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On the transfer of the *Fusarium* toxins deoxynivalenol (DON) and zearalenone (ZON) from sows to their fetuses during days 35–70 of gestation

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Abstract

Eleven pregnant sows with a body weight between 153 and 197 kg were fed a control diet (CON, 0.15 mg DON and 0.0035 mg ZON/kg diet) or a diet containing 15% of *Fusarium* toxin contaminated triticale (MYCO, 4.42 mg DON and 0.048 mg ZON/kg diet) in the period of day 35 and 70 of gestation. The indirect effect of feed intake was separated from the direct effects of the *Fusarium* toxins by the restricted feeding regimen where all sows were fed the same amount of feed (2000 g/d) over the whole study. At the end of experiment, fetuses were delivered by Caesarian section and samples of serum, bile, urine, liver, kidney and spleen of euthanatized sows and fetuses were taken to analyze the concentrations of DON, ZON and their metabolites. Feeding the *Fusarium* toxin contaminated diet to pregnant sows caused neither adverse effects on performance, organ weights and maintenance of pregnancy of sows nor on fetus weight and length. Furthermore, no teratogenic or embryolethal effects could be observed in the MYCO group. Hematological and clinical–chemical parameters of sows and fetuses were not affected by feeding, with the exception of significantly lower GLDH (glutamate dehydrogenase) serum activities in MYCO sows.

The carry over of DON and ZON from the diet to the sow or fetus tissues was calculated by the diet ratio (sum of concentrations of all metabolites in the physiological specimen divided by the dietary toxin concentration), while the fetus ratio was evaluated by the sum of concentrations of all metabolites in the physiological specimen of the fetus divided by that of the sows. DON and deepoxy-DON were found in urine, bile, serum, liver, kidney and spleen of sows of the MYCO group, but not in the bile of fetuses (spleen not analyzed). ZON and its metabolite α-zearalenol (α-ZOL) were detected in urine and bile of sows, while all specimens of fetuses as well as serum and liver of sows were negative for ZON metabolites. The maximum diet ratios for urine and bile in sows of the MYCO group were 0.84 and 0.05 for DON metabolites and 1.2 and 3.8 for ZON metabolites, underscoring the differences in metabolism and excretion of both toxins. The maximum diet ratio of DON and deepoxy-DON into liver, kidney and spleen of MYCO sows were 0.003, 0.007 and 0.003, respectively. The maximum fetus ratio of DON and deepoxy-DON into urine, bile, serum, liver and kidney of fetuses were 0.006, 0, 0.5, 0.88, and 0.33, while the maximum placental ratio (sum of toxin concentrations in the physiological specimen of the fetus divided by the toxin serum concentration of the sow) were 0.64, 0, 0.50, 0.70 and 0.52, respectively. Therefore, it can be concluded that the developing fetus is exposed to DON between the gestation days 35 and 70 when the sows are fed a *Fusarium* toxin contaminated diet. ZON concentration in the MYCO diet was too low to get reliable results for fetus or placental ratios.
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Keywords: *Fusarium* toxin; Deoxynivalenol; Zearalenone; Placental transfer; Fetus; Sow

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1. Introduction

The *Fusarium* toxins deoxynivalenol (DON) and zearalenone (ZON) are of great importance in human and animal nutrition due to their frequent and concomitant occurrence in toxicologically relevant concentrations in cereal grains worldwide (Placinta et al., 1999). Pigs are especially susceptible to these toxins, resulting in guideline values for critical dietary concentrations of 0.9 mg DON/kg for all pig categories; of 0.1 mg ZON/kg for piglets and gilts, and 0.25 mg ZON/kg for sows and fattening pigs (CEC, 2006). Although the monitoring of DON and ZON concentrations in complete pig diets showed that these guideline values were only exceeded occasionally (Meng et al., 2006), there is an ongoing discussion on the effects on fertility and a possible placental transfer of these toxins from the sow to their piglets (Cote et al., 1984; Dänicke et al., in press; Hörügel et al., 2003; Schnurrbusch and Heinze, 2002; Tiemann and Dänicke, 2006). Adverse effects on fertility might be a result of the cytotoxic effects (inhibition of protein synthesis) of trichothecenes such as DON (EFSA, 2004a; Wolf and Hörügel, 1994), whereas ZON and its metabolites α- and β-zearalenol (α-/β-ZOL) interact with the estrogenic receptor affecting the uterus and ovaries both directly or by the superordinated endocrine system (EFSA, 2004b).

