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Effects of diets with cereal grains contaminated by graded levels of two *Fusarium* toxins on selected immunological and histological measurements in the spleen of gilts¹,²

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**ABSTRACT:** Feeding experiments with diets containing *Fusarium* toxin-contaminated wheat were conducted to clarify the pathogenesis of immunological effects of *Fusarium* toxins to porcine spleen cells. Contaminated diets were fed to 36 Landrace prepubertal gilts for 35 d. Concentrations (as-fed basis) of the indicator toxins deoxynivalenol (DON) and zearalenone (ZON), respectively, were 210 and 4 (control—group I), 3,070 and 88 (group II), 6,100 and 235 (group III), and 9,570 and 358 µg/kg (group IV). No signs of hyperestrogenism or uterotrophic effects were observed because of dietary treatments. The feeding of mycotoxin-contaminated diets did not cause gross pathological findings in the spleens of animals. In vivo, no inhibitory effects were detected on concanavalin A-stimulation of blood lymphocytes; however, the proliferation rate of splenocytes was inhibited \( P < 0.05 \) in pigs fed the diet with the highest DON/ZON concentration. With in vitro studies, lower proliferation rates of blood lymphocytes and splenocytes preexposed to DON were detected. Serum IgA concentrations of pigs in group II were increased \( P < 0.05 \) compared with the baseline value before feeding the DON/ZON diet. The histopathological data indicated elevated \( P < 0.05 \) iron staining in the red pulp of spleens in gilts from groups I to IV after 35 d of feeding. The presence of hemosiderin particles in the spleen sections was confirmed by transmission electron microscopic investigation. Together, the results provide evidence of spleen dysfunction (hemosiderosis) in the absence of clinical signs, especially in pigs fed higher concentrations (groups III and IV) of *Fusarium* toxin-contaminated wheat.

**Key words:** deoxynivalenol, *Fusarium* toxin, immunity, pig, spleen, zearalenone

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**INTRODUCTION**

Mycotoxins are biologically active secondary fungal metabolites found as contaminants in feedstuffs and exert toxic effects in animals and human beings. Different effects between the *Fusarium* toxins zearalenone (ZON; Kuiper-Goodman et al., 1987) and deoxynivalenol (DON; Pestka and Casale, 1990; Rotter et al., 1996) have been reported. Deoxynivalenol is one of the most common contaminants of wheat, corn, and barley worldwide (Abouzied et al., 1991; Chelkowski, 1998), and it is produced from *Fusarium graminearum* and *Fusarium culmorum* when the harvest occurs during rainy season or when poor storage conditions are present (Abouzied et al., 1991; Rotter et al., 1996; Döll et al., 2003).

The ability of DON to up- or downregulate immune function has been well established in mouse (Bondy and Pestka, 2000), rat, and human lymphocytes (Atkinson and Miller, 1984). In contrast, Rotter et al. (1994) found no effect of DON-contaminated feed on peripheral lymphocyte mitogen response in pigs. Øvernes et al. (1997), however, observed significantly greater response to phytohemagglutinin in the high-DON group compared with the low-DON group in growing pigs. In mice, dietary exposure to DON upregulates serum immunoglobulin A \((\text{IgA})\) (Yan et al., 1998; Pestka, 2003) and also can lead to decreased immunoglobulin M \((\text{IgM})\) and immunoglobulin G \((\text{IgG})\) concentrations in serum (Forsell et al., 1986; Pestka et al., 1990). Variable results were obtained in pigs (Bergsjø et al., 1992, 1993; Swamy et al., 2002). The effect of DON on the immune system is multifactorial and depends on the concentration and duration of exposure as well as animal species.

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system in mice and rats was confirmed by histopathological findings (Arnold et al., 1986).

Because of the different results reported in the literature, we investigated a possible influence of different concentrations of DON/ZON in diets for prepubertal gilts on 1) cell-mediated immunity of peripheral blood mononuclear cells and splenocytes, 2) serum immunoglobulins, and 3) histopathological and ultrastructural changes in splenocytes.

