The effects of dietary deoxynivalenol (DON) on selected blood biochemical and hematological parameters in pre-pubertal gilts

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Abstract

The aim of this study was to determine whether exposure to low doses of DON leads to changes in serum hematological and biochemical profiles. In the experiment, pre-pubertal gilts (with body weight of up to 25 kg) were administered DON per os at a daily dose of 12 μg/kg BW (group E, n=18) or a placebo (group C, n=21) over a period of 42 days. Blood for analysis was sampled seven times at weekly intervals. Minor, but statistically significant changes were observed in selected indicators, particularly in weeks 2 and 3 in values of biochemical parameters such as ALAT, AspAT, Pn and Fe, and in values of hematological parameters such as RBC, MCV, MCHC, PLT, MPV, WBC, neutrophil, eosinophil, lymphocyte and monocyte counts. In group E, a decreasing trend of the values was observed in most cases, except for RBC, PLT, eosinophil, lymphocyte and monocyte counts. Similar results were obtained in the last two weeks of the experiment. Initially, exposure to DON has a stimulating effect, which is eliminated when adaptive mechanisms are triggered. The results of the study indicate that the intensity of biotransformation processes varies subjected to the body’s energy resources.

Key words: deoxynivalenol, low doses, blood biochemistry, hematology, pre-pubertal gilts

Introduction

The results of hematology and blood biochemistry tests in veterinary laboratories contribute to the effectiveness of medical treatment (Panteghini and Forest 2005). Preventive and clinical measures cannot be initiated when the required medical data are absent or insufficient. The above also applies to diagnoses of pathological conditions caused by exposure to mycotoxins, including deoxynivalenol (DON).

DON is one of the most extensively researched Fusarium mycotoxins that is commonly found in plant material. DON is also widely investigated because it poses a significant health risk for humans and animals (De Angelis et al. 2014). To counteract that risk, maximum levels for DON have been established in
foods of plant and animal origin (Commission Regulation (EC) No. 1126/2007). To date, DON limits have not been set forth in the feed industry.

Structurally, DON is a polar organic compound with the IUPAC name: 12,13-epoxy-3α,7α,15-trihydroxytrichothec-9-en-8-on. The ketone position in C8 is characteristic of class B trichothecenes, and the number and position of hydroxyl and acetyl-ester groups can also determine relative toxicity within cells. Through the epoxide group, DON can bind with a high number of eukaryotic ribosomal subunits and disrupt the activity of peptide transferase, thus disrupting the elongation or shortening of peptide chains. Due to its ability to impair protein synthesis, DON can affect the rate of cellular transport, enzyme metabolism in the cytoplasm and changes in affinity for the active binding site (Waskiewicz et al. 2014). For this reason, DON affects specific tissues and organs. To take effect, DON has to be released from the matrix and absorbed from the intestine into the circulatory system, where it can induce changes in serum biochemical and hematological parameters.

The gastrointestinal system is the first barrier against undesirable substances, such as mycotoxins present in feed. Having passed the intestinal epithelium, mycotoxins reach the blood stream and body organs where they can provoke pathological changes or remain neutral (Maresca and Fantini 2010). Beginning in the intestinal lumen, mycotoxins are biotransformed to produce substances that are frequently more toxic than the parent compound (Frizell et al. 2011). The outcome of the biotransformation process is dependent on the dose. Small doses (beginning with NOAEL) trigger completely different local and systemic changes than those that are generally associated with mycotoxicoses (Calabrese 2005, Gajęcka et al. 2011). The animals were divided into two groups – the experimental group (E) and the control group (C). Every group comprised 18 pre-pubertal gilts. Group E animals were orally administered DON at 12 μg/kg BW. Group C pigs were fed a placebo. In the experimental group, DON was administered at a dose below NOAEL values (Boermans and Leung 2007). The mycotoxin used in the study was purified and standardized in the Department of Chemistry of the Poznań University of Life Sciences under the supervision of Professor Piotr Goliński. The experiment covered a period of 42 days. Blood for hematology tests was sampled from the vena cava cranialis seven times – on the first day of the experiment and on six successive dates at weekly intervals.

