Research Article

Residues of deoxynivalenol (DON) in pig tissue after feeding mash or pellet diets containing low concentrations

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Residues of deoxynivalenol (DON) and its metabolite de-epoxy-DON (DOM) were analyzed in specimens of pigs fed diets containing 0, 25, and 50% contaminated wheat (2.5 mg DON/kg) fed as mash or pellets over the final growing period of 11 wk. Median DON concentrations decreased from bile > kidney > serum > liver = muscle, while DOM was only detected in bile and kidney. Maximum carry over rates were 0.0319 for kidney, 0.0064 for liver, and 0.0043 for muscle, demonstrating that the contribution of animal derived food to the consumers' exposure is very low. The high interindividual variation of DON concentrations in all analyzed specimen of pigs fed diets containing similar concentrations of DON does not allow a diagnostic differentiation of animals fed diets containing DON concentrations of approximately 61% of the guidance level of 0.9 mg DON/kg, and those fed diets containing 137% of this concentration. The different feed forms did not affect residue concentrations in any of the investigated specimens.

Keywords: Carry over / Deoxynivalenol / *Fusarium* / Mycotoxin / Residues

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1 Introduction

Deoxynivalenol (DON) is a mycotoxin produced by fungi of the genus *Fusarium* which are abundant in various cereals. It exhibits immunomodulatory properties and inhibits the synthesis of proteins [1]. The frequent occurrence of toxicologically relevant concentrations of this toxin in grain intended for human and animal consumption may not be completely prevented by good agricultural practice [2]. Hence, exposure of humans and animals may not be completely ruled out. For a risk evaluation, the possibility of an exposure of consumers *via* animal-derived food, by carry over of the toxin from feed, has to be taken into account. Therefore, it is mandatory to acquire relevant data on the carry over of DON.

In addition, concentrations of DON in animal tissues are also of diagnostic interest. Negative feed analyses may not completely exclude DON as a possible cause of observed symptoms, as other sources, as *i. e.*, bedding material, could also contribute to the total exposure of an animal [1]. Furthermore, it seems possible that symptoms of DON exposure are noticed at a point of time when the contaminated feed is completely consumed, especially as the distribution of the mycotoxin is not homogeneous within a batch. Therefore, attempts have been made to utilize, *i. e.*, DON concentrations in serum for diagnostic purposes. Studies over a wide range of concentrations of DON in diets for pigs demonstrated linear dose relationships to serum concentrations [3 – 5]. But these relationships were characterized by a high inter individual variation. Therefore, it seems questionable whether this parameter is sensitive and precise enough to differentiate between pigs receiving diets containing DON concentrations below the guidance value for complementary and complete feeding stuffs for pigs of 0.9 mg/kg diet [6], which are considered safe, and pigs receiving diets exceeding those concentrations.

For those reasons, concentrations of DON were analyzed in various specimens of pigs which received diets containing 0, 25, and 50% of a contaminated wheat containing 2.5 mg DON/kg over a period of approximately 11 wk. To account for possible influences of varying feeding practices, the diets were fed as mash or pellets.

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Abbreviations: DOM, de-epoxy-DON; **DON,** deoxynivalenol; **ZOL,** zearalenol

Table 1. Composition of diets, metabolizable energy, and analyses of selected ingredients of diets (g/kg)

