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Zearalenone, metabolites and their effects on swine reproductive performance: a review

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Abstract

Alterations on estrogenic hormones balance during swine growth can lead to serious problems in the reproductive performance of these animals. One of the factors that may contribute to changes on estrogen hormones balance is the presence of zearalenone (ZON) in ingredients and feed. ZON is produced by fungi of *Fusarium* species (F.*culmorum, F. graminearum* and *F. tricinctum*) under influence of high temperature and relative humidity. When ZON is ingested, it may can be transformed into metabolites (ZAN,α-ZOL,α-ZAL, β-ZAL and β-ZOL) which also have estrogenic properties and lead to fertility reduction. Some ZON toxic effects are related the development of defective sperm and oocytes, abortion, reduced size and weight, changes on progesterone and estradiol levels. ZON and their metabolites have been found in diets of swine, poultry and cattle, causing serious economic losses, in addition to providing risk to human health, as may be present in meat and

liver of animals. This revision presents updated overview regarding ZON, metabolites characteristics and their effects on swine performance.
Keywords: zearalenone, swine, metabolites, toxicity.

Zearalenona, metabólitos e seus efeitos no desempenho da reprodução suína: uma revisão

Resumo

Alterações no nível do hormônio estrogênico durante o crescimento suíno pode levar a sérios problemas no desempenho reprodutivo desses animais. Um dos fatores que pode contribuir para mudanças no nível desse hormônio é a presença de zearalenona (ZON) em ingredientes e rações. ZON é produzida por fungos de espécies *Fusarium* (F*.culmorum, F. graminearum* e *F. tricinctum*), sob influência de alta temperatura e umidade relativa. Quando a ZON é ingerida, ela pode ser transformada em seus metabólitos (ZAN, α-ZOL, α-ZAL, β-ZAL e β-ZOL), os quais também têm propriedades estrogênicas e conduzem a uma redução de fertilidade. Alguns efeitos tóxicos da ZON estão relacionados ao desenvolvimento de defeitos em espermatozóides e ovócitos, aborto, redução do tamanho e peso, mudanças nos níveis de progesterona e estradiol. ZON e seus metabólitos já foram encontrados em rações de suínos, aves e bovinos, ocasionando graves perdas econômicas, além de propiciar risco à saúde humana, uma vez que podem estar presentes na carne e no fígado desses animais. Esta revisão apresenta um panorama atualizado sobre ZON, características de seus metabólitos e efeito sobre o desempenho de suínos.
Palavras-chave: zearalenona, suínos, metabólitos, toxicidade.

Introduction

Mycotoxins are produced by fungi and can be found contaminating either feed ingredients and/or in the final product due to the low grain quality utilized and/or the environmental conditions allowing them to grow. The main toxins affecting animals are aflatoxins, ochatoxin A, fumonisins, zearalenone (ZON) among others. Swine are highly sensitive, especially to ZON toxic effect (Figure 1).

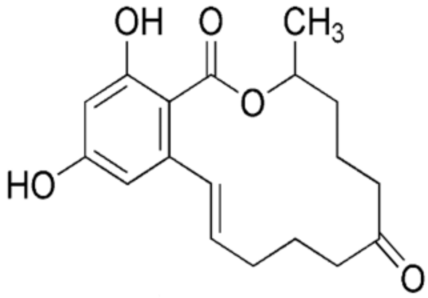

Figure 1. Chemical structure of zearalenone.

ZON is produced by several species of *Fusarium* such as *F. culmorum*, *F. graminearum,F. tricinctum* among others (Gajecka *et al.* 2011; Liu *et al*., 2012) (Table 1) which are prevalent in temperate regions and commonly found in cereals grown in America, Europe, Africa and Asia continents (Creppy, 2002; Tiemann *et al.* 2003). The production of ZON by *Fusarium* is influenced by the interaction of several factors such as moisture content, relative humidity and especially by temperature variation (Table 1).

The highest amounts of ZON produced by *Fusarium* species have been observed below 25^{o}C, at high amplitude of daily temperature and at 16% humidity (Zwierzchowski *et al.* 2005; Nuryono *et al.* 2005; Kinani *et al.* 2008).

ZON can be found in several cereal crops such as maize, barley, wheat, oats, sorghum and sesame seeds, as well as in hay and corn silage (Marin *et al.* 2010). Different animal's species including humans can acquire problems by consuming food contaminated by ZON, and swine are the species most sensitive to this feed contaminant.

Considering that swine are the most ZON sensitive animal species and the need of updating information regarding its derivatives, the purpose of this revision is to present an overview of the relevant work carried out in de last 15 years regarding their characteristics, effects on swine performance, feed contamination and regulation.

