Changes in the spleen and liver of pregnant sows and full-term piglets after feeding diets naturally contaminated with deoxynivalenol and zearalenone

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Abstract

Wheat contaminated naturally with the Fusarium toxins deoxynivalenol (DON) and zearalenone (ZON) was fed to pregnant Landrace sows for 35 days. On day 110, caesarean section was carried out, the offspring were killed immediately after birth, and their livers and spleens examined. At necropsy there were no macroscopic lesions observed in any organ of either sows or piglets. Histopathological evaluation of tissues from sows of the treated group revealed changes in liver and spleen tissues, whereas no significant changes were observed in these tissues in their piglets. Liver damage, as measured by prominent elevated transaminase activities, was not detected in pregnant sows there were individual variations in sensitivity to the Fusarium toxins. In conclusion, it can be assumed that there are no adverse effects on the liver and spleen of full-term piglets when their mothers consumed diets containing up to 9570 and 358 μg DON/ZON per kg diet.

Keywords: Fusarium toxins; Pregnant sows; Full-term piglets; Liver; Spleen

1. Introduction

Mycotoxins are secondary metabolites of moulds that exert toxic effects on animals and human beings. The Fusarium toxins deoxynivalenol (DON) and zearalenone (ZON) both contaminate wheat, maize, and barley worldwide (Abouzied et al., 1991; Chelkowski, 1998). These mycotoxins are produced from Fusarium graminearum, Fusarium culmorum, and Fusarium roseum if harvesting takes place during a rainy season or if poor storage conditions are present (Abouzied et al., 1991; Rotter et al., 1996; Döll et al., 2003).

ZON (Kuiper-Goodman et al., 1987) and DON (Pestka and Casale, 1990; Rotter et al., 1996) have different effects. Among farm animals, pigs are the most susceptible to DON. Its presence in swine feedstuffs decreases feed intake, causes feed refusal, and induces occasional vomiting (Conkova et al., 2003; Dänicke, 2002; Diekman and Green, 1992). In addition to these effects, reproductive changes have been observed in humans and pigs (Alm et al., 2006; Hussein and Brasel, 2001). At the molecular level, DON disrupts normal cell function by inhibiting protein synthesis via binding to the ribosome, and also by activating critical cellular kinases involved in signal transduction related
to proliferation, differentiation, and apoptosis (Middlebrook and Leatherman, 1989; Witt and Pestka, 1990). Macrophages, T cells, and B cells of the immune system are central targets of DON that can be immunostimulatory or immunosuppressive depending on the dose, exposure and timing of functional immune assay (Bondy and Pestka, 2000; Pestka et al., 2004).

In contrast to DON, ZON is biologically potent, but hardly toxic; rather, it affects the reproduction of swine most seriously because it has an oestrogenic effect. The mechanisms of the oestrogenic effect of ZON appear to be mediated via binding of this mycotoxin or its metabolites to the cytoplasmic oestrogen receptor (Katzenellenbogen et al., 1979; Mueller, 2002; Tiemann et al., 2003). This leads to intensification of cell proliferation (Coffey, 2001) resulting in uterine cellular hyperplasia, and cervical and vaginal cellular metaplasia (Gajecki, 2002; Zwierzchowski et al., 2005). In pigs, symptoms of hyperoestrogenism generally appear when contamination of ZON in corn exceeds 1 ppm, but it can occur at concentrations as low as 0.1 ppm (Mirocha et al., 1977).

Previously, in vivo investigations have shown that feeding prepubertal gilts with 9.57 mg DON and 0.358 mg ZON per kg of contaminated wheat caused dysfunction of liver and spleen cells. Although the ZON concentration was above the critical level, no signs of hyperoestrogenism or uterotrophic effects were observed in prepubertal gilts (Tiemann et al., 2006a,b). The upper critical concentrations are 0.05–0.25 mg ZON and 1 mg DON per kg of diet for female pigs, depending on age (BML, 2000).

