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**On the interactions between Fusarium
toxin-contaminated wheat and non-starch-polysaccharide
hydrolyzing enzymes in diets of turkeys on performance,
health and carry over of deoxynivalenol and zearalenone**

Sven Dänicke

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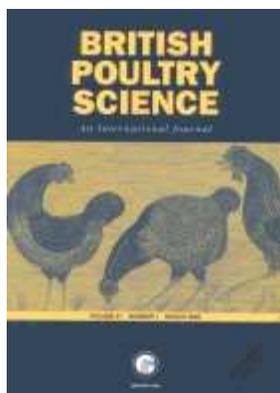
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On the interactions between Fusarium toxin-contaminated wheat and non-starch-polysaccharide hydrolyzing enzymes in diets of turkeys on performance, health and carry over of deoxynivalenol and zearalenone

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On the interactions between effects of Fusarium toxin-contaminated wheat and non-starch-polysaccharide hydrolysing enzymes in turkey diets, on performance, health and carry-over of deoxynivalenol and zearalenone

S. DÄNICKE¹, H. VALENTA, K.-H. UEBERSCHÄR AND S. MATTHES²

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Accepted for publication 31st October 2006

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1
2 28 **Abstract** 1. Diets with increasing proportions of *Fusarium* toxin-contaminated wheat (0, 170,
3
4 29 340 and 510 g CW/kg) were fed to male turkeys (BUT Fig 6) from d 21 to d 56 of age. Each
5
6 30 diet was tested with or without a non-starch-polysaccharide (NSP) hydrolysing enzyme
7
8 31 preparation. Dietary deoxynivalenol (DON) and zearalenone (ZON) concentrations were
9
10 32 successively increased up to approximately 5.4 and 0.04 mg/kg, respectively.

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11 33 2. Weight gain decreased slightly with increasing proportions of CW₂ by 1.6, 0.7 and 3.6 %_w
12
13
14 34 whereas other performance parameters remained unaffected. NSP enzyme supplements to the
15
16 35 diets had no influence.

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17
18 36 3. The weight of the emptied jejunum plus ileum, relative to live weight, decreased in a dose-
19
20 37 related fashion whereby the NSP enzyme exerted an additional weight-decreasing effect. A
21
22 38 similar weight-decreasing NSP enzyme effect was noted for heart weights. Activity of
23
24 39 glutamate-dehydrogenase in serum was significantly increased in groups fed the diets with the
25
26 40 highest CW proportion, whereas γ -glutamyl-transferase remained unaltered.

27
28 41 4. Viscosity in the small intestine was significantly reduced by supplementing the diets with
29
30 42 the NSP enzyme. This effect successively decreased with increasing proportions of the CW.

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31 43 5. Concentrations of DON and of its de-epoxidised metabolite de-epoxy-DON in plasma, liver
32
33 44 and breast meat were lower than the detection limits of 2 ng/ml (plasma) and 4 ng/g,
34
35 45 respectively, of the applied HPLC-method. DON concentration in bile reached up to 13-23
36
37 46 ng/ml whereas de-epoxy-DON concentration was lower than 4 ng/ml.

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38
39 47 6. ZON or its metabolites were not detectable in plasma, liver or breast meat (detection limits
40
41 48 of the HPLC-method were 1 ng/g, 0.5 ng/g and 5 ng/g for ZON, α -zearalenol (ZOL) and β -
42
43 49 ZOL, respectively). Concentrations of ZON and α -ZOL in bile increased with dietary ZON-
44
45 50 concentration. The mean proportions of ZON, α -ZOL and β -ZOL of the sum of all three
46
47 51 metabolites were 19, 77 and 4 %, respectively.

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Keywords: Turkey, *Fusarium* inoculated wheat, deoxynivalenol, zearalenone, non-starch-polysaccharide enzymes¶

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52 INTRODUCTION

1
2 53 The *Fusarium* toxin deoxynivalenol (DON) is of great practical importance as it occurs
3
4 54 frequently on cereal grains (SCOOP TASK 3.2.10 of the European Member States_2003).
5
6 55 Concentrations could be so high that even after dilution by mixing such cereals into diets for
7
8 56 farm animals, the levels may exceed critical dietary DON concentrations (EFSA, 2004a).
9
10 57 Turkeys were not, however, considered in the recent risk evaluation of this toxin by EFSA,
11
12 58 2004a) which might be due to a lack of information in the literature. Indeed, only a few
13
14 59 experiments have been reported. Interestingly, all these experiments revealed slightly negative
15
16 60 effects on performance, which, however, appeared independent of dietary DON dosage,
17
18 61 covering a range between 0.28 and 20 mg/kg (Trenholm *et al.*, 1984; Hamilton *et al.*, 1985;
19
20 62 Manley *et al.*, 1988; Morris *et al.*, 1999; Leitgeb *et al.*, 1999). Besides the fact that different
21
22 63 DON sources were used, which were co-contaminated with other *Fusarium* toxins to a greater
23
24 64 or lesser extent, the question of a critical dietary DON concentration for turkeys could not be
25
26 65 answered up to now. Therefore, the present experiment was designed as a dose response
27
28 66 study using a wheat batch mainly contaminated with DON. Moreover, each dietary DON
29
30 67 level was tested with or without the addition of a non-starch-polysaccharide (NSP)
31
32 68 hydrolysing enzyme preparation as Dänicke *et al.* (2004a) suggested that cell wall alterations
33
34 69 caused by the invasion of the cereal by the *Fusarium* fungus might positively affect the
35
36 70 nutritive value of the infected cereal grain. Thus, it should be tested whether such putative
37
38 71 positive effects could balance the adverse DON effects. Several physiological samples were
39
40 72 collected at the end of the experiment and analysed for mycotoxin residues to contribute to
41
42 73 knowledge on their metabolism and carry-over in turkeys.

