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# On the effects of graded levels of *Fusarium* toxin contaminated wheat in diets for gilts on feed intake, growth performance and metabolism of deoxynivalenol and zearalenone

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A total of 36 gilts (103 ± 6 kg) were divided into four groups and fed diets with increasing proportions of a *Fusarium* toxin contaminated wheat over a period of 35 days. The concentrations of the indicator toxins deoxynivalenol (DON) and zearalenone (ZON) which were analyzed by HPLC methods were 210 and 4, 3070 and 88, 6100 and 235 and 9570 and 358 μg · kg$^{-1}$ diet fed to groups 1 – 4 respectively. Feed was partially refused during the first 21 days of the experiment by groups 2, 3 and 4 where two, three and six out of nine gilts were affected. No signs of hyperestrogenism or uterotrophic effects were observed due to dietary treatments. Blood serum, urine, bile and liver were analyzed for residues of DON, ZON and their metabolites. DON and its de-epoxidized metabolite (de-epoxy-DON) were detected in all analyzed specimens and increased in a significantly linearly related fashion. Alpha-zearalenol (α-ZOL) and β-ZOL could be detected besides the parent toxin ZON, but only in bile and urine. In conclusion, the impact of dietary treatments on the performance parameters was most pronounced in the highest exposed group. The maximum ratio between DON concentration in liver and diet was 0.0013, and suggests that a possible contamination of pig liver with DON is negligible and does not contribute significantly to human DON exposure.

**Keywords:** Deoxynivalenol / Fusarium / Gilt / Metabolism / Zearalenone

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## 1 Introduction

The ubiquitously occurring phytopathogenic species of the mould fungi genus *Fusarium* contaminate plants with their toxins prior to harvest. Among a number of different *Fusarium* toxins, deoxynivalenol (DON) and zearalenone (ZON) are especially of relevance since they can be present in feedstuffs at concentrations which might affect health and performance of farm animals. Pigs are considered as especially sensitive to both toxins and might react with a decrease in feed intake to the presence of DON in feed, and with signs of hyperestrogenism in the case of ZON. However, no safe dietary levels could be established up to now [1, 2]. Difficulties in deriving such safe levels arise from discrepancies in the results obtained by the use of either crystalline standard compounds or by the use of naturally and cocontaminated feed materials.

Not only the different toxin sources contribute to the variable effects reported so far but also the lack of *in vitro* systems combined with *in vivo* studies. Such a combination seems to be essential not only to prove effects observed *in vitro* for their significance *in vivo* but also to explain clinical effects which are observed under feeding conditions. For example, Tiemann *et al.* [3] demonstrated *in vitro* a dose-dependent arresting of endometrial porcine cells in the $G_0/G_1$-phase of the cell cycle by β-zearalenol (β-ZOL), a metabolite of ZON and by DON, but not by α-zearalenol (α-ZOL), another metabolite of ZON. However, the relevance of these findings for pigs fed contaminated diets could not be clarified by this *in vitro* study.

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**Abbreviations: DON**, deoxynivalenol; **ZON**, zearalenone

 

Hence, the aim of the present study was to examine the effects of increasing concentrations of *Fusarium* toxins, provided by wheat primarily contaminated naturally with DON and ZON, in diets for prepubertal gilts on growth performance, organ weights and haematological parameters. Moreover, the fate and metabolization of the so-called indicator *Fusarium* toxins DON and ZON was followed not only in serum as an indicator for the extracellular available toxin pool, but also in several physiological matrices in order to substantiate their significance in affecting health and reproduction. Most studies performed so far on this issue lacked from the concomitant examination of the metabolism of both DON and ZON. However, such a combined procedure seems to be advantageous in the view that both toxins occur often together in naturally contaminated feedstuffs. Thus, such detailed information could contribute for a better interpretation of toxic effects.

In addition, physiological specimens obtained from this study served for further *ex vivo* and *in vitro* studies which are reported elsewhere [4, 5].

## 2 Materials and methods

### 2.1 Experimental design and diets

The experiment was designed according to the simple dose-response principle. The equidistant increase in *Fusarium* toxin concentration was achieved by a stepwise substitution of the clean (uncontaminated) wheat with the *Fusarium* toxin contaminated wheat (Tab. 1) at a constant total wheat proportion of 40%. Thus, the four experimental diets differed only in the proportions of clean and contaminated wheat. Diets were formulated to meet or to exceed the needs for prepubertal gilts; and ME-concentration of the diets was estimated using the prediction equation as proposed by the Committee for Requirement Standards of the Society for Nutritional Physiology [6]. Diets were provided in a coarse meal form.

The experiment was replicated three times with three gilts tested *per* group and replication. Consequently, a total of nine gilts were used to test each diet and each replicated experiment was carried out with 12 gilts.

### 2.2 Gilts, feeding and housing

The experiments commenced when gilts (German Landrace) were 180 days old. Mean initial live weights and SDs were similar for each group (103 ± 6 kg). Gilts were kept individually without bedding on a partially slatted floor. Water was provided for *ad libitum* consumption.

**Table 1.** Composition of the experimental diets (g/kg, 88% dry matter)

| | Group | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Components | | | | |
| Contaminated wheat | 0 | 133.5 | 266.5 | 400 |
| Control wheat | 400 | 266.5 | 133.5 | 0 |
| Barley | 284.3 | 284.3 | 284.3 | 284.3 |
| Dried chips | 100 | 100 | 100 | 100 |
| Soybean meal | 175 | 175 | 175 | 175 |
| Soy oil | 10 | 10 | 10 | 10 |
| Premix[a)] | 30 | 30 | 30 | 30 |
| L-lysine HCl | 0.7 | 0.7 | 0.7 | 0.7 |
| Calculated composition | | | | |
| Crude protein | 165 | 165 | 165 | 165 |
| Crude fibre | 51.5 | 51.5 | 51.5 | 51.5 |
| Lysine | 8.9 | 8.9 | 8.9 | 8.9 |
| Calcium | 7.7 | 7.7 | 7.7 | 7.7 |
| Total phosphorus | 5.7 | 5.7 | 5.7 | 5.7 |
| DON ($mg \cdot kg^{-1}$)[b)] | 0.00 | 2.73 | 5.44 | 8.17 |
| ZON ($\mu g \cdot kg^{-1}$)[b)] | 0 | 89 | 177 | 266 |
| Analyzed composition | | | | |
| Crude protein | 159 | 168 | 158 | 162 |
| DON ($mg \cdot kg^{-1}$)[c)] | 0.21 | 3.07 | 6.1 | 9.57 |
| ZON ($\mu g \cdot kg^{-1}$)[c)] | 4 | 88 | 235 | 358 |

a) Provided *per* kg diet: Ca, 6.3 g; P, 2.1 g; lysine, 0.6 g; Na, 1.8 g; Mg, 0.3 g; vitamin A, 15 000 IE; vitamin $D_3$, 1500 IE; vitamin E, 45 mg; Cu, 22.5 mg; Se, 0.36 mg.
b) Calculated on the basis of the analyzed concentrations of the contaminated wheat (Table 2).
c) Measured by HPLC methods.

Experimental diets were introduced by a stepwise dilution of the uncontaminated control diet with the respective experimental diets over a period of 3 days. Gilts were fed restrictively at an amount of 2 kg daily provided in two equal portions (07:00 and 14:00 h) over a period of 35 days. They were weighed once a week and feed refusals, if occurring, were recorded daily. The outer genitalia of the gilts were daily inspected for obvious signs of estrus.

