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

Ute Tiemann, Sven Dänicke

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

Journal:	*Food Additives and Contaminants*
Manuscript ID:	TFAC-2006-159.R1
Manuscript Type:	Review
Date Submitted by the Author:	28-Sep-2006
Complete List of Authors:	Tiemann, Ute; FBN-Dummerstorf, Reproductive Biology Dänicke, Sven; Federal Agriculture Research Center (FAL)
Methods/Techniques:	Toxicology - cytotoxicity, Toxicology - in-vivo, Toxicology - metabolism
Additives/Contaminants:	Environmental contaminants
Food Types:	Animal feed

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

U. Tiemann [A1] and S. Dänicke [A2]

[A1] Research Institute for the Biology of Farm Animals, Dummerstorf, Germany

[A2] Federal Agriculture Research Center (FAL), Braunschweig, Germany

Abstract

This review summarizes the toxicological data on the effects of the mycotoxins zearalenone (ZON), its metabolites, and deoxynivalenol (DON) on different parameters relating to reproductive and non-reproductive organs in female pigs. *In vivo* 22 mg ZON kg^{-1} in the diet cause alterations in the reproductive tract of swine such as in the uterus, on the follicular and embryo development. ZON and its metabolites have been shown to competitively bind to estrogen receptors in an *in vitro* system. The feeding of pigs with 9 mg DON kg^{-1} diet contaminated diet can act on protein synthesis, humoral and cellular immune response depending on dose, exposure and timing of functional immune assay, and on liver and spleen cell structures. Beside these effects, reproductive alterations were observed in pigs, too. Both *in vivo* and *in vitro* exposure to DON decreased the oocyte and embryo development. *In vitro* application of DON to uterine cells inhibits their proliferation rate and modulates the process of translation at a different molecular level when compared with the *in vivo* application. The histopathological results provide evidence of spleen and liver dysfunction in the absence of clinical signs, especially in pigs fed higher concentrations of *Fusarium* toxin-contaminated wheat. Prepuberal gilts react more sensitively to DON>ZON feeding compared to pregnant sows. In the liver, histopathological changes such as glycogen decrease and interlobular collagen uptake were observed only observed in prepuberal gilts, whereas enhancement of haemosiderin was found in both perpuberal gilts and pregnant sows. This review presents some of the current knowledge on the biological activities of ZON and DON in pig.

Altogether, ZON affects reproduction of pigs most seriously because it possesses estrogenic activity. Whereas DON affects reproduction in pigs via indirect effects such as reduced feed intake, resulting in reduced growth or impairment of function in vital organs like liver and spleen.

Keywords**:** *Fusarium* toxins, zearalenone, deoxynivalenol, reproductive and non-reproductive organs, female pig

---

* Corresponding author: Dr. Ute Tiemann, Research Institute for the Biology of Farm Animals, Wilhelm Stahl-Allee 2, 18196 Dummerstorf, Tel: (49)38208 68 766; Fax: (49)38208 68 752; E-mail: tiemann@fbn-dummerstorf.de

Introduction

Mycotoxins are biologically active secondary fungal metabolites found as contaminants of feedstuffs and exert toxic effects in animals and human beings (Fink-Gremmels, 1999). *Fusarium* toxins, such as deoxynivalenol (DON) and zearalenone (ZON), contaminate wheat, maize, and barley worldwide (Abouzied et al. 1991; Chelkowski, 1998; EFSA, 2004a,b). These mycotoxins are produced mainly by *F. graminearum, F. culmorum,* and *F. roseum* under predisposing environmental conditions during cereal flowering and include humidity and fungus specific optimum temperature range (Oldenburg et al., 2000). *Fusarium* species are probably the most prevalent toxin-producing fungi of the northern temperate regions and are commonly found in cereals grown in America, Europe and Asia. They cause a variety of toxic effects in experimental animals and livestock. Different effects were described for *Fusarium* toxins ZON (Kuiper-Goodman et al. 1987; Wood, 1992) and DON (reviewed by Pestka and Casale, 1990; Rotter et al. 1996).

Zearalenone is biologically potent, but hardly toxic; rather, it sufficiently resembles 17ß-estradiol, the principal hormone produced by the human ovary, to allow it to bind to estrogen receptors in mammalian target cells (Katzenellenbogen et al. 1979). ZON is better classified as a non-steroidal estrogen or mycoestrogen. Metabolism of ZON in monogastric animals takes place in the liver and intestinal mucosa where it is reduced to α- and β-zearalenol (α-ZOL and β-ZOL) (Olsen et al., 1987; Olsen, 1989), a reaction that competes with glucuronidation of the parent molecule. Examination of excretory products indicated that in pig the α-epimer predominates (Farnwoth and Trendholm, 1983; Olsen et al., 1985a,b; Biehl et al., 1993; Zöllner et al., 2002; Döll et al., 2003; Dänicke et al., 2005b). Biotransformation studies with pig liver sub-cellular fractions indicated that α-ZOL is the main hepatic metabolic of ZON in pigs (Malekinejad et al. 2005). Additionally, the authors could detect that another extrahepatic

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

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

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

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

Macrophages, T cells, and B cells of the immune system are central targets of DON that can be immunostimulatory or immunosuppressive depending on dose, exposure and timing of functional immune assay (Rotter et al., 1996; Bondy and Pestka, 2000; Pestka et al. 2004). The presence of DON in pig diets decreases feed intake, causes feed refusal, and induces occasional vomiting (Diekman and Green, 1992). Beside these effects, reproductive alterations were observed in humans and pigs (Hussein and Brasel, 2001; Alm et al. 2006).

