The Impact of Mycotic Contaminated Feed on the Health of Pigs and on the Human Consumer

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Abstract
Mycotoxins are produced by filamentous fungi (molds) that are always present in nature and under the right conditions, have the potential to contaminate almost all raw materials used in pig feed. Mycotoxins are not produced by a single species of mold, which means that different species can produce the same mycotoxins. Molds grow using nutrients from infested plants or raw materials they infest, reducing their nutrient value. This work highlights the negative effects of the presence of microscopic fungi and the toxins released by them in the feed of farm animals, respectively pigs. However, the mycotoxins pose a much greater risk to animals, with pigs being particularly susceptible to this contamination. Most regulatory standards set safe levels of individual mycotoxins. However, the rules do not take into account the cumulative effects of the presence of multiple mycotoxins in feed and the negative effects exerted by human consumption of meat from these animals. In the laboratory, determinations were made on the feed administered to the pigs that showed changes in the state of health, and two species of microscopic fungi were identified, namely Aspergillus and Penicillium. Knowing the negative influence of intoxication states on the health of animals as a result of the administration of moldy feed, the methods of their prevention are of major importance.

Key words: contamination, fungi, intoxication, micotoxins

1. Introduction

One of the most important problems of animal nutrition is the involuntary administration or due to the lack of other feeds of fodder infested with molds. Mycotoxins in cereals represented a problem since ancient times causing numerous epidemics completed with numerous deaths. From social to religious, from medical to historically they have left their mark on all aspects of life. If in the past the lack of information about mycotoxins, determined the people to find explanations of a mystical nature, with the passage of time their implications medical no longer represented an unknown. For the animal body, the consumption of such feeds is harmful due to both the direct action of mycotoxins (toxins produced by molds) and the reduction of the nutritional value of the feed which, although quantitatively sufficient, does not, however, have the appropriate biological value. Mycotoxins are considered to have appeared during the process of phylogenetic evolution of micromycetes as a means of their defense [1]. The consequences of food consumption contaminated with mycotoxins on health to humans are estimated indirectly based on the effect observed in animals. In contrast to the fundamental primary metabolism,

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which is the same for all living beings, the secondary metabolism depends on the species and is very important in the case of molds, leading to the synthesis of a very large number of molecules, including mycotoxins. Mycotoxins are produced in the most diverse substrates (cereals, leaf mass of plants in the vegetation phase, including medicinal plants, legumes, fruits, spices, etc.); they can be present in products of animal origin (milk, fermented cheeses, eggs, meat) in the form of residues from animals fed with contaminated feed or through the subsequent contamination of these products with toxigenic micromycetes. Mold contamination, development and mycotoxin production can occur in the field, during storage, or both. During grain storage, there is a loss of the content of proteins, amino acids, vitamins, etc., which leads to a decrease in the grain's nutritional value. In these conditions they become more vulnerable to the attack of fungi and insects [2, 3]. The presence of molds does not necessarily mean the synthesis of mycotoxins, their production being conditioned by several physical and chemical factors such as changes in temperature, humidity, aeration, the presence of aggressive agents and stress [4]. Mycotoxinogenic molds, which develop in the field (requires a high degree of humidity) belong to the genera Alternaria, Fusarium, Cladosporium, while the storage mycoflora (which requires less moisture) is mainly represented by the genera *Aspergillus* and *Penicillium*. The conditions for the contamination of a substrate by a fungus and the development of mycotoxins are multiple and complex. Knowing the factors involved in the development of fungi is essential for understanding the mechanisms of contamination and can facilitate the prevention of the development of mycotoxins [5]. The major mycotoxins known for the severe poisoning they cause in humans and animals are mainly synthesized by 5 important genera of fungi: *Aspergillus, Penicillium, Fusarium, Alternaria* and *Claviceps* [6, 7]. Research consistently demonstrates the negative impact of mycotoxins on animal performance. However, the impact may not always be evident in pig herds. Loss of homogeneity in age groups, slight changes in daily feed consumption and growth parameters or reduced feed efficiency can all indicate a hidden problem of mycotoxin contamination and lead to significant economic losses. More serious sudden changes, such as increased mortality, could indicate acute contamination and should be investigated immediately. How the general health of pigs is affected is one of the most difficult effects of ingesting mycotoxins to determine. Increasing reformation rates and mortality may point us towards potential mycotoxin contamination problems in mature pig herds. Reduced success of vaccination programs, increased outbreaks of infection due to pathogens, or simply increased drug costs can also be problems generated by mycotoxicosis. Signs of the effect of mycotoxins in animals are often missed until they are already causing performance losses. Detailed and accurate recording of measurement-based data provides a good basis to analyze the situation correctly and develop an effective prevention strategy. A slight change in feed conversion can easily cause serious economic losses and is just one example of the different performance parameters that can draw attention to the potential presence of mycotoxins. It is very important to know that the same mycotoxin can be synthesized by several species of the same genus or different genera of fungi. Also, certain species of fungi can synthesize several types of mycotoxins simultaneously. Sometimes the mere presence of molds can cause unfavourable changes in the taste and/or smell of feed, but in many other cases, their toxic by-products directly affect the appetite of pigs. In extreme cases, the total refusal to consume feed is visible or the pig just plays with its nose in the feed without ingesting that feed. Most of the time, a slight decrease in daily feed intake leads to noticeable performance losses, especially in average daily gain [8]. Mycotoxin contamination is not a static situation; it changes dynamically day by day. Due to the invisible nature of these toxic compounds, even without signs of mold, there may be a threat of contamination, which makes the detection of mycotoxins more complex. The major mycotoxins known for the severe poisoning they cause in humans and animals are mainly synthesized by 5 important genera of fungi: *Aspergillus, Penicillium, Fusarium, Alternaria* and *Claviceps* [9, 10]. In the present paper, it is highlighted that following the accidental consumption by pigs of mycotically contaminated feed, serious changes can occur at the level of the organs, with a high percentage of mortality and an economic impact that cannot be neglected. Much more serious is the fact that the human consumer is endangered, a situation that must be avoided as much as possible.