Up to now, research about the placental transfer of mycotoxins is only fragmentary. Furthermore, the passage of mycotoxins may be dependent on their structure, on the species (type of placenta) and on the time of exposure (gestation period, vascularization of placenta).

Appelgren et al. (1982) studied tissue distribution of radio-labeled ZON in pregnant mice and demonstrated radioactivity in the fetuses on gestational day 17, whereas no radioactivity was identified in the embryos on gestational days 8, 9 or 10. Therefore, the vascularization of the placenta, which occurs between days 10 and 11 of gestation in rodents, seemed to be necessary for the transfer of mycotoxins. Accordingly, ZON and its metabolite α-zearalenol (α-ZOL) could be detected in the placenta and fetus at gestational day 12 and 18 after intravenous (i.v.) or intragastrical (i.g.) application of ZON to pregnant rats (Bernhoft et al., 2001). Following oral exposure, the trichothecenes nivalenol (NIV) and fusarenon-X (FX) were found to be transferred from pregnant mice (on gestational day 17) to fetuses across the placenta, and from lactating to suckling mice via milk (Poapolathep et al., 2004).

In rodents and humans, the haemochorial placenta allows a direct contact of the maternal blood and the fetal chorion, while in pigs the maternal and fetal blood is additionally separated by the endothel cells of the maternal blood vessels, the maternal connective tissue and the endometrial epithel cells (epitheliochorial placenta). Due to the large contact surface and the intensive nutrient exchange a placental transfer of DON and ZON appeared to be likely in swine as well, especially in late pregnancy as recently reported by Dänicke et al. (in press).

Therefore, the aim of the present experiment was to examine the transfer of the *Fusarium* toxins DON and ZON from naturally contaminated wheat from the sow to the fetus, as well as to study their effects on some reproductive traits and hematological parameters. The period of organogenesis between days 35 and 70 of gestation was chosen for this study as the fetuses might show possible teratogenic effects or mortality.

2. Materials and methods

2.1. Experimental design and diets

The toxin source was a batch of triticale contaminated naturally with *Fusarium* toxins. It was included into the experimentally contaminated diet (MYCO) at a proportion of 15%, which resulted in a dietary DON and ZON concentration of 4.42 and 0.048 mg/kg, respectively (Table 1). The *Fusarium* triticale was predominately contaminated with DON (36,000 μg/kg), but contained further trichothecenes to a much lesser extent (μg/kg): nivalenol, 850, scirpentriol, 80, 15-actetyl-DON, 460, and 3-acetyl-DON, 180. Although the acetylated DON metabolites are quickly deacetylated following ingestion (Eriksen et al., 2003) and could, therefore, contribute to the DON concentrations detected in the samples of sows and fetuses, DON intake was not corrected for the acetylated derivates since the proportions of 15-acetyl-DON and 3-acetyl-DON of the total DON intake (DON + 15-acetyl-DON + 3-acetyl-DON) amounted just 1.3 and 0.5%, respectively. The control diet (CON) contained 15% of uncontaminated control triticale. The CON and MYCO diets were fed to 5 and 7 pregnant gilts from day 35 to 70 of gestation. The experiment was terminated by delivering the fetuses by Caesarean section. Physiological samples from sows and fetuses were collected during the procedure.

2.2. Sows, feeding and housing

The experiment was performed with pregnant gilts (German Landrace) with a mean initial live weight of 165.5 ± 10.3 kg and 174.9 ± 22.0 kg for the CON and MYCO group, respectively. Gilts were kept individually without bedding on a partially slatted floor. Water was provided for *ad libitum* consumption. Experimental diets were introduced by a stepwise dilution (25, 50, and 75%) of the uncontaminated control diet with the experimental diet over a period of 6 days. Gilts were fed from day 35 to 70 of gestation restrictively at an amount of

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