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Original article

Evaluation of selected serum biochemical and haematological parameters in gilts exposed *per os* to 100 ppb of zearalenone

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Abstract

Zearalenone (ZEN) widely contaminates animal feed of plant origin. The recommended safe concentrations of ZEN in feeds for various animal species are set mainly based on the mycotoxin's hormonal properties (NOEL). Our growing knowledge about biologically active concentrations of ZEN, molecular mechanisms and cells/tissues targeted by ZEN indicates that the harmful effects exerted by this mycotoxin on animals may be far greater than previously believed. This experiment was performed on pre-pubertal gilts divided into a control group (n=9) and an experimental group (ZEN, n=9). The control group received *placebo*, whereas the experimental group was administered ZEN at a dose of 0.1 mg/kg feed (equivalent to 5 µg/kg BW/day) for 42 days. On days 14, 28 and 42 blood samples were collected from the animals to determine the concentrations of selected zearalenols, serum biochemical and haematological parameters. Conjugated ZEN was found in the blood serum of the experimental gilts. Changes in the analysed biochemical parameters included a transient increase in albumin and cholesterol levels. A statistically significant increase in the concentrations of neutrophilic and acidophilic granulocytes was observed in the white blood cell system. The results indicate that long-term *per os* exposure of pre-pubertal gilts to low doses of ZEN (below NOEL) has a modulatory effect on liver function and white blood cells.

Key words: zearalenone, pre-pubertal gilts, biochemical parameters, haematological parameters

Introduction

Mycotoxins are widely present in feed ingested by animals in different climate zones, which significantly contributes to the spread of mycotoxicoses. Exposure to moderate to high concentrations of mycotoxins produces various clinical symptoms. There is mounting evidence to indicate that exposure to no-observed-effect-level (NOEL) doses of mycotoxins, regarded as safe in modern feed production, may be far more detrimental to animal health and productivity than previously believed (EFSA 2011, Bryden 2012). The above can be attributed to the modulatory effect of mycotoxins on homeostasis which requires healthy functioning and cooperating of the immune, hormonal and nervous systems (D'Mello et al. 1999, Maresca and Fantini 2010).

Fusarium toxins are structurally heterogeneous secondary metabolites which are produced by naturally occurring fungi of the genus *Fusarium*. Zearalenols (ZOLs) are a group of Fusarium toxins that most profoundly affect animal health and productivity. Research studies revealed that feed materials contain up to 6.49 ppm of ZEN (Döll and Dänicke 2011). The toxic effects of ZEN and its metabolites, α -zearalenol (α -ZEL) and β -zearalenol (β -ZEL), have been long associated with their estrogenic activity. The functional and structural abnormalities induced in the animal reproductive system by ZEN and ZOLs have been broadly researched (EFSA 2011). Safe ZEN limits for animal feeds (European Commission, Commission Recommendation 2006) were established based on ZEN's documented adverse influence on the animal reproductive system and its ability to disrupt the endocrine balance.

Safe ZEN limits for animal feeds should be revised in view of the latest data concerning the analysed mycotoxin's biologically active concentrations, targeted cell compartments and molecular pathways of action. Feed contaminated with ZEN induces chronic toxic effects in animals. The immune system is particularly susceptible to mycotoxins, even at concentrations that are generally regarded as non-toxic (Luongo et al. 2008). Zearalenols can influence immune functions at the level of innate and adaptive immune responses (Marin et al. 2011). Prolonged exposure to mycotoxins also influences the organs that metabolize and excrete toxins (liver, kidneys). Increased blood flow and the presence of specialist transporters that accept and accumulate mycotoxins inside cells increase the risk of dysfunctions even during exposure to low doses of those mycotoxins (Wang et al. 2012, Jia et al. 2014). The risk associated with ZEN exposure can be evaluated based on the biological effects observed in mycotoxicoses (Gazzah et al.

