THE CLINICAL AND PATHOMORPHOLOGICAL DIAGNOSIS OF MYCOTOXICOSIS IN DIFFERENT SWINE CATEGORIES

ABSTRACT: The issue of mycotoxins and mycotoxicosis in veterinary medicine is directly connected to the usage of mouldy and/or adversely stored grains (corn, wheat, barley) used in animal feed. In swine production, in our geographical region, the most common are mycotoxicosis caused by zearalenon (F-2 toxin), but aflatoxins, ochratoxin and trichothecenes can also be found. For the known mycotoxins of clinical importance, the response is usually subacute or chronic and the presenting clinical signs are often vague. Mostly the problems are expressed only as alterations of the reproductive cycle, reduced feed intake and slow growth. However, if we consider the clinical signs and pathomorphological picture of mycotoxicosis in different swine categories (breeding animals, suckling and weaned piglets, fatteners), the age dependent changes can be found. Some mycotoxins have hepatotoxic, nephrotoxic and immunosuppressive effects, which further complicate the clinical and pathomorphological picture and diagnosis of mycotoxicosis in swine.

The material for this research included the samples provided from ten swine farms. In different swine categories health disorders, resembling to the problem with mycotoxins were detected. The applied research methods included clinical evaluation and pathomorphological examination and laboratory microbiological feed testing, in order to examine the presence of fungi and some mycotoxins (aflatoxins, zearalenon, ochratoxin A and trichothecenes).

On the basis of the obtained results, it may be concluded that the most frequently detected mycotoxin in the examined feed samples was zearalenon. The presence of mycotoxin in feed was directly connected to the reproductive failures and diagnosed health disorders in the examined swine categories (vulvovaginitis, skin necrosis, pneumonia, gastroenteritis).

KEY WORDS: diagnosis of mycotoxicosis, swine feed, zearalenon

INTRODUCTION

Mycotoxins are secondary metabolites of moulds, and so far, approximately 400 secondary metabolites with toxigenic potential produced by more than 100 moulds have been reported (Kabak and Dobson, 2006). Several fungi, mainly belonging to the genera: Aspergillus, Penicillium, Fusarium and
Alternaria, are detected most frequently. The issue of mycotoxins and mycotoxicoses in veterinary medicine is directly connected to the usage of mouldy and adversely stored grains (corn, wheat, barley) in animal feed (Oswiler, 2006).

In the swine production, in our geographical region, the most common are mycotoxicosis caused by zearalenon (F-2 toxin, ZEA), but also aflatoxins (AF), ochratoxin (OCT) and trichothecenes occur. The problems are expressed mostly only as alterations of the reproductive cycle, reduced feed intake and slow growth (Gonzales and Rodriguez, 2008).

The clinical and patomorphological picture of mycotoxicoses in swine depends on the age and category (breeding animals, suckling and weaned piglets, fatteners). Beside this, some mycotoxins have hepatotoxic, nephrotoxic and immunosuppressive effects (Kabak and Dobson, 2006), which can further complicate the clinical and pathomorphological picture and diagnosis of mycotoxicosis in swine. Immunosuppressive effects of mycotoxins are of special interest and may have significant influence on the occurrence of infective diseases of pigs (Obremski et al., 2008). The connection between clinical cases of common diseases (swine erysipelas, swine disentery and salmonellosis) influenced by AF under experimental conditions are detected (Oswiler, 2006).

MATERIAL AND METHODS

The material for this research included the samples collected from ten swine farms, with different swine categories where health disorders resembling to the problem with mycotoxins were detected. Depending on the specificity of each evaluated case and available material, the applied research methods included: epidemiological and clinical evaluation, pathomorphological examination and laboratory microbiological feed testing, in order to examine the presence of fungi and evaluation to detect the presence of mycotoxins by the method of thin layer chromatography.

RESULTS

In seven examined cases, the presence of ZEA in different swine feed was detected. Depending on the swine category (suckling piglets, weaned piglets, fatteners and breeding animals) the clinical symptoms in diseased animals and patomorphological changes in dead pigs were evaluated.

