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FUNGAL CONTAMINATION AND NATURAL OCCURRENCE OF T-2 TOXIN IN POULTRY FEED

V. Krnjaja, Z. Pavlovski, M. Lukić, Z. Škrbić, Lj. Stojanović, Z. Bijelić, V. Mandić

Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia
Corresponding author: vesnakrnjaja.izs@gmail.com
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Abstract: In this study, a total of 41 poultry (chicken and laying hens) feed samples collected from different farms in Serbia in the beginning of 2014 were investigated for total fungal count, presence of potential toxigenic fungi and natural occurrence of T-2 toxin. The number of total fungi was determined using the plate count method whereas T-2 toxin was detected by enzyme-linked immune sorbent assay (ELISA) method.

Relative high percent of investigated poultry feed samples (43.90%) had the total fungal count 1 - 7 x 10^2 CFU g^{-1}, while in 29.27% of the samples that number was 1.4 - 14 x 10^4 CFU g^{-1}. In regard to potentially toxigenic fungi, species of *Fusarium* genus were isolated in most of poultry feed samples (58.54%), while species from genus *Alternaria* were isolated in least of samples (9.76%). The presence of T-2 toxin was detected in 75.61% of the samples, with concentration of 25.07 - 426.08 μg kg^{-1} (in average, 55.34 μg kg^{-1}). The statistical insignificant negative correlation (r = – 0.05) was obtained between total fungal count and concentrations of T-2 toxin.

In addition, a total fungal count and content of T-2 toxin in the samples were not above the maximum allowed levels, although the presence of species from genus *Fusarium* was found in 58.54% samples. These results indicated that the sanitary and hygienic conditions during the production of poultry feed in Serbia have been at satisfactory level.

Key words: poultry feed, total fungal count, T-2 toxin

Introduction

Trichothecenes are a large group of mycotoxins usually detectable in the various types of cereals, which are the main components of feed for poultry *(Riazipour et al., 2009)*. T-2 toxin belongs to the trichothecene mycotoxins produced by fungi of *Fusarium* genus, especially *F. sporotrichioides* and *F. poae*

(Maragos, 2006). Contamination of agricultural products with potential toxigenic fungi can cause the production of mycotoxins in undesirable concentrations *(Saleemi et al., 2010)*. It is always the risk to use contaminated food with mycotoxins or mouldy food. Even if the microbiological examination does not establish a high incidence of toxigenic moulds, food can be contaminated with mycotoxins, since the presence of the fungi is not always an indication of the presence of mycotoxins *(Krnjaja et al., 2007)*.

Intake of very low concentrations of mycotoxins cause mycotoxicosis that lead to the weakening of the immune system and deteriorating health of animals causing economic losses in the form of reduced production. Mycotoxin residues have a major impact on the production of meat and eggs. Therefore, their presence in animal feed may be a risk for human health. Poultry feed is often contaminated with mycotoxins, and this is the reason of frequent mycotoxicosis *(Oliveira et al., 2007)*. Mouth lesions and associated losses in productivity caused by T-2 toxin are a major concern to the poultry industry. T-2 toxin at concentrations as low as 400 μg kg^{-1} causes oral lesions by affecting the epithelial cells of oral mucous membranes *(Devegowda and Murthy, 2005)*.

The development of the toxigenic fungi causing contamination of animal feed with the mycotoxins is a serious problem that reduces the feed quality. The most important toxigenic fungal genera are *Aspergillus, Fusarium* and *Penicillium*. Almost all *Fusarium* species are capable of producing mycotoxins and differ from the genera *Aspergillus* and *Penicillium* in which only a few species are toxicogenics *(Nijs et al., 1996; Magnoli et al., 1998)*.

Moisture and temperature are two main factors for the development of fungal species and the production of mycotoxins in feed components. The most of toxigenic fungal genera have been isolated from maize grain as the main component of animal feed at 8, 12, 16 and 20% moisture levels stored at 25 and 35ºC *(Niaz et al., 2011)*.

In Serbia there are not sufficient data on the total fungal count, the presence of potentially toxigenic genera of fungi and their connection to the natural occurrence of T-2 toxin in poultry feed, especially when it comes to ready-made mixtures and not individual components. For this reason, the objective of this study was to determine the total fungal count and the natural occurrence of T-2 toxin, as well as their interdependence, and to determine the percentage of potentially toxigenic genera of fungi in poultry feed originating from Serbia.

Materials and Methods

During the first quarter of 2014, total of 41 samples of poultry (chicken and laying hens) feed were collected from different poultry farms in Serbia. Samples were taken from the production line using standard methods *(European*

Commission, 2006) and were kept in plastic bags and stored at 4ºC until analysis. The moisture content of poultry feed samples was determined using a moisture analyzer (Ohaus MB35, USA).

