Toxicon 89 (2014) 26–31

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

The effect of environmental mycotoxins on selected ovarian tissue fragments of multiparous female wild boars at the beginning of astronomical winter

Łukasz Zielonka [a, *], Magdalena Gajęcka [a], Anna Rozicka [b], Michał Dąbrowski [a], Jan Żmudzki [c], Maciej Gajęcki [a]

[a] Department of Veterinary Prevention and Feed Hygiene, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowskiego 13/29, 10-718 Olsztyn, Poland
[b] Arka Anna Rozicka Veterinary Clinic, Iwaszkiewicza 34 A, 10-089 Olsztyn, Poland
[c] Department of Pharmacology and Toxicology, National Veterinary Research Institute in Puławy, Partyzantów 57, 24-100 Puławy, Poland

ARTICLE INFO

Article history:
Received 8 April 2014
Received in revised form 12 June 2014
Accepted 26 June 2014
Available online 9 July 2014

Keywords:
Zearalenone
Deoxynivalenol
Multiparous female wild boar
Ovary
Hydroxysteroid dehydrogenases
Astronomical winter

ABSTRACT

The contamination of plant material with mycotoxins, in particular of the genus *Fusarium*, is common in the natural environment. Multiparous female wild boars are exposed to feed contaminated with zearalenone (ZEN) and deoxynivalenol throughout the year. The aim of this study was to determine the concentrations of the above mycotoxins in multiparous female wild boars and to describe their effect on the histological structure of the ovaries at the beginning of astronomical winter. Toxicological examinations revealed 0.291 ng/ml of ZEN, 0.406 ng/ml of α-zearalenol (α-ZEL), 0.392 ng/ml β-zearalenol (β-ZEL) and an absence of deoxynivalenol (values below the sensitivity of the method) in the blood plasma of multiparous female wild boars. Numerous ovarian follicles at various stages of development, characterized by different degree of damage, were observed. Numerous deformed resting ovarian follicles were noted directly under the epithelium, and signs of follicular atresia and hyalinization were observed. Blood vessels in the medulla of the ovary were dilated, which probably improved the distribution of ZEN in the ovaries. Higher substrate (ZEN) concentrations in the ovaries led to an insignificant increase in the staining intensity of 3β-HSD and 17β-HSD clusters. The observed changes could contribute to prolonging the initial stage of late anestrus in multiparous female wild boars.

1. Introduction

Wild boars have a wide geographic range, and they are often bred for various purposes (meat production, public presentation, hunting and/or gun-dog training) (Andersson et al., 2011; Sales and Kotrba, 2013). Environmental estrogens can exert an uncontrolled influence on ecosystems inhabited by wild boars. In the natural environment, wild boars graze on plants contaminated with undesirable substances, including mycotoxins with estrogenic properties.

In addition to estrogens that occur naturally in the body, animals are also exposed to environmental estrogens and xenobiotics. Environmental estrogens, which include undesirable substances (Gajęcki et al., 2010), are known as endocrine disruptors (EDs) (Crain et al., 2008). Excessive ED levels may lead to pathological changes in the reproductive system, in particular the ovaries. Mycoestrogens such as ZEN are naturally occurring EDs that are produced by molds (Dunbar et al., 2012). Mycoestrogens block estrogen receptors (ERs) or mimic naturally occurring steroid hormones, in particular estradiol (E_2) (Brevini et al., 2005).

* Corresponding author. Tel.: +48 89 5 233 773; fax: +48 89 5 233 618.
E-mail address: lukaszz@uwm.edu.pl (Ł. Zielonka).

http://dx.doi.org/10.1016/j.toxicon.2014.06.021
0041-0101/

According to Shephard (2008), all organisms are constantly exposed to the harmful effects of mycotoxins. Mycotoxins are secondary metabolites of molds, and their biotransformation is generally determined by the host's age and their concentrations in the blood plasma. Mycotoxins are widely spread in the natural environment but at values that are below the sensitivity of the method (VBS). Plasma levels of ZEN and its metabolites, α-ZEL and β-ZEL, are influenced by the rate and form of biotransformation into water-soluble substances in the liver and other tissues. Before biotransformation, mycotoxins are distributed to sensitive tissues, such as the ovaries, and they contribute to pathological changes that affect the reproductive status, behavior and weight gains of animals.

Long-term exposure to ZEN ingested with feed leads to dysfunction of developing ovarian cells (Gajęcka et al., 2004; Skorska-Wyszyńska et al., 2004; Watson et al., 2007) due to disruptions in the physiological levels of steroid hormones (systemic endocrine mechanisms) and their activity (e.g. hydroxysteroid dehydrogenases, HSDs) at the prereceptor level (local intracrine mechanisms).

