MICROBIOLOGICAL MONITORING AND IDENTIFICATION OF CONTAMINANTS IN FEED PRODUCTS PRODUCED BY LOCAL MANUFACTURERS IN THE TASHKENT REGION

Kutlieva Guzal Jumaniyazovna
Candidate of Biological Sciences Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan Tashkent, Uzbekistan
Turaeva Bakhora Ismoilovna
Doctor of Philosophy (PhD) in Biological sciences Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan Tashkent, Uzbekistan
Shonakhunov Tulkin Erkinovich
Doctor of Philosophy (PhD) in Biological sciences Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan Tashkent, Uzbekistan
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Abstract. A study was conducted to assess the microbiological quality of feed products produced by local manufacturers in Tashkent. The analysis included samples of compound feed, cottonseed cake, sunflower cake, soybean cake, and corn grits (V1–V6). Total bacterial contamination, mold content, and the presence of coliform bacteria were determined.

The total bacterial count exceeded the permissible limits (6.2×10^5 – 8.9×10^5 CFU/g), and most samples also showed elevated levels of mold contamination, particularly in sample V6 (6×10^5 CFU/g). Coliform bacteria were detected in 83% of the samples, indicating sanitary violations.

Identification using the MALDI-TOF MS method revealed the dominance of *Bacillus* spp., as well as the presence of opportunistic microorganisms, including *Staphylococcus*, *Klebsiella*, *Enterobacter*, and *Proteus*.

The obtained results indicate heterogeneity in feed quality and highlight the need to strengthen sanitary control during production and storage.

Keywords: Animal feed, microbiological safety, mold fungi, coliform bacteria, MALDI-TOF mass spectrometry**.**

The microbiological composition and abundance of microorganisms in food products are intrinsically interrelated. Environmental conditions of raw materials, processing and manufacturing parameters, as well as packaging and storage conditions, significantly contribute to the contamination of feed with diverse microorganisms.

The consumption of poultry and livestock products derived from animals fed microbiologically uncontrolled feed represents a potential risk to human health, as it may contribute to the development of infectious diseases. Diarrheal diseases, which remain a leading cause of child mortality worldwide, are frequently associated with the consumption of contaminated food.

Accordingly, the establishment of microbiological criteria is essential for ensuring food safety and protecting public health. Microbiological analysis of feed enables the detection of micromycetes and pathogenic bacteria, as well as the prevention of contamination with mycotoxins that may exert adverse effects on animal health and productivity.

Commonly applied analytical approaches include the screening of mycotoxigenic micromycete species contaminating stored feed, along with the quantification of major mycotoxins, such as aflatoxin (AF) and ochratoxin A (OTA).

Microbiological quality control of animal feed is a critical tool for identifying substandard products and serves as an incentive for manufacturers to improve production practices, thereby promoting the prevalence of high-quality feed on the market [1].

In a study by *I. Čabarkapa et al.*, the microbiological safety of 100 animal feed samples was evaluated. The total count of mold fungi in five samples increased from 3.5×10^5 to 8.85×10^5 CFU/g, while the total bacterial count in three samples increased from 1.2×10^7 to 1.37×10^8 CFU/g.

Coagulase-positive *Staphylococcus*, *Escherichia coli*, *Salmonella*, and *Proteus* spp. were not detected in the analyzed samples. Sulfite-reducing *Clostridia* were identified in 18 samples, with counts ranging from 10 to 800 CFU/g [2].

Microbiological examination of cereal products includes the detection of coliform bacteria (coliform bacteria (CB) represent a group of microorganisms differentiated based on morphological and cultural characteristics, encompassing major members of the *Enterobacteriaceae* family and serving as sanitary indicator organisms), pathogenic micromycetes (molds), as well as mesophilic and thermophilic bacteria.

The presence of coliform bacteria, *Bacillus* spp., and *Clostridium* spp. in cereal products indicates non-compliance with hygienic quality standards during production processes. Yeasts and molds are primary spoilage microorganisms responsible for the deterioration of product quality [3].