Effects of a *Fusarium* toxin contaminated diet containing DON and/or ZON on the fertility/reproduction of female pigs need to be viewed in the general context of the toxin effects on porcine health and performance. Before the toxins are able to modify metabolic processes they need to be consumed voluntarily by the pig. Data reported in the literature indicate that the adverse effects of DON contaminated diets on growth performance of growing pigs is primarily caused by depressing the voluntary feed intake (Rotter et al., 1994; Swamy et al., 2002; Goyarts et al., 2005). The mycotoxins obviously act in a concerted manner with regard to metabolic processes and consequently influence the health and fertility/reproduction (Figure 1).

This review aims to give an overview about the effects of feeding with DON>ZON contaminated diet on non-reproductive and reproductive organs which can influence metabolism, health, and fertility/reproduction of female pigs. Figure 1 demonstrates a scheme of the investigations on specific organs and parameters dealt with in the current study.

Furthermore, we have focused on the comparison of *in vivo* and *in vitro* effects of DON and ZON on different parameters.

Protein synthesis

Deoxynivalenol is known to be a potent protein synthesis inhibitor according to research in different *in vitro* and *in vivo* systems (Feinberg and McLaughlin, 1989). However, the significance of this toxic feature was not yet evaluated for the pig. Therefore, an experiment was carried out by Dänicke et al. (2006) to measure porcine tissue protein synthesis employing the so-called flooding dose technique using [$^2H_5$]-phenylalanine as a tracer in dependence on different exposition of DON to pigs exposed to a DON-contaminated diet for ~ 4 wks (chronic) or just uniquely (acute oral) to reflect different feeding scenarios. Moreover, an additional group of pigs was injected with a single intravenous DON-dose (acute intravenous) representing a complete systemic DON-availability. Protein synthesis expressed as fractional synthesis rate (FSR) was significantly reduced in kidneys, spleen and ileum of DON-exposed pigs. FSR of liver, skeletal and heart muscle, mesenteric lymph nodes, duodenum, jejunum, jejunal mucosa cells, pancreas and lung were not affected by DON. Goyarts et al. (2006a) have shown that DON decreased the FSR of albumin as the main protein exported from the liver and as the predominant plasma protein. Pigs exposed to DON 5.7 mg kg^{-1} diet showed an increased protein synthesis capacity (RNA to protein ratio) caused by an elevated liver RNA-concentration (Dänicke et al. 2006) .

Humoral and cellular immune response

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

grains naturally contaminated with DON>ZON for 5 wk the serum IgA concentration of prepuberal pig increased significantly, while serum IgM and IgG concentrations were not altered (Goyarts et al. 2006b; Tiemann et al. 2006a). Similarly, Swamy et al. (2002) observed such effects in pigs fed with DON contaminated corn and wheat. Drocher et al. (2004) demonstrated an IgA elevation in pigs fed a semisynthetic diet without grain components with pure DON (600 μg kg^{-1}) under highly standardized conditions. In contrast, some investigators were not able to show interaction between DON and IgA serum concentrations (Bergsjø et al. 1992, 1993; Döll et al. 2003; Swamy et al. 2003; Dänicke et al. 2004a,b). No differences were observed in serum level of IgA between the pregnant sows fed 9.6 mg DON/0.358 mg ZON kg^{-1} diet and the controls for 35 days (Tiemann unpublished data). Table 1 summarized the data and it can be noteable that the *in vivo* effects of DON on humoral immunity are rather inconsistent. The reason may be methological differences in the experiments carried out by several investigators. *In vitro*, the IgA concentration in the supernatant of ConA stimulated porcine peripheral lymphocytes was dose-dependently inhibited with increasing DON concentrations (Goyarts et al. 2006b).

Mitogen-induced proliferation is a common technique to assess immunotoxicity. The immunosuppressive effect of DON is primarily associated with cellular immune response. Øvernes et al. (1997) observed a significant higher response to phytohemagglutinin in the high DON group compared to the low DON group in growing pigs. In contrast, no effect of DON-contaminated feed on peripheral lymphocyte mitogen response was reported by Rotter et al. (1994); Tiemann et al. (2006a, unpublished data). Compared to bloods lymphocytes, splenocytes were more sensitive to ConA-induced response in perpuberal pigs fed the DON contaminated diet compared to pregnant sows. The DON>ZON feeding of pregnant sows

reduced the proliferative response in splenocytes in approximately the same manner, as observed in prepuberal gilts (Tiemann et al. 2006a, unpublished data).

