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EVALUATION OF INFLUENCE OF \textit{Fusarium} MYCOTOXINS ON INTENSITY OF INTESTINAL SWINE DISEASES

Jasna Prodanov-Radulović\(^1\), Milica Živković-Baloš\(^1\), Sandra Jakšić\(^1\), Igor Stojanov\(^1\), Jelena Petrović\(^1\), Radomir Ratajč\(^1\), Jovan Bojkovski\(^2\)

\(^{1}\) Scientific Veterinary Institute “Novi Sad”, Rumenački put 20, 21000 Novi Sad, Serbia
\(^{2}\) Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobođenja 18, Beograd, Serbia

*Corresponding author: E-mail address: jasna@niv.ns.ac.rs

ABSTRACT

Mycotoxins are structurally diverse fungal metabolites that can contaminate a variety of dietary components consumed by animals and humans. The aim of the paper was evaluation of possible influence of \textit{Fusarium} mycotoxins detected in complete swine feed mixtures in 2015 year on intestinal swine diseases in swine farms located in Vojvodina region. The material for research included the samples from four swine farms, where health disorders i.e. clinical and gross pathological signs resembling to the problem with intestinal infectious diseases in different swine categories were detected. The applied research methods included: epidemiological and clinical evaluation, pathomorphological examination, laboratory bacteriological tissue testing originating from diseased, dead animals. The presence of deoxynivalenol (DON), T-2 toxin and zearalenone (ZEA) in twelve complete swine feed mixtures were analyzed by enzyme-linked immunosorbent assay methods, using Ridascreen\textsuperscript{®} FAST DON (Art.No.R5901), Ridascreen\textsuperscript{®}FAST T2 (Art.No.R5302) and Ridascreen\textsuperscript{®}FAST Zearalenon (Art. No. R5502) test kits (R-Biopharm, Germany). By clinical and pathological examination, in neonatal piglets the lesions dominantly in digestive tract were observed. Applying bacteriological testing \textit{Escherichia coli} and \textit{Escherichia coli haemolytica} were detected. Similarly, enteric infections and alteration of growth performance were notified in weaners and fatteners. In adult swine categories, reduced feed consumption, sometimes distinct feed refusal and vomiting were observed. In all examined samples of complete feed mixtures for different swine categories the concentration of DON exceeded the maximum permitted levels. The obtained results indicate the existence of possible positive interactions between the \textit{Fusarium} mycotoxins and causative agents of intestinal swine diseases.

\textbf{Keywords:} mycotoxins, Fusarium, swine, intestinal health

INTRODUCTION

Mycotoxins are structurally diverse fungal metabolites that can contaminate a variety of dietary components consumed by animals and humans. It is estimated that 25% of the world’s crop production is contaminated by mycotoxins during the pre-harvest period, transport, processing or storage (Greiner \textit{et al.}, 2013; Weaver \textit{et al.}, 2013). The major mycotoxin-producing fungal genera are \textit{Aspergillus}, \textit{Fusarium} and \textit{Penicillium}, mainly producing aflatoxins, zearalenone (ZEN), trichothecenes (TCT), fumonisins, ochratoxins and ergot alkaloids (Piotrowska \textit{et al.}, 2014). Among the mycotoxins produced by the \textit{Fusarium} genus, the broad family of TCT is extremely prevalent (Pinton and Oswald, 2014) as well as ZEN (Wache \textit{et al.}, 2009; Burel \textit{et al.}, 2013).