### 2.3 Collection of blood and tissue samples

Blood was collected by cervical vein puncture at the beginning and at the end of the experiment and prepared for serum or plasma for subsequent haematological investigations and for mycotoxin residue analysis. The day after the 35-day feeding period, pigs were injected after ketamin/xylazin induced anaesthesia (Ursotamin® and Xylazin®, Serumwerk Bernburg, Germany) with a lethal dose of T61 (embutramide/mebezoniumiodide/tetracainhydrochloride, Intervet Unterschleißheim, Germany). At laparotomy urine and bile were collected by puncture of bile and urinary bladder. Spleen, uterus and liver were recovered quickly, their weight recorded and samples were stored respectively for further analysis.

 

The present study was approved by the Committee for Animal Use and Care of the Ministerial Agricultural Department of Mecklenburg-Vorpommern, Germany, according to the German Law for Animal Protection (TierSchG).

## 2.4 Analyses

### 2.4.1 Haematological parameters

Blood samples (from the cranial vena line) were obtained before the pigs were given DON and after 5 wk feeding. Serum was collected within 3 h and the haematological parameters were determined using automated instrumentation (Synchron LX®-System, Beckman and Coulter, Krefeld, Germany).

### 2.4.2 Mycotoxin residues

DON in diets was analyzed by HPLC with diode array detection (DAD) after a clean-up with immunoaffinity column (IAC), (DONprep™, R-Biopharm Rhone, Darmstadt, Germany) according to slightly modified procedure of R-Biopharm Rhone. The detection limit was 0.03 mg · kg$^{-1}$ and the recovery approximately 89%. Further trichothecenes in wheat were analyzed by the Institute of Animal Nutrition of the University of Hohenheim, Germany using a GC-MS method [7].

Physiological samples were analyzed for DON and de-epoxy-DON according to Valenta *et al.* [8] with modifications. Briefly, serum, urine, bile and freeze-dried liver were incubated with β-glucuronidase (Type H-2, β-glucuronidase, EC 3.2.1.31, 114400 U · mL$^{-1}$; arylsulphatase, EC 3.1.6.1, 3290 U · mL$^{-1}$; Sigma, Deisenhofen, Germany) at pH 5.5 and 37°C overnight. Subsequently, serum, urine and bile were extracted with ethyl acetate (bile after pH adjusting to 7) on disposable ChemElut columns (Varian Deutschland GmbH, Darmstadt) and cleaned up with IAC (DONtest™ of VICAM, Klaus Ruttmann GmbH, Hamburg, Germany in case of serum and DONprep™, R-Biopharm Rhone, in case of urine and bile). Freeze-dried liver was extracted with a mixture of ACN and water, defatted with petroleum ether, precleaned with a mixture of charcoal, alumina and celite and cleaned up with IAC (DONprep™, R-Biopharm Rhone). DON and de-epoxy-DON in serum, bile and liver were determined by HPLC with UV detection, in the case of urine by HPLC-DAD. The detection limit for both substances was approximately 2, 10, 4 ng · mL$^{-1}$ and 4 ng · g$^{-1}$ for serum, urine, bile and freeze-dried liver respectively with mean recoveries of 92 – 95 and 88 – 104% for DON and de-epoxy-DON respectively. Further efforts were undertaken to quantify the proportions of DON and de-epoxy-DON in serum and urine which were glucuronated. In doing so, six urine samples and nine serum samples from gilts fed the diets with the higher DON concentrations (groups 3 and 4) were analyzed for the toxins without and after incubation with β-glucuronidase.

Physiological samples (serum, liver, bile, urine) were analyzed for zearalenone (ZON), α-ZOL, β-ZOL, zearalanone (ZAN), α-zearalanol (α-ZAL) and β-zearalanol (β-ZAL) by HPLC with fluorescence detection after treatment overnight at 40°C with 2/0.9 U β-glucuronidase/arylsulphatase (EC 3.2.1.31/EC 3.1.6.1; No. 127698 Roche Diagnostics GmbH, Mannheim, Germany) and cleaning of the extracts by immuno-affinity columns (Easi-Extract™ ZON, R-Biopharm) according to Ueberschär [9] as described by Dänicke *et al.* [10]. Detection limits for all specimens were 2, 2, 5, 100, 50 and 200 ng · g$^{-1}$ for ZON, α-ZOL, β-ZOL, ZAN, α-ZAL and β-ZAL respectively at a sample weight of 5 g. ZON in feedstuffs was analyzed after incubation with 2 U β-glucosidase (EC 3.2.1.21, No. G-0395; Sigma, Taufkirchen, Germany). The mean recovery for ZON, α-ZOL and β-ZOL were 82, 81 and 74% for feedstuffs; 85, 76 and 82% for serum; 88, 76 and 66% for liver; 92, 81 and 104% for bile; and 94, 75 and 85% for urine respectively.

Analytical results were not corrected for recovery.

Mycotoxin concentrations in physiological samples, which were lower than the above, indicated detection limits were considered with a concentration of zero in evaluating the data. This implies that calculated mean values might be lower than the detection limits.

### 2.4.3 Dry matter and crude protein concentration of the diets

Dry matter and Kjeldahl N of the feedstuffs were analyzed according to the methods of the VDLUFA [11]. Crude protein was obtained by multiplying the Kjeldahl N by 6.25.

## 2.5 Statistics

Performance data, organ weights and mycotoxin residual data were tested with a two-way factorial design of ANOVA

$$y_{ijk} = \mu + a_i + b_j + (a \times b)_{ij} + e_{ijk}$$

where $y_{ijk}$ = tested parameter of a gilt k fed a diet type i during a particular replication period j; $\mu$ = overall mean; $a_i$ (fixed effect) = diet type (*i.e.* group effect, diets for groups 1, 2, 3 and 4 contained 0, 13, 27 and 40% of *Fusarium* toxin contaminated wheat); $b_j$ (fixed effect) = replication period (1 – 3); $(a \times b)_{ij}$ = interactions between diet type and replication period; $e_{ijk}$ = error term.

The dependency of haematological parameters at the end of the experimental period on those measured at the beginning of the experiment was considered by the following model

 

$$y_{ijkl} = \mu + a_i + b_j + c_k + (a \times b)_{ij} + (a \times c)_{ik} + (b \times c)_{jk} + (a \times b \times c)_{ijk} + d_{l(a \times b \times c)} + e_{ijkl}$$

where $y_{ijkl}$ = tested parameter of a gilt l fed a diet type *i* during a particular replication period *j* and time point k; $\mu$ = overall mean; $a_i$ (fixed effect) = diet type (*i.e.* group effect, diets for groups 1, 2, 3 and 4 contained 0, 13, 27 and 40% of *Fusarium* toxin contaminated wheat); $b_j$ (fixed effect) = replication period (1 – 3); $c_k$ (fixed effect) = time point of measurement (beginning and end of the experiment); $(a \times b)_{ij}$ = interactions between diet type and replication period; $(a \times c)_{ik}$ = interactions between diet type and time point of measurement; $(b \times c)_{jk}$ = interactions between replication period and time point of measurement; $(a \times b \times c)_{ijk}$ = interactions between diet type, replication period and time point of measurement; $d_{l(a \times b \times c)}$ (random effect) = effect of repeated measurements (beginning and end of the experiment) within the same gilt l, *i.e.* variance component contributed by the gilts; $e_{ijkl}$ = error term.

