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THE FREQUENCY OF PATHOGENIC FUNGI GENERA IN POULTRY FEED

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Feed contamination to fungi can lead to nutrient losses and detrimental effects on animal health and production. The aim of this study was to determine the mycoflora incidence in poultry feeds during a two year period. A total of 230 samples of poultry feeds in 2007 and 235 samples in 2008 were examined for total fungi count and the frequency of pathogenic fungi genera. Total fungi count was 1–9 × 10^4 CFU g^{-1} in the most of investigated poultry feed samples (38.26% and 45.53% in 2007 and 2008, respectively). In comparison of the average total fungi count in samples of poultry feed from two investigation years no statistically significant differences were established. The prevalent fungi genera were *Fusarium* (56.09 and 63.40%) and *Aspergillus* (54.35 and 73.62%), followed by *Rhizopus* (40 and 62.98%), *Penicillium* (30.87 and 48.09%), *Mucor* (30.04 and 31.49%) and the least frequent species were from the genus *Alternaria*, 3.48% and 17.02% in 2007 and 2008, respectively.

Key words: poultry feed; total fungi count; fungi genera

ЗАЧЕСТЕНОСТ НА ПАТОГЕНИТЕ ВИДОВИ ГАБИ ВО ХРАНАТА ЗА ЖИВИНА

Контаминацијата на храната за животни со габи доведува до нарушувања на хранливите вредности и предизвикува штетни ефекти врз здравјето на животните и производството. Целта на овој труд беше во текот на двегодишен период со микробиолошки анализи да се утврди микрофлора која е присутна во храната за живина. Вкупно се испитувани 230 примероци храна за живина во 2007 година и 235 примероци во 2008 година. Испитувани се вкупниот број габи и зачестеноста на патогени родови габи. Во поголем број проучувани примероци на храна за живина вкупниот број на габи изнесуваше 1–9 × 10^4 CFU g^{-1} (38,26% во 2007 и 45,53% во 2008 година). Со споредување на просечните вредности за вкупниот број габи во храната за живина од двете испитувани години не се утврдени статистички значајни разлики. Најзачестени се родовите *Fusarium* (56,09% и 63,40%) и *Aspergillus* (54,35 и 73,62%), потоа следат *Rhizopus* (40 и 62,98%), *Penicillium* (30,87% и 48,09%), *Mucor* (30,04% и 31,49%) и најмалку зачестени видови се од родот *Alternaria,* 3,48% во 2007 година и 17,02% во 2008 година.

Клучни зборови: храна за живина; вкупен број на габи; родови на габи

1. INTRODUCTION

The contamination of agricultural commodities with fungi able to produce toxic metabolites is often unavoidable and of worldwide concern. The relatively high intake of raw materials with the diet of livestock, such as poultry, can lead to nutrient losses and have adverse effects on animal health and on productivity when mycotoxin-contaminated feeds are consumed. Poultry feed is frequently contaminated by mycotoxins. Mycotoxin potential residues may greatly influence meat and eggs production. Presence of mycotoxins in animal feed may also be a threat for human health. (Oliviera et al., 2006).

The intake of very low levels of mycotoxins causes overt mycotoxicosis but also leads to the impairment of immune and acquired resistance to infections causing health problems which lead to economic losses in the form of decreased productivity (Dalcero et al., 1998). Mycotoxicosis can be

with fatal consequences in form of direct losses due to animal mortality. 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 the genus *Aspergillus*, although also some other species of fungi from the genera *Penicillium* and *Rhizopus* produce them. Zearalenone (ZEA) is metabolite of some species of fungi from the genus *Fusarium*. Trihothecenes are produced by different fungi species belonging to different genera, such as: *Trichothecium*, *Stachybotrys*, *Myrothecium*, *Cephalosporium*, *Trichoderma*, *Penicillium*, *Fusarium*, etc. Among fungi which produce trihothecenes, the most important are certain species of the genus *Fusarium*. The fumonisins are a group of economically important mycotoxins and very common contaminants of maize-based foods and feeds throughout the world. The major producers of fumonisins are *Fusarium verticillioides* and closely related species (Muntañola-Cvetković, 1990; Fandohan et al., 2003).