2. Materials and methods

Intensive animal husbandry and exploitation can be adversely affected by mycotoxins present in feed. On the animal organism, mycotoxins act in multiple ways, which cannot be neglected, the most important being nephrotoxic, hepatotoxic, teratogenic, carcinogenic, immunosuppressive and/or immunotoxic. These aspects are all the more important as it has been proven that mycotoxins from the animal body can reach the human consumer [11]. The negative effects of mycotoxins are influenced not only by their growth but also by the period of their ingestion. Also, in mycotoxicosis, there are no therapeutic possibilities to fight, the only effective measure being the prevention of the occurrence of diseases. The research was carried out on 10-15 weeks-old piglets, from the Banat White and Middle White breeds, come from private farms in Timiş County, with a body weight between 23.7-27.9 kg. The breeders noticed that the pigs began to consume reduced amounts of feed, were lethargic, some had vomiting or diarrhea, as well as, were dehydrated and had an appreciable decrease in body weight. Some of the pigs were observed by the breeders when they refused to consume the feed, only rummaging through their food. Pigs with diarrhea pass faeces of irregular consistency, including faeces changing from slightly soft to watery diarrhea with streaks of blood or undigested feed. The situation worsened when some of the affected pigs died (Table 1).

Table 1. Clinical symptoms observed in pigs

Number of pigs	Clinical symptoms		
	General symptoms	Digestive symptoms	Number of deaths
28	lethargy anorexia refusal to move fidgets skin sensitivity	diarrhea dehydration colics rectal prolaps hipersalivation vomiting	9

Suspecting that the main cause of the problems in the pig herds is due to the feed, they were examined, with the collection of samples for the laboratory examination. In the microbiology laboratory, the fodder was analyzed to detect possible molds developed on them, although they did not appear moldy during a general examination. In the laboratory, the samples collected from the fodder were seeded on special culture media for microscopic fungi, namely on Czapek medium and on agar with malt extract. The samples were incubated at 25-30 degrees Celsius for 7 days. All dead piglets were necropsied and all the changes in the tissues and organs were carefully observed.