74 MATERIAL AND METHODS

75 Experimental design and diets

76 All diets contained 510 g wheat/kg of the "Ritmo" variety cultivated at the Mariensee
77 experimental station of the Federal Agricultural Research Centre (FAL), Braunschweig,
78 Germany, which was not inoculated or artificially inoculated with spores of *Fusarium*

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79 *culmorum*. Details of this field experiment were reported by Matthäus *et al.* (2004) and
 80 Dänicke *et al.* (2004b). The increase in the DON-concentration in the diets was achieved by
 81 successive substitution of the uncontaminated wheat by the *Fusarium* toxin contaminated
 82 wheat (CW) (Table 1). Four diets containing 0, 170, 340 and 510 g CW/kg were prepared in
 83 this way. Each of these diets was tested with and without supplementation of an NSP
 84 hydrolysing enzyme preparation (ZY68, Lohmann Animal Health GmbH, KG, Cuxhaven,
 85 Germany; declared activity: endo-1,4- β -xylanase (EC 3.2.1.8) 1000 FXU/g). Diets differed
 86 only in the proportions of both wheat sources and were therefore nearly isoenergetic and
 87 isonitrogenous. Nutrient and energy concentrations were in the range recommended by GfE
 88 (2004). Diets were provided in a pelleted form.

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89 Growth experiment

90 A total of 384 d-old male turkeys of the strain BUT Big 6 were obtained from the breeding
 91 company "Moorgut Kartzfehn", Bösel, Germany. Chicks were evenly distributed in 64 wood-
 92 shaving litter floor pens. Temperature and lighting regimens were in accordance with the
 93 recommendations of the breeder. Birds were fed on a commercial starter diet until d 21 of age
 94 when the experiment started. Turkeys were randomly assigned to the 8 treatments which were
 95 replicated 8 times. Therefore, each treatment comprised a total of 48 turkeys. The average
 96 initial live weight was similar for all groups (581±66g). Feed and water were offered for *ad*
 97 *libitum* consumption. Turkey weights and consumed feed were determined after two weeks of
 98 feeding, and at the end of the experiment at d 56 of age. After the final weighing, 12 turkeys
 99 per treatment were slaughtered by cutting the neck vessels after manual stunning. Mixed trunk
 100 blood was collected for enzyme activity measurements and mycotoxin residue analysis.
 101 Jejunum (from the entry of the main bile and pancreatic ducts to the Meckel's diverticulum),
 102 ileum (from the Meckel's diverticulum to the ileo-caecal junction), liver, spleen, bursa of
 103 fabricii and heart were quickly dissected after inspection of the upper digestive tract (beak,
 104 oral cavity, pharynx, oesophagus). Ingesta from the jejunum and ileum were collected in pre-

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2 105 | cooled tubes, pooled for 4 turkeys and kept on ice before being frozen for later determination
3
4 106 | of viscosity. Bile was sampled by puncturing the gall bladder and pooled for two turkeys.
5
6 107 | Weights of the emptied segments of the small intestine and all other dissected inner organs
7
8 108 | were recorded. Representative samples of breast meat and complete livers (without gall
9
10 109 | bladder) were pooled for 4 birds and kept frozen before being further processed for mycotoxin
11
12 110 | residue analysis.

13
14 111 | **Analyses**
15
16 112 | Diet samples were analysed for Kjeldahl-N and dry matter according to the methods of the
17
18 113 | VDLUFA (Verband Deutscher Landwirtschaftlicher Untersuchungs- und
19
20 114 | Forschungsanstalten) as described by Naumann and Bassler (1993). DON in diet samples
21
22 115 | was analysed by using HPLC (high performance liquid chromatography) with DAD (diode
23
24 116 | array detection) after a clean-up with IAC (immuno-affinity column, VICAM) according to a
25
26 117 | modified VDLUFA-method as described by Valenta *et al.* (2002). The detection limit was
27
28 118 | 0.03 mg/kg and the recovery was approximately 90 % for this matrix.

29
30 119 | Physiological samples were analysed for DON and de-epoxy-DON according to
31
32 120 | Valenta *et al.* (2003), with modifications. Briefly, plasma, bile and freeze-dried liver and
33
34 121 | breast meat samples were incubated with β -glucuronidase (Type H-2, β -glucuronidase, EC
35
36 122 | 3.2.1.31, 114400 U/ml; arylsulphatase, EC 3.1.6.1, 3290 U/ml; Sigma, Deisenhofen,
37
38 123 | Germany) at pH 5.5 and 37°C overnight. Subsequently, plasma and bile were extracted with
39
40 124 | ethyl acetate (bile after adjusting the pH to 7) on disposable ChemElut columns (Varian
41
42 125 | Deutschland GmbH, Darmstadt) and cleaned up with IAC (DONtest™ of VICAM, Klaus
43
44 126 | Ruttman GmbH, Hamburg, Germany, in case of plasma, and DONprep™, R-Biopharm
45
46 127 | Rhone Ltd., Darmstadt, Germany, in case of bile). Freeze-dried liver and breast meat samples
47
48 128 | were extracted with a mixture of acetonitrile and water, defatted with petroleum ether, pre-
49
50 129 | cleaned with a mixture of charcoal, alumina and celite and cleaned up with IAC (DONprep™,
51
52 130 | R-Biopharm Rhone Ltd., Darmstadt, Germany). DON and de-epoxy-DON in plasma, bile,
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1
2 131 liver and breast meat were determined by HPLC with UV detection. The detection limit for
3
4 132 both substances was approximately 2 ng/ml for plasma, and 4 ng/g bile and freeze dried liver
5
6 133 and breast meat, respectively, with mean recoveries of 92-95 % and 88-104 % for DON and
7
8 134 de-epoxy-DON, respectively.

