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SCREENING OF THE SANITARY CONDITION OF COMPOUND FEED MILLS OF UKRAINE

Abstract

The microbiological contamination of finished animal feed largely depends on the sanitary condition of the production facility. The microbiological landscape of production consists of two main factors: the contamination of raw materials of various types and the sanitary condition of equipment and the production environment. The sanitary condition of the equipment directly depends on the presence of material deposits or residues on or inside it, which become a medium for the development of microorganisms. The final microbiological load can have a significant impact on the animal health, especially of the young. Therefore, the purpose of this study was to investigate the sanitary condition of three feed mills in Ukraine: one of them in Odesa region, one in the Kharkiv region, and the last one in Mykolaiv region. We collected samples of deposits and residues from production equipment and the manufacturing environment. All samples were analyzed for two microbiological indicators: total microbial contamination and molds and yeasts. Standard microbiological methods were used for this: first, the samples were suspended in sterile water, then diluted and pour-plated on Petri dishes on MPA (for bacteria) and wort agar (for molds and yeasts). In addition to counting colonies, the presence of certain groups or species (where possible) of microorganisms was also noted, in particular, among fungi, the presence of known mycotoxin producers was noted. The results show that the microbiological burden of the equipment depends on its arrangement and the degree of care: open outdoor receiving equipment was the most contaminated, and significantly increased contamination was recorded in places where the deposits are moistened by atmospheric precipitation (outdoors) or water vapor (indoors). The results show that the proper arrangement of equipment and its proper maintenance is the most important component for obtaining a good sanitary condition of feed production. Based on the results obtained, the mills were offered appropriate recommendations to eliminate deficiencies and improve production.

Keywords: compound feed mill, sanitary condition, microbiological indicators, microbiological landscape.

Literature review

Modern compound feeds contain a wide range of raw materials with varying moisture, protein, sugar, lipid, and other nutrient content. All these substances are a favorable medium for dozens of genera and hundreds of species of microorganisms, including spoilage microbiota, opportunistically pathogenic and pathogenic species for animals [1, 2]. This microbiota is affected by technological processes during production (high temperature, moisture), but it is not completely destroyed, and finished compound feed is subject to secondary contamination from outside, similar to human food products [3]. This microbiota can have a significant impact on the health of young animals, which have not yet developed their own organism's protective forces [4].

One of the most important sources of secondary contamination of raw materials and finished products is the manufacturing equipment and premises, the sanitary condition of which plays a major role in the condition of finished products [5]. Microorganisms on equipment develop in deposits and residues of the material. Therefore, the question arises of researching the sanitary condition of production as a factor in product quality and a prerequisite for the implementation of modern production practices with the HACCP system.

Research goals and tasks

The goal of the study was to determine the role of the production environment of feed mills in the formation of microbiological contamination of finished feed.

The research tasks included:

– microbiological analysis of equipment at feed mills in various regions of Ukraine;

– identification of potential sources of the greatest contamination of feed during the production process.

Objects and subject of research

The *object* of the study was the deposits and residues of material and dust on the surfaces of equipment and floors in feed mills.

The *subject* of the study was the general microbiological indicators of the samples.

To achieve this goal, a microbiological study of residues and deposits was conducted at three feed mills in different regions of Ukraine.

Materials and methods

Feed mill in Odesa Region.

Produces compound feed for all groups of animals.

Sampling points:

- grate above the receiving unit hopper,
- aspiration deposit on the receiving unit,
- external scales for receiving grain, gravity flow at the entrance to the scales,
- loading hole of the bunker with lysine,
- magnetic separator before grinding (hammermill),

- the opening for feeding into the matrix of the pelleting press,
- granulation line sifter, inner wall,
- aspiration deposits on the surface of the granulation line sifter,
- external deposits on the pellet grinder.

Feed mill in Kharkiv Region.

Produces feeds for poultry, pigs and cattle, as well as premixes.

Sampling points:

- receiving unit,
- hammermill,
- portioned grinding unit,
- floor of the 1st storey,
- floor of the 2nd storey,
- premix mixer,
- bunker above the pelleting press,
- separator of the finished feed,
- dispensing unit for the finished product.