A recommended approach for achieving a more objective and comprehensive classification of animal feed is based on the application of the VDLUFA (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten) methodology. This approach has become widely adopted in several European countries, where feed manufacturers and livestock producers rely on microbiological quality assessment as a key indicator for the development of high-quality feed products [4].

This methodology includes the determination of micromycetes, yeasts, and bacteria, taking into account their potential pathogenicity. Depending on microbial counts, which are categorized into seven groups, feed materials are classified into four quality classes. Classes I–III are considered marketable, whereas Class IV is deemed unsuitable for animal feeding. Regular monitoring of microbial populations also enables the identification of additional risks, such as the potential formation of mycotoxins [5].

A wide range of microorganisms is present in animal feed products [6]. Some of these microorganisms exhibit beneficial properties and contribute to improved animal productivity. Such microorganisms (e.g., probiotics) are intentionally incorporated into feed to suppress harmful pathogens. However, feed may also harbor pathogenic microflora capable of negatively affecting animal health through the production of harmful metabolites, including toxins and mycotoxins [7].

In the European Union, legislation establishes microbiological criteria for feed quality based on the principles of the Codex Alimentarius, along with requirements for feed hygiene [8]. The primary objective is to ensure a comprehensive feed safety system for producers,

covering the entire feed supply chain while considering animal health, environmental factors, and minimizing risks to consumer health [9].

In 2022, a study conducted in the Republic of Srpska (Bosnia and Herzegovina) assessed the microbiological safety of animal feed. Samples were analyzed using standardized methods (BAS EN ISO 6579-1, BAS EN ISO 6888-1, BAS EN ISO 7937, BAS EN ISO 4833-1, and BAS ISO 21527-2) to determine the presence of *Salmonella* spp., coagulase-positive *Staphylococcus* and *Staphylococcus aureus*, *Clostridium perfringens*, total microbial count, yeasts, and total micromycetes [10;11].

Feed samples were tested self-control (91.69%) and official control (8.31%). According to established microbiological criteria, 39.94% of the samples were classified as unsatisfactory, of which 95.20% were identified through self-control and 4.80% through official control. The most significant deviations in unsatisfactory samples were associated with elevated total yeast and micromycete counts (36.74%), as well as increased total microbial load (30.03%). Pathogenic bacteria were also detected, including *Salmonella* spp. (0.35%), *Clostridium perfringens* (0.48%), and coagulase-positive *Staphylococcus*, including *Staphylococcus aureus* (0.42%).

The primary reason for classifying feed samples as unsatisfactory was the elevated total microbial count [12;13]. This risk is persistent, highly variable, and often results in indirect losses in livestock production. The microbiological safety of animal feed depends on multiple factors, among which feed quality, climatic conditions, and technological processes of production are the most critical [14;15].

Materials and Methods

Six feed samples (V1–V6) were collected from local producers in Tashkent, including two types of compound feed, cottonseed cake, sunflower cake, soybean cake, and corn grits. Sampling was carried out in accordance with microbiological control requirements. The aim of the study was to assess the sanitary condition and determine the level of microbiological contamination of the samples.

Sample preparation

From each sample (V1–V6), a 1 g portion was aseptically weighed and transferred into sterile containers. The sample was homogenized in sterile 0.9% sodium chloride solution at a ratio of 1:9 to obtain a uniform suspension, ensuring complete dispersion of microorganisms.

Serial dilution preparation

Ten-fold serial dilutions (10^{-1}–10^{-6}) were prepared from the initial suspension using sterile physiological saline by transferring 1.0 mL of suspension into 9.0 mL of diluent in each step.

Inoculation on culture media

Selective and differential culture media were used for the isolation of different microbial groups. Inoculation was performed according to standard microbiological procedures, followed by incubation under optimal temperature conditions.

Total counts of mesophilic aerobic and facultative anaerobic microorganisms were determined by surface plating on Meat Peptone Agar (MPA), followed by incubation at 30–37 °C for 24–48 h.

For the isolation of microscopic fungi and yeasts, Potato Dextrose Agar (PDA) was used, with surface inoculation and incubation at 25–28 °C for 3–5 days.

Selective isolation of yeasts and molds was additionally performed on Sabouraud Dextrose Agar (SDA) under the same incubation conditions (25–28 °C for 3–5 days).