9
10 135 Zearalenone in feed samples was analysed after incubation with 2 U β -glucosidase
11
12 136 (E.C. 3.2.1.21, Sigma, Taufkirchen, Germany, No. G-0395). Results of the analysis of ZON
13
14 137 and its metabolites were not corrected for recovery. Tissue and body fluid samples were
15
16 138 analysed for zearalenone (ZON), α -zearalenol (α -ZOL), β -zearalenol (β -ZOL), zearalanone
17
18 139 (ZAN), α -zearalanol (α -ZAL), β -zearalanol (β -ZAL) by HPLC with fluorescence detection
19
20 140 after treatment with 2/0.9 U β -glucuronidase/arylsulphatase (EC 3.2.1.31/EC 3.1.6.1 Roche
21
22 141 Diagnostics GmbH, Mannheim, Germany, No. 127698) and cleaning of the extracts by
23
24 142 immuno-affinity columns (Easi-ExtractTM Zearalenone, Rhône-Diagnostics, Glasgow, UK)
25
26 143 according to Ueberschär 1999) and as described by Dänicke *et al.* (2001b). Detection limits for
27
28 144 all specimens were 1 ng/g, 0.5 ng/g, 5 ng/g, 100 ng/g, 50 ng/g and 200 ng/g for ZON, α -ZOL,
29
30 145 β -ZOL, ZAN, α -ZAL and β -ZAL, respectively, at a sample weight of 5 g. The mean recovery
31
32 146 for ZON, α -ZOL and β -ZOL was 95, 69 and 55 % for feedstuffs, 79, 59 and 59 % for plasma,
33
34 147 95, 80 and 121 % for bile, and 93, 72 and 63 % for liver and breast meat, respectively.

35
36 148 Plasma chemical parameters were analysed using test-kits supplied by Merck,
37
38 149 Darmstadt, Germany: glutamate dehydrogenase (GLDH, EC 1.4.1.3, Merck-1-Test[®],
39
40 150 1.03373), γ - glutamyltransferase (γ -GT, EC 2.3.2.2, Granutest[®] 3, 1.12189.0001).

41
42 151 Viscosity of jejunal and ileal ingesta was determined by using a Brookfield viscometer
43
44 152 as described by Dusel *et al.* (1998).

45 46 153 **Statistics**

47
48 154 Data were evaluated by a two-factorial design of analysis of variance (ANOVA):

49
50
51 155
$$y_{ijk} = \mu + a_i + b_j + (axb)_{ij} + e_{ijk}$$

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156 where $y_{ijk} = k^{\text{th}}$ observation; $\mu =$ overall mean; $a_i =$ effect of the proportion of contaminated wheat of total
 157 dietary wheat (0, 33, 66 and 100 %); $b_j =$ effect of the NSP hydrolysing enzyme preparation (without, with);
 158 $(axb)_{ij} =$ interactions; $e_{ijk} =$ error term.

159 All statistics were carried out using the Statistica for Windows™ system (StatSoft, 1984).

RESULTS

Growth experiment

162 The experiment took a normal course and no turkey died or had to be removed from the
 163 experiment due to health problems during the 5 weeks of the study. Increasing proportions of
 164 CW in turkey diets decreased live weight gain in a linear-related manner if the first two weeks
 165 are considered ($P_{\text{linear}}=0.040$, Table 2). NSP enzyme supplements to the diets had no effect on
 166 live weight gain. As feed intake remained unaffected in all treatments, the feed-to-gain ratio
 167 was slightly increased due to increasing proportions of CW. This was most pronounced
 168 during the first two weeks of the experiment ($P_{\text{linear}}=0.020$). In this period there was a trend to
 169 an improved feed-to-gain ratio due to the addition of NSP enzyme ($P=0.085$), although this
 170 effect was only obvious at lower CW inclusion rates ($P_{\text{CW} \times \text{ZY68}}=0.098$).

Gross macroscopical findings, organ weights and serum clinical-chemical parameters

172 Gross macroscopical inspection of the upper digestive tract (beak, oral cavity, pharynx,
 173 oesophagus) did not reveal any signs of irritation, inflammation or other pathological changes.
 174 The weight of the emptied jejunum plus ileum, relative to live weight, decreased in a dose-
 175 related fashion ($P_{\text{linear}}=0.002$, $p_{\text{quadratic}}=0.003$), whereby the NSP enzyme exerted an additional
 176 weight-decreasing effect ($P=0.017$, Table 3). A similar weight decreasing NSP enzyme effect
 177 was noticed for heart weights ($P<0.001$). Other organ weights (liver, spleen, Bursa Fabricii)
 178 were not affected. Activity of glutamate-dehydrogenase in serum was significantly increased
 179 in groups fed the diets with the highest CW proportion ($P=0.014$) whereas γ -glutamyl-
 180 transferase remained unaltered.

