Зборник Матице српске за природне науке / Proc. Nat. Sci, Matica Srpska Novi Sad, № 120, 249—257, 2011

UDC 636.4.085.1:582.28
DOI: 10.2298/ZMSPN1120251P

Jasna Z. Prodanov-Radulović, Radoslav Đ. Došen, Igor M. Stojanov, Ivan M. Pušić, Milica M. Živkov-Baloš, Radomir D. Ratajac

Scientific Veterinary Institute "Novi Sad", Rumenački put 20, 21000 Novi Sad, Serbia

INTERACTION BETWEEN MYCOTOXINS AND CAUSATIVE AGENTS OF SWINE INFECTIVE DISEASES

ABSTRACT: Mycotoxins are secondary metabolites of fungi that can contaminate animal feeds at all stages of food production chain. Consumption of feed contaminated with mycotoxins may result in immunosuppression, which represents a predisposing factor for occurence of infectious diseases in livestock. The influence on immune system is of special interest in swine industry. The technology on swine farms demands frequent vaccinations, which may be a problem in the case of immunocompromised animals. The aim of this paper was evaluation of mycotoxin influence on swine farms, as secondary factors for destabilization of animals' immunological system.

Material for this research included the samples from five swine farms, where health disorders, i.e. clinical and patomorphological signs resembling the problem with infectious diseases in different swine categories, were detected. The applied research methods included: epidemiological and clinical evaluation, pathomorphological examination, laboratory testing of bacteriological and virusological tissue originating from dead animals, and microbiological feed testing in order to examine the presence of fungi and mycotoxins.

The obtained results indicated the existence of positive interaction between mycotoxins and causative agents of swine infective diseases. Despite continual pharmaco- and immunoprophylaxis in swine, the health problems of bacterial ethiology (colibacilosis, enteroxemia, dysentery, pneumonia, endometritis) were detected. From an epidemiological point of view, the presence of mycotoxins in animal feed may induce a breakdown of active immunity and occurrence of disease even in properly vaccinated animals.

KEY WORDS: infective diseases, imunoprophylaxis, swine mycotoxicoses

INTRODUCTION

Mycotoxins are secondary metabolites of fungi that can contaminate animal feeds at all stages of food production chain. At global level, it is considered that 25% of the world crop production is contaminated by mycotoxins, which may be a risk factor for human and animal health (Bouhet and Oswald, 2005). Consumption of feed contaminated with mycotoxins may result in immunosuppression, which represent a predisposing factor for infectious diseases

of livestock (M a r i n et al., 2002; O s w a l d et al., 2005). From a public health perspective, increased infections in animals may result in increased animal--to-human transmission of pathogens and/or increased antibiotic concentrations in meat, as a consequence of animal treatment (O s w a l d et al., 2005). The influence of mycotoxins on immune system is of special interest in swine industry. The technology on swine farms demands frequent vaccinations, which may be a problem in the case of immunocompromised animals (O s w e i l e r, 2006; P r o d a n o v et al., 2009). The aim of this paper was to evaluate the influence of mycotoxins in swine production, as secondary factors for destabilization of animals' immunological system.

MATERIALS AND METHODS

Material for this research included the samples from five swine farms, where health disorders, i.e. clinical and patomorphological signs resembling the problem with infectious diseases in different swine categories, were detected. Depending on the specificity of each evaluated case and available material, the applied research methods included: epidemiological and clinical evaluation, pathomorphological examination, standard laboratory testing for detection of the presence of aerobic and anaerobic bacteria, virological testing and microbiological feed testing, in order to examine the presence of fungi and mycotoxins by the method of thin layer chromatography.