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

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

Histopathology of spleen and liver

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

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

Follicle development, oocyte maturation, and embryo development

Edwards et al. (1987) reported that pre-pubertal gilts fed zearalenone displayed first estrus later than controls; however, in other investigations, pre-pubertal gilts consuming a milo diet contaminated with zearalenone exhibited puberty at a younger age without an alteration in conception rates, ovulation rates, or embryonic survival (Rainey et al., 1990).

Zwierzchowski et al. (2005) observed that the amount of ZON to a dose of 200 µg kg^{-1} body weight in immature gilts leads to distribution in the development and maturation of the largest follicles through the activation of an apototic-like process in the granulosa layer of single mature follicles. In contrast, Alm et al. (2006) found that the size distribution of follicles was not affected by feeding of gilts with different *Fusarium*-toxin contaminated diets. In these animals the cumulus cell morphology was not changed by increasing dietary concentrations of both toxins up to 9,570 and 0,358 mg kg^{-1} for DON and ZON, respectively. The authors recovered oocytes from pig ovaries by follicle aspiration after ovario-hysterectomy. At the concentration named above, at the time of recovery, oocytes with compact cumuli showed a

significantly reduced proportion with immature chromatin. Here, the proportion of oocytes having degenerated meiotic chromatin was significantly higher compared to lower concentrations. The oocyte quality was significantly reduced by feeding of a DON>ZON contaminated diet to gilts. The competence of oocytes in compact cumulus oocyte complexes for *in vitro* maturation was likewise significantly reduced by high concentrations of *Fusarium* toxin in feed; this is probably directly related to the reduced proportion of normal germinal vesicle-stage oocytes, i.e., oocytes capable of maturation, in the high toxin groups. These results are in agreement with data reported by Kordic et al. (1992) who observed a doses-dependent effect of ZON on reproductive performance in breeding gilts, with decreased numbers of live embryos as well as other reproductive problems including increased number of dead-born piglets. Little information is available about effects of indirect (i.e., from mother to young) exposure to DON/ZON on the health of piglets. Placenta transfer of mycotoxins can indicate a potential risk for direct effects in the fetus. Long and Diekman (1984) reported that gilts fed 5 to 30 mg kg^{-1} ZON from d 2 to 15 postmating had normal embryonic development, but no fetuses were present in gilts fed 60 or 90 mg kg^{-1} ZON when killed d 40 to 43 post-mating. Embryos recovered on d 14 from gilts fed 60 mg kg^{-1} of ZON from d 7 to 10 post breeding were fragmentous, while those from control gilts were filamentous (Diekman and Long, 1989). Degeneration in blastocysts, affecting both embryonic membranes and the embryonic disk occurring in ZON-treated sows were observed by Long et al. (1992). In contrast, the authors observed no deleterious effects of DON on fetal development when feed containing 8 mg kg^{-1} of purified DON was consumed (summarized Diekman and Green, 1992).

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

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

Intrafollicular steroid synthesis

The feeding of wheat contaminated naturally with DON and ZON to gilts at increasing proportions did not influenced the activity of enzymes involved in progesterone synthesis. *In vivo*-derived porcine granulosa cells were analyzed for the mRNA expression of the mitochondrial enzyme cytochrome P450, cholesterol side-chain cleavage (P450scc) and microsomal enzyme 3β-hydroxysteroid dehydrogenase/isomerase (3β-HSD) by RT-PCR, and additionally for P450scc protein by Western blotting. Neither the expression of the P450scc nor of the 3β-HSD mRNA, nor the abundance of the P450scc protein was significantly influenced by the mycotoxin feeding (Alm et al. 2006).

Cultured primary porcine granulosa cells have been used to test the toxic potential of xenobiotics on reproduction (Haney et al. 1984). *In vitro* addition of α- and β-ZOL at concentrations of 15 and 30 µmol l^{-1} inhibited the FSH-stimulated progesterone synthesis in porcine granulosa cells. The inhibitory effect at a concentration of 30 µmol l^{-1} of both mycotoxins could mainly be due to cell death. The antisteriodogenic effect of 15 µmol l^{-1} α-ZOL or β-ZOL was not due to cytotoxic effects. The enzyme activity of 3β-HSD and the abundance of P450scc protein were reduced by both mycotoxins as well (Tiemann et al. 2003a).

