EFFECT OF EXPERIMENTAL LONG-TERM EXPOSURE TO LOW-DOSE ZEARALENONE MYCOTOXICOSIS ON SELECTED MORPHOMETRIC PARAMETERS OF THE REPRODUCTIVE TRACT IN SEXUALLY-IMMATURE GILTS

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Abstract

The objective of this study was to determine the effect of long-term (48 d), per os animal administration of low zearalenone (ZEA) doses (50% and 100% NOAEL values) on the dynamics of changes in the morphometric parameters of the reproductive organs in sexually-immature gilts. The experiment involved 12 clinically-healthy gilts aged 2 months with initial body weight of ± 40 kg and a determined immune status. The animals were randomly divided into two experimental groups (E1, n=4; E2, n=4) and a control group (C, n=4). Group E1 was administered per os 20 µg of ZEA/kg b.w. for 48 d, group E2 received per os 40 µg of ZEA/kg b.w. for 48 d, and group C was administered per os placebo for 48 d. The mycotoxin was administered daily per os animal in gelatin capsules before morning feeding. The animals were slaughtered at the end of the experiment. No significant morphometric changes were noted in the reproductive system of the gilts, except for an increase in the number of medium-sized ovarian follicles in group E1. This suggests that ZEA at low concentrations may cause hormonal effects (hyperoestrogenism) but it does not exhibit xenobiotic activity.

Key words: gilts, zearalenone, ovary, uterus, morphometry.

Zearalenone (ZEA) is one of the most commonly-occurring fusariotoxins found in maize, maize products, soybeans, and various cereals that are an integral component of plant materials used in food and feedstuff production. Contaminated plant material constitutes a potential risk to the health of humans and animals. ZEA concentrations in cereals vary subject to numerous factors and they change from year to year. The noted concentrations fluctuate between ten to several hundred µg/kg of plant material, but in selected years, values in the range of several µg/kg to 8,000 µg/kg of the material and higher were reported (22). The main vector of ZEA transmission are cereals and maize, but recent studies have revealed that products of animal origin may also play an important role in the indirect distribution of this mycotoxin (21).

ZEA causes hyperoestrogenism in animals, which leads to serious aberrations in their sexual reproduction performance. Pigs, in particular replacement gilts, are the most sensitive species. ZEA and its metabolites found in contaminated feedstuffs administered over long periods may lead to the development of subclinical forms of the disease that are manifested during hyperoestrogenism. The oestrogen-related effects of exposure to dietary ZEA should be analysed, together with other environmental factors as an endocrine disruptor in the evaluation of the risk for to the health of humans and animals. This type of activity affects reproductive system development in gilts supplementing the breeding herd (23).

The objective of this study was to determine the effect of long-term administration of low ZEA doses on the dynamics of changes in the morphometric parameters of the reproductive organs in sexually-immature gilts.

Material and Methods

All the experimental procedures involving the animals were carried out in compliance with the ruling of the Local Ethics Committee for Animal Experimentation.
The experiment was carried out on 12 clinically-healthy gilts aged 2 months with initial body weight of ±40 kg and a determined immune status. The animals were kept in individual cages with ad libitum access to water, and they were fed standard diets tested for the presence of the following mycotoxins: aflatoxin, ochratoxin, ZEA, α-zearalenol, and deoxynivalenol. The animals were divided into two experimental groups and a control group, each comprising four animals. Experimental group 1 (E1) was administered per os 20 µg of ZEA/kg b.w. (Zearalenone Z-0167 Sigma Chemical CO., Germany) for 48 d; experimental group 2 (E2) – was given per os 40 µg of ZEA/kg b.w. for 48 d; control group (C) received orally placebo for 48 d. The animals were slaughtered at the end of the experiment.

The mycotoxin was administered daily in gelatin capsules before morning feeding. ZEA samples were diluted in 300 µl of 96% ethyl alcohol (Polskie Odczynniki Chemiczne SA, Poland) corresponding to a ZEA dose of 20 and 40 µg/kg b.w. (50% and 100% NOAEL - no observable adverse effect levels, i.e. levels at which no harmful changes and clinical symptoms of toxicity were observed). The resulting solution was introduced into feed, placed in gelatin capsules and stored at room temperature for 12 h to evaporate the solvent.

Post-mortem tissue samples were collected from the reproductive system for morphometric analyses on the last day of the experiment.

The results of the experiment are presented as mean values (x) and standard deviations (±SD). The results were processed statistically by an analysis of variance (ANOVA). Where the null hypothesis was rejected, the differences were verified by the Student's t-test. The above analyses were carried out using STATISTICA® software (Statsoft).