RESULTS

Health disturbances in the youngest swine categories were recorded in the first examined farm (suckling piglets and weaners). Clinically, diarrhoea was detected in suckling piglets in the first 3 days of life after farrowing. After the analysis of the farm records several facts were discovered: diarrhoea occured in piglets of normal birth body weight, mortality rate was higher in animals that are in good body condition, and 30% of weaning piglets were small. Therapeutic treatment of piglets by oral and parenteral antibiotics application did not improve health problems. It should be emphasized that the dams were vaccinated during gestation with the aim to prevent disease in piglets (diarrhoea) in the first days of life. By clinical examination of certain number of suckling piglets, the clinical sign of vulvovaginitis (swelling and reddening of the vulva) was discovered. Diarrhoea and signs of pneumonia (cough, nasal secretion, fever) were detected in the weaned piglets. Pathomorphological examination of the dead suckling piglets revealed lesions present predominantly on the mucosal surface of the digestive tract (*Haemorrhagiae mucosae ventriculi*, *Enteritis catharralis acuta*). Besides the lesions in the digestive tract, prominent pathological changes in lungs were also discovered in dead weaners. (*Pneumonia fibrinosa in statu hepatisationis rubrae et griseae*, *Gastroenteritis*

haemorrhagica). Bacteriological testing of tissue samples derived from dead animals revealed the following bacteria: *Escherichia coli var. haemolytica*, *Streptococcus alfa haemolyticus*, *Pasteurella* sp. Despite the fact that all animals were medically treated after bearing, health status control of sows showed reduced appetite, clinical signs of endometritis and agalactia. By pathological control of the reproductive organs of excluded sows in the slaughterhouse, significant percent of endometritis was discovered (presence of liquid muddy content in the uterus with small pieces of destroyd tissue or content that looked like a sour cream). Bacteriological testing of tissue samples from the dams genital organs showed presence of *Staphylococcus haemolyticus*, *Escherichia coli*, *Streptococcus dysgalactiae subsp. equisimilis*. Considering the clinical and pathological symptoms observed, especially the signs of vulvovaginitis in farrowed piglets, a justified suspicion on the presence of mycotoxins in feed was made. Testing of first microbiological feed for piglets detected 3-fold increase in the number of fungi belonging to genera *Fusarium*, *Penicillium*, *Aspergillus*, *Rhizopus*, as compared to the level set by the regulation. By further laboratory testing, an increase of the total number of fungi in the large number of examinated feeds was discovered: corn ($887x10^3$ *Aspergillus*, *Rhizopus*), piglets second feed ($319x10^3$ *Aspergillus*, *Mucor*, *Rhizopus*), feed for pregnant sows ($123x10^3$ *Penicillium*, *Fusarium*) and feed for lactating sows ($526x10^3$ *Aspergillus*, *Penicillium*, *Mucor*). The presence of the following mycotoxins was detected: zearalenon ("ZEA") in the feed for pregnant sows (0.72 mg/kg), aflatoxin AB1 in the piglets first feed (0.018 mg/kg) and ochratoxin OTA in the piglets second feed (0.12 mg/kg).

Health problems on the second examined swine farm included increased incidence of clinical and pathomorphological signs of infective diseases and failed reaction to the applied therapy. By analyzing the existing data on the farm, high mortality in piglets 7 days before weaning was noticed, which did not decrease after medical treatment. With the aim to overcome the problem, the measure of medical treatment of piglets 3 days before weaning (parenteral application of antibiotics) was introduced, but with no results. Disease in the weaned piglets was clinically characterized by severe yellowish diarrhoea, dehidratation, huddling, roughly hair and sporadical coughing. Therapeutic treatment of the diseased animals was multiple: the antibiotics were given through feed, water and parenterally. Applying pathomorphological examination on the dead weaned piglets, the prominant changes of digestive and respiratory tract were detected (*Gastroenterotyphlitis haemorrhagica*, *Poliserositis fibrinosa massiva*, *Pneumonia complex*). By bacteriological testing of tissue samples from dead piglets the following bacteria were isolated: *Escherichia coli haemolytica, Pasteurella* sp**,** *Streptococcus uberis*. By laboratory feed testing of the available first feed for piglets the presence of OCT (0.5 mg/kg) and ZEA (4 mg/kg) was discovered.