The toxicological syndromes caused by ingestion of mycotoxins range from sudden death to reproductive disorders and growth impairment. Consumption of fungal toxins may also decrease resistance to infectious diseases (Wache \textit{et al.}, 2009; Prodanov-Radulovic \textit{et al.}, 2014). Chronic exposure can lead to anorexia, reduced weight gain, as well as nutritional efficiency, neuroendocrine changes and immune modulations (Pestka, 2007). A major problem associated with animal feed contaminated with mycotoxins is not acute disease, but rather the ingestion of low levels of toxins, which may cause an array of metabolic, physiologic and immunologic disturbances (Stojanov \textit{et al.}, 2013; Waśkiewicz \textit{et al.}, 2014).
One of the most commonly detected TCT in cereals is 4-deoxynivalenol (DON), also known as vomitoxin, a mycotoxin to which pigs seem to be the most susceptible species among the domestic animals (Pestka, 2007; Weaver et al., 2013). Because of the high percentage of wheat in pig diets, swine could be at a greater risk of exposure to this toxin. In swine, the initial adverse effect observed after DON exposure is reduced feed intake. Growth (anorexia and decreased nutritional efficiency), immune function (suppression) and reproductive performances (reduced litter size) are also adversely affected by DON (Pestka and Smolinsky, 2005). The intestine is the major site of DON absorption. In the pig, DON is rapidly and efficiently absorbed, most probably in the upper part of the small intestine, and is mainly excreted in the urine, with no accumulation in tissues. Consumption of DON-contaminated feed in pigs impacts the gastrointestinal tract, causing epithelial injuries of the stomach and the intestine, leading to intestinal inflammatory response. In vitro and in vivo studies have also demonstrated that DON inhibits intestinal nutrient absorption, alters intestinal cell functions, and compromises the intestinal barrier function (Wache et al., 2009).

By contrast, the effect of DON on the intestinal microflora has been poorly investigated (Waśkiewicz et al., 2014). Another Fusarium mycotoxin of toxicological significance for pigs is ZEN. Zearalenone is a macrocyclic lactone that is primarily generated, together with over 120 of its derivatives, by Fusarium graminearum, F. culmorum, F. cerealis, and F. equiseti both in the field and during the storage of corn, barley, sorghum, and soya in disadvantageous environmental conditions (Nordkvist and Häggblom, 2014). In animals, ZEN is transformed into α- and β-zearalenol, of which the former binds to estrogen receptors much more strongly than ZEN. Exposure to ZEN leads to changes in the reproductive system of animals, such as edematous uterus, ovarian cysts, and increased follicular maturation (Burel et al., 2013; Piotrowska et al., 2014).

The presence and often co-occurrence of DON and ZEN in cereal pig feed has emerged as a severe health, welfare and reproduction problem worldwide because of multitude of effects by the mycotoxins. In recent surveys it was shown that cereals used in European pig feed are commonly contaminated with DON and/or ZEN as well as other TCT such as T-2 and HT-2 toxins (EFSA, 2013; Nordkvist and Häggblom, 2014). Pigs are considered to be the farm animals which are the most affected by mycotoxins in general (Burel et al., 2013; Wache et al., 2009). Mycotoxins are often present in swine feed in amount that can have detrimental impact on swine health (Prodanov-Radulović et al., 2014).

The aim of the paper was evaluation of possible influence of Fusarium mycotoxins detected in complete swine feed mixtures in 2015 year on intestinal swine diseases in commercial swine farms located in Vojvodina region.

MATERIAL AND METHODS

The material for research included the samples from four swine farms, where health disorders i.e. clinical and pathomorphological signs resembling to the problem with intestinal infectious diseases in different swine categories (suckling and weaned piglets, fatteners, gilts, sows) were detected. Depending on the specificity of each evaluated case and available material, the applied research methods included: epidemiological and clinical evaluation, gross pathological examination, standard laboratory testing for detection the presence of aerobic and anaerobic bacteria and microbiological feed testing.

History of the pig units

The following details were ascertained by the interview and from farm records: number and category of pigs on the unit, production details (farrow-to-finish, nucleus or commercial fattening farm), disease status, current veterinary health plan (vaccination programmes, medication), biosecurity protocols and feeding system used. The animals were observed and inspected for clinical signs of disease and abnormal behaviour. The clinical inspection was
followed by the necropsy of diseased, dead pigs for gross pathological diagnosis and tissue sampling for further laboratory investigation.

**Bacteriology and molecular testing**

Isolation of bacteria from tissue samples deriving from dead pigs was performed by standard aerobic and microaerophilic cultivation. Microscopic examination determined whether the isolated bacteria were Gram positive or not and whether it is a coccoid or rod-like organisms. The determination was carried out by determining the biochemical characteristics of the isolated bacteria (Quinn *et al*., 2011). Beside this, the molecular diagnostic method, a multiplex RT-PCR for detection of *Brachyspira hyodysenteriae* (DNA extracted from feces) (La *et al*., 2006) was applied.