Little information is available on the effects of indirect (i.e., from mother to young) exposure to DON/ZON on the health of piglets. Placental transfer of mycotoxins could present a potential risk for direct effects on the fetus. Indeed, even if the placenta of the sows is epitheliochorial, the feto-maternal contact surface is enlarged by the formation of numerous folds in order to make the exchange of nutrients as easy as possible. Furthermore, later in gestation, intra-epithelial capillaries on both sides of the placenta appear, reducing the physical barrier between fetal and maternal capillaries to one layer of cytoplasm (Clark et al., 1986). Intra-peritoneally administrated deoxynivalenol induced a pattern of skeletal malformations in pregnant mice as reported by Debouck et al. (2001). Intubation of mice on days 8–11 of pregnancy with 5 or more mg DON per kg bodyweight resulted in a high level of embryo lethality, and embryo toxicity was seen at doses of 2.5 and 5 mg/kg (Khera et al., 1982). In contrast, in rats, 5 ppm DON in the diet fed throughout pregnancy did not adversely affect pregnancy or cause birth defects (Morrissey, 1984).

Gilts fed 5–30 ppm ZON from days 2 to 15 after mating had normal embryonic development. However, gilts that received 60 or 90 ppm ZON had no fetuses at days 40–43 after mating (Long and Diekman, 1984). Embryos recovered on day 14 from gilts fed 60 ppm of ZON from days 7 to 10 after mating were fragmented, while those from control gilts were filamentous (Dickman and Long, 1989). The authors observed no deleterious effects of DON on fetal development when feed containing 8 ppm of purified DON was consumed (see Diekman and Green, 1992).

Following on from our previous experiments, we wished to investigate any possible influence of a Fusarium toxin-contaminated feedstuff containing 9.570 and 0.358 mg/kg DON and ZON, respectively, on: (1) Concanavalin A (Con A)-induced splenocyte proliferation (used as an in vitro index of cellular immune function, since lymphocyte stimulation in the presence of mitogen is recommended to test the capacity of cell mediated immunity) (Becker and Misfeldt, 1993); (2) Ca$^{2+}$ and Mg$^{2+}$ATPases in liver tissues; (3) alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) in sera, and (4) histological pattern in liver and spleen tissues of pregnant sows and their offspring. The animals were fed the experimental diets from days 75 to 110 of pregnancy, during which time the immune system develops in the fetus (Roberts and Chapman, 1981).

2. Materials and methods

2.1. Animals

Twelve pregnant Landrace sows (aged 315 days, bodyweight 198 ± 13 kg) were divided into two equal groups fed diets with different proportions of Fusarium toxin-contaminated wheat.

2.2. Dose chosen

A wheat batch contaminated naturally with Fusarium toxins was used as toxin source. This wheat was included in the diet for Experimental Group (2) at a proportion of 40%, which resulted in dietary DON and ZON concentrations of 9.57 and 0.358 mg/kg, respectively. As our earlier studies with gilts had shown that lower toxin levels gave partially inconsistent results, we used this rather high contamination level in the present experiment (Dänicke et al., 2005; Tiemann et al., 2006a,b). The diet for the Control Group 1 contained 40% of a virtually uncontaminated control wheat with 0.210 mg DON and 0.004 mg ZON/kg and can be regarded as a practically representative control, both in terms of diet composition (commonly used feedstuffs) and toxin baseline level commonly found in complete diets for pigs (Meng et al., 2007). Both diets used in the present experiment differed only in the wheat source, i.e. uncontaminated and contaminated, and were formulated to have similar energy and nutrient concentrations.

2.3. Exposure

Sows of both experimental groups were fed 2.0 kg/day from days 75 to 85, 3.0 kg/day from days 86 to 100, and 3.5 kg/day from days 101 to 110 of gestation. The sows were inspected and the feed ration was replaced daily, and the weight of feed portions left uneaten after 24 h was determined.