3. Results and discussions

Following the mycological examination, two species of microscopic fungi were identified in the analyzed feed sample [12]. After 7 days of incubation, it was found that *Penicillium verrucosum* developed on culture media has a white mycelium, with dark green and blue-green coloured conidia. The conidia have smooth walls, initially ellipsoidal in shape, then becoming globular with maturation. Conidiophores are branched, having a brush-like shape. The colour observed on Petri dishes on which *Aspergillus ochraceus* has grown is yellow, varying shades to brown, and the conidiophores have a powdery appearance. The ends of the conidia are globular at first and then the conidial chains adhere and develop two or three divergent columns. The appearance of these conidiophores is granular with pale yellow-brown walls. Conidia are arranged in dry-looking, vertical chains with two or more short columns each (Photo 1). Considering the fact that following the mycological analysis of the harvested feed samples, two species of microscopic fungi were identified, namely *Penicillium verrucosum* and *Aspergillus ochraceus*, it can be concluded that what affected the pigs, killing some of them, are mycotoxins, knowing the fact that ochratoxins are fungal metabolites isolated from the genus *Aspergillus* and *Penicillium*, namely ochratoxin A

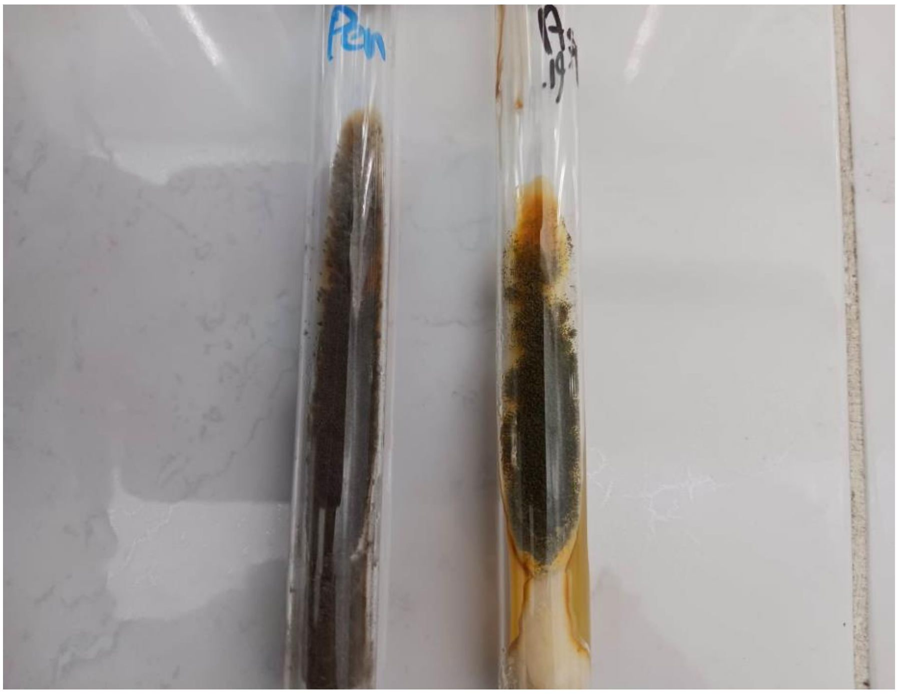

Photo 1. *Penicillium verrucosum* and *Aspergillus ochraceus*

After performing the necropsy examination it was found that the changed appearance of the liver, with the changed color and consistency, the very obvious lobular pattern are morpho-pathological aspects that demonstrate and suggest the presence of extra-lobular hepatic steatosis. There were also lesions of the jaundice, clamps and synechiae that caused the adhesion of the liver to the diaphragm. All these suggest that the liver is the target organ for mycotoxins, being a main indicator of metabolic disorders. Mycotoxins interfere with protein, lipid and carbohydrate metabolism, resulting in the reduction of the body's detoxification capacity (Table 2). Kidneys greatly increased in volume, yellow in colour, with friable consistency, homogenous and bacon-like appearance on section demonstrate the presence of generalized nephrosteatosis. The exaggerated thickening of the walls of the urinary tract and the bladder, the oedema of the bladder also demonstrates the fact that the toxins and metabolic products resulting from the consumption of mycotic contaminated feed, producing toxic products very dangerous for the health and life of the pigs, were eliminated through the urine. The appearance of the spleen, with its greatly increased volume, with a thin, transparent capsule and the mottled-looking parenchyma, with multiple foci of necrosis, suggests a state of hypertrophy, with hyperplasia of the lymphoid follicles induced by tissue changes caused by the cytotoxic action of the mycotoxins added to the feed. Also, haemorrhages and gastrointestinal ulcers were observed in the necropsy examination, in different stages of evolution. All these observed lesions confirm the presence of the pathological effects of the predominantly hepato- and nephrotoxic action of mycotoxins. Mycotoxins affect the cellular and tissue integrity of the pig, leading to an unhealthy imbalance of various physiological systems. They affect the internal organs and result in reduced pig performance, reduced immunity and deterioration of health. Once in the body, ochratoxins are absorbed through the digestive tract, starting from the stomach and continuing into the small intestine, but this process is very slow. Ochratoxin A is partially cleaved in the intestine into Ochratoxin alpha and phenylalanine under the action of caboxypeptidase A and chemotrypsinogen, or by intestinal bacteria and protozoa [10]. Most mycotoxins can cause acute but more often chronic toxicosis in pigs. Many types of toxins are known for their unique toxicological aspects and can cause specific symptoms depending on the species, sex, age, level and duration of contamination.