Estrogenic effects and alterations in uterus

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

In vitro, the affinity of ZON to uterine and oviduct estrogen receptors is greatest in pigs as compared to rats and chickens (Fitzpatrick et al. 1989). The relative binding affinities to the pig uterine cytoplasmic receptor for ZOL show that α-ZOL is more potent than ß-ZOL (Tiemann et al. 2003b). Responsible for the higher binding affinity of α-ZOL to estrogen receptor is the reduction of the 6-keto and also vinyl groups in its molecule, while the ß-epimer is much less active in this regard (Kuiper-Goodman et al. 1987). The mechanism by which α-ZOL inhibits the formation of a hormone-receptor complex can be explained by the fact that α-ZOL exerts its effect by competing with estrogen for cytosolic receptor on cells in target tissue and this fact might upset the delicate balance necessary for normal reproductive activity (Guilette et al. 1996). In contrast to the natural hormones, α-ZOL does not bind the carrier protein (Shrimanker et al. 1985), and consequently may exhibit elevated unbound serum concentrations, thereby increasing its ability to activate the estrogenic pathway (Martin et al. 1978). Compared to α-ZOL, a strong anti-proliferative effect of β-ZOL and DON in porcine endometrial cells was observed by Tiemann et al. (2003b). This finding corresponds to changes in the substructure and to a reduction in viability caused by apoptosis at β-ZOL (Wollenhaupt et al. 2004) or necrosis at DON (Tiemann et al. 2003b). It can be assumed that β-ZOL and DON modulate the process of translation at different molecular level. A molecular target of DON in proliferating eukaryotic cells is the 60 S ribosomal subunit (Middlebrook and Leatherman, 1989; Witt and Pestka, 1990). Following binding of DON to the ribosomal RNA, a rapid activation of mitogen-activated protein kinases (MAPKs) could be observed in a process that has been termed the “ribotoxic stress response” (Zhou et al. 2003; Pestka et al. 2004). The results of Wollenhaupt et al. (2004, 2006a, summerized 2006b) indicated that β-ZOL and DON showed a pronounced impact on MAPKs, which are in mRNA translation, and different signalling pathways can be modulated resulting in the inhibition of cell proliferation after β-ZOL and DON exposure.

Conclusions

Previously, *in vivo* investigation were performed to determine different non-reproductive and reproductive parameters in female pigs after feeding with DON and/or ZON contaminated diets to evaluate the risks of their metabolism, health and fertility/reproduction. From all described parameters, it is notable that the *in vivo* effects of DON on humoral immunity are rather inconsistent. ZON has estrogenic activity and this is reflected by alterations of fertility and reproduction in pigs. This review shows that besides the known effects of ZON on reproductive organs, ingestion of DON also causes reproductive failure showing an impairment of porcine oocyte and embryo development which may probably be related directly to the toxic effect of DON.

Table 1 indicates that *in vivo* effects of DON/ZON were more investigated than *in vitro* effects. The *in vivo* investigations provide information on net effects in whole animals, whereas cell specific answers result from *in vitro* investigations. The summarized results of *in vitro* studies with porcine cell cultures indicate that there are only partial agreements with that of *in vivo* experiments. An explanation could be the fact that sometimes the cells *in vitro* react more sensitively than those *in vivo*, because a direct interaction of chemical substances with the plasma membrane exists which may alter the membrane structure and function. Therefore, *in vitro* experiments may contribute to risk assessments of toxins. Pathophysiological studies are attributable on DON/ZON feeding in pigs and can be important in understanding whether human exposure to DON/ZON may have unfavourable effects, because swine are physiologically similar to humans (Tumbleson and Schook, 1996) and are widely used as models for human disease.

Altogether, ZON affects reproduction of pigs most seriously because it possesses estrogenic activity. Whereas DON affects reproduction in pigs via indirect effects such as reduced feed intake, resulting in reduced growth or impairment of function in vital organs like liver and spleen (Figure 1).

References

Abouzied MM, Azcona JI, Braselton WE, Pestka JJ. 1991. Immunochemical assessment of mycotoxins in 1989 grain foods: Evidence for deoxynivalenol vomitoxin contamination. Applied Environmental Microbiology 57:672-677.

Alm H, Greising T, Brüssow K-P, Torner H, Tiemann, U. 2002. The influence of the mycotoxins deoxynivalenol and zearalenol on the *in vitro* maturation of pig oocytes and *in vitro* culture of pig zygotes. Toxicology In Vitro 16:643–648.

Alm H, Brüssow K-P, Torner H, Vanselow J, Tomek W, Dänicke S, Tiemann U. 2006. Influence of *Fusarium*-toxin contaminated feed on initial quality and meiotic competence of gilt oocytes. Reproductive Toxicology 22:44-50.

Bergsjø B, Marte T, Nafstad I. 1992. Effect of diets with graded levels of deoxynivalenol on performance in growing pigs. Journal of Veterinary Medicine 39:752-758.

Bergsjø B, Langseth W, Nafstad I, Jansen JH, Larsen HJS 1993. The effects of naturally deoxynivalenol-contaminated oats on the clinical condition, blood parameters, performance and carcass composition of growing pigs. Veterinary Research Communications 17:283-294.

Biehl ML, Prelusky DB, Koritz GD, Hartin KE, Buck WB, Trenholm HL. 1993. Biliary excretion and enterohepatic cycling of zearalenone in immature pigs. Toxicology and Applied Pharmacolgy. 121:152-159

Bondy GS, Pestka JJ 2000. Immunomodulation by fungal toxins. Journal of Toxicology and Environmental Health, Part B: Critical Reviews 3:109–143.

Bunner DL, Morris ER. 1988. Alteration of multiple cell membrane functions in L-6 myoblasts by T-2 toxin: An important mechanism of action. Toxicology and Applied Pharmacology 92:113–121.

Chang K, Kurtz H J, Mirocha CJ. 1979 Effects of the mycotoxin zearalenone on swine reproduction. American Journal of Veterinary Research 40:1260–1267.

