# Detection of mycotoxins in cattle feed

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## Abstract

The aim of the study was to study the mycotoxin infestation of various types of cattle feed used in the farms of the Vologda Oblast. Samples for analysis were represented mainly by silos of various compositions (75% of samples), since this is the main type of juicy fodder harvested by farms in the region. The remaining 25% is represented by hay, haylage, grain, single samples of mixed fodder and press cake. Analysis of mycotoxins in feed was performed by enzyme-linked immunosorbent assay using standard test systems. Studies of more than 100 feed samples have shown that most of them contain some degree of mycotoxins. Mycotoxins were found in all studied samples of grain, press cake, compound feed and haylage, but their content is below the maximum permissible concentration. Of the 74 silage samples examined, only two were toxin-free, and almost 40% had mycotoxin content that either exceeded or was in close proximity to the limits for feed products. During the work, it was found that mycotoxins are present in almost all feeds independently harvested by farms. Since such studies of feed harvested in the Vologda Oblast have not been carried out before, the results of this study suggest that, firstly, more thorough control of feed is required, if possible at all stages, secondly, farms should take preventive measures to decontaminate the harvested material, thirdly, it is advisable to introduce adsorbent additives into diets in cases where, for some reason, the use of preventive measures does not give the expected result.

**Keywords:** animal feed; mycotoxins; aflatoxins; zearalenone; ochratoxin-A.

# Introduction

Today, mold toxins are a vast and heterogeneous group of chemicals, there are more than 400 of them, and new ones are constantly being discovered. These compounds appear to have been regularly present in human food and pet food for at least 10,000 years. It should be noted that in a broad sense, all metabolites of mold fungi (and some higher ones, for example, agaritin, a metabolite of agaricaceae) can be considered mycotoxins. Antibiotics produced by molds, which have a toxic effect on other organisms, and in this context are used in medical practice, are also mycotoxins. These compounds provide their producer with competitive advantages in their natural habitat. However, while antibiotics have activity against bacteria (the term is used exclusively for substances that are naturally produced), mycotoxins have a broader toxic effect.

As severe toxicants causing a wide variety of clinical symptoms, mycotoxins thereby largely mask their effects on living organisms (Betina 1984). Many of them have delayed effects that manifest themselves only with long-term exposure (chronic mycotoxicosis) and/or in the form of non-specific pathologies, such as cancer, lesions of internal organs, as well as exhibiting mutagenic effects and systemic disorders (Omotayo et al., 2019). The question of the mutual influence of two or more toxins is poorly studied, and the effect of toxins in concentrations lower than the standards established by regulators is poorly studied (Kolawole et al., 2020).

The increase in the share of silage feed in animal diets caused a wave of severe toxicosis in cattle, expressed in the death of animals, as well as in the form of abortifacient phenomena. Several species of molds of the genus *Penicillium*, in particular *P. roqueforti* and *P. paneum*, were found in the feed samples, producing a variety of toxic substances, including roquefortin, PR toxin, penitrem A, marcfluorines A, B and C, andrazines A and B, patulin and microphenolic acid. All of these toxins have a broad spectrum of action. For example, roquefortin and penitrem A, as well as marcfluorines – neurotoxicants, PR toxin and patulin – are carcinogenic and genotoxicants, etc. Despite the measures taken to reduce infestation, silage feed is contaminated with fungi of the genus *Penicillium* everywhere (Mansfield et al., 2008). The intensity of contamination is directly related to temperature and humidity, with the highest number of detected cases occurring in cooler and wetter areas. However, the number of studies on mycotoxin contamination in grass silage relative to other forms of feed is small (McElhinney et al, 2016).

Infection of plant material can occur both during the growing season (*Alternaria spp.* and *Fusarium spp.*) and after harvesting or harvesting of green mass (*Penicillium spp.*), fungi of the genus *Aspergillus spp.* affect plant substrates at all stages. It has been shown that fungicide treatment of crops does not eliminate mycotoxin contamination of plant accessions (Kosicki et al., 2016).

