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Ute Tiemann, Sven Dänicke

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In vivo and in vitro effects of the mycotoxins zearalenone and deoxynivalenol on different non-reproductive and reproductive organs in female pigs

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3 1 ***In vivo* and *in vitro* effects of the mycotoxins zearalenone and**
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6 2 **deoxynivalenol on different non-reproductive and reproductive organs in**
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9 3 **female pigs – a review**
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14 5 U. Tiemann ^{A1} and S. Dänicke ^{A2}
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19 ^{A1} Research Institute for the Biology of Farm Animals, Dummerstorf, Germany
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22 ^{A2} Federal Agriculture Research Center (FAL), Braunschweig, Germany
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26
27 10 **Abstract**
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30 11 This review summarizes the toxicological data on the effects of the mycotoxins zearalenone
31 12 (ZON), its metabolites, and deoxynivalenol (DON) on different parameters relating to
32 13 reproductive and non-reproductive organs in female pigs. *In vivo* 22 mg ZON kg⁻¹ in the diet
33 14 cause alterations in the reproductive tract of swine such as in the uterus, on the follicular and
34 15 embryo development. ZON and its metabolites have been shown to competitively bind to
35 16 estrogen receptors in an *in vitro* system. The feeding of pigs with 9 mg DON kg⁻¹ diet
36 17 contaminated diet can act on protein synthesis, humoral and cellular immune response
37 18 depending on dose, exposure and timing of functional immune assay, and on liver and spleen
38 19 cell structures. Beside these effects, reproductive alterations were observed in pigs, too. Both
39 20 *in vivo* and *in vitro* exposure to DON decreased the oocyte and embryo development. *In vitro*
40 21 application of DON to uterine cells inhibits their proliferation rate and modulates the process
41 22 of translation at a different molecular level when compared with the *in vivo* application. The
42 23 histopathological results provide evidence of spleen and liver dysfunction in the absence of
43 24 clinical signs, especially in pigs fed higher concentrations of *Fusarium* toxin-contaminated
44 25 wheat. Prepuberal gilts react more sensitively to DON>ZON feeding compared to pregnant
45 26 sows. In the liver, histopathological changes such as glycogen decrease and interlobular
46 27 collagen uptake were observed only observed in prepuberal gilts, whereas enhancement of
47 28 haemosiderin was found in both prepuberal gilts and pregnant sows. This review presents
48 29 some of the current knowledge on the biological activities of ZON and DON in pig.
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3 1 Altogether, ZON affects reproduction of pigs most seriously because it possesses estrogenic
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5 2 activity. Whereas DON affects reproduction in pigs via indirect effects such as reduced feed
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7 3 intake, resulting in reduced growth or impairment of function in vital organs like liver and
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9 4 spleen.
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13 6 **Keywords:** *Fusarium* toxins, zearalenone, deoxynivalenol, reproductive and non-reproductive
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15 7 organs, female pig
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20 9 * Corresponding author: Dr. Ute Tiemann, Research Institute for the Biology of Farm
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22 10 Animals, Wilhelm Stahl-Allee 2, 18196 Dummerstorf, Tel: (49)38208 68 766; Fax:
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25 11 (49)38208 68 752; E-mail: tiemann@fbn-dummerstorf.de
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1 Introduction

