

THE FREQUENCY OF PATHOGENIC FUNGI GENERA IN ANIMAL FEED

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Abstract

*Fungal invasion of feed commodities and subsequent formation of mycotoxins can lead to nutrient losses and detrimental effects on animal health and production. Infections by fungi and mycotoxin production can develop at various stages of crop production: in the field, during harvesting and transportation or storage. Toxigenic fungal spores are present everywhere in soils, air and water. When conditions are favourable, they can germinate, grow and produce the toxin. The aim of this study was to assess the mycoflora incidence in animal feed (maize, ground maize, sunflower and soybean meal, finished mixture etc.) by microbiological analysis during two year period. A total of 142 animal feed samples in 2007 and 155 samples in 2008 were examined for total fungi count and the frequency of pathogenic fungi genera. Total fungi count was $1-9 \times 10^4$ cfu g^{-1} in most of the investigated animal feed samples (66.90 and 74.19% in 2007 and 2008, respectively). In comparison of the average total fungi count in samples of animal feed from two investigation years no statistically significant differences were established. The most prevalent fungi genus was *Fusarium* (100 and 100%) followed by *Aspergillus* (73.94 and 87.10%), *Rhizopus* (64.09 and 83.87%), *Penicillium* (45.78 and 70.32%), *Mucor* (37.32 and 33.55%) while the least frequent species were from genus *Alternaria*, 4.93% and 16.13% in 2007 and 2008, respectively.*

Key words: animal feed, total fungi count, fungi genera

INTRODUCTION

Quality of animal feed is important prerequisite for achieving of optimal production results, as well as preservation of health condition of the animal, especially in intensive industrial production, therefore it is necessary to control raw materials and finished forage mixtures, constantly.

Presence of different microflora in animal feed is sign of increased activity of microorganisms and presence of different metabolism products, of which the most harmful are mycotoxins. Mycotoxins in animal feed causes disease (mycotoxicosis) in animals and humans [9]. Mycotoxicosis can be with fatal consequences in form of direct losses due to animal mortality. Indirect damage of mycotoxicosis is diminished production and reproduction abilities of domestic animals which realize unsatisfactory economical production results. Of numerous mycotoxins, most attention in the world is directed to aflatoxins, ochratoxins, zearalenones, toxins from the

group of trichothecenes and fumonisins. Aflatoxins are the most spread, most dangerous and most studied mycotoxins. Main producers of these mycotoxins are fungi of genus *Aspergillus*, although also some other species of fungi from genera *Penicillium* and *Rhizopus* produce them. Zearalenone is metabolite of some species of fungi from genus *Fusarium*. Triothecenes are produced by different fungi species belonging to different genera, such as: *Trichothecium*, *Stachybotrys*, *Myrothecium*, *Cephalosporium*, *Trichoderma*, *Penicillium*, *Fusarium*, etc. Among fungi which produce triothecenes, the most important are certain species of genus *Fusarium*. The fumonisins are a group of economically important mycotoxins and very common contaminants of maize-based foods and feeds throughout the world [6, 2].

Pathogenic micro organisms and their secondary metabolites (mycotoxins) in general chain of nutrition represent the most important potential risk to animal and human

health. Because of the harmful effect of fungi and their mycotoxins in chain of nutrition of humans and animals, in this paper, total fungi count and the frequency of pathogenic fungi genera in choosed feed samples during two year period was determined.

MATERIAL AND METHOD

In 2007 a total 142 samples and in 2008 a total 155 samples of animal feed (components and finished mixtures) were choosed and investigated for determination of total fungi count and the frequency of fungi genera. Average of moisture content in investigated animal feed samples was 11.42% and 11.06%, in 2007 and 2008, respectively. Samples were investigated in accredited microbiological laboratory of the Institute for Animal Husbandry, Belgrade-Zemun, and originate from the territory of the Republic of Serbia.

20 g of the animal feed samples was weighed into 180 ml sterile pshysiological solution (8.5 g NaCl in 1000 mL distilled water). Four fold dilutions of each of the samples were made and 1 mL of the diluents (10^{-3} and 10^{-4}) were pour plated in duplicate plates on Sabouraud dextrose agar for total fungi counts. All plates were incubated for 5-7 days at 26°C.

Based on macroscopic and microscopic characteristics of pure cultures of obtained isolates from analyzed animal feed samples fungi genera were identified according to

methods of Burgess et al. [1] and Watanabe [10].

All fungal counts were converted to \log_{10} cfu g^{-1} . Significance of differences in total fungi counts established in two investigation years was analyzed statistically using the t-test with level of significance of 0.05 (StatSoft 6).

RESULTS AND DISCUSSIONS

Total fungi count is one of the criteria in evaluation of hygienic quality and it is very important for orientation in lower or higher probability that the feed contains mycotoxins. According to conditions defined in Articles 8 and 9 of the Regulation on maximal quantities of harmful substances and components in livestock feed [8], mixtures and raw materials for animal feed are not in compliance with standards of the hygiene quality if they contain above 300.000 cfu g^{-1} of forage mixture for older animal categories or 50.000 cfu g^{-1} for younger animals.

By analysis of investigated animal feed samples from 1 to 8×10^5 cfu g^{-1} was established. In both years of investigation, 2007 and 2008 majority of samples analyzed contained $1-9 \times 10^4$ cfu g^{-1} (66.90% and 74.19%, respectively) (Table 1). In comparison of the average total fungi count in samples of animal feed from two investigation years no statistically significant differences were established (Table 2).