2013). Serum haematological and biochemical profiles provide information about an animal's health status and bodily responses to external and internal stimuli. Haematological and biochemical parameters fluctuate in both normal and pathological states. They do not always confirm the presence of disease, but they point to cells and tissues that are sensitive to selected substances.

The objective of this study was to determine, based on selected serum haematological and biochemical parameters, whether long-term (42 days) *per os* exposure of pre-pubertal gilts to a ZEN dose of 100 ppb (European Commission, Commission Recommendation 2006) has an impact on the overall health status of the animals.

Materials and Methods

Animals and the experimental procedure

The study was performed on 18 healthy, dewormed female Polish Large White pigs (aged 2 months, body weight 18-20 kg) obtained from a commercial fattening farm in Baldy, Poland. The animals were housed and handled in accordance with resolution No. 24/2009 of the local Ethics Committee (affiliated with the National Ethics Committee for Animal Experimentation of the Polish Ministry of Science and Higher Education). The gilts were housed in pens with *ad libitum* access to water. The animals received blank feed one week before the study, which rules out baseline ZEN levels before the experiment. They were allowed to adapt to the experimental environment for one week before the study. The gilts were administered standard feed (blank feed) which was tested for the following mycotoxins: aflatoxin, ochratoxin, ZEN, α -ZEL and β -ZEL, deoxynivalenol. The pigs were divided into two groups: a control group (n=9) administered *placebo*, and an experimental group (ZEN, n=9) administered zearalenone (SIGMA-ALDRICH, USA, Cat. No. Z2125) at 0.1 mg/kg feed/day. Analytical samples of ZEN were administered daily *per os* in gelatine capsules immediately before morning feeding. Zearalenone samples were diluted in 300 μ l of 96% ethyl alcohol (96% ethyl alcohol, Avantor Performance Materials Poland S.A., Poland) to produce ZEA doses of 0.1 mg/kg feed/day. The resulting solutions were added to feed, placed in gelatine capsules and stored at room temperature for 12 h to evaporate the solvent. Blood samples were collected from pigs before morning feeding and before the administration of ZEN (after fasting for 12 h). Blood samples for analyses of serum biochemical parameters, morphological

blood parameters, and concentrations of ZEN, α -ZEL and β -ZEL were taken from the anterior vena cava on experimental days 14, 28 and 42. Blood was collected into EDTA-treated tubes. Blood plasma was separated from whole blood by centrifugation at 3000 g for 15 minutes at 4°C. Samples were aliquoted and stored at -20°C until further analysis.

Zearalenone, α -zearalenol, β -zearalenol analysis

Blood samples were hydrolysed in a mixture of β -glucuronidase and sulfatase to determine the total concentrations of free and conjugated (glucuronide and sulphate) ZEN and its metabolites in the serum. Blood serum was hydrolysed according to the method of Gajęcka et al. (2013). Non-hydrolysed samples were used to determine the levels of ZEN and its metabolites in free form. Samples treated and not treated with enzymes were extracted by the method proposed by Obremski et al. (2003) to analyse total (free and conjugated) and free ZEN and its metabolites. The extract was diluted in deionized water and loaded onto a zearalenone specific immunoaffinity column (ZearalaTestWB Vicam, Naturan, Poland). Antibody-bound ZEN, α -ZEL and β -ZEL were eluted with methanol which was evaporated in a water bath (40°C), and the residues were dissolved in the mobile phase. The resulting sample for HPLC analysis was concentrated six times in the course of treatment to support a quantitative analysis of ZEN. Quantitative analyses involved HPLC-fluorescence detection (Hewlett Packard 1100, FLD G1321A) at excitation wavelength of $\lambda_{Ex} = 218$ nm and emission wavelength of $\lambda_{Em} = 438$ nm, in a Hypersil ODS column, 5 μ m, 4.6 mm x 250 mm, with a methanol/acetonitrile/water mobile phase (8:46:46; v:v:v) at the flow rate of 1.0 ml/min. Zearalenone and its metabolites were quantified by comparing the peak areas of samples to those of the standard solutions. For recovery tests, serum samples collected (3 ml) before ZEN administration were spiked with ZEN and its metabolites at 1 mg/ml. The rates of ZEN, α -ZEL and β -ZEL recovery from serum samples ranged from 95% to 98%. The detection limits were 2.0 ng/ml for ZEN, 3.0 ng/ml for α -ZEL, and 10.0 ng/ml for β -ZEL. The data used in analyses of ZEN and its metabolites in each sample were not corrected for the recovery rate.

