STUDY ON MICROBIOLOGICAL ANALYSIS OF FEED MILL PROCESSING ENVIRONMENT

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Abstract

The aim of the control of pathogens in feed is to ensure that they are under a critical threshold to minimize the risk to human and animal health. In the production of compound feed, contamination with undesirable substances may occur, which may come from the environment and/or the production process. The aim of the work is to determine the contamination with yeasts and molds of the surfaces of the raw material storage silos of a feed mill in Romania. Sampling to determine yeast and mold contamination were taken from different points of the silos, for a higher accuracy of the results, namely walls, floors, sweep auger, access cover. During 2019 and 2020, 72 samples respectively 48 samples were taken and analyzed to determine the contamination with yeasts and molds. The results of microbiological analyzes performed in the feed mill studyed, showed that all 70 (97.2 %) respectively 36 (100%) samples were positive. Microbiological control of feed mill processing environment must be considered relevant due to the demands of consumers for food safety all over the food chain; introduction of an appropriate system for monitoring and analyzing microbiological contaminants can contribute to the control and prevention of contamination.

Key words: *feed safety, yeasts and molds, compound feed*

INTRODUCTION

Compound feed is vulnerable to the introduction of bacteria throughout the production chain. The aim of the control of pathogens in feed is to ensure that they are under a critical threshold to minimize the risk to human and animal health [1]. In compound feed production, each unit process in production can contribute to feed quality and safety. Equipment such as conveyors, separators, extractors, cells and hoppers, mills, scales, conditioners, granulators, extruders, coolers, could be considered as critical points in the production process from the safety aspect [2,3].

The toxigenic fungi of field crops belong to the genera *Alternaria*, *Aspegillus*, *Cladosporium*, *Helminthosporium*, and *Fusarium* [4,5]. When cereal grains and forages are colonized by molds, there is a significant risk of contamination with their secondary metabolites [6] which may include contaminants such as mycotoxins, these fungal secondary metabolites present in contaminated grains due to favorable environmental conditions in the previous period or post-harvest [7].

The predominant cereal seeds in animal rations are represented by maize grains (*Zea mays L.*), due to their high concentration in energy, but they can present structural defects in the grains such as cracks, foreign particles and impurities, which expose them to fungal contamination , and consequently to the presence of mycotoxins [8,9,10]. In addition to compromising the nutritional value and processing (grinding, extrusion, granulation), the hygienic-sanitary quality of corn kernels can chemically modify the composition of the feed through the

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presence of substrates produced by microorganisms, such as mycotoxins [11].

A high incidence of fungi of the genus *Aspergillus* (aflatoxigenic) was identified in corn kernels stored in conditions of relative humidity between 13%-18% [5]. Zearalenone is the mycotoxin that in high concentrations can contaminate the carcasses of broiler chickens, which implies an anabolic effect in humans, and in production in the case of chickens there is a reduction in feed conversion, a decrease in leukocytes, and a decrease in the size of the crest [12, 13].

MATERIAL AND METHOD

Research has focused on identifying and monitoring possible sources of contamination located in the compound feed production process; these sources have been identified as prone to contamination through heterogeneous mixture formation, microbial contamination and cross-contamination.

The samples that were collected in sterile test tubes, taken from the raw material storage silos, were microbiologically analyzed to determine contamination with yeasts and molds.

The samples were taken from a feed mill in Romania during two years, and the sanitation tests were carried out in the factory laboratory of the unit.

Sampling for the determination of contamination with yeasts and molds was done from different points of the silos, for a higher accuracy of the results; samples were taken from the following points: wall-1 m from the door, floor-10 m from the door, wall-25 m from the door, floor-35 m from the door, sweeper auger, wall-35 m from the door, access cover and floor in the middle of the silo.

The microbiological analysis of the spaces in the silos was carried out in accordance with the standard 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. 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 feeding of animals that have a water activity less than or equal to 0,95 (dry fruits, cakes, jams, dried meat, salted fish, grains, cereals and cereal products, flours, nuts, spices and condiments, etc., by means of the colony count technique at 25 °C ± 1 °C [14].

RESULTS AND DISCUSSION

In order to determine the level of yeast and mold contamination of the surfaces in the studied unit's silos, sanitation tests were carried out during the two years.

In order to carry out the sanitation tests regarding the hygiene status of the feed mill silos, samples were collected from eight different places both in 2019 (n=9) and in 2020 (n=6). The results of the sanitation tests (Table 1) were interpreted in accordance with the provisions of Order no. 976/1998 [15] which provides for hygiene rules regarding the various operations specific to food production, through which a maximum limit of 300 cfu/m^2 was established for the content of yeasts and molds.

In 2019, the highest average value of yeast and mold content was recorded for the sampling site identified on the floor-35 m from the door (104.1 cfu/m^2) followed by the sampling site identified on the wall-1 m from at the door (76.4 cfu/m^2). As in 2019, in 2020 the highest average value was established for the sampling site identified on the floor-35 m from the door (161.5 cfu/m^2), followed by the sampling site identified on the floor-10 m from the door (140 cfu/m^2); for both years of the study, the identified values were below the limit established by the legislation. During 2019 and 2020, 72 samples respectively 48 samples were taken and analyzed to determine the contamination with yeasts

and molds. The results of microbiological analyzes performed in the feed mill studyed, showed that all 70 (97.2%) respectively 36 (100%) samples were positive.

