Scientific Papers. Series D. Animal Science. Vol. LXVIII, No. 2, 2025
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

MICROBIOLOGICAL SAFETY ASSESSMENT OF SOME RAW MATERIALS USED IN COMPOUND FEED PRODUCTION

Dragoş Mihai LĂPUȘNEANU, Silvia Ioana PETRESCU, Mădălina Alexandra DAVIDESCU, Cristina Gabriela RADU-RUSU, Mădălina MATEI, Ioan Mircea POP

"Ion Ionescu de la Brad" Iasi University of Life Sciences,
Mihail Sadoveanu Alley 3, 700490, Iasi, Romania

Corresponding author email: silvia.petrescu@iuls.ro

Abstract

The aim of this study was to microbiologically asses some raw materials (maize and wheat) from a representative feed mill in Romania, in two consecutive years, both for the raw materials received and for those in the unit's stock; the microbiological contaminants that were analyzed were yeasts and molds. Regarding the maize samples taken upon receipt in first year, it was found that 90.9% had positive results, with an average value of 2600.8 cfu/g; in second year the proportion of positive samples was 84.3%, with an average value of 2132.5 cfu/g. Of the total wheat samples taken in first year upon reception, 89.1% were positive, with an average value of 2554.8 cfu/g; in second year the proportion of positive samples increased, reaching 93.3%, with an average value of 2171.4 cfu/g. The application of all measures capable of preventing contamination prevented the contaminated batches from entering the production process, while avoiding the possibility of contamination of other batches of raw materials and finished products (compound feed).

***Key words**: food safety, feed safety, yeasts and molds.*

INTRODUCTION

Compound feed is vulnerable to the introduction of bacteria along the production chain. The presence of pathogens in feed can occur due to the use of contaminated raw materials, during transportation, in the production facility or on the farm. As bacterial contaminants are not evenly distributed in the feed, bacteria present may be damaged or injured and difficulties may arise during microbial analysis (Alali & Ricke, 2012; Matei & Pop, 2022). The unnecessary or unintentional presence of pathogenic microorganisms is referred to as microbiological contamination. Contagious microbes, including bacteria, fungi, yeasts protozoa and even viruses, cause microbial contamination (Chatterjee & Abraham, 2018; Matei et al, 2024).

The toxigenic fungi of field crops belong to the genera *Alternaria, Aspegillus, Cladosporium, Helminthosporium*, and *Fusarium* (Wielogórska et al., 2016; Pinotti et al., 2016). When cereal grains and forages are colonized by molds, there is a significant risk of contamination with their secondary metabolites (Atungulu et al., 2015) which may include contaminants such as mycotoxins, these secondary metabolites of fungi present in contaminated grains due to favorable environmental conditions in the pre- or post-harvest period (Bento et al., 2012).

The predominant cereal grains in animal rations are represented by maize (*Zea mays* L.) kernels, due to their high concentration in eneregy, but they may present structural defects in the kernels such as cracks, foreign particles and impurities, which expose them to fungal contamination and consequently to the presence of mycotoxins (Ubiali et al., 2011; Ruiz et al., 2011; Savi et al., 2016). In addition to compromising nutritional value and processing (milling, extrusion, granulation), the hygienic quality of maize may chemically alter the composition of the forage through the presence of substrates produced by microorganisms, such as mycotoxins (Abdollahi et al., 2010).

A high incidence of fungi of the genus *Aspergillus* (aflatoxigenic) has been identified in maize kernels stored at relative humidity between 13%-18% (Pinotti et al., 2016; Lăpușneanu et al., 2023).

The aim of our study was the microbiological evaluation of some raw materials used in the production of compound feed; the microbiological contamination with yeasts and molds of maize and wheat samples collected during two consecutive years from a feed mill in Romania was analyzed. The maize and wheat used as raw materials in the production of compound feed were analyzed both on arrival at the raw material reception stage and from the unit's stock.