Health problems in the third examined swine farm indicated a potential role of mycotoxins in fattening pigs. Clinically,the fatteners showed signs of broncopneumonia (cough), sporadic and intermitent bloody diarrhoea that was lately intensified, and fever (40–41 °C). Anamnestically, it was discovered

that in the last few days a total of 40 fatteners died. The pathomorphological examination on the dead fattenars was done. The existance of the pathological changes on the respiratory and digestive tract (*Pleuropneumonia actinobacillosa*, *Pericarditis fibrinosa diffusa*, *Gastritis catharrhalis*, *Typhlocolitis haemorrhagica – Dysenteria suum*) was discovered. Bacteriological testing of the tissue samples derived from the dead fatteners isolated the following bacteria: *Escherichia coli haemolytica*, *Pasteurella* sp., *Actinobacillus suis*. The presence of ZEA (0.8 mg/kg) in the samples of corn was microbiologically detected.

Complex health problems that, besides health disorders, included significant reproductive disturbances on the fourth swine farm were noticed. Anamnestic data showed that there were frequent periods of sows delivering mummified piglets, which decreased the litter size. Also, the increased number of rebreeding sows at irregular intervals was discovered. The conception rate has dramatically decreased and the problem with frequent abortions 2 months before was intensified. Besides this, farrowed piglets were nonviable and despite the medical treatment, they lived only 3-4 days after birth. In the pregnant dams, the immunoprophylaxis was carried out with the aim to prevent the outbreak of disease in suckling piglets. Also, the antibiotics were added in the sows feed 7 days before and 7 days after farrowing. Sporadically, the occurrence of severe yellowish diarrhoea in piglets and clinical signs of vulvovaginitis in just born piglets were evident. The clinical signs of diarrhoea and pneumonia were occasionally noticed in the weaners, while the fatteners faced the problem of outbreak of bronchopneumonia (over 80% of the fatteners were coughing and thumping, devloped dispnoa, had nasal secretion, individual vulvovaginitis and rectal prolapses). The pathomorphological examination of the dead fatteners revealed pathological lesions on the organs of respiratory (*Bronchitis catarrhalis*, *Pleuropneumonia actinobacillosa*, *Pneumonia interstitialis*) and digestive tract (*Typhlocolitis haemorrhagica acuta*). By applying bacteriological examination of the tissue samples derived from dead fatteners, the following bacteria were isolated: *Pasteurella* sp, *Actinobacillus suis*, *Mycoplasma* sp. After laboratory testing of swine feed samples, the simultaneous presence of several mycotoxins was established: ZEA (6.4 mg/kg), AB1 (0.0064 mg/kg), OTA (0.032 mg/kg) and trichothecenes: diacetoxyscirpenol ("DAS") 0.25 mg/kg and T-2 toxin (0.1 mg/kg).

The connection between the presence of mycotoxins in swine feed and an outbreak of viral infection of swine, *Morbus Aujeszky* ("MA") was established in the last examined farm. By microbiological testing of the feed for lactating sows the presence of fungi (*Fusarium* sp., *Mucor* sp.) and AB1 (0.02 mg/kg) were detected. Anamnestically, the health disorders in sows and their litters were observed. Epidemiological investigation revealed that 2 months before a total of 50 new sows had been brought on the swine farm. Serologically, the presence of specific antibodies against MA was detected in 6 sows. However, despite the fact that these animals were serologically positive, the origin of that immunological status from the aspect of MA remained unknown: vaccination or infection. On the other hand, the evaluated swine farm stopped with imunoprophylaxisis against MA 8 years before. By clinical examination of the sows, the signs of inapetence, mild apathy and agalactiae were observed.

In suckling piglets the sings of severe distrurbance of the central nervous system (wide open eyes, paddling, trembling, ataxia, paresis and paralysis) were clinically detected. In some cases the whole litter of piglets died within 48 hours. Despite the fact that the sows and piglets were therapeutically treated, there was no evident respond to the applied medication. Clinically, the fatteners also became anorectic, listless and apathic. The pathomorphological changes detected in dead sucklings indicated the lesions characteristic for MA infection (*Necroses miliares hepatis***,** *Haemorrhagiae corticis renis, Tonsillitis diphtheroides necroticans).* By applying virological testing (viral isolation on the susceptible cell culture) of the tissues derived from dead piglets, the *Morbus Aujeszky* virus was isolated.