Feed mill in Mykolaiv Region.

Produces compound feed for livestock, pets, fish, and shrimp.

Sampling points:

- cyclone separator on the 4th storey,
- scalper on the 4th storey for all raw materials,
- spillage under the extruder for "wet extrusion",
- hammermill for grain raw materials,
- mixer for preliminary mixtures,
- bucket elevator at the extruder,
- hammermill on the 1st storey for non-grain raw materials,
- bucket elevator on the 3rd storey,
- raw material distribution pipe on the 3rd storey,
- fan at the air outlet of the hammermill.

In all cases, samples were collected with clean spatulas into prepared containers, avoiding contamination as much as possible. Microbiological tests were performed as soon as possible after the samples were delivered to the laboratory.

Microbiological tests of all collected samples was performed for two indicators: total number of bacteria (total microbial count) and number of mold fungi and yeasts in 1 g of sample.

Weights of 1 g of all samples were transferred into vials with 99 ml of sterile tap water and shaken thoroughly to obtain an initial dilution of 1:100. Next, several tenfold dilutions were made, taking into account the expected degree of microbial contamination: for presumably clean samples – up to 10^4, for presumably dirty samples – up to 10^6. From the last dilution, 1 ml was pour-plated on the nutrient media: for the determination of bacteria – on meat peptone agar (MPA), for the determination of fungi – on malt agar or Sabouraud agar.

Bacterial cultures were incubated at +37°C for 24-48 hours, mold cultures were incubated at +30°C for 48-72 hours, checking for signs of growth until colonies of microorganisms appeared.

After incubation, the number of colonies on the plates was counted and the number of microorganisms in 1 g was calculated by multiplying by the dilution. The morphology of the colonies was also studied, and microscopic examination of the isolated cultures with Gram staining was performed to obtain a picture of the taxonomic composition of the cultures. Among the fungi, the presence of potential mycotoxin producers (at the genus level) was noted.

Results and their discussion

Based on the results of microbiological studies of the number of bacteria and fungi in deposits and residues at feed mills, tables were filled in and charts were generated.

1. Feed mill in Odesa Region

The results of quantitative assessment of microorganisms are presented in Table 1 and Figure 1.

Table 1 – Microbial numbers in the samples from Odesa Region feed mill

Sample number	Microbial number, CFU/g	
	bacteria	fungi
1 - *granulation line sifter, inner wall*	20000	0
2 - *aspiration deposits on the surface of the granulation line sifter*	35000	0
3 - *the opening for feeding into the matrix of the pellet press*	95000	0
4 - *loading hole of the bunker with lysine*	160000	0
5 – grate above the receiving device hopper	2250000	150000
6 – *aspiration deposit on the receiving device*	500000	700000
7 – *external scales for receiving grain, gravity flow at the entrance to the scales*	30000000	2000000
8 – *magnetic separator before grinding (hammermill)*	200000	15000
9 – *external deposits on the pellet grinder*	65000	0

The figure shows that the largest number of microorganisms was found in samples taken from units located outdoors outside the production premises (samples 5, 6, 7). This is explained by the accumulation of soil dust containing numerous microorganisms and the favorable effect of atmospheric precipitation (increased moisture) on the development of microorganisms.

The smallest number of microorganisms was found on the equipment of the granulation and granule crushing lines (samples 1 and 9). This is explained by the high temperature of the pelletizing process, destructive for most microorganisms, after which a noticeably cleaner product (pellets) goes on to further processing.

Another characteristic feature is the detection of mold fungi in all samples from outdoor devices and only in one of the indoor ones – in the magnetic separator (sample 8), where material had accumulated atop of the magnetic block. At the same time, this sample contains the largest number of microorganisms among all samples