Hematological and biochemical analyses

Morphological parameters were determined in blood samples collected into test tubes containing K₂EDTA (2 ml, MEDLAB). Hematological para-

eters were determined immediately after sampling in the ADVIA 2021i haematology analyser (Siemens) using laser-based flow cytometry. In the haematology analyser, a flow cytometry-based system relies on light scatter, differential white blood cell (WBC) lysis, and myeloperoxidase and oxazine 750 staining to provide a complete blood cell count, WBC differential and reticulocyte count. A cyanide-free method is used to measure haemoglobin colorimetrically. The following haematological parameters were determined: white blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and neutrophil (NEUT), lymphocyte (LYMPH), monocyte (MONO), eosinophil (EOS) and basophil (BASO) counts. Blood samples were analysed individually.

Blood samples for biochemical analyses were collected into test tubes containing a clotting activator (9 ml, FLmedical). The activity levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and the concentrations of cholesterol (Chol), glucose (Gluc), triglycerides (Tg), bilirubin (Bil T), albumins (Alb) and total protein (TP) were determined by kinetic, enzymatic and colorimetric methods, respectively, using the ACCESS 200 chemistry analyser (Cormay) and dedicated reagent kits (Cormay). Blood samples were analysed individually.

Statistical analyses

In each sample, the concentrations of ZEN and its metabolites were determined by HPLC in duplicate. The results were presented as means \pm standard deviation (SD). Hematological and biochemical values were presented as means \pm standard deviation ($n=3$). Asterisks indicate significant differences in mean values between the control group and the experimental group on different days of the experiment, which were determined in the paired t -test ($*p<0.05$; $**p<0.01$). Hashes (#) indicate measurements that did not meet the requirements for parametric tests (normal distribution and/or homogeneity of variance).

Results

The serum concentrations of selected zearalenols (ZEN, α -ZEL, β -ZEL) are presented in Table 1. The analysed mycotoxins were not detected in total or free form in serum samples from the control gilts. In serum samples collected from the experimental

Table 1. Total concentrations of zearalenone, α -zearalenol and β -zearalenol in the blood serum of the control pigs and experimental pigs administered zearalenone (ZEN) at 0.1 mg/kg feed/day after 14, 28, 42 days of the experiment.

Concentration ng/ml	Control			ZEN		
	14 d	28 d	42 d	14 d	28 d	42 d
zearalenone	nd	nd	nd	2.15 \pm 0.38	2.06 \pm 0.41	1.92 \pm 0.42
α -zearalenol	nd	nd	nd	nd	nd	nd
β -zearalenol	nd	nd	nd	nd	nd	nd

nd – not detected. Values are expressed as means \pm standard deviation ($n=3$).

Table 2. Serum biochemical parameters (means \pm standard deviation) in the control pigs and experimental pigs administered zearalenone (ZEN) at 0.1 mg/kg feed/day after 14, 28, 42 days of the experiment.