Pathogenic microorganisms and their secondary metabolites (mycotoxins) in the general chain of nutrition represent the most important potential risk to animal and human health. Because of the harmful effect of mycotoxins in the chain of nutrition of humans and animals, in this paper, total fungi count and the frequency of pathogenic fungi genera in samples of poultry feed during two year period were determined.

2. MATERIAL AND METHODS

In 2007 a total 230 samples and in 2008 a total 235 samples of feed intended for nutrition of poultry of all categories were investigated for determination of total fungi count and the frequency of fungi genera. The average of moisture content in the investigated poultry feed samples was 11% and 10.75% in 2007 and 2008, respectively. Samples were investigated in the accredited microbiological laboratory of the Institute for Animal Husbandry, Belgrade–Zemun, and originate from the territory of the Republic of Serbia. The investigation of total fungi count was done using standard microbiological methods according to valid regulations on the hygiene standard and quality of animal feed. Isolation of special fungi genera was done by planting of samples on selective media for fungi, Sabouraud dextrose agar.

Based on macroscopic and microscopic characteristics of pure cultures of obtained isolates from analyzed poultry feed samples fungi genera were identified according to methods of Mihajlović (1983), Nelson et al. (1983), Burgess et al. (1994) and Watanabe (1994).

Significance of differences in the total fungi counts established in two investigation years was analyzed statistically using the t-test with a level of significance of 0.05 (StatSoft 6).

3. RESULTS AND DISCUSSION

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 (Official Journal of SFRY, 1990), mixtures and raw materials for animal feed are not in compliance with the 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 poultry feed samples from 0 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} (38.26% and 45.53%, respectively). In 2007 relative high percent (18.70%) of investigated poultry feed samples were without contamination of fungi (Table 1). In comparison to the average total fungi count in samples of poultry feed from two investigation years no statistically significant differences were established (Table 2).

By applying defined criteria (Official Journal of SFRY, 1990) on all investigated samples of poultry feed collected in 2007 and 2008, it was established that 14.78% and 3.83% of feed samples for young category of poultry wasn't microbiologically correct and 2.61% and 0.81% of feed samples for older category of poultry, whereas 82.61% and 95.32%, in 2007 and 2008, respectively, of feed samples for both stated categories of poultry did satisfy standards of microbiological adequacy. In 2008 a considerably higher percentage (95.32%) of investigated feed samples was establishied which are in compliance with conditions determined in the mentioned Regulation (Table 3).

Table 1

Level of fungal contamination of investigated samples of poultry feed in 2007 and 2008

Fungal counts		Number of samples		Frequency (%)	
CFU g^{-1} *	log_{10}CFU g^{-1}	2007	2008	2007	2008
$1–8 \times 10^5$	5–5.9	230/0	235/1	0	0.43
$1–6 \times 10^5$	5–5.8	230/23	235/17	10	7.23
$1–9 \times 10^4$	4–4.9	230/88	235/107	38.26	45.53
$1–9 \times 10^3$	3–3.9	230/63	235/86	27.39	36.60
$< 1 \times 10^3$	< 3	230/13	235/3	5.65	1.28
0	0	230/43	235/21	18.70	8.94

*Colony forming units per g of sample

Table 2

Average of total fungal counts ($log_{10}CFU\ g^{-1}$) in samples of poultry feed in investigated years

Year of investigation	Average of fungal counts
2007	4.09 ± 0.74
2008	3.90 ± 0.70
	p = 0.11

*Colony forming units per g of sample significant – $p<0.05$; not significant – $p>0.05$

Table 3

Number and percentage of investigated poultry 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	230/34	235/9	14.78	3.83
> 300.000	> 5.5	230/6	235/2	2.61	0.85
Below limiting values		230/190	235/224	82.61	95.32