Chelkowski J. 1998. Distribution of Fusarium species and their mycotoxins in cereal grains. In: Sinha, K.K., Bhatnagar, D. (Eds.), Mycotoxins in Agriculture and Food Safety. Marcel Dekker, New York, USA , pp. 45-66

Dacasto M, Rolando P, Nachtmann C, Ceppa L, Nebbia C. 1995. Zearalenone mycotoxicosis in piglets suckling sows fed contaminated grain. Veterinary and Human Toxicology 37:359–361.

Dänicke S, Brüssow K-P, Valenta H, Ueberschär K-H, Tiemann U, Schollenberger M. 2005a. On the effects of graded levels of *Fusarium*-toxin-contaminated wheat in diets for gilts on feed intake, growth performance and metabolism of deoxynivalenol and zearalenone. Molecular Nutrition Food Research 49:932-43.

Dänicke S, Goyarts T, Döll S, Grove N, Spolders M, Flachowsky G. 2006. Effects of the *Fusarium* toxin deoxynivalenol on tissue protein synthesis in pigs. Toxicology Letters 165:297-311.

Dänicke S, Goyarts T, Valenta H, Razzazi E, Böhm J. 2004a. On the effects of increasing deoxynivalenol (DON) concentrations in pig feed on growth performance, utilization of nutrients and metabolism of DON. Journal of Animal and Feed Science 13:539-556.

Dänicke S, Swiech E, Buraczewska L, Ueberschär K-H. 2005b. Kinetics and metabolism of zearalenone in young female pigs. Journal of Animal Physiology and Animal Nutrition 89:268-276.

Dänicke S, Valenta H, Klobasa F, Döll S, Ganter M, Flachowsky G. 2004b. Effects of graded levels of *Fusarium* toxin contaminated wheat in diets for fattening pigs on growth performance, nutrient digestibility, deoxynivalenol balance and clinical serum characteristics. Archives of Animal Nutrition 58:1-17.

Diekman MA, Green ML. 1992. Mycotoxins and reproduction in domestic livestock. Journal of Animal Science 70:1615-1627.

Diekman MA, Long GG. 1989. Blastocysts development on days 10 or 14 after consumption of zearalenone by sows on days 7 to 10 after breeding. American Journal of Veterinary Research 50:1224-1227.

Döll S, Dänicke S, Ueberschär K- H, Valenta H, Schnurrbusch U, Ganter M, Klobasa F, Flachowsky G. 2003. Effects of graded levels of *Fusarium* toxin contaminated maize in diets for female weaned piglets. Archive of Animal Nutrition 57:311-334.

Döll S, Dänicke S, Schnurrbusch U. 2004. The effect of increasing concentrations of *Fusarium* toxins in piglet diets on histological parameters of the uterus and vagina. Archive of Animal Nutrition 58:413-417.

Drochner W, Schollenberger M, Piepho HP, Gotz S, Lauber U, Tafaj M, Klobasa F, Weiler U, Claus R, Steffl, M. 2004. Serum IgA-promoting effects induced by feed loads containing isolated deoxynivalenol (DON) in growing piglets. Journal of Toxicology and Environmental Health 67:1051-67.

Edwards S, Cantley TC, Day BN. 1987. The effect of zearalenone on reproduction in pigs. II. The effect of puberty attainment and postweaning rebreeding performance. Theriogenology 28:51-58.

EFSA, 2004a. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Deoxynivalenol (DON) as undesirable substance in animal feed. The EFSA Journal 73, 1-41 (available at: http://www.efsa.eu.int/).

EFSA, 2004b. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Zearalenone as undesirable substance in animal feed. The EFSA Journal 89, 1-35 (available at: http://www.efsa.eu.int/).

Ehrlich KC, Daigle KW. 1987. Protein synthesis inhibition by 8-oxo-12,13-epoxytrichothecenes. Biochimica et Biophysica. Acta 923:206-213.

Ellis TN, Beaman BL. 2004. Interferon-gamma activation of polymorphonuclear neutrophil function. Immunology 112:2-12. Review.

EU: Official Journal of the European Union (L 229/7, 17 August 2006)

Farnworth ER, Trenholm HL. 1983. The metabolism of the mycotoxin zearalenone and its effect on the reproductive tracts of young male and female pigs. Canadian Journal of Animal Science 63:967 - 975.

Feinberg B, McLaughlin CS. 1989. Biochemical mechanism of action of trichothecene mycotoxins. In: V R Beasley, editor. Trichothecene mycotoxicosis: Pathophysiological effects. Volume I, CRC Press, Inc. Boca Raton, Florida pp. 27-35.

Fink-Gremmels J. 1999. Mycotoxins: their implications for human and animal health. Veterinary quaterly 21:115-120.

Fitzpatrick DW, Picken CA, Murphy LC, Buhr MM. 1989. Measurement of the relative binding affinity of zearalenone, α-zearalenol and ß-zearalenol for uterine and oviduct estrogen receptors in swine, rats and chickens: an indicator of estrogenic potencies. Comparative Biochemistry and Physiology 94C:691-694.