2 Mycotoxins are biologically active secondary fungal metabolites found as contaminants of
3 feedstuffs and exert toxic effects in animals and human beings (Fink-Gremmels, 1999).
4 *Fusarium* toxins, such as deoxynivalenol (DON) and zearalenone (ZON), contaminate wheat,
5 maize, and barley worldwide (Abouzieed et al. 1991; Chelkowski, 1998; EFSA, 2004a,b).
6 These mycotoxins are produced mainly by *F. graminearum*, *F. culmorum*, and *F. roseum*
7 under predisposing environmental conditions during cereal flowering and include humidity
8 and fungus specific optimum temperature range (Oldenburg et al., 2000). *Fusarium* species
9 are probably the most prevalent toxin-producing fungi of the northern temperate regions and
10 are commonly found in cereals grown in America, Europe and Asia. They cause a variety of
11 toxic effects in experimental animals and livestock. Different effects were described for
12 *Fusarium* toxins ZON (Kuiper-Goodman et al. 1987; Wood, 1992) and DON (reviewed by
13 Pestka and Casale, 1990; Rotter et al. 1996).
14
15 Zearalenone is biologically potent, but hardly toxic; rather, it sufficiently resembles 17 β -
16 estradiol, the principal hormone produced by the human ovary, to allow it to bind to estrogen
17 receptors in mammalian target cells (Katzenellenbogen et al. 1979). ZON is better classified as
18 a non-steroidal estrogen or mycoestrogen. Metabolism of ZON in monogastric animals takes
19 place in the liver and intestinal mucosa where it is reduced to α - and β -zearalenol (α -ZOL and
20 β -ZOL) (Olsen et al., 1987; Olsen, 1989), a reaction that competes with glucuronidation of the
21 parent molecule. Examination of excretory products indicated that in pig the α -epimer
22 predominates (Farnworth and Trendholm, 1983; Olsen et al., 1985a,b; Biehl et al., 1993;
23 Zöllner et al., 2002; Döll et al., 2003; Dänicke et al., 2005b). Biotransformation studies with
24 pig liver sub-cellular fractions indicated that α -ZOL is the main hepatic metabolic of ZON in
25 pigs (Malekinejad et al. 2005). Additionally, the authors could detect that another extrahepatic

1 biotransformation of ZON into α -ZOL exists in porcine granulosa cells (Malekinejad et al.
2 2006). Alpha-ZOL binds more effectively to the receptor than the parent toxin (ZON) and
3 might therefore explain the sensitivity of pigs to this mycotoxin.

4
5 The effect of ZON and its metabolites depends upon the reproductive status (pre-puberal,
6 cycling or pregnant) of the affected animal (Lopez et al. 1988; Diekman and Green, 1992).
7 Pigs, especially gilts before the first oestrus, are the most susceptible to intoxication with
8 higher ZON doses (50-100 mg kg⁻¹) and they can develop symptoms such as vulvae oedema
9 and erythema, impairment of ovulation, conception, implantation, fetus development, and
10 newborn's viability (Mirocha et al. 1977; Weaver et al. 1978; Chang et al. 1979; Long and
11 Diekman, 1986; Price et al. 1993; Dacasto et al. 1995; Yang et al. 1995; Gajecki, 2002). The
12 amount of 22.09 mg kg⁻¹ of ZON in the ration of breeding gilts had an obvious harmful effect
13 on their reproductive performance: a decreased number of corpora lutea, a decreased weight
14 of ovaries, a decreased number of live embryos, an increased number of dead-born piglets,
15 and the occurrence of abortions. These effects were less pronounced in gilts fed mashes
16 containing 2.2 mg kg⁻¹ ZON (Kordic et al. 1992). New guidance values recommended not to
17 exceed 0.1 mg ZON kg⁻¹ for piglets and gilts, 0.25 mg ZON kg⁻¹ for sows and fattening pigs,
18 and 0.9 mg ZON kg⁻¹ diet for pigs in order to avoid adverse effects on reproductive traits (EU,
19 2006). EFSA (2004b) concluded from a recent literature evaluation that safe levels of ZON in
20 feed for pigs can not reliably established at present.

21
22 Deoxynivalenol, a *Fusarium* toxin belonging to the trichothecene group, has been reported to
23 produce a variety of adverse health effects in farm animals, such as inhibition of protein
24 synthesis, reduction of feed intake, and alteration of the immune system. The upper critical
25 concentrations in Germany was set at 0.9 mg DON kg⁻¹ diet for all pigs, as a guideline level in

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3 1 prevention or minimizing strategies (EU, 2006). EFSA (2004a) recommended the collection
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6 2 of more data for refining or establishment of safe dietary DON levels, including the effects of
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8 3 DON on the immune system.

