Detection of trace contaminants in cattle feed in northwest Russia

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Abstract. In our work, we conduct a research of mycotoxin contamination of feed for cattle harvested in 2022–2024. In total, we studied more than 260 samples of feed from a number of farms in the Vologda Region, and carried out more than 500 laboratory analyses of feed. The obtained results showed that almost all studied samples were contaminated with mycotoxins to some extent. The greatest degree of feed contamination of the studied mycotoxins is characterized by the sum of aflatoxins, so exceeding the maximum allowable concentration (MAC) of aflatoxins was detected in about 56% of samples. The next most important contaminant is ochratoxin-A, the share of samples with exceeding MAC is about 31%. Mono-species silages are the most affected by mycotoxins of all the feed samples studied.

1 Introduction

Mycotoxins are secondary metabolites of mold fungi with high toxicity. They can affect the human and animal organism, causing various diseases. The study of mycotoxins is closely related to the agricultural industry, where their harmful qualities are particularly pronounced. The beginning of direct or indirect research on mycotoxins is considered to be the second half of the 19th century, when it was first discovered that mold fungi were a source of increased toxicological threat to humans and animals. Thanks to more research, it has become possible to prove that the toxins that cause various diseases are the effect of mold fungi. Some types of mycotoxins can accumulate in the body, which can lead to chronic diseases. The systematic study of mycotoxins only began in the 20th century. The first mycotoxins, such as aflatoxin and zearalenone, were discovered in the 1960s. Many other types of mycotoxins have been discovered since that time [1].

The problem of feed contamination has a worldwide scale [2–7]. The main source of mycotoxins in the organisms of farm animals is feed, and mold fungi can develop on various types of feed, including grain, hay, silage, and mixed fodder. In addition, animals can be exposed to mycotoxins through air, water, and soil. It is worth noting that mycotoxins can remain in feed even after processing and storage. Russian and foreign studies performed at different times during the 20th century have established the main factors contributing to the spread of mold fungi affecting feed. First of all, we should take into account the regional cultivation features of major feed crops. Mold fungi are often confined to certain

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geographical areas, where they constitute a quantitative majority in the species composition of fungi found in affected forages. High ambient humidity and heat can promote fungal growth; improper harvesting can lead to mold growth; and prolonged storage can lead to the accumulation of mycotoxins [8].

It is necessary to take into account the species composition of plants serving as a basis for feed. This determines the complex of mycotoxins that can be discovered in affected samples [9–11].

Ruminants were previously thought to be poorly susceptible to the effects of mycotoxins. This opinion was based on the metabolic activity of the rumen microflora; however, this statement was true only for cows with an annual milk yield of not more than 5 thousand kilograms. Currently, the rumen microflora of modern cows is proved to be affected serious dysbiotic disorders and loosed the ability to naturally detoxify mycotoxins. Many researchers note that microorganisms' metabolites may be even more dangerous despite their ability in the rumen to neutralize some mycotoxins. Thus, mycotoxins of highly productive ruminants are not neutralized in the rumen, but are absorbed through the intestinal wall, as in monogastric animals [12].

The structure analysis of the Vologda Region sown areas demonstrates that the leading role belongs to forage crops. Silage is the largest volume of harvesting among forages. The demand of animal husbandry in forages by volume is satisfied by 100–120%, but by energy and nutritional value, it is only by 65–80%. Studies of mycotoxin contamination of used fodder by the Vologda Region agricultural enterprises are practically not conducted [13]. Meanwhile, agricultural enterprises face serious economic losses every year due to mycotoxin contamination of plant-based fodder. For example, decreased milk productivity and health problems of cows can lead to daily losses per animal up to 250 rubles (up to 100 thousand rubles per year), even in the absence of obvious symptoms of mycotoxicosis [14].

Moreover, the simultaneous affection of feed with several mycotoxins poses the greatest danger because their combined action enhances the toxic effect on the organism due to synergistic effects and additive properties [15, 16], also causing different clinical symptoms; mycotoxins are able to mask their negative effects on living organisms, which aggravates the problem [17].

The aim of the research is to assess the environmental risk for regional livestock by analyzing the content of mycotoxins in feeds. The task was to carry out mycotoxicological monitoring of different types of feeds, to identify the most affected types of feeds and the most common mycotoxins.

2 Materials and methods

Climatic conditions in the Vologda Region are characterized by temperate continental climate. The severity of the climate increases from the west to the east of the region. The average January temperature ranges from -11°C in the west to -14°C in the east, and the average July temperature ranges from +16°C to +18°C. Precipitation is quite high, ranging from 500 to 650 mm per year, with a maximum in the summer months. Evapotranspiration is much lower, resulting in an abundance of rivers, lakes, and bogs. Snow cover lasts from 165 to 170 days. The growing season lasts about 130 days. Climate of the region is moderately continental with long moderately cold winter and relatively short warm summer favors both preservation of fungi in the soil and their active growth in the mass of harvested fodder in the summer-autumn period; and in the mass of wet silage fodder, where actually all year round, favorable conditions are preserved – in the winter period and until late spring.