Differences in mean values were evaluated for significance using the Tukey test ($p < 0.05$).

Daily feed refusals were not normally distributed and were compared by the Mann – Whitney *U*-test.

Data were analyzed using the SAS package (SAS Institute Inc.) [12].

## 3 Results

### 3.1 Wheat characteristics and dietary DON concentration and ZON concentration

The clean control wheat and the *Fusarium* toxin contaminated wheat were not of the same origin (variety, location). However, their crude protein concentrations differed only slightly (Table 2) and did not influence the crude protein concentration of the diets consistently (Table 1).

The control wheat contained only traces of DON and the concentration of further trichothecene mycotoxins were all lower than the indicated detection limits (Table 2). ZON was also present only in traces. In contrast, the contaminated wheat contained more than 20 mg DON · $kg^{-1}$ when measured by HPLC. Analysis of this wheat by GC-MS for a detailed characterization of the trichothecene mycotoxin pattern revealed an approximately 15% lower DON concentration besides the presence of further B-trichothecene mycotoxins such as 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and nivalenol, and of the A-trichothecene mycotoxin HT-2 toxin.

DON and ZON concentrations of the experimental diets were determined solely by HPLC methods because all physiological matrices were analyzed with this analytical procedure. A comparison of the calculated toxin concentrations (based on HPLC results of the contaminated wheat) with the measured concentrations revealed sufficient agreement. The analyzed DON concentrations were 11, 11 and 15% higher than the calculated ones for diets fed to groups 2, 3 and 4 respectively. The respective figures for ZON concentrations were 0, 25 and 24%.

**Table 2.** Crude protein concentration (g/kg) and mycotoxin pattern (μg/kg) of uncontaminated and *Fusarium* toxin contaminated wheat (88% dry matter)

| | Uncontaminated wheat | Contaminated wheat |
|---|---|---|
| Crude protein | 133 | 126 |
| ZON[a)] | 2 | 665 |
| DON[a)] | 139 | 20 420 |
| DON[b)] | 60 | 17 400 |
| Nivalenol[b)] | <15 | 400 |
| Scirpentriol[b)] | <9 | <9 |
| T2-tetraol[b)] | <8 | <8 |
| Fusarenon-X[b)] | <21 | <21 |
| Monoacetoxyscirpenol[b)] | <3 | <3 |
| 15-Acetyldeoxynivalenol[b)] | <8 | 50 |
| 3-Acetyldeoxynivalenol[b)] | <10 | 30 |
| T2-triol[b)] | <6 | <6 |
| Neosolaniol[b)] | <7 | <7 |
| Diacetoxyscirpenol[b)] | <16 | <16 |
| HT-2 toxin[b)] | <3 | 5 |
| T-2 toxin[b)] | <4 | <4 |

a) Measured by HPLC methods.
b) Measured by a GC-MS method.

### 3.2 Feed intake and live weight gain

Increasing proportions of *Fusarium* toxin contaminated wheat in the diets of gilts resulted in a dose-response related increase of number of individual gilts which refused a part of their daily feed amount (Fig. 1). Feed refusals were recorded for six out of nine gilts of group 4 (diet with the highest degree of contamination), three out of nine and two out of nine of groups 3 and 2 respectively. No feed refusal occurred in group 1 (control group). Not only the number of affected pigs was related to the degree of diet contamination, but also the amount which was refused. Cumulatively, three gilts of group 4 refused less than 5% of their offered feed whereas the other three gilts refused 11, 20 and 41%. In groups 2 and 3, the amount of feed which was not consumed was less than 3% with the exception of one gilt of group 3 which refused 18%. Statistically, the cumulative feed refusals of groups 3 and 4 differed significantly from the control group 1 ($p < 0.05$). Moreover, gilts of group 4 refused significantly more feed than gilts of group 2 ($p = 0.032$) and with a similar trend for gilts of group 3 ($p = 0.098$).

 

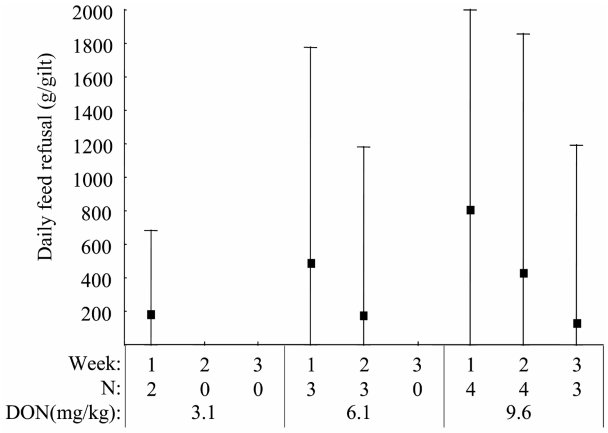


**Figure 1.** Daily feed refusal of gilts of groups 2–4 (no refusal occurred in group 1) during the first 3 wk of the experiments (values are mean values, whiskers indicate minimum and maximum refusals).

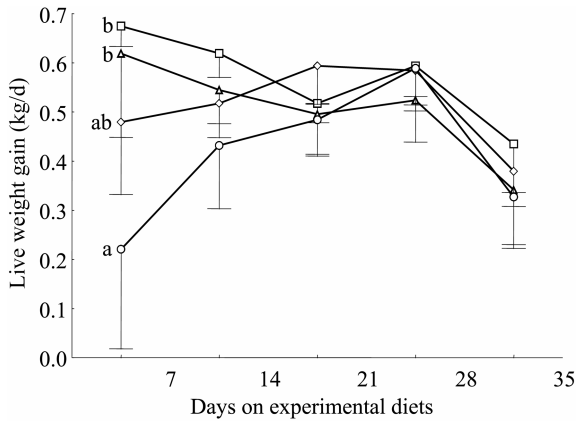


**Figure 2.** Time course of daily live weight gain of gilts fed diets with increasing proportions of *Fusarium* toxin contaminated wheat (ab, mean values with no common superscript are significantly different only during the first week of the experiment ($p < 0.05$)).

| Deoxynivalenol Zearalenone (mg · kg$^{-1}$ diet) | Group | |
|---|---|---|
| 0.21/0.004 | 1 | -□- |
| 3.07/0.088 | 2 | -△- |
| 6.1/0.235 | 3 | -▽- |
| 9.57/0.358 | 4 | -○- |

The effect of experimental diets on feed intake was only detectable at the beginning of the experiment whereas after approximately 3 wk of feeding, all gilts consumed their daily ration of 2 kg completely (Fig. 1).

The dynamics of the mean daily live weight gain over the course of the experiment reflected those described for feed intake. The effects of the experimental diets were much more pronounced at the beginning of the experiment where the feed refusals occurred (Fig. 2). For example, gilts of group 4 gained 67% less than the control group during the first week of the experiment whereas this drop shrunk to 30%, and less than 10% during second and third weeks of the experiment respectively. Cumulatively, the lower live weight and the significantly lower daily live weight gain of gilts of group 4 (Table 3) resulted mainly from the depressed performance during the first 2 wk of the experiment.