Table 2. Morphopathological alterations observed in necropsy examination

The affected organ	Morphopathological changes
Liver	enlarged volume, yellow color; with a friable consistency, and yellow-matte coloring on the section, without luster and with an increased friability; the lobular pattern very obvious; with a reddish center and a yellowish periphery; lesions of jaundice, clamps and synechiae that caused the adhesion of the liver to the diaphragm
Kidney	more enlarged than normal size; grayish-yellowish or generalized yellow color; friable consistency, especially after decapsulation on section dull yellow color, with a homogenous and bacony appearance, indicating generalized nephrosteatosis; in urinary tract, and especially in the bladder, a pronounced thickening of their walls; a pronounced edema in the bladder, as a result of the elimination of toxins and metabolic products through urine
Spleen	increased in volume, with a thin and transparent capsule and the splenic parenchyma had a mottled appearance; on the section, the follicular pattern was evident, with small foci of necrosis, which suggests a state of hypertrophy; hyperplasia of the lymphoid follicles induced by tissue changes caused by a cytotoxic action
Mesenteric lymph nodes	reduced in volume with a small amount of citrine liquid on the surface of the section; moist and glossy appearance to the section
Intestine	villi with reduced height and surface area, ulcers and hemorrhages

Mycotoxins are found in the spores and thallus of mycetes or as their secretion products in the substrates where they develop. These metabolites appear in different stages of grain production (some during grain development, others during their storage). The three genera of mycotoxin-producing fungi, economically important, are *Aspergillus*, *Penicillium* and *Fusarium*. Mycotoxins contaminate agricultural products before or after harvest. In general, humidity and high temperatures favor the multiplication of fungi and the production of mycotoxins. Poor conditions during harvesting, storage, transport and marketing also contribute to the growth of molds and increase the risk of mycotoxin production. Molds can grow before or after harvest, during storage and can contaminate almost all pig feed ingredients, so potential contamination in feed production, transport and feed distribution must be carefully monitored. Sometimes the mold infestation is visible, allowing the breeder to immediately identify the potential risk and apply preventive measures. However, mycotoxins are not visible to the naked eye and require specialized detection techniques.

How the general health of pigs is affected is one of the most difficult effects of ingesting mycotoxins to determine. Increased reformation rate and mortality may point the farm towards potential mycotoxin contamination problems in mature pig herds. Reduced success of vaccination programs, increased outbreaks of infection due to pathogens, or simply increased drug costs can also be problems generated by mycotoxicoses. Rectal prolapse occurs due to frequent diarrhoea, due to abdominal pressure, being one of the most likely indirect symptoms. Lethargy or heightened irritability in larger groups of animals may be symptoms related to mycotoxin contamination. Chewing without feed, hypersalivation and foaming at the mouth and, including, frequent vomiting can draw our attention to a possible situation of contamination with mycotoxins. Increased skin sensitivity leading to skin lesions on the tips of the ears or on the tails can have several causes, but contamination with mycotoxins should not be ruled out either. Mycotoxins present in pig feed always have a negative impact on production in this sector. However, the impact may not always be evident in pig herds.

Loss of homogeneity in age groups, slight changes in daily feed consumption and growth parameters or reduced feed efficiency can all indicate a hidden problem of mycotoxin contamination and lead to significant economic losses [4, 11]. More serious sudden changes, such as increased mortality, could indicate acute contamination and should be investigated immediately. Knowing the negative influence of intoxication states on the reproduction and health of animals as a result of the administration of moldy feed, the methods of their prevention are of major importance. The first measure aims to limit the sources of infection by maintaining hygiene and carrying out routine disinfection in animal shelters and feed storage spaces, with the removal of moldy fodder and not using it even as bedding.