Parameter	Control			ZEN		
	14 d	28 d	42 d	14 d	28 d	42 d
ALT [U/L]	50.0 \pm 6.08	39.0 \pm 14.18	52.33 \pm 12.5	60.33 \pm 10.02	47.0 \pm 8.0	53.0 \pm 9.0
AST [U/L]	58.33 \pm 5.03	46.67 \pm 19.86	65.0 \pm 4.36	71.67 \pm 16.26	42.33 \pm 5.68	43.33 \pm 13.65
ALP# [U/L]	161.3 \pm 44.28	105.33 \pm 16.26	138.0 \pm 93.96	145.67 \pm 41.9	126.0 \pm 39.13	154.33 \pm 80.6
Gluc [mmol/L]	8.0 \pm 1.71	8.10 \pm 2.86	6.23 \pm 1.71	8.23 \pm 2.5	5.93 \pm 1.31	7.1 \pm 0.52
Chol# [mmol/L]	1.97 \pm 0.09	1.24* \pm 0.6	2.01 \pm 0.18	1.94 \pm 0.11	1.71* \pm 0.24	2.0 \pm 0.23
Tg [mmol/L]	0.48 \pm 0.01	0.41 \pm 0.15	0.27 \pm 0.02	0.53 \pm 0.1	0.37 \pm 0.06	0.32 \pm 0.03
Bil T# [μ mol/L]	0.16 \pm 0.21	0.09 \pm 0.05	0.14 \pm 0.12	0.64 \pm 1.07	0.29 \pm 0.29	0.13 \pm 0.17
Alb [g/L]	24.33* \pm 0.65	34.63 \pm 1.9	36.33 \pm 1.66	28.5* \pm 2.35	31.53 \pm 2.5	36.5 \pm 1.71
TP [g/L]	58.9 \pm 4.62	61.6 \pm 2.6	60.17 \pm 1.06	0.23 \pm 1.58	60.1 \pm 3.95	56.77 \pm 4.46

* – statistically significant at $p<0.05$.

Table 3a. Blood haematological parameters (means \pm standard deviation) in the control pigs and experimental pigs administered zearalenone (ZEN) at 0.1 mg/kg feed/day after 14, 28, 42 days of the experiment.

Parameter	Control			ZEN		
	14 d	28 d	42 d	14 d	28 d	42 d
RBC# [10^{12} /L]	5.91 \pm 1.0	6.41 \pm 0.99	6.19 \pm 0.82	5.98 \pm 0.53	6.08 \pm 0.96	7.03 \pm 4.03
HGB# [g/L]	9.37 \pm 1.24	11.03 \pm 1.27	10.53 \pm 1.3	9.53 \pm 0.95	10.4 \pm 2.13	12.93 \pm 5.52
HCT# [L/L]	30.87 \pm 3.25	36.3 \pm 4.96	33.97 \pm 4.74	31.57 \pm 1.76	33.3 \pm 6.34	42.0 \pm 17.44
MCV [fL]	52.6 \pm 4.64	56.73 \pm 2.23	54.83 \pm 2.66	52.93 \pm 4.45	54.6 \pm 3.15	54.2 \pm 4.33
MCH [fL]	15.93 \pm 1.31	17.27 \pm 0.8	17.03 \pm 0.76	15.97 \pm 1.66	16.97 \pm 1.01	16.6 \pm 1.14
MCHC [g/L]	30.27 \pm 1.53	30.43 \pm 0.7	31.07 \pm 0.37	30.13 \pm 1.3	31.07 \pm 0.74	30.7 \pm 0.467

* – statistically significant at $p<0.05$.

Table 3b. Leukocyte parameters (means \pm standard deviation) in the control pigs and experimental pigs administered zearalenone (ZEN) at 0.1 mg/kg feed/day after 14, 28, 42 days of the experiment..