2.4. DON and ZON concentrations

The DON and ZON concentrations in the feed and blood samples were analyzed using high performance liquid chromatography (HPLC) before and at the end of the feeding experiment. The detection limit for DON was 0.03 mg/kg and the recovery approximately 89%. Zearalenone in feedstuffs was analyzed after incubation with 2 U β-glucosidase (EC
2.5. Caesarean section and sampling point

At the end of the experiments, after 35 days of feeding with mycotoxins, venous blood (20 mL) was collected from sows via jugular venepuncture into Kabvette tubes (Sarstedt) containing an anticoagulant (NH$_4$-Heparin). Sows were then anaesthetised with IV ketamine/xylazine (15 mg/kg, Ursoatin; 1.5 mg/kg xylazine) prior to caesarean section. The uterine wall was incised starting at the tip of one uterine horn and each fetus was cautiously removed. Blood samples (2–4 mL) were taken from each fetus by puncturing the umbilical vein and collected into Kabvette tubes. After cutting the umbilical cord, fetuses were euthanased with T61 (embutramide + mebezoniumiodide + tetracainhydrochloride; Intervet Unterschleißheim). Thereafter, the piglets were dried and weighed, and the liver and spleen removed, blotted dry and weighed. A piece of each tissue was cut and submitted immediately for histological investigation. Another piece of spleen was used for the isolation of the splenocytes. Following delivery of the fetus, sows were euthanased with T61 and samples of spleen and liver were recovered for further examination.

All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Agricultural Ministerial Department of Mecklenburg-Vorpommern, Germany.

2.6. Isolation and mitogenic response of splenocytes

Spleen mononuclear cell (SMC) suspensions were prepared from the spleens of pregnant sows (n = 6 per group) and piglets (2–3 per sow) which were aseptically resected as previously described (Tiemann et al., 2006a). Briefly, suspensions of SMCs (1 x 10^6 cells) were aseptically prepared from the spleens of pregnant sows and piglets, and the cell density was adjusted to 1 x 10^6 cells/mL. The suspensions were then washed twice in RPMI 1640 before being added to 96-well flat-bottom micro-titre plates (Nunc) for 72 h at 37°C. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium salt; Sigma) was added to each well and incubated for an additional 4 h at 37°C. The cultures were incubated at 37°C in an atmosphere containing 5% CO$_2$. Thereafter, Con A-stimulated cell proliferation was measured with an assay based on the cellular conversion of yellow tetrazolium salt (MTT; [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]; Sigma) into a blue formazan product by living and metabolically active cells, and was carried out as described by Tiemann et al. (2006a). At the end of the experiments, the proportion of Con A-stimulated response was calculated for each pregnant sow and piglets as follows: (absorbance with Con A/absorbance without Con A) x 100.

2.7. Liver assays

The Ca$^{2+}$- and Mg$^{2+}$-dependent ATPase activities were detected in liver tissue homogenates in sows (n = 6 per group) and piglets (n = 3 per sow) and were measured by monitoring the release of inorganic phosphorus (P$_i$) by colorimetric determination of a phosphomolybdate complex according to O’Brien et al. (1995) and as previously described by Tiemann and Kitchenermeister (1999). ATPase activity was expressed as μmol of P$_i$, released/min/mg protein.

For estimation of the liver enzymes AST and ALT in pregnant sows (n = 6 per group), blood samples (from the external jugular vein) were taken after 5 weeks of feeding with Fusarium toxin-contaminated wheat. In piglets (n = 6–7 per sow), blood samples were taken at birth by caesarean section. Serum was collected from piglets within 3 h and the AST and ALT activities were determined using automated instrumentation (Synchron LX-System, Beckman and Coulter GmbH).

2.8. Analysis of immunoglobulins in serum of sows

An indirect enzyme-linked immunosorbent assay (ELISA) was used as previously described (Tiemann et al., 2006a). All antibodies, reference sera, and tetramethylbenzidine (TMB) were purchased from Natu Tec-Bethyl.

