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CONTAMINATION OF POULTRY FEED BY POTENTIALLY TOXIGENIC FUNGI

Vesna KRNJAJA, Aleksandar STANOJKOVIĆ, Zorica BIJELIĆ, Violeta MANDIĆ, Miloš LUKIĆ, Zdenka ŠKRBIĆ, Veselin PETRIČEVIĆ

Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia

Corresponding author: vesnakrnjaja.izs@gmail.com

Abstract

This study examines the mycological quality of poultry feed in 69 samples (45 samples of chicken feed and 24 samples of feed for layers) in 2014 and in 44 samples of poultry feed (34 samples of chicken feed and 10 samples of feed for layers) in 2015. The total fungal count was determined using a dilution method, and standard mycological methods were used to identify potential toxigenic fungi genera.

The chicken feed contained total fungal count above the allowed limit in 26.67% of samples in 2014, and in 14.71% of samples in 2015, while the total fungal count above the limit in the feed for layers was established only in year 2014, in 8.33% of the samples. Potentially toxigenic fungi species belonging to the genera Aspergillus, Fusarium and Penicillium were identified in both groups of studied poultry feed mixtures during both years. In chicken feed, the highest number of Fusarium positive samples in both years was recorded, 73.3% in 2014 and 64.71% of the samples in 2015. In the feed for layers, in the majority of samples (83.33%), Fusarium species were identified in 2014, while in year 2015, the species of the genera Aspergillus and Fusarium were identified in the majority of samples (90%).

Based on these results it can be concluded that the sanitary and hygiene conditions during the production of poultry feed must be determined by specific strategies for the reduction of the incidence of potentially toxigenic species of the genera Aspergillus, Fusarium and Penicillium. This strategy involves the use of complex and integrated measures to combat, especially during the growing of grain and other plants as the main raw materials, as well as during periods of storage and preservation of basic raw materials and finished mixtures used for poultry feeding.

Key words*: poultry feed, total fungal count, toxigenic fungi.*

INTRODUCTION

Contamination of poultry feed by fungi secondary metabolites – mycotoxins is a major problem in poultry production causing harmful effects on the performance and health, and through consumption of poultry meat also poses danger to human health. The mixtures used in poultry nutrition include mostly grain as a source of carbohydrates, and potentially toxigenic fungi are the main contaminants of grain (Pleadin et al., 2015). In maize, which is the main component of poultry feed (component share of 50-60%; Jokić et al., 2004) about 19 genera of fungi are identified, of which the most common are species of the genera *Aspergillus, Fusarium* and *Penicillium* (Sivakumar et al., 2014). Mycotoxin producing fungal species are widespread in warm and humid environment, their control and decontamination of their metabolites is an important part of the poultry nutrition strategy. Reducing the moisture content (below 14%) in the grain and feed mixtures is one of the basic preventive measures for the control of the fungi (moulds) growth in the process of production and storage of animal feed components (Osho et al., 2007). Considering the growing importance of food safety in the food chain, the adequate animal feed production management is essential because of the connection between feed contaminants levels, their presence in animal products (meat, eggs) and potential risk these contaminants pose to human health (Bhuyan et al., 2015).

Contamination of feed with mycotoxins is a major problem in the production of animal feed because it causes mycotoxicosis in animals. Aflatoxins are the most common mycotoxins in poultry production (Leggieri et al., 2015). Aflatoxin B_1 (AFB_1), produced by *Aspergillus flavus* and *A. parasiticus* (Pildain, 2008), is the most common mycotoxin in maize as a basic component of feed for poultry. The most common adverse effects of aflatoxin in the diet of chickens are reduced body weight and

increased liver and kidney weight in broilers (Zain, 2011). Another important mycotoxin in poultry nutrition is ochratoxin A (OTA) produced by the *Aspergillus* and *Penicillium* species. The prolonged feeding of poultry with feed contaminated with OTA causes reduced egg production, decrease in performance and body weight in poultry (Hassan et al., 2012). *Fusarium* mycotoxins, such as T-2 toxin and diacetoxyscirpenol (DAS) cause the weakening of the immune system and body resistance (Sokolović et al., 2008), necrosis and plaque formation (Zain, 2011). Since mycotoxigenic fungi are capable of producing more than one mycotoxin, and given that usually many different species of fungi develop in the feed at the same time, it is not uncommon that a wide spectrum of mycotoxins occur in feed, especially in feed prepared using multiple components (Streit et al., 2012).

The aim of this paper was to determine the total fungal count and to identify potentially toxigenic fungi in feed for poultry during the two-year period (2014-2015) and also to assess the potential danger of the presence of these contaminants in the food chain.