**MATERIALS AND METHODS**

**Animals and Diets**

Thirty-six prepubertal Landrace gilts (age 180 d, BW 103 ± 6 kg) were allocated to 4 groups (three replicates of 3 gilts each per treatment). Groups were fed diets with an increasing proportion of a *Fusarium* toxin-contaminated wheat (as-fed basis) of the indicator toxins DON and ZON in the diet fed to control gilts (group I) were 210 and 4 μg/kg and to experimental diets (groups II to IV) were 3,070 and 88, 6,100 and 235, and 9,570 and 358 μg/kg, respectively (Dänicke et al., 2005). Toxic concentrations were analyzed in samples collected before and after the end of experiments (after feeding) by HPLC. Other types of mycotoxins were not determined. Each gilt was fed 2 kg/d (as-fed basis) for 35 d, and orts were recorded daily. At the start (before feeding) and at the end of experiments after 35 d feeding with mycotoxins, jugular venous blood (20 mL) from untreated prepubertal gilts were cultured along with Con A: 2.5 μg/mL (Sigma). Viability, as measured by trypan blue exclusion, was 80 to 90%. For assessment of in vitro administration, 100 μL of the MC or SMC (1 × 10^5/well) in complete RPMI 1640 were dispensed into 96-well flat-bottom microtiter plates (Nunc, Wiesbaden, Germany) in quadruplicate for 72 h without or with the T cell-specific mitogen, concanavalin A (Con A: 2.5 μg/mL; Sigma).

**Peripheral Blood Lymphocytes**

Heparinized samples were diluted 1:1 with RPMI 1640 medium (Sigma, Deisenhofen, Germany). Mononuclear cells (MC) were separated over Ficoll (1.0861 g·cm⁻³) at 400 × g for 20 min at room temperature. After centrifugation, lymphocytes were collected from the interface and washed twice in RPMI-1640 medium (10 min at 200 × g). If erythrocytes remained after centrifugation, a Tris buffer (0.16 M NH₄Cl; 0.17 M Tris) was used for lysing these cells. Cells were counted and adjusted to 1 × 10⁶/mL of complete RPMI-1640 medium supplemented with 10% (vol/vol) fetal calf serum, 2 mM (wt/vol) L-glutamine, 0.05 mM (wt/vol) mercaptoethanol, 25 mM (wt/vol) HEPES, antibiotic-antimycotic (100 IU/mL penicillin G, 100 μg/mL streptomycin, 250 ng/mL amphotericin B, final concentrations, vol/vol; Sigma). Viability, as measured by trypan blue exclusion, was 80 to 90%.

**Splenocytes**

Spleen mononuclear cell (SMC) suspensions were prepared from the spleens, which were aseptically resected. A 10-g sample of spleen was placed in sterile Petri dishes containing precooled PBS (pH 7.4) with antibiotic-antimycotic. Single-cell suspensions were obtained by shredding the tissues through a plastic screen with the aid of disposable plastic syringe pistons into ice-cold 17 mM Tris HCl, pH 7.2, containing 136 mM ammonium chloride to lyse red blood cells, and centrifuged at 500 × g for 5 min (Tiemann et al., 1993). Cell pellets were then washed twice in PBS and resuspended in complete RPMI as described earlier. Viability, as measured by trypan blue exclusion, was 80 to 90%.

**Mitogenic Responses of MC and SMC**

Suspensions of MC or SMC (1 × 10^5/well) in complete RPMI 1640 were dispensed into 96-well flat-bottom microtiter plates (Nunc, Wiesbaden, Germany) at 570 nm. For assessment of in vitro administration, 100 μL of the MC or SMC (1 × 10^5) from untreated prepubertal gilts were cultured along with Con A: 2.5 μg/mL and DON (0, 0.235, 0.47, 0.94, 1.88, and 3.76 μM, final concentrations) in complete RPMI 1640 for 48 h. For all in vitro experiments, DON was dissolved in dimethylsulfoxide (final concentration, 0.2%). No differentiation of the cells was observed in the presence of 0.2% dimethylsulfoxide. The Con A-induced blood lymphocyte or splenocyte proliferation was measured with the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) assay (described later), which is accepted as an indicator of cell viability (Sugita-Konishi and Pestka, 2001; Uzarzski et al., 2003).