2.9. Histopathology

For microscopic examination, small pieces of liver and spleen were removed immediately from the euthanased sows (n = 5–6 per group) and piglets (n = 3 per sow), and fixed in 10% buffered formalin. After routine processing, the tissue was embedded in paraffin. For general orientation the sections were stained with haematoxylin–cosin (HE); for glycogen detection with periodic acid-Schiff (PAS); for detection of iron particles with Berlin-Blue; and for detection of collagen fibres with Masson Goldner’s trichrome staining. In our experiments, the percentage of the stained areas was estimated by the AnalySIS-trame grabben CSIS system (AnalySIS 3.4, Sirius) as previously described (Tiemann et al., 2006a, b). Fifteen randomly selected slides with separate images completely filled with tissue were stored in a computer. For randomisation, each slide was divided into 15 single segments and a distinct image was used from each segment for evaluation. The definition of the segment was performed to obtain three images in horizontal and five images in vertical orientation. The observer was blind to the treatment of the animals. Using AnalySIS 3.4, the threshold (brightness, colour) was adjusted to detect the stained areas for PAS and Berlin-Blue, respectively. The adjustment of the microscope, camera and the software settings were standardized and stored in the computer so that they remained constant. In order to analyze the image database, a self-written AnalySIS macro was used to open the corresponding settings, detect the stained areas and calculate the percentage of stained areas in relation to the whole area for each image as well as to save the results as a file.

The same equipment was used to measure the Masson Goldner’s trichrome-stained collagen fibres. Each lobule was neatly outlined by an envelope of fibrous connective tissue. The thickness of the fibres of the lobule boundaries was determined after calibrating the images. A total of five distances between the two sides of the fibre in the shortest direction were measured for each image using AnalySIS 3.4 and the mean value per image was calculated.

To examine ultrastructural alterations in liver, small pieces were removed immediately from euthanased sows (n = 2 per group) and piglets (n = 3 per sow), and trimmed in 2.5% glutaraldehyde. The fixed cells were dehydrated in a graded series of ethanol and embedded in LR White (Resin Company Ltd.). Ultra-thin sections (50–100 nm) were cut on an ultramicrotome (Ultracut SWS, Leica) and mounted on gold grids. The thin sections were stained with uranyl acetate (10% in ethanol, weight/volume) and lead citrate (0.2% in distilled water, weight/volume), pH 12, and finally examined and micrographed in a Zeiss EM 902 A electron microscope.

2.10. Statistical analysis

The data are expressed as means ± SEM. All data were analyzed using one-way analysis of variance (ANOVA) using the SigmaStat for Windows, Version 1.0 (Jandel Scientific). If the differences were significant, a Student–Newman–Keuls test was used for post-ANOVA multiple comparisons (P < 0.05).

3. Results

The mean feed refusal over the course of the study was 0.03 ± 0.05% in the control (Group 1) and 32.0 ± 6.34% in the group fed the contaminated diet (Group 2), respectively. As a result of the initial feed intake depression, the mean live weight gain was only 84% in Group 2 compared to Control Group 1. Furthermore, the mean weight of piglets whose mothers were fed the Fusarium toxincareni- contaminated diet was significantly reduced (18%, P < 0.05). The liver and spleen weights were not altered in sows. In piglets in Group 2, the weights of the spleen were significantly reduced compared to those of Group 1 (S. Dünicke et al.,
unpublished data). At necropsy there were no macroscopic lesions observed in any organ of sows or piglets exposed to the *Fusarium* toxin. No signs of hyperoestrogenism or uterotrophic effects were monitored in connection with dietary treatment. The numbers of corpora lutea of follicles were approximately the same in both groups.

### 3.1. Mitogenic response to spleen mononuclear cells SMCs

Splenocytes from the DON/ZON-exposed sows of Group 2 showed a suppressed response to mitogen (76%, $P < 0.05$) compared to controls. No inhibitory effect was registered on the stimulation in SMCs derived from piglets of the low or high mycotoxin-fed groups (Fig. 1).

### 3.2. Immunoglobulin concentration

No significant differences were observed in serum levels of IgA, IgM and IgG between the pregnant sows of both groups after 35 days feeding (Table 1).

### 3.3. Enzymes in liver and serum

Five weeks of feeding *Fusarium* toxin-contaminated diets to pregnant sows did not affect the Ca$^{2+}$ and Mg$^{2+}$ ATPases in the liver. In piglets these enzyme activities were significantly lower compared to those of their mothers (Fig. 2).