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181 [Table 4](#)).

182 **Viscosity of intestinal ingesta**

183 Viscosity of both jejunal and ileal ingesta was significantly reduced by supplementing the
 184 diets with the NSP enzyme ($P=0.004$) with a generally higher viscosity level of the ileal
 185 ingesta ([Figure 1](#)). However, this effect successively decreased with increasing proportions of
 186 the CW as indicated by the significant CW effects ($P=0.005$ and 0.001 for jejunal and ileal
 187 ingesta) and the significant interactions between CW and the NSP enzyme ($P_{CW \times ZY68} < 0.001$
 188 for jejunal and ileal ingesta, [Figure 1](#)).

189 **Mycotoxin residues and carry over**

190 Concentrations of DON and of its de-epoxidised metabolite de-epoxy-DON in plasma, liver
 191 and breast meat were lower than the detection limits of 2 ng/ml (plasma) and 4 ng/g,
 192 respectively, of the applied HPLC-method. DON concentration in bile reached up to 13-23
 193 ng/ml whereas de-epoxy-DON concentration was lower than 4 ng/ml.

194 ZON or its metabolites were not detectable in plasma, liver and breast meat (detection
 195 limits of the HPLC-method were 1 ng/g, 0.5 ng/g and 5 ng/g for ZON, α -ZOL and β -ZOL,
 196 respectively). Concentrations of ZON and α -ZOL in bile increased in a quadratic fashion
 197 ($P_{quadratic} < 0.001$) with dietary ZON-concentration, whereas that of β -ZOL generally remained
 198 at a lower and dietary ZON-independent level ([Table 5](#)). The proportions of α -ZOL and β -
 199 ZOL of the sum of all metabolites (ZON+ α -ZOL+ β -ZOL) varied independent of dietary ZON
 200 concentrations of between one and six percent, and 74 to 83%, respectively. The proportion of
 201 ZON increased significantly with dietary ZON concentration from 13 to 24% ($P_{linear} = 0.001$).
 202 The addition of the NSP enzyme did not alter the concentration or profile of ZON and its
 203 metabolites in bile although a significant interaction occurred between the CW and NSP
 204 enzyme for the α -ZOL concentration ($P_{CW \times ZY68} = 0.036$).

205 **DISCUSSION**

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2 206 The CW fed in the present experiment was of the same origin as that tested earlier on Pekin
3
4 207 ducks. This experiment, as well as the chemical and mycotoxin composition of the CW, were
5
6 208 reported by Dänicke *et al.* (2004b). Briefly, the control wheat and the CW contained ($\mu\text{g}/\text{kg}$
7
8 209 dry matter): DON, 190, 7790; nivalenol, 24, 45; scirpentriol, <9, 67; T-2-tetraol, <8, <8;
9
10 210 fusarenon-X, <21, <21; monoacetoxyscirpenol, <3, <3; 15-acetyl-DON, <8, 12; 3-acetyl-
11
12 211 DON, <10, 1573; T-2-triol, <6, <6; neosolaniol, <7, <7; diacetoxyscirpenol, <16, <16; HT-2
13
14 212 toxin, <3, <3; T-2 toxin, <4, <4 and zearalenone, 2, 100. The DON concentrations and
15
16 213 increments of the diets fed in the present experiment were based on the DON analysis of the
17
18 214 CW. It becomes obvious that the analysed dietary DON concentrations exceeded that of the
19
20 215 calculated values by approximately 40 % on average, whereby the increments between DON
21
22 216 concentrations were not equidistant. This problem is not new and might to a large extent be
23
24 217 attributable to sampling and sample preparation errors (Friend *et al.*, 1983; Young *et al.*,
25
26 218 1983; Hamilton *et al.*, 1985; Dänicke *et al.*, 2004c). It remains to be examined to what extent
27
28 219 a feed mix- and pellet- (heat and moisture treatment) related conversion of the substantial
29
30 220 amounts of 3-acetyl-DON (approximately 17% of the sum of DON plus 3-acetyl-DON) could
31
32 221 have contributed to the observed discrepancies. That diets were mixed properly can be
33
34 222 deduced from the slight and approximately equidistant increase in the analysed crude protein
35
36 223 concentration of the diets, which was due to the higher crude protein concentration of the CW
37
38 224 compared to the control wheat (142 *vs.* 131 g/kg dry matter). Moreover, turkeys responded to
39
40 225 increasing dietary CW inclusion rates with a linear decrease in live weight gain. This was
41
42 226 most pronounced during the first two weeks of the experiment, where the decrease compared
43
44 227 to the control group amounted to 0.8, 1.6 and 4.4% for the groups fed diets containing 33, 66
45
46 228 and 100% CW of total dietary wheat, respectively. This decrease corresponded to a
47
48 229 concomitant increase in feed-to-gain ratio of 1.1, 1.8 and 2.6%. A comparison of the present
49
50 230 results with literature reports can only be undertaken with caution in view of the fact that
51
52 231 different genetic turkey origins and different age periods were tested. This is further

