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**On the effects of Fusarium toxin contaminated wheat and feed intake level on biotransformation and carry over of deoxynivalenol in dairy cows**

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1     ***On the effects of Fusarium toxin contaminated wheat and feed intake level on***  
2     ***the biotransformation and carry over of deoxynivalenol in dairy cows***

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21     *An experiment was carried out to examine the effects of feeding Fusarium toxin contaminated*  
22     *wheat (8.21 mg deoxynivalenol, DON, and 0.09 mg zearalenone, (ZON), per kg dry matter,*  
23     *DM) at different feed intake levels on biotransformation and carry over of DON in dairy*  
24     *cows. For this purpose, fourteen ruminal and duodenal fistulated dairy cows were fed a diet*  
25     *containing 60 % concentrate with a wheat portion of 55 % (Fusarium toxin contaminated*  
26     *wheat [mycotoxin period] or control wheat [control period]) and ration was completed with*  
27     *maize- and grass silage (50:50) on a DM basis. Daily DON intakes ranged from 16.6 up to*  
28     *75.6 mg in the mycotoxin period at DM intakes of 5.6 up to 20.5 kg.*

29     *DON was almost completely biotransformed to de-epoxy DON (94-99 %) independent of the*  
30     *DON/feed intake, and the flow of DON and de-epoxy DON at the duodenum related to DON*  
31     *intake ranged between 12 and 77 % when the Fusarium toxin contaminated wheat was fed. In*  
32     *the serum samples, de-epoxy DON was detected in the range of 4 up to 28 ng/ml in the*  
33     *mycotoxin period, while concentrations of DON were all below the detection limit. The daily*  
34     *excretion of DON and de-epoxy DON in the milk of cows fed the contaminated wheat varied*  
35     *between 1 and 10 µg and 14 and 104 µg, respectively. The total carry over rates as the ratio*  
36     *between daily excretion of DON and de-epoxy DON into milk and DON intake were in the*

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3 37 range of 0.0001 up to 0.0002 and 0.0004 up to 0.0024, respectively. Total carry over rates of  
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5 38 DON as DON and de-epoxy DON into the milk increased significantly with increasing milk  
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7 39 yield. In the urine samples, de-epoxy DON was the predominant substance as compared to  
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9 40 DON with a portion of the total DON plus de-epoxy DON concentration up to 96 % when the  
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11 41 *Fusarium* toxin contaminated wheat was fed, whereas the total residues of DON plus de-  
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13 42 epoxy DON in faeces ranged between 2 and 18 % of DON intake in the mycotoxin period. The  
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15 43 degree of glucuronidation of de-epoxy DON was found to be approximately 100 % in serum.  
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17 44 From 33 up to 80 % of DON and from 73 up to 92 % of de-epoxy DON and from 21 up to 92  
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19 45 % of DON and from 86 up to 100 % of de-epoxy DON were glucuronidated in the milk and  
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21 46 urine, respectively.  
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23 47 It is concluded that DON is very rapidly biotransformed to de-epoxy DON in the rumen and  
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25 48 only negligible amounts of DON and de-epoxy DON were transmitted into the milk within the  
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27 49 range of 5.6 up to 20.5 kg/d DM intake and milk yields (fat corrected milk, FCM) between 10  
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29 50 and 42 kg/d.  
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35 53 **Keywords:** Deoxynivalenol, *Fusarium*, ruminants, level of feed intake, milk, carry over  
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**Keywords:** Deoxynivalenol, *Fusarium*, ruminants, level of feed intake, milk, carry over

## Introduction

Deoxynivalenol (DON) is one of the most frequently recognised mycotoxins in Europe produced by several *Fusarium* species on a variety of cereal grains (Bottalico and Perrone 2002). In animal livestock, ingestion of DON can cause numerous adverse toxicological responses associated with reduced performance and immune function (Rotter and Prelusky 1996). In contrast to pigs, which react most sensitively to DON, ruminants appear to be relatively tolerant, which is attributed to the metabolisation of the toxin by microbes in the rumen. Several *in vitro* studies with bovine rumen fluid have demonstrated that DON is biotransformed extensively to the less toxic compound de-epoxy DON (King *et al.* 1984, Swanson *et al.* 1987, He *et al.* 1992). Consequently, the diversion of cereal batches suspected to be contaminated with DON to ruminants is a useful means of utilising grain that is unsuitable for humans or monogastric farm animals. But, in combination with higher DON concentrations in cereals and roughages during so called *Fusarium* years (Oldenburg *et al.* 2000), the influence of DON on the health and performance of ruminants should not be underestimated. However, little research has been published on the effects of DON on ruminants. Most of the reports were given as field or case studies and experiments under

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3 71 controlled conditions were only performed with small numbers of animals using either  
4 72 purified DON or naturally contaminated feedstuffs (for reviews see Höltershinken *et al.* 1996,  
5 73 Whitlow and Hagler 1999, Bauer 2000, Dänicke *et al.* 2000, Seeling and Dänicke 2005).  
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7 74 Thus, the described effects are quite variable between the different studies and it is difficult to  
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9 75 draw conclusions for the practical feeding. Furthermore, high-producing dairy cattle in early  
10 76 lactation also appear more sensitive to DON than low-producing dairy cattle (Whitlow and  
11 77 Hagler 1999). But no studies are available considering factors other than dosage and duration  
12 78 of toxin exposure, which could cause these differences in susceptibility. Additionally, the  
13 79 information about the carry over of DON and de-epoxy DON into the milk of dairy cows  
14 80 feeding practically relevant concentrations are still rare. While no transmission of DON or de-  
15 81 epoxy DON to milk was observed in a study of Charmley *et al.* (1993), trace amounts of  
16 82 DON and de-epoxy DON could be detected by Prelusky *et al.* (1984) and Cote *et al.* (1986).  
17  
18 83 Possibly, the feed intake (passage rate) as indicator for the feed retention in the rumen could  
19 84 alter the ruminal metabolisation of DON. A rapid passage rate as an effect of increased feed  
20 85 intake may limit the available time for a metabolisation of DON to de-epoxy DON in the  
21 86 rumen. Due to a modified ratio of DON to de-epoxy DON at the duodenum, the toxicity as  
22 87 well as the carry over of these substances in the milk could be altered. Therefore, the aim of  
23 88 the present study was to examine the effect of varying levels of feed intake using *Fusarium*  
24 89 toxin contaminated or non contaminated wheat on biotransformation and carry over of DON  
25 90 by dairy cows. The paper is part of a comprehensive study where non contaminated control  
26 91 wheat and *Fusarium* toxin contaminated wheat were fed to ruminants at different feed intake  
27 92 levels. In further papers the influence on ruminal fermentation and milk composition as well  
28 93 as the biotransformation of the mycotoxin ZON were described (Seeling *et al.* 2005a, Seeling  
29 94 *et al.* 2005b).  
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## 96 **Material and methods**

### 97 ***Treatments, experimental design and animals***

50 98 The experimental design and procedures were described previously (Seeling *et al.* 2005a,  
51 99 Seeling *et al.* 2005b). In brief, fourteen dairy cows of the “German Friesian breed” equipped  
52 100 with permanent ruminal and duodenal cannulas were selected for this experiment. The cows  
53 101 had a mean live weight of  $626 \pm 71$  kg. The animals were in different stages of lactation:  
54 102 Cows 4-14 and Cows 5-14 lactated in the mycotoxin period and in the control period,  
55 103 respectively, whereas the rest did not lactate. They were tethered using neck straps, housed in

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3 104 a stanchion stable system equipped with individual feedbunks and had free access to water.  
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5 105 All cows were fed a diet containing 60 % concentrate and 40 % maize- and grass silage  
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7 106 (50:50) on a DM basis (Table 1). The only cereal component of the concentrate was either 55  
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9 107 % uncontaminated control wheat (control period) or 55 % *Fusarium* toxin contaminated  
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11 108 wheat (mycotoxin period). Further components of the concentrate were 25 % soy bean meal,  
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13 109 16 % dried sugar beet pulp, 2 % soy oil and 2 % mineral feed. The composition of the dietary  
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15 110 ration was constant for all animals, only the daily amounts were adjusted individually  
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17 111 according to the current performance so that total dry matter intakes of the cows ranged from  
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19 112 5.6 up to 20.5 kg. Each cow was fed both the uncontaminated control wheat and the *Fusarium*  
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21 113 contaminated wheat in two subsequent periods. The concentrate was offered at 0500 h, 0800  
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23 114 h, 1500 h and 1800 h in equal-sized meals. Silages were given twice daily in equal portions at  
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25 115 0800 h and 1500 h. Cows were milked at 0500 h and 1500 h and milk yields were recorded at  
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27 116 each milking. Weights of the cows were recorded before and after the experimental periods.  
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### 118 ***Measurement and sample collection***

119 Each experimental period lasted for four weeks. Following an adaptation period of three  
120 weeks to the diets, duodenal samples were collected during the last week in order to estimate  
121 the duodenal flow using chromium oxide ( $\text{Cr}_2\text{O}_3$ ) as marker (Rohr *et al.* 1984). The marker  
122 was given in four boluses into the rumen. Marker administration started ten days prior to the  
123 duodenal sampling period. Spot samples of duodenal chyme were taken at 2 h intervals from  
124 Monday to Friday yielding a total of 60 samples for each cow. The whole procedure followed  
125 the principles as described by Rohr *et al.* (1979). Blood samples (25 ml) were drawn from  
126 each cow in each treatment one day in the 3rd week at 1000 h. Serum was separated by  
127 centrifugation at 2000 x g for 20 min at 15°C 2 h after sampling and was kept at -20°C until  
128 analysis. Milk sampling from each lactating cow took place through six consecutive milkings  
129 in the third week of each period. The samples were pooled based on the weight of milk  
130 produced at the morning and evening milking and frozen at -20°C, then freeze-dried and  
131 stored at -20°C until further analysis. Faecal samples from the rectum as well as spontaneous  
132 urine samples were collected in the last week of each experimental period. Faeces and urine  
133 were immediately frozen at -20°C after sampling. Representative samples of the concentrates,  
134 silages and feed refusals, if they occurred, were collected daily and pooled over the five-day  
135 duodenal sampling trial. For proximate analyses, these samples were dried at 60°C and  
136 ground to pass through a 1-mm screen.