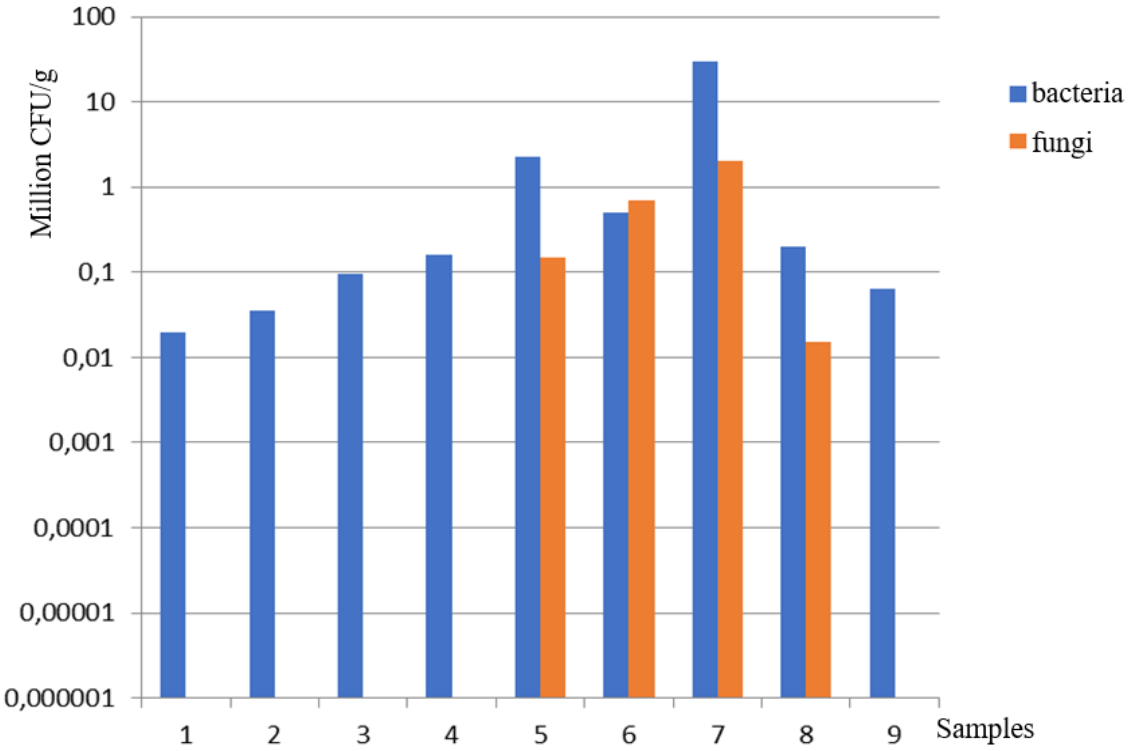

Fig. 1. Microbiological contamination of deposits and residues at the feed mill in the Odesa Region

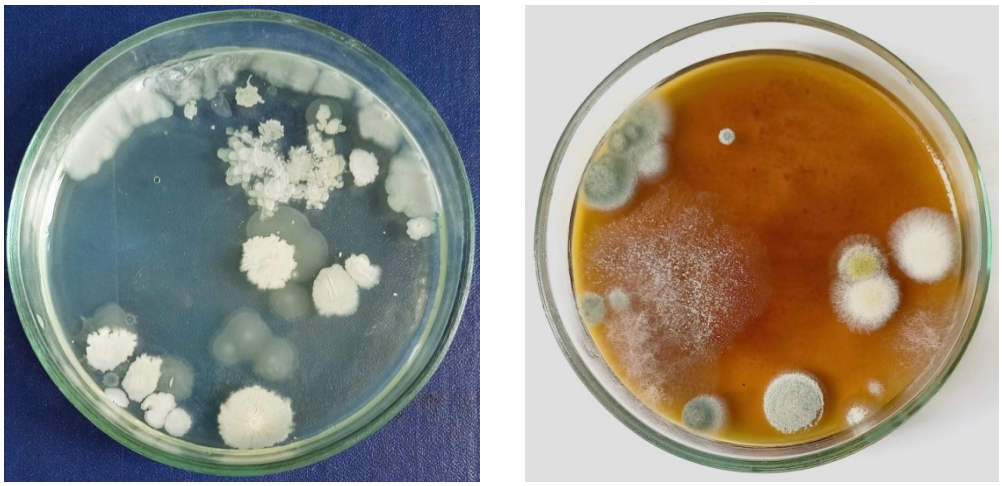

Fig. 2. Bacterial and fungal cultures recovered from the tested samples

from indoor devices. This is explained by the location of this separator at the beginning of the production process (initial grinding), when the raw material still contains a large number of microorganisms.