**Mycotoxicological feed testing**

The presence of DON, T-2 toxin and ZEA in twelve complete swine feed mixtures were analyzed by enzyme-linked immunosorbent assay methods (ELISA), using Ridascreen®FAST DON (Art. No. R5901), Ridascreen®FAST T2 (Art. No. R5302) and Ridascreen®FAST Zearalenon (Art. No. R5502) test kits (R-Biopharm, Germany).

**RESULTS AND DISCUSSION**

The first examined farm represents the modern commercial swine farm, located in Južnobački district in Vojvodina. In the time of examination, on the farm the following swine categories were included: 850 sows, 7 boars, 120 growing gilts, 290 breeding gilts, 1560 suckling piglets, 5051 weaned piglets and 7050 fatteners. The farm represents the one-site production system (farrow-to-finish) i.e. all production stages occurring at one site. The farm have organized own veterinary services and swine health control program include vaccination against *Classical Swine Fever* (CSF), *Porcine Parvovirus* (PPV), *Mycoplasma hyopneumoniae*, *Circovirus type 2* (PCV-2), *Erysipelas* and sows vaccination against enteric bacterial infections (*Clostridium perfringens* and *Escherichia coli*). The last mentioned vaccination of dams is applied during gestation with the aim to prevent disease in piglets in the first days of life. In the case of disease outbreak, the affected categories are therapeutically treated (parenteral injection for clinically diseased animals and water/feed medication for in-contacts). Recently, the health disturbances in the female breeding categories on the farm were registered: different levels of decreased feed consumption, in some animals even complete feed refusal (anorexia). In some cases vomiting in sows was detected. Clinically, the diarrhea in weaned piglets around weaning (28-35 day of age) was notified. After supervision of the farm records it was discovered that diarrhea occurs in the piglets of normal birth body weight and on the weaning there is 30% of small piglets. Therapeutic treatment of piglets by antimicrobials did not improve health problems. Performing further health control in the weaned piglets the signs of pneumonia (dyspnea, cough, serous to purulent nasal discharge) were detected. The gross pathological examination of the dead weaned piglets revealed lesions dominantly on the mucosal surface of the digestive tract (*Haemorrhagiae mucosae ventriculi, Enteritis catharralis acuta et haemorrhagica*). Also, in some cases the gross pathological changes in lungs were discovered (*Pleuroneumonia, Pneumonia fibrinosa*). By bacteriological testing on tissue samples deriving from dead animals the following bacteria was detected: *Escherichia coli* (*E.coli*), *E. coli haemolytica*, *Arcanobacterium pyogenes*, *Pasteurella multocida*. Microbiological testing of complete feed mixture for piglets (grover) detected significant increase in the number of fungi genera *Fusarium* (200000 CFU/g), as compared to the level set by the regulation (<50000 CFU/g) (Official Gazette RS, 2010). Applying further laboratory testing, the presence of DON in the feed for pregnant and lactating sows was detected.
In the second and third evaluated swine farms, the presence of DON in the feed for fatteners was detected. These two farms represent one-site production system i.e. on the farm there is only fattener production, capacity 2000 animals. The pigs are delivered from two large farrow-to-finish, commercial swine farms at the body weight around 20-25 kg. Anamnestically and clinically, the health problems included increased incidence of gastrointestinal diseases. Analysing the existing data on the seconds and third farm, the high incidence of morbidity in fatteners was noticed, which did not decreased after medical treatment.

