.6% and the genus Aspergillus Mich. – 24.7%, representatives of the genus Penicillium Linc. were 9.5%, Fusarium Linc. – 3.3, other genera – 36.9%.

It was found that the main contaminant of feed was mold of the genus Aspergillus Mich., from which species: Asp. flavus, Asp. amstelodami, Asp. niger, Asp. sydowi, Asp. fumigatus, Asp. candidus, Asp. oryzae, Asp. ochraceus were identified. They were most often isolated from compound feeds, mono-feeds, silage, haylage, hay, corn, grain mixtures. The genera Mucor and Rhizopus were identified in compound feeds, mono-feeds, wheat, sunflower, straw, hay, and silage.

From micromycetes of the genus Penicillium Linc. most often species Pen. lanosum, Pen. commune, Pen. stoloniferum were found in compound feeds, haylage, hay, grain mixtures, corn, straw. The genus Fusarium Linc. species Fusarium moniliforme, Fusarium moniliforme, var. lactis were isolated from mono-feeds, compound feeds, grain mixtures, corn, barley.

According to mycotoxicological studies, 35 samples of feed with the highest degree of contamination by microscopic fungi, the presence of aflatoxin B1, zearalenone, sterigmatocystin and patulin were established within the regulated indicators in accordance with the requirements of the "List of maximum levels of undesirable substances in feeds" (The Order of the Ministry of Agrarian Policy and Food of Ukraine № 131 dated 19.03.2012, as amended by the order of the Ministry of Economic Development and Trade № 550 dated 11.10.2017).

However, in the feed sample obtained from the dairy farm of Kharkiv region, the presence of zearalenone was determined at a concentration of 0.88 mg/kg, that is 76% higher than the MAL of this toxin [8]. Thus, it was established that during the mycological monitoring of 87 samples of feed and feed materials used for feeding cattle in 2018 and the first half of 2019, 73.6% of feeds were of poor quality, that is more than two thirds of the total.

According to our data obtained in previous years, this trend has been continuing for the last 4 years. Thus, if in 2014 and 2015 substandard feed by the degree of contamination with microscopic fungi was 51–52%, in 2016 it was already 79%, and in 2017 – 88% [13–15]. Due to the weather conditions in 2019, for the first time in recent years, the main contaminants of feed were micromycetes of the family Mucoraceae (25.6%) and the family Aspergillus Mich. (24.7%) [16].

Also, due to the high content of zearalenone in the feed sample, special attention should be paid to the members of the family Fusarium Linc. Although a relatively small number of them was isolated – 12 isolates (3.3%), but they led to toxin formation. Toxin-forming taxa of micromycetes of the following species were identified: Asp. flavus, Asp. amstelodami, Asp. niger, Asp. sydowi, Asp. fumigatus, Asp. candidus, Asp. oryzae, Asp. ochraceus, Pen. lanosum, Pen. commune, Pen. stoloniferum, Fusarium moniliforme, Fusarium moniliforme, var. lactis.
Toxin-forming taxa of micromycetes were represented by species Asp. flavus, Asp. amstelodami, Asp. niger, Asp. sydowi, Asp. fumigatus, Asp. candidus, Asp. oryzae, Asp. ochraceus, Pen. lanosum, Pen. commune, Pen. stoloniferum, Fusarium moniliforme, Fusarium moniliforme, var. lactic. During mycotoxicological studies of 35 feed samples in the 1st feed sample showed the presence of zearalenone at a concentration of 0.88 mg/kg, which is 76% higher than the MAL.

Aflatoxin B1, sterigmatocystin, patulin and zearalenone in other test samples were outside the detection of the method and below the MAL. This trend persisted during 2014–2017, the degree of contamination with microscopic fungi of substandard feed was 51–88%. Prospects for further research are the systematic control of contaminants of biotic origin (micromycetes, mycotoxins) of feed materials and feeds used for feeding cattle, to prevent their negative impact on animal health and productivity and reduce economic losses in livestock.

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