Table 1. Majors zearalenone fungi producing species and their optimal development temperature

Fusarium species	Temperature (°C)		
	Optimal	**Minimum**	**Maximum**
F. avenaceum	25	-3	31
F. crockwellense (F. cerealis)	21	0	30
F. equiseti	21- 30	-3	>35
F. graminearum	25	0	31
F. oxysporum (F. redolens)	25-30	5	37
F. tritinctum	22-23	0-10	31- 32.5
F. culmonorum	25	0	31

(Samson and Hoekstra, 2004)

Zearalenone and metabolites formation and characteristics

When a contaminated feed is ingested by the animal, ZON is absorbed and metabolized by the intestine and liver tissues through redox reactions producing several metabolites: zearalanone (ZAN), α-zearalenol (α-ZOL), α-zearalanol (α-ZAL), β-zearalanol (β-ZAL) and β-zearalenol (β-ZOL) which have similar chemical structures estrogenic hormons. Figure 2 presents ZON and metabolites chemical structures with their similarities to the natural estrogens (estradiol, estrone and estriol). ZON and metabolites lead to negative reproductive effects by changing estrogen hormone natural balance. They are considered endocrine disruptors since they regulate hormonal activity at the pre-receptor level (Penning *et al.* 2004).

Swine have been found to convert ZON predominantly to α-ZOL in the liver, the small intestines and even in granulose cells (Fink-Gremmels and Malekinejad, 2007). According to studies carried out by Malekinejad *et al.* (2006a) there are differences between animal species in the hepatic biotransformation of ZON. The authors demonstrated that swine seem to convert ZON mainly into α-ZOL, whereas in cattle β-ZOL is the dominant hepatic metabolite.

In several animal species and probably in humans, α- and β-ZOL are produced through ZON reduction, in the liver by 3-α/β hydroxysteroid dehydrogenases (Ayed *et al.,* 2011). These enzymes play an essential role in the homeostasis of the natural occurring steroid hormones (Figure 3). They catalyze not only the final step in the biosynthesis of androgens, estrogens, and progesterone, but also convert the receptor active keto-steroids into their less active reduced forms, thus regulating the hormone activity at the pre-receptor level (Malekinejad *et al.,* 2006b).

The formation of α-ZAL from ZON or α-ZOL is controversial because α-ZAL is also the active component in commercial anabolic growth promoters. Which has been allowed their use in some countries including the United States, but are banned in the European Union (Sorensen and Elbaek, 2005). Complications in pharmacokinetic distribution and secondary effects attributed to other unidentified factors can make it difficult to decipher the direct toxicity mechanism of α-ZOL to the cells (Yang *et al.,* 2007). The major metabolites of α-ZAL are ZAN and β-ZAL by oxidoreduction and a stereoisomerism reactions, respectively (Zheng *et al.,* 2011).

Important to emphasize that, although these metabolites are produced mainly by the animal metabolism, they can be also produced by fungi, however at much lower concentrations though. The biotransformation of ZON by *Fusarium* produces mainly α-ZOL and β-ZOL (Kuiper-Goodman *et al.*, 1987).