We obtained fodder samples from different farms of the Vologda Region; they were the material of natural moisture content for this type of sample (dry samples for hay and straw, with natural moisture content for plant samples – for green mass, with increased moisture content – for harvested fodder: silage, haylage). We classified the samples by type (silage, haylage, hay, straw, green mass), and compared its composition, place and time of harvesting and preparation for each sample. Sampling was carried out on the basis of GOST R ISO 6497-2011. When conducting studies, we considered only clean samples from mycotoxins in which the content of ochratoxin-A, zearalenone, DON, T-2 toxin and the sum of aflatoxins B1, B1, G1, G2 were below the lower detection limit of the test systems. MAC levels, which are reflected in the veterinary and sanitary requirements of the Customs Union (approved by the decision of the Commission of the Customs Union of the Eurasian Economic Union 317, dated June 18, 2010), regulate the content of mycotoxins in such feeds as wheat, barley, oats, corn, soybeans, etc., ignoring succulent and roughage ones. The values of MAC levels for the above-mentioned crops have insignificant differences, so we used these norms in our work, as well as the norms of MAC values for mycotoxins recommended by the Grain Association of the European Union (RIDA News, III/01).

We analyzed mycotoxins according to GOST 31653-2012 “Feed. Method of immunoenzymatic mycotoxin determination” with the help of standard test systems produced by ComProdService (Belarus), using the analyzer of immunoenzymatic reactions AIFR-01 UNIPLAN (Picon, Russia) in the CUC “Center for Agricultural Research and Biotechnology” of VolRC RAS.

Each sample was dried to a constant weight and pulverized twice regardless of its type (dry or wet): the first pulverization was to a particle size of no more than 2–3 mm, the second – to a size of no more than 0.1–0.2 mm. The crushed sample was immediately subjected to extraction with a mixture of methanol and water in volume ratios of 70 : 30 (in case of extraction of aflatoxins, zearalenone, ochratoxin-A, and T-2 toxin) or water in case of extraction of deoxynivalenol. Quantitative ratios of the sample weight in grams to the extraction mixture volume in ml are 1 : 5 for aflatoxins, zearalenone, ochratoxin-A, T-2 toxin, and 1 : 20 – for deoxynivalenol. The extraction time with stirring on a laboratory shaker is 5–7 minutes. After extraction, the mixture was centrifuged at 1,600 G, which made it possible to exclude from the sample preparation process the stage of filtration through neutralized “blue ribbon” filters, which increases the sample preparation time, as a possible cause of additional loss of toxins due to their degradation in contact with air and solvent.

After centrifugation, we took up to 5 ml of supernatant and used it for further analysis within 2 hours. The analysis was performed according to the protocol provided by the manufacturer of the assay kits. Each test was accompanied by the construction of calibration dependence on the basis of optical densities of five standard solutions of the tested toxins. The accuracy of measurements is ensured by twofold repetition and averaging of the results, as well as by twofold reading of optical densities in the wells of the tablet.

3 Results and discussion

Our work presents a study of mycotoxin contamination of cattle feeds harvested 2022–2024. In total, we analyzed more than 260 feed samples from a number of the Vologda Region farms, and carried out 509 tests on prepared feed in a year: for the content of aflatoxins, it was 155 tests; for ochratoxin-A – 132 tests; for zearalenone – 105 tests; for dioxynivalenol (DON) – 89 tests; for T-2 toxin – 28 tests. About half of the samples were analyzed for simultaneous content of several mycotoxins.

The research identifies the following toxins: aflatoxin group (aflatoxins B1, B2, G1, G2), ochratoxin-A, zearalenone, deoxynivalenone, and T-2 toxin. These toxins form the basis of the complex of mycotoxins found in feed according to available foreign and Russian studies. These toxins are also subject to strict controls; they particularly constitute the bulk of detected lesions in agricultural products. It is also important to note that precise standardized methods are available for these toxins, allowing their discovery in test samples with high precision and stability.

The obtained results showed that 99% of the studied samples were contaminated with mycotoxins to some extent: all analyzed samples contained aflatoxins and zearalenone; 97% of them contained ochratoxin-A, and less than one third of samples had DON. Figure 1 shows the share of samples contaminated with mycotoxins exceeding the maximum allowable concentration, with 56.77% of samples exceeding MAC of aflatoxins and 31.1% exceeding ochratoxin.