	Group (Grower diets)			Group (Finisher diets)		
	1[b]/2[c]	3[b]/4[c]	5[b]/6[c]	1[b]/2[c]	3[b]/4[c]	5[b]/6[c]
Ingredients						
Control wheat	500	250	0	500	250	0
Contaminated wheat	0	250	500	0	250	500
Barley	248	248	248	376	376	376
Soybean meal (44% XP)	200	200	200	80	80	80
Soybean oil	20	20	20	15	15	15
Dicalciumphosphate	3.5	3.5	3.5	1	1	1
Sodium chloride	1	1	1	0.5	0.5	0.5
L-lysine HCL	2.5	2.5	2.5	2.5	2.5	2.5
Premix[a]	25	25	25	25	25	25
Calculated composition						
Crude protein	168	168	168	143	143	143
ME (MJ/kg)	13.1	13.1	13.1	13.1	13.1	13.1
Lysine	10.1	10.1	10.1	8.1	8.1	8.1
Methionine + cystine	5.7	5.7	5.7	5	5	5
Threonine	5.9	5.9	5.9	4.8	4.8	4.8
Tryptophan	2.1	2.1	2.1	1.8	1.8	1.8
Calcium	9.4	9.4	9.4	8.5	8.5	8.5
Phosphorus	6.4	6.4	6.4	5.7	5.7	5.7
Analyzed composition						
Crude protein	185/190	196/200	200/199	147/146	150/152	153/151
DON (mg/kg)	0.09/0.13	0.62/0.65	1.07/0.95	0.05/0.07	0.57/0.55	1.23/1.13

a) Provided per kg of diet: Ca 6.1 g; P 1.5 g; Na 1.4 g; Mg 0.3 g; Fe 100 mg; Cu 25 mg; Mn 50 mg; Zn 100 mg; I 1.3 mg; Se 0.4 mg; Co 0.5 mg; vitamin A 10000 IU; vitamin D_3 1000IU; vitamin E 30 mg; vitamin B_1 18.8 mg; vitamin K_3 1.3 mg; nicotinic acid 12.5 mg; pantothenic acid 8.4 mg; choline chloride 125 mg.
b) Mash diets.
c) Pellets.

2 Materials and methods

2.1 Experimental design and animals

Concentrations of DON were investigated in samples of 30 pigs derived from a growth experiment described elsewhere [7].

In brief, a complete two by three two-factorial design was applied to examine three different concentrations of DON in the diet and a possible effect of a pelleting process. The inclusion of DON into the diets was facilitated by a stepwise exchange of control wheat by *Fusarium* infected wheat at a constant proportion of 50% wheat in the diet. The diet composition was optimized to meet or exceed the nutritional requirements of fattening pigs as recommended by the German Society of Nutritional Physiology [8] (Table 1). The diet containing no *Fusarium* infected wheat was produced first, followed by the diets containing the infected wheat with increasing concentrations to avoid conveyance of toxins. These three diets were offered as coarse mash diets or pellet diets while pelleting was performed at a maximum temperature of 80°C. Therefore, a total of six experimental treatments was tested.

Furthermore a two-phase feeding regimen was chosen to account for the changing needs of protein of the pigs. The grower diets were fed for 6 wk (covering a mean live weight range from 27 to 72 kg) and the finisher diets were fed for the following 5 wk of the experiment (mean live weight range from 72 to 111 kg).

Treatments and experiments were conducted according to the European Community regulations concerning the protection of experimental animals and the guidelines of the Regional Council of Braunschweig, Lower Saxony, Germany.

2.2 Growth experiment

In the growth experiment, a total of 96 male, castrated crossbred (dam line: Large White × German Landrace, db Classic; sir line: db.77, Bundeshybridzuchtprogramm (BHZP)) pigs with a mean live weight of 24.6 ± 2.8 kg were allotted to floor pens without bedding according to the Danish system of stalling. Animals were kept in single pens with a trough for *ad libitum* access to feed and a nipple drinker for *ad libitum* access to water. During the first week all the pigs were fed the control diet to allow them to become accustomed to the stalling. After this pretrial period the pigs were randomly assigned to the experimental diets at a mean live weight of 27.3 ± 2.4 kg.

On days 78 and 79, a total of 30 pigs (five *per* treatment group) was slaughtered by cutting the jugular vein after electrical stunning. Samples of blood for the preparation of serum, bile, liver, kidney, and muscle (*M. longissimus*) were taken and frozen at −20°C for further processing or analyses.