Bacteria of the coliform group were isolated using Endo agar, while staphylococci were detected using Mannitol Salt Agar (MSA). Plates were incubated at 37 °C for 24 h.

Incubation

Petri dishes with inoculated media were incubated in a thermostat. Sterility controls included uninoculated media to verify aseptic conditions.

Enumeration and evaluation of results

After incubation, colonies were counted on plates containing 30–300 colonies, and results were recalculated using the dilution factor. Microbial loads were expressed as colony-forming units per gram (CFU/g).

Microbial identification

- On MPA: determination of total bacterial contamination [21;22].
- On PDA and SA: assessment of fungal morphotypes (yeasts and molds) [16–20].
- On Endo agar: differentiation of lactose-positive (metallic sheen) and lactose-negative bacteria [24].
- On MSA: detection of staphylococci [23].

Interpretation of results

The obtained results were compared with regulatory sanitary microbiological standards (GOST 10444.15-94, GOST 10444.12-88) to assess the level of contamination and sanitary quality of the samples.

In addition, MALDI-TOF MS analysis enabled refinement of the taxonomic profile of the detected microorganisms and identification of both commensal microflora and opportunistic pathogens of sanitary significance.

Results and Discussion

The results of the microbiological examination of feed samples (V1–V6), presented in Table 1, indicate microbial growth on all used culture media, including Potato Dextrose Agar (PDA), Meat Peptone Agar (MPA), Sabouraud Agar (SA), Endo agar, and Mannitol Salt Agar (MSA).

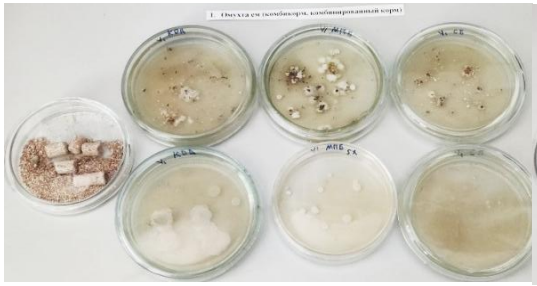
Growth of colonies on Potato Dextrose Agar (PDA) and Sabouraud Agar indicates contamination of several samples with microscopic fungi and yeasts. The most pronounced fungal contamination was observed in samples V3 and V5, which may suggest an elevated presence of mold and yeast-like microorganisms in these feed batches.

Microbial growth on Meat Peptone Broth confirms the presence of a bacterial component in all examined samples. In particular, samples V1, V2, and V6 exhibited the most intensive bacterial growth, indicating a high level of contamination with mesophilic aerobic and facultative anaerobic microorganisms.

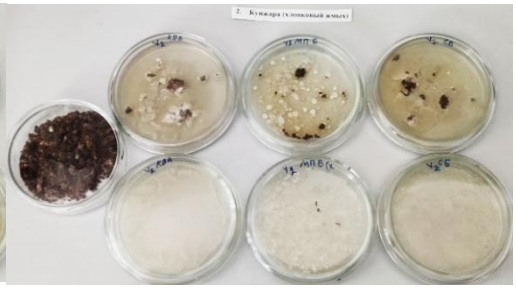
The observed differences in the nature and intensity of growth across the culture media reflect the heterogeneity of the microbiological composition of the investigated samples. The obtained data suggest possible violations of temperature and humidity storage conditions, as well as non-compliance with sanitary and hygienic requirements during production and transportation of feed products, which warrants further detailed investigation.

Table 1.