In the clinical cases of ZEA mycotoxicosis in the suckling piglets, the occurrence of neonatal diarrhoea already in the first 3 days of life after farrowing were detected. These health problems did not improve after the medical treatment with antibiotics. In great number of just farrowed piglets the most prominent clinical sign was vulvovaginitis (swelling and reddening of the vulva). Beside this, in two cases, a large number of small, weak and splayleg piglets were noticed. Applying patomorphological examination on the dead suckling piglets the prominent changes on mucosal surfaces of the digestive tract (Gas-
trotenteritis haemorrhagica), fewer number of pale kidneys, necrotic and distrophic processes on liver tissue were detected. By laboratory testing of the available swine feed grains from the stables intended for different swine categories, the presence of ZEA in different concentrations was detected (from 0.72 to 6.4 mg/kg).

The ZEA mycotoxicosis in the weaned piglets was clinically characterised with signs of pneumonia, slow growth, vulvovaginitis and necrosis of the tails, sporadically with diarrhoea and rectal prolapses. The patomorphological examination of the dead weaners revealed the following lesions: bleeding on the mucosal surface of the digestive tract (Gastroenterotyphlocolitis haemorrhagica), pleuropneumonia and pneumonia with the sings of purple to gray areas of consolidation, hepatomegalia, focal nephritis and rectal prolapses. Etiologically the pneumonia was caused by Actinobacillus pleuropneumoniae, Haemophilus suis, and Mycoplasma hyopneumoniae. Another problem that was frequently observed was digestive infection caused by enteropathogenic Escherichia coli, which might have been promoted by the presence of mycotoxin (ZEA 0.8 mg/kg) and a high number of different fungi species in the weaners feed (Fusarium, Penicillium, Aspergillus, Rhisopus). During evaluation of the farm storage facility, approximatelly 20% of mouldy wheat was found. Having in mind this fact and the clinical symptoms observed in the piglets (necrosis of tails and ears, vomiting and diarrhoea) a justifed suspicion on the presence of ergot alkaloids was made.

In the fatteners, clinical symptoms included vulvovaginitis, bronchopneumonia, bloody diarrhoea, and sporadically rectal prolapses. One of the characteristics of this swine category was transient feed refusal. The patomorphological examination revealed the lesions on the mucosa of digestive organs (Gastroenteritis haemorrhagica, Dysenteria suum), pleuropneumonia and pericarditis. After laboratory testing of the corn samples the presence of ZEA from 0.5 to 0.8 mg/kg was detected.

Only in one case the presence of ZEA was detected and the following clinical symptoms were noticed in the suckling piglets and sows: reduced feed intake, agalactia and endometritis. On the another farm, the clinical symptoms in sows consisted of reproductive disorders with the increased number of rebreeding (= 20%), lower rate of conception (from 93% to 77%), increased number of deadborn (0.85 per litter) and mummified piglets, and decreased litter size. However, it should be noted that apart from the presence of ZEA in feed (6.4 mg/kg), the infection with parvovirus was also diagnosed on the farm, which further complicated the diagnosis.

On two swine farms, in the feed samples the presence of two mycotoxins, ZEA (0.1—4 mg/kg) and OCT-A (0.080—0.12 and 0.5 mg/kg), were simultaneously detected. The following feed samples were tested: corn, piglets first and second feed, feed for pregnant sows and boars. The piglets showed the following clinical signs: feed refusal, reduced growth, diarrhoea, pneumonia. The pathomorphological changes were not different from the aforementioned signs observe on the other farms that consumed feed contaminated with ZEA. However, in sows the reproductive disorders were clinically evident: rebreeding (27%), infertility (20%), anestrous (10.6%) and frequent endometritis.
The presence of AFB1 in the first feed for piglets (0.018 mg/kg) was detected only in one case. Microbiological feed testing detected 3-fold increase in the number of fungi from genera *Penicillium*, *Aspergillus*, *Rhizopus* as compared to the level set by the regulation. In the weaned piglets clinical and patomorphological lesions characteristic for the enteropathogenic *Escherichia coli* infection were diagnosed. It can be assumed that this was provoked by the presence of mycotoxin in feed.