2.3 Analyses

Samples from wheat batches and diets were analyzed for crude nutrients according to the methods of the "Verband Deutscher Landwirtschaftlicher Untersuchungs und Forschungsanstalten" (VDLUFA) [9]. DON in experimental diets was determined by HPLC with DAD after clean-up with immunoaffinity columns (IAC, DONprep™, R-Biopharm AG, Darmstadt Germany) according to the manufacturer's procedure with modifications as described in ref. [10]. The detection limit was 30 ng/g and the mean recovery was approximately 95%. Freeze-dried liver, kidney, muscle, bile, and serum samples were analyzed for DON and de-epoxy-DON (DOM) by HPLC with UV detection after incubation with β-glucuronidase (EC 3.2.1.31; Type H-2, Sigma–Aldrich Chemie, Taufkirchen, Germany) [4, 11]. The detection limit was 4 ng/g for freeze-dried liver, kidney, and muscle and 4 ng/mL for bile and 2 ng/mL for serum with a mean recovery between 87–95 and 86–97% for DON and DOM, respectively.

ZON, α-zearalenol (α-ZOL), and β-zearalenol (β-ZOL) in diets were determined by HPLC with fluorescence detection after overnight treatment with 2 U β-glucuronidase [12, 13]. The detection limit of ZON, α-ZOL, and β-ZOL was 1, 1, and 4 ng/g, respectively. The mean recovery for ZON, α-ZOL, and β-ZOL was 82, 81, and 74%.

Further *Fusarium* mycotoxins were analyzed in wheat samples by the Institute of Animal Nutrition of the University of Hohenheim using a GC-MS method [14].

Results of analyses of DON, ZON, and its metabolites were not corrected for recovery.

Mycotoxin concentrations in physiological samples which were lower than the above-mentioned detection limits were considered to be zero. In addition, for freeze dried material the detection limit is given for freeze-dried material, but values are reported in fresh material. Therefore, calculated mean values might be lower than the detection limit.

2.4 Calculations and statistics

The carry over factor was calculated as follows:

$$\text{Carry over factor} = \frac{\text{Toxin concentration of tissue (µg/kg)}}{\text{Toxin concentration of diet (µg/kg}}$$

Data on live weight and feed intake were evaluated for statistically significant differences employing the Tukey-test. All other data were not normally distributed. Therefore, differences were evaluated by the nonparametric Mann–Whitney *U*-test ($p < 0.05$). Values are given as median and range. All statistics were carried out using Statistica for windows [15].

3 Results

3.1 Mycotoxin contamination of wheat and diets

The control wheat contained only traces of DON, ZON, and nivalenol. The *Fusarium* infected wheat contained mainly DON (2.5 mg DON/kg) while only traces of ZON and 3-acetyldeoxynivalenol (2% of the concentration of DON) were detected. All other determined *Fusarium* toxins were below the indicated detection limit (Table 2). The concentrations of DON in the diets (Table 1) were in the range expected considering the respective rate of inclusion of the *Fusarium* contaminated wheat. DON concentrations in mash diets do not differ from the ones in pellets.

Table 2. *Fusarium* toxins (µg/kg DM) in inoculated and control wheat

	Control wheat	Contaminated wheat
Zearalenone	1.4	1.1
DON	45	2500
15-Acetyldeoxynivalenol	<8	<8
3-Acetyldeoxynivalenol	<10	50
Nivalenol	20	<15
Fusareon-X	<21	<21
Scirpentriol	<9	<9
Monoacetoxyscirpenol	<3	<3
Diacetoxyscirpenol	<16	<16
T-2 toxin	<4	<4
T2 Triol	<6	<6
T2 Tetraol	<8	<8
Neosoloniol	<7	<7
HT-2	<3	<3

3.2 Performance and intake of DON

Feeding the experimental diets over a period of 11 wk did not lead to significant differences of the live weight of these pigs or to effects on the feed intake (Table 3). The mean daily DON intake is given for the last week of the experiment as it is most relevant for toxin residues in pig tissues. The DON intake of the pigs of group 5 (50% contaminated wheat, mash) was highest with a median of 42.5 µg/kg LW/day. The DON intake of group 6 (50% contaminated wheat, pellets) was only 85% of that of the pigs fed the same diet as mash. However, summing up the groups receiving the same proportion of contaminated wheat in the diet it is obvious that the groups receiving 50% contaminated wheat in the diets ingested two times as much DON as the pigs