**Blood tests** – Blood samples for serum indicator tests were collected from pre-pubertal gilts into test tubes containing no anticoagulant. After clotting, they were centrifuged, collected and freeze stored (-20°C). Laboratory analyses were performed with the use of the Accent-200 automatic biochemical analyzer (Cormay, Poland) Reagents were supplied by the manufacturer. Serum glucose concentration was analyzed by the colorimetric glucose oxidase method with measurements at 510 nm/670 nm wavelength. Total cholesterol concentrations were analyzed by the colorimetric method with cholesterol esterase and oxidase – CHOD/PAP, and measurements at 510 nm/670 nm wavelength absorbance. Alanine aminotransferase activity (AlAT) was analyzed by the IFCC colorimetric kinetic method without pyridoxal phosphate activation, and measurements at 340 nm/450 nm wavelength. Aspartate transferase activity (AspAT) was analyzed by the IFCC method and measurements at 340 nm/450 nm wavelength. Total protein concentrations were analyzed by the use of the Biuret test, with colorimetric measurements at 546 nm/670 nm wavelength. Inorganic phosphorus concentrations (P_{in}) was analyzed by the colorimetric method with molybdenum ions and measurements at 340 nm/405
nm wavelength. Iron concentrations were analyzed by the colorimetric method with ferene and measurements at 630 nm/670 nm wavelength. Alkaline phosphatase activity (AP) was analyzed by the IFCC kinetic method, with colorimetric measurements of the rate of p-nitrophenol synthesis at 405 nm/670 nm wavelength. Urea concentrations were analyzed by the colorimetric kinetic method with urease and glutamate dehydrogenase and measurements at 340 nm/450 nm wavelength. Total bilirubin concentrations were analyzed by vanadium oxidation with colorimetric measurements at 546 nm/670 nm wavelength. Blood urea nitrogen (BUN) was measured automatically in the analyzer, according to the enzymatic method with urease and glutamate dehydrogenase.

**Ionogram** – measurements were performed in lithium heparin whole blood using the Siemens RKZ Rapidlab 348 analyzer with Na, K and Cl ion-selective electrodes.

**Hematology tests** – Blood samples of 2 ml were collected from the pre-pubertal gilts into test tubes containing EDTAK2 (Sigma Aldrich) as anticoagulant. The samples were thoroughly mixed and analyzed to determine: red blood cell (RBC) counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentrations (MCHC), mean corpuscular hemoglobin (MCH), hematocrit, and white blood cell (WBC) counts in a Medonic hematology analyzer according to the procedure recommended by the manufacturer. Measurements were performed in EDTAK2 whole blood by flow cytometry and laser in the Siemens Advia 2120i hematology analyzer equipped with: (i) optical peroxide biosensor for measuring dispersed light and light absorbed by individual cells by hydrodynamic focusing on a cell stream in a flow through cuvette; (ii) laser optics for measuring high-angular and low-angular light dispersion and absorption by individual cells, where the laser diode was the source of light – the measurement was performed to evaluate red blood cells, platelets and lobulation of nuclei in white blood cells; (iii) hemoglobin colorimeter – for measuring lamp voltage corresponding to the amount of transmitted light; (iv) PEROX and BASO reagents – for generating differential cyograms.

**Statistical analysis** – The results were grouped based on: (i) duration of the experiment in group C and group E on a given sampling date; (ii) sampling dates for a given factor. The significant differences between sampling dates were determined for group C (\(P<0.05\), \(\star\star P<0.01\)) and group E (\(P<0.05\), \(\star\star P<0.01\)), and significant differences between group C and group E (\(P<0.05\), \(\star\star P<0.01\)) were determined on selected dates. The results were processed in the Statistica application (Statistica 9.0). The differences between groups (factor or sampling date) were determined by ANOVA. The use of ANOVA was justified by the Brown-Forsythe test for the equality of group variances. When differences between groups were statistically significant (\(P<0.01\) – highly significant differences, \(0.01<P<0.05\) – significant differences, \(P>0.05\) – no differences), Tukey’s HSD test was used to identify the groups that were significantly different.