Cumulative feed to gain ratio was only slightly influenced by dietary treatments ($p = 0.102$) and increased from 3.6 to 4.4, 4.0 and 5.7 kg · kg$^{-1}$ for groups 1–4, which corresponded to a relative increase of 22, 10 and 58% for groups 2, 3 and 4 when compared to the control group 1.

**Table 3.** Effects of increasing proportions of *Fusarium* toxin contaminated wheat fed over a period of 5 wk on live weight, weight gain and weights of spleen and uterus of gilts

| DON/ZON, mg/kg diet | Live weight, kg | Live weight gain, kg *per* day | Spleen | | Uterus | |
|---|---|---|---|---|---|---|
| | | | g | g/kg | g | g/kg |
| 0.21/0.004 | 122.0 | 0.568[c)] | 341 | 2.75 | 305 | 2.45 |
| 3.07/0.088 | 120.7 | 0.505[c)] | 439 | 3.64 | 295 | 2.42 |
| 6.1/0.235 | 121.2 | 0.511[c)] | 523 | 4.30 | 299 | 2.39 |
| 9.57/0.358 | 117.1 | 0.410[c)] | 486 | 4.11 | 271 | 2.23 |
| Probabilities | | | | | | |
| Group | 0.649 | 0.029 | 0.107 | 0.080 | 0.993 | 0.995 |
| Linear | 0.287 | 0.006 | 0.041 | 0.026 | 0.795 | 0.813 |
| Quadratic | 0.633 | 0.596 | 0.218 | 0.229 | 0.914 | 0.921 |
| Replication[a)] | 0.028 | 0.016 | 0.005 | 0.016 | 0.075 | 0.073 |
| Group × replication | 0.974 | 0.419 | 0.856 | 0.886 | 0.894 | 0.880 |
| PSEM[b)] | 5.0 | 0.060 | 52 | 0.74 | 81 | 1.06 |

a) Replication: Each group was tested three times with three pigs in each group, *i. e.* nine pigs *per* group in total.
b) PSEM, pooled standard error of means.
c) Mean values with no common superscript are significantly different within columns ($p < 0.05$).

 

**Table 4.** Effects of increasing proportions of *Fusarium* toxin contaminated wheat fed over a period of 5 wk on differential blood count of gilts

| DON/ZON, mg · kg$^{-1}$ diet | Group | Leukocytes, 10$^9$/L 10 – 22[a)] | | Lymphocytes, % 49 – 85[a)] | | Basophils, % 0 – 2[a)] | | Eosinophils, % 0 – 6[a)] | | Monocytes, % 0 – 5[a)] | | Neutrophils, % 10 – 39[a)] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | B[b)] | E[c)] | B[b)] | E[c)] | B[b)] | E[c)] | B[b)] | E[c)] | B[b)] | E[c)] | B[b)] | E[c)] |
| 0.21/0.004 | 1 | 15.8 | 15.1 | 57.2 | 63.1 | 0.9 | 0.5 | 3.5 | 2.7 | 8.0 | 6.4 | 30.4 | 27.3 |
| 3.07/0.088 | 2 | 16.8 | 13.2 | 59.9 | 59.6 | 0.6 | 0.6 | 3.2 | 4.2 | 8.0 | 7.1 | 28.3 | 28.5 |
| 6.1/0.235 | 3 | 15.2 | 14.3 | 59.2 | 61.5 | 0.5 | 0.4 | 2.4 | 2.6 | 7.7 | 6.6 | 30.2 | 28.9 |
| 9.57/0.358 | 4 | 17.3 | 14.3 | 57.7 | 63.7 | 0.5 | 0.6 | 2.9 | 2.3 | 7.1 | 7.3 | 32.6 | 26.1 |
| Probabilities | | | | | | | | | | | | | |
| Group | | 0.884 | | 0.996 | | 0.152 | | 0.149 | | 0.970 | | 0.983 | |
| Replication[d)] | | 0.025 | | 0.010 | | 0.021 | | 0.712 | | 0.002 | | 0.054 | |
| Age | | 0.026 | | 0.134 | | 0.175 | | 0.840 | | 0.137 | | 0.167 | |
| Group × replication | | 0.432 | | 0.292 | | 0.117 | | 0.171 | | 0.546 | | 0.288 | |
| Group × age | | 0.551 | | 0.722 | | 0.053 | | 0.132 | | 0.718 | | 0.614 | |
| Replication × age | | 0.050 | | 0.108 | | 0.140 | | 0.698 | | 0.018 | | 0.290 | |
| Group × replication × age | | 0.276 | | 0.790 | | 0.250 | | 0.199 | | 0.961 | | 0.714 | |
| PSEM[e)] | | 11.4 | | 3.4 | | 0.1 | | 0.5 | | 0.9 | | 2.8 | |

a) Reference ranges according to Ref. [19].
b) Beginning of the experiment.
c) After 5 wk feeding the experimental diets.
d) Replication: Each group was tested three times with three pigs in each group, *i. e.* nine pigs *per* group in total.
e) PSEM, pooled standard error of means.

### 3.3 Intake of DON and ZON

The mean intake of these toxins was calculated on the basis of the live weight data of the last 14 days of the experiment where the 2 kg diet offered daily was consumed by all gilts. DON intake increased from 3.6 (3.1–3.9) to 52.4 (47.6–60.6), 104.2 (91.2–119.2) and 169.1 (148.4–193.5) μg · kg$^{-1}$ live weight *per* day; and that of ZON from 0.06 (0.06–0.08) to 1.5 (1.4–1.7), 4.0 (3.5–4.6) and 6.3 (5.6–7.2) μg · kg$^{-1}$ live weight *per* day in gilts fed diets containing 210/4, 3070/88, 6100/235, 9570/358 μg DON/ZON *per* kg diet.

### 3.4 Estrus control

Outer signs of estrus, such as reddened and/or swollen vulva, were observed irregularly among the gilts. Neither the onset nor the duration of the signs could be related to dietary treatments.

### 3.5 Haematological parameters

Neither the total counts of leukocytes nor their percent distribution (lymphocytes, basophils, eosinophils, neutrophils, monocytes) were altered by dietary treatment (Table 4). In contrast, replicating the experiment significantly influenced the leukocyte counts and the proportions of lymphocytes, basophils and monocytes. Moreover, the leukocyte counts were significantly lower as the gilts aged. All interactions were insignificant with the exception of the interactions between replication and age for the monocytes proportion.

Haemoglobin concentration and haematocrit were significantly higher in gilts of group 4 compared to group 1, and seemed to increase from group 1 to group 4 (Table 5). Similar trends could be seen for the erythrocytes count ($p = 0.112$). The significantly lowest mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were found for gilts of group 3. Mean corpuscular haemoglobin concentration (MCHC) was not influenced by dietary treatment. Replication of the experiment exerted a significant effect on the erythrocytes indices MCH, MCHC and MCV. Also, most of the red blood cell parameters, with the exception of the MCHC, were significantly higher in older gilts. Significant interactions between replication and age were detected for haemoglobin concentration, haematocrit, erythrocytes count and MCV. Experimental diets (group effect) exerted their effects on MCH with significant differences in particular replications of the experiments (significant group by replication interaction).

### 3.6 Organ weights

There was a trend for a dose-response related increase in the absolute and relative weights of the spleen ($p = 0.107$ and $p = 0.08$, Table 3). The relative weights of the uteri remained unaffected with increasing proportions of *Fusarium* toxin contaminated wheat (Table 3).