Table 3. Effects of feeding diets containing increasing concentrations of DON as mash or pellet on live weight, feed intake, and DON intake of pigs

Group	DON (mg/kg diet)	Diet form	Live weight (kg)	Feed intake[a)] (kg/day)	DON intake[b)] (μg/kg LW/day)
1	0.05	Mash	112.2 (107.6–114.4)	2.84 (2.68–3.11)	1.5^{E} (1.2–1.6)
2	0.07	Pellet	114.2 (113.4–119.4)	2.85 (2.63–2.96)	2.3^{D} (2.2–2.5)
3	0.57	Mash	113.0 (107.0–114.8)	2.90 (2.82–3.13)	20.0^{C} (16.9–21.1)
4	0.55	Pellet	113.8 (109.6–115.6)	2.79 (2.69–3.21)	17.3^{C} (14.1–18.7)
5	1.23	Mash	111.6 (107.2–113.2)	2.88 (2.84–2.99)	42.5^{A} (36.3–43.6)
6	1.13	Pellet	109.6 (108.4–112.2)	2.76 (2.55–3.05)	36.2^{B} (32.5–38.9)

Data in one column with no common letter are significantly different (Mann–Whitney *U*-test, $p = 0.05$).
a) Mean feed intake in the complete feeding experiment.
b) Derrived from the mean feed intake in the last week of the experiment and the dietary concentration.

Table 4. Concentrations of DON and DOM and the derived carry over factor of DON in body fluids (serum and bile) of pigs fed diets containing low concentrations of DON either as mash feed or as pellets ($n = 5$; median (range))

Group	DON (mg/kg diet)	Diet form	Serum			Bile		
			DON (ng/mL)	DOM (ng/mL)	Carry over factor[a)]	DON (ng/mL)	DOM (ng/mL)	Carry over factor[a)]
1	0.05	Mash	0^{B} –	0 –	0^{C} –	5.0^{C} (0–23.0)	0.0^{B} (0–6.0)	0.1087^{AB} (0–0.5000
2	0.07	Pellet	0^{B} –	0 –	0^{C} –	11.0^{BC} (0–52.0)	0.0^{AB} (0–11.0)	0.1486^{AB} (0–0.7027)
3	0.57	Mash	5.2^{A} (2.0–12.2)	0 –	0.0091^{A} (0.0031–0.0213)	42.0^{AB} (22.0–60.0)	14.0^{A} (0–30.0)	0.0734^{AB} (0.0385–0.1049)
4	0.55	Pellet	3.2^{A} (2.0–5.2)	0 –	0.0058^{AB} (0.0029–0.0095)	56.0^{A} (34.0–59.0)	9.0^{A} (0–16.0)	0.1022^{A} (0.0620–0.1077)
5	1.23	Mash	7.0^{A} (0–7.0)	0 –	0.0057^{AB} (0.0000–0.0057)	59.0^{AC} (0–125.0)	17.0^{A} (0–44.0)	0.0478^{B} (0–0.1012)
6	1.13	Pellet	4.0^{A} (0–7.0)	0 –	0.0035^{B} (0.0000–0.0062)	36.0^{AC} (0–144.0)	15.0^{AB} (0–43.0)	0.0318^{AB} (0–0.1273)

Data in one column with no common letter are significantly different (Mann–Whitney *U*-test, p=0.05).

a) Carry over factor $= \frac{\text{Toxin concentration of tissue } (\mu g/kg)}{\text{Toxin concentration of diet } (\mu g/kg)}$

receiving diets with 25% contaminated wheat and approximately 20-times as much as the control groups.