The cultures were incubated at 37°C in an atmosphere containing 5% CO₂. Thereafter, Con A-stimulated cell proliferation was measured with an assay based on the cellular conversion of MTT yellow tetrazolium salt; (Sigma) into a blue formazan product by living and metabolically active cells and was carried out as described by Tiemann et al. (1996). The cells were pulsed with MTT (0.5 mg/mL) for 4 h at 37°C and then solubilized to dissolve the dark blue crystals in lysis buffer (10 g/L SDS in 0.01 N HCl) overnight. The optical density was measured by a microplate reader (Dynatech, Denkendorf, Germany) at 570 nm. For in vivo assessment, the percentage of Con A-stimulated response was calculated as follows: (absorbance with Con A/absorbance without Con A) × 100 for each pig at the start (before feeding) and at the end of experiments.
For in vitro assays, the percentage of control response was calculated as follows: (absorbance of DON with Con A treatment/absorbance of Con A without DON) × 100.

IgA Production of SMC

Suspensions of SMC (6 × 10^5/well) in complete RPMI 1640 were dispensed into 24-well flat-bottom microtiter plates (Nunc) in triplicate for 48 h at 37°C. Cultures were unstimulated or stimulated with Con A: 2.5 μg/mL and DON (0, 0.235, 0.47, 0.94, 1.88, and 3.76 μM, final concentrations). Supernatant fractions were collected after 2 d and stored at −20°C until analysis.

Analysis of Immunoglobulins in Serum and Supernatant Fractions

An indirect ELISA was used. All antibodies, reference sera, and tetramethylbenzidine (TMB) were purchased from Natu Tec-Bethyl (Frankfurt a. Main, Germany). Microtiter plates (Nunc C bottom Immunoplate 96-well) were coated for 60 min at room temperature, with goat anti-pig IgG (E100-104) diluted 1:100, goat anti-pig IgM (E100-100) diluted 1:100, and goat anti-pig IgA (E100-102) diluted 1:100 in 50 mM carbonate buffer, pH 9.6. Coated plates were washed 3 times with washing solution (50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0) to remove excess capture antibodies. Wells were incubated with 200 μL of blocking (postcoat) solution (50 mM Tris, 0.14 M NaCl, 1% BSA, pH 8.0) for 30 min to block nonspecific protein binding and then washed 3 times with washing solution. For determination of immunoglobulins (IgG, IgM, or IgA), reference sera or serum samples were diluted (50 mM Tris, 0.14 M NaCl, 1% BSA, 0.05% Tween 20, pH 8.0), and 100 μL was added to appropriate wells. Serum dilutions were 1:15,000 for IgA and 1:40,000 for IgM and IgG. For IgA estimation in cultures, supernatant fractions were used undiluted. Concentrations of IgG, IgM, and IgA in the reference sera were 0.45, 4.7, and 16.5 mg/mL, respectively. Standard curves were diluted from the reference sera for IgA and IgM ranging from 15.625 to 1,000 ng/mL and for IgG from 7.8 to 500 ng/mL. The concentrations of Ig in the test serum samples were determined using these standard curves. Plates were incubated at room temperature for 60 min and then washed 5 times in washing solution. Then, 100 μL of peroxidase-conjugated goat anti-pig IgG (A100-104P) diluted 1:150,000, peroxidase-conjugated goat anti-pig IgM (A100-100P) diluted 1:40,000, or peroxidase-conjugated goat anti-pig IgA (A100-102P) diluted 1:100,000 in solution for samples was added to each well. Plates were incubated at room temperature for 60 min and washed 5 times with washing solution. After incubation, unbound peroxidase-conjugates were removed, and each well was washed 5 times with washing solution. Bound peroxidases were determined with TMB (0.1 mg/mL) and H₂O₂ (0.006%) in 0.1 M acetate, pH 6.0; and 100 μL of this substrate was transferred to each well. After 15 to 60 min incubation, the TMB reaction was stopped by addition of 100 μL of 2 M H₂SO₄ to each well, and the optical density was measured at 450 nm with a microplate reader (Anthos Instruments, Salzburg, Austria).