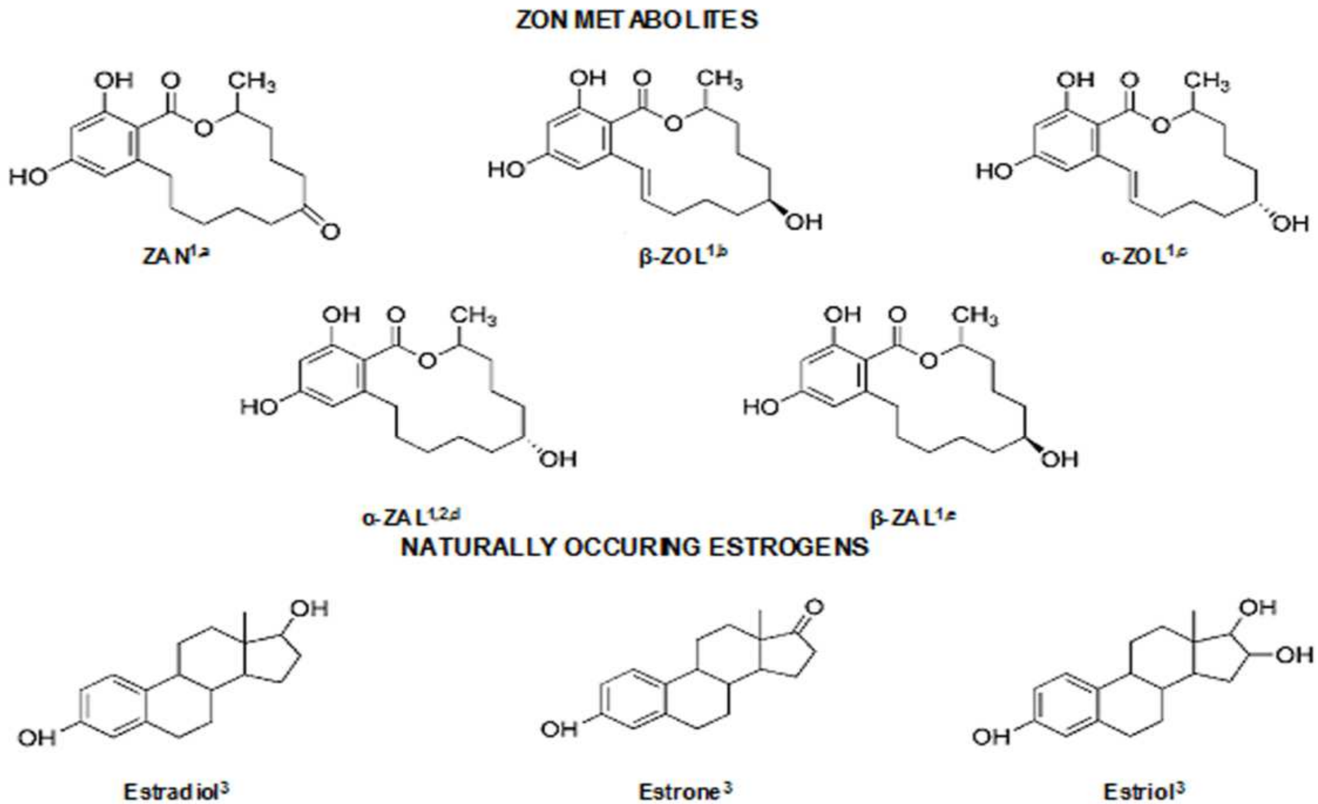

Figure 2. Molecular structures of ZON, its metabolites and the naturally occurring estrogens.[a]ZAN: zearalanone;[b]β-ZOL: β-zearalenol;[c]α-ZOL: α-zearalenol;[d]α-ZAL: α-zearalanol; [e]β-ZAL: β-zearalanol; ([1]Produced by fungi and in the animal body; [2]used as hormone in animals. [3]only produced in the body).