When determining the main taxonomic composition of the isolated cultures, aerobic gram-positive spore-forming forms (genus Bacillus) prevailed (70-75%) among bacteria. Bacilli are found in large numbers in grain mass and other plant raw materials, and in the form of spores they withstand high-temperature processing of grain raw materials and the granulation process of finished feeds (Fig. 2).

However, a significant number of non-spore-forming bacteria were also detected, both gram-positive and gram-negative. Cocci predominated among gram-positive non-spore-forming bacteria, and actinomycetes were also detected among the cultures.

Among fungi (Fig. 2), about 80% of species were from the soil mycobiota: genera *Penicillium*, *Aspergillus*, *Rhizopus*, *Mucor*, *Trichoderma*, and others. Among *Aspergillus* species, potential mycotoxin producers were noted, in particular, *A. flavus* and *A. fumigatus*.

Thus, the production equipment of the plant in the Odesa region contains quite large amounts of microorganisms in deposits and residues, although not uniformly.

The sanitary condition of outdoor raw material receiving facilities is unsatisfactory — the presence of large numbers of bacteria and mycelial fungi poses a risk of contamination of raw materials and the emergence of mycotoxins and dangerous pathogenic species or strains of microorganisms.

Similarly, the presence of mold fungi in the deposits in the magnetic separator is a rather negative factor: this indicates the possibility of fungi and mycotoxins entering the ground components and feed mixture.

Mycotoxins are known to not be destroyed by heating to high temperatures, so no thermal process used in the production of compound feed (grain processing, granulation) can destroy them to a safe level.

2. Feed mill in Kharkiv Region

The results of quantitative assessment of microorganisms are presented in Table 2 and Fig. 3.

The figure shows that the largest number of microorganisms was found in three samples – on the storey floors (samples 5 and 8) and in the bunker above the pellet mill (sample 1). The latter is explained by the fact that water vapor from the pellet press enters this bunker during the pelletizing operation, creating a moist and warm environment inside the bunker that is very favorable for the growth of microorganisms. At the same time, this explains the detection of mold fungi in fairly large quantities here, while they were absent in other samples, which indicates a high level of equipment maintenance.

At the same time, no bacteria were detected in the finished feed separator at all – its sterility is explained by the fact that the feed arrives here after being treated at high temperatures (granulation) along with effective maintenance of the equipment.

The receiving unit at this mill (sample 4), unlike the plant in the Odesa Region, is located indoors and

Table 2 – Microbial numbers recovered from samples from Kharkiv Region feed mill

Sample number	Microbial number, CFU/g	
	bacteria	fungi
1 – *bunker above the pelleting press*	2000000	2000000
2 – *hammermill*	100000	0
3 – *dispensing unit for the finished product*	50000	0
4 – *receiving unit*	30000	0
5 – *floor of the 2nd storey*	7000000	0
6 – *separator of the finished feed*	0	0
7 – *portioned grinding unit (ZN#3060)*	60000	0
8 – *floor of the 1st storey*	700000	0
9 – *premix mixer*	40000	0