Gajecki M. 2002. Zearalenone – undesirable substances in feed. Polish Journal of Veterinary Science 2:117–122.

Goyarts T, Dänicke S, Grove N. 2006a. Effects of the *Fusarium* toxin deoxynivalenol from naturally contaminated wheat on the *in vivo* protein synthesis of peripheral blood lymphocytes and plasma proteins in the pig. Food and Chemical Toxicology in press

Goyarts T, Dänicke S, Tiemann U, Rothkötter H-J. 2006b. Effect of the *Fusarium* toxin deoxynivalenol (DON) on IgA, IgM and IgG concentrations and proliferation of porcine blood lymphocytes. Toxicology In Vitro 20, 858-867.

Guilette Jr LJ, Arnold SF, McLachlan JA. 1996. Ecoestrogens and embryos - Is there a scientific basis for concern? Animal Reproduction Science 42:13-24.

Haney A, Hughes S, Hughes CJ. 1984. Screening of potential reproductive toxicants by use of porcine granulosa cell cultures. Toxicology 30:227-241.

Hussein SH, Brasel JM. 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology 167:101–134.

Jone C, Erickson L, Trosko JE, Chang CC. 1987. Effect of biological toxins on gap-junctional intercellular communication in Chinese hamster V79 cells. Cell Biology and Toxicology 3:1–15.

Katzenellenbogen BS, Katzenellenbogen JA, Mordecai D. 1979. Zearalenones: characterization of the oestrogenic potency and receptor interactions of a series of fungal beta-resorcylic acid lactones. Endocrinology 105:33–40.

Kordic B, Pribicevic S, Muntanola-Cvetkovic M, Nicolic P, Nicolic B. 1992. Experimental studies of the effects of known quantities of zearalenone on swine reproduction. Journal of Environmental Pathology, Toxicology and Oncology 11:53-55.

Kuiper-Goodman T, Scott PM, Watanabe H. 1987. Risk assessment of the mycotoxin zearalenone. Regulatory Toxicology and Pharmacology 7:253-306.

Long GG, Diekman MA. 1984. Effect of purified zearalenone on early gestation in gilts. Journal of Animal Science 59:1662-70.

Long GG, Diekman MA. 1986. Characterization of effects of zearalenone in swine during early pregnancy. American Journal of Veterinary Research 47:184–187.

Long GG, Turek J, Diekman MA, Scheidt AB. 1992. Effect of zearaleone on days 7 to 10 post-mating on blastocyst development and endometrial morphology in sows. Veterinary Pathology 29:60–67.

Lopez TA, Odriozola ER, Milano GD, Sobredo JC, Donadio LH, Rocco JA. 1988. Hyperestrogenism in swine due to natural poisoning with zearalenone. Revista Argentina de Microbiologia 20:119-123.

Malekinejad H, Colenbrander B, Fink-Gremmels J. 2006. Hydroxysteroid dehydrogenases in bovine and porcine granulosa cells convert zearalenone into its hydroxylated metabolites α-zearalenol and β-zearalenol. Veterinary Research Communications 30:445-453.

Malekinejad H, Maas-Bakker RF, Fink-Gremmels J. 2005. Bioactivation of zearalenone by porcine hepatic biotransformation. Veterinary Research 36:799–810.

Martin PM, Horwitz KB, Ryan DS, McGuire WL. 1978. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. Endocrinology 103:1860-1867.

Middlebrook JL, Leatherman DL. 1989. Binding of T-2 toxin to eukaryotic cell ribosomes. Biochemical Pharmacology. 38:3103–3110.

Mirocha CJ, Pathre SV, Chrisensen CM. 1977. Zearalenone. In: Rodricks JV, Hesseltine CW, Mehlmann MA, editors. Mycotoxins in Human and Animal Health. Pathotox Publ., Park Forest, IL.

Obremski K, Gajecki M, Zwierzchowski W, Zielonka L, Otrocka-Domagala I, Rotkiewicz T, Mikolajczyk A, Gajecka M, Polak M. 2003. Influence of zearalenone on reproductive system cell proliferation in gilts. Polish Journal of Veterinary Science 6:239–245.

Oldenburg E, Valenta H, Sator Ch. 2000. Risikofaktoren für die Fusariumtoxinbildung und Vermeidungsstrategien bei der Futtermittelerzeugung und Fütterung. In: Dänicke s, Oldenburg E, editors. Landbauforschung Völkenrode, SH 216, pp. 5-34.

Olsen M. 1989. Metabolism of zearalenone in farm animals. In: Chelkowski J, editor. *Fusarium*: Mycotoxins, Taxonomy and Pathogenicity. Amsterdam: Elsevier. p. 167.

Olsen M, Malmöf K, Pettersson H, Sandholm K, Kiessling KH. 1985a. Plasma and urinary levels of zearalenone and alpha-zearalenol in a prepubertal gilt fed zearalenone. Acta Pharmacologica et Toxicologica 56:239-243.

Olsen M, Pettersson H, Sandholm K, Visconti A, Kiessling KH. 1987. Metabolism of zearalenone by sow intestinal mucosa in vitro. Food and Chemical Toxicololgy 25:681-683.

