HISTOPATHOLOGY OF SELECTED ORGANS OF THE REPRODUCTIVE TRACT OF PIGS SUPPLIED WITH FEED CONTAINING ZEARALENONE DESTROYER

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Received for publication February 09, 2009

Abstract

The efficacy of zearalenone destroyer in feed fortified with different amounts of zearalenone (ZEA) and the impact of the destroyer itself on selected organs of the reproductive tract of gilts was examined. The gilts were divided into five groups: group I (n=8) – non-treated control; group II (n=8) – receiving 200 µg of ZEA/kg b.w.; group III (n=8) – fed feeding stuff with zearalenone destroyer and with 200 µg of ZEA/kg b.w.; group IV (n=8) – fed feeding stuff with zearalenone destroyer and with 150 µg of ZEA/kg b.w.; group V (n=8) – fed feeding stuff with the neutral residues of zearalenone destroyer. After slaughter (day 8), the animals were autopsied and the segments of the uterus, ovaries, uterine tube, vulva, and vagina were examined histopathologically. It was found that the addition of the zearalenone destroyer to feed fortified with zearalenone limits the oestrogenic mode of action of this mycotoxin, and residues of the destroyer have no impact on the structure and functioning of the organs of the reproductive tract.

Key words: swine, zearalenone, zearalenone destroyer, reproductive tract, histopathology.

The presence of zearalenone (ZEA) in animal feeding stuff often causes hyperoestrogenism, particularly in pigs, which presents a great problem in breeding and rearing of animals. The physiological activity of ZEA and the oestrogenic correlation between this substance and 17β-oestradiol may explain its competitive mode of action in achieving the specific binding spots at oestrogenic receptors (9, 11).

Pigs, in particular gilts before the first heat, are most vulnerable to intoxication among other species. Oedema and reddening of the vulva, increase in the uterus weight and, in very severe cases, prolapsed of the vaginal walls and prolapsed of the anus are noted among the typical clinical signs. Even the low concentration of this mycotoxin in feed may lead to the disturbances in reproduction, mainly to retarded occurrence of the first heat, pseudogestation, early abortions, stillbirths, less numerous litters, delivering weak piglets and piglets with congenital splayleg syndrome, and agalactia in sows. Oedema and hyperplasia of the uterine cells and metaplasia of vaginal and uterine cervix cells are observed among microscopical lesions (3, 4, 8).

It is very difficult to fix the doses of naturally occurring zearalenone that provoke the specific reaction of the swine reproductive system. This is connected with animals’ individual reaction as well as with the frequent occurrence of ZEA together with other mycotoxins, such as substances with similar chemical structure and mode of action (6, 10).

The presence of ZEA in feedstuffs questions the suitability of using such kind of raw material for manufacturing animal feed. Commonly this kind of material is assigned for breeding other less susceptible animals, mostly ruminants. The negative effect on animal body may also be reduced by “diluting” the material.

There are some trials undertaken that test on using different kinds of detoxicants that operate either by binding mycotoxins on hepatoenteric path or by forming non-absorbable conglomerates. The detoxicants may also block the activity of mycotoxins by destroying their chemical structure (1). As far as the chemical effect (destruction) on the degradation of the lactone cycle is concerned, the answers to several questions have to be provided, such as: what is the efficacy of this process and what is its impact on an animal organism and, by entering the food chain, also on humans. In comparison with the efficacy that may be determined in vitro by applying chromatographic analysis, the answers to the other questions have to be found through performing tests on animals.

The aim of the study was to define whether feeding stuff fortified with the different amounts of ZEA given alone or together with ZEA destroyer to gilts has
any negative effect on their selected reproductive organs. We also tried to determine the effect the destroyer residues on these organs.

**Material and Methods**

The activities connected with the experiment on animals were carried out according to the operative procedures and legislation in Poland that define the conditions and methods of performing tests on animals.

The experiment was carried out on 40 gilt pigs of mixed breed (Polish White Large x Polish White Earpendent), 120-125 d of age, with the average body weight of 49.2 ±3.6 kg. The condition and nutrition status of the animals was good. During the research, the animals were kept in the separate cages.