### Results

A statistical analysis of the morphometric parameters of the reproductive system of gilts revealed statistically-significant differences (P<0.05) only in respect of the number of medium-sized (4-6 mm) ovarian follicles (Table 1). The highest values (29.61) were noted in group E1 and the lowest (8.16) – in group E2 (Table 1).

The mean values obtained in the study showed no significant differences in the morphometric parameters of the ovaries. However, in all cases the lowest values were observed in group E2. In the group of large ovarian follicles, the differences between the mean values exceeded 50%, but they were statistically non-significant.

As regards the morphometric parameters of the uterus (uterine weight, length of the right uterine horn, length of the left uterine horn), the mean values were higher in group E2, compared with groups C and E1 (Table 1). The highest uterine weight was noted in group E2 (average difference of 5 g). Only the length of the uterine body was found to be lower in group E2, compared with groups C (1.4 cm) and E1 (3.7 cm) (Table 1).

### Table 1

The results of a statistical analysis of the mean morphometric parameters of the ovaries and uterus of gilts in the experimental groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>E1</th>
<th>E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian weight (g)</td>
<td>2.22±1.13</td>
<td>3.11±0.69</td>
<td>2.13±1.77</td>
</tr>
<tr>
<td>Ovarian length (mm)</td>
<td>19.40±5.64</td>
<td>20.67±1.50</td>
<td>17.50±6.35</td>
</tr>
<tr>
<td>Ovarian width (mm)</td>
<td>13.30±5.56</td>
<td>15.17±1.33</td>
<td>10.50±4.12</td>
</tr>
<tr>
<td>Ovarian volume (cm³)</td>
<td>1.80±1.23</td>
<td>2.67±0.52</td>
<td>2.00±1.15</td>
</tr>
<tr>
<td>Number of small ovarian follicles (1-3 mm)</td>
<td>67.26±8.41</td>
<td>67.54±30.36</td>
<td>60.12±40.52</td>
</tr>
<tr>
<td>Number of medium-sized ovarian follicles (4-6 mm)</td>
<td>10.09±0.92 b</td>
<td>29.61±15.67 a</td>
<td>8.16±6.42 b</td>
</tr>
<tr>
<td>Number of large ovarian follicles (&gt;6 mm)</td>
<td>8.68±0.53</td>
<td>8.24±2.85</td>
<td>4.05±0.97</td>
</tr>
<tr>
<td>Uterine weight (g)</td>
<td>103.26±75.23</td>
<td>103.16±33.64</td>
<td>108.42±39.55</td>
</tr>
<tr>
<td>Length of the right uterine horn (cm)</td>
<td>27.67±5.08</td>
<td>30.63±5.01</td>
<td>33.17±4.25</td>
</tr>
<tr>
<td>Length of the left uterine horn (cm)</td>
<td>27.45±4.42</td>
<td>30.08±3.75</td>
<td>30.43±2.97</td>
</tr>
<tr>
<td>Length of the uterine body (cm)</td>
<td>7.73±3.66</td>
<td>10.05±1.63</td>
<td>6.33±0.42</td>
</tr>
</tbody>
</table>

a,b,c: homogeneous groups significant at P<0.05 ± - SD
Discussion

Pigs are particularly sensitive to ZEA intoxication due to the specific features of this mycotoxin biotransformation in their bodies. The metabolite profile resulting from the chemical transformation of ZEA poses a greater risk for pigs than for other farm animals (9). The transformation pathway of this mycotoxin has been most thoroughly studied in pigs at the pre-systemic (gastrointestinal lumen) and systemic (intestine, liver) level (12). The process of ZEA transformation in target cells (7) has recently attracted researchers’ interests, yet the relevant knowledge continues to be scant.

In mammals, ZEA exhibits hormonal activity, which is determined by its spatial chemical structure, concentration, and time of exposure (6, 17). The reproductive system is a target tissue for ZEA hormonal effects (4). ZEA is capable of modulating the level of enzymatic protein expression not only by interacting with oestrogen receptors (ERs), but also as a substrate by way of competition in the absence of or at low concentrations of the endogenous substrate. Its hormonal activity in the gonads is determined by concentrations at which the cellular ERs are reached, as well as by interactions with a given ER type. The above is due to differences in ERα and ERβ affinity for ZEA, as well as the specific function of each type of ERs and/or their mutual interactions in target cells after ligand binding (8, 11). A different ERα profile relative to ERβ is observed in the ovarian follicles of pigs, subject to their degree of maturity (18, 19). The regulatory effect of oestrogens on folliculogenesis and steroidogenesis in the ovarian follicles, in particular medium-sized follicles, is attributed mostly to ERβ in respect of which ZEA demonstrates weak oestrogenic activity (16).