Histological Assessment

At postmortem examination, samples of spleen (n = 3) were collected from all groups and fixed in 10% buffered formalin. The tissue pieces were then dehydrated through graded alcohols and embedded in paraffin wax. Sections were cut 5 μm thick and stained with hematoxylin and eosin for histopathological examination and with Berlin-Blue for iron loading. Quantitative computation of iron staining was done by the AnalySIS-trame grabben CSIS system (AnalySIS 3.4, SIS, Münster, Germany) and based on the results obtained by Masuda et al. (1993). Those authors observed a linear correlation between the mean optical density of the tissue section measured with a commercial image analyzer and the iron concentration measured with an atomic absorption spectrophotometer. In our experiments, the percentage of the stained areas in Berlin-Blue stain was calculated using the image analyzing system. Images were captured by a digital, Peltier-cooled, color camera system (ColorView 12, Kappa, Gleichen, Germany) adapted to a light microscope (Zeiss, Jena, Germany) with a 50-fold lens. Fifteen randomly selected separate images per slide filled by tissue were stored on computer. Using AnalySIS 3.4, threshold (brightness, color) was adjusted to detect the stained areas for Berlin-Blue. The adjustment of microscope, camera, and software settings was standardized, computer-stored, and constant for all measurements. For analyzing the image database, a self-written AnalySIS macro was used to open the corresponding settings, detect the stained areas, calculate the percentage of stained areas in relation to the whole area for each image, and to save the results as a file.

Ultrastructural alterations and the presence and location of hemosiderin particles in the spleen were examined by transmission electron microscopy. The fixed cells were dehydrated in a graded series of ethanol and embedded in LR White (Resin Company Ltd., London). Thin sections (50 to 100 nm) were cut on an ultramicrotome (Ultracut SWS, Leica, Germany) and mounted on gold grids. The thin sections were stained with uranyl acetate (10% in ethanol; wt/vol) and lead citrate (0.2% in distilled water; wt/vol), pH 12, and finally examined and micrographed in a Zeiss EM 902 A electron microscope (Jena, Germany).

Statistical Analyses

Data were analyzed by the Student-Newman-Keuls test following one-way ANOVA using SigmaStat Statistical Analysis System (Jandel Scientific, San Rafael, CA). Data are means ± SEM derived from fourfold deter-
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Figure 1. Proliferation of peripheral blood mononuclear cells (MC) exposed in vivo to mycotoxin-contaminated diets (panel A). Proliferation of MC obtained from prepubertal gilts fed deoxynivalenol/zearalenone-contaminated diets for 35 d (mg/kg; as-fed basis): I (0.21/0.004), II (3.07/0.088), III (6.10/0.235), and IV (9.57/0.358). The yellow tetrazolium salt response was evaluated in quadruplicate for each animal (n = 9 per group). In panel B, proliferation of MC from 3 untreated prepubertal gilts was evaluated after in vitro exposure to deoxynivalenol. Bars represent means ± SEM. In vivo: yellow tetrazolium salt values of concanavalin A (Con A)-stimulated MC were related to the adequate values without Con A. In vitro: the control (0) was considered to be 100%. *Significant differences between the control and experimental group, P < 0.05.

RESULTS

In the current study, feed was partially refused during the first 21 d of the experiment by groups II, III, and IV, where 2, 3, and 6 of the 9 gilts were affected. Mean daily live weight gain was reduced by 0.568, 0.505, 0.511, and 0.410 kg/d (P < 0.05) for groups I to IV, respectively, which resulted from the initial feed intake depression (Dänicke et al., 2005). There was a trend for a dose-response-related increase in the relative weights of the spleen (P = 0.08, data not shown), but no macroscopic lesion was observed in any organ of the Fusarium toxin-exposed gilts at terminal necropsy. No signs of hyperestrogenism or uterotrophic effects were observed attributable to dietary treatments.