Olsen M, Pettersson H, Sandholm K, Kiessling KH. 1985b. Quantitative liquid chromatographic method using fluorescene detection for determining zearalenone and its metabolites in blood plasma and urine. Journal of the association of official analytical chemists 68:632-635.

Øvernes G, Matre T, Sivertsen T, Larsen HJS, Langseth W, Reitan LJ, Jansen JH. 1997. Effects of diets with graded levels of naturally deoxynivalenol-contaminated oats on immune response in growing pigs. Journal of Veterinary Medicine A 44:539-550.

Pestka JJ. 2003. Deoxynivalenol-induced IgA production and IgA nephropathy-aberrant mucosal immune response with systemic repercussions. Toxicology Letters 140-141: 287-295.

Pestka JJ, Casale WL. 1990. Naturally occurring fungal toxins. Advances in Environmental Science & Technology 23:613 - 638.

Pestka JJ, Zhou HR, Moon Y, Chung YJ. 2004. Cellular and molecular mechanisms for immune modulation by deoxynivalenol and other trichothecenes: unraveling a paradox. Toxicology Letters 153:61-73.

Price WD, Lowell RA, McChesney DG. 1993. Naturally occurring toxins in feedstuffs. Journal of Animal Science 71:2556–2562.

Rainey MR, Tubbs RC, Bennett LW, Cox NM. 1990. Prepubertal exposure to dietary zearalenone alters hypothalamo-hypophysial function but does not impair post-pubertal reproductive function of gilts. Journal of Animal Science 6:2015-2020.

Rotter BA, Prelusky DB, Pestka JJ. 1996. Toxicology of deoxynivalenol (vomitoxin). Journal of Toxicology and Environmental Health 48:1–34.

Rotter BA, Thompson BK, Lessard M, Trendholm HL, Tryphonas H. 1994. Influence of low-level exposure to *Fusarium* mycotoxins on selected immunological and hematological parameters in young swine. Fundamental and Applied Toxicology 23:117-124.

Shrimanker K, Salter LJ, Patterson RL. 1985. Binding of steroid hormones and anabolic agents to bovine sex-hormone binding globulin. Hormone and Metabolic Research 17: 454-457.

Swamy HVLN, Smith TK, Mac Donald EJ, Boermans HJ, Squires EJ. 2002. Effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on swine performance, brain, regional neurochemistry, and serum chemistry and the efficacy of a

polymeric glucomannan mycotoxin adsorbent. Journal of Animal Science 80:3257-3267.

Swamy HVLN, Smith TK, MacDonald EJ, Karrow NA, Woodward B, Boermans HJ. 2003. Effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on growth and immunological measurements of starter pigs, and the efficacy of a polymeric glucomannan mycotoxin adsorbent. Journal of Animal Science 81:2792-2803.

Takayama H, Shimada N, Mikami O, Murata H. 2005. Suppressive effect of deoxynivalenol, a *Fusarium* mycotoxin, on bovine and porcine neutrophil chemiluminescence: an *in vitro* study. Journal of Veterinary Medicine A 67:531-3.

Thompson WL, Wannemacher RW Jr. 1986. Structure-function relationship of 12,13-epoxytrichothecene mycotoxins in cell culture: comparison to whole animal lethality. Toxicon 24:985-994.

Tiemann U, Brüssow K-P, Jonas L, Pöhland R, Schneider F, Dänicke S. 2006a. Effects of diets with cereal grains contaminated by graded levels of two *Fusarium* toxins on selected immunological and histological parameters of spleen in gilts. Journal of Animal Science 84:236-245.

Tiemann U, Brüssow K-P, Küchenmeister U, Jonas L, Kohlschein P, Pöhland R, Dänicke S. 2006b. Influence of diets with cereal grains contaminated by graded levels of two *Fusarium* toxins on selected enzymatic and histological parameters of liver in gilts. Food and Chemical Toxicology 44:1228-1235.

Tiemann U, Tomek W, Schneider F, Vanselow J. 2003a. Effects of the mycotoxins α- and β-zearalenol on regulation of progesterone synthesis in cultured granulosa cells from porcine ovaries. Reproductive Toxicology 17:673-681.

Tiemann U, Viergutz T, Jonas L, Schneider F. 2003b. Influence of the mycotoxins α- and β-zearalenol and deoxynivalenol on the cell cycle of cultured porcine endometrial cells. Reproductive Toxicology 17:209–218.

Tumbleson ME, Schook LB. 1996. In: Advances in swine in biochemical research. New York, Plenum Press. p 99.

Ueno Y. 1983. General toxicology. In: Trichothecenes: Chemical, Biological and Toxicological Aspects, Amsterdam, Elsevier. pp 135-146.

Weaver GA, Kurtz HJ, Mirocha CJ, Bates FY, Behrens JC, Robison TS, Gipp WF. 1978. Mycotoxin-induced abortions in swine. Canadian of Veterinary Journal 19:1–74.

Witt MF, Pestka JJ. 1990. Uptake of the naturally occurring 3-α-hydroxy isomer of T-2 toxin by a murine B-cell hybridoma. Food and Chemical Toxicology 28:21-28.