137



138 ***Analysis***

139 Analyses of crude nutrients of feedstuffs were done on dry samples according to the methods  
140 of the 'Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten'  
141 (VDLUFA; Bassler, 1976). After DM determination duodenal digesta and faeces were freeze-  
142 dried and ground to pass through a 1 mm sieve. Aliquots of the duodenal digesta from each  
143 day were pooled according to daily DM flow calculated from Cr<sub>2</sub>O<sub>3</sub> concentration. Chromium  
144 concentration in duodenal digesta and faeces was determined by atomic spectrophotometry  
145 according to Williams *et al.* (1962).

146 DON in feedstuffs was determined by high performance liquid chromatography (HPLC) with  
147 diode array detection (DAD) after extraction by acetonitrile-water (83:16, v/v) and clean up  
148 with immuno-affinity columns (IAC; DONtest HPLC columns, VICAM, Watertown, USA)  
149 according to a modified VDLUFA method as described by Valenta *et al.* (2002). The  
150 detection limit was 0.03 mg/kg. The mean recovery for feedstuffs was 74 %. ZON in  
151 feedstuffs was analysed by HPLC after incubation with 2 U β-glucosidase (E.C. 3.2.1.21; No.  
152 G-0395, Sigma, Taufkirchen, Germany) according to Ueberschär (1999). The mean recovery  
153 was 86 %. The detection limit was 1 ng/g. Determination of the trichothecenes (nivalenol  
154 (NIV), scirpentriol, T2-tetraol, fusarenon-X (FUS-X), monoacetoxyscirpenol (MAS), 15-  
155 acetyldeoxynivalenol (15-AcDON), 3-acetyldeoxynivalenol (3-AcDON), T2-triol,  
156 neosolaniol (NEO), diacetoxyscirpenol (DAS), HT-2 toxin (HT2) and T-2 toxin (T2)) was  
157 carried out according to a gas chromatography-mass spectrometry (GC-MS) method as  
158 described by Schollenberger *et al.* (2005).

159 Freeze-dried duodenal digesta and faeces were analysed for DON and de-epoxy DON by  
160 HPLC-DAD after incubation with β-glucuronidase, extraction with acetonitrile-water and  
161 clean up by immuno-affinity columns (IAC; DONtest HPLC columns, VICAM, Watertown,  
162 USA) according to Valenta *et al.* (2003). Serum and urine samples were analysed for DON  
163 and de-epoxy DON by HPLC-DAD after incubation with β-glucuronidase, extraction with a  
164 ChemElut<sup>®</sup> cartridge (Varian, Darmstadt, Germany) by ethyl acetate and clean up with IAC  
165 following a HPLC method by Valenta *et al.* (2003). In contrast to the described method by  
166 Valenta *et al.* (2003) incubation with β-glucuronidase was modified. Acetate buffer, pH 5.5,  
167 was added to the faeces and urine samples and phosphate buffer, pH 6.8, was added to the  
168 digesta and serum samples. After addition of β-glucuronidase (type H-2, min. 100000 U/ml,  
169 sulfatase up to 7500 U/ml, Sigma-Aldrich, Taufkirchen, Germany), the mixture was incubated  
170 overnight at 37 °C. The detection limits for both toxins were approximately 0.02 mg/kg  
171 (freeze-dried faeces and digesta) and 4 ng/ml (serum) and 10 ng/ml (urine). The recoveries for



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3 172 DON and de-epoxy DON were determined to be 83 % and 92 % (duodenal chyme), 92 % and  
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5 173 86 % (faeces), 85 and 97 % (serum) and 88 and 88 % (urine), respectively. To confirm the  
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7 174 results in serum, four serum samples were incubated, extracted and purified as described  
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9 175 above and analysed by HPLC-mass spectrometry (HPLC-MS) according to Razzazi-Fazeli *et*  
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11 176 *al.* (2003).

12 177 Six contaminated serum samples (Cows 4, 6, 8, 10, 12 and 14) and six contaminated urine  
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14 178 samples (Cows 3, 4, 5, 9, 10, 14) of cows fed the contaminated wheat were parallelly  
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16 179 investigated with and without enzymatic ( $\beta$ -glucuronidase) digestion in order to estimate the  
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18 180 degree of glucuronidation of DON and de-epoxy DON in serum and urine.

19 181 Freezed dried milk samples were analysed for DON and de-epoxy DON at first without  
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21 182 incubation with  $\beta$ -glucuronidase by a GC-MS method after extraction by water/ethanol  
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23 183 (90/10), cleaning up by means of ChemElut<sup>®</sup> (Varian, Darmstadt, Germany) and Mycosep<sup>®</sup>  
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25 184 columns according to Schothorst *et al.* (2005). Trimethylsilyl (TMS) ether was used for  
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27 185 derivatisation and residues were dissolved in iso-octan. Beside DON and de-epoxy DON, the  
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29 186 trichothecenes FUS-X, NIV, 3-AcDON, 15-AcDON, DAS, NEO, HT2 and T2 in the milk  
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31 187 samples were determined with the same GC-MS method. The detection limits for DON, FUS-  
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33 188 X, NIV, 3-AcDON, 15-AcDON, DAS, NEO, HT2 and T2 were 0.1, 0.16, 0.25, 0.22, 0.18,  
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35 189 0.30, 0.33, 0.22 and 0.16  $\mu\text{g}/\text{kg}$ . The recoveries were determined to be 91, 96, 44, 104, 97,  
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37 190 104, 109, 106 and 111 %. The samples were further analysed by HPLC-UV after incubation  
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39 191 with  $\beta$ -glucuronidase, extraction with acetonitrile-water and clean up by immuno-affinity  
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41 192 columns (DONprep, r-biopharm) according to the procedure described above for freeze-dried  
42  
43 193 faeces with slight modifications after detecting de-epoxy DON predominately as glucuronide  
44  
45 194 conjugate in the serum. The recoveries for DON and de-epoxy DON were determined to be  
46  
47 195 72 % and 89 %. The detection limits were 0.5  $\mu\text{g}/\text{kg}$ . All milk samples of cows fed the  
48  
49 196 contaminated wheat were parallelly investigated without enzymatic ( $\beta$ -glucuronidase)  
50  
51 197 digestion in order to establish the degree of glucuronidation of DON and de-epoxy DON  
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53 198 using the HPLC-UV method. To confirm the results in the milk, the milk samples of all cows  
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55 199 fed the contaminated wheat were again analysed by the GC-MS method described above for  
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57 200 milk samples with enzymatic ( $\beta$ -glucuronidase) digestion. Acetate buffer, pH 5.2, was added  
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59 201 to the milk samples. After addition of  $\beta$ -glucuronidase (Helix Pomatia Juice from BioSeptra;  
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202 activities > 100,000 FU/ml), the mixture was incubated overnight at 37 °C.

203 Results of mycotoxin analysis were not corrected for recovery.

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205

206 ***Calculations and statistics***

207 DM flow of the duodenal chyme and DM amount of the faeces were calculated by dividing  
 208 the amount of Cr dosed by the concentration of Cr of the duodenal digesta and faeces,  
 209 respectively. Concentrations of DON and de-epoxy DON in duodenal digesta and faeces were  
 210 calculated by multiplying mycotoxin concentration in duodenal digesta and faeces (on DM  
 211 basis) with the respective DM flow/DM amount.

212 Two carry over rates into milk were calculated as follows:

213

Total carry over rate into milk

$$214 = \frac{\text{Daily excretion of DON and/or de - epoxy DON in milk } (\mu\text{g/d})}{\text{Daily DON intake } (\mu\text{g/d})}$$

215

Duodenal carry over rate into milk

$$216 = \frac{\text{Daily excretion of DON and/or de - epoxy DON in milk } (\mu\text{g/d})}{\text{Daily DON and/or de - epoxy DON flow at the duodenum } (\mu\text{g/d})}$$

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218 Fat corrected milk (FCM) was calculated as following:

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$$220 \text{ FCM (kg/d)} = ((\% \text{ milk fat} * 0.15) + 0.4) * \text{kg milk yield}$$

221

222 Where applicable, dose-response relationships were evaluated by simple linear regression  
 223 analysis. All statistics were carried out with the Statistica for the Windows<sup>TM</sup> operating  
 224 system (StatSoft Inc., 1994).

225

226 **Results**

227 ***Wheat and diets***

228 The contents of crude nutrients and amino acids as well as the contents of analysed *Fusarium*  
 229 toxins for control and *Fusarium* contaminated wheat are summarised in Table 2. Only slight  
 230 differences in crude nutrients could be detected between control and contaminated wheat. The  
 231 *Fusarium* toxin contaminated wheat contained on average 8 mg DON and 0.09 mg ZON per  
 232 kg DM, while 0.25 mg DON and 0.05 mg ZON per kg DM were detected in the control  
 233 wheat. Maize silage which was used as roughage both in the control and in the toxin rations  
 234 also contained low levels of both toxins. In comparison with the control wheat, slightly higher  
 235 concentrations of 15-acDON, 3-acDON, scirpentriol and HT2 were detected in the *Fusarium*  
 236 contaminated wheat.