Table 1. The results of mycotoxicological testing swine feed samples from four examined farms

<table>
<thead>
<tr>
<th>Swine Farm</th>
<th>Complete feed mixture for:</th>
<th>Detected level of investigated mycotoxins (µg/kg)</th>
<th>Reference value (µg/kg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number 1</td>
<td>Pregnant gilts and sows</td>
<td>DON 3140</td>
<td>&lt; 900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T-2 &lt; 33</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZEA 500.13</td>
<td>&lt; 500</td>
</tr>
<tr>
<td>Number 2</td>
<td>Lactating sows</td>
<td>DON 3890</td>
<td>&lt; 900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZEA 282.90</td>
<td>&lt; 500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T-2 &lt; 33</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td>Fatteners</td>
<td>DON 2940</td>
<td>&lt; 900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T-2 &lt; 33</td>
<td>/</td>
</tr>
<tr>
<td>Number 3</td>
<td>Fatteners</td>
<td>ZEA 197.01</td>
<td>&lt; 500</td>
</tr>
<tr>
<td></td>
<td>Weaned piglets 20–30 kg body weight</td>
<td>DON 2000</td>
<td>&lt; 900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T-2 36.99</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZEA &gt; 400</td>
<td>&lt; 200</td>
</tr>
<tr>
<td>Number 4</td>
<td>Breeding animals 30–60 kg body weight</td>
<td>DON 1770</td>
<td>&lt; 900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T-2 &lt; 33</td>
<td>/</td>
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<tr>
<td></td>
<td></td>
<td>ZEA &gt; 400</td>
<td>&lt; 200</td>
</tr>
<tr>
<td></td>
<td>Breeding animals 60–100 kg body weight</td>
<td>DON 2500</td>
<td>&lt; 900</td>
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<tr>
<td></td>
<td></td>
<td>T-2 41.18</td>
<td>/</td>
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<tr>
<td></td>
<td></td>
<td>ZEA 300.23</td>
<td>&lt; 500</td>
</tr>
<tr>
<td></td>
<td>Fatteners</td>
<td>DON 4340</td>
<td>&lt; 900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T-2 &lt; 33</td>
<td>/</td>
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<tr>
<td></td>
<td></td>
<td>ZEA 551.84</td>
<td>&lt; 500</td>
</tr>
<tr>
<td></td>
<td>Fatteners</td>
<td>DON 3890</td>
<td>&lt; 900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T-2 &lt; 33</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZEA 628.27</td>
<td>&lt; 500</td>
</tr>
<tr>
<td></td>
<td>Lactating sows</td>
<td>DON 2590</td>
<td>&lt; 900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T-2 &lt; 33</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZEA 442.52</td>
<td>&lt; 500</td>
</tr>
<tr>
<td></td>
<td>Pregnant sows</td>
<td>DON &gt; 6000</td>
<td>&lt; 900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T-2 37.56</td>
<td>/</td>
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<tr>
<td></td>
<td></td>
<td>ZEA &gt; 800</td>
<td>&lt; 500</td>
</tr>
</tbody>
</table>

* maximum permissible level according to Serbian regulations (Official Gazette RS, 2014)

Therapeutic treatment of the diseased animals was intensive and multiple: the antibiotics were given through feed, water and parenterally. Clinically, the bloody diarrhoea and perineal staining in most of fatteners was detected. In some cases, the diarrhoea was greyish black, with blood and mucus flecks. A reduced feed consumption, loss of weight and insufficient weight gain were also notified. Therapeutic treatment with antibiotics only temporarily improved health problems. However, reappearance of diarrhea often occurs after removal of antimicrobials from the water or feed. Applying gross pathological examination on the dead weaners and finishers, the prominent changes on the digestive tract (Gastroenteritis
haemorrhagica, Typhlocolitis haemorrhagica, Ulcus oesophagogastricum) and respiratory tract (Pleuritis adhaesiva diffusa) were detected. By bacteriological testing from the tissue samples deriving from the dead fatteners, E. coli and E. coli haemolytica were isolated. Applying RT-PCR method on the fecal samples derived from weaners and finishers, Brachyspira hyodysenteriae was detected.