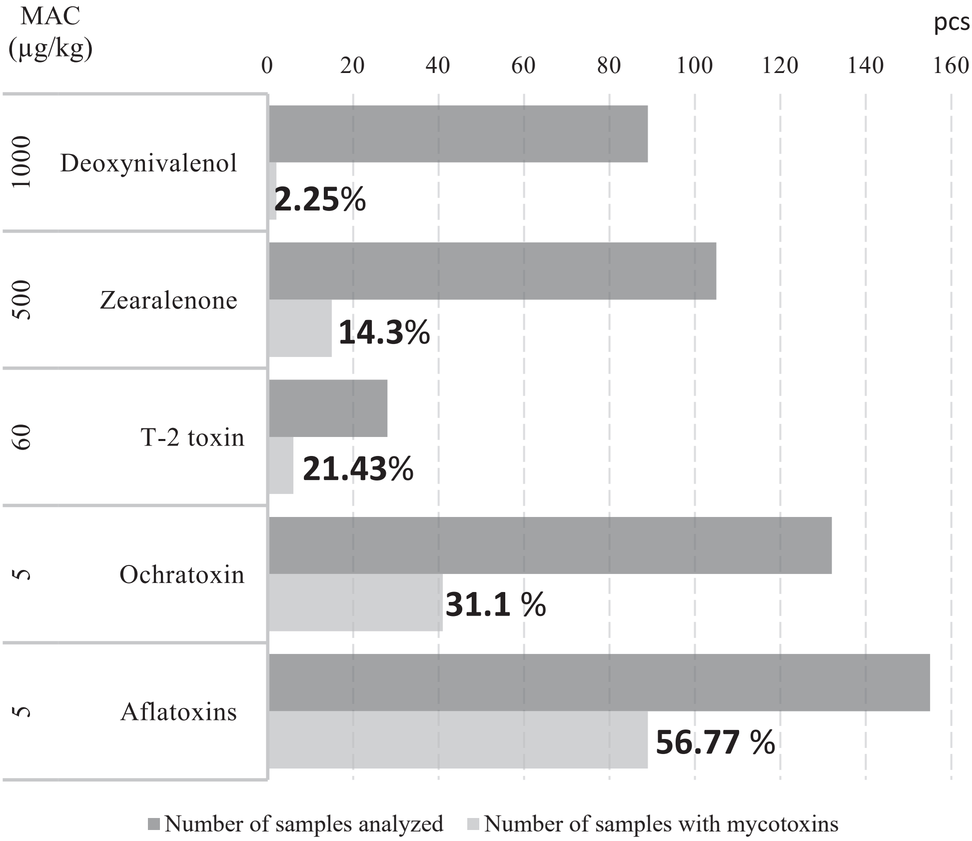

Fig.1. Proportion of samples containing the tested mycotoxins in amounts exceeding MAC

The high contamination degree of harvested fodder is also related to the nature of use of agricultural areas: mold fungi spread widely in the soil and surely infect any grown material with no regular mechanical tillage or change of cultivated crops. This correlates well with the fact that the bulk of the areas from which material for fodder harvesting is collected are represented by perennial grasses forming artificial perennial meadows.

The main contaminants whose content exceeds the established MAC are aflatoxins and ochratoxin-A, which make the main contribution to contamination even in case of simultaneous testing of samples for all the specified toxins. Consequently, the bulk of toxin producers is represented by one genus of mold fungi, the fungi Aspegillus and, in particular, *A. flavus*, *A. parasiticus* as aflatoxin producers, and *A. ochraceus* as the main and most important producer of ochratoxin-A. Fungi of the genus Fusarium and Gibberella affect harvested forages to a much lesser extent, therefore the frequency of contamination with T-

2 toxin and zearalene is lower. In favor of the low prevalence of fungi of the genus Fusarium, it is the almost complete absence in the samples of significant amounts of deoxynivalenol, the main producers of which are also fungi of the genus Fusarium. Therefore, we can preliminary conclude about the low prevalence and lower probability of fusariums in the Vologda Region. However, it is premature to make solid conclusions, since the other toxins stay outside of the study. All these toxin producers are extremely widespread fungi living in soil, on the surface of fruits and seeds, in plant residues, thus the infection of the studied substrates inevitably occurs during vegetation, harvesting (mowing), drying in the open air, at the time of storage and processing.

Silages are of major interest for study, as they are the main form of harvested fodder by all agricultural enterprises of the Vologda Region. In addition to being an independent feed, silages are included in various feed mixtures; other forms of feed are used less frequently or as additives to silages. The technology of silage preparation creates conditions maximally favorable for the development of various mold fungi, so this type of feed is quite often contaminated with various mycotoxins [18–20].

We have studied silages of different compositions prepared on the territory of the Vologda Region. In total, we identified 10 types of compositions on the basis of predominance of one or another component (or several components). All the source material for silage preparation is grown by the agricultural enterprises of the region independently; basically, similar agrotechnics, methods of harvesting the material and methods of its preparation are applied.