Quantitative determination of fungal colonies were done on solid medium (Sabouraud maltose agar) using the pour-plate method. First, 20 g of the sample was homogenized with 180 ml of normal saline (NaCl, 8.5 g/l) in the course of a few minutes on the orbital shaker (GFL 3015, Germany). Serial dilutions to 10^{-4} concentration were made and 1 ml of dilutions to 10^{-2}, 10^{-3} and 10^{-4} each, and applied on Sabouraud maltose agar in Petri plates (9 cm in diameter). The Petri plates kept in incubator (Memmert, Germany) at 25ºC for 5-7 days. Total fungal count was presented as colony-forming units (CFU) per gram of sample. Identification of fungal genera was done based on morphological characteristics according to fungal key of *Watanabe (1994)*.

The presence of T-2 was detected by ELISA assay according to the instructions Celery Techna ® ELISA kits on an ELISA reader (Biotek EL x 800TM, USA). The limit of detection was 25 μg kg^{-1} for T-2 toxin.

Correlation between individual values obtained for grain moisture content, total fungal count and concentration of T-2 toxin was determined using Pearson's correlation coefficient (Microsoft Office Excel 2007).

Results

By analyzing samples of poultry feed it was established that the number of fungi ranged from 0 to 14 x 10^4 CFU g^{-1}. Most of samples (43.90%) had a total fungal count from 1 to 7 x 10^2 CFU g^{-1}, whereas 29.27% of samples contained from 1.4 to 14 x 10^4 CFU g^{-1}. No fungi were detected in 7.32% of the samples (Table 1). Statistically insignificant positive correlation (r = 0.02) was determined between the moisture content and the total number of fungi, showing that with the increase of moisutre content also the total fungal count slightly increased. The moisture content of the poultry feed samples ranged from 8.04 to 12.67% with an average of 10.92%.

Table 1. Level of fungal contamination of investigated poultry feed samples

Fungal counts (CFU g^{-1*})	Number of samples	Frequency (%)
1.4-14 x 10^4	12/41	29.27
1.1-9 x 10^3	8/41	19.51
1-7 x 10^2	18/41	43.90
0	3/41	7.32

*Colony forming units per g of sample

Mycological survey of investigated poultry feed samples using Sabouraud maltose agar medium showed the presence of six fungal genera, *Alternaria*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus*. In most samples the species from genus *Fusarium* (58.54% positive samples) were isolated, followed by species from genera *Penicillium* (51.22% positive samples) and *Aspergillus* (46.34% positive samples), whereas species from genera *Alternaria*, *Mucor* and *Rhizopus* were present in 9.76, 10.07 and 14.63% of samples, respectively (Table 2).

Table 2. Fungal genera in investigated poultry feed samples

Fungal genera	Number of samples	Frequency (%)
Alternaria	4/41	9.76
Aspergillus	19/41	46.34
Fusarium	24/41	58.54
Mucor	7/41	10.07
Penicillium	21/41	51.22
Rhizopus	6/41	14.63

Mycotoxicological analysis showed the presence of 75.61% T-2 positive samples, and T-2 toxin was detected in 31 of the analysed samples of poultry feed. Concentrations of T-2 toxin were from 25.07 to 426.08 μg kg^{-1} with an average concentration for all investigated samples of 55.34 μg kg^{-1} (Table 3). Between the concentrations of T-2 toxin, and the moisture content and the concentration of T-2 toxin, and the total number of fungi, statistically insignificant negative correlations r = – 0.07 and r = – 0.05, respectively, were established.

Table 3. Concentration T-2 toxin in investigated poultry feed samples

Item	T-2 toxin
Sample size[a]	31/41
Incidence %	75.61
Range (μg kg^{-1})	25.07 - 426.08
Mean[b] (μg kg^{-1})	55.34

[a] Number of positive samples/Number of total samples
[b] Mean concentration in positive samples

Discussion

The assesment of total fungal count in animal feed is important criteria in the determination of hygienic quality and a necessary tool for assessing the potential risks and dangers of the increased presence of mycotoxins. According to the Regulation on quality of animal feed *(Official Gazette of the Republic of Serbia, 4/2010)*, mixtures and forage raw materials do not correspond to the

hygienic quality if they contain more than 200,000 spores in 1 g of mixture for older animals or 50,000 spores in feed for young animals. In Serbia the maximum allowed level of T-2 toxin is 500 μg kg^{-1} *(Official Gazette of the Republic of Serbia, 4/2010)*.