3.3 DON residues of diagnostic interest

DON was detected in serum of pigs receiving diets containing contaminated wheat but not in serum of pigs receiving diets containing 0% contaminated wheat (Table 4). Serum concentrations of pigs fed diets containing contaminated wheat ranged from 0 to 12.2 ng/mL and were similar in all groups independent of the inclusion rate or the diet form. DOM was not detected in any of the serum samples. The median carry over factors ranged from 0.0035 to 0.0091 in the groups fed diets containing 25 or 50% contaminated wheat, with the highest in group 3 (25% contaminated wheat, mash) and the lowest in group 6 (50% contaminated wheat, pellet).

DON and DOM were detected in bile samples of all groups. The variation of bilary DON concentrations within the groups was very high with, *i.e.*, values ranging from 0 to 144.0 ng/mL for samples of group 6. Therefore, there were no statistically significant differences between groups receiving contaminated wheat and the groups receiving the control diets (Table 4). The carry over of DON into the bile was the highest among all analyzed specimens. It appears that median carry over factors decrease with increasing proportions of contaminated wheat in the diets as they are 0.108 and 0.1486 for the groups 1 and 2 and 0.0734, 0.1022, 0.0478, and 0.0318 for groups 3, 4, 5, and 6, respectively.

Depicting the concentration of residues detected in serum and bile in relation to DON intake (Fig. 1) underlines that there is no clear dose relationship.

3.4 DON residues in eatable tissue

The concentrations of DON and DOM in muscle, liver, and kidneys are shown in Table 5. Among these specimens the kidney was the only organ where DOM could be detected.