MATERIALS AND METHODS

To ascertain the yest and molds content in maize and wheat grain from the feed mill reception stage, 66 respectively 37 analyzes were carried out in first year, and 95 respectively 45 analyzes in second year; to determine the yest and molds content of maize and wheat grain from the feed mill stock, 20 respectively 35 analyzes were carried out in first year, and 11 respectively 9 analyzes in second year.

Yeasts and molds were detected in accordance with SR ISO 21527-2:2009. This part of ISO 21527 specifies a horizontal method for the enumeration of viable osmophilic yeasts and xerophilic moulds in products intended for human consumption or animal feed having a water activity less than or equal to 0.95 (dried fruits, cakes, jams, dried meat, dried fish, salted fish, grains, cereals and cereal products, flours, nuts, spices and spices, etc.) by the colony counting technique at 25°C ± 1°C. Prepare surface inoculated plates using a specified selective culture medium. Depending on the expected number of colonies, a certain amount of sample (if the product is liquid) or initial suspension (in the case of other products) or decimal dilutions of the sample/suspension shall be used. Additional plates may be prepared under the same conditions, using decimal dilutions of the test sample or initial suspension. The plates are then incubated aerobically at 25°C ± 1°C for 5 to 7 days. The colonies/propagules are then counted and, if necessary (to distinguish yeast from bacterial colonies), the identity of any doubtful colonies is confirmed by examination with a binocular loupe or microscope. The number of yeasts and moulds per gram or per milliliter of sample is calculated from the number of colonies/propagules/germs obtained on plates chosen at dilution levels that produce countable colonies. Molds and yeasts are counted separately if necessary.

The analyzed data were subjected to statistical processing and interpretation. Minimum and maximum values were determined and position and variance estimators were calculated. In particular, the arithmetic mean ($\bar{x}$) and the standard deviation (s) were calculated for the samples that gave positive results.

The results obtained were subjected to comparison with the values regulated by the Romanian legislation. Interpretation of these results led to conclusions on feed and food safety.

RESULTS AND DISCUSSIONS

For the quantitative determination of total fungi count (TFC) in maize grains and wheat grains, determinations were carried out on samples taken in two consecutive years, both for the raw materials received and for those in the unit's stock. In accordance with Order No 249 of March 31, 2003, the maximum permissible total number of potentially toxin-producing fungal species (*Aspergillus, Penicillium, Fusarium, Alternaria*) is 5000 ucf/g for cereal grains used in the production of compound feed.

The results obtained on the quantitative determination of the total number of fungi in maize kernels sampled at reception and in the stock of the studied unit during the two years are presented in Table 1.

With regard to the samples of grain maize taken at reception in the first year, it was found that 90.9% were positive, with an average value of 2600.8 cfu/g; in the second year the proportion of positive samples was 84.3%, with an average value of 2132.5 cfu/g.

Comparative analysis of the results recorded at the reception of grain maize for the years under study shows that, only in the first year, 6% of the samples analyzed had values exceeding the maximum limit allowed by the legislation, which led to the rejection of the lots in question.

Of the total samples of grain maize taken in the first year from the studied plant stock, 80% were positive with an average of 2237.5 cfu/g; in the second year, the samples were 100% positive with an average of 3872.7 cfu/g. Considering that the standard deviation for the analyzed grain maize from the stock in first year indicates that the identified values are close to or even reach the maximum limit of TFC, it is recommended to increase the number of samples analyzed to control the growth of toxigenic fungi. Mycological study of the samples indicated the presence of three genera of potentially toxigenic fungi (Figure 1).

Table 1. Results on the total fungal number in maize grain from reception and stock

Specification		First Year						Second Year					
		n	Positive (%)	Min. (cfu/g)	Max. (cfu/g)	x̄	s	**n**	Positive (%)	Min. (cfu/g)	Max. (cfu/g)	x̄	s
Maize grain	Recep.	66	90.9	200	8000	2600.8	1816.1	95	84.2	200	5000	2132.5	1534.8
	Stock	20	80	300	4000	2237.5	1333.6	11	100	2000	5000	3872.7	1265.2

n - number of samples analyzed; x̄ - mean; s - standard deviation; Min. - minimum value identified; Max. - maximum value identified.

