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Zearalenone and its metabolites in the tissues of female wild boars exposed *per os* to mycotoxins

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ABSTRACT

The study was performed on 18 clinically healthy female wild boars with initial body weight of 35 ± 2 kg. The animals were divided into two experimental groups (group I and group II) and one control group (group C) of 6 female wild boars per group. Group I animals were administered *per os* pure zearalenone (ZEN) at 150 μg/kg BW every two months for 7 subsequent days, whereas group II animals received feed naturally contaminated with ZEN at 50 μg/kg BW/day. Female wild boars were exposed to ZEN over a period of 1 year. Control group animals were fed a placebo. Tissue samples (dorsal muscles, left lobe of liver, left kidney, spleen, apical part of the cardiac muscle, cranial lobe of lung, left ovary, central part of the left horn of the uterus) were collected on the last day of the experiment within 3 min after slaughter. In group I, the highest ZEN levels were noted in the spleen (19.813 ng/g), cardiac muscle (18.105 ng/g) and kidneys (14.555 ng/g). In group II, the highest concentrations of ZEN were observed in muscle tissue (12.033 ng/g), uterus (10.821 ng/g) and kidneys (10.463 ng/g). The highest values of the carry-over factor were noted in the same tissues. In the examined female wild boars, *per os* exposure to natural sources of the parent substance or a combination of ZEN and its metabolites led to different concentrations of ZEN in the analyzed tissues. Zearalenone concentrations were compatible with CF values in both experimental groups.

1. Introduction

Mouldy feed has been long known as an etiological factor of many diseases affecting humans and livestock (Cortinovis et al., 2014). Mycotoxins produced by fungi do not always cause pathological changes (Gajęcka et al., 2013a, b), but they may induce conditions that differ from the norm in humans (Wang and Li, 2015) and animals (Morgavi and Riley, 2007; De Saeger and van Egmond, 2012; Zielonka et al., 2014). Fungi of the genus *Fusarium* are ubiquitous in feed components and feeds. In their natural habitat, Fusarium fungi produce NOAEL (no observable adverse effect level) amounts of zearalenone (ZEN) which, when ingested over prolonged periods of time, can produce subclinical disease states in accordance with the low-dose hypothesis (Vandenberg et al., 2012) and the principle of hormesis (Frizzell et al., 2011). The principle of

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hormesis states that when ingested in NOAEL amounts, many toxic substances that act as endocrinological disruptors (EDs) (Vandenberg et al., 2012), including ZEN, may exert stimulating/adaptive effects in animals, including pigs and female wild boars (Heberer et al., 2007; Dobrzyński and Fornalski, 2011; Zielonka et al., 2014). The resulting mycotoxicoses may induce chronic subclinical states where symptoms are too weakly expressed and too non-specific to support effective diagnosis. The question that remains to be answered is whether those states are induced by the mycotoxin alone or in combination with other agents, such as environmental factors and/or disruption of homeostasis in animals exposed to toxic substances (stress overload, dysfunctions, specific stage of the productive or reproductive cycle, phase of somatic development such as sexual maturation).

Wild animals are a good model for this type of research because their exposure to environmental factors remains fairly similar throughout the year (Keuling et al., 2009). As the result, the bodily systems of female wild boars, including their immune systems, undergo constant "training" and adaptation. Livestock animals, such as pigs, are protected against possible environmental stressors. Pigs (*Sus scrofa* f. *domestica*) are most sensitive to the presence of ZEN and its metabolites in feed (Gajęcki et al., 2010). Pigs, members of the family Suidae, are descendent from the wild boar, and they have been domesticated several thousands of years ago (Morgavi and Riley, 2007). For this reason, physiological data relating to pigs and wild boars can be extrapolated for research purposes without the risk of a major error (Zielonka et al., 2015a).

Health problems affecting wild boars and zoonotic diseases spread by wild boars should be analyzed in view of the factors found in the local environment (Zielonka et al., 2014) and the ecotone (Boadellaa et al., 2012). Wild boars rarely reside in a single habitat, and their populations are on the rise around the world, which decreases their physical tolerance and competitiveness in forest ecosystems (van Ginkel et al., 2013; Liu et al., 2013). As a result, wild boars increasingly often search for food in fields, meadows and human settlements (Keuling et al., 2008, 2010). The ecotone is a narrow transitional zone between different ecosystems which has unstable boundaries and is used as an indicator of global climate change (Wasson et al., 2013).