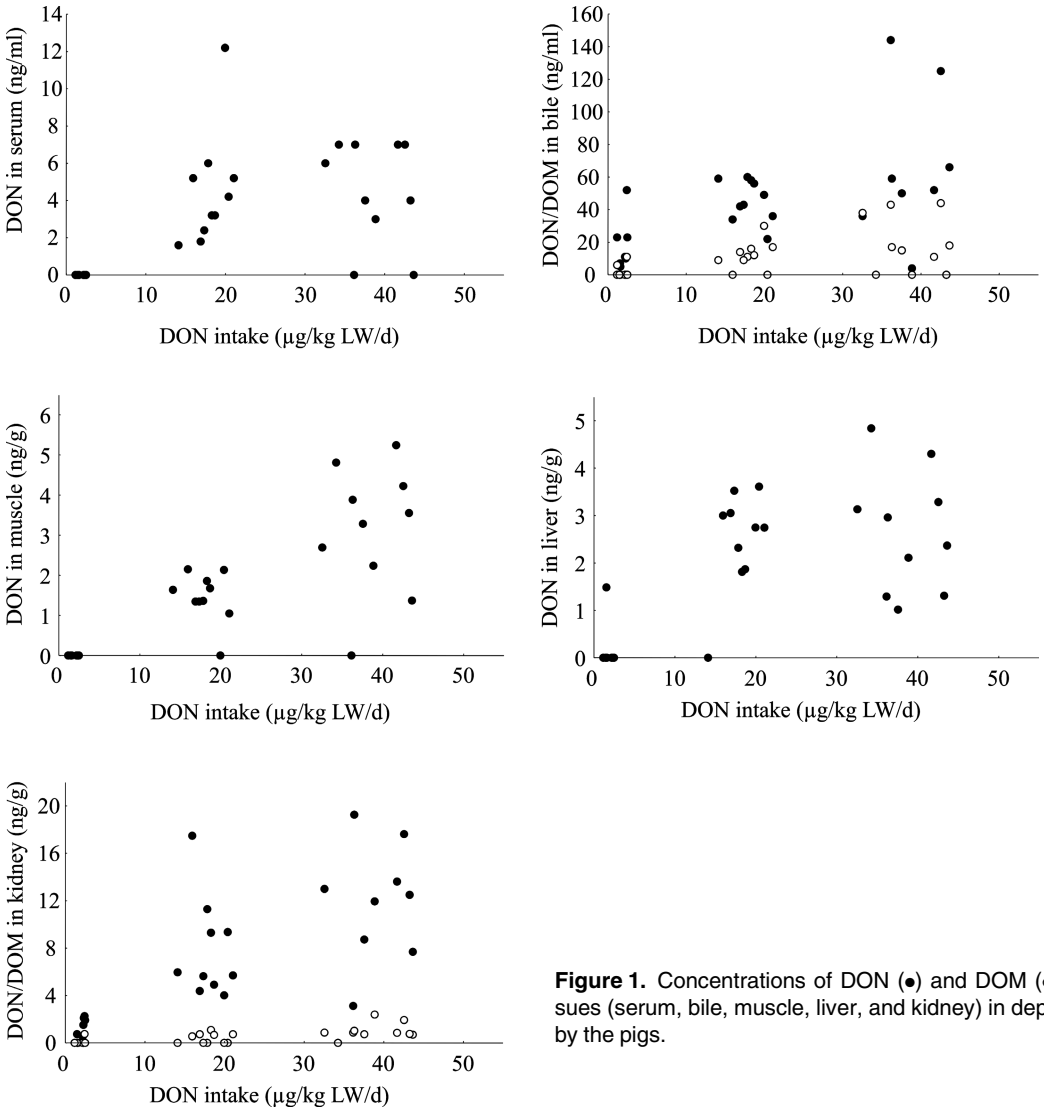

Figure 1. Concentrations of DON (•) and DOM (○) in body fluids and tissues (serum, bile, muscle, liver, and kidney) in dependence on DON intake by the pigs.

In muscle, DON was only detected in samples of pigs fed diets containing 25 and 50% of contaminated wheat resulting in a significant difference of these groups to the control groups. Within the DON dosed groups group differences only reached significance between group 3 (25% contaminated wheat, mash) and group 5 (50% contaminated wheat, mash). The carry over factors were similar for all groups fed diets containing contaminated wheat with the highest in group 5 (0.0033).

The concentrations of DON in the liver were not clearly dose dependent (Fig. 1). The DON concentrations were similar in the livers of the pigs receiving diets containing 25 or 50% DON, irrespective of the inclusion rate. Differences between groups fed mash or pellets were also not detected. The carry over factor for the groups receiving the diets containing 25% *Fusarium* contaminated wheat was higher than the carry over factors for the groups receiving diets containing 50% contaminated wheat.

The highest DON concentrations in the analyzed edible tissues were detected in kidneys with a maximum concentration of 19.3 ng/g in a sample of group 5. Therefore, the carry over factor for kidneys is also higher than for muscle or liver. But as in the other tissues, no clear dose relationship to the toxin intake could be shown (Fig. 1).

4 Discussion

The aim of the present study was to investigate the carry over of DON under practically relevant conditions. Therefore, low dietary concentrations, 61 and 137% of the guidance value of 0.9 mg/kg complete feeding stuffs for pigs [6], were used. An investigation of mixed feed for pigs in Germany demonstrated that median concentrations ranged from 0.07 to 0.2 mg/kg while the 90th percentile was between 0.35 and 0.51 and maximum reached concentra-

Table 5. Concentrations of DON and DOM and the derived carry over factor of DON in edible tissue (muscle, liver, and kidneys) of pigs fed diets containing low concentrations of DON either as mash feed or as pellets ($n = 5$; median (range))