Parameter	Control			ZEN		
	14 d	28 d	42 d	14 d	28 d	42 d
WBC [10^9 /L]	11.86 \pm 2.85	15.79 \pm 0.89	16.04 \pm 1.76	17.59 \pm 2.6	16.65 \pm 2.2	14.68 \pm 2.09
NEUT [10^9 /L]	2.57** \pm 0.94	3.47* \pm 1.14	6.48 \pm 2.33	6.98** \pm 0.84	7.36* \pm 1.72	5.47 \pm 1.09
LYMPH [10^9 /L]	10.04 \pm 0.57	10.56 \pm 2.0	8.13 \pm 1.02	8.97 \pm 1.32	9.13 \pm 0.53	7.87 \pm 0.82
MONO [10^9 /L]	0.53 \pm 0.05	0.75 \pm 0.15	0.45 \pm 0.12	0.84 \pm 0.31	0.7 \pm 0.1	0.64 \pm 0.16
EOS [10^9 /L]	0.12** \pm 0.01	0.23 \pm 0.02	0.16** \pm 0.04	0.29** \pm 0.04	0.34 \pm 0.15	0.35** \pm 0.04
BASO# [10^9 /L]	0.09 \pm 0.01	0.08 \pm 0.04	0.08 \pm 0.04	0.14 \pm 0.08	0.07 \pm 0.02	0.04 \pm 0.02

* – statistically significant at $p<0.05$. ** - statistically significant at $p<0.01$.

animals, free-form concentrations of ZEN, α -ZOL, β -ZOL and total concentrations of α -ZOL and β -ZOL were below the sensitivity of the method. In serum samples collected from the experimental pigs, total ZEN concentrations were detectable on days 14, 28 and 42.

Serum biochemical parameters are presented in Table 2. Statistically significant differences were observed in cholesterol levels which were higher in the experimental pigs (1.71 ± 0.24) than in the control animals (1.24 ± 0.6) on day 28. Significantly higher albumin levels were observed in the serum of the experimental gilts (28.5 ± 2.35) than in the control animals (24.33 ± 0.65) on day 14.

Blood haematological parameters are presented in Table 3a. No significant differences in the values of the analysed parameters (RBC, HGB, HCT, MCV, MCH, MCHC) were determined between the experimental and control pigs. Leukocyte parameters are given in Table 3b. Statistically significant differences were observed in the counts of neutrophilic and acidophilic granulocytes. On day 14, neutrophil counts were highly significantly higher ($p < 0.01$) in the experimental group (6.98 ± 0.84) than in the control group (2.57 ± 0.94). Neutrophil counts decreased in the experimental pigs on day 28, but remained significantly higher ($p < 0.05$) than in the control group. Eosinophil counts in the blood serum of the experimental animals were highly significantly higher ($p < 0.01$) than in the control group on days 14 and 42.

Discussion

The risk of zearalenone mycotoxicosis is still high due to the lack of effective detoxification methods and widespread contamination of animal feeds with ZOLs. In addition to its estrogenic potency, ZEN also exerts immunotoxic, hepatotoxic, nephrotoxic, genotoxic and cytotoxic effects (EFSA 2011). The scope and intensity of changes taking place outside the reproductive system are largely influenced by the dose, duration of exposure, level of somatic and sexual maturity, and the metabolic profile resulting from the biotransformation of ZEN. The intestinal mucosa and the liver participate in key reactions of ZEN metabolism: (i) reduction of ZEN to ZOLs, (ii) formation of a glucuronic acid conjugate from the UDP-glucuronyltransferase (UGT) catalysed reaction, (iii) active transport (Liehr et al. 1998). Symptoms of zearalenone-induced mycotoxicosis were confirmed by the presence of total zearalenone in the blood serum of experimental gilts intoxicated with ZEN. The presence of only conjugated ZEN in the serum suggests that most of the ingested ZEN dose

