

*Jasna Z. PRODANOV-RADULOVIĆ\**,  
*Igor M. STOJANOV<sup>1</sup>, Milica M. ŽIVKOV-BALOŠ<sup>1</sup>,*  
*Sandra M. JAKŠIĆ<sup>1</sup>, Ivan M. PUŠIĆ<sup>1</sup>,*  
*Jovan A. BOJKOVSKI<sup>2</sup>*

<sup>1</sup> Scientific Veterinary Institute “Novi Sad”,  
Rumenački put 20, Novi Sad 21000, Republic of Serbia

<sup>2</sup> Faculty of Veterinary Medicine, University of Belgrade,  
Bulevar oslobođenja 18, Belgrade 11000, Republic of Serbia

## IMPACT OF *Fusarium* MYCOTOXINS ON SWINE HEALTH – FIELD OBSERVATIONS

**ABSTRACT:** Mycotoxins are structurally diverse fungal metabolites that can contaminate a variety of dietary components consumed by animals and humans. The aim of this paper was to present the field observations of clinical and pathological consequences on swine health in the cases when *Fusarium* mycotoxins were detected in swine feed. The material for research included the samples from swine farms located in the region of Vojvodina, where health disorders resembling intestinal problems in different swine categories were detected. The applied research methods included: epidemiological and clinical evaluation, gross pathology examination, bacteriological tissue testing originating from diseased dead animals. The presence of deoxynivalenol (DON), T-2 toxin and zearalenone (ZEA) in thirteen complete swine feed mixtures were analyzed by enzyme-linked immunosorbent assay methods, using Ridascreen®FAST DON, Ridascreen®FAST T2, and Ridascreen®FAST Zearalenon test kits (R-Biopharm, Germany). By clinical and pathological examination, the lesions predominantly located in digestive tract were observed in different swine categories. The problem of persistent enteric infections in suckling piglets and alteration of growth performance were notified in weaners and fatteners. In adult 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, but also the presence of other *Fusarium* mycotoxins was detected. The obtained results indicate the existence of feed mixtures contamination with low levels of *Fusarium* mycotoxins and their possible positive interaction with etiological agents present in swine farms.

**KEYWORDS:** swine health, *Fusarium* mycotoxins, Vojvodina

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\* Corresponding author. E-mail: [jasna@niv.ns.ac.rs](mailto:jasna@niv.ns.ac.rs)

## INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by certain fungi, which can cause a variety of adverse effects on both humans and animals (Osweiler, 2006; Kabak, 2012). Depending on classification, 300–400 mycotoxins are known today (Streit *et al.* 2012; Weaver *et al.* 2013). In many parts of the world, mold-contaminated feedstuffs and resultant mycotoxin levels in the diet constitute a considerable health hazard (Jackson and Cockcroft, 2007; Streit *et al.* 2012). It is estimated that 25% of the world's crop production is contaminated by mycotoxins during the pre-harvest period, transport, processing or storage (Greinier and Applegate, 2013; Weaver *et al.* 2013). The major mycotoxin-producing fungal genera are *Aspergillus*, *Fusarium* and *Penicillium*, mainly producing aflatoxins (AF), zearalenone (ZEN), trichothecenes (TCT), fumonisins, ochratoxins and ergot alkaloids (Piotrowska *et al.* 2014). Among the mycotoxins produced by *Fusarium* genus, the broad family of TCT is extremely prevalent (Pinton and Oswald, 2014) as well as ZEN (Wache *et al.* 2009; Burel *et al.* 2013).

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 (Prodanov-Radulović *et al.* 2014; Waśkiewicz *et al.* 2014). Consumption of fungal toxins in swine as a species may decrease resistance to infectious diseases (Prodanov-Radulović *et al.* 2011; Prodanov-Radulović *et al.* 2014). Also, chronic exposure can lead to anorexia, reduced weight gain, as well as nutritional efficiency and neuroendocrine changes (Pestka, 2007; Prodanov-Radulović *et al.* 2013). The aim of this paper was to present the field observations of clinical and pathological consequences on the swine health, in the cases when *Fusarium* mycotoxins were detected in complete swine feed mixtures.