Mitogenic Response to MC and SMC

Results of the blood lymphocyte stimulation test are shown in Figures 1 and 2. No inhibitory effect was detected on the stimulation in MC derived from pigs of the low, medium, and high mycotoxin-fed groups compared with the corresponding samples, which were taken before the first feeding of DON/ZON, or to the very low DON/ZON group I (Figure 1A). For in vitro studies (Figure 1B), the effects in MTT response produced by preexposure of MC from untreated pigs to a range of 0.235 to 3.76 μM DON were evaluated. Significant differences, with respect to the control (0), were caused by a dose-dependent decrease in the frequency of Con A-stimulated cells. The first decrease occurred at 0.47 μM (66%; P < 0.05). At the highest concentration used, cell proliferation was only 24.9% (P < 0.05) of that in unexposed controls (Figure 1B). Figure 2A indicates that splenocytes from the DON/ZON-exposed pigs in the highest dose group showed a suppressed response (75.5%; P < 0.05) compared with group I. In vitro, the first decrease was detected at 0.47 μM (86%; P < 0.05). At 3.76 μM DON, cell proliferation was only 40.2% (P < 0.05) of the unexposed controls (Figure 2B).

Immunoglobulin Concentrations

Only in gilts from group II was there an increase of serum IgA concentration (167.6%; P < 0.05; Figure 3A) compared with the baseline values and with the other groups. No differences were observed in levels of IgM (Figure 3B) and IgG (Figure 3C) in all groups after 35 d of feeding. In splenocytes, addition of DON in vitro did not stimulate the IgA production (data not shown).
Figure 2. Proliferation of splenocytes (SMC) exposed in vivo to mycotoxin-contaminated diets (panel A). Proliferation of SMC obtained from prepuberal gilts fed deoxynivalenol/zearalenone-contaminated diets for 35 d (mg/kg; as-fed basis): I (0.21/0.004), II (3.07/0.088), III (6.10/0.235), and IV (9.57/0.358). The response to yellow tetrazolium salt (MTT) was evaluated in quadruplicate for each animal (n = 5 to 6 per group). In panel B, proliferation of SMC from 3 untreated prepuberal gilts was evaluated after in vitro exposure to deoxynivalenol. Bars represent means ± SEM. In vivo: MTT values of concanavalin A (Con A)-stimulated SMC were related to the adequate values without Con A. In vitro: the control (0) was considered to be 100%. *Significant differences between the control and experimental group, P < 0.05.

Histopathological Findings

Animals in groups I and II exhibited no significant histopathological differences among spleen samples. Numerous spherical collections of blue-staining cells in the hematoxylin-and-eosin view represent the white pulp (Figure 4). The more red tissue between these focal aggregates of lymphoid tissue is referred to as the red pulp. In microscopic sections, white pulp is equivalent to the lymphocyte population of the spleen, in the form of the periarteriolar lymphocyte sheath (PALS). In all groups, there was approximately the same number of PALS in the spleens. Iron staining occurred as diffuse, nongranular cytoplasmic staining (blue reactivity) of iron complexes and as granules (hemosiderin) in the macrophages of the red pulp. Microscopic examination showed increased deposition of hemosiderin in the spleens of gilts from groups I to IV after 35 d of feeding. Figure 5 demonstrates the mean result of 15 images each of 3 gilts of each treatment group. In groups III and IV, respectively, the area of the blue-colored portion was 20 times greater and 78.9 times greater than in group I. In groups I and II, similar amounts of hemosiderin stores were found in splenocytes. A representative photomicrograph indicates a minimal blue staining in the spleen section of pigs in group I (Figure 6A). Hemosiderin deposition was increased (P < 0.05) in the spleen of pigs in group IV (Figure 6B). The histopathological findings were supported by the electron microscopic data. Ultrastructurally, the cells of group IV indicated hemosiderin particles, but the rough endoplasmic reticulum and the mitochondria were not changed. A representative photomicrograph is shown in Figure 7.