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13 5 Macrophages, T cells, and B cells of the immune system are central targets of DON that can
14
15 6 be immunostimulatory or immunosuppressive depending on dose, exposure and timing of
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17 7 functional immune assay (Rotter et al., 1996; Bondy and Pestka, 2000; Pestka et al. 2004).
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19 8 The presence of DON in pig diets decreases feed intake, causes feed refusal, and induces
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21 9 occasional vomiting (Diekman and Green, 1992). Beside these effects, reproductive
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23 10 alterations were observed in humans and pigs (Hussein and Brasel, 2001; Alm et al. 2006).
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30 12 Effects of a *Fusarium* toxin contaminated diet containing DON and/or ZON on the
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32 13 fertility/reproduction of female pigs need to be viewed in the general context of the toxin
33
34 14 effects on porcine health and performance. Before the toxins are able to modify metabolic
35
36 15 processes they need to be consumed voluntarily by the pig. Data reported in the literature
37
38 16 indicate that the adverse effects of DON contaminated diets on growth performance of
39
40 17 growing pigs is primarily caused by depressing the voluntary feed intake (Rotter et al., 1994;
41
42 18 Swamy et al., 2002; Goyarts et al., 2005). The mycotoxins obviously act in a concerted
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44 19 manner with regard to metabolic processes and consequently influence the health and
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46 20 fertility/reproduction (Figure 1).
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54 22 This review aims to give an overview about the effects of feeding with DON>ZON
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56 23 contaminated diet on non-reproductive and reproductive organs which can influence
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58 24 metabolism, health, and fertility/reproduction of female pigs. Figure 1 demonstrates a scheme
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60 25 of the investigations on specific organs and parameters dealt with in the current study.

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3 1 Furthermore, we have focused on the comparison of *in vivo* and *in vitro* effects of DON and
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6 2 ZON on different parameters.
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10 4 **Protein synthesis**

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13 5 Deoxynivalenol is known to be a potent protein synthesis inhibitor according to research in
14
15 6 different *in vitro* and *in vivo* systems (Feinberg and McLaughlin, 1989). However, the
16
17 7 significance of this toxic feature was not yet evaluated for the pig. Therefore, an experiment
18
19 8 was carried out by Dänicke et al. (2006) to measure porcine tissue protein synthesis
20
21 9 employing the so-called flooding dose technique using [²H₅]-phenylalanine as a tracer in
22
23 10 dependence on different exposition of DON to pigs exposed to a DON-contaminated diet for
24
25 11 ~ 4 wks (chronic) or just uniquely (acute oral) to reflect different feeding scenarios. Moreover,
26
27 12 an additional group of pigs was injected with a single intravenous DON-dose (acute
28
29 13 intravenous) representing a complete systemic DON-availability. Protein synthesis expressed
30
31 14 as fractional synthesis rate (FSR) was significantly reduced in kidneys, spleen and ileum of
32
33 15 DON-exposed pigs. FSR of liver, skeletal and heart muscle, mesenteric lymph nodes,
34
35 16 duodenum, jejunum, jejunal mucosa cells, pancreas and lung were not affected by DON.
36
37 17 Goyarts et al. (2006a) have shown that DON decreased the FSR of albumin as the main
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39 18 protein exported from the liver and as the predominant plasma protein. Pigs exposed to DON
40
41 19 5.7 mg kg⁻¹ diet showed an increased protein synthesis capacity (RNA to protein ratio) caused
42
43 20 by an elevated liver RNA-concentration (Dänicke et al. 2006) .
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54 22 **Humoral and cellular immune response**

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56 23 DON affects the humoral immunity (Bondy and Pestka, 2000). An overview on the
57
58 24 mechanisms of IgA production induced by DON is given by Pestka (2003). The effects of
59
60 25 DON on immunoglobulin levels of serum in swine are rather inconsistent. After feeding of