Identification of potentially toxigenic fungi in our research in most of the samples showed species from genera *Fusarium, Penicillium* and *Aspergillus*. The values for total fungal count and content of T-2 toxin in the investigated poultry feed samples have not exceeded maximum allowed limit confirmed by the Regulation (data not presented). In Serbia, similar results were reported by *Živković et al. (2005)*, *Bočarov-Stančić et al. (2011)*, *Janić-Hajnal et al. (2013)* and *Kapetanov et al. (2013)*. According to *Kapetanov et al. (2013),* the total number of *Fusarium* colonies was 15 x 10^4 and the concentrations of T-2 toxin was 480 μg kg^{-1} in investigated chicken feed. In the analysis of mycotoxins in samples of 40 different protein feed performed by *Bočarov-Stančić et al. (2011)*, in three samples of soybean and its processed products (meal and cake), only T-2 toxin was detected, and microbiological analysis identified the fungi of the genera *Aspergillus*, *Fusarium* and *Mucor*, of which species *F. solani* was the producer of T-2 toxin. The concentration of T-2 toxin did not exceed 375 μg kg^{-1}. According to the data presented by *Janić-Hajnal et al. (2013),* in 52% of samples of maize as the most important component of poultry feed, the sum of T-2 and HT-2 toxins has been detected. In a positive samples the concentration of T-2 toxin was from 25 to 200 μg kg^{-1}. Mycotoxicological analysis of 57 samples of poultry feed performed by *Živković et al. (2005)* detected T-2 toxin in 19 samples at a concentration of < 300 μg kg^{-1}, in 18 samples at a concentration of 500 μg kg^{-1} and in three samples at a concentration of 1000 μg kg^{-1}.

According to data from other countries with similar geographical and climatic conditions, in the 45 examined poultry feed mixtures from western Poland in 2010, *Cegielska-Radziejewska (2013)* have isolated as most common fungal species of genera *Aspergillus* and *Rhizopus* and total fungal count was from 5.5 x 10^1 to 7.0 x 10^3 CFU g^{-1} (average 7.0 x 10^2 CFU g^{-1}) and T-2 toxin was not detected. Mycotoxicological examination of 50 samples of poultry feed mixtures in Slovakia T-2 toxin was detected in 90% samples with an average concentration of 13 μg kg^{-1} (range 1-130 μg kg^{-1}) *(Labuda et al., 2005)*. Similarly, in Croatia, *Pleadin et al. (2012)* have not detected high concentrations of T-2 toxin (in average 18.2 μg kg^{-1}) in the investigated poultry feed.

Conclusion

For successful poultry production, it is necessary to ensure both healthy and high-quality fresh components that are included in the feed mixtures, and ready-made mixtures without contaminants that may cause adverse effects in the

production chain. The obtained results revealed the presence of contaminants such as potentially toxigenic fungi and the T-2 toxin, but the levels of these contaminants did not exceed allowed limits. Since the *Fusarium* species were isolated in most samples (58.54% positive samples) and the T-2 toxin was present in 75.61% of investigated samples, it is necessary to emphasize the need for continuous monitoring of the quality of animal feed as an important preventive measure to prevent conditions for increased production of mycotoxins.

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Kontaminacija gljivama i prirodna pojava T-2 toksina u hrani za živinu

V. Krnjaja, Z. Pavlovski, M. Lukić, Z. Škrbić, Lj. Stojanović, Z. Bijelić, V. Mandić

Rezime

U radu su proučavani ukupan broj gljiva, prisustvo potencijalno toksigenih rodova gljiva i prirodna pojava T-2 toksina u 41 uzoraka hrane za živinu (piliće i nosilje) koji su sakupljeni iz različitih farmi u Srbiji početkom 2014. godine. Ukupan broj gljiva određen je primenom metode razređenja a T-2 toksin je detektovan primenom imunoadsorpcione enzimske metode (ELISA).

Relativno visok procenat proučavanih uzoraka hrane za živinu (43,90%) imao je ukupan broj gljiva 1 - 7 x 10^2 CFU g^{-1}, dok je u 29,27% uzoraka ukupan broj bio 1,4 - 14 x 10^4 CFU g^{-1}. Od potencijalno toksigenih gljiva u ispitivanim uzorcima hrane za živinu u najvećem broju uzoraka (58.54%) izolovane su vrste iz roda *Fusarium*, dok su vrste iz roda *Alternaria* bile izolovane u najmanjem broju uzoraka (9.76%). Prisustvo T-2 toksina detektovano je u 75,61% ispitivanih uzoraka sa koncentracijom od 25,07 - 426,08 μg kg^{-1} (prosek 55.34 μg kg^{-1}). Statistički neznačajna korelacija (r = – 0.05) utvrđena je između ukupnog broja gljiva i koncentracija T-2 toksina.

Ukupan broj gljiva i sadržaj T-2 toksina u ispitivanim uzorcima nisu bili iznad maksimalno dozvoljenih količina iako je ustanovljeno prisustvo vrsta iz roda *Fusarium* u 58.54% uzoraka. Ovi rezultati ukazuju da su u Srbiji sanitarno-higijenski uslovi za proizvodnju hrane za živinu na zadovoljavajućem nivou.