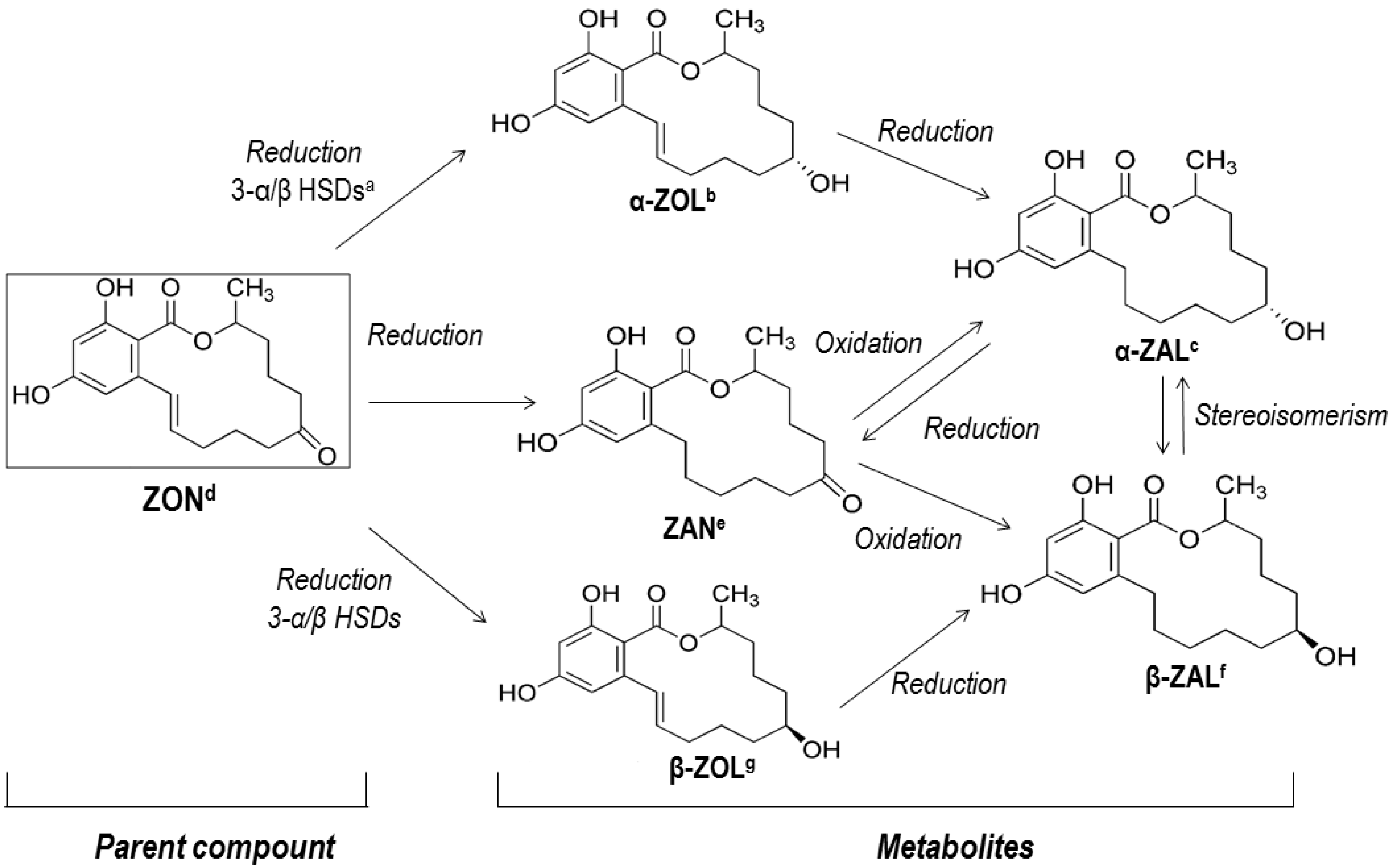

Figure 3. Biotransformation of zearalenone in its metabolites by swine liver and intestinal tissues cells ([a] 3-α/β hydroxysteroid dehydrogenases; [b] α-zearalenol; [c] α-zearalanol; [d] zearalenone; [e] zearalanone; [f] β-zearalanol; [g] β-zearalenol).

Zearalenone and metabolites physical-chemical properties

Chemically, ZON ($C_{18}H_{22}O_5$) is a resorcyclic acid lactone, described as 6-[10-hydroxy-6-oxo-*trans*-1-undecenyl]-B-resorcyclic acid lactone (Zinedine *et al.*, 2007). It is white in color, crystalline in structure, has a melting point of 164-165 °C and molecular weight of 318.36. It is insoluble in water, however soluble in aqueous alkali and various organic solvents (Döll andDänicke, 2011). The name is derived partly from the generic name of the *host plant* infected by *Fusarium* (corn = *Zea*) and partly from its chemistry (*ral* = from resorcylic acid lactone, *en* = from double bond at C-1-2, and *one* = from ketone) (Urry *et al.,* 1966).

ZON is a stable, either during the storage/milling and processing/cooking conditions, and does not decompose at high temperatures (Atoui *et al*., 2012). The fluorescence properties of ZON are sensitive to the toxin environment and is modulated by solvent, pH, and water quenching phenomena (Appell and Bosma, 2011). ZON and some of its derivatives develop a blue-green fluoresce under ultraviolet radiation (360 nm) and is even more intense when irradiated at 260 nm (Agag, 2004). However the fluorescence decreases with the double bond C_{11}-C_{12} reduction in the ZOLs metabolites (Miles *et al*., 1996).

The five metabolites chemical structure differences are related to the (a) double bonds between C_{10} and C_{11} and (b) hydroxyl or ketone group at C_6. Their molecular formula vary with the hydrogens number, being for ZAN, α-ZAL, β-ZAL, α-ZOL and β-ZOL of $C_{18}H_{24}O_5$, $C_{18}H_{26}O_5$, $C_{18}H_{26}O_5$, $C_{18}H_{24}O_5$ and $C_{18}H_{24}O_5$, respectively. Other differences are the isomery for the α and β metabolites. Due to those differences the properties slightly differ: density (1.148 to 1.174), boiling point (576 to 599°C) and refractive index (1.526 to1.548) (Table 2).

Table 2. Physical-chemical properties of zearalenone and its metabolites

Compound	Molecular formula	Mass (g/mol)	Density (g/cm^3)	Boiling point (°C)	Refractive index	CAS Number
Parent						
ZON	$C_{18}H_{22}O_5$	318.364	1.169	600.396	1.539	17.924-92-4
Metabolites						
ZAN	$C_{18}H_{24}O_5$	320.380	1.148	576.8	1.526	5.975-78-0
α-ZAL	$C_{18}H_{26}O_5$	322.396	1.153	576	1.535	26.538-44-3
β-ZAL	$C_{18}H_{26}O_5$	322.396	1.153	576	1.535	42.422-68-4
α-ZOL	$C_{18}H_{24}O_5$	320.380	1.174	599	1.548	36.455-72-8
β-ZOL	$C_{18}H_{24}O_5$	320.380	1.174	599	1.548	71.030-11-0

CAS (2011)

Toxic effects of zearalenone and metabolites on swine

The toxic effects of ZON and its metabolites, described as having estrogenic properties, are related to the chemical structure of this mycotoxin, which is similar to the naturally occurring estrogens, estradiol, estrone and estriol (Gromadzka *et al*., 2009). Therefore they reproductive system is the major target of ZON toxicity.