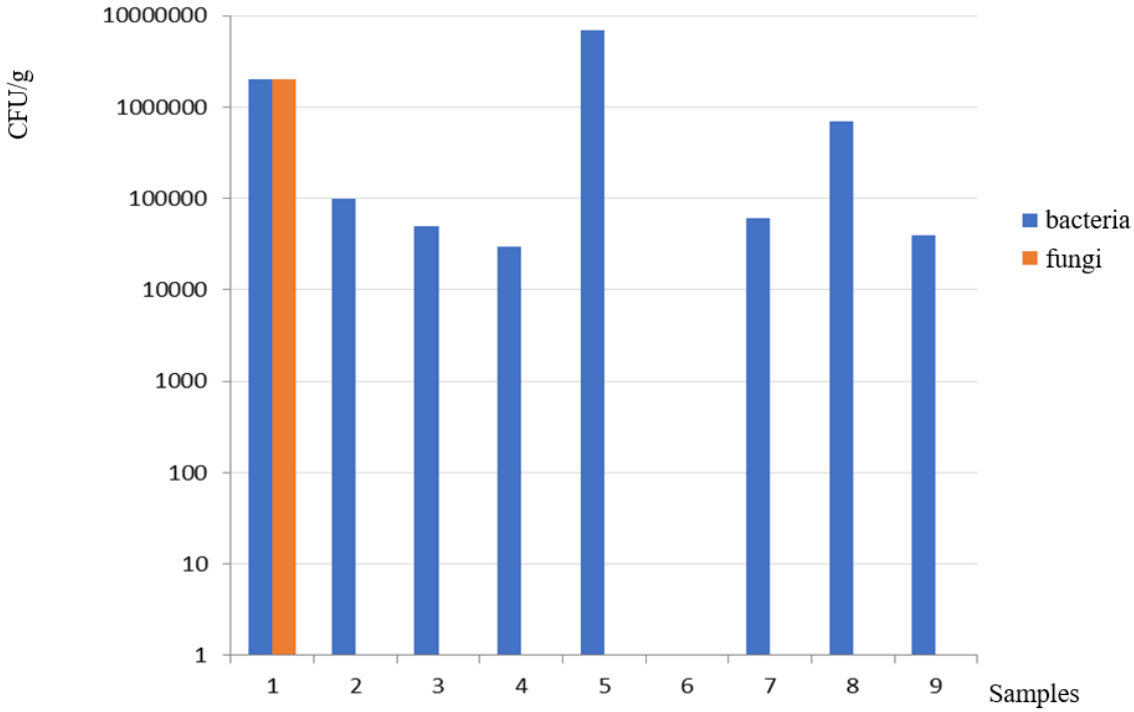

Fig. 3. Microbiological contamination of samples from the Kharkiv Region feed mill

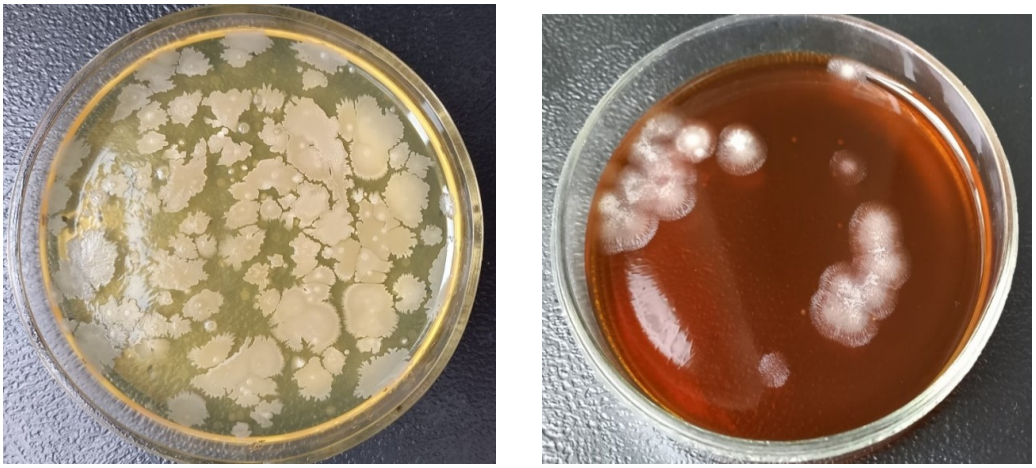

Fig. 4. Bacterial and fungal cultures recovered from the tested samples

protected from atmospheric precipitation and is adequately maintained, so the deposits on it contain significantly fewer microorganisms.

Determination of the main taxonomic composition of the isolated bacteria (Fig. 4) showed that this composition is similar to that in samples from the Odesa Region mill. Among the bacterial cultures, about 75% were spore-forming gram-positive aerobic rods (genus *Bacillus*) from grain and non-grain plant raw materials. However, slime-producing gram-negative rods were also found.

The fungi found in sample 1 belonged to the ascomycetes genera *Penicillium* and *Aspergillus*.