According to the DSM[1] report, in 2022, out of 27300 samples examined for mycotoxins, 81% were found to contain mycotoxins in quantities above the lower limit of detection of the techniques used, and 57% of the cases detected 2 or more toxins in one sample. A Cargill[2] report indicates that 52% of the 311,000 samples tested contain 3 or more mycotoxins.

On average in the European Union, more than half of wheat and bran samples, 92% of all maize samples, and 75% of feed of various types contain mycotoxins in some form.

A report by Cargill and Biomin recognizes Russia as a country with low risks caused by mycotoxins. In 2022, 6648 tests were conducted, in which 38% of the samples contained mycotoxins at concentrations above the detection limit of the analysis techniques used, and 19% of the samples showed high mycotoxin contents.

It is worth noting that both the Cargill and Biomin reports focus mainly on cereals. Aside are bulk feeds, which accounted for less than 4% of all samples examined in the Cargill report, Biomin does not provide results for this type of sample at all.

Occurring in all types of feed used in agriculture, including feed for cattle, small animals, birds, fish, etc., mycotoxins are nevertheless found in different parts of the world in different ratios. Among the more than 400 toxins, the "big six" of the most significant toxins stand out, represented by the aflatoxin group, zearalenone, deoxynivalenol, fumonisin, ochratoxin-A and T2 toxin (Betina 1984).

It is quite difficult to estimate the direct and indirect economic losses resulting from the damage of mold fungi to crop products, as well as toxic effects in farm animals. At various stages of U.S. agricultural production, mycotoxins cause $10 billion in losses. A similar estimate for the EU is about €5 billion (i.e. about 2% of the total GDP generated by EU agriculture), and both estimates appear to be quite inaccurate. Most likely, the problem has a similar scale in Russia. The problem is of a similar scale in Russia. Reduced productivity and health problems can lead to a shortfall of up to 250 rubles per animal per day (Yildirim et al. 2019). This figure does not include possible economic losses due to a decrease in the quality of the final agricultural product, its culling, a decrease in the productivity of farm animals and their premature retirement.

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[1] The DSM Report (formerly known as the Biomin World Mycotoxins Report) is published annually (https://annualreport.dsm.com/ar2022/services/downloads.html)

[2] The DSM Report (formerly known as the Biomin World Mycotoxins Report) is published annually (https://annualreport.dsm.com/ar2022/services/downloads.html)

Due to the accelerated globalization of the food and animal feed market, the rate of spread of contaminated materials and, as a result, mycotoxin producers is increasing, and so is the number of samples contaminated with mycotoxins in the total mass of products (Streit et al, 2013; Kononenko & Burkin, 2014, Dorozhkin et al., 2022; Sakthi Priya et al., 2023).

On the other hand, the relatively poor knowledge of the spread of mycotoxins in Russia as a whole and in the North-West of Russia as a region that is very favorable for the growth of mold fungi in harvested fodder material (the climate is warm and humid in summer, with abundant precipitation and high humidity in autumn and relatively warm winter), which leads to an underestimated assessment of risks by harvesting farms.

Many species of cereals and leguminous fodder grasses serve as a source of a complex complex of mycotoxins entering the body of animals, which is subject to changes during the growing season and has its own characteristics of composition and ratio of individual components in different crops (Burkin & Kononenko, 2015).

Dairy specialization of livestock breeding in the regions of the North-West of the Central European zone of the country requires large volumes of harvesting juicy and dry fodder. The actual harvesting is often carried out in conditions that are far from favorable. In many farms, there is no control of harvested raw materials for mycotoxins and measures are not taken to prevent contamination and decontamination of feed.

Taking into account the scientific and economic significance of this area of research, the Laboratory of Bioeconomics and Sustainable Development of the VolRC RAS in 2022 began work on the study of the spread of mycotoxins in harvested feed in the Vologda Oblast, before that such studies had not been conducted in the region.

The aim of the study was to study the mycotoxin infestation of various types of harvested feed in the farms of the Vologda Oblast.

# Material and methods

Mycotoxin analysis was carried out in accordance with GOST 31653–2012 “Feed. Method of enzyme-linked immunosorbent determination of mycotoxins” with the help of standard test systems manufactured by ComProdService (Belarus) and R-Biopharm (Germany), using the analyzer of enzyme-linked immunosorbent reactions AIAR-01 UNIPLANE (Pikon, Russia) at the Center for Agricultural Research and Biotechnology of the VolRC RAS (location: 59.29234361089507, 39.672556460134906).

