Part 3. Biosafety

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ASSESSMENT OF THE SAFETY AND QUALITY OF COMBINED FEED FOR POULTRY FARMS IN UKRAINE IN 2023–2025

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Summary. In the context of developing market relations in the livestock sector of agricultural production, ensuring high productivity of animals and poultry and obtaining high-quality products at the lowest cost is the main task. Therefore, violations of feed safety and quality indicators and the search for ways to correct them are relevant. The aim of this study was therefore to identify violations of the most important safety and quality indicators for compound feed in Ukraine's poultry industry between 2023 and 2025. To determine the degree of microfungal contamination, 47 samples of compound feed for agricultural poultry were tested. The results showed that 78.7% of the feed was substandard, while 21.3% was of good quality. The main contaminants in the feed were representatives of the genera Aspergillus (38.3%), Penicillium (16.4%), Fusarium (4.7%), and Mucoraceae (13.7%). Potentially toxinproducing taxa of the genus Aspergillus were represented by A. flavus, A. fumigatus, A. niger, A. glaucus, and A. oryzae; Penicillium — P. lanosum and P. commune; Fusarium — F. moniliforme; the family Mucoraceae — Mucor and Rhizopus, and the genus Trichothecium — T. roseum. Monitoring of the quality indicators of 58 feed samples revealed the following: in compound feeds, there was a decrease in crude protein content by an average of 16.3%, crude fiber content by 33.8%, crude fat content by 15.6%, total calcium concentration by 13.3%, and inorganic phosphorus by 13.4%; in concentrated feed, there was a decrease in crude protein content by an average of 7.0% and an increase in crude fiber content by 11.3%. These results suggest that the sanitary conditions of the feed for agricultural poultry are unsatisfactory, which is probably due to non-compliance with processing regimes for compound feed components and the consequences of military operations in Ukraine

Keywords: mold fungi, contamination, compound feed components

Introduction. Feed and feed additive safety involves minimizing or eliminating risks at all stages of production, distribution, disposal, and destruction. Risk management to prevent possible harmful effects is carried out during the cultivation and harvesting of feed, and at all stages of the technological processes involved in its production, transportation, and storage.

The use of raw materials from regions affected by particularly dangerous animal diseases for the production of feed and feed additives is prohibited. In such regions, the mandatory removal and destruction of animals, products, and raw materials of animal origin that pose a particular danger to the health and life of animals and humans is carried out.

The harvesting, storage, and transportation of raw materials used in the production of feed and feed supplements must comply with the requirements set out in regulatory acts and normative documents relating to veterinary medicine and food safety (VRU, 2018).

Feeding poultry is an important part of modern poultry production technology. Animal productivity is 55–60% determined by the level and adequacy of feeding, while breed accounts for 25–30%, and keeping methods and technology account for 15–20%. The main condition for achieving high productivity is to organize a complete, high-quality and balanced feeding regimen using diets that meet the birds' needs in terms of essential nutrients

and biologically active substances (VRU, 2018; Arroyo-Manzanares et al., 2014; Sentin, 2005).

If feeding conditions do not meet the bird's physiological needs, all types of metabolism will be profoundly disturbed, resulting in decreased resistance and productivity, as well as clinically apparent diseases. Statistics show that a significant proportion of animal diseases are non-contagious and associated with feed poisoning. Providing animals with safe, high-quality feed ensures genetically determined productivity and high feed conversion efficiency (Sentin, 2005; Cegielska-Radziejewska, Stuper-Szablewska and Szablewski, 2013).

Of the many biological contaminants that pollute feed and food products, microscopic fungi are of particular concern. They are potential producers of mycotoxins, which most often affect grain feed and pose a real threat to human and livestock health. Feed quality indicators also remain an important area of research as changes in climatic conditions and agricultural production technologies lead to changes in quality and safety (Cegielska-Radziejewska, Stuper-Szablewska and Szablewski, 2013; Harčárová et al., 2018; Vasjanovych, Ruda and Jangol, 2017; Kutsan et al., 2020; Kyryliuk, 2019).