DISCUSSION

The achieved results support the existence of positive interaction between the mycotoxins and causative agents of swine infective diseases. The continuos intake of small amounts of mycotoxins leads to chronic intoxication which is clinically characterized by the loss of weight, insufficient weight gain and increased susceptibility to infectious diseases (D i e k m a n and G r e e n , 1992; O s w e i l e r , 2006). The clinical toxicological syndroms caused by ingestion of moderate to high amounts of mycotoxins and their effects on immune system have been well characterized (M e i s s o n n i e r et al., 2008; P r o d a n o v et al., 2009). From an epidemiological point of view, mycotoxins may cause breakdown of active immunity and occurrence of disease even in properly vaccinated animals (M a r i n et al., 2002; T a r a n u et al., 2005).

The presence of mycotoxin in feed can be directly connected to the detected health disturbances on the examined swine farms. In our research we discovered persistant presence of various infections which had poor or no reaction at all to the applied antimicrobial therapy (gastroenteritis, pneumonia, dysenteria suum, endometritis). Also, the chronic disturbances, such as slow growth, malnutrition and persistant presence of infections of low intensity suggest the potential presence of mycotoxins. Normal immune function is expected to be restored after exposure to the toxin ends (O s w e i l e r , 2006). The consumption of mycotoxin-contaminated feed leads to the induction of teratogenic, cancerogenic, oestrogenic, neurotoxic and immunosuppressive effect in the organism (K a b a k et al., 2006). As a consequence of immunosuppressive action of mycotoxins, clinical and pathological lesions corresponding to infectious diseases of different ethiology occured (O s w a l d et al., 2005; O s w e i l e r , 2006). The obtained results offer an example of immunosuppressive effect, i.e. the occurence of enteroxemia in piglets, despite the fact that dams were vaccinated twice during the gestation. The enterotoxemia is caused by pathogenic bacterial strains and occurs frequently as a cause of mortality in the examined production phase. It can be provoked by feed quality, i. e. the presence of mycotoxins. On the other hand, young animals are much more

sensitive to the effects of mycotoxins in comparison to the adults. Also, dietary deficiencies of protein, selenium and vitamins have been sugested as predisposing factors for mycotoxicoses (K a b a k et al., 2006). Piglets of the nursing sows, exposed to AB1, may be immunocompromized because residues of AF-M1 occur in milk (O s w e i l e r, 2006). The biggest challenge with mycotoxicoses is the non-specific nature of symptoms in the affected animals. Consequently, the health disorders due to mycotoxins in the feed are difficult to diagnose (O s w e i l e r, 2006; P r o d a n o v et al, 2008). The acute symptoms are relatively more specific in pigs, for example zearalenone-induced reproductive disorders, fumonisins-induced pulmonary oedema and deoxynivalenol ("DON")--induced feed refusal (M e i s s o n n i e r et al., 2008). Mycotoxin mixtures, i.e. the combinations of several micotoxins are likely to occur naturally and may influence the immunity in an additional or synergistic manner. An example is combination of AB1 and T-2 toxin, AB1 and OTA. The latest combination was detected on the first examined swine farm. Economic losses that occur as a consequence of interaction of several mycotoxins are still unknown because in low concentrations several mycotoxins may interact in a way that is difficult to detect. Combinations of several and more moderate concentrations of different mycotoxins, which individually may appear in a level too low to be concerned with, can cause cumulative toxicoses, which affect the ability of the pigs organism to fight diseases (D i e k m a n and G r e e n, 1992; O s w e i l e r, 2006).

In the second evaluted case, the presence of OCT and ZEA in the feed was detected. Consequently, an evident decrease in the swine immunity against infective diseases (of the respiratory and digestive tract) was noticed on the farm, and there was no positive respond to the applied antibiotic therapy. Also, the occurrence of diarrhoea and an increased percent of waste piglets can be connected with the presence of mycotoxins in the feed, because the piglets display clinical feed refusal. Some effects caused by the consumption of mycotoxin contaminated feed were experimentally detected after long exposure (10-15 days). This long exposure is likely to occur in the field, as animals might eat the same batch of feed for an extended period of time (O s w e i l e r, 2006). The gastrointestinal tract represents the first barrier against ingested food contaminants and natural toxins. Upon the ingestion of mycotoxin contaminated feed, intestinal epithelial cells could be exposed to a high concentration of toxin (B o u h e t and O s w a l d, 2007). Stability of the intestinal flora appeared to be an important factor for animal health (O s w a l d et al., 2005). Finally, while systemic immunity is the focus of most investigations, it is highly probable that mycotoxins have their greatest effect on mucosal lymphoid tissue before they are absorbed and subsequently metabolized (B o u h e t and O s w a l d, 2005; O s w a l d et al., 2005). More recently, it has been experimentally demonstrated that the ingestion of OTA contaminated feed increases the susceptibility of pigs to natural infection by *Salmonella cholerasuis, Serpulina hyodysenteriae, Campylobacter coli*. Furthermore, it was discovered that, after the oral intake of purified fumonisin B1, susceptibility of animals to intestinal infections significantly increased. Although *Escherichia coli* is a normal inhabitant of intestinal microphlora, pathogenic bacterial strains poses