According to some studies, in the compound feed industry there may be a high probability of microbiological contamination in the different production sectors, caused by the handling of raw materials and finished products [16]. Coradi et al. [17] carried out a study on microbiological contamination in different sectors of a compound feed factory, and for raw material storage silos the percentage of positive samples was 89.5% with an average of 2.9x104 cfu/m^2.

A study regarding the microbiological contamination of a processing environment of a feed mill in which the samples were taken from different points in the production flow, (mixers, granulators, sieves, mills, hoppers, coolers, as well as from the hoppers of the machines used to transporting feed) revealed that all the results were negative [18,19]. A study occurred during the years 2019 and 2020; in 2019, 191 samples of raw materials were analyzed and in 2020, 143 samples. Among the tested samples of raw materials, the mean values of the yeasts and molds for maize, wheat, soybean, and sunflower meal were 1.3 x 10^3, 9.5 x 10^2, 6.4 x 10^2, and 7.4 x 10^2 cfu/g in 2019 and 1.5 x 10^3, 1.0 x 10^3, 5.2 x 10^2, and 7.1 x 10^2 cfu/g in 2020 [20]. As a result, the contamination of raw material storage silos from a feed mill can have an impact on the subsequent contamination of raw materials.

Table 1 Results of sanitation tests (microbiological examination) from the feed mill silos to determine yeasts and molds (year 2019 and 2020)

Place of sampling	2019 Year						2020 Year					
	n	Positive (%)	x̄	s	Min. (cfu/m^2)	Max. (cfu/m^2)	n	Positive (%)	x̄	s	Min. (cfu/m^2)	Max. (cfu/m^2)
Wall-1 m from the door	9	100	76.4	61.1	16	200	6	100	82.8	67.9	4	160
Floor-10 m from the door	9	100	66.3	34.4	4	110	6	100	140	88.6	25	250
Wall-25 m from the door	9	100	55.3	24.4	13	80	6	100	104.5	67.5	1	200
Floor-35 m from the door	9	100	104.1	97.9	4	300	6	100	161.5	46.5	89	200
Sweep auger	9	100	59.3	42.1	6	150	6	100	127	61.2	25	200
Wall-35 m from the door	9	100	50.5	44.6	2	150	6	100	103.6	89.1	1	210
Access cover	9	88.8	58.6	47.3	4	140	6	100	65.5	64.3	1	180
Silo middle floor	9	88.8	73.2	72.0	1	200	6	100	76.1	50.4	5	150

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

Figure 1 presents an overview of the averages obtained in the two years of the study, in order to be able to more clearly observe the differences between the results; it can be seen that for all sampled silo surfaces, higher average values of yeast and

mold contamination were identified in 2020. The higher average values obtained in 2020 may be due to improper conditioning of raw materials stored in silos.

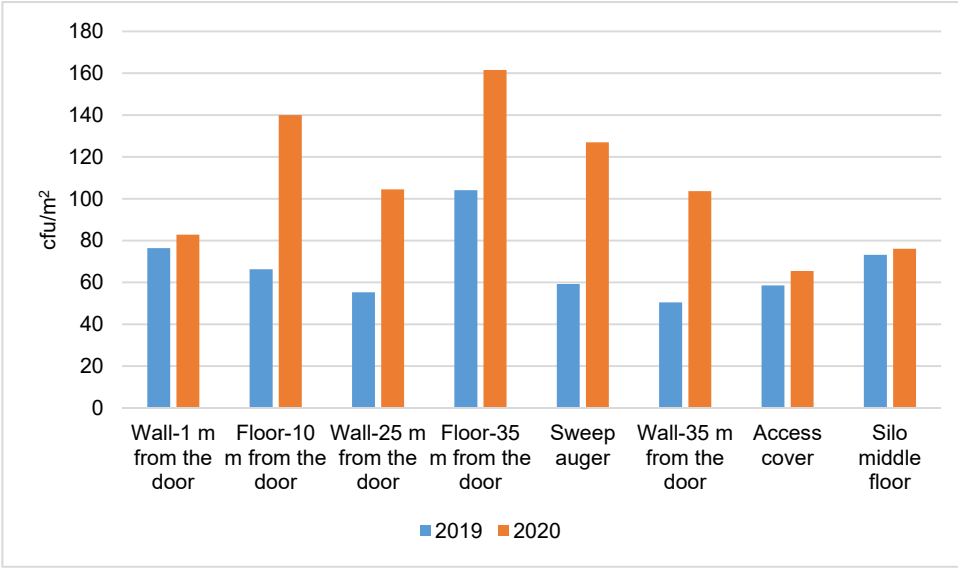

Fig. 1 Overview of the averages obtained in the two years of the study for yeast and molds silo contamination

CONCLUSIONS

The stages of production, transportation, and distribution of compound feed can be affected by a few deviations from their safety guidelines, leading to accidental or intentional contamination, which can have a negative impact on the health of animals and the security of food intended for human consumption.

Sampling to determine yeast and mold contamination were taken from different points of the silos, for a higher accuracy of the results, namely walls, floors, sweep auger, access cover. During 2019 and 2020, 72 samples respectively 48 samples were taken and analyzed to determine the contamination with yeasts and molds. The results of microbiological analyzes performed in the feed mill studyed, showed that all 70 (97.2 %) respectively 36 (100%) samples were positive.

As feed raw materials are potentially contaminated by several fungi at a time, and completed feed is made from various commodities, animals can be exposed, through its rich cereal diet, to high concentrations of mixtures of mycotoxins [21,22]. From this point of view, microbiological control of feed mill processing environment must be considered relevant and mandatory due to the demands of consumers for food safety all over the food chain.

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