MATERIALS AND METHODS

The material for mycological analysis consisted of a total 69 samples of poultry feed mixtures in 2014 (45 samples of feed for chickens and 24 samples of feed for layers) and a total 44 samples in 2015 (34 samples of feed for chickens and 10 samples of feed for layers) originating from poultry farms in the vicinity of Belgrade in Serbia. Samples in the amount of 1 kg were collected successively (multiple times) in both study years. Mycological analysis was performed immediately after laboratory admission of the samples or the samples were stored for 2-3 days controlled temperature prior to the analysis. The moisture content of the tested samples was determined using a laboratory moisture meter (OHAUS MB35, USA), and mycological analysis was performed according to the method ISO 21527-2 (2008).

Based on morphological characteristics, macroscopic (appearance of colonies) and microscopic (appearance of spores), potentially toxigenic fungi genera were identified in conformity with taxonomic criteria for genera and species of fungi according to Watanabe (2002). The frequency of positive, i.e. samples contaminated by toxigenic fungi, was calculated according to the formula: Fr (%) = The number of samples were a fungal genus occurred/the total number of samples x 100.

Statistical analysis was performed with nonparametric test, using the SPSS software (IBM, Statistic 20). To determine the normality, the Shapiro-Wilk (SW) test was used, and to determine homogeneity of variance, the Levene's test. Because the Shapiro-Wilk test showed significant difference compared to the normal distribution, the significance of differences was tested using the Mann-Whitney U - test.

The correlation among individual values for moisture content, total fungal count and the frequency of fungal positive samples was determined using the Pearson correlation coefficient.

RESULTS AND DISCUSSIONS

The total fungal count and contamination of poultry feed samples with potentially toxigenic fungi of genera *Aspergillus, Fusarium* and *Penicillium* were examined in this paper.

Mycological analysis of samples of feed for chickens and layers established the total fungal count in the range from 1 x 10^1 to 2.41 x 10^5 cfu g^{-1}. In both study years, the highest number of samples of feed for layers was established with the total fungal count from 1-9 x 10^4 cfu g^{-1}, i.e. 58.30% of the samples in 2014, and 50% in 2015. Also, established is relatively high percentage of samples of feed for layers with the total fungal count of 1-2.41 x 10^5 cfu g^{-1}, i.e. 20% (2015) to 20.83% (2014) was established (Table 1).

The total fungal count is a parameter for hygiene and safety of animal feed, and, in the Republic of Serbia, according to the Regulation on the quality of animal feed (Službeni glasnik RS, 4/2010, 113/2012 and 27/2014), it should not exceed the value of 5 x 10^4 cfu g^{-1} for younger categories of farm animals and 2 x 10^5 cfu g^{-1} for adult animal categories. In the present study, in 26.67% of samples in 2014, and in 14.71% of samples in 2015 of chicken feed, and in 8.33% of samples of feed for layers in 2014 (Table 2), the total fungal counts above

the limit were established. Similar to these results, Dalcero et al. (1998) have found that the total fungal counts were above the permitted levels in samples of poultry feed in several months during the study period from May/1996 to May/1997 in Argentina. Likewise, in Iraq, Shareef (2010) has determined average total fungal count of 7 x 10^5 cfu g^{-1} in samples of poultry feed collected during a two-year period (2005-2007). In contrast, according to Oliveira et al. (2006), the total fungal count in the tested samples of poultry feed originating in Brazil did not exceed 1 x 10^4 cfu g^{-1}. Also, in Poland, the mean amount of moulds and yeasts in the analysed samples of feed for chickens was 7 x 10^2 cfu g^{-1} (Cegielska-Radziejwska et al., 2013).

In the analysed samples of chicken feed, an average total fungal count was statistically significantly higher ($P \leq 0.05$) in 2014 compared to 2015, while in case of the samples of feed for layers, there was no statistically significant difference in the total fungal count between the examined years (Table 3).

Table 1. Level of fungal contamination of investigated poultry feed samples in 2014 and 2015

Fungal counts		Frequency (%)			
		Year 2014		Year 2015	
cfu g^{-1} *	log_{10}cfu	1	2	1	2
1-2.41 x 10^5	5-5.38	13.33	20.83	11.76	20
1-9 x 10^4	4-4.95	31.11	58.30	14.71	50
1-9 x 10^3	3-3.95	20	4.17	2.94	0
< 1 x 10^3	< 3	35.56	16.70	70.59	30

*Colony forming units per g of sample; 1- samples of feed for chickens; 2- samples of feed for layers

Table 2. Frequency of investigated poultry feed samples in 2014 and 2015 with total fungal count within limit values according to Regulation of Republic of Serbia

Fungal counts		Frequency (%)			
		Year 2014		Year 2015	
cfu g^{-1} *	log_{10}cfu	1	2	1	2
> 50,000 (5 x10^4)	> 4.7	26.67	33.33	14.71	50
> 200,000 (2 x 10^5)	> 5.3	2.22	8.33	0	0

*Colony forming units per g of sample; 1- samples of feed for chickens; 2- samples of feed for layers

Table 3. Mean of total fungal counts (log_{10}cfu g^{-1}) in samples of feed for chickens and feed for layers in investigated years 2014 and 2015