References

BOČAROV-STANČIĆ A.S., SALMA N.M., PANTIĆ V.R., ADAMOVIĆ M.J., MILJKOVIĆ A.D., SUZIĆ S.V. (2011): Microbiological and mycotoxicological correctness of protein feed ingredients in Vojvodina. Jour. Nat. Sci., Matica srpska Novi Sad, 120, 211-218.

CEGIELSKA-RADZIEJEWSKA R., STUPER K., SZABLEWSKI T. (2013): Microflora and mycotoxin contamination in poultry feed mixtures from western Poland. Annals of Agricultural and Environmental Medicine, 20, 1, 30-35.

DEVEGOWDA G., MURTHY T.N.K. (2005): Mycotoxins: effects on poultry performance and health. Pp. 25-56. *In*: Diaz D.E. (eds), The mycotoxin blue book. Nottingham University Press, Nottingham, United Kingdom.

EUROPEAN COMMISSION (2006): Commision regulation (EC) No. 401/2006. Official Journal of the European Union, L70, 12.

JANIĆ-HAJNAL E.P., KOS J.J., MASTILOVIĆ J.S. (2013): Presence of T-2 and HT-2 toxins in maize. Jour. Nat. Sci., Matica srpska Novi Sad, 124, 131-136.

KAPETANOV M.C., POTKONJAK D.V., STOJANOV I.M., ŽIVKOV-BALOŠ M.M., JAKŠIĆ S. (2013): Clinical and pathomorphological diagnostics of mycotoxicosis in parent poultry flock caused by T-2 trichothecene. Jour. Nat. Sci., Matica srpska Novi Sad, 124, 137-143.

KRNJAJA V., LEVIĆ J., TOMIĆ Z., STOJANOVIĆ LJ., TRENKOVSKI S., NEŠIĆ Z., MARINKOV G. (2007): The presence of potential toxigenic fungi in animal feed with particular rewiev on species of genera *Aspergillus* and *Fusarium*. Biotehnology in Animal Husbandry, 23, 102, 95-103.

LABUDA R., PARICH A., BERTHILLER F., TANČINOVÁ D. (2005): Incidence of trichothecenes and zearalenone in poultry feed mixtures from Slovakia. International of Food Microbiology, 105, 19-25.

MAGNOLI C., DALCERO A.M., CHIACCHIERA S.M., MIAZZO R., SAENZ M.A. (1998): Enumeration and identification of *Aspergillus* group and *Penicillium* species in poultry feeds from Argentina. Mycopathologia, 142, 27-32.

MARAGOS C.M. (2006): Measurment of T-2 and HT-2 toxins in eggs by high-performance liquid chromatography with fluorescence detection. Journal of Food Protection, 69, 11, 2773-2776.

NIAZ I., DAWAR S., SITARA U. (2011): Effect of different moisture and storage temperature on seed borne mycoflora of maize. Pak. J. Bot., 43, 5, 2639-2643.

NIJS M., ROMBOUTS F., NOTERMANS S. (1996): *Fusarium* moulds and their mycotoxins. J. Food Safety, 16, 15-58.

OFFICIAL GAZETTE OF THE REPUBLIC OF SERBIA (2010): Regulation on the quality of animal feed, No. 4.

OLIVEIRA G.R., RIBEIRO J.M., FRAGA M.E., CAVAGLIERI L.R., DIREITO G.M., KELLER K.M., DALCERO A.M., ROSA C.A. (2007): Mycobiota in

poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. Mycopathologia, 162, 355-362.
PLEADIN J., PERŠI N., VULIĆ A., ZADRAVEC M. (2012): Survey of mycotoxin feed contamination in Croatia. Biotechnology in Animal Husbandry, 28, 2, 167-177.
RIAZIPOUR M., IMANI FOOLADI A.A., RAZZAGHI-ABYANEH M. (2009): Natural occurence of T-2 toxin in domestic and imported rice. Iranian J. Publ. Health, 38, 4, 111-116.
SALEEMI M.K., KHAN M.Z., KHAN A., JAVED I. (2010): Mycoflora of poultry feeds and mycotoxins producing potential of *Aspergillus* species. Pak. J. Bot., 42, 1, 427-434.
ŽIVKOVIĆ G.R., ŽIVANOV N.M., ŽIVKOVIĆ J.Z., MILOJEVIĆ M.J., TEODOSIN J.M., PEĆANAC S.L., MILIĆ D.V., BOČAROV-STANČIĆ A.S., ĐEKIĆ J.P. (2005): Mycotoxins and their impact on poultry production. Jour. Nat. Sci., Matica srpska Novi Sad, 108, 197-205.
WATANABE T. (1994): Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and key to species. Lewis Publishers, Boca Raton, Boston, London, Washington D.C. pp. 410.

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