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2 232 | complicated by the use of contaminated feedstuffs with more or less-characterised *Fusarium*
3
4 233 | toxin patterns. Keeping these drawbacks in mind, it seems that turkeys might respond to the
5
6 234 | presence of DON in feed somewhat differently than broilers, which were often found to react
7
8 235 | with an increase in live weight gain when DON-contaminated diets were fed (for review see
9
10 236 | Dänicke *et al.*, 2001a). In contrast, turkeys seem to respond with a decrease in live weight
11
12 237 | gain independent of dietary DON concentration. The decrease in live weight gain relative to
13
14 238 | the control amounted to 5 and 3%, corresponding to 0.28 and 0.55 mg DON/kg diet
15
16 239 | (Hamilton *et al.*, 1985), 8.3 and 0.4%, corresponding to 2.2 and 4.4 mg DON/kg diet (Manley
17
18 240 | *et al.*, 1988), 1.3%, corresponding to 4.4 mg DON/kg diet (Manley *et al.*, 1988) and just
19
20 241 | 3.1% at a dietary DON concentration of 20 mg/kg (Morris *et al.*, 1999). Thus, the impact of
21
22 242 | DON-contaminated diets on turkey growth can not conclusively be evaluated, mainly due to
23
24 243 | the different study conditions.

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25
26 244 | An interesting observation of the present study was a decrease in the effect of the NSP
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28 245 | enzyme supplementation in lowering intestinal viscosity with increasing inclusion rates of the
29
30 246 | CW. This effect is probably due to the physico-chemical alterations of the CW, caused by the
31
32 247 | fungal invasion, compared to the non-infected control wheat. These changes include an
33
34 248 | increase in the activities of protease, amylase and cell wall degrading enzymes (Matthäus *et*
35
36 249 | *al.*, 2004). Moreover, the water extract viscosity of the infected wheat was lower than that of
37
38 250 | the control wheat, despite an increase in soluble NSP. The latter relationship was surprising as
39
40 251 | former experiments on the variability of wheat NSP revealed a positive correlation between
41
42 252 | soluble NSP and water extract viscosity on the one hand and between water extract viscosity
43
44 253 | and intestinal viscosity on the other hand (*e.g.*, Bedford and Classen, 1992; Dusel *et al.*,
45
46 254 | 1997).

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47
48 255 | To test the hypothesis that NSP enzymes generated by the fungus invasion could act in
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50 256 | a similar manner to supplemented NSP enzymes, Dänicke *et al.* (2004a) tested the effects of
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52 257 | feeding either an uninfected control wheat or CW in absence and presence of an added NSP
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2 258 enzyme on performance and intestinal viscosity of Pekin ducks. They found that
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4 259 supplementation of the diet with the NSP enzyme was not necessary when CW was in the diet
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6 260 and intestinal viscosity was considered as response criterion. Thus, the results of the present
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8 261 experiment support this hypothesis as an increase in the dietary proportion of CW can be
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10 262 directly related to an increase in cell wall degrading activities in the diet, and leaves less
11
12 263 room, or even no room, for the action of an added NSP enzyme preparation. A high intestinal
13
14 264 viscosity in growing birds is generally considered as anti-nutritive and is inversely related to
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16 265 performance (for reviews see Bedford and Schulze1998; Dänicke *et al.*, 1999).

17
18 266 | Whether the net effect of the viscosity-lowering effects of *Fusarium* infected wheat is
19
20 267 | positive with regard to performance depends on the potential negative effects of the
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22 268 | concomitantly present DON. The treatment-related effects on the observed intestinal viscosity
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24 269 | were only partly reflected by performance. This might be due to a less pronounced
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26 270 | relationship between intestinal viscosity and performance in turkeys, or to a more pronounced
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28 271 | susceptibility to DON when compared with broilers. Deleted: to

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30 272 | Another typical effect of the presence of NSP-degrading enzyme activities in the diets
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32 273 | - either provided by the fungus or as feed additive - was the decrease in the relative weight of
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34 274 | the small intestine (Simon1998; Dänicke *et al.*, 2000b). It was shown that an increased
35
36 275 | intestinal viscosity increases the mass of small intestinal tissue through stimulated protein
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38 276 | synthesis (Dänicke *et al.*, 2000a). A close relationship between intestinal viscosity and the
39
40 277 | relative weight of the small intestine also becomes obvious from the present experiment. Also
41
42 278 | notable is the decrease in heart weight after NSP enzyme supplementation which, however,
43
44 279 | appeared to be independent of the CW proportion of the diets. Whether this decrease is a
45
46 280 | reflection of a lower blood requirement of the reduced weight of the small intestine can not be
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48 281 | answered conclusively.

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50 282 | Feeding of the diet containing the highest CW proportion resulted in a significant
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52 283 | increase in the serum activity of the glutamate dehydrogenase by approximately 1 U/L when Deleted: L

1
2 284 compared with the control groups. This increase would suggest a DON-related enhancement
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4 285 of hepatocyte necrosis, whereby larger amounts of the enzyme are released into circulation.
5
6 286 Literature findings on DON hepatotoxicity in poultry do not provide an integrative picture.
7
8 287 For example, Dänicke *et al.* (2003) failed to detect any clinical-chemical signs of
9
10 288 hepatocellular damage in broilers fed diets containing up to 14 mg DON/kg diet for 5 weeks,
11
12 289 whereas Kubena *et al.* (1987) found γ -glutamyltransferase to be increased in rearing female
13
14 290 chickens fed on a diet containing 18 mg DON/kg over a period of 168 d. In ducks, this
15
16 291 enzyme was significantly decreased when the diet contained 30% CW, but increased to the
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18 292 level of the control group when the diet contained 60% CW (Dänicke *et al.*, 2004b). Besides
19
20 293 the fact that different poultry species might respond differently and more dependent on
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22 294 exposure it needs to be stressed that a general evaluation of such enzyme activities is difficult
23
24 295 because of the lack of respective reference values. Thus, significant treatment differences do
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26 296 not necessarily mean a general liver health problem.