ZON binds to estrogen receptors, causing functional and morphological changes in the responsive reproductive organs (Shier *et al*., 2001). It also inhibits protein and DNA synthesis and triggers lipid peroxidation and cell death (Ayed-Boussema *et al*., 2011). ZON has been shown to be immunotoxic and genotoxic, and to induce DNA-adduct formation *in vitro* cultures of bovine lymphocytes . It has a rather low oral acute toxicity; however the sub chronic and chronic toxicities are dominated by its estrogenicity. ZON is rapidly absorbed after oral administration. ZON has low acute toxicity after either oral or interperitoneal administration in mice, rats and guinea swine (oral LD_{50} values of >4000 up to >20,000 mg/kg bw) (JECFA 2000). It intoxication is associated with decreased fertility, to reduced litter size, changed weight of adrenal, thyroid, pituitary glands in offspring and change in serum levels of progesterone and estradiol (Table 3).

Swine during growth, when affected by ZON, develop several changes such as reduction of fertility, reduced litter size, low weight, as well as progesterone and changes on estradiol serum levels (Table 3).

Estrogenic effects of ZON on *gilts* and *sows* include edematous uterus, ovarian cysts, increased follicular maturation and number of stillborns and decreased fertilization rate (Zain, 2011) (Figure 4). It can also be observed intensification of cell proliferation in the uterus and oviduct, swelling of the vulva and mammary glands, pseudo pregnancy through prolonged estrus intervals (Fink-Gremmels and Malekinejad, 2007; Mizutani *et al*., 2011; Briones-Reyes *et al*., 2007).

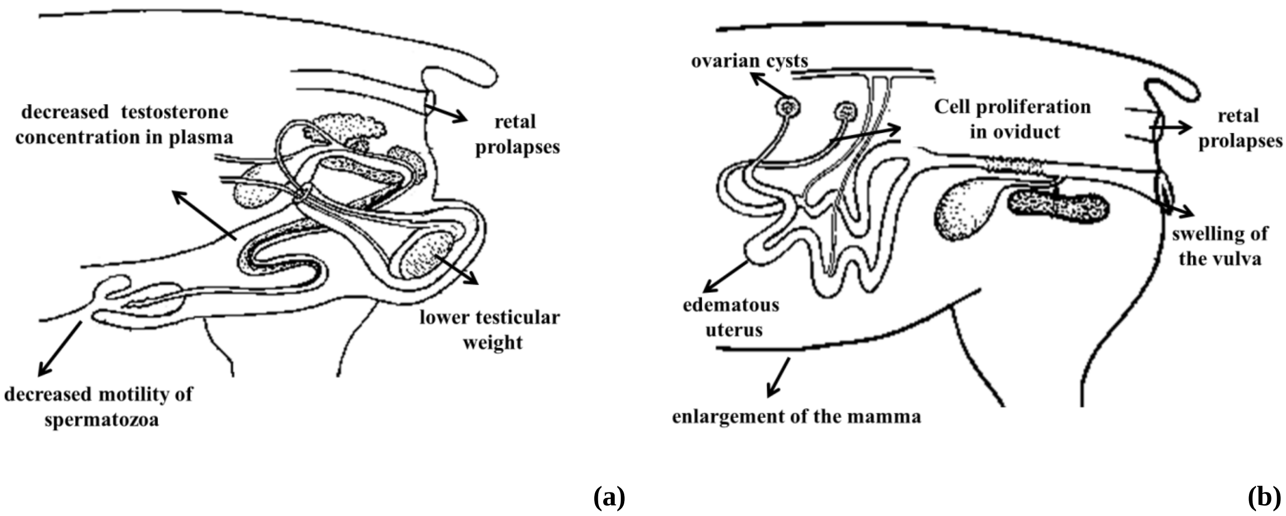

Figure 4. Swine reproduction organs alterations by ZON feed contamination: (a) *boars* and (b) *gilts/sows.*

Gilts: gilts are more sensitive than sows (Edwards *et al*., 1987). Research indicated that feeding feed to *gilts* contaminated with low concentrations of ZON (0.235 to 0.358 mg/kg) significantly reduced the intrinsic quality of the oocyte collected from these animals (Alm *et al*., 2006). After administration of a single dose of ZON at 200 μg/kg body weight (μg/kg bw) in sexually immature gilts, α-ZOL was the main metabolite present in the blood at nanogram levels (Benzoni *et al*., 2008). *Sows:* in the sows reproduticve cycle, ZON contamination at levels of 5 to 10 mg/kg in feed causes, after weaning, a prolonged cycle or even anestrus (Meyer *et al*., 2000).