3. Feed mill in Mykolaiv Region

The results of quantitative assessment of microorganisms are presented in Table 3 and Fig. 5.

The table and figure show that, in general, the level of bacterial contamination was quite high (about 1 million CFU/g), which is explained by the nature of production: the use of fish meal, meat-and-bone meal, and blood meal. These components are very favorable substrates for microorganisms due to their high content of easily accessible nutrients (proteins, lipids, mineral elements) and moisture. At the same time, a lower level of fungal contamination was observed, and in some samples molds were not detected at all.

In three samples (Nos. 3, 7, and 10), the number of microorganisms was significantly lower than in the other samples, which can be explained by a number of factors:

- the fan creates a dry environment that is unfavorable for microorganisms,

Table 3 – Microbial numbers recovered from the samples of Mykolaiv Region feed mill

Sample number	Number (CFU/g)	
	bacteria	fungi
1 – *cyclone on the 4th storey*	1000000	10000
2 – *scalper on the 4th storey for all raw materials*	2000000	20000
3 – *spillage under the extruder for "wet extrusion"*	3300	500
4 – *hammermill for grain raw materials*	500000	0
5 – *mixer for preliminary mixtures*	1200000	0
6 – *bucket elevator at the extruder*	1300000	0
7 – *hammermill on the 1st storey for non-grain raw materials*	4000	200
8 – *bucket elevator on the 3rd storey*	200000	0
9 – *raw material distribution pipe on the 3rd storey*	2000000	40000
10 – *fan at the air outlet of the hammermill*	3000	2000