1
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3 1 grains naturally contaminated with DON>ZON for 5 wk the serum IgA concentration of
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5
6 2 prepuberal pig increased significantly, while serum IgM and IgG concentrations were not
7
8 3 altered (Goyarts et al. 2006b; Tiemann et al. 2006a). Similarly, Swamy et al. (2002) observed
9
10 4 such effects in pigs fed with DON contaminated corn and wheat. Drocher et al. (2004)
11
12 5 demonstrated an IgA elevation in pigs fed a semisynthetic diet without grain components with
13
14 6 pure DON (600 $\mu\text{g kg}^{-1}$) under highly standardized conditions. In contrast, some investigators
15
16 7 were not able to show interaction between DON and IgA serum concentrations (Bergsjø et al.
17
18 8 1992, 1993; Döll et al. 2003; Swamy et al. 2003; Dänicke et al. 2004a,b). No differences were
19
20 9 observed in serum level of IgA between the pregnant sows fed 9.6 mg DON/0.358 mg ZON
21
22 10 kg^{-1} diet and the controls for 35 days (Tiemann unpublished data). Table 1 summarized the
23
24 11 data and it can be notable that the *in vivo* effects of DON on humoral immunity are rather
25
26 12 inconsistent. The reason may be methodological differences in the experiments carried out by
27
28 13 several investigators. *In vitro*, the IgA concentration in the supernatant of ConA stimulated
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30 14 porcine peripheral lymphocytes was dose-dependently inhibited with increasing DON
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32 15 concentrations (Goyarts et al. 2006b).
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42 17 Mitogen-induced proliferation is a common technique to assess immunotoxicity. The
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44 18 immunosuppressive effect of DON is primarily associated with cellular immune response.
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46 19 Øvernes et al. (1997) observed a significant higher response to phytohemagglutinin in the high
47
48 20 DON group compared to the low DON group in growing pigs. In contrast, no effect of DON-
49
50 21 contaminated feed on peripheral lymphocyte mitogen response was reported by Rotter et al.
51
52 22 (1994); Tiemann et al. (2006a, unpublished data). Compared to bloods lymphocytes,
53
54 23 splenocytes were more sensitive to ConA-induced response in perpuberal pigs fed the DON
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56 24 contaminated diet compared to pregnant sows. The DON>ZON feeding of pregnant sows
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1 reduced the proliferative response in splenocytes in approximately the same manner, as
2 observed in prepuberal gilts (Tiemann et al. 2006a, unpublished data).

3
4 Mitogen-induced proliferation was impaired at low concentrations after *in vitro* exposure to
5 porcine lymphocytes or splenocytes (Goyarts et al. 2006b; Tiemann et al. 2006a). Both
6 authors found a reduced proliferative response which may be due to the capacity of DON to
7 inhibit protein synthesis (Ueno, 1983; Thompson and Wannemacher, 1986; Ehrlich and
8 Daigle, 1987). The latter assumption is supported by the findings of Goyarts et al. (2006a)
9 who measured the overall protein synthesis of porcine peripheral blood lymphocytes *in vivo*
10 by using a stable isotope labelled amino acid. Other toxic mechanisms that have been
11 associated with trichothecenes include impaired membrane function (Bunner and Morris,
12 1988), altered cellular communication (Jones et al. 1987), deregulation of calcium homeostasis
13 (Yoshino et al. 1996), and such as described above.

14
15 The capacity of DON to affect porcine neutrophils, critical mediators between innate and
16 acquired immunity (Ellis and Beaman, 2004), at pharmacological concentrations was
17 investigated by Takayama et al. (2005).

18 19 **Histopathology of spleen and liver**

20 After 5 weeks of feeding, the histopathological effects on spleens and livers in gilts (Tiemann
21 et al. 2006a,b) and pregnant sows (unpublished data) can be mostly observed at a dietary DON
22 concentration of 9.57 mg kg⁻¹, originating from naturally contaminated wheat containing only
23 minor traces of ZON (0.358 mg kg⁻¹ diet), but in absence of clinical signs (Dänicke et al.
24 2005a, unpublished data). The authors observed the appearance of a dysfunction in porcine
25 liver and spleen which can be seen as hemosiderosis. Pre-puberal gilts react more sensitively

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3 1 to DON>ZON feeding. In the liver, histopathological changes such as glycogen decrease and
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5 2 interlobular collagen uptake were observed only in prepuberal gilts. In both animals an
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7 3 enhancement of haemosiderin in liver and spleen was found. These results were supported by
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9 4 ultrastructural investigations and can be due to a dysfunction in both spleen and liver cell
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11 5 circulation. Because there was a statistically significant, but only a modest elevation of the
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13 6 liver enzyme activities for aspartate aminotransferase (AST), alanine aminotransferase (ALT),
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15 7 the authors suggest that the doses of mycotoxins used did not cause hepatic fibrosis. The
16
17 8 observed histological alterations in liver and spleen, however, were not clinically manifested.
18
19 9 There were no adverse effects on liver and spleen of piglets when their mothers consumed
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21 10 diets containing up to 9,570 and 358 μg DON/ZON kg^{-1} diet (unpublished data).
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30 12 **Follicle development, oocyte maturation, and embryo development**