### 3.7 Mycotoxin residues

#### 3.7.1 DON and de-epoxy-DON

DON and its metabolite de-epoxy-DON were detected in all specimens analyzed (Table 6, sum of glucuronic acid

 

**Table 5.** Effects of increasing proportions of *Fusarium* toxin contaminated wheat fed over a period of 5 wk on some haematological parameters of gilts

| DON/ZON, mg/kg diet | Group | Haemoglobin, mmol/L 6.7 – 9.2[a)] | | Haematocrit 33 – 45[a)] | | Erythrocytes, $10^6$/µL 5.8 – 8.1[a)] | | MCV, fL 50 – 65[a)] | | MCHC, mmol/L 19 – 22[a)] | | MCH, fmol 1 – 1.3[a)] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | B[b)] | E[c)] | B[b)] | E[c)] | B[b)] | E[c)] | B[b)] | E[c)] | B[b)] | E[c)] | B[b)] | E[c)] |
| 0.21/0.004 | 1 | 7.0 | 8.1[d)] | 0.35 | 0.40[d)] | 6.3 | 7.0 | 57.3 | 57.7[d)] | 19.8 | 20.1 | 1.13 | 1.17[d)] |
| 3.07/0.088 | 2 | 8.0 | 8.6[d)] | 0.40 | 0.43[d)] | 7.1 | 7.3 | 58.2 | 59.2[d)] | 20.2 | 19.9 | 1.17 | 1.18[d)] |
| 6.1/0.235 | 3 | 7.6 | 8.6[d)] | 0.39 | 0.43[d)] | 7.0 | 7.8 | 55.5 | 56.1[d)] | 19.8 | 20.1 | 1.10 | 1.12[d)] |
| 9.57/0.358 | 4 | 8.1 | 8.9[d)] | 0.41 | 0.45[d)] | 7.0 | 7.5 | 57.7 | 59.0[d)] | 19.9 | 20.0 | 1.15 | 1.18[d)] |
| Probabilities | | | | | | | | | | | | | |
| Group | | 0.042 | | 0.030 | | 0.112 | | 0.042 | | 0.963 | | 0.010 | |
| Replication[e)] | | 0.340 | | 0.066 | | 0.451 | | 0.007 | | 0.001 | | <0.001 | |
| Age | | 0.001 | | 0.002 | | 0.023 | | <0.001 | | 0.453 | | 0.003 | |
| Group × replication | | 0.826 | | 0.779 | | 0.514 | | 0.191 | | 0.232 | | 0.010 | |
| Group × age | | 0.853 | | 0.948 | | 0.814 | | 0.258 | | 0.340 | | 0.587 | |
| Replication × age | | 0.045 | | 0.013 | | 0.034 | | <0.001 | | 0.211 | | 0.570 | |
| Group × replication × age | | 0.351 | | 0.295 | | 0.633 | | 0.279 | | 0.279 | | 0.790 | |
| PSEM[f)] | | 0.3 | | 0.02 | | 0.3 | | 0.7 | | 0.2 | | 0.01 | |

a) Reference ranges according to Ref. [19].
b) Beginning of the experiment.
c) After 5 wk feeding the experimental diets.
d) Mean values with no common superscript are significantly different within columns ($p < 0.05$).
e) Replication: Each group was tested three times with three pigs in each group, *i. e.* nine pigs *per* group in total.
f) PSEM: pooled standard error of means.

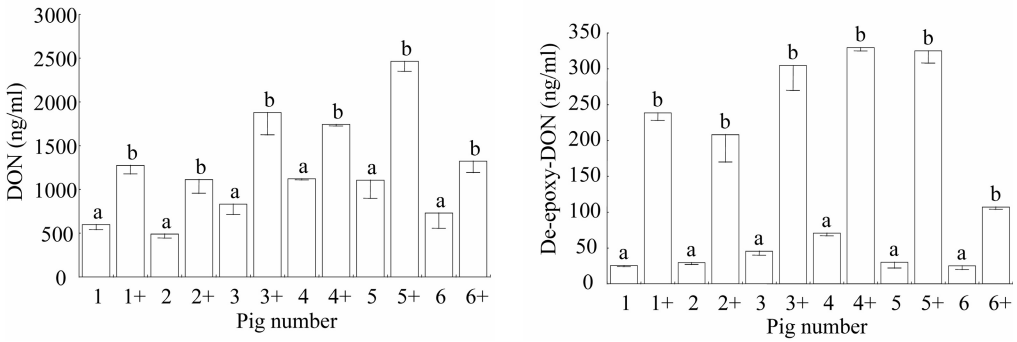


**Figure 3.** DON (left) and de-epoxy-DON (right) concentration in urine of gilts of groups 3 and 4; without and after incubation with β-glucuronidase (+).

conjugated plus unconjugated compounds). A significantly linear increase in concentrations due to increased dietary DON concentration could be detected in each case. The effect of incubation of urine and serum samples with β-glucuronidase prior to HPLC analysis revealed a significant increase in concentration (Figs. 3, 4) for each individual pig sample with one exception of a serum sample where the increase was not significant (Fig. 4, Pig 9). Data for de-epoxy-DON in serum are not shown since its concentration was lower than the detection limit when analyzed without enzymatic pretreatment. Therefore, the total de-epoxy-DON concentration in serum actually represents just the conjugated part.

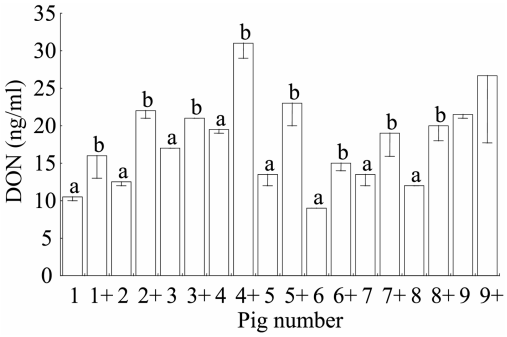


**Figure 4.** DON concentration in serum of gilts of groups 3 and 4; without and after incubation with β-glucuronidase (+).

### 3.7.2 ZON and its metabolites

ZON and its metabolites were generally analyzed after treatment with glucuronidase/sulphatase. Thus, all presented concentrations include the sum of unconjugated plus conjugated metabolites.

Neither ZON nor its metabolites could be detected in the serum and livers of gilts where concentrations were all lower than the detection limits. Dose-response related increases in the concentrations of ZON, α-ZOL and β-ZOL were found in bile and urine (Tab. 7, Fig. 5) whereas ZAN, α-ZAL and β-ZAL concentrations were lower than the indicated detection limits for these specimens.