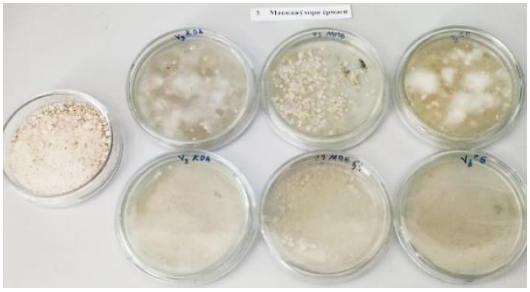
Growth of microorganisms in feed samples (V1–V6) on culture media: Potato Dextrose Agar (PDA), Meat Peptone Agar (MPA), and Sabouraud Agar (SA)



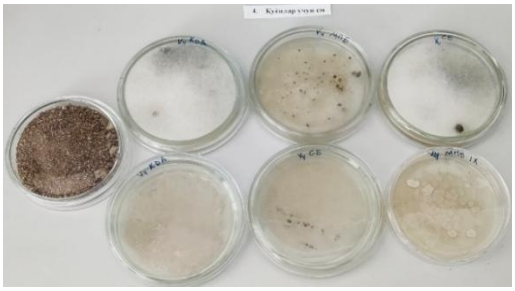
V1 (compound feed)



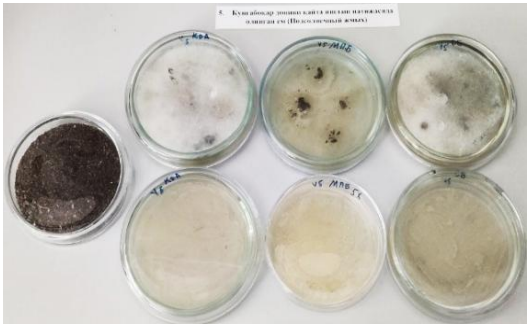
V2 (cottonseed cake)



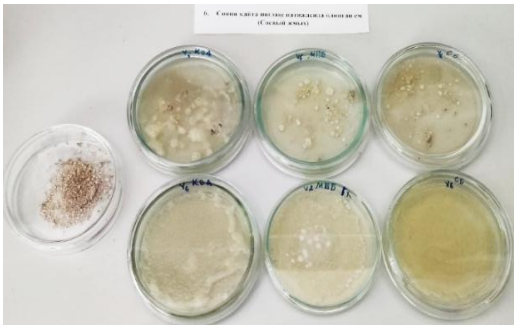
V3 (corn grits)



V4 (rabbit compound feed)



V5 (sunflower cake)



V6 (soy cake)

The analysis of microbiological parameters of feed samples (V1–V6) revealed varying levels of contamination across three key indicators: total bacterial count, mold content, and the presence of coliform bacteria (CB) (Table 2).

The total bacterial count in all investigated samples ranged from 6.2×10^5 to 8.9×10^5 CFU/g. These values indicate an acceptable level of bacterial contamination in all batches of the examined feed products.

However, mold contamination in all samples exceeded the permissible limit (5×10^4 CFU/g). The highest levels were recorded in samples V4 and V5 (9.2×10^5 CFU/g), as well as in sample V6 (3×10^5 CFU/g), exceeding the established standard. Fungal contamination poses a potential risk of mycotoxin accumulation and may adversely affect feed safety.

The presence of coliform bacteria, which is not permitted under current sanitary regulations, was detected in samples V1, V3, V4, V5, and V6. Only sample V2 complied with regulatory requirements for this parameter.

Thus, the analysis demonstrated that none of the investigated samples met the established standards for total bacterial contamination. In addition, most samples failed to

comply with sanitary and hygienic requirements regarding mold content and the presence of coliform bacteria, which are considered opportunistic enterobacteria.

The combined presence of these violations suggests possible non-compliance with technological conditions during production, transportation, or storage of feed. These findings highlight the necessity of strengthening microbiological control at all stages of the production chain to ensure the biological safety of feed products.

The analysis of microbiological parameters of feed samples (V1–V6) revealed varying levels of contamination across three key indicators: total bacterial count, mold content, and the presence of coliform bacteria (CB) (Table 2). The obtained data indicate a systematic violation of sanitary and hygienic standards and highlight the need for in-depth identification of the detected contaminants.

Accordingly, in the next stage of the study, qualitative detection of staphylococci was performed using Mannitol Salt Agar (MSA) and assessment of mannitol fermentation. This approach not only confirmed the presence of staphylococcal contamination but also enabled preliminary identification of the species affiliation of the isolated strains.