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32 13 Edwards et al. (1987) reported that pre-pubertal gilts fed zearalenone displayed first estrus
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34 14 later than controls; however, in other investigations, pre-pubertal gilts consuming a milo diet
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36 15 contaminated with zearalenone exhibited puberty at a younger age without an alteration in
37
38 16 conception rates, ovulation rates, or embryonic survival (Rainey et al., 1990).
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41 17 Zwierzchowski et al. (2005) observed that the amount of ZON to a dose of 200 μg kg^{-1} body
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43 18 weight in immature gilts leads to distribution in the development and maturation of the largest
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45 19 follicles through the activation of an apoptotic-like process in the granulosa layer of single
46
47 20 mature follicles. In contrast, Alm et al. (2006) found that the size distribution of follicles was
48
49 21 not affected by feeding of gilts with different *Fusarium*-toxin contaminated diets. In these
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51 22 animals the cumulus cell morphology was not changed by increasing dietary concentrations of
52
53 23 both toxins up to 9,570 and 0,358 mg kg^{-1} for DON and ZON, respectively. The authors
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55 24 recovered oocytes from pig ovaries by follicle aspiration after ovario-hysterectomy. At the
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57 25 concentration named above, at the time of recovery, oocytes with compact cumuli showed a
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1 significantly reduced proportion with immature chromatin. Here, the proportion of oocytes
2 having degenerated meiotic chromatin was significantly higher compared to lower
3 concentrations. The oocyte quality was significantly reduced by feeding of a DON>ZON
4 contaminated diet to gilts. The competence of oocytes in compact cumulus oocyte complexes
5 for *in vitro* maturation was likewise significantly reduced by high concentrations of *Fusarium*
6 toxin in feed; this is probably directly related to the reduced proportion of normal germinal
7 vesicle-stage oocytes, i.e., oocytes capable of maturation, in the high toxin groups. These
8 results are in agreement with data reported by Kordic et al. (1992) who observed a doses-
9 dependent effect of ZON on reproductive performance in breeding gilts, with decreased
10 numbers of live embryos as well as other reproductive problems including increased number
11 of dead-born piglets. Little information is available about effects of indirect (i.e., from mother
12 to young) exposure to DON/ZON on the health of piglets. Placenta transfer of mycotoxins can
13 indicate a potential risk for direct effects in the fetus. Long and Diekman (1984) reported that
14 gilts fed 5 to 30 mg kg⁻¹ ZON from d 2 to 15 postmating had normal embryonic development,
15 but no fetuses were present in gilts fed 60 or 90 mg kg⁻¹ ZON when killed d 40 to 43 post-
16 mating. Embryos recovered on d 14 from gilts fed 60 mg kg⁻¹ of ZON from d 7 to 10 post
17 breeding were fragmentous, while those from control gilts were filamentous (Diekman and
18 Long, 1989). Degeneration in blastocysts, affecting both embryonic membranes and the
19 embryonic disk occurring in ZON-treated sows were observed by Long et al. (1992). In
20 contrast, the authors observed no deleterious effects of DON on fetal development when feed
21 containing 8 mg kg⁻¹ of purified DON was consumed (summarized Diekman and Green,
22 1992).

23
24 *In vitro*, the maturation of porcine oocytes was investigated after addition of α - and β -ZOL
25 and DON. All three substances negatively affected oocyte maturation and degeneration rates

1 in a dose-dependent manner, but to different extents (DON> α -ZOL> β -ZOL). In addition, α -
2 ZOL directly affected the early embryonic development *in vitro* of *in vivo*-derived porcine
3 embryos (Alm et al. 2002).

5 **Intrafollicular steroid synthesis**

6 The feeding of wheat contaminated naturally with DON and ZON to gilts at increasing
7 proportions did not influenced the activity of enzymes involved in progesterone synthesis. *In*
8 *vivo*-derived porcine granulosa cells were analyzed for the mRNA expression of the
9 mitochondrial enzyme cytochrome P450, cholesterol side-chain cleavage (P450_{scc}) and
10 microsomal enzyme 3 β -hydroxysteroid dehydrogenase/isomerase (3 β -HSD) by RT-PCR, and
11 additionally for P450_{scc} protein by Western blotting. Neither the expression of the P450_{scc}
12 nor of the 3 β -HSD mRNA, nor the abundance of the P450_{scc} protein was significantly
13 influenced by the mycotoxin feeding (Alm et al. 2006).