### 237 ***Mycotoxin flow at the duodenum***

238 The daily flow of the sum of DON and de-epoxy DON at the duodenum ranged between 2.1  
239 and 35.6 mg after feeding the *Fusarium* toxin contaminated wheat and between 0.4 and 4.4  
240 mg after feeding the control wheat (Table 3). The predominant substance was de-epoxy DON  
241 with a daily flow at the duodenum of 2.1 up to 35.1 mg and of 0.4 up to 4.0 mg in the  
242 mycotoxin and control period, respectively, whereas the daily flow of DON varied between  
243 0.04 to 2.2 mg as well as 0.01 to 1.3 mg. The flow of DON and de-epoxy DON at the  
244 duodenum relative to the DON intake varied between 12 and 77 % when the *Fusarium*  
245 contaminated wheat was fed and between 32 and 153 % in the control period. Thereby, the  
246 portion of DON and de-epoxy DON of the total DON and de-epoxy DON flow were in the  
247 range of 1 and 6 % as well as 94 and 99 % in the mycotoxin period (Figure 1) and between 3  
248 and 35 % as well as 65 and 97 % in the control period, respectively.

249

### 250 ***Mycotoxin residues in serum***

251 The metabolite de-epoxy DON was detected in all serum samples of the cows in the  
252 mycotoxin period. The presence of de-epoxy DON was confirmed by HPLC-MS (Razzazi-  
253 Fazeli *et al.* (2003). Values ranged between 4 and 28 ng/ml (Figure 2). DON concentrations  
254 were all below the detection limit. DON could not be detected in the serum samples of the  
255 control period, whereas the concentrations of de-epoxy DON ranged between below the  
256 detection limit and 5 ng/ml. Furthermore, based on the analysis of six samples with and  
257 without incubation with  $\beta$ -glucuronidase, the degree of glucuronidation of de-epoxy DON  
258 was found to be 100 %. It has to be stressed, however, that it is possible that due to the low  
259 concentrations of conjugated de-epoxy DON in the serum samples a slight amount of non-  
260 conjugated de-epoxy DON could not be detected.

261

### 262 ***Mycotoxin residues in milk***

263 Using the HPLC-UV method without  $\beta$ -glucuronidase incubation, de-epoxy DON was  
264 determined in only one sample (Table 4), whereas the metabolite was detected in the milk of  
265 all cows fed the contaminated wheat using the same method with  $\beta$ -glucuronidase incubation.  
266 Based on the analysis of the one sample, the degree of glucuronidation of de-epoxy DON was  
267 found to be approximately 77 %. DON was both with and without enzyme incubation in all  
268 milk samples below the indicated detection limit using the HPLC-UV method. Concentrations

269 of de-epoxy DON in milk of cows fed the *Fusarium* toxin contaminated wheat ranged  
270 between 1.6 and 2.7 µg/kg (HPLC-UV-method with β-glucuronidase incubation).

271 Both DON and de-epoxy DON were detected using the more sensitive GC-MS method  
272 without and with enzyme incubation (Table 4). However, it has to be stressed that DON levels  
273 were barely above the detectable limit. Values of the further analysed trichothecenes in the  
274 milk were all below the detection limit. Concentrations of DON and de-epoxy DON in the  
275 milk of the cows fed the *Fusarium* toxin contaminated wheat ranged between 0.1 and 0.3 and  
276 1.5 and 3.1 µg/kg, respectively (GC-MS method with enzyme incubation; Table 4). The  
277 degree of glucuronidation of DON and de-epoxy DON was found to be in the range between  
278 33 and 62 % and 73 and 92 %, respectively. Based on the daily average weights of milk  
279 produced (Table 3), the calculated daily excretion of DON and de-epoxy DON in milk of the  
280 cows fed the contaminated wheat ranged up to 10 µg/d and between 14 up to 104 µg/d,  
281 respectively. The daily amounts of DON and de-epoxy DON in the milk increased  
282 significantly with increasing DON intake ( $y = -4,512 + 2,098e^{-4**} \cdot x$ ,  $r^2 = 0.548$ ,  $**P < 0.01$   
283 (DON),  $y = -21,956 + 0.002^* \cdot x$ ,  $r^2 = 0.407$ ,  $*P < 0.05$  (de-epoxy DON)). The total carry over  
284 rates of ingested DON as DON and de-epoxy DON into milk ranged between 0.0001 and  
285 0.0002 and 0.0004 and 0.0024, respectively and did not correlate with the DON intake, as  
286 determined in the intake range 33.8 to 75.6 mg DON per cow per day. The total carry over  
287 rates of ingested DON as DON and de-epoxy DON are significantly linearly correlated with  
288 the milk yield (Figure 3). The daily flows of DON and de-epoxy DON at the duodenum are  
289 significant linearly correlated with the DON and de-epoxy DON amount in the milk (Figure  
290 4). The duodenal carry over rates of DON and de-epoxy DON into the milk ranged up to  
291 0.013 and between 0.0003 and 0.0007. The daily milk yield did not correlate with the  
292 duodenal carry over rates of DON and de-epoxy DON. The de-epoxy DON concentrations  
293 analysed with the GC-MS method were found to be up to 0.13 % higher as the results  
294 obtained with the HPLC-UV method.

295 DON and de-epoxy DON could not be detected in the milk samples of the control period.

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### 297 ***Mycotoxin excretion in urine and faeces***

298 DON and de-epoxy DON were detected in both urine and faeces of the cows in the control as  
299 well as in the mycotoxin period. Values of DON and de-epoxy DON in urine samples of the  
300 mycotoxin period ranged between 0.1 and 0.3 µg/ml and 1.1 and 4.0 µg/ml, respectively  
301 (Figure 5), whereas values in the control period were in the range of below the detection limit  
302 and 0.2 µg/ml and between 0.1 and 0.4 µg/ml, respectively. The portion of DON and de-

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3 303 epoxy DON of total DON plus de-epoxy DON concentration in urine in the mycotoxin period  
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5 304 varied between 4 % and 18 % and 82 % and 96 %, respectively. Based on the analysis of six  
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7 305 samples with and without incubation with  $\beta$ -glucuronidase, the degree of glucuronidation of  
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9 306 DON and de-epoxy DON in urine samples was found to be in the range of 21 % and 92 % and  
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11 307 86 % and 100 %. Daily DON and de-epoxy DON excretion in faeces of cows ranged between  
12  
13 308 89 and 1526  $\mu$ g and 256 and 3978  $\mu$ g in the mycotoxin period, whereas values in the control  
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15 309 period varied between 4 and 232  $\mu$ g and 74 and 586  $\mu$ g (Figure 6). Thereby, the portions of  
16  
17 310 DON and de-epoxy DON of DON intake were in the range of 0.4 up to 8.7 % and 1.2 up to  
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19 311 16.9 % when the *Fusarium* toxin contaminated wheat was fed and in the range of 0.2 up to  
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21 312 11.1 % and 3.1 up to 18.5 % feeding the control wheat. The total residues of DON plus de-  
22  
23 313 epoxy DON in faeces ranged between 2 % and 18 % and between 8 % and 27 % of DON  
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25 314 intake in the mycotoxin and control period, respectively. DON and de-epoxy DON accounted  
26  
27 315 for from 5 % up to 44 % and from 56 % up to 95 % of the residues of both substances in  
28  
29 316 faeces when feeding the contaminated wheat and for 2 % up to 74 % and 27 % up to 98 % of  
30  
31 317 the residues of both substances in faeces when feeding the control wheat.  
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### 319 Discussion

34 320 Contradictory results have been obtained regarding the effects of DON on feed intake of  
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36 321 ruminants. For example, Noller *et al.* (1979) found a slight decrease of daily feed intake with  
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38 322 a concomitant decrease of body weight when feeding *Gibberella zeae* infected corn in  
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40 323 complete mixed rations to lactating dairy cows. Similarly, Trenholm *et al.* (1985) noticed that  
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42 324 feed intake was slightly reduced when naturally contaminated grain (DON concentration up to  
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44 325 6.4 mg/kg) was added to the ration of nonlactating cows. In contrast, the feeding of diets  
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46 326 containing DON concentrations of 6 or 12 mg/kg (Charmley *et al.* 1993) and up to 14 mg/kg  
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48 327 concentrate (Ingalls 1996) did not affect the feed intake. In the present study, the average  
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50 328 DON concentrations of the daily rations were 3.4 mg/kg and 0.3 mg/kg (at a reference DM  
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52 329 content of 88 %) in the mycotoxin and control period, so that the critical DON concentration  
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54 330 of 5 mg per kg of the total daily ration at a reference dry matter content of 88 % as issued for  
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56 331 dairy cows by the German Ministry of Consumer Protection, Food and Agriculture (BML  
57  
58 332 2000) was not exceeded. With regard to the feed intake, the change in feeding the control  
59  
60 333 wheat to feeding the *Fusarium* contaminated wheat did not show any distinctive features.  
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335 Due to the detoxifying ability of the rumen microflora, ruminants appear to have a higher  
tolerance towards harmful substances such as DON. Several *in vitro* studies using bovine



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3 336 rumen fluid have shown that the toxin is de-epoxidised to the less-toxic metabolite de-epoxy  
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5 337 DON (King *et al.* 1984, Swanson *et al.* 1987, He *et al.* 1992). However, it is possible that a  
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7 338 modified toxin profile could alter the toxicity possibly responsible for the variable effects  
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9 339 observed when DON was fed to ruminants in practically relevant concentrations. So, the  
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11 340 present study was conducted to evaluate whether the feed intake, as an indicator for the feed  
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13 341 retention in the rumen, could alter the metabolisation and, therefore, the proportion of DON  
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15 342 and de-epoxy DON reaching the duodenum and eventually the milk.