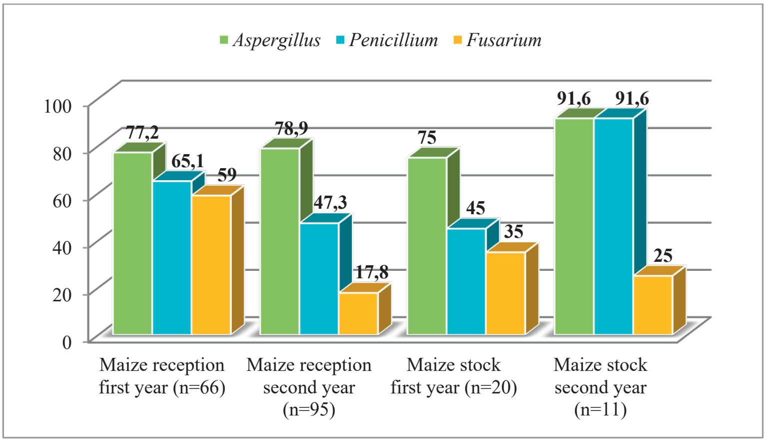

Figure 1. Proportion (%) of potentially toxigenic fungal genera in maize grain on receipt and stock

As distinguished in the figure above, the genus *Aspergillus* was found in both years of the study in more than 70% of the analyzed maize sample categories (reception and stock). In the maize analyzed at reception in the first year, the genus *Penicillium* was identified in 65.1% of the samples, while in the second year the proportion of isolates decreased to 47.3%; the same difference was recorded for the genus *Fusarium*, with its identification in 59% of the samples in the first year and 17.8% in the second year.

The results of the quantitative determinations of the total number of fungi in the wheat grains taken at reception and in the stock of the studied unit during the two years of study are presented in Table 2.

Table 2. Results on the total fungal number in wheat grain from reception and stock

Specification		First Year						Second Year					
		n	Positive (%)	Min. (cfu/g)	Max. (cfu/g)	x̄	s	**n**	Positive (%)	Min. (cfu/g)	Max. (cfu/g)	x̄	s
Wheat grain	Recep.	37	89.1	200	6000	2554.8	1798.3	45	93.3	200	5000	2171.4	1278.2
	Stock	35	97.1	200	4000	2272	1450.4	9	88.8	3000	4000	3375	517.5

Of the total samples of wheat grains taken at reception in the first year, 89.1% were positive, with an average value of 2554.8 cfu/g; in the second year the proportion of positive samples increased to 93.3%, with an average value of 2171.4 cfu/g. The analyses performed at reception in the first year revealed an exceeding of the maximum admissible limit of TFC (2.7% of the total samples analyzed), which led to the rejection of the lot.
The values of the mycological analysis for 97.1% of the positive samples of wheat grains taken from stock in the first year averaged 2272 cfu/g; in the second year the average value was 3375 cfu/g for 88% of the positive samples.
With regard to the averages established for the values identified from the analyses carried out on the wheat samples from the reception and from the stock of the studied unit, it can be seen that they exceeded in each case the value of 2000 cfu/g for both years of research, a value which suggests a high mycological load.
The mycological study of the samples indicated the presence of three genera of potentially toxigenic fungi (*Aspergillus, Penicillium and Fusarium*), and their isolation frequency can be seen in Figure 2.

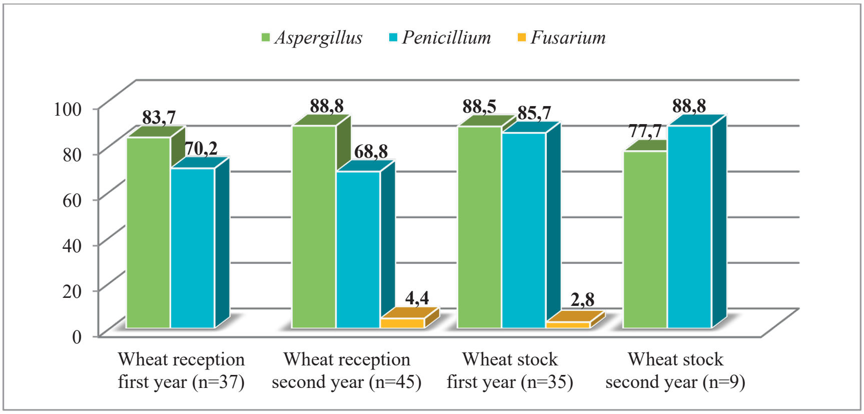

Figure 2 Proportion (%) of potentially toxigenic fungal genera in wheat grain on receipt and stock

