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FUNGAL CONTAMINATION OF PIG FARM FEEDS

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Abstract: The aim of this study was to establish the total fungal (mould) count in 79 commercial pig farm feed samples (42 samples for piglets, 29 samples for fattening pigs and 8 samples for sows) collected from the Serbian feed producers during the three-year period (2017–2019), as well as to evaluate the percentage of contamination samples by fungi, especially species from *Aspergillus*, *Fusarium* and *Penicillium* genera. Using standard mycological methods, the total count and identification of fungi were determined. Total fungal count was ranging from 1×10^2 to 1.41×10^5 cfu g^{-1} in the feed samples for piglets. Furthermore, in the feed samples for fattening pigs it ranged from 1×10^2 to 2.54×10^5 cfu g^{-1}, and from 1×10^2 to 1.93×10^5 cfu g^{-1} in the feed samples for sows. It has established the impermissible limit of total fungal count in 3.45% and 4.76% of a feed for fattening pigs and feed for piglets, respectively. Statistical analysis of the total number of fungi did not establish significant differences between the examined feed groups. *Fusarium* species were present in the most feed samples for fattening pigs (65.52%), followed by the feed for sows (62.50%) and piglets (47.62%). *Aspergillus* species were determined in 59.52, 58.62 and 37.50% feed samples for piglets, fattening pigs and sows, respectively. *Penicillium* species contaminated the lowest percentage of feed samples for fattening pigs (27.59%) and the highest percentage of feed samples for sows (37.50%). In a small number of samples *Alternaria*, *Mucor,* and *Rhizopus* species were identified. Based on these results, it can be concluded that the investigation of fungal contamination is an important indicator of a hygienic condition of feed intended for the nutrition of farm pigs. In addition, the percentage of fungal contamination of the examined samples indicates a potentially high risk to animal health. Due to that, a regular mycological evaluation is necessary to assess nutritional quality as one of the fundamental criteria for feed safety.

Key words: pig farm feed, total fungal count, fungal species

Introduction

Cereal grains, as the main component of the feed mixture, are an excellent substrate for development of fungi (moulds). Fungal species from genera *Aspergillus*, *Fusarium* and *Penicillium* and their secondary metabolites (mycotoxins) are inevitable contaminants in cereals and feeds. These fungi produce mycotoxins causing adverse effects in animal health and production (*Harčárová et al., 2018; Chiotta et al., 2020*).

High moisture content and water activity influence negatively on microbial stability of grains and in association with high temperature contribute favorably to the fungal growth and mycotoxin synthesis (*Bakutis et al., 2006; Giorni et al., 2012*). It was reported that temperatures ranging from 25 to 35°C were optimal for *Aspergillus* species growth and toxin production. Unlike *Aspergillus* species, *Fusarium* species have ability to grow at wide temperature values, ranging from 4–30°C, but production of toxins does not occur until temperature reaches bellow 15°C (*Manstretta and Rossi, 2016; Mannaa and Kim, 2017*). *Fusarium* species growth was also influenced by moisture content which must be in the ranges of 5–25%, while water activity must be from 0.87–0.99 (*Gebremeskel et al., 2019; Camardo Leggieri, 2017*).

Striving to provide human and animal healthy diet, the implementation of feed safety strategy is a priority. Intensive livestock production has high demands for animal feed hygiene. Total fungal count in animal feed is one of the fundamental parameters of feed quality. The quality of feed and raw cereal grains has an impact on animal health and growth (*Biagi, 2009; Dänicke et al., 2007*). According to the Serbian *Regulation on the quality of animal feed*, the limit values for total fungal count in 1g of feed mixtures was determined and it ranges from 50 000 and 200 000 in 1g of the feed mixture for young animals and adults, respectively.

Since there is not much data in the literature on fungal contamination of pig feed, the main aim of this research was to evaluate the total fungal count and the percentage contamination of pig feed samples by fungi, especially with potentially toxigenic fungi from *Aspergillus*, *Fusarium* and *Penicillium* genera. Given that fungal contamination is common in animal feed, these investigations should provide valuable information of the risk assessment for these contaminants in feed produced for farm pigs.

Materials and Methods

A total of 79 commercial pig feed samples (42 samples for piglets, 29 samples for fattening pigs and 8 samples for sows) collected from different Serbian feed producers during three years (2017–2019) were examined by mycological

tests. The sample size was approximately 1 kg. The moisture content of examined samples was determined using a moisture analyzer (OHAUS MB35, Parsippany, NJ, USA).