the virulence determinants which are involved in septicaemia development (O s w a l d et al., 2005). The presence of mycotoxin in feed for pregnant sows has an influence on the occurrence of embrional and fetal death, and decreased immunological defence in piglets. Reproduction disorders, fetal mummification and stillbirths may be connected with mycotoxin contaminated feed (P r o - d a n o v et al., 2009). Nutritional effects associated with feed refusal may also contribute to the observed decreased efficacy of therapeutic treatments and vaccination (O s w a l d et al., 2005).

Immune functions suppressed by mycotoxins may decrease resistance to infectious diseases, reactivate chronic infections and reduce vaccine and therapeutic efficacy (O s w a l d et al., 2005). In the last examined case, where the outbreak of MA on the farm was examined, mycotoxin (AF) in the feed can be connected with the possible reactivation of chronic (latent) infection in sows. It has been discovered that aflatoxin decrease resistance to bacterial, fungal, viral and parasitic diseases in swine. Subsequently, vaccinations against various infectous diseases may be less effective in animals exposed to mycotoxins (D i e k m a n and G r e e n, 1992). For example, AB1 interferes with the development of acquired immunity in swine after erysipelas vaccination (O s w a l d et al., 2005). Even when present in low doses, AF alters the immune response and this may predispose pigs to infectious diseases (M a r i n et al., 2002; T a r a n u et al., 2005). The titers of specific antibodies after vaccination are good indication of humoral immunoresponses. It has been demonstrated experimentally that DON and ZEA impaired the production of specific antibodies after vaccination against classical swine fever (C h e n et al., 2008). Therefore, the presence of mycotoxins in the feed may lead to a breakdown in vaccinal immunity and the occurrence of disease even in properly vaccinated animals (O s w a l d et al., 2005). It should be remembered that detected concentrations of mycotoxins in the feed are approximations, because sampling is never completely representative. Mycotoxin can be identifeid by chemical analyses, but sometimes causative cereal that initiated the problem is no longer available or representative sample (O s w e i l e r , 2006). On the other hand, when discussing the mycotoxin problem, feed dilution may reduce the exposure, but wet or contaminated grain can introduce new fungi, and develop the conditions that eventually may lead to the entire mixture being contaminated (O s w a l d et al., 2005). Mycotoxin contamination may not be suspected until most or all of the contaminated feed is consumed. A wise product practice is to save a representative sample of feed purchased, and keep it under stable conditions until swines are marketed. In case of any doubts regarding the feed quality, those samples may be valuable in documenting whether specific feeds were involved in the problem (O s w e i l e r , 2006).

REFERENCES

Bouhet, S., Oswald, I. P. (2005): *The effects of mycotoxins, fungal food contaminants, on the intestinal epithelial cell-derived innate immune response*. Vet Immunol Immunopathol, 108 (1-2): 199-209.

Bouhet, S., Oswald, I. P. (2007): *The intestine as a possible target for fumonisin toxicity*. Mol Nutr Food Res, 51(8): 925-931.

Chen, F., Ma, Y., Xue, C., Ma, Y., Xie, Q., Wang, G., Bi, Y., Cao, Y. (2008): *The combination of deoxynivalenol and zearalenone at permitted feed concentrations causes serious physiological effects in young pigs*. J. Vet. Sci., 9 (1): 39-44.