Table 2.
Microbiological characteristics of feed samples (V1–V6)

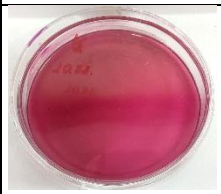
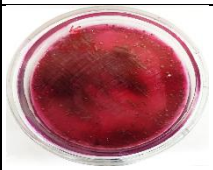
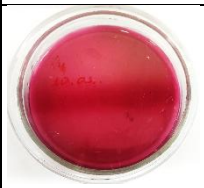
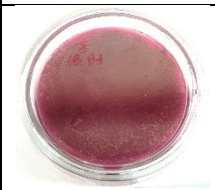
version	bacterial count (CFU/g)	Molds, (CFU/g)	coliform bacteria (in 0.1 g)
V1	$6.2x10^5$	$6.2x10^4$	-
V2	$7.1x10^5$	$6,5x10^4$	+
V3	$6.5x10^5$	$6,2x10^4$	+
V4	$8.9x10^5$	$9,2x10^5$	+
V5	$6.5x10^5$	$9,2x10^5$	+
V6	$7.3x10^5$	$6x10^5$	+
Норма	$5x10^5$	$5x10^4$	-

Note: CFU – colony-forming units; CB – coliform bacteria (bacteria of the coliform group); "+" – present; "–" – absent.

In samples V1, V3, and V4, growth of lactose-positive colonies of *Escherichia coli* with a characteristic metallic sheen was observed. The CFU levels did not exceed the established regulatory limits, indicating satisfactory sanitary conditions for these samples (Table 3).

In sample V6, growth of lactose-negative colonies characteristic of opportunistic *Proteus* spp. was detected. In samples V2 and V5, although lactose-positive growth was observed, the microbial load exceeded permissible levels, indicating fecal contamination and suggesting violations of sanitary conditions during production, storage, or transportation. These samples require further investigation for the presence of pathogenic enterobacteria (Table 3).

Table 3.
Microbial growth of samples V1–V6 on Endo agar with colony characteristics and assessment of lactose fermentation (positive/negative)

V	colony morphology	lactose positivity / negativity	interpretation	Figure
V1	Round, smooth colonies with a metallic sheen and a pinkish hue	**lactose** +	Presence of enterobacteria corresponding to the normal intestinal microbiota	
V2	Colorless colonies with a slightly elevated surface and smooth, even edges	**lactose** -	Increased bacterial load indicating fecal contamination	
V3	Round, smooth colonies with a metallic sheen and a pinkish hue	**lactose** +	Moderate presence of enterobacteria	
V4	Round, smooth colonies with a metallic sheen and a pinkish hue	**lactose** +	Normal intestinal microbiota, compliant with sanitary standards	
V5	Small pink colonies with regular (smooth) edges	**lactose** +	Elevated bacterial contamination indicating fecal contamination	
V6	Colorless colonies with a slightly elevated surface and smooth, regular margins	**lactose** –	Presence of lactose-negative bacteria, possibly opportunistic pathogens	

During microbiological monitoring of six feed samples from local producers in the Tashkent region (V1–V6), staphylococcal contamination was detected in all examined samples without exception. For the identification of the isolates, a classical phenotypic approach was applied, including cultivation on Mannitol Salt Agar (MSA) and assessment of mannitol fermentation ability (Table 4).

A comprehensive analysis of microbiological parameters (Tables 2–3) revealed a systematic non-compliance of the investigated samples with current sanitary and hygienic standards across all key indicators. The total bacterial count in all samples exceeded the permissible limit (5×10^5 CFU/g): the lowest deviation was observed in samples V1, V3, and V5 ($6.2–6.5 \times 10^5$ CFU/g), while the highest was recorded in sample V4 (8.9×10^5 CFU/g), representing a clear exceedance of the standard.

A similar pattern was observed for mold contamination: the permissible limit (5×10^4 CFU/g) was exceeded in all six samples, with sample V6 showing a critical deviation of 7.3×10^5 CFU/g, indicating a substantial exceedance of the regulatory threshold.

The coliform bacteria (CB) indicator, serving as an indicator of fecal contamination and violations of sanitary and hygienic production practices, met regulatory requirements in only one sample (V1). In samples V2–V6, coliform bacteria were detected, directly indicating breaches in the technological chain of feed raw material storage or transportation.