Total fungal count was determined using the dilution method according to ISO 21527-2 (2008). To prepare an initial suspension (primary dilution), 20 g of sample was added to 180 mL 0.1% of peptone water broth (PWB) and homogenized for 10 minutes on an orbital shaker (GFL 3015, Germany). A series of dilutions (10^{-2}, 10^{-3} and 10^{-4}) was prepared by transffering 1 mL of primary dilution (10^{-1}) into 9 mL of PWB. From prepared dilutions, 1 mL of suspension was transferred by sterile pipette and spread over the surface of Dichloran Glycerol agar (DG18) in Petri plates (Ø90) which were then kept in an incubator (Memmert, Germany) at 25ºC for 5 days. Total fungal count was expressed as colony-forming units per gram of sample (cfu g^{-1}).

Based on macroscopic and microscopic observations, the determination of fungal genera was done according to the descriptions of *Watanabe (2002*). The results were shown as the percentage of samples contaminated with fungal species per examined feed group.

Statistical data were analysed with the non-parametric test, independent-samples Kruskal-Wallis Test, using the SPSS software (IBM, Statistic 20). The correlation among individual values for total fungal count and the moisture content was determined using the Pearson correlation coefficient.

Results and Discussions

The total fungal count and fungal contamination of pig farm samples were studied. These parameters are good indicators of feed quality and essential for feed safety management. Constant evaluations of hygienic feed quality can reduce detrimental health effects in animals.

According to Serbian *Regulation on the quality of animal feed*, the total fungal count that has been recorded were above the permissible limit for older (2×10^5 cfu g^{-1}; 5.3 log10cfu g^{-1}) and younger categories of pigs (5×10^4 cfu g^{-1}; 4.7 log10cfu g^{-1}) in 3.45% and none of the feed samples for fattening pigs and sows, respectively and in 4.76% of the feed samples for piglets (Table 1).

Total fungal count was ranging from 1×10^2 (2 log10cfu g^{-1}) to 1.41×10^5 cfu g^{-1} (5.15 log10cfu g^{-1}) in the feed samples for piglets, from 1×10^2 (2 log10cfu g^{-1}) to 2.54×10^5 cfu g^{-1} (5.40 log10cfu g^{-1}) in the feed samples for fattening pigs and from 1×10^2 (2 log10cfu g^{-1}) to 1.93×10^5 cfu g^{-1} (5.29 log10cfu g^{-1}) in the feed samples for sows. The mean moisture content was 10.60, 13.38 and 9.79% in feed samples for piglets, fattening pigs and sows, respectively. Regarding total fungal count, there was no statistically significant difference between examined groups of pig farm feed samples (Table 2).

Table 1. Percentage of contaminated pig feed samples with total fungal count above the regulation limits

Total fungal count		Percentage (%) / Number of samples		
cfu g^{-1}	log_{10}cfu g^{-1}	Feed for piglets	Feed for fattening pigs	Feed for sows
$> 2 \times 10^5$	> 5.3	0/42	3.45/29	0/8
$> 5 \times 10^4$	> 4.7	4.76/42	24.14/29	25/8

*Colony forming units per g of sample

Table 2. Median, minimum and maximum total fungal count and mean moisture content in examined pig feed samples

Item	Total fungal count (cfu g^{-1} / log_{10}cfu g^{-1})			Mean moisture content (%)
	Median	Minimum	Maximum	
Feed for piglets [a]	6×10^2/ 2.78	1×10^2 / 2	1.41×10^5 / 5.15	10.60
Feed for fattening pigs [b]	2.10×10^2 / 3.32	1×10^2 / 2	2.54×10^5 / 5.40	13.38
Feed for sows	2.80×10^2/ 3.45	1×10^2 / 2	1.93×10^5 / 5.29	9.79
Level of significance	ns	-	-	-

[a] Animals from 15 to 25 kg; [b] Animals from 30 to 90 kg; ns - not significant at $P \geq 0.05$

In this study, considering mean moisture contents, the highest moisture content was in the samples of feed for fattening pigs (13.38%), followed by feed for piglets (10.60%) and sows (9.79%). Likewise, the maximum value of total fungal count (2.54×10^5 cfu g^{-1}; 5.40 log_{10}cfu g^{-1}) was in samples of feed for fattening pigs, feed group with the highest mean moisture content (Table 2).