16 343 In the present experiment, the proportion of DON and de-epoxy DON at the duodenum  
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18 344 relative to the DON intake varied between 12 % and 77 % when the *Fusarium* contaminated  
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20 345 wheat was fed (Table 3) and between 32 % and 153 % when the control wheat was fed. The  
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22 346 higher recovery of DON and de-epoxy DON at the duodenum in the control period is possibly  
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24 347 related to a higher variation coefficient of analytical results which is often found at lower  
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26 348 DON concentrations (Whitaker *et al.* 2000). In the mycotoxin period, the recovery of DON  
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28 349 and de-epoxy DON as a percentage of DON intake was relatively constant at a low level (12-  
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30 350 23 %) at the lower feed/DON intakes, while an increased level of feed/DON intake resulted in  
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32 351 higher differences and recoveries (35-77 %). Furthermore, DON was almost completely  
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34 352 biotransformed to de-epoxy DON, whereby the de-epoxy DON portion of the duodenal flow  
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36 353 of both substances amounted up to 99 % in the mycotoxin period (Figure 1) and up to 97 % in  
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38 354 the control period. Likewise, Dänicke *et al.* (2005) who fed fistulated dairy cows with  
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40 355 *Fusarium* contaminated wheat (8.05 and 7.15 mg DON/kg wheat DM) over a 35 d period  
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42 356 found, that 89 % of the duodenal flow of both toxins were de-epoxy DON. Furthermore, 4-28  
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44 357 % of ingested DON were recovered at the duodenum in the form of DON and de-epoxy DON  
45  
46 358 at total daily DM intakes between 4 and 12 kg, which corresponds well with the recoveries of  
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48 359 12-35 % obtained at daily DM intakes between 6 and 12 kg in the present study. The slightly  
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50 360 higher recovery of 12-35 % compared to 4-28 % might possibly be caused by differences of  
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52 361 diet composition because, in contrast to the present experiment, the wheat portion of daily  
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54 362 ration amounted 50 % (on a DM basis) in the study of Dänicke *et al.* (2005), and rations were  
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56 363 completed by hay or grass silage. Dänicke *et al.* (2005) suggested that the low recovery of  
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58 364 DON as the sum of DON and de-epoxy DON at the duodenum would indicate either a  
59  
60 365 complete degradation of the toxin in the rumen or an absorption by mucosa of the rumen. This  
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367 presumption is supported by Prelusky *et al.* (1985) who calculated from the areas under the  
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369 plasma-time concentration curves a mean pharmacokinetic bioavailability of 7.5 % following  
administration of a single oral dose of 5 mg DON/kg b.w. and a single intravenous dose of 0.5  
mg DON/kg b.w. to sheep. Furthermore, along with the oral dose, a cobalt-EDTA marker,

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3 370 which indicates the liquid phase, was added in the rumen. Due to the intensive disappearance  
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5 371 rate of DON from the rumen which was two to three times faster than the marker the authors  
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7 372 also suggested a complete degradation and/or absorption of DON from the rumen. However,  
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9 373 conversion of DON to de-epoxy DON in the rumen was found to occur only up to 15 %  
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11 374 (Prelusky *et al.* 1985). In the present study, and in the experiment by Dänicke *et al.* (2005), a  
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13 375 high proportion of the flow of DON and de-epoxy DON at the duodenum was the metabolite  
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15 376 de-epoxy DON (up to 99 % and 89 %). Due to the low variation of the proportion of de-epoxy  
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17 377 DON to DON of total DON plus de-epoxy DON flow at the duodenum between the different  
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19 378 DON/feed intakes in the current experiment, it can be concluded that DON is very rapidly  
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21 379 biotransformed to de-epoxy DON. Moreover, the lower biological recovery of DON plus de-  
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23 380 epoxy DON at the lower DON/feed intakes could possibly indicates a complete degradation  
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25 381 of the toxin due to a higher ruminal retention time. Furthermore, de-epoxy DON was the  
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27 382 major component detected in the serum samples of cows fed the contaminated wheat in the  
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29 383 present study, while concentrations of DON were all below the indicated detection limit  
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31 384 (Figure 2). Moreover, the substance was only found as glucuronide conjugate, which implied  
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33 385 that the toxin will go directly to the liver where it undergoes an extensive glucuronidation in  
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35 386 the first-pass. In contrast, Sabater Vilar (2003) found a mean DON content of 32 µg/l using an  
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37 387 ELISA-method in blood serum of cows belonging to a Dutch farm. However, no data was  
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39 388 given by the authors regarding the applicability of the applied ELISA-method for  
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41 389 determination of DON in serum. The author supposed that the rumen was not able to degrade  
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43 390 the amount of DON to which the animals were exposed. The estimated individual daily DON  
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45 391 intake was 4.5 mg at an average daily DM intake of 20 kg, which is similar to the  
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47 392 concentration of 0.3 mg DON/kg DM control diet in the present study. However, in the  
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49 393 current experiment only trace amounts of de-epoxy DON were detected in the serum of the  
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51 394 cows fed the control wheat, whereas concentrations of DON were in all samples below the  
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53 395 detection limit. Accordingly, Prelusky *et al.* (1987) indicated that following an intravenous or  
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55 396 a large single oral dose of DON, only a small fraction was present in the blood plasma as de-  
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57 397 epoxy DON (Prelusky *et al.* 1984, Prelusky *et al.* 1985, Prelusky *et al.* 1990). However a  
58  
59 398 more prolonged oral exposure to a DON contaminated diet (330 and 880 mg/kg diet) for three  
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400 399 days resulted in higher amounts of de-epoxy DON in the plasma. The authors supposed an  
401  
402 400 efficient biotransformation of DON due to an extended exposure of DON to the rumen  
403  
404 401 microflora. A similar pattern of DON and de-epoxy DON was observed in the serum and in  
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406 402 the milk samples obtained from the present study. De-epoxy DON was detected as  
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408 403 predominant substance in the milk of the cows fed the contaminated wheat mainly occurring



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3 404 as glucuronide conjugate (Figure 1; Table 4). However, trace amounts of DON were detected  
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5 405 beside the metabolite de-epoxy DON with the GC-MS method (Table 4), although, it needs to  
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7 406 be stressed that the detected DON levels were barely above the detection limit. In general,  
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9 407 knowledge about the carry over of DON and de-epoxy DON into the milk of dairy cows is  
10  
11 408 still deficient. While Charmley *et al.* (1993) reported no transmission of DON and de-epoxy  
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13 409 DON in the milk of cows with a daily DON intake up to 104 mg (conjugated DON and de-  
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15 410 epoxy DON were not analysed), Prelusky *et al.* (1987) found maximum total DON residue  
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17 411 levels of 222 ng/ml and 135 ng/ml, predominately as conjugated de-epoxy DON in milk of  
18  
19 412 sheep fed extremely high oral doses of 4.0 g and 1.5 g pure DON over a 72 hr period. Only  
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21 413 very low amounts of free and conjugated DON (< 4 ng/ml) were detected in the milk  
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23 414 following a large single oral dose of 1.7 mg DON/kg b.w. to lactating dairy cows (de-epoxy  
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25 415 DON was not analysed; Prelusky *et al.* 1984). Based on the levels of milk production, the  
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27 416 carry over rate of DON into the milk was almost negligible, only about 0.0001 % of the  
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29 417 administered dose. In the present experiment the total carry over rate of DON as DON and de-  
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31 418 epoxy DON transmitted into milk ranged between 0.0001 and 0.0002 and 0.0004 and 0.0024  
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33 419 (GC-MS with enzyme incubation; Table 4). The results indicate a large difference in carry  
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35 420 over especially of de-epoxy DON between animals with lower and higher milk yields. For the  
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37 421 mycotoxin aflatoxin M<sub>1</sub> (AFM<sub>1</sub>, metabolite of aflatoxin B<sub>1</sub>) it is supposed that there is a  
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39 422 passive concentration-dependent permeability of AFM<sub>1</sub> from blood to the alveolar cells of the  
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41 423 mammary gland (Veldman *et al.* 1992). Possibly such a concentration-dependent permeability  
42  
43 424 exists for DON and de-epoxy DON, which could explain the significantly increasing total  
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45 425 carry over results of ingested DON as DON and de-epoxy DON in relation to the increased  
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47 426 milk production. Also, different permeabilities of the alveolar cell membranes might be  
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49 427 responsible for the variation in de-epoxy DON carry over. Furthermore, Veldman *et al.*  
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51 428 (1992) assumed that udder condition could also influence the carry over of AFM<sub>1</sub> because  
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53 429 udder infections can increase the permeability of the udder. Therefore, increased udder  
54  
55 430 permeability at the higher milk yields could also be a reason for the higher DON and de-  
56  
57 431 epoxy DON contents in the milk. Further factors which could influence the DON and de-  
58  
59 432 epoxy DON levels in milk might be equilibration between blood and other compartments,  
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433 DON biotransformation, entero-hepatic circulations, rates of urinary and faecal elimination  
434  
435 and rates of absorption from different areas of the gastrointestinal tract (Prelusky *et al.* 1984).  
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437 In the present study the daily DON and de-epoxy DON flows at the duodenum are  
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439 significantly linearly correlated with the daily DON and de-epoxy DON amounts in the milk  
440  
441 (Figure 4).