Group	DON (mg/kg diet)	Diet form	Muscle			Liver			Kidney		
			DON (ng/g)	DOM (ng/g)	Carry over factor[a)]	DON (ng/g)	DOM (ng/g)	Carry over factor[a)]	DON (ng/g)	DOM (ng/g)	Carry over factor[a)]
1	0.05	Mash	0^{C} –	0 –	0^{B} –	0.0^{B} (0–1.5)	0 –	0.0000^{ABC} (0–0.0323)	0.0^{D} (0–0.7)	0^{C} –	0.0000^{C} (0–0.0161)
2	0.07	Pellet	0^{C} –	0 –	0^{B} –	0^{B} –	0 –	0^{C} –	1.9^{C} (0.5–2.3)	0.0^{BC} (0 – 0.8)	0.0259^{A} (0.0073 – 0.0306)
3	0.57	Mash	1.3^{B} (0–2.1)	0 –	0.0024^{A} (0–0.0037	2.8^{A} (2.3–3.6)	0 –	0.0048^{A} (0.0041 – 0.0063)	5.7^{B} (4.0–11.3)	0.0^{BC} (0 – 0.7)	0.0100^{AB} (0.0070 – 0.0197)
4	0.55	Pellet	1.7^{AB} (1.3–2.2)	0 –	0.0031^{A} (0.0025– 0.0039	1.9^{A} (0–3.5)	0 –	0.0034^{AB} (0–0.0064)	6.0^{AB} (4.9–17.5)	0.6^{ABC} (0 – 1.1)	0.0109^{AB} (0.0090– 0.0319)
5	1.23	Mash	4.1^{A} (3.6–5.2)	0 –	0.0033^{A} (0.0029– 0.0042	3.0^{A} (1.3–4.3)	0 –	0.0024^{B} (0.0011– 0.0035)	13.6^{A} (7.7–19.3)	0.9^{A} (0.7 – 1.9)	0.0110^{AC} (0.0062– 0.0156)
6	1.13	Pellet	2.7^{AB} (0–4.8)	0 –	0.0024^{A} (0–0.0043)	2.1^{A} (1.0–4.8)	0 –	0.0019^{B} (0.0009– 0.0043)	8.7^{ABC} (0–13.0)	0.9^{AB} (0–2.4)	0.0077^{BC} (0–0.0115)

Data in one column with no common letter are significantly different (Mann–Whitney *U*-test, $p = 0.05$).

a) Carry over factor $= \dfrac{\text{Toxin concentration of tissue } (\mu g/kg)}{\text{Toxin concentration of diet } (\mu g/kg)}$

tions of up to 2.3 mg DON/kg feed during the years 2001–2003 [16]. Hence, the diets incorporated in the trial represent the situation in practice.

It is known that feed and food processing can have an effect on the DON concentrations in the final product [17]. Therefore, the two feed forms were chosen to represent various feeding practices. Crystalline DON has a high thermal stability, especially at acetic (till 170°C) to neutral pH (up to 120°C). But it is recommended to study the effects of processing on the toxin in the respective matrix since the physical environment can lead to enhanced or decreased stability [17]. But the pelleting of the feed did not affect the DON concentrations. This result is in accordance to the findings of Trigo-Stockli *et al*. [18], pelleting feeds for shrimp containing similar concentrations of DON as in the present study. Furthermore, it seems possible that differences in the feed form could modulate the bioavailability of DON as it could affect the liberation of the toxin from the matrix and thereby influence residue concentrations in animal tissue. However, there were no distinct differences in DON concentrations in the analyzed specimens that could be ascribed to the different feed forms.

The most prominent effect of DON on pigs is a reduction of the voluntary feed intake of the animals causing decreased live weight gain [1]. But the groups of pigs in the present investigation did not differ significantly in feed intake over the complete feeding period and live weight at the time of sampling. These animals were randomly selected from a growth experiment including a total of 96 animals. Effects of DON in the feed and the pelleting of the diets on the performance, organ weights, selected clinical chemical parameters, lymphocyte proliferation and nutrient digestibility are discussed elsewhere [7].