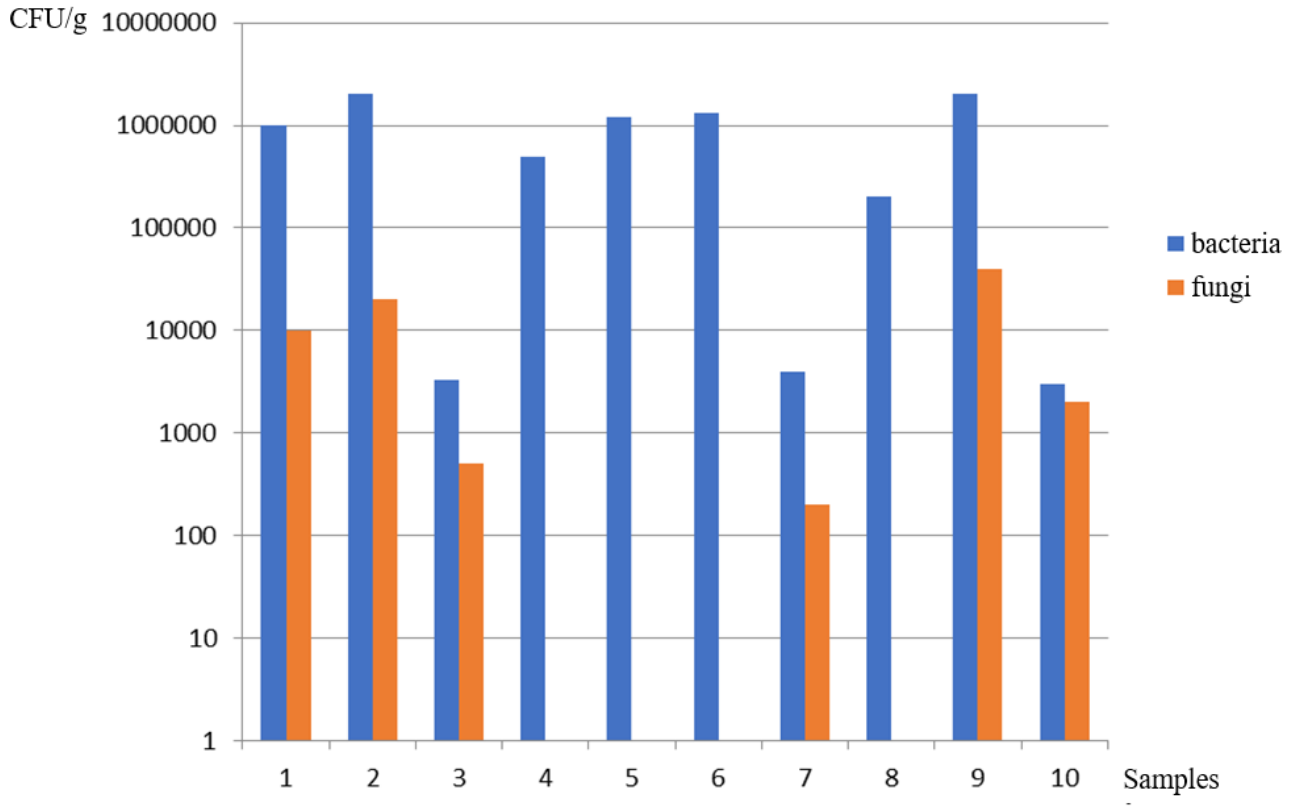

Fig. 5. Microbiological contamination of deposits and residues at the Mykolaiv Region feed mill

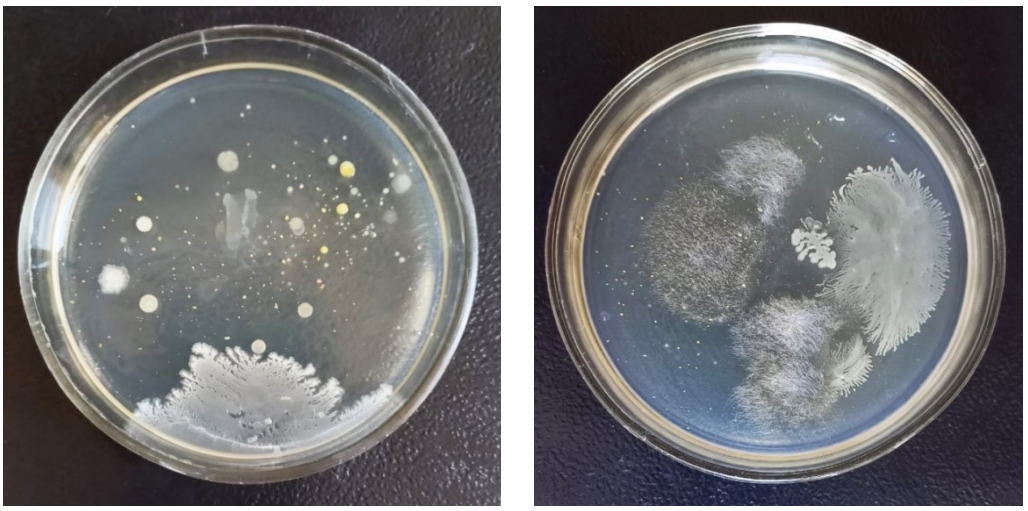

Fig. 6. Bacterial and fungal cultures obtained from the samples of Mykolaiv Region feed mill

- the spillage under the extruder was the dry extrudate (disinfected by high extrusion temperature),

- non-grain, non-animal raw materials are less favorable for microorganisms.

The taxonomic composition assessment showed that most bacteria belonged to the group of spore-forming aerobic rods from the *Bacillaceae* or *Paenibacillaceae* families (Fig. 6). This is typical microbiota of grain, dry grain products, and meal.

At the same time, there were also cocci and non-spore-forming rods present.

Almost all of the isolated fungi belonged to the zygomycetes of the order Mucorales (Fig. 6).

Comparison of plants.

Comparison of the results of studies of samples taken at the same points at different plants reveals differences in some cases and similarities in others. Thus, as mentioned above, the receiving units at mills in the Odesa and Kharkiv regions have different structures, which results in their different microbiological load. At the feed mill in the Odessa region, the receiving unit located outdoors is exposed to atmospheric precipitation, and the deposits on it are mixed with soil dust, while at the mill in the Kharkiv region, the receiving unit is indoors and there are no such impacts. Accordingly, the microbiological load of the receiving unit at the mill in the Kharkiv region is hundreds of times lower than the corresponding indicator at the mill in the Odesa region.