**Results**

**Analysis of mycotoxin levels in feed**

The values obtained were below the sensitivity of the test (LOD 5 μg/kg).

**Blood biochemistry tests**

The results of a statistical analysis of glucose concentrations are presented in Fig. 1A. Glucose levels tended to decrease in both groups, and a more marked decrease was observed in group E. In each week of exposure, excluding week 2, higher glucose concentrations were reported in group C. At \(P<0.05\), differences were observed in group C between week 1 vs. weeks 3 and 5, and between groups in week 5 when higher values were determined in group C. At \(P>0.01\), differences were found in group C between week 1 vs. weeks 2, 4 and 6, and between groups in week 1.

The results of a statistical analysis of total cholesterol concentrations are presented in Fig. 1B. Cholesterol levels were higher in group E throughout the experiment, in particular in the last three weeks of exposure. At \(P<0.05\), differences were observed in group C between weeks 2 and 6, and in group E between week 6 vs. weeks 0 and 1. At \(P<0.01\), differences were observed between groups in weeks 5 and 6.

The results of a statistical analysis of ALAT activity are presented in Fig. 1C. Higher activity levels were found in group E throughout the experiment, but a steady decrease in ALAT values was observed in both groups in successive weeks. At \(P<0.05\), differences were observed between groups in weeks 3 and 4. At \(P<0.01\), differences were determined in group C between week 1 vs. weeks 5 and 6, and between groups in weeks 1, 2, 5 and 6.

The results of a statistical analysis of AspAT activity are presented in Fig. 1D. In the first four weeks and in the last week of the experiment, enzyme activity was higher in group C. At \(P<0.05\), differences were observed in group C between weeks 0 and 3, between week 2 vs. weeks 4, 5 and 6, and between groups in
Fig. 1. Selected biochemical indices of the blood serum in the examined gilts.

week 1. At $P<0.01$, differences were found in group C between weeks 0 and 2, and between groups in weeks 2 and 4.

Differences in total protein concentrations are presented in Fig. 1E. No statistically significant differences were observed between groups in any week of exposure. A growing trend in total serum protein levels was noted in both groups. At $P<0.05$, differences were reported in group C between week 0 vs. weeks 4, 5 and 6, and between week 1 vs. weeks 4, 5 and 6. No differences were observed at $P<0.01$.

Significant differences in $P_{in}$ concentrations are presented in Fig. 1F. Higher $P_{in}$ values were observed in group C in weeks 2, 3, 4 and 5, whereas in the
remaining three weeks of exposure $P_{\text{in}}$, levels were higher in group E. At $P<0.05$, differences were determined in group C between weeks 0 and 5, between weeks 1 and 4, and between groups in weeks 1 and 4. At $P<0.01$, differences were observed in group C between week 0 vs. weeks 2, 3, 4 and 6, between week 1 vs. weeks 2, 3 and 6, and between groups in weeks 2 and 3.

Iron (Fe) concentrations are shown in Fig. 1G. Fe levels were higher in group C in the first four weeks of exposure, and in group E in the last two weeks of the experiment. A decreasing trend in iron concentrations was observed in group C, whereas a growing trend was noted in group E. At $P<0.05$, differences were reported in group C between week 1 vs. weeks 5 and 6, and between groups in week 4. At $P<0.01$, differences were observed between groups in weeks 1, 2 and 3.

Significant differences in sodium concentrations are presented in Fig. 1H. Sodium levels were higher in group E in the first two weeks, and a reverse trend was observed in successive weeks of exposure. At $P<0.05$, differences were reported in group C between week 6 vs. weeks 0 and 1, and between groups in week 2. At $P<0.01$, differences were observed between groups in week 6.

The results of a statistical analysis for potassium are presented in Fig. 1I. No significant differences in potassium levels were observed between weeks of exposure. However in weeks 3, 4 and 6 potassium concentrations tended to increase in group E. At $P<0.05$, differences were reported in group E between week 6 vs. weeks 0 and 1, and between groups in week 2. At $P<0.01$, differences were observed in group E between groups in weeks 0 and 3, between weeks 1 and 2, and between groups in week 3.