Concerning the proportion of genera identified, it is distinguished that the genus *Aspergillus* was found in almost more than 80% of the total wheat samples analyzed in both years of study, followed by the genus *Penicillium*, identified in more than 70% of the analyzed samples. The frequency of isolation of *Aspergillus* was slightly higher in the first year (88.8%) than in the second year (83.7%) for wheat grains taken from the reception, and slightly higher in the first year (70.2%) than in the second year (68.2%) for *Penicillium*. *Fusarium* was isolated in 2.8% of the wheat samples in the first year and in 4.4% of the wheat samples in the second year.
In other studies, which have investigated microbiological contamination of raw materials and compound feed in maize and wheat grain, the presence of *Aspergillus* genus has been identified in over 50% of analyzed samples total fungal count ranging from 400 to 4900 cfu/g. In addition, besides the genera of potentially toxigenic fungi identified in our study, the genera *Cladosporium*, and *Alternaria* were also identified for the two cereal categories (Lăpușneanu et al. 2023; Lapusneanu et al., 2024). Krnjaja et al. (2017) analyzed 127 maize samples for determinate fungal contamination and the total fungal count ranged from 100 to 30 000 cfu/g and identified the most frequent genera as the *Fusarium* genera (92.22%). In a study conducted by Lapusneanu et al. (2021) the content in the genus *Aspergillus* was predominant (65.45%) in maize and wheat grain, followed by the content in the genus *Fusarium* (41.81%). The literature states that *Aspergillus* fungi are the main producers of aflatoxins and ochratoxin A (Marin et al., 2013; Völkel et al., 2011).

CONCLUSIONS

The present study showed that maize and wheat grain can be a potential source of potential toxigenic fungi in compound feed.

Our results showed that only a small proportion of the samples analyzed contained levels above the maximum legal limit.

It is important for feed mills to establish adequate control measures for yeast and mold contamination of raw materials to ensure feed and food safety; given that in our study, in the reception of raw materials, exceedances of the permissible limits of yeast and mold content were identified at the reception stage, the batches were rejected and did not enter the production process.

The application of all measures capable of preventing contamination prevented the contaminated batches from entering the production process, while avoiding the possibility of contamination of other batches of raw materials and finished products (compound feed).

Therefore, further investigations are needed at feed mills to identify the various contaminants to ensure feed and food safety.

REFERENCES

Abdollahi, M.R., Ravindran, V., Wester, T.J., Ravindran, G. & Thomas, D.V. (2010). Influence of conditioning temperature on the performance, nutrient utilisation and digestive tract development of broilers fed on maize- and wheat-based diets. *British Poultry Science, 51*(5), 648–657.

Alali, W.Q. & Ricke, S.C. (2012). *The ecology and control of bacterial pathogens in animal feed*. In *Animal Feed Contamination-Effects on Livestock and Food Safety*, 1st ed., Fink-Gremmels, J., Ed. Cambridge, USA: Woodhead Publishing House, 35–55.

Atungulu, G.G., Zhong, S., Thote, A., Okeyo, A. & Couch, A. (2015) Microbial Prevalence on Freshly-Harvested Long-Grain Pureline, Hybrid, and Medium-Grain Rice Cultivars. *Applied Engineering in Agriculture*, *31*, 949–956.

Bento, L.F., Caneppele, M.A.B., Albuquerque, M.C.F., Kobayasti, L., Caneppele, C. & Andrade, P.J. (2012) Occurrence of fungi and aflatoxins in corn kernels. *Revista do Instituto Adolfo Lutz*, *71*, 44–49.