Another similar point is hammermills and grinding units. At the mill in the Kharkiv region, the microbiological load of samples from a separate hammermill (sample 2) was 40% higher than that of the grinding unit (sample 7), which can be explained by the different nature and degree of processing of raw materials in these machines. At the same time, a comparison with a pellet grinder at the mill in the Odesa Region shows that deposits of deeply processed material (pelleted feed) are less favorable for the development of microorganisms, but require maintenance of the equipment. A similar picture is shown by comparison with the mill in the Mykolaiv Region, where a sample from the grain raw material hammermill (sample 4) had a significantly higher microbiological load than the sample from the hammermill for non-grain raw materials (sample 7). This is explained by the nature of the raw materials and equipment maintenance.

In addition, premix and preliminary mixture mixers were common to both mills in the Kharkiv and Mykolaiv regions. However, the microbiological load of such sample was significantly lower (30 times) at the mill in the Kharkiv Region than at the mill in the Mykolaiv Region, which can be explained by the difference in the composition of the preliminary mixtures: as mentioned above, the mill in the Mykolaiv Region produces compound feed for fish and pets using a large amount of animal raw materials (blood meal, meat and bone meal, fish meal, etc.).

Conclusions

Thus, an analysis of the sanitary conditions of three feed mills in Ukraine was conducted, and certain patterns were identified when comparing the results. The following was established:

– the use of outdoor receiving units poses a significant risk of dangerous microorganisms, including toxigenic molds that produce mycotoxins, entering feed raw materials;

– the nature of raw materials significantly affects the microbiological landscape of the feed mill: the use of animal raw materials (fish meal, blood meal, meat and bone meal) significantly increases microbiological contamination of equipment due to its nutritiousness;

– proper installation of equipment is required: moistening of finished dry loose or pelleted feed in bunkers or other containers with water vapor is unacceptable;

– systematic maintenance of plant equipment is required.

Based on these results, it is recommended that Ukrainian feed mills take appropriate measures to eliminate the identified problems.

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ДОСЛІДЖЕННЯ САНІТАРНОГО СТАНУ КОМБІКОРМОВИХ ВИРОБНИЦТВ УКРАЇНИ

Анотація

Мікробіологічне обсіменіння готових комбікормів значною мірою залежить від санітарного стану виробництва. Мікробіологічний ланшафт виробництва складається з двох основних факторів: обсіменіння сировини різних видів та санітарний стан обладнання й виробничого оточення. Санітарний стан обладнання безпосередньо пов'язаний з наявністю на ньому або всередині нього відкладень або відносів матеріалу, що стають середовищем для розвитку мікроорганізмів. Кінцеве мікробіологічне навантаження може проявляти істотний вплив на здоров'я тварин, перш за все – молодняка. Тому метою даної роботи було дослідження санітарного стану трьох комбікормових заводів України: одного заводу в Одеській області, одного заводу в Харківській області та одного заводу в Миколаївській області. На заводах відбиралися зразки відкладень та відносів з виробничого обладнання та оточення. Всі зразки досліджувалися за двома мікробіологічними показниками – загальне мікробне обсіменіння та плісеневі гриби і дріжджі. Для дослідження користувалися стандартними мікробіологічними методами: робили спочатку змиви, потім розведення зразків та висівали на чашки Петрі під МПА (бактерії) та сусло-агар (плісеневі гриби й дріжджі). Окрім підрахунку колоній, також відзначали наявність певних груп або видів (за можливості) мікроорганізмів, зокрема, серед грибів – присутність відомих продуцентів мікотоксинів. Отримані результати показують, що мікробіологічне навантаження обладнання залежить від його облаштування та ступеню догляду: відкрите зовнішнє приймальне обладнання було найбільш забрудненим, значно посилене забруднення реєструвалося в місцях, де відкладення зволожуються атмосферними опадами (під відкритим небом) або водяною парою (в приміщеннях). Отримані результати показують, що правильне облаштування обладнання та належний догляд за ним є найважливішою складовою для отримання доброго санітарного стану комбікормового виробництва. За отриманими результатами заводам запропоновано відповідні рекомендації щодо усунення недоліків та покращення стану виробництва.

Ключові слова: комбікормове виробництво, санітарний стан, мікробіологічні показники, мікробіологічний ландшафт.

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