DISCUSSION

In the current study, gilts fed higher concentrations of *Fusarium* toxin-contaminated wheat (groups III and IV) for 35 d revealed some immunological and histological changes in the spleen compared with animals fed lower concentrations of DON/ZON (groups I and II); however, the alterations observed were not manifested clinically. We found in the in vivo experiments that Con A-stimulated proliferation of blood lymphocytes was not inhibited by increasing DON/ZON concentrations in the diets. This result agrees with the findings of Rotter et al. (1994) and Øvernes et al. (1997). In contrast, we found a significantly lower Con A-induced response in splenocytes from pigs in group IV. We suggest that different sensitivity to mycotoxins exists in both tested cell types. This finding supports the results reported by Robbana-Barnat et al. (1988), who found a different sensitivity of thymic lymphocytes and splenocytes in the blastogenic response to mitogens after administration of a DON-containing diet to mice for 1 wk.

In our in vitro assays the addition of DON to MC or SMC for 48 h (Figure 1B, 2B) caused an inhibition (P < 0.05) of Con A response at 0.47 μM, and it was different from those observed in vivo (Figure 1A, 2A). Most in vitro experiments with DON show depressed mitogen-
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**Figure 3.** Serum concentrations of immunoglobin (Ig) A (A), IgM (B), and IgG (C) in prepubertal gilts fed mycotoxin-contaminated diets. Immunoglobulin concentrations were determined in samples obtained before the first feeding and after 35 d feeding of deoxynivalenol/zearalenone-contaminated diets (mg/kg; as-fed basis): I (0.21/0.004), II (3.07/0.088), III (6.10/0.235), and IV (9.57/0.358). Immunoglobulins for individual gilts were analyzed by ELISA. Bars represent means ± SEM. Nine pigs were investigated per group. *ab*Bars with different letters differ, *P* < 0.05.

induced proliferation as described for human peripheral blood lymphocytes (Mekhanca-Dahel et al., 1990; Johannisson et al., 1999; Berek et al., 2001), human macrophages (Sugita-Konishi and Pestka, 2001), and murine splenic lymphocytes (Mekhanca-Dahel et al., 1990). The in vitro results show that the active concentrations are very low (0.5 to 1 μM). In our experiments, the changes in the Con A-stimulated proliferation of MC or SMC exposed in vitro to DON demonstrated that the in vitro cell response of DON is not an accurate predictor of toxicity in the animal as a whole; however, for the toxin evaluation, the in vitro model can only hint at the reaction mechanisms of mycotoxins in biological cells. In the current study, the decreased proliferative response may be due to the capacity of DON to inhibit protein synthesis, as described by Ueno (1983), Thompson and Wannemacher (1986), and Ehrlich and Daigle (1987). Furthermore, DON and other trichothecenes bind to ribosomes via a mechanism known as the ribotoxic stress response (Pestka et al., 2004).

Exposure of experimental animals and humans to trichothecenes, T-2 toxin, and DON (Atroshi et al., 1994) has been associated with altered measures of humoral-associated immunity. We observed increased serum IgA concentrations in gilts in group II, whereas serum IgM and IgG concentrations were not altered. The significant increase in serum IgA level is in agreement with Swamy et al. (2002), who found increased IgM concentrations in pigs fed mycotoxin-contaminated grains. Elevated Ig concentrations might be due to the presence of fusaric acid and other unidentified *Fusarium* mycotoxins in the diet. In contrast to the results of Swamy et al. (2002) and the current study, Bergsøe et al. (1992) did not detect a significant effect on IgA concentrations after feeding oats contaminated with 4.82 ppm DON to pigs for 6 wk, perhaps because of the use of different methodology. Bergsøe et al. (1992) used radial immunodiffusion, whereas in the current study we used the ELISA technique, which has a greater sensitivity and accuracy. One explanation for the stimulatory effect of DON on the serum IgA concentration in our study in group II only may be altered plasma membrane permeability (Zanic-Grubisic et al., 1995). This could result in increased antigen sampling and presentation, with a subsequent elevation of antigen-directed B-cell response. An overview of the mechanisms of IgA production induced by DON is given by Pestka (2003). Whether the elevation of blood IgA concentrations observed in the current study is specific or nonspecific remains unclear. To identify the specific effect of DON on IgA production, we used splenic lymphocyte cultures; however, in vitro, DON was unable to stimulate an increase in IgA production, perhaps because the heterogeneous cell population masked any potential response. Yan et al. (1998) reported that macrophages and their interaction with T cells are critical for the upregulation of IgA production in mice exposed orally to DON. The effects of macrophages are likely to be mediated by secretion of soluble mediators such as...
interleukin (IL-6). An explanation that DON at higher concentrations in the diets (groups III and IV) did not increase the IgA production as in group II may be because DON has the capacity to rapidly alter the regulatory function of macrophages.