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3 438 DON and de-epoxy DON residues were also analysed in urine and faeces samples. In the  
4  
5 439 current study de-epoxy DON was compared to DON the predominant substance in urine  
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7 440 (Figure 5), which was supposed to be the most important excretion route in a study of Cote *et*  
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9 441 *al.* (1986). The portion of de-epoxy DON of the total DON plus de-epoxy DON concentration  
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11 442 in urine amounted to 96 % when the *Fusarium* toxin contaminated wheat was fed.  
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13 443 Furthermore, DON, as well as de-epoxy DON residues in urine appeared mainly as  
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15 444 glucuronide conjugate (66 % and 98 %), which implied that glucuronic acid conjugation  
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17 445 appears to be an important metabolic pathway. However, although all animals were in a  
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19 446 steady feeding state, the urine concentrations of the present study should not be overvalued  
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21 447 because samples were collected spontaneously without consideration of water intake and the  
22  
23 448 kinetic behaviour of DON. Yet, the analyses show that DON and de-epoxy DON were also  
24  
25 449 detected in the urine of the cows fed the control wheat with lower concentrations of DON in  
26  
27 450 the diet. Prelusky *et al.* (1986) found that only 7 % of DON and metabolites were eliminated  
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29 451 with the urine and 55 % up to 75 % of the DON dose appeared as unconjugated forms of  
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31 452 DON and de-epoxy DON in the faeces. However, the assumption that DON seems to  
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33 453 principally be excreted via the faeces can not be confirmed by the present data. Only from 2  
34  
35 454 % up to 18 % of consumed DON were excreted as DON plus de-epoxy DON in faeces when  
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37 455 the *Fusarium* contaminated wheat was fed. The method of DON administration possibly  
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39 456 influence the urinary and faecal excretion rates of DON and de-epoxy DON because, in  
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41 457 contrast to the current experiment, Prelusky *et al.* (1986) administered a large single oral dose  
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43 458 of DON at a level of 5 mg/kg b.w. Furthermore, for the present experiment it has to be  
44  
45 459 stressed that the faeces samples were also taken spontaneously without consideration of the  
46  
47 460 kinetic behaviour of DON, however all animals were in a steady feeding state and the daily  
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49 461 amount of faeces from the cows was estimated using Cr<sub>2</sub>O<sub>3</sub> as marker.  
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51 462

#### 47 463 Conclusion

49 464 It can be concluded from this experiment that after the feeding of DON contaminated diets  
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51 465 (approximately 3.4 mg DON and 0.3 mg DON per kg complete ration, at a reference DM  
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53 466 content of 88 %, in the mycotoxin and control period) to dairy cows, DON is very rapidly  
54  
55 467 biotransformed to de-epoxy DON in the rumen due to the low variation of the portion of de-  
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57 468 epoxy DON of the total flow of DON plus de-epoxy DON at the duodenum between the  
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59 469 different DON/feed intakes. Moreover, the lower biological recovery of DON plus de-epoxy  
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470 DON at the duodenum at lower DON/feed intakes could possibly indicate a complete  
471 471 degradation of the toxin due to a higher ruminal retention time. Furthermore, excretion of

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3 472 DON and de-epoxy DON occurred through both urinary and faecal routes, with urinary  
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5 473 seeming to be the most important. Although very little amounts of DON and de-epoxy DON  
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7 474 were transmitted into the milk of mycotoxin fed animals, the increasing carry over rates of  
8  
9 475 DON and de-epoxy DON with an increasing milk yield need to be examined further.

10 476

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#### 22 23 483 **References**

24  
25 484 BASSLER, R. (Ed.), 1976, Die chemische Untersuchung von Futtermitteln, Methodenbuch. Bd.  
26 485 3 mit Ergänzungslieferungen 1983, 1988 und 1993. VDLUFA, Darmstadt, Germany.

27  
28 486 BAUER, J., 2000, Mykotoxine in Futtermitteln: Einfluss auf Gesundheit und Leistung. In:  
29 487 Handbuch der tierischen Veredlung. 25. Auflage, Kammlage-Verlag, Osnabrück , 169-  
30 488 192.

31  
32  
33 489 BML (The German Federal Ministry of Nutrition, Agriculture and Forestry), 2000,  
34 490 Orientation values for critical concentrations of deoxynivalenol and zearalenone in diets  
35 491 for pigs, ruminants and gallinaceous poultry. *VDM* 27/00, 2-3.

36  
37 492 BOTTALICO, A. and PERRONE, G., 2002, Toxigenic *Fusarium* species and mycotoxins  
38 493 associated with head blight in small-grain cereals in Europe. *European Journal of Plant*  
39 494 *Pathology* 108, 611-624.

40  
41  
42 495 CHARMLEY, E., TRENHOLM, H. L., THOMPSON, B. K., VUDATHALA, D., NICHOLSON, J. W. G.,  
43 496 PRELUSKY, D. B. and CHARMLEY, L. L., 1993, Influence of level of deoxynivalenol in  
44 497 the diet of dairy cows on feed intake, milk production, and its composition. *Journal of*  
45 498 *Dairy Science* 76, 3580-3587.

46  
47  
48 499 COTE, L. M., DAHLEM, A. M., YOSHIZAWA, T., SWANSON, S. P. and BUCK, W. B., 1986,  
49 500 Excretion of deoxynivalenol and its metabolites in milk, urine, and faeces of lactating  
50 501 dairy cows. *Journal of Dairy Science* 69, 2416-2423.

51  
52 502 DÄNICKE, S., VALENTA, H. and UEBERSCHÄR, K. H., 2000, Risikoabschätzung und  
53 503 Vermeidungsstrategien bei der Fütterung. In: Dänicke, S. and Oldenburg, E. (Eds.)  
54 504 Risikofaktoren für die *Fusarium*toxinbildung in Futtermitteln und  
55 505 Vermeidungsstrategien bei der Futtermittelerzeugung und Fütterung. *Landbauforschung*  
56 506 *Völkenrode*, Sonderheft Nr. 216, 5-34.

57  
58  
59 507 DÄNICKE, S., MATTHÄUS, K., LEBZIEN, P., VALENTA, H., UEBERSCHÄR, K.-H. and  
60 508 FLACHOWSKY, G., 2005, On the effects of *Fusarium* toxins on the fermentation and

- 1  
2  
3 509 nutrient utilization in rumen of dairy cows. *Journal of Animal Physiology and Animal*  
4 510 *Nutrition* (in press).
- 5  
6  
7 511 HE, P., YOUNG, L. G. and FORSBERG, C., 1992, Microbial transformation of deoxynivalenol  
8 512 (vomitoxin). *Applied and Environmental Microbiology* **58**, 3857-3863.
- 9  
10 513 HÖLTERSINKEN, M., MAIWORM, K. and SCHOLZ, H., 1996, Mykotoxikosen beim Rind –  
11 514 Probleme auch in Norddeutschland? *Collegium Veterinaria* **26**, 9-14.
- 12  
13 515 INGALLS, J. R. (1996) Influence of deoxynivalenol on feed consumption by dairy cows.  
14 516 *Animal Feed Science and Technology* **60**, 297-300.
- 15  
16  
17 517 KING, R. R., MCQUEEN, R. E., LEVESQUE, D. and GREENHALGH, R., 1984, Transformation of  
18 518 deoxynivalenol (vomitoxin) by rumen microorganisms. *Journal of Agricultural and*  
19 519 *Food Chemistry* **32**, 1181-1183.
- 20  
21 520 NOLLER, C. H., STOB, M. and TUIITE, J., 1979, Effects of feeding *Gibberella zeae*-infected corn  
22 521 on feed intake, body weight gain, and milk production of dairy cows. *Journal of Dairy*  
23 522 *Science* **62**, 1003-1006.
- 24  
25  
26 523 OLDENBURG, E., VALENTA, H. and SATOR, C., 2000, Risikoabschätzung und  
27 524 Vermeidungsstrategien bei der Futtermittelerzeugung. In: Dänicke, S. and Oldenburg, E.  
28 525 (Eds.) Risikofaktoren für die Fusariumtoxinbildung in Futtermitteln und  
29 526 Vermeidungsstrategien bei der Futtermittelerzeugung und Fütterung. *Landbauforschung*  
30 527 *Völkenrode, Sonderheft Nr. 216*, 5-34.
- 31  
32  
33 528 PRELUSKY, D. B., TRENHOLM, H. L., LAWRENCE, G. A. and SCOTT, P. M., 1984, Transmission  
34 529 of deoxynivalenol (vomitoxin) to milk following oral administration to dairy cows.  
35 530 *Journal of Environmental Science and Health* **B19**, 593-609.
- 36  
37 531 PRELUSKY, D. B., VEIRA, D. M. and TRENHOLM, H. L., 1985, Plasma pharmacokinetics of the  
38 532 mycotoxin deoxynivalenol following oral and intravenous administration to sheep.  
39 533 *Journal of Environmental Science and Health* **B20**, 603-624.
- 40  
41  
42 534 PRELUSKY, D. B., VEIRA, D. M., TRENHOLM, H. L. and HARTIN, K. E., 1986, Excretion profiles  
43 535 of the mycotoxin deoxynivalenol, following oral and intravenous administration to  
44 536 lactating sheep. *Fundamental and Applied Toxicology* **6**, 356-363.
- 45  
46  
47 537 PRELUSKY, D. B., VEIRA, D. M., TRENHOLM, H. L. and FOSTER, B. C., 1987, Metabolic-fate  
48 538 and elimination in milk, urine and bile of deoxynivalenol following administration to  
49 539 lactating sheep. *Journal of Environmental Science and Health* **B22**, 125-148.
- 50  
51 540 PRELUSKY, D. B., HARTIN, K. E. and TRENHOLM, H. L., 1990, Distribution of deoxynivalenol  
52 541 in cerebral spinal fluid following administration to swine and sheep. *Journal of*  
53 542 *Environmental Science and Health* **B25**, 395-413.
- 54  
55  
56 543 RAZZAZI-FAZELI, E., BÖHM, J., JARUKAMJORN, K. and ZENTEK, J., 2003, Simultaneous  
57 544 determination of major B-trichothecenes and the de-epoxy-metabolite of deoxynivalenol  
58 545 in pig urine and maize using high-performance liquid chromatography-mass  
59 546 spectrometry. *Journal of Chromatography* **B796**, 21-33.