undergoes glucuronidation. Songsermsakul et al. (2013) reported 100% glucuronidation of ZOLs in the blood serum and urine. In the present experiment, free or conjugated α -ZEL and β -ZEL were not determined in the blood serum of gilts administered ZEN at 100 ppb. The noted absence could be attributed to low sensitivity of the analytical method and/or the intensity and type of ZEN transformations. Hydroxylation reactions compete with conjugation reactions and transport mechanisms in the apical and basolateral compartments of enterocytes and hepatocytes. *In vitro* studies revealed that the concentrations of ZEN metabolites differed on the apical and basolateral surfaces of the Caco-2 cell monolayer subject to dose. The above indicates that facilitated diffusion and active transport mechanisms are used differently in various metabolites, depending on their concentrations (Videmann et al. 2008). Döll et al. (2003) did not observe ZEN, α -ZEL or β -ZEL in the blood serum of weaned piglets exposed to ZEN doses of 10-420 ppb for 35 days. The above ZOLs were identified in the liver, bile and urine samples. Research indicates that serum ZEN concentrations are robust indicators of the degree of toxicity in cells and tissues exposed to high doses of ZEN over a short period of time (Obremski et al. 2003). Glucuronidation is a process whereby xenobiotics are usually deactivated but, in some cases, they can also be bioactivated. Glucuronides can react with plasma albumins to form covalent adducts with potentially immunomodulatory properties (Ding et al. 1993, Ritter 2000).

The liver, which participates in the metabolism of ZOLs, is particularly exposed to the toxic effects of ZEN. Zearalenone's hepatotoxicity is determined mainly by the dose, and it is manifested by abnormal function and histology of the liver (Marin et al. 2013). Hepatocyte activity is evaluated based on the activity levels of serum enzymes (Jiang et al. 2010). Changes in serum biochemical parameters indicative of hepatocyte damage (ALT, AST, AP) were not observed in gilts exposed to ZEN (100 ppb). In this experiment, ZEN had a modulatory effect on liver function. The liver is responsible for protein synthesis. In both animal groups, total protein and albumin concentrations were within the norm. Albumin levels increased in the experimental animals (Table 2). Al-Seeni et al. (2011) also reported a temporary increase in albumin concentrations in zearalenone-induced mycotoxicosis. There are no published references to diseases that are accompanied by increased serum albumin levels. Elevated albumin concentrations are generally attributed to dehydration resulting from decreased feed or water intake (Al-Seeni et al. 2011). In view of the antioxidant properties of albumins and ZEN's ability to induce oxidative stress, an increase in albumin con-

centrations could be part of the body's response to disturbance of the oxidative homeostasis (Abid-Essefi et al. 2009, Tawerna et al. 2013). Systemic cholesterol is synthesized mainly in the liver. In females, endogenous oestrogens have a positive effect on the serum lipid profile. Exogenous oestrogens such as ZEN and its metabolites also influence the lipid profile subject to dose (Dai et al. 2004). In this experiment, ZEN had no significant influence on Tg levels, but it induced a transient increase in total cholesterol concentrations (Table 2). Allan et al. (2001) demonstrated that total cholesterol levels were highly correlated with the concentrations of high-density lipoproteins (HDL) in pigs. In view of the antioxidant properties of HDL (Barter et al. 2004) and ZEN's oxidative potential, it can be assumed that cholesterol concentrations increase in response to ZEN-induced changes in oxidative status. According to Gazzah et al. (2013), ZEN influences lipid metabolism in the liver, which could increase cholesterol concentrations in the blood serum.