Simultaneous presence of several mycotoxins (ZEA, AFB1, AFG1, OCT) was established only in one examined case. The above mentioned mycotoxins were detected in the feed for pregnant and lactating sows (ZEA 0.8 mg/kg; AFB1 0.008 mg/kg; AFG1 0.02 mg/kg; OCT 0.2 mg/kg), in the supplemental feed for fatteners (ZEA 4 mg/kg; AFB1 0.008 mg/kg; AFG1 0.016 mg/kg; OCT 0.5 mg/kg), corn (ZEA 4 mg/kg; AFB1 0.008 mg/kg; AFG1 0.002 mg/kg; OCT 0.2 mg/kg), sunflower pellets (ZEA 4 mg/kg; AFB1 0.016 mg/kg; AFG1 0.008 mg/kg; OCT 1.0 mg/kg), soyabean pellets (ZEA 2.0 mg/kg; AFB1 0.016 mg/kg; AFG1 0.008 mg/kg; OCT 1.0 mg/kg) and animal yeast (ZEA 0.8 mg/kg; AFB1 0.016 mg/kg; AFG1 0.02 mg/kg; OCT 3 mg/kg). Clinical signs were noticed in the piglets and sows. In the sows, gravidity period and farrowing time were prolonged, cases of agalactia (sudden loss of milk and lying on the udder), a small number of stillbirths and mummified piglets were noticed. The newborn piglets were described as weak, nonviable, with diarrhoea. The diseased piglets lived only 4 days after birth. They probably died due to hypoglicemia, because sows did not have enough milk or the piglets were too weak and did not have enough strenght for milk suckling. Sporadically, the occurence of splayleg was observed. By patomorphological examination of the dead suckling piglets the following lesions were evident: pale kidneys, hepatic damage (yellowish color), gastric ulcers, diffuse haemorrhage on internal organs and the brain.

**DISCUSSION**

On the basis of the obtained results, it may be concluded that the most frequently detected mycotoxin in the examined feed samples was ZEA. The presence of mycotoxin in feed was directly connected to the reproductive failures and health disorders (vulvovaginitis, skin necrosis, pneumonia, gastroenteritis) diagnosed in the examined swine categories. Zearalenone is a mycotoxin which acts as estrogen, binding competitively to estrogen receptors of the uterus, mammary gland, liver and hypothalamus (Gajecki, 2002). Pigs are the most susceptible to the presence and negative effects of ZEA (Diekmann and Green, 1992; Obremski et al., 2003). In our research, the perinatal hyperestrogenic syndrome was a constant clinical sign in the suckling piglets. This is certainly the consequence of mycotoxins presence in the feed for sows, i.e. during the pregnancy and the presence of its excreted metabolite in milk of the exposed sows.

The toxic effect of mycotoxins depends on a number of factors: the intake levels, duration of exposure, toxin species, mechanisms of action, meta-
bolism and defense mechanism. The consumption of mycotoxin-contaminated feed leads to the induction of teratogenic, cancerogenic, oestrogenic, neurotoxic and immunosuppressive effects in the organism (Kabak et al., 2006). The influence on immune system is of special interest in the swine industry. The technology on swine farms demands frequent vaccinations, especially in piglets and sows which may be a problem in the case of immunocompromised animal. From the obtained results, several examples of immunosuppressive effect can be presented. For instance, the occurrence of enteroxemia in piglets, despite the fact that dams were vaccinated twice during gestation.

Aflatoxins (B1, B2, G1, G2) are recognised as immunomodulating agents, and when AFB1 is metabolised by mammals it occurs in milk as M1. It is assumed that AFB1 is the most toxic fraction (Oswieler, 2006; Zivkova-Balo et al., 2008). Gross lesions associated with porcine aflatoxicosis include liver fatty degeneration and necrosis: clay-colored liver with hemorrhages; the fibrosis develops, characterised by a firm hard liver with accentuated lobular pattern (Oswieler, 2006). In our research, it was discovered the persistent presence of various infections, which reacted poorly or failed to react on the applied antimicrobial therapy (endometritis, pneumonia). Although the examined feed samples did not contain trichothecenes, their strong tendency to induce feed refusal and vomiting in swine should be stressed, which makes them somehow self-limiting as toxins (Obremski et al., 2008).

The ochratoxin is a nephrotoxic and immunosuppressive mycotoxin, and principal effects are manifested on proximal renal tubules. However, the clinical signs which include polydipsia, polyuria, reduced growth and lower feed efficiency can not be associated with sole ochratoxicosis. The true control should be carried out in the abattoir. It is assumed that pale and firm kidneys (nephrosis and interstitial fibrosis) and gastric ulcers are commonly associated with ochratoxin in endemic areas (Oswieler, 2006). Our results indicate the occurrence of slow growth, the increased feed consumption and significant difference in the weight of fatteners as a consequence of mycotoxin feed contamination.