Diekman, M. A. and Green, M. L. (1992): *Mycotoxins and reproduction in domestic livestock*. J Anim. Sci., 70:1615-1627.

Kabak, B., Dobson, A. D., Var, I. (2006): *Strategies to Prevent Mycotoxin Contamination of Food and Animal Feed*. Critical Reviews in Food Science and Nutrition, 46:593-619

Marin, D. E., Taranu, I., Bunaciu, R. P., Pascale, F., Tudor, D. S., Avram, N., Sarca, M., Cureu, I., Criste, R. D., Suta, V. and Oswald, I. P. (2002): *Changes in performance, blood parameters, humoral and cellular immune responses in weanling piglets exposed to low doses of aflatoxin*. J Anim. Sci., 80: 1250-1257

Meissonnier, G. M., Pinton, P., Laffitte, J., Cossalter, A. M., Gong, Y. Y., Wild, C. P., Bertin, G., Galtier, P., Oswald, I. P. (2008): *Immunotoxicity of aflatoxin B1: impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression*. Toxicol. Appl. Pharmacol., 231(2): 142-149.

Oswald, I. P., Marin, D. E., Bouhet, S., Pinton, P., Taranu, I. and Accensi, F. (2005): *Immunotoxicological risk of mycotoxins for domestic animals*. Food Additives & Contaminants, 22(4): 354-360.

Osweiler, G. D. (2006): *Occurrence of Mycotoxins in Grains and Feeds*. In: Straw, E. B., Zimmerman, J. J., D. Allaire, S., Taylor, D. J., Eds, Diseases of Swine, Ed 9th, Blackwell Publishing, Ames, Iowa, 915-930.

Prodanov, J., Došen, R., Pušić, I., Stojanov, I., Ratajac, R., Živkov-Baloš, M. (2009): *The clinical and pathomorphological diagnosis of mycotoxicosis in different swine categories*. Proc. Nat. Sci. Matica Srpska, Novi Sad, 116: 281-287.

Taranu, I., Marin, D. E., Bouhet, S., Pascale, F., Bailly, J. D., Miller, J. D., Pinton, P. and Oswald, I. P. (2005): *Mycotoxin Fumonisin B_1 Alters the Cytokine Profile and Decreases the Vaccinal Antibody Titter in Pigs*. Toxicological Sciences, 84: 301-307.

ИНТЕРАКЦИЈА МИКОТОКСИНА СА УЗРОЧНИЦИМА ИНФЕКТИВНИХ ОБОЉЕЊА СВИЊА

Јасна З. Проданов-Радуловић, Радослав Ђ. Дошен, Игор М. Стојанов, Иван М. Пушић, Милица М. Живков-Балош, Радомир Д. Ратајац

Научни институт за ветеринарство „Нови Сад“, Руменачки пут 20, 21000 Нови Сад, Београд

Резиме

Микотоксини као секундарни метаболити плесни контаминирају анимална хранива у свим стадијумима њихове производње. Конзумација хранива контаминираних микотоксинима може имати за последицу имуносупресивно дејство у организму, што представља фактор предиспозиције за инфективна обољења. Дејство на имунолошки систем је од посебног значаја за свињарство, јер технологија на фармама свиња подразумева примену многобројних вакцинација, што представља проблем код имунолошки компромитованих јединки. Циљ рада је био испитивање утицаја микотоксина у свињарству, као секундарних фактора у дестабилизацији имунолошког система животиња.

Материјал за испитивање је обухватао пет фарми свиња, на којима су регистровани здравствени проблеми односно утврђено је присуство клиничких и патоморфолошких знакова инфективних обољења различитих категорија свиња. У оквиру примењених метода, обављена су епизоотиолошка и клиничка испитивања, патоморфолошки преглед угинулих јединки, вирусолошко и бактериолошко испитивање узорака органа угинулих јединки и микробиолошко испитивање узорака хране у циљу установљавања присуства плесни и микотоксина. Постигнути резултати испитивања указују на постојање позитивне интеракције између микотоксина и узрочника инфективних обољења свиња. Са епидемиолошког аспекта, значајно је да микотоксини у храни за животиње могу довести до пробоја активног имунитета и избијања обољења иако је имунопрофилакса обављена на прописан начин.