- 1  
2  
3 547 ROHR, K., BRANDT, M., CASTRILLO, O., LEBZIEN, P. and ASSMUS, G., 1979, Der Einfluss eines  
4 548 teilweise Ersatzes von Futterprotein durch Harnstoff auf den Stickstoff- und  
5 549 Aminosäurenfluss am Duodenum. *Landbauforschung Völkenrode* **29**, 32-40.
- 7  
8 550 ROHR, K., BRANDT, M., LEBZIEN, P. and SCHAFFT, H., 1984, Measurement of duodenal flow  
9 551 in dairy cows by either total collection or spot sampling, using a special cannula.  
10 552 *Canadian Journal of Animal Science* **64** (Suppl.), 116-117.
- 12  
13 553 ROTTER, B. A. and PRELUSKY, D. B., 1996, Toxicology of deoxynivalenol. *Toxicology of*  
14 554 *Environmental and Health* **48**, 1-34.
- 15  
16 555 SABATER VILAR, M., 2003, Assessment and intervention of food- and feed-borne  
17 556 mycotoxins. PhD Thesis, Department of Veterinary Pharmacology, Pharmacy and  
18 557 Toxicology, Utrecht University, The Netherlands.
- 20  
21 558 SCHOLLENBERGER, M., MÜLLER, H.-M., RÜFLE, M., SUCHY, S., PLANCK S. and DROCHNER,  
22 559 W., 2005, Survey of *Fusarium* toxins in foodstuffs of plant origin marketed in Germany.  
23 560 *International Journal of Food Microbiology* **97**, 317-326.
- 25  
26 561 SCHOTHORST, R. C., JEKEL, A. A., VAN EGMOND, H. P., VAN KLAVEREN, J., BOON, P., TJOE NIJ,  
27 562 E. I. M., 2005, Determination of trichothecenes in duplicate diets for young children by  
28 563 capillary gas chromatography with Mass Spectrometric Detection. *Food Additives and*  
29 564 *Contaminants* **22/1**, 48-55.
- 31  
32 565 SEELING, K., LEBZIEN, P., DÄNICKE, S., SPILKE, J., SÜDEKUM, K. H. and FLACHOWSKY, G.,  
33 566 2005a, Effect of level of feed intake and of *Fusarium* toxin contaminated wheat on  
34 567 rumen fermentation as well as blood and milk parameters in cows. *Journal of Animal*  
35 568 *Physiology and Animal Nutrition*, (in press).
- 36  
37 569 SEELING, K., DÄNICKE, S., UEBERSCHÄR, K. H., LEBZIEN, P. and FLACHOWSKY, G., 2005b, On  
38 570 the effects of a *Fusarium* toxin contaminated wheat and the feed intake level on  
39 571 metabolism and carry over of zearalenone in dairy cows. (submitted).
- 41  
42 572 SEELING, K. and DÄNICKE, S., 2005, Relevance of the *Fusarium* toxins deoxynivalenol and  
43 573 zearalenone in ruminant nutrition: A review. *Journal of Animal and Feed Science* **14**, 3-  
44 574 40.
- 45  
46 575 STATSOFT INC., 1994, Statistica for the Windows™ Operating System. Tulsa OK, USA.
- 47  
48 576 SWANSON, S. P., NICOLETTI, J., ROOD, H. D., BUCK, W. B., COTE, L. M. and YOSHIZAWA, T.,  
49 577 1987, Metabolism of three trichothecene mycotoxins, T-2 toxin, diacetoxyscirpenol and  
50 578 deoxynivalenol, by bovine rumen microorganisms. *Journal of Chromatography* **414**,  
51 579 335-342.
- 53  
54 580 TRENHOLM, H. L., THOMPSON, B. K., HARTIN, K. E., GREENHALGH, R. and MCALLISTER, H. J.,  
55 581 1985, Ingestion of vomitoxin (deoxynivalenol)-contaminated wheat by nonlactating  
56 582 dairy cows. *Journal of Dairy Science* **68**, 1000-1005.
- 57  
58 583 UEBERSCHÄR, K. H., 1999, Einfluß von Zearalenon auf Wachstum und Rückstände in den  
59 584 Geweben von Mastkaninchen. VDLUFA-Kongreßband 1999, Halle/Saale, *VDLUFA-*  
60 585 *Schriftenreihe*, **52/1999**, 425-428.



- 1  
2  
3 586 VALENTA, H., DÄNICKE, S. and WOLFF, J., 2002, Vergleich einer HPLC- und einer ELISA-  
4 587 Methode zur Bestimmung von Deoxynivalenol in Mühlenstäuben, Kleien und Getreide.  
5 588 VDLUFA-Kongressband 2002, *VDLUFA-Schriftenreihe* **58**, 675-679.  
6  
7  
8 589 VALENTA, H., DÄNICKE, S. and DÖLL, S., 2003, Determination of deoxynivalenol and de-  
9 590 epoxy deoxynivalenol in animal tissues by liquid chromatography after cleanup with an  
10 591 immunoaffinity column. *Mycotoxin Research* **19**, 51-55.  
11  
12 592 VELDMAN, A., MEIJS, J. A. C., BORGGREVE, G. J. and HEERES-VAN DER TOL, J. J., 1992, Carry-  
13 593 over of aflatoxin from cows food to milk. *Animal Production* **55**, 163-168.  
14  
15  
16 594 WHITLOW, L. W. AND HAGLER, W. M., 1999, An association of mycotoxins with production,  
17 595 health and reproduction in dairy cattle and guidelines for prevention and treatment. In:  
18 596 Lyons T. P., Jaques K. A. (Eds.). Proceedings of Alltechs 15<sup>th</sup> Annual Symposium,  
19 597 Nottingham University Press, Nottingham, UK, 1999, 401-419.  
20  
21 598 WILLIAMS, C. H., DAVID, D. J. and IISMAA, O., 1962, The determination of chromic oxide in  
22 599 faeces samples by atomic absorption spectrophotometry. *Journal of Agricultural*  
23 600 *Sciences* **59**, 381-385.  
24  
25  
26 601 WHITAKER, T. B., HAGLER, W. M., GIESBRECHT, F. G. and JOHANSSON, A. S., 2000, Sampling,  
27 602 sample preparation, and analytical variability associated with testing wheat for  
28 603 deoxynivalenol. *Journal of AOAC International* **83**, 1285-1292.  
29  
30 604  
31  
32  
33  
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Table 1. Composition and mycotoxin concentrations of the concentrates and silages

		Concentrate <sup>1)</sup>		Maize silage	Grass silage
		Contaminated	Control		
Dry matter	(g/kg)	877	885	395	314
Nutrients	(g/kg DM)				
Crude protein		208	210	76	137
Neutral detergent fibre		222	226	404	543
Mycotoxins					
Deoxynivalenol	(mg/kg DM)	5.2	0.1	0.6	< DL
Zearalenone	(µg/kg DM)	59.4	21.7	62.4	2.0

1) 55 % (*Fusarium* contaminated) wheat, 25 % soybean meal, 16 % dried sugar beet pulp, 2 % soybean oil, 2 % mineral feed\*;

\* Per kg mineral feed: Ca, 175 g; Na, 100 g; P, 50 g; Mg, 30 g; Fe, 1.5 g; Mn, 2 g; Zn, 6 g; Cu, 1.2 g; I, 30 mg; Co, 20 mg; Se, 40 mg; vitamin A 1,000,000 IU; vitamin D<sub>3</sub> 100,000 IU; vitamin E, 2000 IU;

DL = detection limit;

n.a. = not analysed



Table 2. Analyses of selected ingredients and *Fusarium* toxins in *Fusarium* contaminated and control wheat

	Contaminated wheat	Control wheat
Dry matter (g/kg)	862.4	875.6
<i>Nutrients (g/kg dry matter)</i>		
Organic matter	980.8	979.9
Crude ash	19.2	20.1
Starch	659.1	696.9
Crude fibre	29.7	22.4
Crude protein	135.3	143.4
<i>Amino acids</i>		
Cysteine	2.3	2.6
Methionine	1.9	1.9
Aspartic acid	6.9	6.3
Threonine	3.3	3.4
Serine	4.9	5.7
Glutamic acid	21.0	23.3
Proline	9.4	11.4
Glycine	4.5	4.9
Alanine	4.3	4.4
Valine	4.7	5.0
Isoleucine	3.8	4.1
Leucine	7.0	7.9
Tyrosine	1.0	2.0
Phenylalanine	5.0	5.8
Histidine	2.9	3.1
Lysine	4.5	4.4
Arginine	5.4	5.8
<i>Mycotoxins (µg/kg dry matter)</i>		
Deoxynivalenol <sup>1</sup>	8210	250
Zearalenone <sup>1</sup>	91	51
Nivalenol <sup>2</sup>	46	40
Scirpentriol <sup>2</sup>	23	< 8
T2-tetraol <sup>2</sup>	< 7	< 7
Fusarenon-X <sup>2</sup>	< 19	< 19
Monoacetoxyscirpenol <sup>2</sup>	< 3	< 3
15-acetyldeoxynivalenol <sup>2</sup>	116	11
3-acetyldeoxynivalenol <sup>2</sup>	46	11
T2-triol <sup>2</sup>	< 5	< 5
Neosolaniol <sup>2</sup>	< 6	< 6
Diacetoxyscirpenol <sup>2</sup>	< 14	< 14
HT-2 toxin <sup>2</sup>	12	< 3
T-2 toxin <sup>2</sup>	< 4	< 4

<sup>1</sup> Measured by high-performance liquid chromatography (HPLC)-methods

<sup>2</sup> Measured by a gas chromatography mass spectrometry (GC-MS)-method

Table 3. Dry matter (DM) intake, deoxynivalenol (DON) intake, milk yield, daily flow of DON and de-epoxy DON and DON plus de-epoxy DON flow as percentage of DON intake at the duodenum of individual lactating cows in the mycotoxin period