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28 297 As edible tissues, liver and breast meat were virtually free of DON, ZON and their
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30 298 metabolites considering the indicated limits of detection of the applied HPLC methods. Thus,
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32 299 the general conclusions drawn by EFSA (2004a,b) concerning the negligible carry over of
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34 300 DON and ZON also apply for turkeys, which were not considered. The only physiological
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36 301 specimen with detectable mycotoxin residues was the bile. The observation that especially
37
38 302 ZON and its metabolites accumulate in bile agrees with our earlier findings in laying hens,
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40 303 broilers and ducks (Dänicke *et al.*, 2004b; 2002; 2001b). Comparatively, the turkey seems to
41
42 304 differ from laying hens and ducks in its ZON-metabolising pattern as the mean proportions of
43
44 305 ZON, α -ZOL and β -ZOL amounted to 19, 77 and 4%, respectively, whereas the respective
45
46 306 figures in laying hens were 75, 15 and 10 %, and in ducks 80, 16 and 4%. Therefore, the
47
48 307 turkey seems to have a higher capacity to metabolise dietary ZON than other poultry species.
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50 308 This conclusion is supported by the results of Olsen *et al.* (1986) who found the proportion of
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52 309 α -ZOL of the sum of α -ZOL + ZON in liver to be approximately 91% in male turkeys

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2 310 exposed to a diet containing 800 mg ZON/kg for two weeks. Only trace amounts of β -ZOL
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4 311 were detected in this study. From the data presented by Olsen *et al.* (1986) a carry-over factor
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6 312 of approximately 0.004 can be estimated as the ratio between the concentration of ZON plus
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8 313 α -ZOL in liver and ZON concentration in the diet. Applying this factor to the highest ZON
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10 314 concentration of the present experiment of 0.044 mg ZON/kg diet would mean an expected
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12 315 liver concentration of approximately 0.16 ng ZON plus α -ZOL/g, which is below the
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14 316 detection limit of our HPLC-method. Therefore, when practical ZON concentrations are fed
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16 317 to turkeys, the expected trace concentrations of ZON residues are negligible from a consumer
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18 318 protection point of view.

19
20 319 Entero-hepatic cycling of ZON and its metabolites is another interesting feature of
21
22 320 ZON metabolism. In this regard it differs markedly from DON. The ratio between metabolite
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24 321 concentrations in bile and diet can be used as an indicator of the extent of the importance of
25
26 322 the entero-hepatic cycling. The mean ratio for ZON amounted to 3.2, whereas that of DON
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28 323 was just 0.003 in the present experiment. The respective ratios for gilts were reported to be
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30 324 4.0 and 0.03 (Dänicke *et al.*, 2005). Whether the obviously more intensive toxin cycling in
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32 325 pigs contributes to their higher susceptibility to DON and ZON as compared to poultry can
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34 326 not be finally evaluated.

35 327 **Conclusions**

36 328 A stepwise increase in dietary DON concentration up to 5 mg/kg resulted in a dose-related but
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38 329 transitory decrease in live weight gain. More dose-response studies covering longer fattening
39
40 330 periods are necessary to establish critical dietary DON concentrations for turkeys. The carry-
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42 331 over of DON and ZON into edible tissues can be considered as negligible from a consumer
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44 332 protection point of view.

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47
48 335 located in Celle, and at the Institute of Animal Nutrition of the Federal German Agricultural

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Table 1. *Composition of the diets (g/kg)*

	Group							
	1[-]	1[+]	2[-]	2[+]	3[-]	3[+]	4[-]	4[+]
<u>Components:</u>								
Wheat	510	511.8	340	341.8	170	170	-	-
Contaminated wheat	-	-	170	170	340	341.8	510	511.8
Soybean oil	14	14	14	14	14	14	14	14
Soybean meal	313.6	311.6	313.6	311.6	313.6	311.6	313.6	311.6
Soybean protein	100	100	100	100	100	100	100	100
Dicalcium phosphate	26.6	26.6	26.6	26.6	26.6	26.6	26.6	26.6
Calcium carbonate	11.9	11.9	11.9	11.9	11.9	11.9	11.9	11.9
Sodium chloride	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
L-lysine-HCL	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
DL-methionine	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
L-threonine	1	1	1	1	1	1	1	1
Enzyme preparation ZY68 ¹	-	0.2	-	0.2	-	0.2	-	0.2
Premix ²	10	10	10	10	10	10	10	10
<u>Calculated composition:</u>								
Crude protein	250	250	252	252	253	253	255	255
AME _N (MJ/kg)	11.49	11.49	11.49	11.49	11.49	11.49	11.49	11.49
Lysine	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2
Methionine + cystine	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6
Methionine	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
Threonine	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6
Calcium	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
Total phosphorus	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2
Sodium	2	2	2	2	2	2	2	2
DON (mg/kg)	0.10	0.10	1.39	1.39	2.68	2.68	3.97	3.97
ZON (µg/kg)	1	1	18	18	34	34	51	51
<u>Analysed composition:</u>								
Dry matter	905	908	909	901	898	901	905	903
Crude protein	247	250	254	258	257	264	263	266
DON (mg/kg)	0.10±0.03	0.11±0.03	2.09±0.47	1.96±0.61	4.66±0.29	4.59±0.33	5.42±0.09	5.17±0.30
ZON (µg/kg)	16	23	39	30	42	36	44	41
Endo-1,4-β-Xylanase (FXU/kg)		240		230		270		280