A thorough organoleptic examination of the fodder regarding the degree of mycotic contamination is also necessary. The organoleptic examination must be completed with the laboratory mycological examination, necessary especially when, following the organoleptic examination, changes in the general appearance of the fodder are noticed and when the question arises as to whether they can be introduced into the animals' diet without endangering their health. Human consumption of pork contaminated with mycotoxins can have very serious consequences. Most mycotoxins are resistant to high temperatures (baking, boiling and in some cases they also resist frying). Many of these also withstand industrial meat processing. If crops used for feed are contaminated then products like milk and meat will contain toxins or biotransformation products. In the case of pigs, the ochratoxin present in the feed accumulates in their meat. Due to the fact that mycotoxins are resistant to processing, they can be found even in highly processed foods, constituting a real danger to the health of the human consumer. A greater risk of poisoning is especially in the case of people with low immunity due to chronic diseases, the elderly or children. Depending on the type of toxins ingested, in food poisoning the evolution is acute or chronic. The main symptoms of poisoning can be in humans: headache, a sudden increase in temperature, vomiting, nausea, belching, flatulence, diarrhoea, abdominal pain and muscle weakness [10, 12, 13]. In recent years, studies have been carried out on the methods of detoxification of mycotoxins by biological methods compared to chemical or physical methods. The advances made in the field of biotechnology in recent years, as well as the fact that microbial populations can have unlimited catabolic capacities, will probably determine in the not too distant future a change in this trend in favour of the biological methods. Unlike physical and chemical methods, biological detoxification is still insufficiently defined. Thus, feed additives that bind mycotoxins, additives that improve taste, and other nutritional interventions have been grouped under biological decontamination methods [14-16]. The detoxification of mycotoxins was resorted to with the help of lactic bacteria. In 2005 Malgorzata and Zakowska analyzed 29 strains of lactic acid bacteria belonging to the genera *Lactobacillus* and *Lactococcus*, for their ability to remove ochratoxin A from the liquid medium. All tested strains were able to reduce the concentration of ochratoxin A and many of them were insensitive to the toxin. The highest adsorption (over 50%) of the initial ochratoxin A content was obtained with *Lb. acidophilus CH-5, Lb. rhamnosus GG, Lb. plantarum BS*, *Lb. brevis* and *Lb. Sanfranciscensis* [17]. Similar results were reported for the ability of wine LAB to bind ochratoxin A. *Lactobacillus species* and *Oenococcus oeni* strains. Another alternative is the fungal degradation of mycotoxins by yeasts. Yeasts have a huge potential to be used in reducing the effects of mycotoxins in grain-based foods and animal feed. t is far too early to interpret aspects of the binding mechanisms and further studies are needed to understand the interaction between mycotoxins and the different components of the yeast wall. The possible use of *S. cerevisiae* as binding agents for mycotoxins in human and animal food depends on the stability of the complex in the gastrointestinal tract and the time spent. In general, *S. cerevisiae* shows a low adhesion to the intestine, but which has a high degree of specificity [18-20]. Nutrition experiments with live yeast and cell wall showed that the addition of S. cerevisiae to the diet led to a decrease in the toxicity of mycotoxins, indicating the stability of the yeast-mycotoxin complex in the gastrointestinal tract [21, 22].

4. Conclusions

Following laboratory examinations of the feed given to animals with clinical signs of intoxication, it was revealed that they were contaminated with microscopic fungi of the species *Penicillium verrucosum* and *Aspergillus ochraceus*. The conclusion can be drawn is that what affected the pigs, killing some of them, are mycotoxins, knowing the fact that ochratoxins are fungal metabolites isolated from the genus *Aspergillus* and *Penicillium*, namely ochratoxin A.

The necropsy of dead animals with clinical signs of intoxication revealed anatomopathological changes characteristic of mycotoxin poisoning.

Liver lesions confirm that this is the target organ for mycotoxins, being an indicator of the metabolic disorders produced by them. Mycotoxins interfere with protein, lipid and carbohydrate metabolism, resulting in the reduction of the body's detoxification capacity. Metabolic disorders induced by mycotoxins are also reflected in the degree of utilization of feed.

The average daily gain achieved by the animals was much lower. Because mycotoxins are resistant to processing, they can be found even in highly processed foods, constituting a real danger to the health of the human consumer.

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