Poultry feed is most susceptible to the growth of microscopic fungi. The main source of fungal microflora in feed is its plant-based components, primarily grains (Creppy, 2002; Kwiatek and Kukier, 2008). During the growing season, harvesting, transportation, storage, and preparation for feeding, feed is affected by moldy saprophytes, which give it a darker color and an unpleasant odor. In particular, during storage, the mycobiota of mill waste, grain, and mixed feed consists of facultative parasites and epiphytes capable of toxin production, namely representatives of the genera Fusarium, Penicillium, Aspergillus, Alternaria, Botrytis, Helmintosporium, Nigrospora, Diplodia, Sclerotinia, Trichoderma, Trichothecium, Cephalosporium, Acremoniella (Monopodium), Mucor, Rhizopus, etc. (Kriuchkova, 1999; Pidoplichko M. and Bilai, 1946; Shareef, 2010; Oliveira et al., 2006).

During the development of mold fungi, the physical properties of feed change, and organic substances decompose to form toxic compounds that can cause poisoning if consumed by poultry. Broilers in particular show clinical signs such as decreased weight gain and appetite, digestive disorders, salivation, constipation or diarrhoea, liver and kidney damage, anorexia, decreased feed conversion efficiency, reduced egg production and poor eggshell quality (Rosa et al., 2006; Cegielska-Radziejewska, Stuper-Szablewska and Szablewski, 2013; Alkhursan, Khudor and Abbas, 2021).

The quality of agricultural raw materials has a significant impact on human health. The production of livestock products depends on various factors, such as the level of breeding work, the adoption of modern animal husbandry technologies, and, most importantly, the development of a high-quality feed base. Developing feed quality control is one of the most important tasks in modern animal nutrition science. Constant monitoring of feed quality violations and finding ways to correct them is important because, in the context of market relations developing in the livestock sector of agricultural production, the main task is to ensure animals and poultry are highly productive and to produce highquality products at the lowest cost (Ibatullin and Zhukorskyi, 2016).

Poultry farming is particularly susceptible to excessive contamination by microscopic fungi and a decline in feed quality, since compound feed consists mainly of grains and food supplements such as concentrated protein, soybean meal, vitamins and minerals.

Therefore, under martial law, it is particularly relevant to conduct systematic mycological studies of feed and feed raw materials to detect mold saprophytes, as this will not only determine their taxonomic affiliation and identify species that produce toxins, but also contribute to the creation of a system of measures to prevent feed toxicosis in poultry.

Taking the above into account, the research **aimed** to determine the safety and quality of compound feed for poultry farms in various regions of Ukraine between 2023 and 2025, based on priority indicators.

Materials and methods. Feed testing was conducted at the Laboratory of Toxicology, Safety and Quality of Agricultural Products and the Laboratory of Clinical Biochemistry in the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine).

Forty-seven samples of compound feed for agricultural poultry from farms in Vinnytsia, Poltava, Sumy, and Kharkiv regions were tested for mycological contamination.

The samples were examined in accordance with the generally accepted methods of mycological analysis. In particular, the following were determined:

- the degree of contamination of feed with microscopic fungi, by primary isolation under culturing conditions in agar and Chapek nutrient media; the total number of fungal spores in 1 g of feed, isolated into a pure culture (Obrazhei et al., 1998).
- the species affiliation of microscopic fungal isolates by comparing their cultural and morphological characteristics (e. g. features of growth on different nutrient media, size, shape, width, edge and center structure, growth intensity, surface characteristics, color of colonies and reverse, mycelium, etc.) with descriptions in microfungal identification guides and museum strains of test cultures (Bilai, 1977; Pidoplichko N. and Milko, 1971; Pidoplichko N., 1972; Bilai and Koval, 1988; Danshina, Danshin and Timchuk, 1985).

The research results were interpreted by the 'List of Maximum Permissible Levels of Undesirable Substances in Feed and Feed Materials for Animals' (MAPFU, 2012).

Feed quality indicators (n = 58) were monitored and analyzed on farms in Vinnytsia, Poltava, Sumy, and Kharkiv regions.

The quality of feed was studied based on the crude protein content, which was determined using the Kjeldahl method (ISO, 2005), moisture content (ISO, 1999b), crude fiber content (ISO, 2000), crude fat (ISO, 1999a), calcium (ISO, 1985), phosphorus (ISO, 1998).

Results and discussion. Forty-seven samples of compound feed for agricultural poultry were subjected to mycological monitoring and determination of the degree of contamination with microscopic fungi (Fig. 1).