ALT and AST activities were approximately in the same range in sera of sows in both groups (Fig. 3a), but in their piglets these enzyme activities were expressed at a very low level. However, an elevation in serum ALT (119.2%; $P > 0.05$) and AST (129.1%; $P < 0.05$) was observed in piglets in Group 2 compared to those of the Control Group 1 (Fig. 3b).

### 3.4. Morphology of hepatocytes and splenocytes

Generally, feeding pregnant sows with *Fusarium* toxin-contaminated diets caused some changes in liver and spleen cells as observed by means of light and electron microscopy in sows of Group 2, but not in their piglets.

#### 3.4.1. Hepatocytes

In pregnant sows in both groups, the liver architecture was preserved similarly, with hepatocytes separated by

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**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>DON/ZON (mg/kg; as-fed basis)</th>
<th>IgA (mg/mL)</th>
<th>IgM (mg/mL)</th>
<th>IgG (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.21/0.004</td>
<td>0.809 ± 0.106</td>
<td>5.83 ± 0.239</td>
<td>11.0 ± 0.239</td>
</tr>
<tr>
<td>2</td>
<td>9.57/0.358</td>
<td>0.729 ± 0.138</td>
<td>5.50 ± 0.417</td>
<td>11.8 ± 1.900</td>
</tr>
</tbody>
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Immunoglobulins for individual gilts were analyzed by ELISA. Values represent means ± SEM, $n = 6$ per group.
sinusoids and connective tissue. The morphology of hepatocytes in fetuses on day 110 of gestation was in accordance with images described by Bielanska-Osuchowska (1996).

The morphometric data on hepatocytes from the pregnant sows in Group 2 showed that Fusarium toxin feeding had no significant effect on volume density of glycogen. There was a big variation in the glycogen level of sows within the control and experimental groups. In piglets in both groups, a large amount of glycogen and some fatty infiltration could be found in all hepatocytes.

Histological examination of the liver showed only a slight differences in thickness of interlobular connective tissue in the sows of Group 2 (5.37 ± 0.248 μm; n = 5) compared to those in the control Group 1 (4.96 ± 0.231 μm; n = 6) (P = 0.258). In the piglets, the liver exhibited lobules with distinct centrolobular and perportal areas, but the interlobular connective tissue was not developed.

Iron staining demonstrates iron complexes as particles in the hepatocytes. The term “iron particles” is used here because the histological and ultrastructural features of iron and ferritin are indistinguishable. Fig. 4 demonstrates the mean result of 15 images of Berlin-Blue stained area in hepatocytes of each of six sows in Group 1 and five of Group 2. Generally, the staining was very low in the hepatocytes of sows in both groups, but the mean value was significantly greater in sows in Group 2 compared to those of Group 1. In piglets in Groups 1 and 2 the mean part of iron particles was not significantly different. The histopathological findings were supported by electron-microscopic data. Ultrastructurally, the splenocytes of sows in Group 2 exhibited iron particles; the rough endoplasmic reticulum, however, were not changed.

3.4.2. Splenocytes

Pregnant sows in Group 2 had an increased amount of iron particles in the red pulp of their spleens, but there was marked variation between individuals. Fig. 6 demonstrates the mean result of 15 images from Groups 1 (n = 6) and 2 (n = 5). In sows in Group 2, the mean value of the area of iron particles was greater (P < 0.05) to that of the control animals. The histopathological finding was supported by the electron-microscopic data. Ultrastructurally, the splenocytes of sows in Group 2 exhibited iron particles; the rough endoplasmic reticulum and the mitochondria, however, were not changed. A representative
photomicrograph is shown in Fig. 7. In piglets in Groups 1 and 2, no staining of iron particles was found in splenocytes.

Fig. 5. Electron micrograph of hepatic cell in sows. Group 1 (Panel a): Cell shows well-developed rough endoplasmic reticulum (rER) and mitochondria (M). Group 2 (Panel b): Cell undergoes the stage of necrosis occurring in increase of fatty vacuole (V), but intact mitochondria (M). Group 2 (Panel c): Degranulated rough endoplasmic reticulum and large foci of smooth endoplasmic reticulum (sER) without ribosomes, accumulation of large autophagosomes filled with dense membranes (arrows), but mitochondria were no affected. Magnification 14,000×.