 

**Table 6.** Concentration of DON and its metabolite de-epoxy-DON in serum, liver, bile and urine of gilts exposed to increasing proportions of *Fusarium* toxin contaminated wheat over a period of 5 wk

| DON/ZON, mg/kg diet) | Group | Serum, ng/mL | | Liver, ng/g | | Bile, ng/mL | | Urine, ng/mL | |
|---|---|---|---|---|---|---|---|---|---|
| | | DON | De-epoxy-DON | DON | de-epoxy-DON | DON | de-epoxy-DON | DON | de-epoxy-DON |
| 0.21/0.004 | 1 | 1.0[a)] | 0.1[a)] | 1.6[a)] | 0.3 | 9.8[a)] | 3.9[a)] | 299[a)] | 29[a)] |
| 3.07/0.088 | 2 | 4.1[a)] | 1.3[a)] | 2.9[a)] | 1.0 | 53.8[a)] | 39.9[a)] | 690[a)] | 150[a)] |
| 6.1/0.235 | 3 | 14.3[a)] | 2.8[a)] | 5.8[a)] | 4.8 | 134.0[a)] | 72.1[a)] | 804[a)] | 177[a)] |
| 9.57/0.358 | 4 | 21.6[a)] | 4.1[a)] | 8.2[a)] | 2.8 | 223.8[a)] | 103.2[a)] | 1572[a)] | 289[a)] |
| Probabilities | | | | | | | | | |
| Group | | <0.001 | 0.001 | <0.001 | 0.064 | <0.001 | <0.001 | 0.004 | <0.001 |
| Linear | | <0.001 | <0.001 | <0.001 | 0.048 | <0.001 | <0.001 | 0.001 | <0.001 |
| Quadratic | | 0.241 | 0.929 | 0.552 | 0.257 | 0.208 | 0.824 | 0.397 | 0.897 |
| Replication[b)] | | 0.124 | 0.3 | 0.625 | 0.341 | 0.035 | 0.696 | 0.428 | 0.180 |
| Group × replication | | 0.861 | 0.954 | 0.753 | 0.383 | 0.224 | 0.541 | 0.731 | 0.745 |
| PSEM[c)] | | 3.0 | 1.07 | 0.9 | 1.2 | 17.7 | 10.9 | 370 | 57 |

a) Mean values with no common superscript are significantly different within columns ($p < 0.05$).
b) Replication: Each group was tested three times with three pigs in each group, *i. e.* nine pigs *per* group in total.
c) PSEM: pooled standard error of means.

**Table 7.** Concentration of ZON and its metabolites in bile and urine (ng/g) of gilts exposed to increasing proportions of *Fusarium* toxin contaminated wheat over a period of 5 wk

| DON/ZON (mg/kg diet) | | Bile | | | Urine | | |
|---|---|---|---|---|---|---|---|
| | Group | β-ZOL | α-ZOL | ZON | β-ZOL | α-ZOL | ZON |
| 0.21/0.004 | 1 | 0.9[a)] | 29.2[a)] | 19.5[a)] | 0.0[a)] | 16.4[a)] | 10.4[a)] |
| 3.07/0.088 | 2 | 0.3[a)] | 43.6[a)] | 33.2[a)] | 0.4[a)] | 18.3[a)] | 16.7[a)] |
| 6.1/0.235 | 3 | 9.1[a)] | 162.0[a)] | 116.3[a)] | 0.3[a)] | 22.1[a)] | 15.4[a)] |
| 9.57/0.358 | 4 | 17.8[a)] | 309.5[a)] | 268.3[a)] | 3.3[a)] | 57.9[a)] | 43.1[a)] |
| Probabilities | | | | | | | |
| Group | | <0.001 | <0.001 | <0.001 | 0.012 | 0.001 | 0.007 |
| Linear | | <0.001 | <0.001 | <0.001 | 0.017 | 0.002 | 0.007 |
| Quadratic | | 0.006 | 0.001 | 0.002 | 0.094 | 0.028 | 0.118 |
| Replication[b)] | | 0.704 | 0.402 | 0.730 | 0.835 | 0.680 | 0.449 |
| Group × replication | | 0.608 | 0.057 | 0.746 | 0.857 | 0.764 | 0.897 |
| PSEM[b)] | | 2.6 | 30.7 | 34.0 | 1.3 | 12.4 | 11.4 |

a) Mean values with no common superscript are significantly different within columns ($p < 0.05$).
b) Replication: Each group was tested three times with three pigs in each group, *i.e.* nine pigs *per* group in total.
c) PSEM, pooled standard error of means.

## 4 Discussion

The feed intake depressing effect of the *Fusarium* toxin contaminated wheat can be attributed mainly to the presence of DON. This anorectic effect can be attributed mainly to the presence of DON in the diet since DON is well-known to depress voluntary feed intake in pigs when given either in the form of crystalline standard or from contaminated feedstuffs (for reviews, see D'Mello *et al.* [13], Dänicke *et al.* [14], EFSA [1]). Nivalenol and ZON as the toxins which occurred in the contaminated wheat next to DON probably do not influence feed intake at these concentrations as indicated by observations in young male pigs [15] and gilts [16] where purified toxins were used. The present study revealed a high degree of individual variation since three gilts of group 4 obviously did not recognize the presence of DON in their diets at all, whereas another three gilts only partially consumed their feed for a period of approximately 5 – 7 days, where the magnitude of refusal varied between 10 and 50%. The remaining gilts of this group responded rather sensitively to the dietary DON concentration of 9.7 mg · kg$^{-1}$. One of these gilts required approximately 3 wk to get customized to the diet. Especially at the beginning of this adaptation period there were some days with a complete feed refusal (Fig. 1). Therefore, the whole spectrum in responsiveness to dietary DON was observed in the present experiment. During the last 2 wk of the experiments all gilts consumed their ration of 2 kg offered daily which presumably concealed further feed intake regulatory effects of DON which are usually observed under *ad libitum* feeding conditions. The significant effects of dietary treatments on cumulative weight gain (Tab. 3) mainly resulted from the pronounced differences in daily live weight gain observed during the first week of the experiment (Fig. 2), which in turn reflected the relationships described for feed intake. In fact, the mean value differences in live weight gain were caused by those individual gilts which refused their diet. Pair feeding and restricted feeding experiments have shown that effects of feeding a *Fusarium* toxin contaminated feedstuff on weight gain and feed to gain ratio can be attributed almost solely to its anorectic properties [17, 18].

 

Most of the examined haematological parameters were characterized by a high individual variation. However, the majority of the individual values were in the reference range as given by Kraft and Dürr [19], and minimum and maximum limits were exceeded only slightly by 10–20% with the exception of the proportion of monocytes which were systematically higher in all animals. These deviations probably reflected the interlaboratory variance component. Only a few of the analyzed parameters could be identified to be influenced significantly by dietary treatments (haemoglobin concentration, haematocrit and derived indices). On the other hand, the variation in the differential blood count which was caused by replicating the experiments was in most cases significantly greater than that of the other factors (group, age). Literature reports on the effects of dietary DON on total leukocyte counts and their percent of distribution are contradictory and might be due to the use of different genetic pig strains, and especially the feeding of DON *via* contaminated feedstuffs, which are usually characterized by variations in the *Fusarium* toxin pattern which might modulate biological responses. For example, Swamy *et al.* [20] observed a dose-response related increase in total lymphocyte counts in young growing pigs when dietary DON concentration increased up to 5.8 mg · $kg^{-1}$. However, this effect was only significant after 7 days of feeding the experimental diets and disappeared after 21 days. In contrast, Rotter *et al.* [21] reported an initial decrease in leukocyte count after 7 days feeding the experimental diets (up to 3 mg DON · $kg^{-1}$) whereas after 28 days the opposite was found. In this regard it is interesting to note that a feeding regimen (*ad libitum* or restrictively) caused a greater variance in total leukocyte count than the DON contamination of the diet (6–7 mg · $kg^{-1}$) [18]. In the present study, the variance caused by replicating the experiments and by age related changes was greater than that by dietary *Fusarium* toxin contamination.