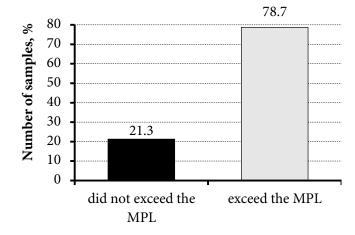


Figure 1. Degree of contamination of compound feed for agricultural poultry with micromycetes in 2023–2025.

It was established (Fig. 1) that 37 samples (78.3%) exceeded the permissible level of contamination with mold fungi (more than 5.0×10^4 CFU/g of feed), while 10 samples (21.3%) had permissible mycological contamination. When the maximum permissible level (MPL) was exceeded, the total contamination of feed with microscopic fungi ranged from 7.50×10^4 CFU/g of feed to 260.0×10^4 CFU/g of feed.

When determining the composition of the mycobiota of poultry feed, 342 isolates of mold and yeast-like fungi were isolated and identified (Fig. 2). The main isolates

were mold saprophytes of the genera *Aspergillus* — 131 isolates, *Penicillium* — 56 isolates, Mucoraceae — 47 isolates, *Fusarium* — 16 isolates. Representatives of other genera accounted for 92 isolates.

Species identification of isolated mold fungi, taking into account the presence of toxin-producing taxa (Table 1), a large number of which in feed can contribute to increased feed toxigenicity due to the accumulation of secondary metabolites — mycotoxins (Vasjanovych, Ruda and Jangol, 2017; Kutsan et al., 2020; Kyryliuk, 2019; Creppy, 2002), was the next stage of our research.

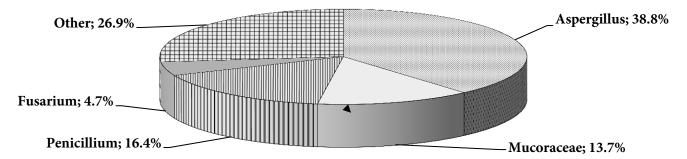


Figure 2. Generic composition of mycobiota in compound feed used in the poultry industry in 2023–2025.

Table 1 — Species composition of toxin-producing micromycetes isolated from feed and feed raw material samples

| Species of toxin-producing micromycetes, name of toxic metabolite | Number of isolates | Total number of isolates within the genus, % |
|---|--------------------|--|
| Genus Aspergillus | | |
| A. <i>flavus</i> — aflatoxins B ₁ , B ₂ , G ₁ , G ₂ , H ₁ , H ₂ , sterigmatocystin, penitrins, tremogens, ochalates, etc. | 48 | 36.6 |
| A. fumigatus — aflatoxin, fumigatin, fumitoxins A–D, fumitremorgins, etc. | 27 | 20.6 |
| A. niger — aflatoxin, ochalates | 16 | 12.2 |
| A. glaucus — aflatoxin, patulin, etc. | 11 | 8.4 |
| A. oryzae — aflatoxin, oryzoxolin, maltoricin | 10 | 7.6 |
| A. amstelodami — aflatoxin, sterigmatocystin | 6 | 4.6 |
| Other species | 13 | 10.0 |
| Total | 131 | 100.0 |
| Genus Penicillium | | |
| P. lanosum — citrinin | 24 | 42.8 |
| P. commune — ochratoxin, penitrem, aflatoxin, etc. | 19 | 33.9 |
| Other species | 13 | 23.3 |
| Total | 56 | 100.0 |
| Family Mucoraceae | | |
| Genus Rhizopus — aflatoxin, toxic properties | 26 | 55.3 |
| Genus <i>Mucor</i> — toxic properties | 21 | 44.7 |
| Total | 47 | 100.0 |
| Genus Fusarium | | |
| F. moniliforme — moniliformin, vomitoxin, T-2 toxin, etc. | 16 | 100.0 |
| Genus Trichothecium | | |
| <i>T. roseum</i> — roseotoxins and trichothecenes | 11 | 100.0 |

Based on the identification of the species of the isolated microscopic fungi (Table 1), it was determined that the predominant species of the genus *Aspergillus*, were the toxic species *A. flavus*, *A. fumigatus*, *A. niger*,

A. glaucus, A. oryzae. The most frequently identified species among the representatives of the genus *Penicillium* were *Pn. lanosum* and *P. commune*. The Mucoraceae family was represented by the *Mucor* and

Rhizopus genera. The species *F. moniliforme* was isolated from the genus *Fusarium*.