The economic losses caused by fungi of the genus *Fusarium* were first recognized in the 1970s. Fusarium fungi produce various metabolites, including ZEN, that contaminate cereal crops, in particular corn (Rasmussen et al., 2010; Storm et al., 2014). Despite those risks, corn acreage continues to increase. Climate change, in particular longer and warmer autumns, contributes to fungal proliferation (Paterson and Lima, 2011) and increases the probability that female wild boars will be exposed to ZEN contained in corn grown in fields and stored in forest feeding grounds.

Zearalenone and its metabolites, α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL), are oestrogen-like substances, but unlike steroids, they do not have the base structure of a sterane. There are numerous mechanisms (Barton, 2012; Frizzell et al., 2011) by which EDs such as ZEN can influence hormonal systems and induce adverse changes in female wild boars by: (i) competing with endogenous oestrogens for binding sites for oestrogen receptors (ERs) and androgen receptors, which can lead to changes in *m*RNA expression and protein synthesis and minimize the endogenous effects of steroids, (ii) binding with the receptor without activating it – the presence of substances on the receptor prevents bonding of endogenous hormones (antagonistic effects) (Soni et al., 2014; Zielonka et al., 2014), (iii) binding with transport proteins in the blood, which decreases the concentrations of endogenous hormones in the bloodstream, (iv) disrupting metabolic processes by influencing synthesis/degradation and the release of endogenous hormones.

The above mechanisms were investigated by analysing the accumulation and carry-over of ZEN in various bodily tissues of female wild boars, animals that inhabit ecotones between forests, farm fields and human settlements and feed on products characterized by different levels of contamination. In this study, female wild boars were administered *per os* pure ZEN from natural sources or feed naturally contaminated with this mycotoxin. The results can be used to evaluate the probability and severity of infections caused by ZEN in female wild boars (Signorini et al., 2012).

2. Materials and methods

2.1. Ethical principles

All experimental procedures involving animals were carried out in compliance with the Polish legal regulations determining the terms and methods for performing experiments on animals (opinion of the local Ethics Committee for Animal Experimentation No. 35/2010 22.02 2010).

2.2. Experimental animals and feed analysis

The experiment was conducted at the Centre for Research into Forests and Game Breeding of the Wrocław University of Environmental and Life Sciences, Poland, on 18 clinically healthy female wild boars with initial body weight of 35 ± 2 kg. The animals were penned in groups with *ad libitum* access to water. The administered feed was tested for the presence of mycotoxins: ZEA, α-ZEL and DON. Mycotoxin levels in the diets were estimated by common 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. The following chromatographic conditions were applied: Poroshell 120 SB-C18 column, 2.7 μm, 3.0 × 100 mm (Agilent P/N 695975-302), temperature – 40 °C, phase A (10 mM AcNH4/H2O), phase B (10 mM AcNH4/MeOH + ACN), gradient: 5%B to 100%B in 15 min, flow – 0.8 ml/min, time of analysis – 15 min, sample volume – 1 μl, source: ESI, gas temperature – 300 °C, gas flow – 5 l/min, nebulizer – 45, capillary – 3500 V, Hewlett Packard liquid chromatograph (HPLC), 1050 and 1100, with fluorescent and/or UV detection (Zwierzchowski et al., 2004).

2.3. Experimental design

The animals were divided into two experimental groups (group I and group II) (n = 6) and one control group (C; n = 6) (Smith et al., 2005; Heberer et al., 2007). All groups were fed ground corn. The presence of ZEN, α-ZEL and β-ZEL was not determined in corn administered to group I and group C. Group I female wild boars were orally administered ZEN at 150 μg/kg BW/every other day. Group II animals were administered feed naturally contaminated with ZEN at ≈ 50 μg/kg BW/daily. Group C female wild boars were fed a placebo of gelatin capsules containing ground corn without ZEN.

ZEN for group I animals was synthesized and standardized by the Department of Chemistry of the Poznań University of Life Sciences under the supervision of Professor Piotr Goliński. The experiment covered a period of 1 year. Doses of contaminated feed were administered *per os* daily in gelatine capsules before the morning feeding. Mycotoxin samples were diluted in 300 μl 96% ethyl alcohol (96% ethyl alcohol, SWW 2442-90, Polskie Odczynniki Chemiczne SA) to obtain the required doses (subject to body weight). The resulting solutions were stored at room temperature for 12 h to evaporate the solvent. The animals were weighed every