In contrast, haemoglobin concentration and haematocrit increased significantly with dietary inclusion of the *Fusarium* toxin contaminated wheat. Although no significant interactions between age and group were observed the differing initial values at the beginning of the experiment should be considered when discussing the significant group effects.

Haemoglobin concentration and haematocrit are known to be influenced by changes in plasma volume which for its part might be related to water intake relative to live weight. Since water intake was not measured in the present study, a relative polyglobulia as a result of a decreased plasma volume [19] cannot be ruled out. Another explanation for the increased haemoglobin concentration and haematocrit could be an absolute polyglobulia which results from an erythrocytosis. This possibility should not be completely excluded although erythrocyte counts were only slightly ($p = 0.112$) increased in *Fusarium* toxin exposed gilts besides the significant increases in the other parameters. It is known that certain kidney damages might stimulate erythropoetin secretion and thus erythropoesis [19] on one hand, and that DON is suspected to damage the kidney on the other hand [22]. Therefore, the findings of the present study with regard to the red blood count require further examination. Although DON residues in kidneys were not measured, this organ was highly exposed to this toxin, which might be deduced from its high concentration in urine (Tab. 6). It is known from other studies where DON balances were measured quantitatively that urine is the main excretion route for this toxin [23–25]. The percent excretion *via* the urinary route varied between 54 and 82%, 28 and 57% and 44 and 60% respectively, depending on the methods which were used. The contribution of faecal and bilary DON excretion of total DON excretion is of less importance (20 and 2.5% respectively [23]). The 5–24-fold lower concentration of DON plus de-epoxy-DON in bile than urine as calculated from the present data might be a reflection of these mentioned excretory relationships. The degree of metabolization of DON to de-epoxy-DON was not influenced by increasing dietary DON concentrations and seemed to be higher in liver and bile (16–45 and 28–43% respectively) than in serum and urine (9–24 and 8–18% respectively). De-epoxy-DON is formed by microbes

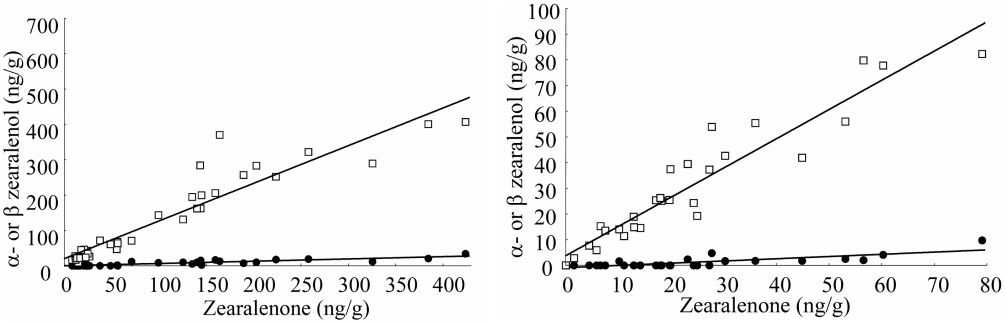


**Figure 5.** Relationships between the ZON concentration in bile (left) and urine (right) and its metabolites α- and β-ZOL. (-□-) α-ZOL (bile) = 20.4 + 1.1 *x*, $r^2 = 0.877$ (-□-) (α-ZOL (urine) = 3.8 + 1.1*x*, $r^2 = 0.896$ (-●-) (β-ZOL (bile) = 0.1 + 0.1*x*, $r^2 = 0.762$ (-●-) β-ZOL (urine) = –0.9 + 0.1*x*, $r^2 = 0.627$

 

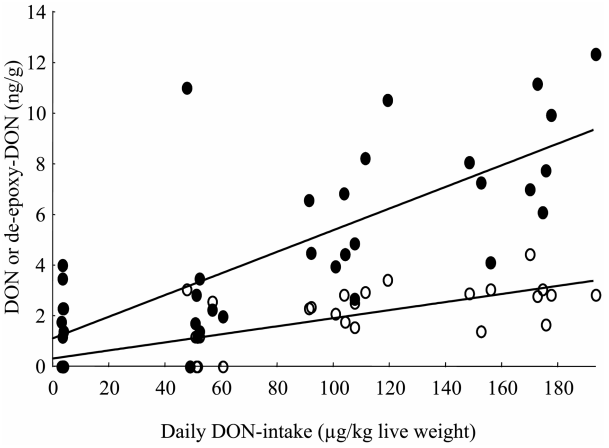


**Figure 6.** DON and de-epoxy-DON concentrations in liver in dependence on DON intake by the gilts.
DON = 1.1 + 0.043 *x*, $r^2$ = 0.572 (-●-)
de-epoxy-DON = 0.31 + 0.016 *x*, $r^2$ = 0.569 (-○-)

which are present in faeces and digesta of pigs [26, 27]. Moreover, Dänicke *et al.* [28] have shown an increase in the de-epoxy-DON concentration relative to the DON concentration in digesta collected from the duodenum up to the rectum of pigs. Thus, it is assumed that the systemic occurrence of this metabolite results mainly from the absorption of intestinally formed de-epoxy-DON. To the authors' knowledge it is not known if DON is also de-epoxydized by the liver or other tissues after being absorbed. It must be clarified whether the higher de-epoxy-DON proportions in bile and liver indicate such a possibility. Since de-epoxidation of DON can be viewed as a detoxification reaction, its contribution for the protection of the pig seems rather limited in the view of the low or moderate de-epoxidation degrees. Moreover, the evaluation of the relationship between the concentrations of DON and the detoxified de-epoxy-DON in the liver in dependence on the intake of DON by the gilts is interesting from a carry-over and consumer protection point of view. Only DON residues in edible tissues of pigs were reported so far according to a recent literature review by EFSA [1]. An approximately threefold higher increase in DON concentration in the liver in dependence on DON intake was estimated from the data of the present study when compared with the increase in de-epoxy-DON concentration (Fig. 6). Although DON increased more steeply than de-epoxy-DON, its maximum concentration corresponded to a carry-over factor (ratio between DON concentration in liver and in the diet) of approximately 0.0013 which is low and does not significantly contribute to human DON exposure.

A further detoxification might be expected from conjugation reactions which might occur in the liver prior to toxin excretion. Approximately 50% of the DON excreted in urine was conjugated with glucuronic acid (36–56%) whereas approximately 84% of de-epoxy-DON occurred in this form (77–91%) (Fig. 3). The degree of conjugation of DON in serum was approximately 33% (19–45%) (Fig. 4) whereas that of de-epoxy-DON could not be evaluated since its concentrations in enzymatically untreated serum samples were all lower than the detection limits. An inadequate enzymatic treatment might be the reason why earlier studies failed to detect de-epoxy-DON in blood of pigs although it was present in urine [1].