Table 1 Level of fungal contamination of investigated samples of animal feed in 2007 and 2008

Fungal counts		Number of samples		Frequency (%)	
cfu g^{-1} *	\log_{10} cfu g^{-1}	2007	2008	2007	2008
$1-8 \times 10^5$	5-5.90	-	155/1	-	0.65
$1-6 \times 10^5$	5-5.78	142/22	155/22	15.49	14.19
$1-9 \times 10^4$	4-4.95	142/95	155/115	66.90	74.19
$1-9 \times 10^3$	3-3.95	142/25	155/17	17.61	10.97

*Colony forming units per g of sample

Table 2 Average of total fungal counts (\log_{10} cfu g^{-1}) in samples of animal feed in investigated years

Year of investigation	Average of fungal counts
2007	4.45 ± 0.52
2008	4.49 ± 0.51
	$p = 0.45$

*Colony forming units per g of sample

significant - $p < 0.05$; not significant - $p > 0.05$

By applying defined criteria [8] on all investigated samples of animal feed collected in 2007 and 2008, it was established that 14.09% and 7.10% of feed samples for young category of animals wasn't microbiologically correct and 1.41% and 1.29% of feed samples for older category of animals, whereas 84.51% and 91.61%, in 2007 and

2008, respectively, of feed samples for both stated categories of animals did satisfy standards of microbiological adequacy. In 2008 considerably higher percentage (91.61%) of investigated feed samples was established which are in compliance with conditions determined in mentioned Regulation (Table 3).

Table 3. Number and percentage of investigated animal feed samples in 2007 and 2008 with total fungi count within limit values according to Regulation

Fungal counts		Number of samples		Frequency (%)	
cfu g ⁻¹ *	log ₁₀ cfu	2007	2008	2007	2008
> 50.000	> 4.7	142/20	155/11	14.09	7.10
> 300.000	> 5.5	142/2	155/2	1.41	1.29
Bellow limiting values		142/120	155/142	84.51	91.61

*Colony forming units per g of sample

By microbiological analysis of investigated animal feed samples, six fungi genera have been isolated and identified: *Alternaria*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus*. In 2007 and 2008, considering average values of their presence in percentage in all choosed animal feed samples beside the most present fungi genus *Fusarium* (100% and 100%) were also fungi species from genera *Aspergillus* (73.94%

and 87.10%), *Rhizopus* (64.09% and 83.87), *Penicillium* (45.78% and 70.32%), *Mucor* (37.32% and 33.55%) and *Alternaria* (4.93% and 16.13%). In 2008, the frequency of most identified fungi genera was considerably higher than the frequency of fungi genera in 2007, especially for genera *Alternaria*, *Aspergillus*, *Penicillium* and *Rhizopus* (Table 4). In most of the samples more than one fungi genus were identified.

Table 4. Frequency in percentage of fungi genera in investigation animal feed samples in 2007 and 2008

Fungal genera	Frequency (%)	
	2007	2008
<i>Alternaria</i>	4.93	16.13
<i>Aspergillus</i>	73.94	87.10
<i>Fusarium</i>	100	100
<i>Mucor</i>	37.32	33.55
<i>Penicillium</i>	45.78	70.32
<i>Rhizopus</i>	64.09	83.87

Results of the investigation of animal feed intended for nutrition of pigs and poultry obtained by Marković et al. [3] for the period of 1995-2004, show that mixtures for younger categories contained from 100 to 3.400.000 fungi g⁻¹, and even 35.71% of samples contained prohibited number of fungi. According to these authors, mixtures for grown animals contained from 800 to 8.000.000 fungi g⁻¹, and 7.54% of samples was microbiologically improper. Mrđen et al. [5] established that because of the presence of fungi (*Aspergillus*, *Penicillium*, *Fusarium*

etc.) 22.7% of samples of forage mixture were microbiologically improper, 20.8% of samples of components of plant origin (corn, barley meal), collected mainly in region of Vojvodina, and 1,8% of samples of components of animal origin, mainly from import. Due to the presence of species from genera *Aspergillus* (78.3%), *Penicillium* (58.7%) and *Fusarium* (43.5%), Mašić et al. [4] established that in year 2001 43.5-48.1% of mixtures for pigs and 14.0-17.2% of mixtures for initial and final fattening of pigs, pregnant and suckling sows, were improper.

Šefer et al. [7] established that of 160 analyzed samples of forage mixtures used in poultry nutrition only 57 (35.6%) were in accordance with provisions of the Regulation on quality of livestock feed, and in mixtures for grown animals maximum 3×10^6 cfu g⁻¹ was established. The most frequent isolated fungi species are of genus *Mucor* (76.5%), followed by species of genera *Aspergillus* (58.3%), *Penicillium* (29.6%) and *Fusarium* (15.6%) [7].

CONCLUSIONS

Feeds and feedstuffs are excellent media for the growth of fungi and so, very high standard of hygiene is necessary to avoid feed contamination. One of the best ways to control feed contamination and mycotoxin problem is to investigate frequency of fungi genera in feeds. Based on our mycological analyses of samples of animal feeds the following can be concluded:

By application of standard microbiological methods in analysis of investigated animal feed samples it was established that 1.41% and 1.29% of samples for older categories of animals and 14.09% and 7.10% of samples for young categories of animals, in 2007 and 2008, respectively, were not in accordance with regulated conditions relating to correctness of livestock feed.

In investigated samples of animal feed six fungi genera have been isolated and identified, *Alternaria*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus* of which genus *Fusarium* had the highest presence (100% and 100%), and genus *Alternaria* the lowest 4.93% and 16.13%, in 2007 and 2008, respectively.

Although in stated results relatively low percentage of samples with prohibited number of fungi was established, frequency of fungi genera which are significant producers of mycotoxins is not negligible/minor. Due to this fact, regular microbiological but also mycotoxicological analysis should be necessary methods for determination of quality and safety of animal feed.

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