Feed samples for chickens	cfu g^{-1} (log10) ± S.E.	Mediana
Year 2014	3.59 ± 0,18	3.48
Year 2015	2.99 ± 0,22	2.30
Level of significance	*	
Feed samples for layers	cfu g^{-1} (log10) ± S.E.	Mediana
Year 2014	4.23 ± 0,22	4.58
Year 2015	4.06 ± 0,38	4.69
Level of significance	ns	

cfu g^{-1} - colony forming units per g of sample; * - significant - $P<0.05$; ns - not significant - $P>0.05$

Table 4. Frequency of contaminated samples with potentially toxigenic fungi from *Aspergillus*, *Fusarium* and *Penicillium* genera

Fungal genus	Frequency of fungal contaminated samples (%)			
	Year 2014		Year 2015	
	1	2	1	2
Aspergillus	44.44	79.17	55.88	90
Fusarium	73.33	83.33	64.71	90
Penicillium	53.33	54.17	17.65	30

1- samples of feed for chickens; 2- samples of feed for layers

High fungi colony counts in 2014 year can be explained through the influence of suitable climate conditions which were optimal for the development of toxigenic mould during the growing and harvesting phase of maize. An important prerequisite for the development of toxigenic fungi in the grain before and after harvest are suitable conditions of temperature and humidity. According to data from Paterson and Lima (2011), mild temperatures and wet weather during the growth of maize favour the development of *Fusarium* species. Likewise, Asselt et al. (2012) have found that high rainfall and wind speed during the silking of maize contribute to intensive *Fusarium* infection of the grains. Furthermore, one of the most important preconditions for the infection of grain during storage is the moisture content of the grain. Maize moisture content of ≤15% is suitable for safe storage. According to Kana et al. (2013) moulds are capable to develop on dry surfaces and on feeds containg not more than 13% of moisture.

In both groups of investigated poultry feed samples, species of the genera *Aspergillus, Fusarium* and *Penicillium* were identified. In addition to these fungal genera, in a small number of samples genera *Mucor, Rhizopus* and *Alternaria* were identified. In both study years, in most samples (64.71-90%), *Fusarium* species were identified, and, in 2015, the same number (90%) of *Fusarium* and *Aspergillus* positive samples of feed for layers were identified (Table 4). Similarly, Dalcero et al. (1998) have found the highest incidence of the genera *Aspergillus* (85%) and *Fusarium* (70%) of the 130 samples of poultry feed, whereas according to Rosa et al. (2006), Shareef (2010) and Cegielska-Radziejwska et al. (2013), the most common in the investigated samples of poultry feed were identified species of the genus *Aspergillus*.

In samples of feed for chickens, the average moisture content was 11.57% in 2014 and 11.44% in 2015, while the average moisture content in samples of feed for layers was 10.96% in 2014 and 10.90% in 2015. Based on the data of the Pearson correlation analysis between the moisture content of the samples and the total fungal count, a negative correlation was established in the group of samples of feed for chickens in both study years, 2014 (r=-0.35) and 2015 (r=-0.18), while the correlation in the group of samples of feed for layers was positive, in both years 2014 (r=0.23) and 2015 (r=0.26). These data are similar to the data of Rosa et al. (2006).

Considering the correlation between the most common fungi in contaminated samples in 2014, a positive correlation was established between *Aspergillus* and *Fusarium* positive samples of feed for chickens (r=0.17) and layers (r=0.38), while in 2015, this correlation was positive in samples of feed for layers (r=0.06), but not in samples of chicken feed (r=-0.42) (data not presented). Similar to these results, *Scudamore et al. (1997)* have found that aflatoxins produced by *Aspergillus* species and fumonisins produced by *Fusarium* species appear jointly mostly in the maize samples. Also, *Oliveira et al. (2006)* have found a simultaneous occurrence of AFB_1 and *Fusarium* mycotoxins in poultry feed mixtures.

CONCLUSIONS

Based on the obtained results of the total fungal count and contamination of samples by potentially toxigenic fungi, it can be concluded that the mycological analysis are justified and necessary for the assessment of health and hygienic correctness of poultry feed.

In regard to the two groups of samples of investigated poultry feed, 26.67% of samples of feed for chickens had total fungal count above allowed limit, in 2014, and 14.71% of the samples in 2015, while much smaller number of samples (8.33%) of the feed for layers had total fungal count above allowed limit, and only in 2014.

In both studied groups of poultry feed, potentially toxigenic fungi of the genera *Aspergillus, Fusarium* and *Penicillium* were identified, with the majority of the samples contaminated with *Fusarium* species, followed by species of the genus *Aspergillus*. In most of the studied poultry feed mixtures, a positive correlation between *Aspergillus* and *Fusarium* positive samples was established.

These results suggest the need for continuous, primarily, mycological but also mycotoxicological analysis of the quality of poultry feed, in order to provide health stability of poultry products for human consumption.

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