Chatterjee, A. & Abraham, J. (2018). *Microbial contamination, prevention and early detection in food industry.* In *Microbial Contamination and Food Degradation*. Grumezescu, A.T., Holban, A.M., Eds.; Amsterdam, NL: Academic Press Publishing House, 21–47.

Krnjaja, V., Stanojković, A., Stanković, S.Ž., Lukić, M., Bijelić, Z., Mandić, V. & Mićić, N. (2017). Fungal contamination of maize grain samples with a special focus on toxigenic genera. *Biotechnology in Animal Husbandry*, *33*, 233–241.

Lapusneanu, D.M., Pop, I.M., Pop, C., Radu-Rusu, C.G., Musca, A. & Zaharia R. (2021). Study on analysis of biological hazards associated with compound feed producing in relation on food safety. *Scientific Papers. Series D. Animal Science, LXIV*(1), 175–181.

Lăpușneanu, D.M., Radu-Rusu, C.G., Matei, M., Petrescu, S.I. & Pop, I.M. (2024). Aflatoxins occurrence and levels in maize from a romanian feed mill. *Scientific Papers. Series D. Animal Science, LXVII*, (1), 110-114.

Lăpușneanu, D.M., Simeanu, D., Radu-Rusu, C. G., Zaharia, R., & Pop, I. M. (2023). Microbiological Assessment of Broiler Compound Feed Production as Part of the Food Chain - A Case Study in a Romanian Feed Mill. *Agriculture*, *13*(1), 107.

Marin, S., Ramos, A.J., Cano-Sancho, G. & Sanchis, V. (2013). Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food and Chemical Toxicology*, *60*, 218–237.

Matei, M., & Pop, I.M. (2022). Monitoring of dairy farms to assess the potential level of pollution of animal feed and animal production. *Scientific Papers. Series D. Animal Science*, *LXV*(2), 129–136.

Matei, M., Petrescu, S.I., Flocea, E.I., Lăpușneanu, D.M., Simeanu, D., & Pop, I.M. (2024). Variation in mineral oil hydrocarbons content of milk during processing. *Scientific Papers. Series D. Animal Science, LXVII*(1), 490–499.

Pinotti, L., Ottoboni, M., Giromini, C., Dell'Orto, V. & Cheli, F. (2016). Mycotoxin contamination in the EU feed supply chain: A focus on cereal byproducts. *J. Toxins*, *8*, 45.

Ruiz, M.J., Macáková, P., Juan-García, A. & Font, G. (2011) Cytotoxic effects of mycotoxin combinations in mammalian kidney cells. *Food and Chemical Toxicology*, *49*(10), 2718–2724.

Savi, G.D., Piacentini, K.C., Marchi, D. & Scussel, V.M. (2016). Fumonisins B1 and B2 in the corn-milling process and corn-based products, and evaluation of estimated daily intake. *Food Additives & Contaminants: Part A, 33*(2), 339–345.

SR ISO 21527-2:2009. Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Enumeration of Yeasts and Moulds - Part 2: Colony Count Technique in Products with Water Activity Less than or Equal to 0,95. Romanian Standards Association: Bucharest, Romania, 2009.

Ubiali, D.G., Boabaid, F.M., Borges, N.A., Caldeira, F.H.B., Lodi, L.R., Pescador, C.A., Souza, M.A. & Colodel, E.M. (2011). Acute poisoning with Crotalaria spectabilis (Leg. Papilionoideae) seeds in pigs, *Pesquisa Veterinária Brasileira., 31*(4), 313–318.

Völkel, I., Schröer-Merker, E., & Czerny, C.P. (2011). The carry-over of mycotoxins in products of animal origin with special regards to its implications for the european food safety legislation. *Food Science & Nutrition*, *2*, 852–867.

Wielogórska, E., Mac Donald, S., & Elliott, C.T. (2016) A review of the efficacy of mycotoxin detoxifying agents used in feed in light of changing global environment and legislation. *World Mycotoxin J.*, *9*, 419–433.