Sampling was carried out by the farms of the agro-industrial complex of the Vologda Oblast in accordance with the recommendations received, developed on the basis of GOST R ISO 6497–2011 in the Laboratory of Bioeconomics and Sustainable Development of the VolRC RAS. The received sample was dried to a constant mass, crushed to a particle size of no more than 0.2 mm, and the sample was extracted and prepared according to the protocol of the test system manufacturer. During the storage period of the dry sample and during sample preparation, the impact of light on the sample was minimized. The finished extract was transferred to cryotubes with a volume of 2 ml and, if necessary, stored at a temperature of -30°C before the analysis (as a rule, no more than 2-3 days). As the degradation of mycotoxins under these conditions in the finished samples is negligible, storage had little or no effect on the result (Diaz et al., 2012). In the studies, samples in which the content of ochratoxin-A, zearalenone and the sum of aflatoxins $B_1$, $B_2$, $G_1$, $G_2$ was below the lower limit of detection of test systems (less than 2 μg/kg) were considered free of mycotoxins. Unfortunately, the MPC 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 European Economic Community dated 18.06.2010 No. 317) regulate the content of mycotoxins in such feeds as wheat, barley, oats, corn, soybeans, etc., ignoring silage. The values of the MPC levels for the above crops do not have significant differences, so we were guided by these standards in our work.

The weather conditions of the growing seasons of 2022 and 2023 (the period of forage harvesting) did not differ significantly from each other in terms of temperature and precipitation. In general, 2022 can be characterized as moderately humid and warm with a rather cold May (for the growing season: the average monthly temperature is 1 ºC higher than the average values of the last 10 years, the total precipitation is lower by 8.7 mm). In 2023, the growing season turned out to be moderately warm (at the level of average temperatures of previous years) with dry May and August, but a rather wet July (for the growing season: the average monthly temperature is higher by 0.3 ºC compared to the average values of the last 10 years, the total precipitation is lower by 11.7 mm).

# Results and Discussion

Studies of more than 100 cattle feed samples have shown that most of them contain some degree of mycotoxins. Samples for analysis were represented mainly by silos of various compositions (75% of samples, including legumes and cereals, forbs, clover silos), since this is the main type of juicy fodder harvested by farms in the Vologda Oblast.

The remaining 25% are represented by hay, haylage, grain, single samples of compound feed and cake received by farms from large producers who carry out their own control of mycotoxin content, for this reason sample of compound feed and cake did not contain toxins. The 18 grain samples examined for zearalenone also did not show a high zearalenone content (less than 20 μg/kg at MPC = 1000 μg/kg), with the exception of one barley sample (250.7 g/kg). Since all the cereal samples are obtained from large producers, it is obvious that the production was carried out under conditions that prevent the growth of fungi of the genus Fusarium, as well as product controls.

All haylage samples contained aflatoxins in amounts ranging from 3.37 to 8.81 μg/kg (at MPC = 20 μg/kg). 1., the content of ochratoxin-A did not exceed 2.2 μg/kg (MPC = 5 μg/kg).

Among the 74 silage samples, only 2 were toxin-free. Eight samples exceeded the upper limit of detection in cases of ochratoxin-A and another 8 samples exceeded the maximum permissible concentrations for the sum of aflatoxins and ochratoxin-A. The results of the silo studies are shown in Figures 2-5.

