REPRODUCTIVE TOXICITY, METABOLISM AND IMPACT OF SELECTED MYCOTOXINS ON FARM ANIMALS

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ABSTRACT

The worldwide contamination of foods and feeds with mycotoxins is a significant problem. The economic impact of mycotoxins include loss of animal life, increased health care and veterinary care costs, reduced livestock production, disposal of contaminated foods and feeds, and investment in research and applications to reduce severity of the mycotoxin problem. In the field of reproductive toxicology mycotoxins are one of the most analyzed groups of toxins. For observation of effects of mycotoxins on animal organism *in vitro*, can be used different types of cells (e.g. porcine ovarian granulosa cells). Granulosa cells (GCs) are the most important cells in the ovary that undergo serious changes morphologically and physiologically during the processes of follicular proliferation, differentiation, ovulation, lutenization and atresia. Since now, several studies showed the influence of mycotoxins on these processes in granulosa cells. This review briefly summarizes the latest data in the literature of mycotoxins effects on farm animals and their reproductive functions.

Keywords: zearalenone, deoxynivalenol, farm animals, porcine

INTRODUCTION

The reproductive health of animals could be affected by a number of endogenous as well as exogenous factors, such as exposure to heavy metal **(Kolesarova et al., 2010a; Massanyi et al., 2010; Kňažická et al., 2010; Schneidgenová et al., 2007),** essential elements **(Kolesarova et al., 2010b; Kolesarova et al., 2011; Kolesarovsa et al., 2011a)** and mycotoxins **(Maruniaková et al., 2010; Medveďová et al., 2011)**. Mycotoxins are toxic products produced by naturally occurring metabolic processes in fungi. The four main genera of mycotoxin-producing fungi are *Aspergillus* spp., *Fusarium* spp., *Penicillum* spp. and *Claviceps* spp. **(Diekman and Green, 1992; Schollenberger, et al., 2007)**. Mold growth can occur at various stages during the production process of animals and plants. Mycotoxins can invade the seeds before the actual harvest, while the crop is still on the field, or alternatively, mold growth can occur during storage at the feed mill or on the farm. As a result, high numbers of mycotoxins may already be present in the ingredients before these are received in feed mills or farms **(Abouzied et al., 1991; Biro et al., 2009)**. Mycotoxins may cause various toxic effects or mycotoxicosis. Symptoms caused by mycotoxin contamination depend on the level and type of mycotoxins, but also on several factors such as animal species, sex, environment, nutritional status and other toxic entities **(Chang et al., 1979; Diekman et al., 1992)**. However, they are not transmissible between animals and contaminated feed is mostly the cause. Diagnosis of mycotoxicosis is often very difficult because the effects of mycotoxins in animals are diverse, varying from specific to unspecific symptoms like immune suppression, diarrhea, hemorrhages or reduced performance **(Pestka et al., 2004)**. From the point of view of animal production, there are five important classes of mycotoxins, i.e., trichothecenes, zearalenone, ochratoxins, aflatoxins and fumonisins **(Kanora and Maes, 2009)**.

ZEARALENONE (ZEA)

Structure and production of ZEA

Zearalenone ($C_{18}H_{22}O_5$) (Figure 1) is a non-steroidal estrogenic mycotoxin produced by several *Fusarium* spp. It has been implicated in numerous mycotoxicoses in farm animals, especially in pigs **(Malekinejad et al., 2007)**. It is a white crystalline solid and solubility in water is about 0.002 g.100 ml^{-1}. ZEA is slightly soluble in hexane and progressively more so in benzene, acetonitrile, methylene chloride, methanol, ethanol and acetone **(Desjardins and Proctor, 2007)**. It is also soluble in aqueous alkali **(Dojin et al., 2003)**. It can be a contaminant of both corn and wheat and may survive thermal food processes. ZEA was shown to be produced on corn by *Fusarium*. *Fusarium* isolates from bananas can also produce ZEA **(Jiménez et al., 1997)**. The concentrations in food and feed vary over a wide range, depending on climatic conditions **(Müller et al., 1997)**.