Fig. 6. Effects of mycotoxin feeding on pig spleen histopathology (n = 6, Group 1; n = 5, Group 2) after day 35 (from days 75 to 110 of gestation) feeding with a DON/ZON-contaminated diet (mg/kg; as-fed basis): Groups 1 (0.21/0.004) and 2 (9.57/0.358). Spleen sections of each group were prepared and stained with Berlin-Blue. The staining was quantified by measuring the stained areas in relation to the whole area for each image. Bars represent mean ± SEM for Group 1 (n = 90 images) for and Group 2 (n = 75 images). Values with different superscripts are significantly different (P < 0.05).

4. Discussion

The higher concentration of Fusarium toxin-contaminated diet (Group 2; 9.57 mg DON and 0.35 mg ZON per kg diet) fed between gestation days 75 and 110 caused

Fig. 7. Transmission electron microscopy of spleen of Group 2 – iron particles in phagosomes (white arrow). Magnification 14,000×.
significant histological alterations in the liver and spleen cells of pregnant sows when compared with control animals fed the very low concentration of DON/ZON (Group 1). Piglets from pregnant sows (Group 2) showed normal development, no higher mortality or effects on liver or spleen cells. This result agrees with the data reported by Long and Diekman (1984) for gilts fed 5–30 mg/kg ZON from days 2 to 15 after mating.

No deleterious effects of DON on fetal development were observed when feed containing 8 mg/kg purified DON was consumed by sows (see Diekman and Green, 1992). Conversely, Kordic et al. (1992) found that 22.09 mg/kg of ZON in the ration of breeding gilts had an obviously harmful effect on reproductive performance with decreased numbers of corpora lutea, decreased weight of ovaries, decreased number of live embryos, increased number of dead-born piglets, and the occurrence of abortions. These effects were less pronounced in gilts fed mash containing 2.2 mg/kg ZON. However, in both groups the uterotrophic effect of ZON was obvious. One possible explanation for these observed differences could be the fact that mycotoxins other than ZON, or possible synergistic actions between several mycotoxins, may be involved in the effects reported by Kordic et al. (1992).

Pregnant sows in Group 2 differed in their responses and lesions to DON/ZON feeding for a 5-week period. The changes observed in the liver and spleen, however, were not clinically manifested. The high DON/ZON feeding (Group 2) of pregnant sows reduced the proliferative response in splenocytes in approximately the same way as was seen in prepubertal gilts (Tiemann et al., 2006a). The reduced proliferative response in the splenocytes of pregnant sows may be due to the capacity of DON to inhibit protein synthesis (Ueno, 1983; Thompson and Wannenmacher, 1986; Ehrlich and Daigle, 1987). Since the response to Con A in splenocytes of piglets in Group 2 was not impaired, this could be caused by a weak placental transfer of mycotoxins in utero. Ongoing studies will address this subject.

The activities of liver Ca$^{2+}$ and Mg$^{2+}$ATPases were not altered and the ultrastructure of the mitochondria was not impaired after the 5-week feeding period. This finding agrees with data obtained from prepubertal gilts (Tiemann et al., 2006a) showing that DON/ZON feeding, in the concentrations we used, did not act on the electron transport system of the liver cells. On the other hand, in some other parameters measured in sera (immunoglobulins, liver enzymes), we observed age-related differences in the sensitivity to DON/ZON feeding. In gilts the activity of the liver enzymes ALT and AST, two markers of putative cellular damage (Kaneko et al., 1997), was more strongly impaired (Tiemann et al., 2006b) when compared with pregnant sows in the present experiment and fed the same diet. Indeed, young pigs are more sensitive to aflatoxicosis as reported by Clarkson (1980).