## MATERIAL AND METHODS

The material for research included the samples from swine farms located in the region of Vojvodina, where health disorders i.e. clinical and gross pathological signs resembling the problems with permanent intestinal infectious diseases in different swine categories were detected. The applied research methods included: epidemiological and clinical evaluation, gross pathology examination and laboratory testing. The following details were determined by inspecting farm records: number and category of pigs, type of production (farrow-to-finish, fattening farm), disease status, current veterinary health plan (vaccination, medication). The clinical inspection of live animals was followed by the necropsy of dead pigs for gross pathological diagnosis and tissue sampling for further bacteriological testing. Isolation of bacteria from tissue samples deriving from dead pigs was performed by standard aerobic and microaerophilic cultivation (Quinn *et al.* 2011). Beside this, the molecular diagnostic method, a multiplex

RT-PCR for detection of *Brachyspira hyodysenteriae* and *Lawsonia intracellularis* (DNA extracted from feces) (La *et al.* 2006) was applied. The presence of deoxynivalenol (DON), T-2 toxin, and ZEA in thirteen complete swine feed mixtures were analyzed by enzyme-linked immunosorbent assay methods, 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, farrow-to-finish production system with the following production capacity: 750 sows, 7 boars, 120 growing gilts, 290 breeding gilts, 1,260 suckling piglets, 4,550 weaned piglets and 6,030 fatteners. The farm organizes its own veterinary services, and swine health control program includes vaccination according to the Law Regulations (*Classical Swine Fever*) and against most frequently diagnosed diseases in the region (*Porcine parvovirus*, *Mycoplasma hyopneumoniae*, *Circovirus – PCV2*, *Erysipelas*) and vaccination against *Clostridium perfringens* and *Escherichia coli*. The last mentioned vaccination of dams is applied twice during late gestation (30–42 and 15–20 days prior to farrowing date) with the aim to prevent enteric disease in piglets in the first days of life. In the case of disease outbreak, the affected categories are therapeutically treated with antimicrobials (parenteral injection for clinically diseased animals and water and/or feed medication for in-contacts). Recently, the following health disturbances in the female breeding categories in the farm were registered: different levels of decreased feed consumption and lethargy in sows and gilts, while in some animals even complete feed refusal was notified (anorexia). Vomiting in sows was also detected. Clinically, the diarrhea in weaned piglets around weaning (28–32 day of age) was notified. However, therapeutic treatment of piglets by antimicrobials did not improve health problems. The gross pathological examination of the dead weaned piglets revealed lesions predominantly in the digestive tract (*Haemorrhagiae mucosae ventriculi*, *Gastritis ulcerosa multiplex*, *Enteritis catharralis acuta et haemorrhagica*). By bacteriological testing of tissue samples deriving from dead animals the following bacteria were detected: *E. coli*, *E. coli haemolytica*, *Cl. perfringens*, and *Salmonella typhimurium*. Microbiological testing of complete feed mixture for piglets (Grover) revealed significant increase in the number of fungi genera *Fusarium* (200,000 CFU/g), as compared to the level set by the regulation (<50,000 CFU/g) (*Official Gazette RS*, 2010). Applying further laboratory testing of complete mixtures for breeding categories, the presence of DON in the feed for pregnant and lactating sows was detected. Additionally, in complete feed mixtures for weaned piglets (body weight 15–25 kg) the presence of elevated values of ZEA and DON was discovered (Table 1).

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

Swine Farm	Complete feed mixture for category:	Detected level of investigated mycotoxins ( $\mu\text{g}/\text{kg}$ )		Reference value ( $\mu\text{g}/\text{kg}$ )*
Number 1	Lactating sows	DON	3,890	< 900
		ZEA	282.90	< 500
		T-2	< 33	/
	Pregnant gilts and sows	DON	3,140	< 900
		T-2	< 33	/
		ZEA	500.13	< 500
	Weaned piglets (15–25 kg body weight)	DON	4,249	<900
		ZEA	>400	<200
		T-2	< 33	/
Number 2	Fatteners	DON	2,940	< 900
		T-2	< 33	/
		ZEA	197.01	< 500
Number 3	Fatteners	DON	4,240	< 900
		ZEA	287.01	< 500
Number 4	Weaned piglets (20–30 kg body weight)	DON	2,000	< 900
		T-2	36.99	/
		ZEA	> 400	< 200
	Weaned piglets (20–30 kg body weight)	DON	5,620	< 900
		T-2	< 33	/
		ZEA	> 400	< 200
	Breeding animals (30–60 kg body weight)	DON	1,770	< 900
		T-2	< 33	/
		ZEA	> 400	< 200
	Breeding animals (60–100 kg body weight)	DON	2,500	< 900
		T-2	41.18	/
		ZEA	500.23	< 500
	Fatteners	DON	4,340	< 900
		T-2	< 33	/
		ZEA	551.84	< 500
	Fatteners	DON	3,890	< 900
		T-2	< 33	/
		ZEA	628.27	< 500
	Lactating sows	DON	2,590	< 900
		T-2	< 33	/
		ZEA	442.52	< 500
Pregnant sows	DON	> 6,000	< 900	
	T-2	37.56	/	
	ZEA	> 800	< 500	