The results of a statistical analysis for chlorine concentrations are shown in Fig. 1J. Excluding the first two weeks, chlorine levels were insignificantly higher in group C throughout the experiment. At $P<0.05$, differences were reported in group E between week 2 vs. weeks 0 and 4. At $P<0.01$, differences were observed in group C between weeks 2 and 6, in group E between week 2 vs. weeks 1, 3, 5 and 6, and between groups in week 6.

No statistically significant differences were observed in total bilirubin levels, urea concentrations, AP activity or BUN values, and the values were within the reference ranges (Winnicka 2008).

**Hematology tests**

The results of a statistical analysis of red blood cell (RBC) counts are presented in Fig. 2A. RBC counts values were higher in group E in weeks 1, 2 and 3, whereas no differences between groups were found in the remaining weeks of the experiment. At $P<0.05$, differences were reported between groups in weeks 2 and 3.

The results of a statistical analysis of mean corpuscular volume (MCV) are shown in Fig. 2B. Insignificantly higher values were determined in group C throughout the entire experiment, excluding week 6. No significant differences within groups were observed between weeks of exposure. At $P<0.05$, differences were reported between groups in week 2. At $P<0.01$, differences were observed between groups in week 3.

Differences in mean corpuscular hemoglobin concentrations (MCHC) are presented in Fig. 2C. Excluding weeks 2 and 4, the lowest MCHC values were observed in both groups, and the values observed in group E were lower. At $P<0.05$, differences were found in group E between week 3 vs. weeks 2 and 4, and between groups in week 3.

Significant differences in mean platelet (PLT) counts are shown in Fig. 2D. PLT values were higher in group E throughout the experiment, excluding weeks 1 and 6. A decreasing trend in PLT counts was noted in group C in successive weeks. At $P<0.05$, differences were reported in group E between week 4 vs. weeks 0 and 2. At $P<0.01$, differences were observed in group E between week 6 vs. weeks 0 and 2, and between groups in weeks 2 and 5.

Differences in mean platelet volume (MPV) are presented in Fig. 2E. No significant differences were observed within the analyzed groups. Except for week 6, MPV values were higher in group C throughout the experiment. Neither group exhibited clear increasing or decreasing trends. Differences between groups were found in week 3 at $P<0.05$ and in weeks 2 and 5 at $P<0.01$.

The results of a statistical analysis of white blood cell (WBC) counts are shown in Fig. 2F. Higher values were noted in C throughout the experiment, excluding weeks 3 and 4. At $P<0.05$, differences were reported in group C between weeks 1 and 6, in group E between weeks 0 and 6 and between weeks 2 and 3, and between groups in weeks 1, 2 and 3.

The results of a statistical analysis of the mean percentage of neutrophils in WBC counts are presented in Fig. 2G. A decreasing trend in neutrophil percentages was found in both groups throughout the experiment. No significant differences were observed within groups. Significant differences between groups were reported in weeks 1 and 5 at $P<0.05$ and in week 4 at $P<0.01$.

The results of a statistical analysis of the mean percentage of eosinophils in WBC counts are shown in Fig. 2H. In group C, eosinophil percentages
increased in the first three weeks and decreased in successive weeks of the experiment. A growing trend was observed in group E throughout the study. At $P<0.05$, differences were reported between groups in weeks 5 and 6 due to clearly opposite trends towards the end of the experiment. At $P<0.01$, differences were observed in group C between week 2 vs. weeks 4, 5 and 6, between week 0 vs. weeks 2 and 3, and between week 1 vs. weeks 2 and 3.

The results of a statistical analysis of the mean percentage of lymphocytes in WBC counts are presented in Fig. 2I. A minor increasing trend in lymphocyte percentages was observed in both groups. No significant differences were found within groups. Differences were reported between groups in week 4 at $P<0.05$.

The results of a statistical analysis of the mean percentage of monocytes in WBC counts are pres-
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ent in Fig. 2J. Higher values were determined in group E on most sampling dates, excluding in weeks 1 and 3. At P<0.05, differences were reported in group C between weeks 1 and 2, and between groups in week 2.