The recommendation of maximum limited moisture content in cereal grains (a main component of feed) as good storage practice is 14.5% (*Fleurat-Lessard, 2015*). However, in stored grains, fungal development at lower moisture ($a_w \leq 0.70$) was found (*Magan et al., 2003*). In addition to the moisture content as a key factor for fungal growth on pre- and post-harvest grains, environmental relative humidity and temperature during storage are also important factors (*Ezekiel et al., 2020*). *Kukier and Kwiatek (2011)* have also reported that total fungal count in cereal grains was strongly dependent on weather conditions during the growing season.

Analogous to our results, *Milićević et al. (2010)* have established total fungal count ranging from 1×10^5 to 40×10^5 cfu g^{-1} in 18 pig feed samples collected from different provinces in Serbia, of which 39% samples exceeded the limit according to the Serbian legislation. They have also reported that with the storage period of pig feed extending, the moisture content is increasing. Similarly, by analysing 756 pig and poultry feed samples from Serbia over a ten-year period (1995–2004), *Marković et al. (2005)* have detected fungal count above the permissible limit in 35.1% feed samples for young animals and in 7.54% of feed samples for adults. These authors have pointed out that the high number of

contaminated feed samples was a consequence of inadequate environmental conditions (temperature and humidity levels) during storage. Furthermore, in the mycological analysis of pig feed samples from northwest of Croatia, *Pleadin et al. (2012)* have determined the total number of fungi ranged from 1×10^3 to 1×10^5 cfu g^{-1}. Then, *Almeida et al. (2009)* have established the mean number of fungi of 6.6×10^2 cfu g^{-1} (range from 2.7×10^1 cfu g^{-1} to 2.7×10^3 cfu g^{-1}) in pig feed samples from Portugal. In Argentina, *Pereyra et al. (2011)* analysed mycobiota contamination in 90 samples of raw materials (milled maize, whole soybean, wheat bran and soybean pellets) and finished feed for fattening pigs (suckling pig, piglet, weaner, growing and boar). It has been observed that high temperatures affected a greater amount of total fungal count in milled maize and all finished feed samples.

In this study, the examined pig farm feed samples were contaminated by fungal species that mainly belong to *Fusarium*, *Aspergillus* and *Penicillium* genera. *Fusarium* species contaminated the most feed samples for fattening pigs (65.52%) and the least samples for piglets (47.62%). *Aspergillus* species were identified in the most feed samples for piglets (59.52%), followed by feed samples for fattening pigs (58.62%) and sows (37.50%). The least percentage of examined feed samples was contaminated with *Penicillium* species ranging from 27.59% of feed for fattening pigs to 37.50% of feed for sows (Table 3). In addition to potentially toxigenic fungi from *Fusarium*, *Aspergillus* and *Penicillium* genera, *Alternaria*, *Mucor* and *Rhizopus* species were also identified but in a small percentage of examined samples (data not presented).

Table 3. The percentage of contaminated pig feed samples with *Aspergillus*, *Fusarium* and *Penicillium* species

Fungal species	Percentage of contaminated samples (%) / Number of samples		
	Feed for piglets	Feed for fattening pigs	Feed for sows
Aspergillus spp.	59.52/42	58.62/29	37.50/8
Fusarium spp.	47.62/42	65.52/29	62.50/8
Penicillium spp.	28.57/42	27.59/29	37.50/8

Contrary to our results, *Milićević et al. (2010)* registered the most number of pig feed samples contaminated with *Penicillium* species (94.4%). *Fusarium* species were present in 55.5% and *Aspergillus* species in 22% of pig feed samples. Other fungi from *Alternaria* and *Mucor* genera were represented in a smaller amount, while *Paecilomyces* spp. were registered in 44.4% of pig feed samples (*Milićević et al., 2010*). Similar to that, *Marković et al. (2005)* and *Pleadin et al. (2012)* have also reported that *Penicillium* species were the most common in analysed feed samples. Furthermore, according to the reports of *Almeida et al. (2009)* in Portugal and *Pereyra et al. (2011)* in Argentina, *Fusarium*, *Aspergillus* and *Penicillium* were also the prevalent fungal genera in commercial pig feeds,

while other fungi belonged to *Phoma, Rhizopus* and *Paecilomyces* genera (*Almeida et al., 2009).*