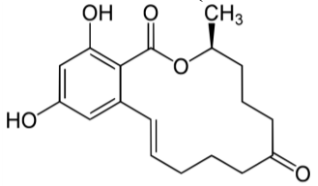

Figure 1 Chemical structure of ZEA (Yikrazuul, 2009)

Metabolism and mechanism of ZEA effect

Zearalenone is rapidly absorbed after oral administration. Although the degree of absorption is difficult to measure owing to extensive biliary excretion, it appears to be extensively absorbed in rats, rabbits, and humans **(Kuiper-Goodman et al., 1987)**. In pigs exposed intravenously or orally, an initial absorption and distribution phase was followed by a reduced plasma concentration and 45% of the administered dose was recovered in the urine during the first 48 h, 22% was recovered in the faeces **(Biehl et al., 1993)**. Both intestinal mucosa and gut microflora of pigs metabolize ZEA to alpha-zearalenol and to the glucuronides of both compounds. Healthy human intestinal microflora cultured in a continous flow system was unable to degrade ZEA **(Akiyama et al., 1997)**. Zearalenone and its

metabolites are excreted mainly by the bile in most animal species except rabbits, in which urine is the main route. Most of an administered dose is excreted within 72 h **(MacDougald et al., 1990)**.

Zearalenone has a resorcyclic acid lactone structure and can cross cell membranes binding to the cytosolic E_2 receptors and forming a zearalenone E_2 receptor complex (ZEA-E_2R). This complex is transferred into the cell nucleus and binds to specific nuclear E_2 receptors activating the gene responsible for mRNA synthesis (normally generated by E_2). These estrogen-like effects cause anabolic and reproduction activity. ZEA interacts not only with both types of estrogen receptors but is also a substrate for hydroxysteroid dehydrogenases, which convert it into two stereo-isomeric metabolites, alpha-zearalenol and beta-zearalenol. Alpha-hydroxylation results in an increase of estrogenic potency and explains the species specific sensitivity towards ZEA intoxications, whereas glucuronidation capacity inactivates ZEA. In comparison with other species, pigs have a low glucuronidation capacity making them more sensitive to ZEA **(Malekinejad et al., 2005; Fink-Gremmels, 2008)**.

Toxic effect of ZEA

In the field of reproductive toxicity, ZEA has been associated with hyperestrogenism and other reproductive disorders, such as impaired fertility in farm animals **(Diekman and Green, 1992)**.

At the molecular level a higher estrogenic potency of the ZEA metabolite α-ZOL has been demonstrated **(Malekinejad et al., 2005)**. ZEA and α-ZOL potentially inhibited oocyte maturation in maturing oocytes (**Malekinejad et al., 2007)**. Authors have observed an increase of the percentages of oocytes with an aberrant nucleus even before 30h of ZEA administration. Also after 30h combined exposure to ZEA and DON led to the highest percentage of oocytes exhibiting nuclear malformations. In accordance with assessment of cytotoxicity of ZEA and its metabolites, **Othmen et al. (2008)** have demonstrated that ZEA can provide inhibition of protein and DNA synthesis. They also observed higher induction of Hsp 27 and Hsp 70 stress protein expression. ZEA can induce early puberty of gilts (at 70 days of age) if fed at a level of 2 ppm for 45 to 90 days **(Rainy et al., 1990)**. However, the first heats of these gilts are mostly infertile without ovulation. Recent research indicated that feeding mixture for gilts contaminated with low concentrations of ZEA, significantly reduced the intrinsic quality of the oocyte collected from these animals **(Alm et al., 2002)**. The level of contamination has dose dependent effects on granulosa cells, steroidogenesis and gene expression **(Malekinejad et al., 2005; Ranzenigo et al., 2008)**.
These findings support an idea that the effects of mycotoxins lead to abnormality in embryo development of pigs.

During pregnancy, ZEA reduces embryonic survival when administered above a threshold and sometimes decreases foetal weight **(D'Mello et al., 1999)**. ZEA may affect the uterus by decreasing luteinising hormone (LH) and progesterone secretion and by altering the morphology of uterine tissues **(Etienne and Dourmad, 1994)**.

In gilts, experimental administration of zearalenone or contaminated feed at relatively low doses (1.5 to 2 ppm) leads to a swelling and thickening of the vaginal and vulvar wall, an increased uterus mass and atrophic ovaries, but without a standing reflex **(Kanora and Maes, 2009)**.