Our results showed that in piglets recovered on day 110 of gestation, the ALT and AST activities were much lower compared with their mothers, but they were in the same range as reported by Grün and Ix (1973). The significant elevation of AST observed in piglets of Group 2 was also within the physiological range defined by Bostedt and Reinhardt (1980). We cannot conclude from our data that the increase in both enzymes, which appeared in the serum of piglets in Group 2 after DON/ZON exposure of their mothers, may be attributable to a dysfunction of the hepatocytes, because signs of necrosis and microscopic changes were not observed.

We did not detect an influence of Fusarium toxin-contaminated feeding on liver glycogen in sows and their piglets. High variability of liver glycogen concentrations existed in sows of Groups 1 and 2. In their piglets, we noticed that the glycogen concentration was very high compared with their mothers. In the last 3–4 weeks of gestation, rapid accumulation of glycogen in porcine fetal liver and muscle occurs because an increase in body glycogen stores at birth may improve the chance of survival of newborn piglets (Okai et al., 1978). Liver glycogen plays an important role in glucose homeostasis during farrowing and in the period before ingestion of colostrum (Mersmann, 1974).

Our histological findings indicated a significant elevation of iron particles in splenocytes and hepatocytes of sows fed Diet 2, but less in both cell types of their piglets. The results confirm the data reported by Furugouri (1973), where at birth there was more iron in the liver, but less or none in the spleen of piglets. The enhanced iron particles in the spleen and liver cells of sows (Group 2) were found without any signs of anemia. This finding was supported by the fact that no decrease in red blood cells, haemoglobin concentration, or haematocrit was found in the high mycotoxin group (S. Dänicke et al., unpublished data). These data are in accordance with those described by Kraft and Dürr (1999).

The deposition of iron particles (including haemosiderin) without marked tissue damage is referred to as haemosiderosis (Nyska et al., 1989; Wixom et al., 1980). The exact reason for the slight haemosiderosis cannot be ascertained from the present data, however it could be due to an increase in erythrocyte turnover, which could be influenced by DON. Trichotheccenes up-regulate cytokine production in murine models in vivo. The results reported by Zhou et al. (1997, 1998) indicated that exposure of DON in mice rapidly induces gene expression for a wide range of cytokines. Overexpression of certain cytokines (e.g., TGF-beta, TNF-alpha, and IFN-alpha) results in shortened survival of red blood cells, suppression of erythroid progenitor cells, and impaired iron utilization (Birgegard et al., 2005; Sing et al., 1989; Wang et al., 1995). In consequence, an accumulation of an excess of iron in macrophages of red pulp can occur. Popovic and Templeton (2004) explained the occurrence of haemosiderosis by an accumulation of an excess of iron in hepatocytes, because transferrin is already saturated with iron and is not able to bind any more. In the current study, using light microscopic examination, we observed an
excess of iron in spleen and liver cells in the sows in Group 2, which was, however, more apparent in prepubertal gilts fed the same diet (Tiemann et al., 2006a,b). The influence of DON/ZON feeding reflects our ultrastructural findings in liver cells which indicate that the most prominent features are proliferation of smooth endoplasmic reticulum, loss of ribosomes from the rough endoplasmic reticulum, loss of glycogen, and an increase of fatty vacuoles. These data agree with our previously published results (Tiemann et al., 2006b) but in the splenocytes of sows in Group 2 no abnormalities were observed in the ultrastructure (e.g., rough endoplasmic reticulum, mitochondria).

5. Conclusions

This study provides evidence that feeding of Fusarium toxin-contaminated wheat to pregnant sows for 35 days is capable of eliciting hepaticcellular effects such as iron enhancement and ultrastructural organelle changes in their livers. Based on the appearance of more fatty vacuoles, it can be postulated that the metabolism of lipid peroxidation can be impeded with subsequent effect on the liver cell function. However, liver fibrosis was not detected. Furthermore, a significant decrease in cellular immune response to Con A and the appearance of haemosiderosis were observed in splenocytes. Based on our previously reported data, prepubertal gilts react more sensitively to DON/ZON feeding compared to pregnant sows. As a further conclusion of our present study, it can be stated that there is no danger of liver and spleen intoxication of piglets if their mothers do not consume more than 9570 and 358 µg DON/ZON per kg diet during the third trimester of pregnancy.

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