The gilts were divided into five groups: group I (n=8) – non-treated control; group II (n=8) – receiving 200 µ/kg b.w of ZEA (Zearalenone [17924-92-4] ICN Pharmaceuticals, Inc. USA); a dose 100% higher than the lowest ZEA dose causing the apparent signs of hyperoestrogenism; group III (n=8) – receiving the feeding stuff with ZEA destroyer added in the manufacturing process and with 200 µ of ZEA/kg b.w.; group IV (n=8) – given the feeding stuff with ZEA destroyer added in the manufacturing process and with 150 µ of ZEA/kg b.w.; and group V (n=8) – fed the feeding stuff with the neutral residues of the ZEA destroyer. The gilts were given daily app. 3 kg of the prepared feeding stuffs in two portions at 6.30 a.m. and 3.00 p.m. The regular access to water was provided.

The material used for feed manufacturing did not contain ZEA or other mycotoxins, such as ochratoxin A, aflatoxin, and deoxynivalenol, which was determined by high performance liquid chromatography (HPLC) technique in the laboratory of the Department.

The destroyer used in the experiment had been previously verified in *in vitro* tests and its influence was based on the short-time effect of alkaline medium, which was achieved by adding sodium carbonate (Na2CO3), and ZEA in feed.

On day 8 of the experiment, the animals were slaughtered, autopsied, and segments of the uterus, ovaries, uterine tube, vulva, and vagina were immediately taken for the histopathological examination. The tissues were fixed in 10% neutralised formalin and embedded in the paraffin blocks. The microtome sections were stained with haematoxylin and eosin and then examined under the light microscope.

The histopathological examination was carried out in the Department of Pathological Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn.

**Results**

No histopathological changes were observed in the ovaries, uterus, vulva, and vagina in the pigs of control group (group I) and in the pigs supplied with feed containing only the ZEA destroyer (group V).

Different lesions were observed in the groups that were supplied with feeding stuff fortified with zearalenone together with the destroyer added in the manufacturing process (groups III and IV). Within the group with the lower amount of ZEA and destroyer (group IV), we observed the lesions that may indicate the low oestrogenic influence of the xenobiotic on the reproductive organs. These were: oedema of the vaginal submucosal tissue (Fig. 1), minor hyperaemia and oedema of the uterus (Fig. 2), and up to focal disintegration of the granular layer of the ovary (Fig. 3).

Within the group with the higher amount of ZEA together with the destroyer in feeding stuff (group III), these lesions are more apparent and lead up to the degradation of the ovarian follicles (Fig. 4).

The group of animals supplied with the feeding stuff fortified with ZEA, but without the additive of the destroyer (Group II), some evident lesions were noted.

Macroscopically, an increase in the size of the reproductive organs was observed. Microscopically, conspicuous thickening of the endometrium, proliferation of the endometrial glands, and significant damage of the ovarian follicles (Fig. 5) were seen.

**Discussion**

The results of histopathological examination enable us to assume that the ZEA destroyer added to feed does not affect the morphology and functioning of the reproductive organs. The addition of the destroyer to feed fortified with ZEA limited the oestrogenic mode of action of this xenobiotic. However, its efficacy to a major degree depends on the amount of ZEA in feed. The destroyer mode of action is based on chemical destruction of lactones cycle and because of this there is a theoretical risk of the reversed chemical reaction – re-closure of lactones cycle after acidification of feed. However, the experiments carried out as well as the previous tests *in vitro* (5) rather suggest that ZEA present in feed is not entirely inactivated and the extent of its destruction also depends on its concentration. Repeated lactorisation of open-chain form of ZEA, which is rather an uncompleted reaction of its inactivation, is quite possible. Compounds with similar chemical structure and with comparable oestrogenic mode of action may also be formed.

According to the data included in FAO/WHO report entitled “Safety evaluation of certain food additives and contaminants” from 1999, it may be stated that the highest acceptable doses ruled in some countries should be updated. We should also pay attention to the differences in the size of the doses of ZEA, published in the report, that produce visible effects on the structure and functioning of the reproductive organs. The reason for these divergences is usually correlated with the differences in the mode of ZEA action obtained in the way of chemical synthesis compared to the one obtained as natural contamination.
The physiological status of the animals assigned for the research is also very important, particularly physiological cycles, body weight, and way of feeding (7). According to some authors, even very low doses of ZEA (1 ppm) may lead to disturbances in the fertility of pigs and cattle. Alternatively, some papers point out the positive effect of the low doses of ZEA present in feed on physiological processes and stress its role in steroidogenesis. Thus a kind of chemopreventive function may be attributed to ZEA (2).

The amount of ZEA left in feed, which contains the destroyer, is unfortunately sufficient for producing pathomorphological changes in the selected organs of the reproductive tract.
Acknowledgments: The authors thank Professor T. Rotkiewicz and Dr I. Otrocka-Domagała for critical reading of this manuscript.

References