Wollenhaupt K, Jonas L, Tiemann U, Tomek W. 2004. Influence of the mycotoxins α- and β-zearalenol (ZOL) on regulators of cap-dependent translation control in pig endometrial cells. Reproductive Toxicology 19:189–199.

Wollenhaupt K, Dänicke S, Brüssow K-P, Tiemann U. 2006a. *In vitro* and *in vivo* effects of deoxynivalenol (DNV) on regulators of cap dependent translation control in porcine endometrium. Reproductive Toxicology 21:60–73.

Wollenhaupt K, Tomek W, Tiemann U. 2006b. Comparison of the molecular effects of the mycotoxins β-zearalenol (β-ZOL) and deoxynivalenol (DON) in porcine endometrial cells (PEC). Review. Acta Veterinaria Hungarica in press.

Wood G E. 1992. Mycotoxins in food and feeds in the United States. Journal of Animal Science 70:3941–3949.

Yang HH, Aulerich RJ, Helferich W, Yamini B, Chou KC, Miller ER, Bursian SJ. 1995. Effects of zearalenone and/or tamoxifen on swine and mink reproduction. Journal of Applied Toxicology 15:223–232.

Yoshino N, Takizawa M, Akiba H, Okumura H, Tashiro F, Honda M, Ueno Y. 1996. Transient elevation of intracellular calcium ion levels as an early event in T-2 toxin-induced apoptosis in human promyelotic cell line HL-60. Nature Toxins 4:234–241.

Zhou HR, Lau AS, Pestka JJ. 2003. Role of double-stranded RNA-activated protein kinase R (PKR) in deoxynivalenol-induced ribotoxic stress response. Toxicology Science 74:335-344.

Zöllner P, Jodlbauer J, Kleinova M, Kahlbacher H, Kuhn T, Hochsteiner W, Lindner W. 2002. Concentration levels of zearalenone and its metabolites in urine, muscle tissue, and liver samples of pigs fed with mycotoxin-contaminated oats. Journal of Agricultural and Food Chemistry 50:2494-2501.

Zwierzchowski W, Przybylowicz M, Obremski K, Zielonka L, Skorska-Wyszynska E, Gajecka M, Polak M, Jakimiuk E, Jana B, Rybarczyk L, Gajecki M. 2005. Level of zearaleonene in blood serum and lesions in ovarian follicles of sexually immature gilts in the course of zearalenone microtoxicocis. Polish Journal of Veterinary Science 8:209-218.

Legend

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).

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

Parameter	*in vivo*	*in vitro*		References
	Fusarium 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

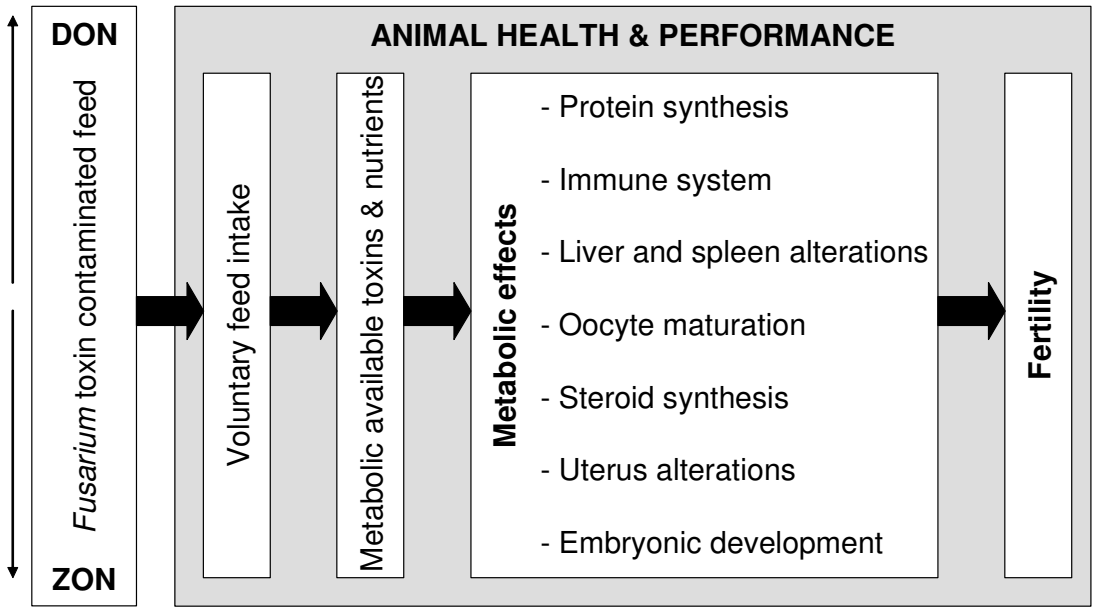

Figure 1. The effects of a *Fusarium* toxin contaminated diet containing predominantly deoxynivalenol (DON) and zearalenone (ZON) on the fertility of female pigs need to be viewed in the general context of the toxin effects on animal health and performance. Before the toxins are able to modify metabolic 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).