In cows, infertility, reduced milk production and hyperestrogenism have been associated with ZEA or with *Fusarium* producing this mycotoxin **(D'Mello et al., 1999)**.

In male pigs, ZEA can depress serum testosterone, weights of testes and spermatogenesis, while inducing feminization and suppressing libido **(D'Mello et al., 1999; Zinedine et al., 2007)**.

DEOXYNIVALENOL (DON)

Structure and production of DON

DON ($C_{15}H_{20}O_6$) (Figure 2) is also called Dehydronivalenol, 4-Deoxynivalenol, 4-Desoxynivalenol or Vomitoxin. DON, stable at a temperature of 120 ºC, is not decomposed under mild acidic conditions. Its three hydroxyl groups can be derivatized (e.g., esterified) **(JECFA, 2001a)**. DON is one of the more polar trichothecenes with a molecular weight of 296.32. DON is optically active, has great stability during storage/milling and in the processing and cooking of food, and does not degrade at high temperatures **(USDA GIPSA, 2008)**. DON at low levels (<1 ppm) is frequently encountered but can sporadically occur at levels as high as 5 to 20 ppm even in human foods such as corn meal and granola **(Abouzied et al., 1991)**.

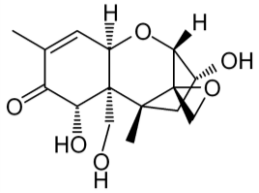

Figure 2 Chemical structure of DON (Panoramix303, 2007)

Metabolism and mechanism of DON effect

DON can be acutely or chronically toxic, or both, depending on the kind of toxin and the dose. Also, it could be rapidly absorbed after oral administration passively throughout the gastrointestinal tract and actively in the kidneys, liver, muscle, adipose tissue and reproductive tissues. To je z vasho clanku –doplnit autorov **(Marquardt and Frohlich, 1992)**.

Acute high dose toxicity of trichothecenes is characterized by "radiomimetic" effects such as diarrhoea, vomiting, leukocytosis, haemorrhage, and circulatory shock and death, whereas chronic low dose toxicity is characterized by anorexia, reduced weight gain, diminished nutritional efficiency, neuroendocrine changes and immunologic effects. Basically, trichothecenes bind to eukaryotic ribosomes and inhibit protein synthesis by blocking translation and inhibiting the elongation of peptide chains. DON sequentially induces mitogen-activated protein kinases (MAPKs) phosphorylation (activation), transcription factor activation and cyclooxygenase-2 (COX-2) mRNA expression. The process in which compounds bind to ribosomes and rapidly activate MAPKs and apoptosis is known as "ribotoxic stress response". The plasma

elimination half-life of DON in pigs is about four hours **(Larsen et al., 2004)**.

Toxic effect of DON

DON can cause reproductive alterations resulting in decreased oocyte and embryo development of pigs **(Tiemann and Danicke, 2007; Ranzenigo et al., 2008)**. Prepubertal gilts react more sensitively to DON>ZEA feeding compared to pregnant sows. The effects of DON and its relationship to reproduction in pigs is a more indirect effect, mainly linked to the reduced feed intake and with subsequent dysfunction of vital organs like the liver and spleen **(Kanora and Maes, 2009)**.

In porcine cumulus oocyte complexes, DON decreased maturation (telophase 1 and metaphase 2) rates and increased degeneration rates after 48 hours culture *in vitro* **(Alm et al., 2002)**. **Schoevers et al. (2010)** have found that DON impairs oocyte developmental competence by interfering with microtubule dynamics during meiosis, and by disturbing oocyte cytoplasmic maturation (Figure 3). The outcome of the analysis of the specific stages of meiosis in relation with (a) cumulus cell viability, (b) spindle malformation, (c) altered fertilization, and (d) diploidy of embryos indicate that DON affects oocyte developmental at different levels of oocyte maturation **(Schoevers et al. (2010)**. The effects of DON on some intraovarian factors have been examined previously **(Ranzenigo et al., 2008)**.