Table 3. Zearalenone toxic effect on swine growth at different stages and time of exposure versus levels of contamination

Swine	Age (days)	Weight (kg)	Level of contamination (µg/kg)	Toxic effects	Reference
Piglets					
	NI	6	250	Uterus hyperemia and blood vessel dilatation Concentrations of serum protein and albumin decreasing	Feng *et al*., 2008
Boar					
	77	23- 54	40	Reduced libido, associated with a decreased testosterone concentration in plasma	Berger et al., 1981
Gilts					
	60	40	20-40	Endometrial hyperaemia and advanced hyalinization of the endometrial connective tissue	Gajecka *et al.*, 2011
	75	26.50	0-2000	Increased uterus weight and vulva width	Wang *et al.*, 2010
	180	103	4-358	Histopathological alterations with different degrees on glycogen reduction and increase of hemosiderine particles in the liver cells	Tiemann *et al.*, 2006
	180	103	210-9570	Regulation of oocyte	Alm *et al.*, 2006
	NI	50	20-40	ZON do not induce apoptosis in porcine ovaries, and the inhibition of proliferation must be associated with other mechanisms	Wasowicz *et al.*, 2005
Sows					
	315	198	358	Changes in liver and spleen tissues	Tiemann*et al.*, 2008
	NI	153-197	3.5- 48	Lower serum activities	Goyarts *et al.*, 2007

NI: not informed

However, little information is available on the negative effects of low concentrations of ZON (1.05 mg/kg diet) on nutrient availability, quantitative data about vulva and testis, histological damages on genital organs, and serum hormones in gender-dependent manner so far (Jiang *et al*., 2012).

Piglets: studies have examined the possible impact of ZON during pregnancy and litter. When gilts/sows ingest ZON at doses of 100, 200, or even 400 μg/kg bw during pregnancy, the result is fetal death and/or reduction of neonatal weight (Gajecka *et al*., 2011). The influence of ZON on litter size can be explained by a negative impact on fertilization, but also by embryonic and fetal death of the piglets (Kanora and Maes, 2009). The piglets can be exposed in the utero as well as via sow's milk. Clinical symptoms indicative of exposure to ZON are essentially the same as described for adult animals (Fink-Gremmels and Malekinejad, 2007). Some piglets symptoms are reddening and swelling of the vulva, necrosis of the tail, enlargement of the mamma, weakness or splay-leg and vaginal and/or rectal prolapses (Etienne and Dourmad, 1994; Malekinejad *et al*., 2005).

Boars: in toxicological experiments the effects of ZON on reproductive performance have been observed in *boars* such as lower testicular weight and decreased motility of spermatozoa (Figure 4). A reduced libido, associated with a decreased testosterone concentration in plasma. Moreover, exposure of boar semen to ZON or α-ZOL at concentrations of 40 to 80 μg mL^{-1} of diluted semen induced significant reductions in sperm motility, viability and binding ability to zone pellucid (Tsakmakidis *et al.,* 2007). β-ZOL exclusively affected motility parameters (Benzoni *et al*., 2008).

The metabolites α-ZAL, ZAN and β-ZAL which have endocrine-related biological activity, are less biologically active than α-ZAL (Zheng *et al.,* 2011). Although these metabolites have different structures, they cause similar effects in swine (Table 4). Among these metabolites, α-ZOL, α-ZAL, and β-ZAL have relatively higher estrogenic activity than that of ZON as follows: α-ZOL >α-ZAL >β-ZAL > ZON >β-ZOL (Shier *et al.,* 2001).

Table 4. Zearalenone and its metabolite toxic effects of swine

Compoud	Toxic effects	Reference
Parent		
ZON	Functional and morphologic changes in the reproductive organs	Shier *et al*., 2001
Metabolites		
ZAN	Estrogenic potency	Marin *et al*., 2010
α-ZAL	Fetal development deffects	Trout *et al*., 2007
β-ZAL	Estrogenic potency	Marin *et al*., 2010
α-ZOL	Fertilization ability of boar sperm defects	Benzoni *et al*., 2008
β-ZOL	Increasing maturation of swine oocytes in vitro defects	Frizzell *et al*., 2011

Regulation forzearalenone worldwide

The exposure to contaminated food, the kinetic parameters including absorption, distribution in the body, metabolism and excretion, determine the doses and the toxin concentrations at target sites (Fink-Gremmels and Malekinejad, 2007). Concerned with the estrogenic action of ZON, several countries established levels of tolerance to avoid control of food contaminating. For human consumption some countries including Austria, Brazil, France, Italy, Romania, Russia, and Uruguay, have specific regulations for ZON, ranging from 0.03 to 1000 μg /kg, applied to either specific foodstuffs or all foods (FAO, 2004; Brazil, 2011). For feeding gilts, sows, piglets and swine only 8 countries joined a specific legislation for ZON (Table 5). European Committee has limited its concentration to 100 μg ZON/kg in piglets and gilts diets (EC, 2006). The lowest limit allowed in Ukraine was determined by combined feed for sows (pregnant, feeding), breeding boars, and piglets younger than 2 months which is 40 μg/kg. Canada is the country that has the highest limit allowed to feed for gilts and sows with 3000 μg/kg. The same limit was set by Ukraine for combined feed for swine (for pork) over 50 kg of weight. European regulation does not consider residues from animal products in the assessment of ZON exposure for humans, assuming that "secondary human exposure resulting from meat, milk and eggs is expected to be low, contributing only marginally to the daily intake" (EFSA, 2004).