Cow	DM intake (kg/d)	DON intake ( $\mu\text{g/d}$ )	Milk yield (kg/d)	Flow of DON at the duodenum ( $\mu\text{g/d}$ )	Flow of de-epoxy DON at the duodenum ( $\mu\text{g/d}$ )	DON plus de-epoxy DON (% of DON intake)
1	5.6	18800	-*	35	2104	12
2	5.6	16570	-*	108	3650	23
3	6.0	22620	-*	66	4059	18
4	8.9	33840	9.6	225	4698	15
5	11.4	38090	17.8	352	12170	33
6	12.3	41040	15.4	185	5745	15
7	12.6	47580	18.0	163	16539	35
8	13.1	38780	19.7	526	12065	36
9	15.3	42430	32.7	831	27659	67
10	16.5	43510	29.1	1804	31505	77
11	16.2	47900	23.7	362	24342	51
12	17.8	47030	35.9	2193	32778	74
13	19.9	75610	31.8	503	35064	47
14	20.5	60760	42.7	663	26503	45

\* Cows did not lactate

Table 4. DON and de-epoxy DON concentrations ( $\mu\text{g}/\text{kg}$ ) in the milk of individual lactating cows in the mycotoxin period determined with a HPLC-UV and a GC-MS method with and without  $\beta$ -glucuronidase incubation

Cow	(HPLC-UV <sup>1</sup> )				(GC-MS <sup>2</sup> )			
	with $\beta$ -glucuronidase incubation		without $\beta$ -glucuronidase incubation		with $\beta$ -glucuronidase incubation		without $\beta$ -glucuronidase incubation	
	DON	De-epoxy DON	DON	De-epoxy DON	DON	De-epoxy DON	DON	De-epoxy DON
1	*	*	*	*	*	*	*	*
2	*	*	*	*	*	*	*	*
3	*	*	*	*	*	*	*	*
4	< 0.5	1.6	< 0.5	< 0.5	0.11	1.8	< 0.1	0.2
5	< 0.5	1.6	< 0.5	< 0.5	0.13	1.7	< 0.1	0.3
6	< 0.5	2.1	< 0.5	< 0.5	0.13	2.1	< 0.1	0.2
7	< 0.5	1.2	< 0.5	< 0.5	0.15	1.5	0.10	0.2
8	< 0.5	1.7	< 0.5	< 0.5	0.15	2.1	< 0.1	0.2
9	< 0.5	1.6	< 0.5	< 0.5	0.15	2.2	< 0.1	0.4
10	< 0.5	2.7	< 0.5	0.6	0.22	2.9	0.13	0.6
11	< 0.5	1.8	< 0.5	< 0.5	0.22	3.1	< 0.1	0.3
12	< 0.5	1.5	< 0.5	< 0.5	0.23	1.5	0.14	0.4
13	< 0.5	1.5	< 0.5	< 0.5	0.26	2.7	0.11	0.5
14	< 0.5	0.8	< 0.5	< 0.5	0.22	2.0	0.11	0.3

\* Cows did not lactate

<sup>1</sup> Measured by a high-performance liquid chromatography (HPLC) method (detection limit 0.5  $\mu\text{g}/\text{kg}$ ),

<sup>2</sup> Measured by a gas chromatography mass spectrometry (GC-MS)-method (detection limit 0.1  $\mu\text{g}/\text{kg}$ )

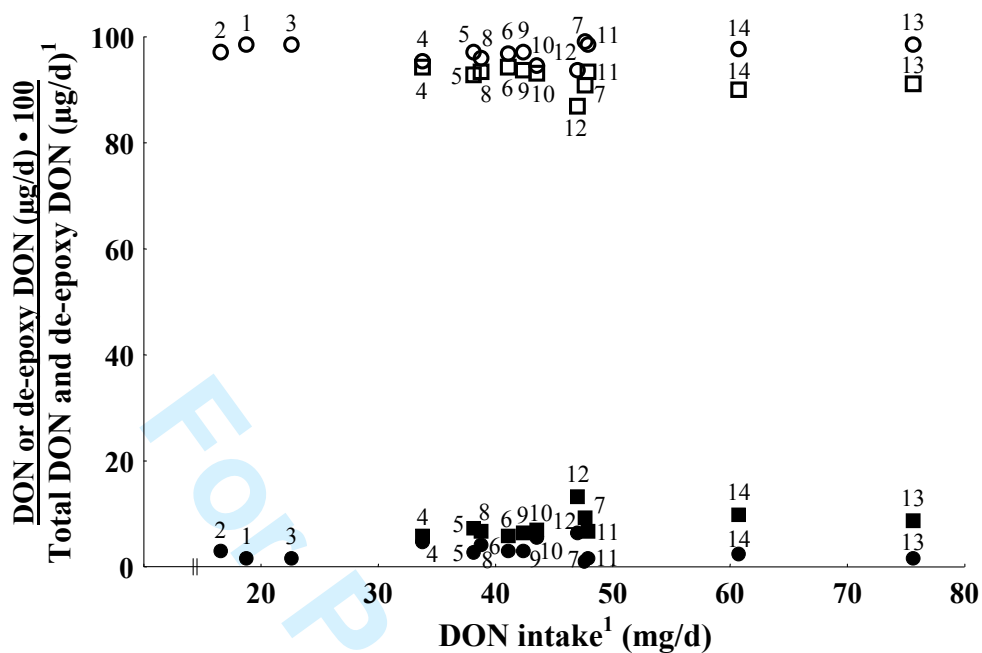


Figure 1. Relationship between deoxynivalenol (DON) intake (mg/d) and percentage of DON and de-epoxy DON of the total DON plus de-epoxy DON flow at the duodenum as well as percentage of DON and de-epoxy DON of the total DON plus de-epoxy DON amount in the milk of dairy cows in the mycotoxin period

Metabolite profile of ● DON and ○ de-epoxy DON at the duodenum

Metabolite profile of ■ DON and □ de-epoxy DON in the milk

Numbers over the symbols denote the individual cows

<sup>1</sup>Measured by a high-performance liquid chromatography (HPLC) method

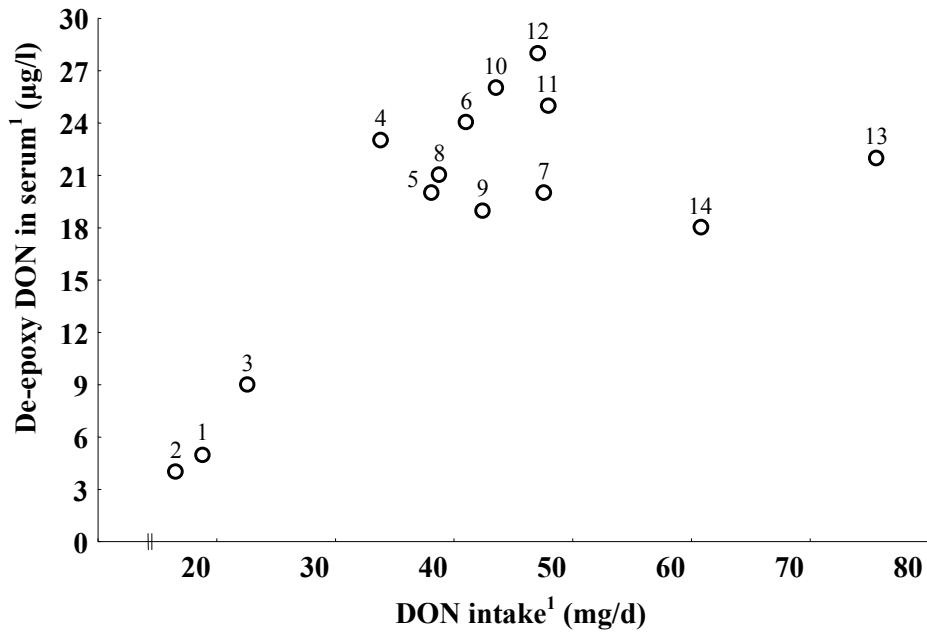


Figure 2. Relationship between deoxynivalenol (DON) intake (mg/d) and de-epoxy DON residues in serum of dairy cows in the mycotoxin period  
 Numbers over the symbols denote the individual cows  
<sup>1</sup>Measured by a high-performance liquid chromatography (HPLC) method

Review Only

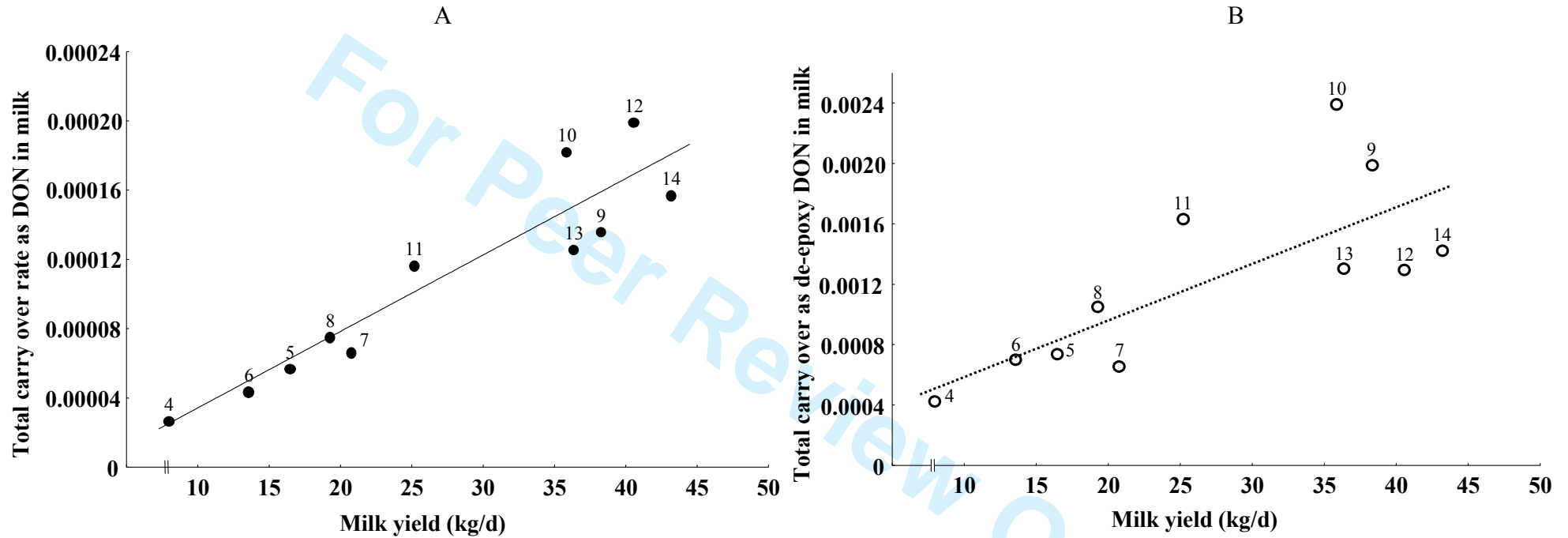


Figure 3. Relationship between milk yield (kg/d) and total carry over rate of DON as DON (A) and de-epoxy DON (B) into milk of dairy cows in the mycotoxin period