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¹ZY68, Lohmann Animal Health GmbH, KG, Cuxhaven, Germany; declared activity: endo-1,4-β-xylanase (EC 3.2.1.8) 1000 FXU/g.

²Premix provided per kg of diet: 4.8 mg retinol, 100 µg cholecalciferol, 40 mg dl-α-tocopheryl acetate, 2.3 mg thiamin, 9.5 mg riboflavin, 20 mg calcium-D-pantothenate, 50 mg nicotinic acid, 7.5 mg pyridoxin-hydrochloride, 1.7 mg folic acid, 35 µg cyanocobalamin, 4 mg menadione, 200 µg biotin, 650 mg choline chloride, 80 mg Zn, 50 mg Fe, 120 mg Mn, 25 mg Cu, 0.55 mg Co, 1.2 mg J, 0.25 mg Se, 100 mg butylhydroxytoluol.

Table 2. *Effects of Fusarium toxin contaminated wheat proportion and non-starch-polysaccharide hydrolysing enzyme addition on performance of BUT Big 6 males*

Group	Contaminated wheat (CW, % of total wheat)	Enzyme (ZY68, g/kg)	Live weight gain (n=8, g/d)			Feed intake (n=8, g/d)			Feed-to-gain ratio (n=8, g/g)		
			d 21-35	d 36-56	d 21-56	d 21-35	d 36-56	d 21-56	d 21-35	d 36-56	d 21-56
1[-]	0	-	68.1	126.1	102.9	107.3	224.1	177.4	1.582	1.774	1.723
1[+]	0	0.2	67.7	124.7	101.9	104.2	219.2	173.2	1.539	1.760	1.701
2[-]	33	-	65.4	122.6	99.7	104.5	218.7	173.0	1.597	1.783	1.734
2[+]	33	0.2	69.3	123.5	101.9	108.3	223.3	177.3	1.558	1.808	1.740
3[-]	66	-	66.4	124.5	101.2	106.5	224.8	177.5	1.609	1.811	1.758
3[+]	66	0.2	67.2	125.6	102.2	105.0	225.0	177.0	1.569	1.796	1.736
4[-]	100	-	65.0	121.4	98.8	102.8	216.3	170.9	1.584	1.784	1.731
4[+]	100	0.2	64.8	120.9	98.5	104.6	215.4	171.1	1.618	1.782	1.738
<u>ANOVA (probabilities)</u>											
CW			0.185	0.275	0.190	0.668	0.184	0.262	0.136	0.172	0.106
Linear			0.040	0.136	0.064	0.355	0.305	0.290	0.020	0.313	0.089
Quadratic			0.452	0.684	0.569	0.407	0.152	0.179	0.842	0.047	0.071
ZY68			0.300	0.997	0.735	0.870	0.943	0.994	0.085	0.907	0.454
CW x ZY68			0.422	0.927	0.823	0.421	0.714	0.620	0.098	0.601	0.554
PSEM			1.5	2.4	1.8	2.3	4.1	3.2	0.018	0.017	0.014

Table 3. Organ weights of male BUT big 6 males as influenced by *Fusarium toxin* contaminated wheat proportion and non-starch-polysaccharide hydrolysing enzyme addition (g/kg body weight, n=12)

Group	Contaminated wheat (CW, % of total wheat)	Enzyme (ZY68, g/kg)	Jejunum plus ileum	Spleen	<i>Bursa fabricii</i>	Liver	Heart
1[-]	0	-	18.4	1.2	1.2	16.5	4.2
1[+]	0	0.2	17.5	1.0	1.2	15.5	3.9
2[-]	33	-	16.2	1.0	1.2	15.8	4.2
2[+]	33	0.2	15.4	1.0	1.1	14.9	3.7
3[-]	66	-	16.3	1.1	1.2	14.5	4.1
3[+]	66	0.2	15.3	1.0	1.1	16.3	3.9
4[-]	100	-	16.7	1.0	1.2	15.2	4.2
4[+]	100	0.2	15.5	1.1	1.2	14.6	4.0
<u>ANOVA (probabilities)</u>							
CW			<0.001	0.647	0.998	0.086	0.622
Linear			0.002	0.804	0.882	0.020	0.566
Quadratic			0.003	0.592	0.945	0.773	0.234
ZY68			0.017	0.183	0.440	0.564	<0.001
CW x ZY68			0.575	0.209	0.659	0.005	0.455
PSEM			0.6	0.1	0.1	0.4	0.1

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466 **Table 4.** *Effects of Fusarium toxin-contaminated wheat proportion and non-starch-*
 467 *polysaccharide hydrolysing enzyme addition on serum activities of the γ -glutamyltransferase*
 468 *and glutamate dehydrogenase of BUT big 6 males (d 56, n=12)*