Yet, most importantly, tissue response, including the ovarian response, is determined by the ZEA dose. By affecting the transcription activity of nuclear receptors, ZEA and its metabolites modify the metabolism of endogenous and exogenous compounds at the ovarian level. The above receptors control the expression and the activity of phase I and II enzymes (1). ZEA hormonal activity is manifested subject to the mycotoxin concentration and affinity to selected receptors. At low concentrations, ZEA may display only hormonal effects, while at high concentrations it may show both oestrogenic and xenobiotic activity, thus affecting the enzymes involved in its bioconversion (10).

The morphometric parameters of ovary samples collected on the last day of the experiment did not exhibit statistically-significant differences between groups, except for the number of medium-sized ovarian follicles. In relation to the growing concentrations of endogenous oestrogens, the low mycotoxin concentrations applied in the study probably did not cause a significant dysfunction in the mechanisms controlling ovarian development. In view of the low ZEA concentrations and the long time of administration of the mycotoxin, the observed intoxication could be interpreted as chronic low-dose ZEA-induced mycotoxicosis. A factor which enhanced the ZEA effect on the ovaries of experimental gilts were gonad receptors, for which ZEA showed a high affinity. The above-facilitated ZEA absorption and it probably reinforced its presence in ovarian structures at low concentrations. Despite the above, it was the main mechanism responsible for the effect of ZEA, an exogenous hormonal substance, on the reproductive system (20).

The changes in gonad morphology during experimental ZEA-induced mycotoxicoses in sexually-immature gilts, noted by other authors, were mostly indicative of ZEA agonistic or antagonistic oestrogenic activity (5, 13). Methodological discrepancies (dosage) and the sexual maturity of experimental animals should be taken into account during the evaluation of macroscopic differences in gonads. Some authors applied high ZEA doses or administered them over short periods. The administration of 200 or 400 µg/kg b.w. over 7 d resulted in a significant decrease in ovarian weight and volume and a significant increase in uterine weight in both experimental groups. The above was not observed during long-term administration of threshold ZEA doses (15).

ZEA influence on the homeostasis of the reproductive system in gilts, including the uterus in very young animals, differs in its effects on the ovary and has not been fully explained to date. The biological activity of sex steroids at the cellular level in the uterus is determined not only by active hormone concentrations or the level of expression of its specific receptor, but also by the level of the expression and activity of enzymes biotransforming the hormone to the target cell (14). The attainment of sexual maturity in pigs is a multi-stage process marked by changes in gonadotropin and steroid hormone concentrations and the rapid anatomical development of reproductive system tissues. The growth of uterine tissues is noted at higher doses of endogenous and exogenous oestrogens, including ZEA. In general, the above hormones or hormone-like substances affect the size and the number of cells stimulating the synthesis of proteins, rRNA, tRNA, mRNA, and DNA (3). Oestrogens contribute to the proliferation of the endometrium, the overgrowth and elongation of endometrial glands, and the rhythmic contraction activity of the myometrium. According to Rybarczyk (15), the administration of threshold ZEA doses during long-term intoxication (which is usually the case in the natural environment) did not stimulate proliferative processes in the uterus. Significant uterine hyperaemia and blood extravasation was reported. In the above study, the uterus was swollen, but the weight of the uterine muscles did not increase. In our experiment, a morphometric analysis revealed an insignificant increase in uterine weight, but only in group E2 in comparison with groups C and E1, which could be attributed to the effects of phytooestrogen (2) and hyperoestrogenism, i.e. the combined effects of endogenous and diet-dependent oestrogens (ZEA). This statistically non-significant increase in uterine weight in group E2 confirms the results of previous studies.
The analysis of the morphometric parameters of the ovaries and uterus of sexually-immature gilts administered low ZEA doses (50% and 100% NOAEL values) over a long period (48 d) revealed no significant changes in the reproductive system of experimental animals, except for an increase in the number of medium-sized ovarian follicles in group E1. This suggests that ZEA at low concentrations may cause hormonal effects (hyperoestrogenism) but it does not exhibit xenobiotic activity.

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References

15. Rybarczyk L.: Experimental asymptomatic zearalenone mycotoxicosis in gilts. Doctoral dissertation. Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland, 2009.