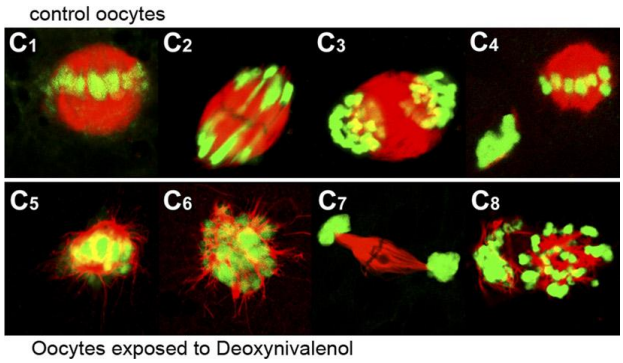

Figure 3

Confocal laser scanning photomicrographs of chromosomes and microtubules in porcine oocytes. Red: microtubules; Green: chromatin; Yellow: overlap. Control oocytes: Metaphase I with chromatin inside the spindle (C1), Anaphase I with chromatin being pulled towards the spindle poles (C2), Telophase I with chromatin located at both ends of the spindle (C3) and Metaphase II with chromatin inside spindle and polar body with remnants of microtubules (C4). Oocytes matured in the presence of 2 _M DON: reduced microtubule formation at Prometaphase I (C5), disorganized chromatin and microtubule structures during Metaphase I (C6), arrested at Telophase I with reduced size of the spindle and with condensed chromatin at the spindle poles (C7) and aberrant Metaphase II plate with condensed chromatin and disorganised microtubules (C8); (Schoevers et al. (2010).

In our previous study the results indicate, (1) a direct effect of DON on secretion of growth factor IGF-I and steroid hormone progesterone, (2) expression of markers of proliferation (cyclin B1 and PCNA) but not on the (3) expression of marker of apoptosis (caspase-3) in porcine ovarian granulosa cells (**Medveďová et al., 2011)** (Figure 4). DON caused significantly more nuclear aberrations than ZEA before 30h incubation **(Malekinejad et al., 2007)**.

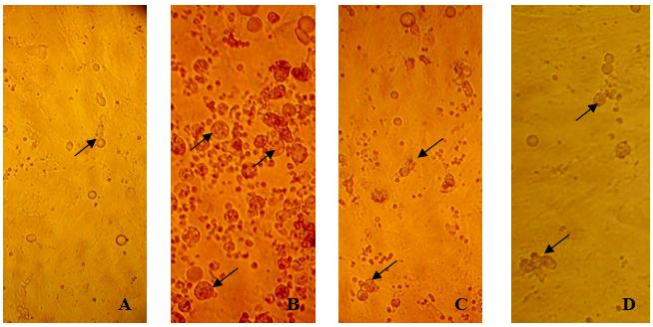

Figure 4

Visible mitosis of porcine ovarian GCs (Medveďová et al., 2011).

In swine herds exposed to DON in feed, reproductive problems such as swollen vulvas, vaginal and rectal prolapses, infertility or decreased fertility, prolonged estrous cycle or failure to return to estrus, abortion, and small litter size were reported in 50% field cases **(Cote et al., 1984)**.

CONCLUSION

Mycotoxins are ubiquitous and toxics, they globally present a potential danger for animal and human reproductive system. It is thus of great interest to develop studies focusing on mycotoxins absorption, metabolism, or eventual storage and elimination, in order to better understand their bioavailability and also their transfer rate to animal products. Animals differ with regard to sensitivity to ZEAs and DONs effects with pigs being highly susceptible and poultry and ruminants being relatively resistant. Studies in farm animals should be initiated that allow the establishment of a safe levels of ZEA and DON in feed materials and compounded feeds, particularly for pigs of different age groups, as they are considered to be the most sensitive animal species, followed by dose-effect studies in other farm animals **(Larsen et al., 2004; Zinedine et al., 2007; Pestka, 2007; Kanora and Maes, 2009).** Tissue and cell cultures are of increasing interest in the evaluation of toxicological risks of contaminated compounds. Our studies are in progress to deepen understanding the mode of action of ZEA and DON on releasing hormones, apoptosis and cell proliferation in granulosa cells and the influence of ZEA and DON on reproductive function in the pig.

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