Group	Contaminated wheat (CW, % of total wheat)	Enzyme (ZY68, g/kg)	γ -glutamyltransferase (U/L)	Glutamate dehydrogenase (U/L)
1[-]	0	-	2.3	5.8
1[+]	0	0.2	2.8	5.9
4[-]	100	-	2.2	6.9
4[+]	100	0.2	2.2	6.8
<u>ANOVA (probabilities)</u>				
CW			0.092	0.014
ZY68			0.229	0.988
CW x ZY68			0.331	0.883
PSEM			0.2	0.4

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Table 5. *Effects of Fusarium toxin-contaminated wheat proportion and non-starch-polysaccharide hydrolysing enzyme addition on the concentration of zearalenone (ZON) and its metabolites β -zearalenol (β -ZOL) and α -ZOL in bile of BUT big 6 males (day 56, n=6)*

Group	Contaminated wheat (CW, % of total wheat)	Enzyme (ZY68, g/kg)	Concentration (ng/g)				Metabolite profile (% of the sum of β -ZOL+ α -ZOL+ZON)		
			β -ZOL	α -ZOL	ZON	β -ZOL+ α -ZOL+ZON	β -ZOL	α -ZOL	ZON
1[-]	0	-	3.0	20.4	6.3	29.7	4.1	83.1	12.8
1[+]	0	0.2	4.7	37.7	10.5	53.0	3.9	78.8	17.2
2[-]	33	-	7.0	104.6	26.7	138.3	4.5	76.4	19.1
2[+]	33	0.2	9.9	122.4	28.1	160.3	5.9	76.1	18.0
3[-]	66	-	3.5	128.5	31.1	163.1	2.0	79.1	18.9
3[+]	66	0.2	2.1	82.4	24.1	108.5	1.6	76.0	22.3
4[-]	100	-	4.9	97.1	29.0	131.0	3.6	74.4	22.0
4[+]	100	0.2	3.0	91.4	29.2	123.6	1.4	75.1	23.5
<u>ANOVA (probabilities)</u>									
CW			0.097	<0.001	<0.001	<0.001	0.276	0.376	0.007
Linear			0.535	<0.001	<0.001	<0.001	0.239	0.136	0.001
Quadratic			0.347	<0.001	0.003	<0.001	0.848	0.693	0.630
ZY68			0.867	0.643	0.919	0.744	0.815	0.482	0.161
CW x ZY68			0.720	0.036	0.507	0.091	0.826	0.881	0.505
PSEM			2.4	11.6	3.6	16.5	1.8	3.2	1.9

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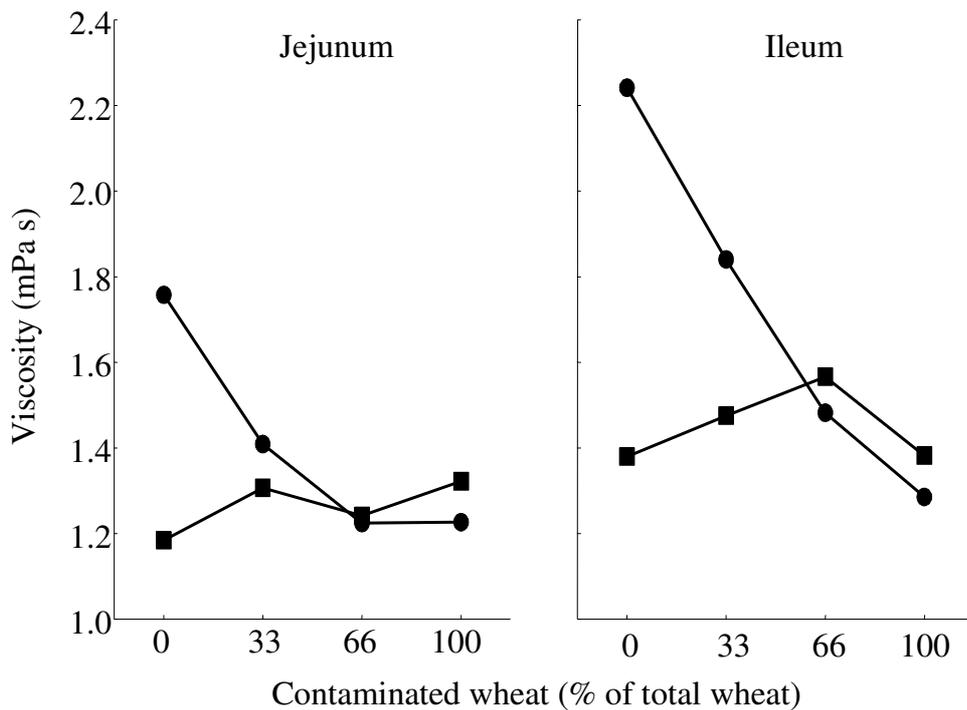


Figure 1. *Effects of Fusarium toxin-contaminated wheat proportion (CW) and non-starch-polysaccharide hydrolysing enzyme addition (ZY68) on the viscosity of the supernatants of small intestinal ingesta of BUT big 6 males (day 56, n=3, ANOVA probabilities, jejunum: CW=0.005, ZY68=0.004, CW x ZY68<0.001; ileum: CW=0.001, ZY68=0.001, CW x ZY68<0.001).*

- without enzyme
- 0.2 g ZY68/kg diet

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