No significant differences were observed in mean hemoglobin (Hb), hematocrit (Ht), mean corpuscular hemoglobin (MCH), basophil and large unsustained cell (LUC) values, which remained within the reference ranges (Winnicka 2008) in both groups and between the groups.

Discussion

The results of this study indicate that feed contaminated with even very low amounts of mycotoxins can contribute to changes in blood homeostasis in pre-pubertal gilts. Changes were observed in the results of hematology in pre-pubertal gilts administered DON at a daily dose of 12 μg/kg BW for 42 days.

DON is resistant to high temperature, which contributes to the risk of DON contamination of food and feed. Food and feed production processes have a minor influence on DON levels in the end product. The bioavailability of DON can differ in various forms of feed, which can affect the release of the analyzed mycotoxin from the matrix and its retention in animal tissues. A study of samples collected from the same pre-pubertal gilts (Waśkiewicz et al. 2014) demonstrated that the degree of tissue accumulation (liver and intestinal sections) of DON is determined not only by the dose, but also by the duration of exposure. The longer the exposure period, the higher the accumulation levels of DON in the examined tissues, which ultimately supported the determination of the carry-over factor (Völkel et al. 2011) after different exposure times. Maximum values of carry-over factors were determined at 0.037 (week 6) in the ileum (small intestine), 0.049 (week 5) in the ascending colon, 0.046 (week 6) in the cecum (large intestine), and 0.081 (week 1) in the liver. Similar values of the carry-over factor (liver) were reported by Danicke and Brzezina (2013) despite the fact that the animals used in their experiment were fed a different diet and were characterized by lower body weight. Possible variations in the results of hematological tests of animals exposed to different doses of DON are difficult to ascertain due to limited literature. Nevertheless, our findings differ from the results of previous studies on pigs. Rotter et al. (1994) observed an increase in the percentages of segmented neutrophils and the remaining leukocytes in pigs fed diets contaminated with DON at 0.75-3 mg/kg of feed for 28 days. Swamy et al. (2002) noted a decrease in plasma Ca, P, and Cl concentrations in piglets fed a diet contaminated with DON at 5.5 mg/kg of feed for 21 days. In other studies (Danicke et al. 2004, Accensi et al. 2006, Pintón et al. 2008), the administration of feed contaminated with DON at 0.84 to 10 mg/kg did not lead to changes in hematological profiles.

The variations observed in this study could be attributed to the size of the DON dose (NOAEL) and/or the kinetics of DON bioavailability. The latter can be divided into several factors, including mycotoxin extraction from the feed matrix and its conversion to an available form through absorption, distribution, accumulation in tissues and excretion (Danicke and Brzezina 2013). The above can produce two types of effects. Firstly, the described processes require considerable energy expenditure (Alonso-Pozos et al. 2003, De Angelis et al. 2014), which can lead to significant weight loss (unpublished data – the analyzed animals were characterized by lower body weight, but no significant differences were observed). Secondly, peripheral blood, sampled from the vena cava cranialis, can respond differently to various doses of DON. The vena cava cranialis is situated remotely from the liver (detoxification) and the kidneys (excretion), organs that are directly adjacent to the gastrointestinal system. The results of hematological profiles during exposure to low doses of DON and at relatively low carry-over factor of DON to the intestines (towards the end of the experiment) and the liver (highest values were found in week 1) (Waśkiewicz et al. 2014) led to minor, but statistically significant changes in the values of selected indicators. The first changes were observed in weeks 2 and 3 in biochemical indices, such as ALAT (Fig. 1C), AspAT (Fig. 1D) P (Fig. 1F) and Fe (Fig. 1G), and other hematological indices, including RBC (Fig. 2A), MCV (Fig. 2B), MCHC (Fig. 2C), PLT (Fig. 2D), MPV (Fig. 2E), WBC (Fig. 2F), neutrophils (Fig. 2G), eosinophils (Fig. 2H), lymphocytes (Fig. 2I) and monocytes (Fig. 2J).