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

In the second and third evaluated swine farms, the presence of DON in the feed for fatteners was detected (Table 1). These two farms represent one-site production system (fatteners) and have the capacity of 2,000–3,000 animals. Anamnestically and clinically, the health problems included increased incidence of gastrointestinal diseases. Analyzing the available data on the farms, the high incidence of morbidity in fatteners was noticed (intermittent diarrhea), which did not decrease after medical treatment. Therapeutic treatment of the diseased animals was intensive and multiple (antibiotics were given through feed, water and in some cases, parenterally). Clinically, the bloody diarrhea and perineal staining in most of the fatteners was notified. In some cases, the diarrhea was greyish black, with blood and mucus flecks. A reduced feed consumption, loss of weight and insufficient weight gain were also present. Therapeutic treatment with antibiotics only temporarily improved health problems. On post-mortem examination, all dead pigs were in poor condition. Applying gross pathological examination on the dead fatteners, the prominent changes on the digestive tract (*Gastroenteritis haemorrhagica*, *Typhlocolitis haemorrhagica*, *Ulcus oesophogogastricum*) were detected. The large bowel was full of liquid feces and blood. The surface of mucosa was diphtheric, serosal, dark purple and edematous. In some cases, the mucosal proliferation causing erosions in the ileum was discovered. Some animals died suddenly because of blood clot in the lumen of the large intestine. By bacteriological testing from the tissue samples deriving from the dead fatteners, only *E. coli* and *E. coli haemolytica* were isolated. However, applying molecular diagnostic method (multiplex RT-PCR) on the fecal samples derived from finishers, *B. hyodysenteriae* and *L. intracelullaris* were detected.

The last examined farm represents the commercial swine farm, with one-site production system (farrow-to-finish), located in the South Bačka District in Vojvodina. At the time of examination, farm had the following production capacity: 1,250 sows, 25 boars, 205 breeding gilts, 2,365 suckling piglets, 6,073 weaned piglets, and 5,150 fatteners. The farm organizes its own veterinary services and swine health control program includes vaccination against the same diseases as first presented farm. In the case of health disturbance, the animals are therapeutically treated (when necessary parenteral injections, but mostly in-feed and in-water medication are applied). By clinical examination, in neonatal piglets the clinical signs of vulvovaginitis and diarrhea almost immediately after farrowing (first days of life) were detected. Applying gross pathological examination of dead suckling piglets, prominent lesions predominantly in the upper part of digestive tract were discovered (*Gastritis ulcerosa multiplex*, *Haemorrhagiae mucosae ventriculi*, *Enteritis haemorrhagica*). By bacteriological testing, only *E. coli* and *E. coli haemolytica* were detected. Similarly, enteric infections and alteration of growth performance were notified in weaners and a number of fatteners. In breeding swine categories, reduced feed consumption, sometimes distinct feed refusal and vomiting were periodically 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 maximum 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 results of examined complete mixture samples showed that the exposure of pigs to DON and ZEN occurred at different levels. A 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 (Osweiler, 2006; Prodanov-Radulović *et al.* 2011). The continuous intake of small amounts of mycotoxins can lead 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; Weaver *et al.* 2013). Congestion and erosions of the gastric and intestinal mucosae have been described following chronic DON exposure in pigs. However, reporting of intestinal lesions has been inconsistent and not systematically correlated with the clinical signs (Greiner and Applegate, 2013; Pinton and Oswald, 2014). 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, 2014). Unfortunately, the toxicity of combinations of mycotoxins cannot always be predicted based upon their individual toxicities (Wache *et al.* 2009; Burel *et al.* 2013). Clinically observed vulvovaginitis (swelling and reddening of the vulva) in newborn piglets is a consequence of the presence of an oestrogenic mycotoxin (ZEA), produced by *Fusarium* fungus (Osweiler, 2006; Jackson and Crackcroft, 2007). It is characteristic that the clinical signs appear within a few days of pigs being exposed to the mycotoxin and disappear within a few days of the toxin being absent from the food (Jackson and Cockcroft, 2007). Female piglets on the sow are most frequently affected: toxins pass into the milk and hence to suckling piglets (McOrist, 2014). This condition is called the perinatal hyperestrogenic syndrome and represents the consequence of ZEA presence in feed for pregnant sows and its excreted metabolite in their milk (Prodanov-Radulović *et al.* 2011; Prodanov-Radulović *et al.* 2013).