*Colony forming units per g of sample

By microbiological analysis of the investigated poultry 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 investigated poultry feed samples the most present were fungi species from the genera *Fusarium* (56.09% and 63.40%) and *Aspergillus* (54.35% and 73.62%), followed by *Rhizopus* (40% and 62.98), *Penicillium* (30.87% and 48.09%), *Mucor* (30.04% and 31.49%) and *Alternaria* (3.48% and 17.02%). In 2008, the frequency of all identified fungi genera was considerably higher than the frequency of fungi genera in 2007, especially for the genera *Alternaria, Aspergillus, Fusarium, 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 poultry feed samples in 2007 and 2008

Fungal genera	Frequency (%)	
	2007	2008
Alternaria	3.48	17.02
Aspergillus	54.35	73.62
Fusarium	56.09	63.40
Mucor	30.04	31.49
Penicillium	30.87	48.09
Rhizopus	40	62.98

Šefer et al. (1998) 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 the genus *Mucor* (76.5%), followed by species of genera *Aspergillus* (58.3%), *Penicillium* (29.6%) and *Fusarium* (15.6%) (Šefer et al., 1998).

In Argentina, the species belonging to *Aspergillus* (85%) and *Fusarium* (70%) genera were the most frequent in 130 investigated poultry feed samples and fungal counts ranged from 6.6×10^3 to 6.3 x 10^5 CFU g^{-1} (Dalcero et al., 1998). Results of Oliveira et al. (2006) showed that fungal counts in poultry feed samples were $2.18 – 3.27 \times 10^3$ CFU g^{-1}. *Penicillium* spp. (41.26%) and *Aspergillus* spp. (33.33%) had the highest isolation frequencies followed by *Fusarium* spp. (20.63%). In Nigeria, according to data Oscho et al. (2007) of the 50 investigated poultry feed samples no one was found completely free from fungi contaminant and the highest frequency of occurrence had *Rhizopus* spp. (44%), followed by *Fusarium* spp. (42%), *Aspergillus flavus* (40%) and *A. niger* (38%).

The mould spore plate count often has been used to estimate the potential risk that feed may pose to poultry health. This measurement, however, was found to be highly variable between batches of feed manufactured at different geo-

graphical locations. However, this test can be less expensive than analyses for mycotoxin contamination, while yielding a number corresponding to the number of viable mould spores per unit of feedstuff (Brothers and Wyatt, 2000). In general the fungal propagules are a helpful indicator to determine feeds'hygienic quality, these counts should not exceed the values of 1×10^5 CFU g^{-1} (Dalcero et al., 1998). Based on obtained results, the sole determination of total fungi count is insufficient to estimate the quality of poultry feed. Identification of fungi genera indicates potential presence of mycotoxins, therefore for complete analysis of poultry feed it is necessary to determine also the content of mycotoxins.

4. CONCLUSION

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

By application of standard microbiological methods in analysis of investigated poultry feed samples it was established that 2.61% and 0.81% of samples for older categories of poultry and 14.78% and 3.83% of samples for young categories of poultry, in 2007 and 2008, respectively, were not in accordance with regulated conditions relating to the correctness of livestock feed.

In investigated samples of poultry feed six fungi genera have been isolated and identified, *Alternaria, Aspergillus*, *Fusarium*, *Mucor, Penicillium* and *Rhizopus* of which genera *Fusarium* and *Aspergillus* had the highest presence (56.09% and 63.40%) and (54.35% and 73.62%), respectively, and the genus *Alternaria* the lowest 3.48% and 17.02%, in 2007 and 2008, respectively.

It must be pointed out that fungi species from genera *Fusarium* and *Aspergillus* are the most frequent producers of different and very dangerous mycotoxins (zearalenones, trihothecenes, fumonisins, aflatoxins, ochratoxins) in poultry feed.

Although in stated results relatively low percentage of samples with a 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 poultry feed.

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