The results showed that DON and ZEN exposure to the pigs occurred at different levels from examined complete mixture samples. Research investigating the influence of mycotoxins on the animal susceptibility to infectious diseases focuses mainly on exposure to single major mycotoxins. However, limited information is available on the interaction between low levels of mycotoxins and causative agents of swine infectious diseases (Prodanov-Radulović et al., 2014). The main effect of DON ingestion in pigs, is a decrease of both feed intake and weight gains. These effects are observed for contamination level above 1 mg/kg feed and are dependent on the age of animals and the feeding period (Wache et al., 2009). The gastrointestinal tract is the first barrier against feed contaminants as well as the first target for mycotoxins. DON affects the integrity of intestinal epithelium through alterations in cell morphology and differentiation in the barrier function. The intestine is a preferential immune site where immune regulatory mechanisms simultaneously defend the body against pathogens, but also maintain tissue homeostasis to avoid immune-mediated pathology in response to environmental challenges (Burel et al., 2013; Waśkiewicz et al., 2014).

The last examined farm represent the modern commercial swine farm, located in Južnobački district in Vojvodina. In the time of examination, on the farm the following swine categories were included: 1550 sows, 30 boars, 285 breeding gilts, 2765 suckling piglets, 6573 weaned piglets and 5650 fatteners. The farm represent the one-site production system (farrow-to-finish) i.e. all production stages occurring at one site. The farm have organised own veterinary services and swine health control programm include: the vaccination against CSF, PPV, Mycoplasma hyopneumoniae, PCV-2, Erysipelas and sows vaccination against Cl.perfringes and E. coli. In the case of health disturbance, the animals are therapeutically treated (parenteral injections, in-feed and in-water medication). By clinical and pathological examination, in neonatal piglets the clinical sign of vulvovaginitis and lesions dominantly in digestive tract were discovered. Applying bacteriological testing E. coli and E.coli haemolytica were detected. Similarly, enteric infections and alteration of growth performance were notified in weaners and fatteners. In breeding swine categories, reduced feed consumption, sometimes distinct feed refusal and vomiting were observed. In all examined samples of complete feed mixtures for different swine categories the concentration of DON and ZEA exceeded the maximum permitted levels (Table 1). According to Serbian Regulation (Official Gazette RS, 2014) there is no maximal permitted level for T2 toxin. Indicative levels for the sum of T-2 and HT-2 in compound feed according to Commission recommendation of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products (EC, 2013) is 250 µg/kg.

The continuos intake of small amounts of mycotoxins may leads to chronic intoxication which is clinically characterized by the loss of weight, insufficient weight gain and increased susceptibility for infectious diseases (Wache et al., 2009; Prodanov-Radulovic et al, 2014). The reduction in weight gain as a consequence of reduced feed consumption is strongly associated with the exposure of farm animals to DON, with pigs being one of the most sensitive species (Piotrowska et al., 2014). The reporting of intestinal lesions has been inconsistent and not systematically correlated with the clinical signs. Congestion and erosions of the gastric and intestinal mucosae have been described following chronic DON exposure in pigs. The intestinal mucosa is the first biological barrier encountered by natural toxins, and consequently, it could be exposed to high amounts of dietary toxins. The mycotoxins may induce intestinal pathologies, including necrosis of the intestinal epithelium. They also disturb the barrier function, potentially leading to the increased translocation of pathogens and an increased susceptibility to enteric infectious diseases (Pinton and Oswald,
Unfortunately, the toxicity of combinations of mycotoxins cannot always be predicted based upon their individual toxicities. Recent data suggest that the type of interaction depends not only on the type of toxin and their ratio, but also on the concentration of the toxin-mixture at a constant ratio (Wache et al., 2009; Nordkvist and Häggblom, 2014). Our results are in agreement with other studies, showing a transient strong effect of DON on feed intake in pigs and occurrence of clinical signs of gastrointestinal disturbances (vomiting, anorexia). On the investigated swine farms, we noticed the presence of various persistant infections of gastrointestinal tract, which react poorly or do not react on the applied antimicrobial therapy. 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 (Prodanov-Radulovic et al., 2014).

**CONCLUSIONS**

The obtained results indicate the presence of DON above the maximum permissible level according to Serbian National Regulations in all examined samples of complete swine mixtures. Beside this, on investigated swine farms the existence of possible positive interactions between the *Fusarium* mycotoxins and causative agents of intestinal swine diseases may be suggested. Certainly, more comprehensive research is needed to understand the impact of mycotoxin combinations and to determine when synergistic interactions occur.

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