The analysis of the data revealed not only trends, but also statistically significant differences. In group E, most indicators were characterized by a decreasing trend, excluding RBC, PLT, eosinophils, lymphocytes and monocytes. Similar results were obtained in the last two weeks of the experiment. The above could be explained by the fact that initial doses of DON (NOAEL) led to a period of slow adaptation (lasting two to three weeks) to the mycotoxin, which can be regarded as a specific food tolerance process (Maresca and Fantini 2010).

The observed activity levels of liver enzymes (ALAT and AspAT) support the food tolerance hypothesis. Aminotransferases are reliable and specific indicators of liver disease in humans and animals.
They are released into the blood stream proportionally to the number of damaged cells, and they are used as markers of inflammation (Tiemann et al. 2006). In group E, AIAT values were characterized by a decreasing trend, but they were significantly higher than in group C throughout the experiment (Fig. 1C). In group E, the highest levels of AspAT activity were noted in weeks 2 and 3, but they were lower than in group C (Fig. 1D). The above results point to pathological processes in the liver (higher AspAT values), but also an improvement in hepatic function (lower AIAT values) relative to the control group (Wang et al. 2014). Such discrepancies are noted on a daily basis (balance between catabolism and anabolism), and they neutralize the symptoms observed.

The food tolerance hypothesis is also indirectly validated by glucose levels in weeks 1 and 5 (Fig. 1A), which were significantly higher in group C. Glucose concentrations were also lower in group E in nearly all remaining weeks of the experiment. In the light of the observations made by De Angelis et al. (2014), the above scenario probably led to the conversion of native mycotoxins (at low doses) into glycosyl derivatives. Glycosyl derivatives were synthesized during interactions with glucose released from feed in the small intestine. DON is absorbed most rapidly and in largest amounts in the small intestine at approximately 51% of the ingested dose, and this process required significant energy expenditure (Alonso-Pozos et al. 2003). The above could explain the drop in peripheral blood glucose levels in group E animals.

The exposure to DON increased RBC counts (Fig. 2A) and led to a significant decrease in MCV and MCHC values (Figs. 2B, 2C), which is indicative of iron-deficiency anemia (Fig. 1C). The above condition is accompanied by an increase in MCH values, which could be exacerbated by maturation processes in pre-pubertal gilts. The situation observed could result from a higher demand for oxygen in selected tissues exposed to DON, including the juxtaglomerular apparatus, which regulates mycotoxin excretion from the body. The results obtained could also be attributed to an inflammation of the intestinal wall, as demonstrated by Tarasiuk, who observed histological changes indicative of a local inflammation in the jejunum. The discussed changes in hematological tests results were accompanied by a significant increase in the percentage of eosinophils at the beginning and end of the exposure period (Fig. 2H) both between and within groups. The presence of the connective tissue with vessels and immune system cells – lymphocytes, plasmocytes and acidophilic granulocytes – was observed in the villous stroma and between the crypts. In this case, the results were compatible.

Our previous study of the same group of pre-pubertal gilts (Gajęcka et al. 2013) demonstrated that low doses of DON (below NOAEL) in feed inhibit inflammatory processes in the gastrointestinal tract, in particular in the small intestine and the colon, due to a decrease in mRNA expression of genes controlling isomerase NOS-1 and NOS-2 (Grześk et al. 2011). This inhibitory effect could be caused by the “escape” of signal molecules, such as NO and DON, from stimulation of the local and general immune system. A similar situation is observed when Treg cells are induced by pathogens (Silva-Campa et al. 2012) during chronic infections, which could be regarded as a case of food tolerance (Maresca and Fantini 2010). Our findings seem to suggest that low (below NOAEL) doses of DON could deliver therapeutic effects (Alassane-Kpembi et al. 2013).

Further work is needed to deepen our understanding of mechanisms that underlie changes in the hematological profiles of pre-pubertal gilts induced by exposure to DON at NOAEL doses. Low levels of exposure to DON produce completely different changes in the metabolic profile than those resulting from higher doses of the toxin. Initially, exposure to DON has a stimulating effect, which is eliminated when adaptive mechanisms are triggered. The results of the study indicate that the intensity of biotransformation processes varies subject to the body’s energy resources.

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References