Zearalenone exerts toxic effects on both red and white blood cells. Maaroufi et al. (1996) reported a significant increase in HCT, HGB, MCV and WBC values in rats exposed to ZEN doses of 1.5-5.0 mg/kg BW. Similar changes were observed by Abbes et al. (2006) in mice administered ZEN doses of 40 and 500 mg/kg BW and by Šperanda et al. (2006) in piglets. Boeira et al. (2014) reported a statistically significant decrease in RBC counts and an increase in WBC counts in mice exposed to a ZEN dose of 40 mg/kg BW. In the present experiment, no significant changes in RBC counts or parameters were noted in gilts administered ZEN at 100 ppb (equivalent to 5.0 µg ZEN/kg BW/day). Similar results were reported by Teixeira et al. (2011) in piglets administered 750 ppb of ZEN for 21 days, and by Jiang et al. (2010) in piglets exposed to a ZEN dose of 1 ppm. Zearalenone has a more uniform effect on white blood cells, and it usually increases the counts of WBC and/or individual granulocyte fractions (Maaroufi et al. 1996, Abbes et al. 2006, Boeira et al. 2014). No changes in WBC counts were observed in gilts exposed to ZEN (100 ppb), and similar results were reported in piglets by Teixeira et al. (2011) and Jiang et al. (2010). In our study, an analysis of white blood cells revealed that ZEN significantly increased the counts of neutrophils and eosinophils. Those results cannot be compared with the findings of the previously cited authors who did not determine the numbers of different leukocyte types. Endogenous oestrogens modulate the effector cells of specific and non-specific immune systems: directly – by interacting with oestrogen receptors, or indirectly – by inducing the production of soluble mediators or the expression of their receptors (Chi-

ghizola and Meroni 2012). Supraphysiological levels of 17β-estradiol (E2) during pregnancy or hormone replacement therapy cause neutrophilia and/or an increase in WBC counts (Bergström et al. 2006, Lurie et al. 2008). Neutrophil counts generally increase in acute infectious and non-infectious inflammations, during intoxication and in certain metabolic diseases. An increase in the counts of acidophilic granulocytes is observed in parasitic diseases, allergies and mycotoxicoses. Eosinophils can provoke an inflammatory response to environmental allergens, such as fungi, by identifying toxic metabolites (Matsuwaki et al. 2011). Some substances released by activated eosinophils act as alarmins that initiate host defence via toll-like receptors type 2 (TLR2) (Yang et al. 2008). Interleukin-3 and IL-5 stimulate the production of eosinophils in the bone marrow and prolong their lifespan in peripheral tissues. In an *in vitro* study, ZEN provoked EL4 cells (thymoma cell line) to secrete IL-2 and IL-5 (Marin et al. 1996), as in an *in vivo* study, the mycotoxin stimulated the release of IL-2 in ileal Peyer's patches in gilts (Obremski 2014). The observed increase in the numbers of neutrophilic and acidophilic granulocytes during ZEN-induced mycotoxicosis indicates that ZEN probably elicits an inflammatory response. According to Taranu et al. (2015), ZEN stimulates the expression of TLR and certain cytokines involved in the inflammatory response and the recruitment of immune system cells. Marin et al. (2013) administered ZEN to piglets at a dose of 316 ppb and observed that the mycotoxin was capable of inducing an inflammation in the bloodstream by increasing the production of proinflammatory cytokines. Similar findings were reported by Obremski (2014) who noted an increase in IFN-α levels in ileal Peyer's patches in pigs receiving 100 ppb of ZEN. Stimulation of cells of the innate immune system (NEUT, EOS) can also lead to the production of mediators of oxidative stress, which disrupts cell homeostasis and the maintenance of immunocompetence (Yu et al. 2009). Zearalenone and other ZOLs increase the expression of early markers of oxidative stress which are also endogenous TLR ligands (El Golli-Bennour and Bacha 2011, Dunbar et al. 2012). Acting via TLRs in various tissues, endogenous ligands can contribute to the induction of a „sterile” inflammatory response (Yu et al. 2010).

There is growing evidence to indicate that low, non-cytotoxic doses of ZEN can induce changes in the expression of mRNA and proteins participating in metabolic, reproductive and immune processes. The immunomodulatory effects are particularly visible when the function or efficiency of the immune system is compromised. In this study, an analysis of blood biochemical and haematological parameters in

pre-pubertal gilts administered 100 ppb of ZEN for 42 days has revealed that the evaluated mycotoxin exerts a modulatory effect on liver function and leukocytes. The results indicate that even low doses of ZEN can sensitize the immune system and exert negative effects on the animal's health under supporting conditions.

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