The concentration of all DON metabolites (de-epoxy-DON, DON and their conjugates) in bile varied only between 30 and 120% of that of all ZON metabolites (ZON, α-ZOL and β-ZOL and their conjugates) although the ZON concentrations of the diets 1–4 were 53-, 35-, 26- and 27-fold lower than the respective DON concentrations. These relationships underline the differences in metabolism between DON and ZON (Fig. 7). The latter undergoes an intensive enterohepatic cycling [29, 30] and its metabolites accumulate in the bile. Bile concentrations of these metabolites were shown to be linearly related to the ZON concentration of piglet diets [31]. Moreover, the concentration of ZON metabolites in bile was approximately twofold to eightfold higher than the respective concentrations in urine although the majority of ZON metabolites is excreted quantitatively *via* urine [29, 30]. That no ZON residues were detected in serum of the gilts is consistent with the findings of Döll *et al.* [31] who were not able to detect ZON metabolites in serum of piglet fed diets up to a ZON concentration of 420 µg · $kg^{-1}$. It needs to be stressed that the maximum dietary ZON concentration in the present study (358 µg · $kg^{-1}$) corresponded to a relatively low maximum ZON intake of 7.2 µg · $kg^{-1}$ live weight *per* day, which is much lower than reported in other studies. For example, an oral exposure of 192 µg ZON · $kg^{-1}$ live weight *per* day of a prepubertal gilt corresponded to a maximum ZON plus α-ZOL concentration of 10.4 ng · $mL^{-1}$ plasma [32]. If these figures are used for a rough calculation, it would mean that the approximately 27-fold lower ZON intake as calculated for the present study would correspond to a ZON plus α-ZOL concentration in plasma/serum of 0.4 ng · $mL^{-1}$ which is approximately 13-fold lower than the detection limit of the used HPLC method. Alpha-ZOL was found to be the main metabolite of ZON which is consistent with other findings [31, 32]. The proportions between the metabolites were nearly similar in bile and urine where the proportions between α-ZOL, β-ZOL and ZON of the sum of these metabolites were 56 (52–59), 2 (0–3) and 42 (39–45)% in bile, and 57 (52–61), 1 (0–3) and 42 (39–47)%. The relationships between ZON and α- or β-ZOL in both matrices were linear (Fig. 5) and an increase in ZON concentration of 1 ng · $g^{-1}$ corresponded to similar increases of 1.1 ng · $g^{-1}$ for α-ZOL and 0.1 ng · $g^{-1}$ for β-ZOL. Whether the similarity in the metabolite profile in bile and urine indicates that no further extraintestinal and/or extrahepatic metabolization occurred before these metabolites were excreted *via* urine

 

needs to be examined further. The predominance of α-ZOL among the metabolites of ZON in pigs is of special importance in the view that this metabolite is characterized by a higher affinity to the cytosolic estrogen receptor than its mother toxin or β-ZOL [3, 33] on one hand, and by the higher affinity of α-ZOL to the receptors of pigs than to those of poultry or rats [33] on the other hand. However, although β-ZOL increased in bile and urine in a dose-dependent manner – and probably in other matrices – in the present study, critical concentrations in target tissues were probably not exceeded to evoke clinical signs of hyperestrogenism or to exert uterotrophic effects. Critical dietary concentrations which were reported in the literature depend mainly on the ZON source and the age of the pigs, but other experimental conditions seem also to play a role. Bauer *et al.* [34] observed an intensified formation of tertiary follicles in 20 kg weighing female piglets at a dietary crystalline ZON concentration of 50 μg · $kg^{-1}$, and obvious signs of hyperestrogenism at 250 μg · $kg^{-1}$. Lusky *et al.* [35] reported histopathological signs of hyperestrogenism in growing pigs when diets were supplemented with 250 μg ZON *per* kg diet. However, Döll *et al.* [36] were not able to find marked effects on the general appearance of uterus sections or conspicuous alterations in morphometric parameters of piglets fed diets containing 420 μg ZON *per* kg diet originating from contaminated maize, although the weights of the uteri were significantly increased, whereas a ZON concentration of 220 μg · $kg^{-1}$ failed to induce an uterotrophic effect. Coenen and Boyens [37] demonstrated the uterotrophic effects of crystalline ZON in ovarectomized piglets at both tested dietary ZON concentrations of 180 and 360 μg · $kg^{-1}$. These literature findings together with the failure to observe obvious signs of hyperestrogenism in the present study at a maximum dietary ZON concentration of 358 μg · $kg^{-1}$ lead one to conclude that this obviously falls into a critical range where hyperestrogenism sometimes occurs and sometimes does not.

All discussed *Fusarium* toxin associated effects observed in the present study were related to some extent to the specific toxin or metabolite pattern which partially reflects the biotransformation capacity of the liver for DON and ZON. On the other hand, the liver itself is also a target organ for these toxins as reported by Tiemann *et al.* [4] who demonstrated for the livers of gilts of groups 3 and 4 a mild form of fibrosis which was manifested by biochemical parameters and histopathological observations without destruction of the morphological liver structure. Thus, biotransformation of these toxins by the liver might be related to their hepatotoxic effects.

Although the weights of the spleens of the gilts relative to live weight were only slightly influenced by dietary treatments ($p = 0.08$), the dose-dependent linear increase in their weights should not be overlooked ($p = 0.026$). Tiemann *et al.* [5] examined the function and histological and

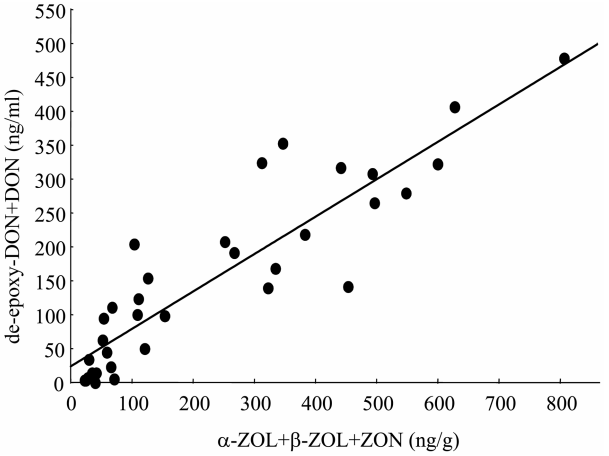


**Figure 7.** Relationships between the concentrations of α-ZOL + β-ZOL + ZON and DON + de-epoxy-DON in bile (unconjugated + conjugatedmetabolites; $y = 24 + 0.55x$, $r^2 = 0.845$).

ultrastructural parameters of spleen samples obtained from the present study. It was found that the proliferation of the splenocytes was significantly inhibited in gilts of group 4. Moreover, an increase in the size and frequency of B cell germinal centres and haemosiderin deposition was found. Thus, the hypertophy of this immune organ with increasing dietary DON concentrations could be interpreted as a compensatory mechanism to account for the depression in function.

In conclusion, restrictively fed gilts refused feed when dietary *Fusarium* toxin contaminated wheat was present in their diets. This effect was dose-dependent with regard to the total feed amount which was refused and to the duration of refusal. During the last 2 wk of the 5 wk experimental period all gilts consumed similar feed amounts. Daily live weight gain reflected the effects observed for feed intake. No signs of hyperestrogenism or other uterotrophic effects were seen even after feeding the diet with the highest ZON concentration of 358 μg · $kg^{-1}$ diet.

Linear relationships were found between dietary mycotoxin concentration and mycotoxin residues in different physiological samples. DON was detected besides its de-epoxidized metabolite de-epoxy-DON in serum, bile, liver and urine. Both metabolites were found in their free form and as their glucuronate conjugates. ZON and its metabolites α- and β-ZOL were found only in bile and urine.

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