The daily DON intake in the week before slaughtering reflects the various inclusion rates of contaminated wheat into the diets. The significantly different DON intakes of the groups receiving 50% *Fusarium* infected wheat in the diets as mash or pellets may not be solely attributed to the minor difference in DON concentration in the diet but is also due to differences in voluntary feed intake between those two groups in the last week of the feeding experiment (results not shown). However, these group differences in DON intake are not reflected in the DON concentrations in the serum. Attempts have been made to utilize the concentrations of DON in physiological samples as a biomarker for the exposure to DON. Studies in young pigs (approx. 32 kg bw), fattening pigs (approx. 68 kg bw) and gilts (approx. 120 kg bw) feeding graded levels of contaminated cereals in the diets up to DON concentrations of 3.9, 3.9, and 9.6 mg DON/kg feed, respectively, revealed linear relationships between the serum concentrations and the DON intake or the DON concentration in the feed [3–5]. Other specimens, such as, *e.g.*, bile fluid, demonstrated a very high variation of DON concentrations, proving that these concentrations are not useful as biomarkers. Therefore, it was concluded that the serum concentrations are the most suitable parameter to demonstrate differences in the systemic availability of DON, due to varying dietary concen-

trations or large differences in bioavailability as intended by the use of so called mycotoxin binders under experimental conditions [19]. However, the present results emphasize that the differences between groups have to be sufficiently large, as the differences in DON intake of the groups fed 25 or 50% contaminated wheat in the diets were not reflected by the serum concentrations. In addition, this clearly shows that it is impossible to distinguish between animals which received a diet containing DON concentrations well below the guidance level and those exceeding the guidance level by 37%, by the analysis of DON in the serum, even under experimental conditions. This is also true for the DON concentrations in the further analyzed specimen as they were characterized by a high interindividual variation. No clear statistical significant differences between the groups receiving 25 and 50% contaminated wheat in the diets could be detected. DON residues in bile and kidneys of pigs fed the diets containing only control wheat did not even allow a clear differentiation of animals fed contaminated diets. Hence, it has to be pointed out that the feed analysis is the most precise and feasible tool to assess the dietary exposure of animals to DON.

In the present study, the concentrations of DON decreased from bile > kidney > serum > liver = muscle. This is in general agreement with findings of Goyarts *et al*. [20], analyzing DON residues in pigs fed diets containing 6.7 mg DON/kg over a period of 12 wk. But in contrast to the present experiment, the authors reported higher concentrations in liver than in muscle. Also Schneweis *et al*. [21] detected higher concentrations of DON in the livers than in muscle of pigs fed diets containing 63.5% of varying wheat batches containing up to 1 mg DON/kg for 4 wk.

Only bile and kidney contained detectable concentrations of DOM, the metabolite of DON while in trials incorporating higher concentrations of DON in the diets DOM could also be detected in muscle, liver, and serum [4, 20].

The carry over factors reported in the present investigation are in general agreement with the aforementioned study by Goyarts *et al*. [20]. The large differences in DON concentrations in the diets (0.57/0.55 and 1.23/1.13 mg DON/kg in the present study and 6.68 mg DON/kg for [20] did not lead to marked differences in carry over factors. However, comparing the recent mean carry over factors for liver it has to be noted that the factors for the groups 3 and 4 (25% contaminated wheat in the diet) are higher than the ones of groups 5 and 6 (50% contaminated wheat in the diet). But the higher variation of analysis results at lower concentrations could have contributed to this observation.

To illustrate the significance of DON originating from pig-derived foodstuffs for the exposure of the consumer, the amounts of foodstuff necessary to reach the tolerable daily intake of 1 µg DON/kg bw as suggested by the Scientific Committee on Food [22] assuming a standard consumer with 70 kg bw and a feedstuff contamination of 1 mg DON/kg was calculated using the highest carry over factor determined for the animals fed contaminated diets. According to this calculation one would have to consume 2.2 kg kidney, 10.9 kg liver, or 16.3 kg muscle to reach the tolerable daily intake while 70 g of the respective grain (assumed as feed for the pig) would result in the equal exposure. Furthermore, the determined carry over factors are likely to overestimate the actual carry over under practical conditions as feed was not withdrawn from the pigs in the experiment while under practical conditions a withdrawal period of several hours is common practice. Considering the rather short elimination half-life of DON determined in chronically exposed pigs of 6.3 h [23], it can be assumed that residue concentrations are even lower under practical conditions.

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The authors have declared no conflict of interest.

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