Table 5. Swine feed zearalenone tolerance levels for established for from different countries by FAO

Country	Products for swine			
	Complete feeding stuffs	Tolerance (µg/kg)	Feed	Tolerance (µg/kg)
Austria	NI	NI	Breeding-swine	50
Canada	NI	NI	Gilts and sows	3000
Cyprus	Piglets	1000	NI	NI
	Swine other than piglets	1500	NI	NI
Estonia	Swine and other young farm animals	100	NI	NI
Lithuania	NI	NI	Piglet	300
	NI	NI	Swine	100
Serbia/ Montenegro	NI	NI	Swine (until 50kg)	500
	NI	NI	Other type of swine	1000
Slovenia	NI	NI	Swine	1000
Ukraine	Sows, breeding boars, piglets	40	NI	NI
	Swine fed for pork lighter than 50 kg	2000	NI	NI
	Swine fed for pork over 50 kg of weight	3000	NI	NI

NI: not informed; (FAO, 2004)

Residues of zearalenone and its metabolites in foods of animal origin

Several studies have reported contamination of ZON in food for animals and humans consumption world wide (Table 6) at levels varying as low as 3 up to 165.000 µg/kg which have caused serious economic losses worldwide, apart from the risk of being transferred to meat/liver, food of human consumption. However, when it comes to detection of ZON metabolites, data are still poorly reported and studied (Table 7). The amount of detectable ZON in animal tissues depends on the contamination of feed, treatment of animals with ZON or ɑ- ZOL duration of exposure to the toxin, the persistence of ZON in the animal and species variation in response to the mycotoxin (JECFA, 2000)..

The great concern of ZON metabolites residues in food is due to its biotransformation in the animal organism originating their residues in meat and liver manly. In Puerto Rico, residues of estrogenic compounds in red meat and poultry remain two of the most likely causes of premature thelarche (Saenz de Rodriguez and Toro-Sola, 1982).

Table 6. Levels of zearalenone detected internationally in feed for animal and human consumption

Animal/ Country	Samples		Zearalenone (μg/kg)		Contaminated Samples (%)		References
	Year (s)	Number	Mean	Range	LOD	Method	
Swine							
Portugal	NI	30	NI	104-356	50	TLC	Martins *et al*., 2008
Lithuania	1999	25	32	NI- 77	10	LC	Garaleviciene *et al.*, 2002
Vietnam	2005	24	86	10-295	10	ELISA	Thieu *et al*., 2008
Argentina	2005	240	ND	ND	100	TLC	Pereyra *et al*., 2008
Argentina	2008	10	ND	ND	100	LC	Pereyra *et al*., 2010
Brazil	1994-2010	105.509	74.1	NI-17.000	NI	NI	Mallmann *et al*, 2011
Cattle							
Spain	NI	11	ND	ND	NI	LC	Jaimez *et al*., 2004
Turkey	NI	40	175.26	51.61-1023.25	NI	ELISA	Aksoy *et al.*, 2009
Poultry							
Indonesian	2001	18	32.2	10.1–122	3	LC	Nuryono *et al.*, 2005
Slovak	2003-2004	50	21	ND-86	7	LC	Labuda *et al.*, 2005
Brazil	2003-2004	480	NI	100-7000	NI	TLC	Oliveira *et al.*, 2006
Lithuania	1999	27	27	NI-83	10	LC	Garaleviciene *et al.*, 2002

NI: not informed; ND: not detect; LC: liquid chromatography of high performance; ELISA: enzyme-linked immunoabsorbent assay TLC: thin layer chromatography.