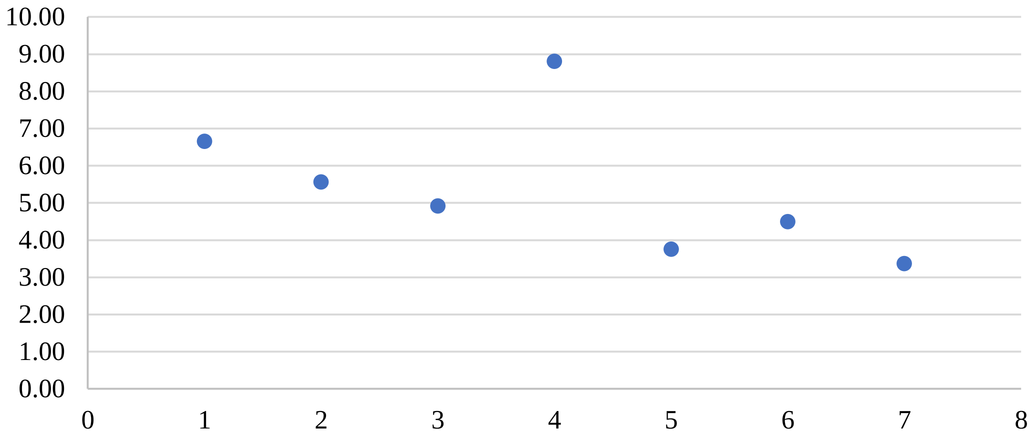


**Fig. 1:** The amount of aflatoxins in haylage.

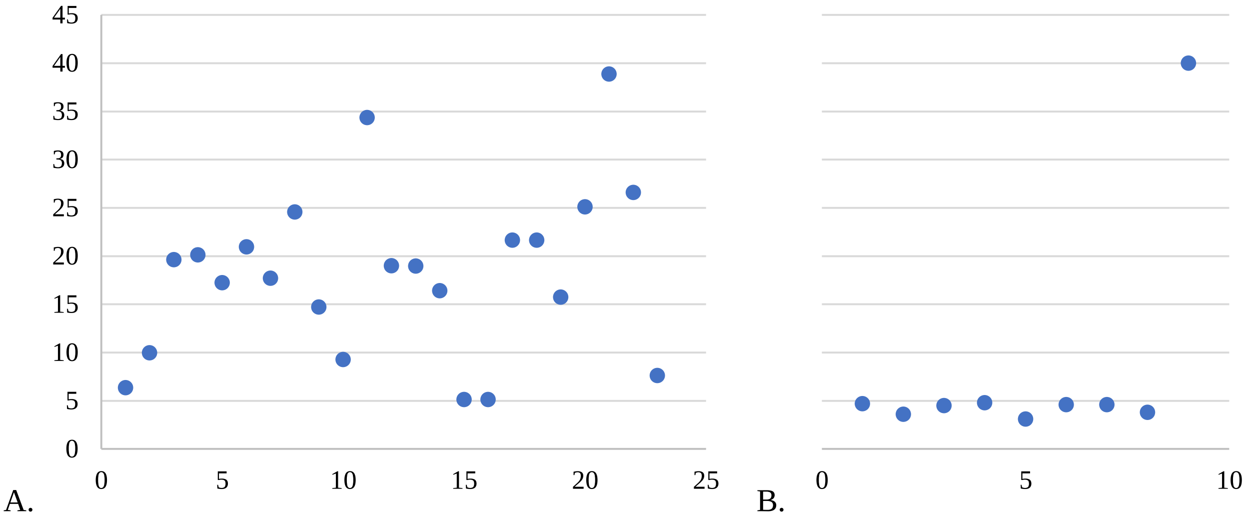


**Fig. 2:** The sum of aflatoxins (A) and ochratoxin-A (B) in legume-cereal silage.

The results of the study, carried out on a number of different samples, make it clear that mycotoxins are present in almost all feed harvested by farms themselves, and in many cases their content either exceeds the established norms for feed products or is in close proximity to them (almost 40% of the samples studied). As is known, the producers of aflatoxins and ochratoxin-A are fungi of the genera *Aspergillum* and *Penicillum*, for the development of which silage conditions are favorable (Yildirim et al. 2019). Some representatives of these genera successfully develop at a pH of 2.5 and a lactic acid content of up to 3.5%. It is possible that the silage mass was populated by representatives of these fungi and led to its contamination with these mycotoxins. In addition, a contribution to feed contamination cannot be ruled out on the part of the plants that served as raw materials for harvesting, there are studies showing the widespread infestation of seeded grasses on the vine by mycotoxin-producing fungi (Burkin & Kononenko, 2015).