In groups II and IV, we found increased accumulation of hemosiderin particles in macrophages of splenocytes. Hemosiderin deposition without marked tissue damage and organ dysfunction is referred to as hemosiderosis (Nyska et al., 1989). In the current study, the image analyzer provided a sensitive, quantitative method for evaluation of the degree of splenic hemosiderosis that was dose related. In the spleens of pigs in groups III and IV, increased hemosiderosis was found without signs of anemia. This finding was supported by the fact that no decreases of red blood cells, hemoglobin concentration, and hematocrit were found in the highest dose group (Dänicke et al., 2005). Our results agree with the data published by Kraft and Dürr (1999). The exact reason for the hemosiderosis cannot be ascertained from the data presented; however, it could be due to an increased
ern red blood cells, suppression of erythroid progenitor cells, and impaired iron use (Sing et al., 1989; Wang et al., 1995; Birgegard et al., 2005). As a consequence, an accumulation of an excess of iron (hemosiderin) in macrophages of red pulp can occur, perhaps because transferrin is already saturated with iron. It can be assumed that this excess of iron inhibits the iron transporter transferrin (Underwood, 1975) and is responsible for observed effect. Additional studies will be required to evaluate transferrin receptors in spleens. Furthermore, the saturation of serum ferritin and transferrin iron should be monitored to determine how DON affects the erythropoietic system. We suggest that the effect in splenocytes in groups III and IV seemed to be secondary to erythrocyte toxicity because there were no observed abnormalities in the ultrastructure (e.g., rough endoplasmic reticulum, mitochondria) of splenocytes.

Excess iron accumulates in parenchymal organs and threatens cell viability. Indeed, when iron-buffering capability is overwhelmed, oxidative stress-induced cell damage and fibrogenesis may arise. Andrews (1999) reported that an excess of hepatic iron can cause oxidative effects, augmenting cellular damage and resulting in the development of liver cancer. It is unknown to date how much stored iron or iron overload is necessary to cause disease (Gordenk et al., 1994; Stal, 1995). The alterations in the red pulp found in association with DON (ZON) exposure in pigs were similar to the changes in murine spleen (Pestka et al., 1990).

We concluded that after 5 wk of feeding, significant effects on the spleen in gilts can be mostly observed at a dietary DON concentration of 9.57 mg/kg originating from naturally contaminated wheat containing only minor traces of ZON (0.358 mg/kg of feed). This finding was reflected by a decreased cellular immune response to Con A in splenocytes of gilts. Our histopathological findings support the appearance of a dysfunction in the porcine spleen, which can be observed as hemosiderosis. Altogether, these results provide evidence of spleen dysfunction in the absence of clinical signs. Pathophysiology attributable to DON/ZON in pigs can be important in understanding whether human exposure to DON/ZON might have unfavorable effects because swine are physiologically similar to humans (Tumbleson and Schook, 1996) and are widely used as models for human disease.

**Figure 7.** Transmission electron microscopy of spleen (group IV; panel A) hemosiderin particles in phagosomes (white arrow) and intact mitochondria (M). Panel B is the intact rough endoplasmatic reticulum with ribosomes (rER). Magnification = 14,000x.

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