It should be noted that, over the past three years, representatives of the genus *Trichothecium*, specifically *T. roseum*, have increasingly been identified in experimental compound feeds.

T. roseum is known to be saprotrophic and is found in soils in many countries. The main habitats of *T. roseum* are uncultivated soils, forest nurseries, forest soils under beech trees, teak, cultivated soils with legumes, citrus plantations, heaths, dunes, salt marshes, and garden compost. There are about 222 different host plants of *T. roseum* in the world.

T. roseum has also been isolated from barley, wheat, oats, corn, apples, grapes, beans, forest nuts, pistachios, peanuts, meat products, cheese, and coffee. *T. roseum* levels in foods other than fruits are usually low (Domsch, Gams and Anderson, 1980).

T. roseum produces a wide range of secondary metabolites, including mycotoxins such as roseotoxins and trichothecenes, which are the main mycotoxins found in agricultural products and animal feed. It can act as both a secondary and opportunistic pathogen, causing pink rot on various fruits and vegetables, and thus has an economic impact on the agricultural industry. Secondary metabolites of *T. roseum*, in particular trichothecinol A, are being investigated as potential antimetastatic drugs. *T. roseum* is a source for the production of enzymes (Freeman and Morrison, 1949; Zhang et al., 2010).

Thus, mycological monitoring of 47 samples of compound feed for agricultural poultry revealed an increase in the percentage of poor-quality feed in 2023–2025 compared to our previous studies (Yaroshenko and Kolchyk, 2022), reaching 78.7%. Feed that did not exceed the maximum permissible level of contamination with micromycetes accounted for 21.36%.

The main contaminants in the feed were mold fungi from the genera Aspergillus (38.3%), Penicillium (16.4%), Fusarium (4.7%), Mucoraceae (13.7%), and representatives of other genera accounted for 26.9%. Potentially toxin-producing taxa of the genus Aspergillus were represented by A. flavus, A. fumigatus, A. niger, A. glaucus, and A. oryzae; Penicillium — P. lanosum and P. commune; Fusarium — F. moniliforme; the family Mucoraceae — Mucor and Rhizopus, and the genus Trichothecium — T. roseum.

Thus, the data obtained indicate the unsatisfactory sanitary condition of feed for agricultural poultry. This is

probably due to non-compliance with processing regimes for compound feed components (e.g., granulation and extrusion), as well as the consequences of military operations in Ukraine.

Therefore, poultry farms must conduct systematic mycological studies of stored feed at least once every two months to promptly identify spoilage and prevent negative effects on animal health.

According to the literature, mycological contamination of feed significantly impacts its zoochemical composition, including the destruction of proteins and vitamins, the acceleration of fat oxidation processes, and the reduction of feed digestibility (Chhaya, O'Brien and Cummins, 2021).

We therefore monitored the quality of feed (both concentrated and mixed feed for different age groups of poultry) from farms in various regions of Ukraine. It was found that 68% of the feed complied with current regulatory documents, while 32% was of poor quality for feeding (Klitsenko, 2001).

Analysis of the obtained data showed that the highest percentage of quality violations was found in compound feed intended for young laying hens aged between one and eight weeks (Fig. 3).

Chemical analysis of compound feed for young laying hens aged 1–8 weeks revealed an increase in moisture content of 5.2%, an increase in crude fibre content of 48.7%, and a decrease in crude protein content of 18.0%, as well as decreases in crude fat content of 13.4%, total calcium concentration of 12.6%, and inorganic phosphorus content of 17.5%. When analysing compound feed for young laying hens aged 9–17 weeks, a decrease was observed in the content of crude protein by 17.0%, crude fat by 9.4%, total calcium by 20.8% and inorganic phosphorus by 15.6%, as well as an increase in the content of crude fibre by 18.9%. The moisture content was within the limits established by DSTU 4120-2002 (DSSU, 2003).

In samples of compound feed for young laying hens aged 18-22 weeks, a decrease in the content of all the studied nutrients was determined: crude protein by 14.0%, crude fat by 24.0%, crude fibre by 6.6%, total calcium concentration by 6.5%, inorganic phosphorus by 7.2% and moisture by 11.0%. According to the literature, a decrease in calcium and phosphorus concentrations affects bone tissue formation and calcification, resulting in changes to other systems in the animal's body.