During the last decade, the occurrence of mycotoxins in feed materials increased, and this may be a result of changes in agricultural practice but also the consequence of climate changes (Stojanov *et al.* 2013; Weaver *et al.* 2013). Most of the known mycotoxins have a short biological half-life and do not accumulate in animal tissues. They are, therefore, not a hazard for consumers of pig meat (McOrist, 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, diarrhea) (Greiner and Applegate, 2013; Wache *et al.* 2009). In the investigated swine farms, we noticed the presence of various persistent infections of intestinal tract of different etiology (enterotoxigenic, salmonellosis, swine dysentery, proliferative ileitis), which react poorly or do not react to the applied antimicrobial therapy.

## CONCLUSION

In the investigated swine farms, the existence of possible positive interactions between *Fusarium* mycotoxins and causative agents of intestinal swine diseases may be suggested. A more comprehensive research is needed to understand the impact of mycotoxin combinations and to determine when synergistic interactions occur.

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## УТИЦАЈ РАЗЛИЧИТИХ МИКОТОКСИНА НА ЗДРАВЉЕ СВИЊА – ЗАПАЖАЊА С ТЕРЕНА

Јасна З. ПРОДАНОВ-РАДУЛОВИЋ<sup>1</sup>, Игор М. СТОЈАНОВ<sup>1</sup>,  
Милица М. ЖИВКОВ-БАЛОШ<sup>1</sup>, Сандра М. ЈАКШИЋ<sup>1</sup>, Иван М. ПУШИЋ<sup>1</sup>,  
Јован А. БОЈКОВСКИ<sup>2</sup>

<sup>1</sup> Научни институт за ветеринарство „Нови Сад“,

Руменачки пут 20, Нови Сад 21000, Република Србија

<sup>2</sup> Универзитет у Београду, Факултет ветеринарске медицине,  
Булевар ослобођења 18, Београд 11000, Република Србија

**РЕЗИМЕ:** Микотоксини су структурно различити метаболити плесни који могу да контаминирају компоненте које се користе у исхрани животиња и људи. Циљ рада био је да се прикажу теренска запажања клиничких и патолошких последица по здравље свиња у случајевима када су *Fusarium* микотоксини утврђени у храни за ову врсту. Материјал за испитивање је обухватао узорке пореклом с фарми свиња у Војводини, на којима су регистровани здравствени проблеми интестиналног тракта код различитих категорија. Примењене методе испитивања су обухватале: епизоотиолошка и клиничка испитивања, макроратоморфолошки преглед угинулих јединки, бактериолошко и молекуларно испитивање узорака органа и ткива пореклом од оболелих, угинулих јединки и микробиолошко испитивање хране у циљу контроле присуства плесни. Присуство деоксиниваленола, Т-2 токсина и зеараленона је испитивано у 14 узорака различитих комплетних смеша за свиње применом имуноензимске технике (Ridascreen®FAST DON, Ridascreen®FAST T2, Ridascreen®FAST Zearalenon, R-Biopharm, Germany). Клиничким и патолошким прегледом, код различитих категорија свиња, утврђене су лезије доминантно у дигестивном тракту. Проблем упорних ентералних инфекција код прасади на сиси и промене у порасту су забележене код залучене прасади и товљеника. Код одраслих категорија свиња, утврђена је умањена конзумација хране, понекад изражено одбијање хране и повраћање. У свим испитаним узорцима комплетних смеша за различите категорије свиња концентрација микотоксина DON била је већа од максимално дозвољених вредности. У испитаним узорцима хране утврђено је и присуство других *Fusarium* микотоксина. Постигнути резултати указују на контаминацију хранива с ниским вредностима *Fusarium* микотоксина и на њихову могућу позитивну интерреакцију с узрочницима болести присутним на фармама свиња.

**КЉУЧНЕ РЕЧИ:** здравље свиња, *Fusarium* микотоксини, Војводина