14
15 Cultured primary porcine granulosa cells have been used to test the toxic potential of
16 xenobiotics on reproduction (Haney et al. 1984). *In vitro* addition of α - and β -ZOL at
17 concentrations of 15 and 30 $\mu\text{mol l}^{-1}$ inhibited the FSH-stimulated progesterone synthesis in
18 porcine granulosa cells. The inhibitory effect at a concentration of 30 $\mu\text{mol l}^{-1}$ of both
19 mycotoxins could mainly be due to cell death. The antisteriodogenic effect of 15 $\mu\text{mol l}^{-1}$ α -
20 ZOL or β -ZOL was not due to cytotoxic effects. The enzyme activity of 3 β -HSD and the
21 abundance of P450_{scc} protein were reduced by both mycotoxins as well (Tiemann et al.
22 2003a).

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2 **Estrogenic effects and alterations in uterus**

3 After 5 wk feeding of pre-puberal pigs with a diet containing a concentration of DON which
4 was more than nine times, and of ZON only 0.43 times, higher than the upper critical
5 concentrations, hyperestrogenism, uterotrophic effects (Tiemann et al. 2006b) or an
6 impairment of several enzymes on endometrial cell metabolism were not observed
7 (Wollenhaupt et al. 2006a). The data was supported by the fact that the quantity of
8 progesterone and estradiol receptors in the uterus was not affected by DON>ZON feeding. In
9 agreement to these results, Döll et al. (2004) reported that after 5 wk feeding of prepuberal
10 piglets with the concentrations of ZON up to 0.42 mg and DON up to 3.9 mg kg⁻¹ originating
11 from *Fusarium* toxin contaminated maize, the mean weight of the uteri of animals receiving
12 the most highly contaminated diet was significantly increased at the time of slaughtering.
13 Although an uptake in uterus weight occurred the surface epithelium of the uterus, the uterine
14 glands and the vaginal epithelium were not altered by the treatment (Döll et al. 2004).
15 Enhancement of ZON (56 mg kg⁻¹) and DON (4.99 mg kg⁻¹) in the diet of pigs caused
16 histopathological changes of uterine tissue such as hyperplasia, hypertrophy, and metaplasia of
17 the myometrium (Lopez et al. 1988). Application of ZON in crystalline form in the dose of
18 200–400 µg kg⁻¹ body weight day⁻¹ for a period of 7 days to immature gilts caused an
19 intensified cell proliferation which was expressed with the increase of PCNA (Proliferating Cell
20 Nuclear Antigen) index. This can be due to estrogenic effects and was observed in the
21 oviduct and uterus (Obremski et al. 2003). Higher concentrations of ZON, 2.2 mg kg⁻¹ and
22 22.09 mg kg⁻¹ in the ration of breeding gilts had an obvious harmful effect on their
23 reproductive performance, and the uterotrophic effect of ZON was obvious in both groups
24 (Kordic et al. 1992).

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2
3 1 *In vitro*, the affinity of ZON to uterine and oviduct estrogen receptors is greatest in pigs as
4
5 2 compared to rats and chickens (Fitzpatrick et al. 1989). The relative binding affinities to the
6
7 3 pig uterine cytoplasmic receptor for ZOL show that α -ZOL is more potent than β -ZOL
8
9 4 (Tiemann et al. 2003b). Responsible for the higher binding affinity of α -ZOL to estrogen
10
11 5 receptor is the reduction of the 6-keto and also vinyl groups in its molecule, while the β -
12
13 6 epimer is much less active in this regard (Kuiper-Goodman et al. 1987). The mechanism by
14
15 7 which α -ZOL inhibits the formation of a hormone-receptor complex can be explained by the
16
17 8 fact that α -ZOL exerts its effect by competing with estrogen for cytosolic receptor on cells in
18
19 9 target tissue and this fact might upset the delicate balance necessary for normal reproductive
20
21 10 activity (Guillette et al. 1996). In contrast to the natural hormones, α -ZOL does not bind the
22
23 11 carrier protein (Shrimanker et al. 1985), and consequently may exhibit elevated unbound
24
25 12 serum concentrations, thereby increasing its ability to activate the estrogenic pathway (Martin
26
27 13 et al. 1978). Compared to α -ZOL, a strong anti-proliferative effect of β -ZOL and DON in
28
29 14 porcine endometrial cells was observed by Tiemann et al. (2003b). This finding corresponds
30
31 15 to changes in the substructure and to a reduction in viability caused by apoptosis at β -ZOL
32
33 16 (Wollenhaupt et al. 2004) or necrosis at DON (Tiemann et al. 2003b). It can be assumed that
34
35 17 β -ZOL and DON modulate the process of translation at different molecular level. A molecular
36
37 18 target of DON in proliferating eukaryotic cells is the 60 S ribosomal subunit (Middlebrook
38
39 19 and Leatherman, 1989; Witt and Pestka, 1990). Following binding of DON to the ribosomal
40
41 20 RNA, a rapid activation of mitogen-activated protein kinases (MAPKs) could be observed in a
42
43 21 process that has been termed the "ribotoxic stress response" (Zhou et al. 2003; Pestka et al.
44
45 22 2004). The results of Wollenhaupt et al. (2004, 2006a, summerized 2006b) indicated that β -
46
47 23 ZOL and DON showed a pronounced impact on MAPKs, which are in mRNA translation, and
48
49 24 different signalling pathways can be modulated resulting in the inhibition of cell proliferation
50
51 25 after β -ZOL and DON exposure.