This, firstly, confirms the thesis that mycotoxins and their producers are ubiquitous, affect all types of feed and, therefore, their toxic effects can affect the welfare of animals, including chronic poisoning in small doses.
Secondly, it shows the importance of monitoring the quality of harvested feed in terms of mycotoxin content.

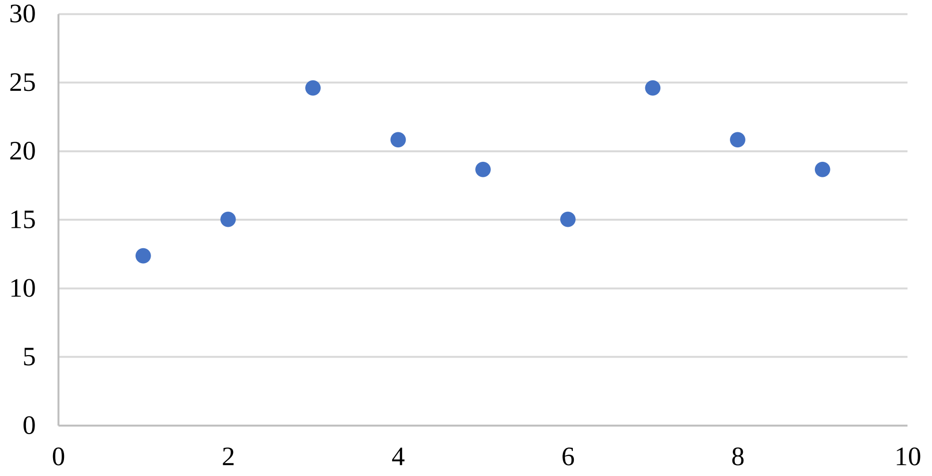


**Fig. 3:** Aflatoxin sum content in cereal silage.

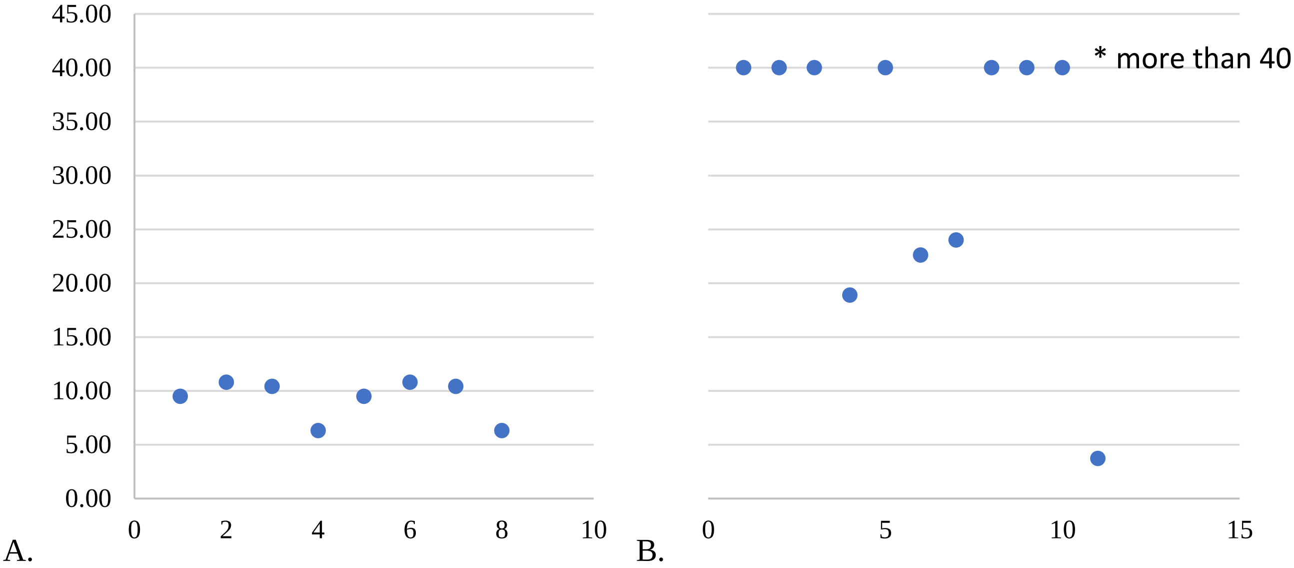


**Fig. 4:** The sum of aflatoxins (A) and ochratoxin-A (B) in forb silage.

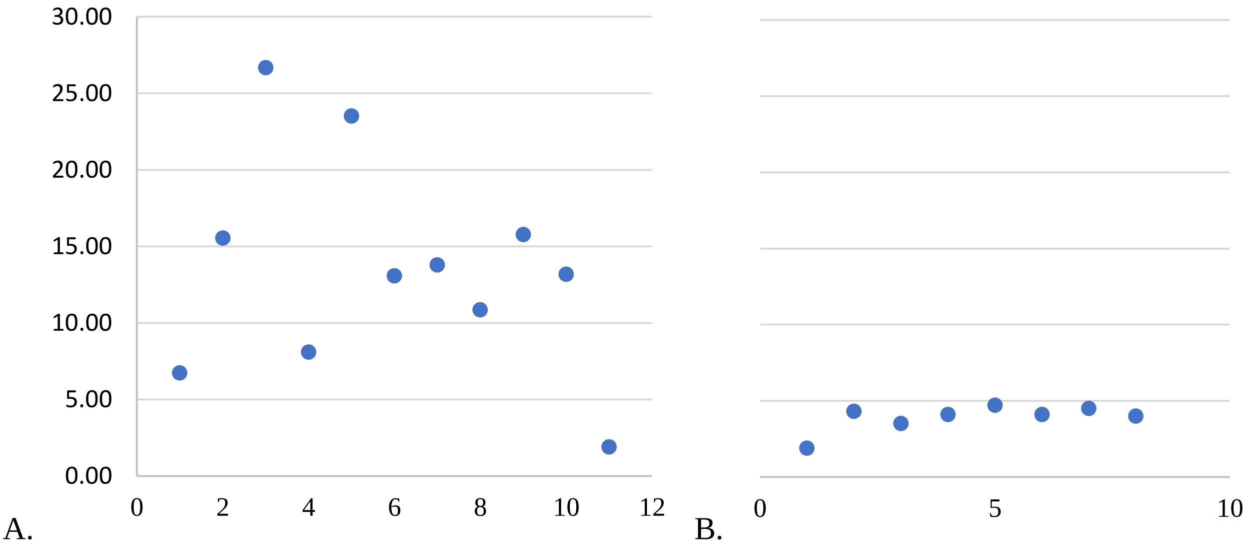


**Fig. 5:** Sum of aflatoxin (A) and ochratoxin-A (B) in silage from natural land.

Thirdly, at this stage already we can note the importance of farms taking measures of feed decontamination (including preventive decontamination carried out before the examination of samples or in the absence of it) and of reducing the toxic impact on animals. In order to minimize the risks of animal consumption of feed contaminated with mycotoxins, farm specialists need to monitor compliance with technological instructions when harvesting and storing feed, constantly control the humidity and temperature of feed stocks. But it is equally important to evaluate feed ingredients imported from different regions in a timely manner, monitor quality indicators according to the data of quality and safety documents of raw materials, and regularly send feed samples to the laboratory to do testing for the presence of main mycotoxins level. An effective measure to prevent the negative impact of mycotoxins is the use of adsorbent feed additives and complexes in the diets of all cattle groups with proven declared efficacy of their action. Adsorbent feed additives can include various components with sorbent properties – zeolites, bentonite clays, diatomite, activated carbon, yeast cell walls. Each of these components has specific sorption properties for a particular mycotoxin, for example, bentonite and diatomite are best at binding aflatoxins, and yeast cell walls are best at binding zearalenone. Some components, such as activated charcoal, bind vitamins contained in premixes together with mycotoxins, so its use on a regular basis is not desirable (Cha et al., 2021; Kemboi et al., 2023).

## Conclusion

Thus, the conducted studies made it possible to obtain data on the contamination of harvested feed by agricultural enterprises of the region with mycotoxins. The research plan of the laboratory for the next years involves a significant expansion of the research not only in terms of the number of analyzed samples, but also in the study of the dynamics of the accumulation of the main toxins in feeds of various composition, time, and method of preparation.

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