Against the background of the overall microbial load, particular attention should be given to the total staphylococcal contamination of the entire sample set. The analysis revealed that the dominant species in samples V1–V4 and V6 was *Staphylococcus gallinarum*, a coagulase-negative species primarily associated with feed.

In sample V2, which exhibited the highest total bacterial count (7.1×10^5 CFU/g), the *S. gallinarum* isolate simultaneously demonstrated intense mannitol fermentation (++), which may indirectly indicate increased metabolic activity of the strain. A similar pattern was observed in sample V4 (8.9×10^5 CFU/g, fermentation ++), suggesting that high total microbial load and enhanced staphylococcal fermentative activity may represent interrelated indicators of more pronounced microbial degradation of the feed.

In sample V5, *Staphylococcus condimenti* was identified, a species originally described as a contaminant of fermented food products but increasingly reported in feed raw materials. Notably, sample V5, despite the detection of coliform bacteria and exceedance of regulatory limits for all total microbial load indicators, exhibited moderate mannitol fermentation (+), which may indicate species-specific differences in the biochemical activity of the isolates.

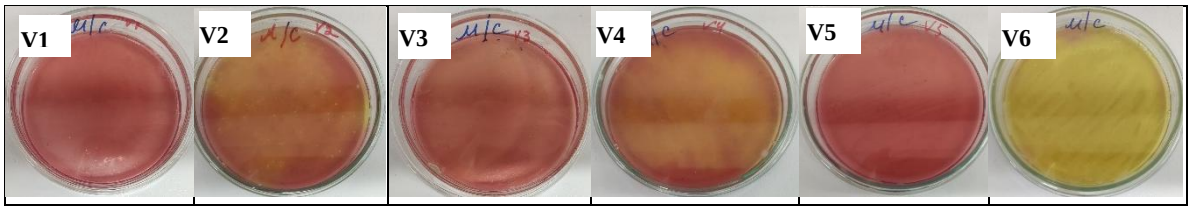
Sample V6 is of the greatest sanitary and epidemiological concern. Despite a relatively moderate exceedance of the total bacterial count (7.3×10^5 CFU/g), it exhibited critically high mold contamination (6×10^5 CFU/g), the presence of coliform bacteria, and staphylococcal contamination by *S. gallinarum*.

Such a combination of multiple contaminant types within a single sample indicates a complex pattern of microbiological deterioration and a potentially high risk of mycotoxin formation.

Thus, none of the six investigated feed samples from local producers in the Tashkent region fully complied with the established microbiological standards. The identified complex of contaminants—including elevated mold levels, the presence of coliform bacteria, and widespread staphylococcal contamination—indicates systemic violations of sanitary and hygienic conditions during production, storage, or transportation of feed and highlights the need for corrective measures at the level of technological control in manufacturing enterprises.

Table 4.

Qualitative detection of staphylococci on culture medium in feed samples and mannitol fermentation (F/M)



Staphylococcus gallinarum	*Staphylococcus gallinarum*	*Staphylococcus gallinarum*	*Staphylococcus gallinarum*	*Staphylococcus condimenti*	*Staphylococcus gallinarum*
(F/M)+	**(F/M)++**	**(F/M)+**	**(F/M)++**	**(F/M)+**	**(F/M)+++**

Microorganism identification by MALDI-TOF MS revealed a diverse taxonomic composition of the microbiota in the investigated samples (V1–V6), with a predominance of Gram-positive spore-forming bacteria of the genus *Bacillus* and the presence of opportunistic microorganisms.

Across all samples, members of the genus *Bacillus* (*B. subtilis*, *B. vallismortis*, *B. atrophaeus*, *B. licheniformis*) were dominant. These species are not considered of direct sanitary concern; however, they may contribute to product spoilage under improper storage conditions.

Opportunistic microorganisms were detected in all samples, including staphylococci (*Staphylococcus gallinarum*, *S. condimenti*) and Gram-negative enterobacteria, namely *Klebsiella variicola*, *Enterobacter cloacae*, and *E. cancerogenus*. The highest taxonomic diversity of opportunistic microbiota was observed in samples V1 and V2, where representatives of the genera *Staphylococcus*, *Klebsiella*, and *Enterobacter* were simultaneously detected, indicating an elevated sanitary risk. In samples V3–V6, the opportunistic fraction was mainly represented by *Staphylococcus* and *Bacillus*.