In reactive tissues, including the ovaries, pairs of HSDs interconvert steroid hormones from metabolically related inactive substances, and they regulate the number of ligands that enable bonding with trans-active nuclear receptors in all organisms (Kisiela et al., 2012). HSDs, in particular 3β-HSD and 17β-HSD, act as molecular switches that control the modulation of steroid hormone prereceptors in the endoplasmic reticulum of eukaryotes (Marchais-Oberwinkler et al., 2011) and prokaryotes (Kisiela et al., 2012). In eukaryotes, 3β-HSD fulfills this role in relation to progesterone (P_4), and 17β-HSD – in relation to E_2. The activity of HSDs is also influenced by other substances, including xenoestrogens, phytoestrogens, fungal species, mycoestrogens (such as zearalenone) and drugs (Kristan and Rižner, 2012).

The aim of this study was to determine the effect of environmental mycotoxins (ZEN and deoxynivalenol) on the histological structure of the ovaries in multiparous female wild boars at the beginning of astronomical winter.

2. Materials and methods

2.1. Material sampling

Samples for laboratory analyses were obtained from multiparous female wild boars inhabiting north-eastern Poland. The animals were hunter-harvested in the last quarter of the year (astronomical winter). At least five animals were harvested in every hunting season over a period of two years (five in 2011 and five in 2012).

Blood samples for the determination of ZEN, α-ZEL, β-ZEL and DON concentrations were collected immediately after hunter-harvesting in each quarter of 2011 and 2012. The samples were immediately transferred to chilled centrifuge tubes containing heparin and were centrifuged at 3000 rpm for 20 min at 4 °C. The resulting plasma was placed in 3 ml Eppendorf tubes and freeze-stored at −20 °C until analysis.

2.2. Mycotoxicological analysis

The concentrations of ZEN, α-ZEL, β-ZEL and DON in the blood plasma were determined in the Department of Veterinary Prevention and Feed Hygiene, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, by combined separation techniques with the use of immunoaffinity columns (Zearala-Test™ Zearalenone Testing System, G1012, VICAM, Watertown, USA and DON-Test™ DON Testing System, VICAM, Watertown, USA) and high-performance liquid chromatography (HPLC)/mass spectrometry (MS) based on the method proposed by Gajęcka et al. (2013).

2.3. Statistical analysis

The concentrations of ZEN and its metabolites (Fig. 1) in the blood plasma of multiparous female wild boars were expressed in terms of mean values ($\overline{X}$) and standard deviation (SD) for each sample. Data was analyzed in the Statistica application (StatSoft Inc., USA). Mean values were compared by one-way ANOVA for repeated measures to account for the applied ZEN dose and its administration period (Fig. 1). The equality of variances in the compared groups was tested by the Brown–Forsythe test. The significance of differences between groups was estimated by Tukey's HSD post-hoc test ($P < 0.05$ or $P < 0.01$).

2.4. Histological analysis

Post-mortem samples of the ovaries were collected immediately after hunter-harvesting. Samples for histological analysis were prepared and processed at the Arka Anna Rozicka Veterinary Clinic. Sections sampled for histopathological analyses were fixed in 10% formalin, neutralized and buffered to pH 7.4, and embedded in paraffin blocks. Microtome sections were stained with haematoxylin and eosin (HE) and PAS according to the method proposed by McManus. Ovarian cross-sections were examined in minimum 20 fields of view at 100× magnification. Microscopic images were analyzed under a light microscope (100 W halogen lighting was used in the Olympus BX50 microscope) at 400× magnification.

2.5. Immunohistochemistry analysis

2.5.1. Localization of 3β-HSD

Immunohistochemistry was performed on paraplast sections which were deparaffinized and rehydrated for

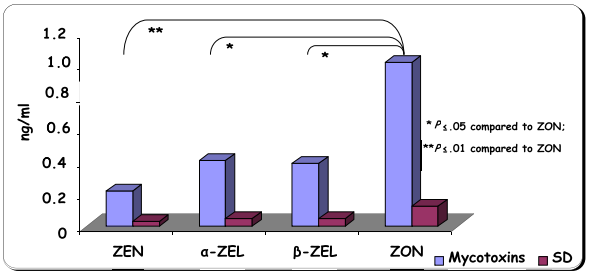

Fig. 1. Concentrations of ZEN and its metabolites in the blood plasma of multiparous female wild boars.