For the known mycotoxins of clinical importance in the swine production, the response is usually subacute or chronic and the presenting signs are often subtle and vague (Gonzales and Rodriguez, 2008). As a consequence of immunosuppressive action of mycotoxins, clinical and pathological lesions correspond to the infective diseases of different etiology (Obremski et al., 2008). Dietary deficiencies of protein, selenium and vitamins have been suggested as predisposing factors in the mycotoxicoses. Combinations of several mycotoxins may potentiate the action of one other, or at least exert an additional effect (Oswieler, 2006). The obtained results strongly support the interaction between the mycotoxins and infective agents.

In the last five years, the swine industry in our country was exposed to the most unfavourable conditions. As a consequence, most of the pig producers blend mycotoxin-contaminated feed with the sound one in such a proportion that animals consume it without any obvious adverse effects on the growth and reproduction. However, economic losses that occur due to usage of blended feed probably stay unknown because low concentrations of several
mycotoxins may interact in ways that are difficult to detect reducing the performance. The continuous intake of small amounts leads to chronic intoxication which is characterised by the loss of weight and insufficient weight gain, fertility disorder or increased susceptibility to infectious diseases. As feedstuffs are mostly contaminated with several different mycotoxins simultaneously, it may be assumed that mycotoxicoses are multicausal (multitoxic) diseases (Diekman and Green, 1992; Oswald, 2006).

Because of detrimental effects of mycotoxins, a number of strategies have been developed to decontaminate and detoxify mycotoxin-contaminated feed. They may include inhibition of mycotoxin adsorption in the gastrointestinal tract. One of the most recent approaches to the prevention of mycotoxicoses is the addition of non-nutritional adsorbents in the feed that bind mycotoxins in the gastrointestinal tract and reduce their bioavailability. The activated carbons, aluminosilicate, zeolites, bentonites and certain clays are well known. A novel strategy to control the problem of mycotoxicoses in animals is the application of microorganisms, yeasts (Eubacterium-BBSH 797; Trichosporon MTV, 115) capable of biotransforming mycotoxins into nontoxic metabolites (Kabak et al., 2006). The basic preventive measures in order to protect the animals are usage of healthy feed and proper storage and condition management of animal feed. Certainly, if mycotoxicosis occurs or is suspected, the first action should be the change of the source of feed. Mycotoxicoses are generally a herd problem and not amenable to individual treatment. Practical preventive program should be the part of every swine management program.

REFERENCES


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КЛИНИЧКА И ПАТОМОРФОЛОШКА ДИЈАГНОСТИКА МИКОТОКСИКОЗА КОД РАЗЛИЧИТИХ КАТЕГОРИЈА СВИЊА

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Резиме
Примена плеснивих и/или неадекватно ускладиштенх житарица у исхрани животиња (кукуруз, пшеница, јеча м) директно се повезује са проблемом микотоксина и микотоксикоза у ветеринарској медицини. На нашем географском подручју у свињарској производњи су најчешће микотоксикозе узроковане зеараленоном (F-2 токсин), али су значајни и афлатоксин, охратоксин и трихотецин. За микотоксикозе које су од клиничког значаја ток обољења је најчешћу субакутан или хроничан и клинички знаци су нејасни. У највећем броју случајева здравствени проблеми се огледају у поремећају репродуктивног циклуса, успореном расту и смањеној конзумацији хране. Међутим, када је у питању клиничка и патоморфолошка манифестација микотоксикоза у свињарској производњи постоји изражена специфичност узрста (приплодне јединке, прасад на сиси и одгого и товљеници). Поред тога, поједини микотоксини имају изражен хепатотоксинични, нефротоксинични и имуносупресивни ефекат, што даље компликује клиничку и патоморфолошку манифестацију обољења и дијагностику микотоксикоза код свиња.

Материјал за испитивање је обухватао десет фарми свиња, на којима су ре- гистровани здравствени проблеми код различитих категорија свиња а који су указивали на потенцијални проблем микотоксикозе. У оквиру примењених метода обављени су клиничка испитивања, патоморфолошки преглед угнутих јединки и микробиолошко испитивање хране у циљу установљавања присуства плесни и микотоксина (афлатоксин, зеараленон, охратоксин A и трихотецин). Постигнути резултати испитивања указују да је у храни за свиње најчешће установљено присуство микотоксина зеараленона. Присуство микотоксина у храни доводи се директно у везу са поремећајем репродуктивне ефикасности и различитим поремећајима здравственог статуса код испитаних категорија свиња (влувовагинити, некрозе коже, пнеумоније и гастроентерити).