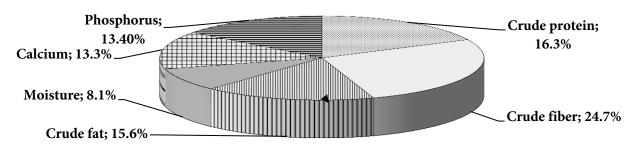


Figure 3. Analysis of the quality of compound feed for young laying hens.

When analyzing compound feed for 1–3-week-old broilers (Fig. 4), a decrease in crude protein content by an average of 30.2% was found in all samples, an increase in fiber content by 43.7% and crude fat by 16.2% relative to the reference level. According to the literature, an

increase in fat in feed with a simultaneous decrease in protein can cause disorders in the digestive system, leading to the development of fatty liver dystrophy (Loza, 2012).

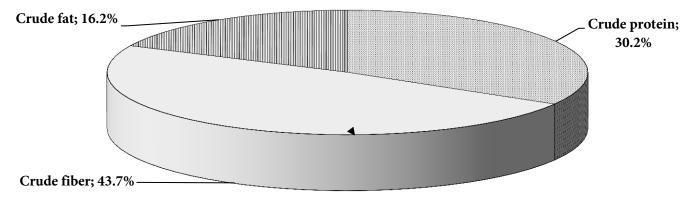


Figure 4. Analysis of the quality of compound feed for 1–3-week-old broilers.

As a result of establishing changes in the quality of compound feed for all age groups of poultry, the quality of the constituent components was monitored. The results show that 10.0% of the tested samples (including soybean and sunflower meal, wheat and corn grain) did not comply with current regulatory documents. In soybean meal samples, for example, an average decrease in crude protein content of 10.0% was noted. Sunflower meal samples showed an average decrease in crude protein content of 8.0% and an average increase in crude fibre content of 11.3%. Chemical analysis of grain samples revealed a slight decrease in crude protein content in wheat (3.0%) and an increase in crude fibre content (5.8%), and a decrease in crude protein content in corn grain (30.0%) and an increase in crude fibre content (16.8%). Based on these findings, it is evident that the components of the compound feed contain lowquality protein, which can negatively impact the digestibility and absorption of the feed. Additionally, poor-quality components are difficult to dose accurately, leading to an imbalance of essential nutrients (Kulik et al., 2020).

Therefore, monitoring feed quality through chemical analysis enables the creation of diets with high nutritional value, ensuring the provision of all necessary nutrients and facilitating rapid live weight gain and increased poultry productivity.

Conclusions. 1. Mycological monitoring of 47 samples of compound feed for agricultural poultry showed that the percentage of substandard feed increased in 2023–2025 and amounted to 78.7%. Feed that did not exceed the maximum permissible level of contamination with micromycetes accounted for 21.36%.

2. The main feed contaminants were representatives of mold fungi of the genera *Aspergillus* (38.3%), *Penicillium* (16.4%), *Fusarium* (4.7%), Mucoraceae (13.7%),, and representatives of other genera accounted for 26.9%. Potentially toxin-producing taxa of the genus *Aspergillus* were represented by *A. flavus*, *A. fumigatus*, *A. niger*, *A. glaucus*, and *A. oryzae*; *Penicillium* — *P. lanosum* and *P. commune*; *Fusarium* — *F. moniliforme*; the family Mucoraceae — *Mucor* and *Rhizopus*, and the genus *Trichothecium* — *T. roseum*.

3. As a result of monitoring the quality of feed, noncompliance with regulatory documents was established for the following indicators:

— compound feed: a decrease in crude protein content by an average of 16.3%, crude fibre content by 33.8%, crude fat content by 15.6%, total calcium concentration by 13.3%, and inorganic phosphorus by 13.4%;

— concentrated feed, there was a decrease in crude protein content by an average of 7.0%, and an increase in crude fibre content by 11.3%.

4. The results obtained indicate the unsatisfactory sanitary condition of feed intended for agricultural poultry. This is not only associated with non-compliance with processing regimes for compound feed components, but also with the consequences of military operations in Ukraine.

Prospects for further research lie in the systematic monitoring of safety and quality indicators for feed raw materials and feed used in poultry farming to prevent their negative impact on animal health and productivity and reduce economic losses in the poultry industry.

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