Three approaches to the cultivation of grasses for ensiling are practically widespread. The first approach is the use of natural phytocenoses (meadows) with appropriate species composition (such silages are called mixed-grass silages and usually with an indication of the type of phytocenosis, e.g. meadow mixed-grass). This category includes grasslands of all types. The second approach is the cultivation of perennial herbaceous plants, initially sown artificially. Various perennial cereals (timothy-grass, meadow fescue, oat-grass, bromegrass, etc.), legumes (medick, clover, lupines, galega, etc.) and their mixtures in different proportions are used as perennial herbaceous plants (with predominance of cereals silages are designated as cereal-legume silages, with predominance of legumes – legume-grass silages). And the third one os annual artificially sown herbaceous plants. This type of material is usually cultivated in crop rotation and often has higher yields than perennials.

It is worth noting that annual, and often more than once mowing in natural phytocenoses changes the species composition toward its impoverishment and predominance of plants resistant to regular mowing (which include cereals and legumes, such as various clovers). In fact, after some time, the species composition of such phytocenoses begins to approach the species composition of perennial agrocenoses formed by cereals and legumes. The reduction of species diversity, in turn, creates a favorable environment for the development of mold fungi. While mold fungi do not pose any threat to the phytocenosis as a whole, being efficient reductants and utilizing dying organic residues, for agriculture such changes result in increased contamination of harvested fodder.

Table 1 presents the results of silages of different compositions for five controlled mycotoxins. We should say that the most affected are mono-species silages. When considering the total contamination, we can find that legume and cereal mono-species silages are the most contaminated in total for the five toxins studied. Both compositions are predominantly contaminated with aflatoxins (the content of other mycotoxins does not exceed the maximum allowable concentration). Grass silages are contaminated to a lesser extent, but aflatoxins are the predominant toxins in them as well; other authors obtained similar data [21–22].

Table 1. Average content of mycotoxins in silage of different composition (μg/kg).

Mycotoxin	Legume -grass	Leguminous	Legume-legume	Cereal	Divergent grass	Other silages	MAC (μg/kg)
Deoxynivalenol	71.0	below detection limit	below detection limit	150.0	89,0	110.0	1000
Zearalenone	170.03	329.80	187.71	161.35	184,50	151.60	500
T-2 toxin	33.50	43.40	25.80	32.41	33,51	37.27	60
Ochratoxin	4.51	5.70	3.86	6.27	4,45	4.21	5
Sum of aflatoxins	13.24	20.56	10.56	20.79	19,70	7.66	5

4 Conclusion

In recent decades, the problem concerning mycotoxicosis has become more visible to veterinarians, zootechnicians, and administrators of livestock farms. Determination of mycotoxins in feed has become easier and cheaper due to the development of analytical techniques. However, many farms are unaware of the threat posed by mycotoxins. They rarely conduct mycotoxin testing of feed in advance, preferring to do it only after the appearance of mycotoxicosis symptoms. According to our data, the composition of mycotoxin contamination of fodder practically does not depend on the specific territory of the Vologda Region, where the studied material was collected and harvested, as well as on the environmental conditions at the time of collection and harvesting. The main production of toxins occurs after wet forages are put into silage. Even dry forages (hay, straw) show that the main period of accumulation is during storage. A specific microbiota capable of active toxin formation is formed during fermentation and subsequent storage of plant matter under anaerobic conditions.

Mycotoxin producers occurring in the Vologda Region penetrate into the harvested material with almost equal probability, which confirms the conclusion about their wide and uniform prevalence. Such uniform contamination allows predicting with high accuracy both the composition of contaminants and the degree of their contamination depending on the conditions and terms of harvesting.

Accumulation of mycotoxins in feed can be prevented by the following methods: the first one is the use of quality: if it is possible, raw materials should be selected with no visible signs of mold and fungus. The second method is the correct harvesting technology: it is important to observe optimal conditions for harvesting feed, such as temperature, humidity, and laying time. The third method is regular quality control: it is necessary to carry out regular quality checks of feeds for mycotoxins. The fourth method is proper storage: feed should be stored in dry, well-ventilated rooms to avoid the formation of mold and fungi. The fifth one is optimal use of feeds: it is significant to avoid long storage of feeds, especially wet feeds, and to use them in accordance with specialist recommendations. The next method is the use of preservatives: the addition of chemical or biological preservatives can help to prevent the development of mold and fungi. Feeds are recommended to be tested for mycotoxins on a regular basis, especially before the start of the harvest season and before the usage of feeds. The frequency of studies depends on many factors, including the type of feed, conditions of its storage and use, and the risk of mycotoxin contamination. The data of toxicological analysis will allow specialists of agricultural enterprises to develop a set of measures to decontaminate feed and measures to reduce the toxic effect of mycotoxins on animals.

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