Table 7. Residues of zearalenone metabolites found in animal and human foods

Food	Metabolites	Samples		ZON (μg/kg)			Method	References
		Type	Number	Mean	Range	(%)		
Animals								
	β-ZOL	Milk	53	NI	ND- 73.24	28	LC	Meucci *et al.*, 2011
	α-ZOL	Feed	7	ND	ND	ND	LC	Saeger *et al.*, 2003
Humans								
	ZOLs	Grasses	33	4.9	1.1-15	18	LC	Zheng *et al.*, 2011
	ZOLs	Beer	15	ND	ND	ND	LC	Maragou *et al.*, 2008
	α-ZOL	Corn	25	NI	36-71	24	LC	Cerveró *et al.*, 2007

NI: not informed;LC: liquid chromatography

In a study with piglets fed diets with increasing ZON concentrations (0.01, 0.06, 0.15, 0.22, 0.42 μg/kg) for 5 weeks, the mean total ZON (parent) and α-ZOL (metabolite) concentrations detected in the liver were 1.8/0.3, 0.2/0.1, 2.1/1.1, 2.9/1.7 and 5.3/2.8 μg/kg for ZON/α-ZOL, respectively (Döll *et al.*, 2003). In a similar study carried out with swine (fed a diet containing 700 μg ZON/kg for 18 days), the maximum level of parent and metabolites (α-ZOL and β-ZOL) were 3.1, 12 and 4.8 µg/kg detected in the liver of while α-ZAL and α-ZOL contents of up to 13.3 and 14.5 µg/kg were detected in the meat, respectively (EFSA, 2011).

Measures to control spread of zearalenone

ZON is formed in the grains by *Fusarium* during the pre-harvest period; therefore it is necessary to control those fungi growing conditions in order to reduce toxins production. Applying control measures to reduce *Fusarium* proliferation can decrease the ZON formation and swine feed contamination. *Fusarium* species are probably the most prevalent toxin-producing fungi of temperate regions. Thus those typical temperatures, humidity and their abrupt variation allow *Fusarium* fungi growth (Creppy, 2002). Wheat, triticale and maize grains are vulnerable to *Fusarium* infection and are more contaminated with ZON than to other cereal (Döll and Dänicke, 2011). Environmental and other conditions allow fungal colonization, therefore the control should start from temperature reduction, relative humidity evaluation and insect infestation control (Zain, 2011). Moreover, detoxification strategies for contaminated foods and feeds to reduce or eliminate the toxic effects of ZON by chemical, physical, and biological methods are crucial to improve food safety and prevent economic losses (Zinedine *et al*., 2007). Despite this, measures to *prevent* ZON accumulation are the most effective approach to reduce exposure to that mycotoxin (Habschied *et al.,* 2011).

Methods of detection of Zearalenone and metabolites

Several analytical methods have been developed to separate and detect these macrocyclic lactone mycotoxins in different samples such as foods, animal feeds and complex biological matrices (Andres *et al*., 2008). Many popular detection methods rely on the native fluorescence of ZON; a property associated with the electron rich resorcylic acid moiety of this toxin. These fluorescence based methods include LC methods coupled with fluorescence detection, capillary electrophoresis, and immunoassays (Appell and Bosma, 2011). High-performance liquid chromatography with fluorescence detection is usually chosen for the determination of ZON, α and β-ZOL due to their natural fluorescence (Saeger *et al.*, 2003). Other methods including gas chromatography coupled with flame ionization detection or with mass spectrometric detection, TOF (time of the flight) mass spectrometric and enzyme-linked immunosorbent assays (ELISA) (Pérez-Torrado *et al*., 2010; Andres *et al*., 2008). Although ELISA is selected for rapid qualitative screening, it mostly fails in providing accurate quantitative results and a definite confirmation of the toxin. Better suited to the purpose is the commonly used GC or LC combined with different detectors, given their good performances in terms of accuracy, precision, sensitivity, and reproducibility (Liu *et al*., 2012). Regarding LC coupled to tandem mass spectrometry a multi-toxins method develop by Driehuis et al. (2010) included ZON and its metabolites, allowing them to be quantify all together.

Conclusion

ZON and its metabolites are important contaminants that affect animal's health, and through pork, can by transfer to humans. Although there is lack of information regarding ZON and its metabolites toxic effects in humans, data on reproductive performance in animals have being registered and the possibility of their effects in humans can not the excluded.

Oxidation and reduction reactions can cause ZON biotransformation, through enzymes such as 3-α/β HSDs into α - and β-ZOL, α - and β-ZAL and ZAN. Swine are the most sensitive species to these toxins estrogenic effects in different intensities among piglets, gilts, sows and boars. The age, weight and period of exposure affect the degree of toxicity and the decrease on fertility. Reduction of litter size, stillbirth and weight change are the symptoms observed. Only eight countries in the world have specific regulation for ingredient and feed for swine at levels varying from 40 to 3000 µg/kg. Levels that may soon be changed, due to numerous studies demonstrating the negative effect on swine health and performance, to levels even lower. There is a need for improvements in the levels tolerable in food for these contaminants. The application of measures for fungi proliferation control of *Fusarium* and production of ZON is of fundamental importance. Temperature and humidity in the period of harvest of grains and cereals should be controlled; application of *Fusarium* resisted grain variety also should by applied. The use of toxins absorbents are another measure being adopted, but still with the need of further studies to better applicability and efficiency.

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