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6 2 **Conclusions**
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9 3 Previously, *in vivo* investigation were performed to determine different non-reproductive and
10
11 4 reproductive parameters in female pigs after feeding with DON and/or ZON contaminated
12
13 5 diets to evaluate the risks of their metabolism, health and fertility/reproduction. From all
14
15 6 described parameters, it is notable that the *in vivo* effects of DON on humoral immunity are
16
17 7 rather inconsistent. ZON has estrogenic activity and this is reflected by alterations of fertility
18
19 8 and reproduction in pigs. This review shows that besides the known effects of ZON on
20
21 9 reproductive organs, ingestion of DON also causes reproductive failure showing an
22
23 10 impairment of porcine oocyte and embryo development which may probably be related
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25 11 directly to the toxic effect of DON.
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32 13 Table 1 indicates that *in vivo* effects of DON/ZON were more investigated than *in vitro*
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34 14 effects. The *in vivo* investigations provide information on net effects in whole animals,
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36 15 whereas cell specific answers result from *in vitro* investigations. The summarized results of
37
38 16 *in vitro* studies with porcine cell cultures indicate that there are only partial agreements with
39
40 17 that of *in vivo* experiments. An explanation could be the fact that sometimes the cells *in vitro*
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42 18 react more sensitively than those *in vivo*, because a direct interaction of chemical substances
43
44 19 with the plasma membrane exists which may alter the membrane structure and function.
45
46 20 Therefore, *in vitro* experiments may contribute to risk assessments of toxins.
47
48 21 Pathophysiological studies are attributable on DON/ZON feeding in pigs and can be important
49
50 22 in understanding whether human exposure to DON/ZON may have unfavourable effects,
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52 23 because swine are physiologically similar to humans (Tumbleson and Schook, 1996) and are
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54 24 widely used as models for human disease.
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3 1 Altogether, ZON affects reproduction of pigs most seriously because it possesses estrogenic
4 activity. Whereas DON affects reproduction in pigs via indirect effects such as reduced feed
5 intake, resulting in reduced growth or impairment of function in vital organs like liver and
6 spleen (Figure 1).
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52 Legend

53
54 22 **Figure 1.** The effects of a *Fusarium* toxin contaminated diet containing predominantly
55 23 deoxynivalenol (DON) and zearalenone (ZON) on the fertility of female pigs need to be
56 24 viewed in the general context of the toxin effects on animal health and performance. Before
57 25 the toxins are able to modify metabolic processes they need to be consumed voluntarily by the
58 26 pig. The effects on feed intake, which are mediated by DON, not only determine the amount

1 of toxins entering the organism but also the metabolically available nutrients which might also
2 markedly modulate processes involved in fertility. Although the primary molecular targets of
3 DON (inhibition of protein synthesis) and ZON (interference with the oestrogen receptor) are
4 different, they obviously act in a concerted manner with regard to health and fertility (further
5 details in text).