The presence of opportunistic microorganisms in all investigated samples indicates secondary contamination of the feed and probable violations of sanitary and hygienic requirements during production, storage, or transportation, thereby necessitating strengthened microbiological control measures (Table 5).

Table 5.

Distribution of microorganisms in feed samples (V1–V6) with assessment of sanitary risk

№	Sample	Genus	Strain	**Taxonomic group**	Sanitary significance
1	V1	*Staphylococcus*	*gallinarum*	Gram-positive cocci	opportunistic pathogenic
2		*Bacillus*	*subtilis*	Gram-positive spore-forming bacilli	normal microbiota
3		*Klebsiella*	*variicola*	Gram-negative enterobacteria	opportunistic pathogenic
4	V2	*Enterobacter*	*cancerogenus*	Gram-negative enterobacteria	opportunistic pathogenic
5		*Klebsiella*	*variicola*	Gram-negative enterobacteria	opportunistic pathogenic
6		*Staphylococcus*	*gallinarum*	Gram-positive cocci	opportunistic pathogenic
7		*Enterobacter*	*cloacae*	Gram-negative enterobacteria	opportunistic pathogenic
8	V3	*Bacillus*	*subtilis*	Gram-positive spore-forming bacilli	normal microbiota

9		*Staphylococcus*	*gallinarum*	Gram-positive cocci	opportunistic pathogenic
10		*Bacillus*	*vallismortis*	Gram-positive spore-forming bacilli	normal microbiota
11	V4	*Bacillus*	*vallismortis*	Gram-positive spore-forming bacilli	normal microbiota
12		*Staphylococcus*	*gallinarum*	Gram-positive cocci	opportunistic pathogenic
		Bacillus	*subtilis*	Gram-positive spore-forming bacilli	normal microbiota
	V5	*Bacillus*	*subtilis*	Gram-positive spore-forming bacilli	normal microbiota
		Staphylococcus	*condimenti*	Gram-positive cocci	opportunistic pathogenic
		Bacillus	*vallismortis*	Gram-positive spore-forming bacilli	normal microbiota
	V6	*Bacillus*	*atrophaeus*	Gram-positive spore-forming bacilli	normal microbiota
		Bacillus	*licheniformis*	Gram-positive spore-forming bacilli	normal microbiota
		Staphylococcus	*gallinarum*	Gram-positive cocci	opportunistic pathogenic

Note: Microorganism identification was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Conclusions. The microbiological monitoring of six feed samples from local producers in the Tashkent region revealed systematic non-compliance of all investigated batches with current sanitary and hygienic standards. The total bacterial load ($6.2 \times 10^5 – 8.9 \times 10^5$ CFU/g) exceeded the permissible limit (5×10^5 CFU/g) in all samples without exception. Mold contamination also exceeded the regulatory threshold (5×10^4 CFU/g) in all cases, reaching a critical level of 6×10^5 CFU/g in sample V6, indicating a substantial risk of mycotoxin accumulation. Coliform bacteria, representing opportunistic enterobacteria, were detected in five out of six samples, suggesting fecal contamination and violations of sanitary conditions during production, storage, or transportation.

Microorganism identification using phenotypic methods and MALDI-TOF MS demonstrated that, alongside dominant commensal flora of the genus *Bacillus*, all samples contained opportunistic microorganisms, including *Staphylococcus gallinarum* (V1–V4, V6), *S. condimenti* (V5), as well as Gram-negative enterobacteria (*Klebsiella variicola*, *Enterobacter cloacae*, and *E. cancerogenus*), predominantly in V1 and V2. Staphylococcal contamination was universal, affecting all investigated feed samples.The obtained results highlight the necessity of strengthening microbiological control across all stages of the production chain, revising storage conditions for feed raw materials, and implementing continuous monitoring using modern microorganism identification techniques to ensure the biological safety of feed products in the Tashkent region

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