—●— DON,  $y = -1,277 e^{-5} + 4.429 e^{-6***} \cdot x$ ,  $r^2 = 0.873$ ,  $***P < 0.001$

---○--- de-epoxy DON,  $y = -2.31 e^{-4} + 3.709 e^{-5**} \cdot x$ ,  $r^2 = 0.559$ ,  $**P < 0.01$

Numbers over the symbols denote the individual cows

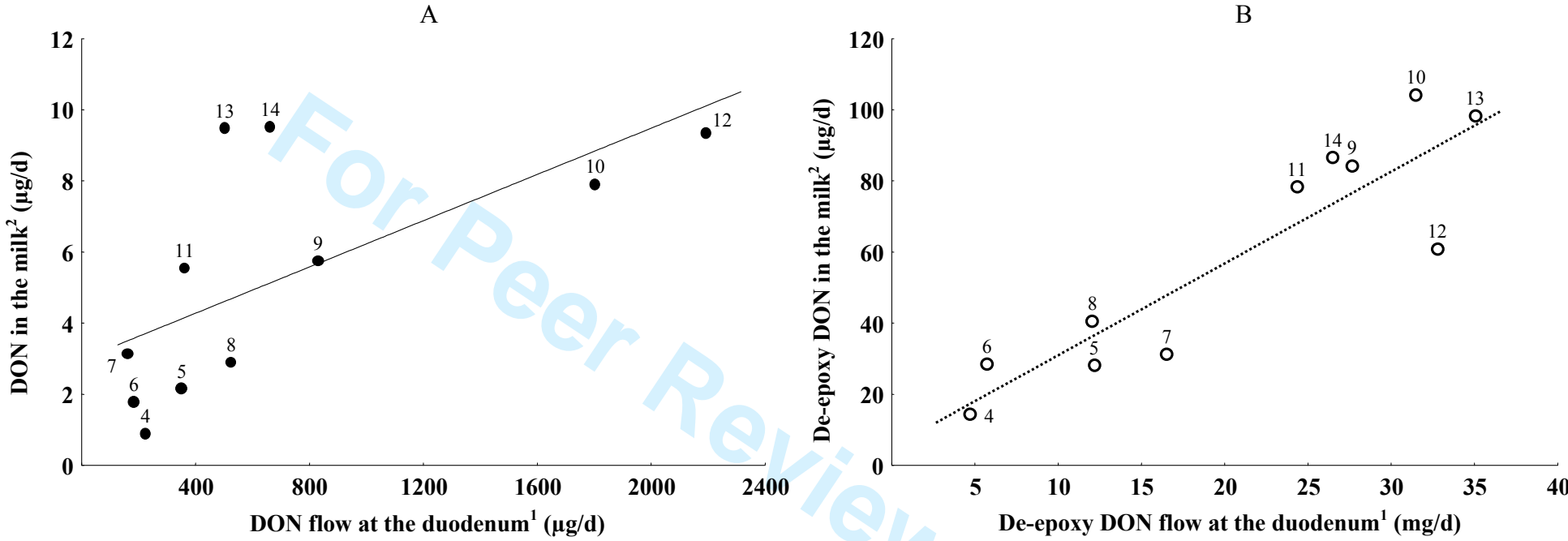


Figure 4. Relationship between DON and de-epoxy DON flow at the duodenum and DON (A) and de-epoxy DON (B) excretion in milk (µg/d) of dairy cows in the mycotoxin period

—●— DON,  $y = 3.038 + 0.003 \cdot x$ ,  $r^2 = 0.417$ ,  $*P < 0.05$   
 ...○... de-epoxy DON,  $y = 4.623 + 0.003^{***} \cdot x$ ,  $r^2 = 0.814$ ,  $***P < 0.001$

Numbers over the symbols denote the individual cows

<sup>1</sup>Measured by a high-performance liquid chromatography (HPLC) method with β-glucuronidase incubation

<sup>2</sup>Measured by a gas chromatography mass spectrometry (GC-MS) method with β-glucuronidase incubation



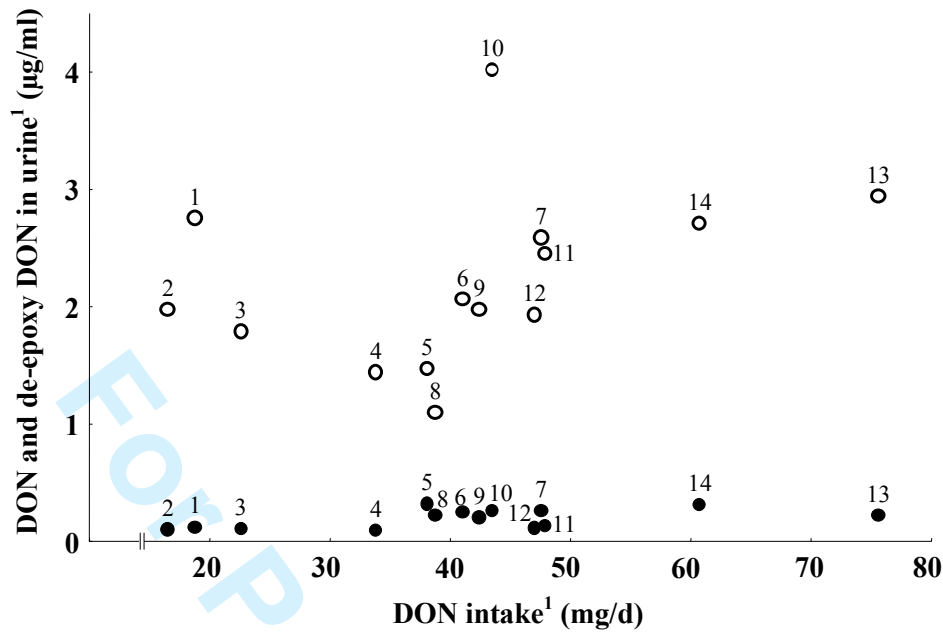


Figure 5. Relationship between deoxynivalenol (DON) intake and DON and de-epoxy DON residues in urine of dairy cows in the mycotoxin period

● DON; ○ de-epoxy DON

Numbers over the symbols denote the individual cows

<sup>1</sup>Measured by a high-performance liquid chromatography (HPLC) method

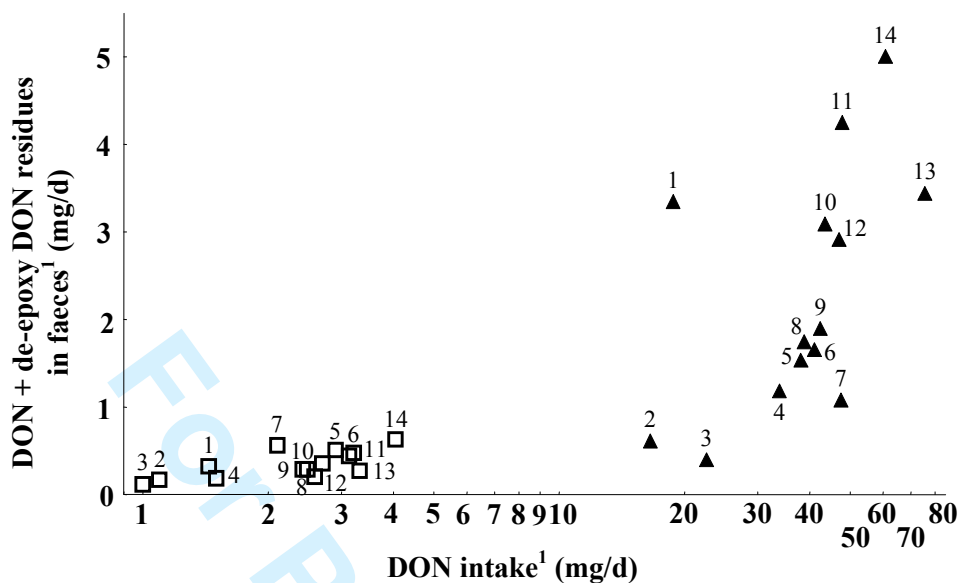


Figure 6. Relationship between deoxynivalenol (DON) intake and DON plus de-epoxy DON residues in faeces of dairy cows in the mycotoxin period and control period

□ Control period, ▲ Mycotoxin period

Numbers over the symbols denote the individual cows

<sup>1</sup>Measured by a high-performance liquid chromatography (HPLC) method