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Table 1. Effects of deoxynivalenol (DON) and zearalenone (ZON) on selected reproductive and non-reproductive parameters in pigs

Parameter	<i>in vivo</i>	<i>in vitro</i>		References
	<i>Fusarium</i> toxin	DON	ZON	
Protein synthesis	+ (DON) + (DON)			Dänicke et al., 2006 Goyarts et al., 2006a
Immune response	- (DON) - (DON) - (DON) + (DON) + (DON) + (DON) - (DON) + (DON) - (DON) + (DON>ZON)	+ +		Bergsjø et al., 1992, 1993 Dänicke et al., 2004a,b Döll et al., 2003 Drochner et al., 2004 Goyarts et al., 2006b Øvernes et al., 1997 Rotter et al., 1994 Swamy et al., 2002 Swamy et al., 2003 Takyayama et al., 2005 Tiemann et al., 2006a
Histopathology liver and spleen	+ (DON>ZON)			Tiemann et al., 2006a,b
Follicular development	- (DON>ZON) + (ZON)			Alm et al., 2006 Zwierzchowski et al., 2005
Oocyte maturation	+ (DON>ZON)	+	+	Alm et al., 2002 Alm et al., 2006
Embryo development	+ (DON>ZON) + (ZON) + (ZON) + (ZON)	+	+	Alm et al., 2002 Alm et al., 2006 Diekman and Long, 1989 Kordic et al., 1992 Long et al., 1992
Steroid synthesis	- (DON>ZON)		+	Alm et al., 2006 Tiemann et al., 2003a
Uterine effects	- (DON>ZON) + (ZON) + (ZON>DON) + (ZON) - (DON>ZON) - (DON>ZON)	 + +	 + +	Döll et al., 2004 Fitzpatrick et al., 1989 Kordic et al., 1992 Lopez et al., 1988 Obremski et al., 2003 Tiemann et al., 2003b Tiemann et al., 2006b Wollenhaupt et al., 2004 Wollenhaupt et al., 2006

‘-’ marked no effect; ‘+’ marked significant effect

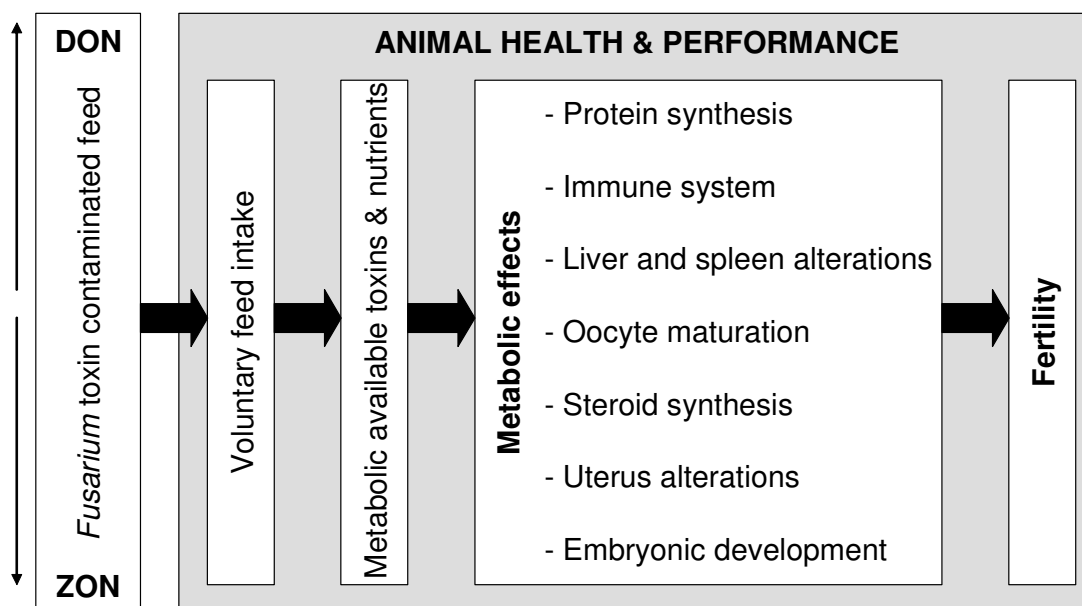


Figure 1. The effects of a *Fusarium* toxin contaminated diet containing predominantly deoxynivalenol (DON) and zearalenone (ZON) on the fertility of female pigs need to be viewed in the general context of the toxin effects on animal health and performance. Before the toxins are able to modify metabolic processes they need to be consumed voluntarily by the pig. The effects on feed intake, which are mediated by DON, not only determine the amount of toxins entering the organism but also the metabolically available nutrients which might also markedly modulate processes involved in fertility. Although the primary molecular targets of DON (inhibition of protein synthesis) and ZON (interference with the oestrogen receptor) are different, they obviously act in a concerted manner with regard to health and fertility (further details in text).