Moderate positive correlations were found between the total fungal count and moisture content, in the feed samples for piglets (r = 0.50) and fattening pigs (r = 0.40). A fairly strong negative correlation was observed between the total fungal count in feed for sows and moisture content (r = -0.79) (data not presented). Fungal growth is affected by many abiotic factors, such as moisture, temperature, nutrient availability, oxygen and pH. Of these, temperature and especially moisture are considered as the most significant factors for fungal proliferation. It was expected that the total fungal count increases with higher moisture content. However, some fungal species require a certain limit of moisture content for optimal growth *(Christensen and Kaufmann, 1965).* In addition, a weak positive correlation between feed microbial count and moisture content was found by *Vlachou et al. (2004).*

Conclusion

Based on obtained results in this study, the total fungal count was above the permissible limit in 3.45% and 4.76% feed for fattening pig and piglet samples, respectively. Potentially toxigenic fungi from *Fusarium, Aspergillus* and *Penicillium* genera were identified in the most samples. The most of feed samples for fattening pig (65.52%) were contaminated by *Fusarium* species, while *Aspergillus* and *Penicillium* species contaminated the most samples of piglets (59.52%) and sows (37.50%) respectively. A small number of examined pig farm feed samples were contaminated with *Alternaria, Mucor* and *Rhizopus* species.

These results indicate a potential risk in animal health, but also potential reduction in animal production. Hence, continuous mycological analysis of animal feed and risk assessment of fungal contaminants must be basic measures in feed safety strategy. Since cereals are the main components of animal feed, field and storage monitoring of grains/seeds is also a basic measure to reduce fungal contamination in the food/feed chain. Measures such as moisture content reduction, maintenance of low temperature, ventilation and fumigation should be used in the storage of cereal grains. During storage, additionally, adsorbents may reduce the incidence of fungal contaminants.

Kontaminacija hrane za farmske svinje gljivama

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Rezime

Cilj ovog istraživanja bio je da se utvrdi ukupan broj gljiva (plesni) u 79 komercijalnih uzoraka hrane za farmske svinje: za prasad (42 uzoraka), tovne svinje (29 uzoraka) i krmače (8 uzoraka), sakupljenih od različitih proizvođača stočne hrane u Srbiji tokom trogodišnjeg perioda (2017–2019), kao i da se oceni procenat kontaminiranosti uzoraka gljivama, posebno vrstama iz rodova *Aspergillus*, *Fusarium* and *Penicillium*. Primenom standardnih mikoloških metoda određen je ukupan broj i identifikacija gljiva.
Ukupan broj gljiva bio je u rangu od 1×10^2 do 1.41×10^5 cfu g^{-1} u uzorcima hrane za prasad, od 1×10^2 do 2.54×10^5 cfu g^{-1} u uzorcima hrane za tovne svinje i od 1×10^2 do 1.93×10^5 cfu g^{-1} u uzorcima hrane za krmače. Ustanovljen je nedozvoljen ukupan broj gljiva u 3,45% uzoraka hrane za tovne svinje i u 4,76% uzoraka hrane za prasad. Statističkom analizom ukupnog broja gljiva nisu utvrđene značajne razlike između ispitivanih grupa hrane.
Fusarium vrste bile su prisutne u najvećem broju uzoraka hrane za tovne svinje (65,52%), zatim u uzorcima hrane za krmače (62,50%) i prasad (47,62%). *Aspergillus* vrste bile su prisutne u 59,52% uzoraka hrane za prasad, 58,62% uzoraka hrane za tovne svinje i 37,50% uzoraka hrane za krmače. *Penicillium* vrste su kontaminirale najmanji broj uzoraka hrane za tovne svinje (27,59%) a najveći broj uzoraka hrane za krmače (37,50%). U malom broju uzoraka identifikovane su *Alternaria*, *Mucor*, and *Rhizopus* vrste.
Na osnovu dobijenih rezultata može se zaključiti da je ukupan broj gljiva odličan pokazatelj higijenske ispravnosti hrane za farmske svinje. Pored toga, procenat kontaminiranih uzoraka gljivama ukazuje na potencijalno visok rizik za zdravlje životinja. Stoga, redovna mikološka analiza je neophodna za ocenu nutritivnog kvaliteta kao jednog od osnovnih kriterijuma za bezbednost hrane za životinje.

Ključne reči: hrana za farmske svinje, ukupan broj gljiva, vrste gljiva

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