Animal (2013), **7:4**, pp 699–703 © The Animal Consortium 2012
doi:10.1017/S1751731112002066

animal

Soybean isoflavone reduces the residue of zearalenone in the muscle and liver of prepubertal gilts

D. F. Wang[1,2], H. L. Zhou[1], G. Y. Hou[1], D. S. Qi[2†] and N. Y. Zhang[2]

[1]*Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Ministry of Agriculture, Danzhou, Hainan 571737, PR China;*
[2]*Department of Animal Nutrition and Feed Science, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China*

(Received 10 October 2011; Accepted 16 August 2012; First published online 22 November 2012)

The aim of the present study was to evaluate the protective effects of isoflavone (ISO) against zearalenone (ZEA) residues in the muscle and liver tissues of prepubertal gilts. Seventy 75-day-old, prepubertal, female pigs (Duroc × Landrace × Yorkshire, 26.5 ± 0.60 kg) were allocated randomly to seven diet treatments for 21 days as follows: one control group (fed the basal diet) and six groups fed the basal diet with the addition of either 0.5 or 2.0 mg/kg ZEA plus either 0, 300 or 600 mg/kg ISO. The results showed that the diet with 2.0 mg/kg ZEA added caused an increase of ZEA residue level in muscle tissue ($P < 0.05$*), and that the addition of both 0.5 and 2.0 mg/kg ZEA increased the residue level of ZEA in the liver of prepubertal gilts (*$P < 0.05$*). Addition of 600 mg/kg ISO to 2.0 mg/kg ZEA-contaminated diet decreased the ZEA residue level in liver tissue (*$P < 0.05$*), and the addition of 300 or 600 mg/kg ISO to the 2.0 mg/kg ZEA-contaminated diet decreased the residue levels of ZEA in muscle tissue (*$P < 0.05$*). Western blot analysis demonstrated that feeding ZEA to prepubertal gilts increased their protein expression of 3α/3β-hydroxysteroid dehydrogenase (HSD;* $P < 0.05$*), and that the addition of 300 or 600 mg/kg ISO to the 2.0 mg/kg ZEA-contaminated diet decreased the protein expression of 3α/3β-HSD (*$P < 0.05$*), compared with the addition of 2.0 mg/kg ZEA alone. The results demonstrated that muscle and liver tissues retain residual ZEA when pigs are fed a diet contaminated with high concentrations of ZEA, and that the concentration of ZEA in muscle and liver tissues increased with increased amounts of ZEA in the feed. In diets contaminated with high levels of ZEA, the addition of ISO may accelerate the biotransformation and degradation of ZEA and its metabolites, and reduce the residues of ZEA in liver and muscle tissues of prepubertal gilts.*

Keywords: soybean isoflavone, prepubertal gilts, zearalenone residue, 3α/3β-HSD expression

Implications

Maize and soybean are two of the principle ingredients in animal feed, and as such zearalenone (ZEA) and soybean isoflavones (ISOs) are often found together in maize–soybean meal-based feed. Our study shows that diets containing ISO may accelerate the biotransformation and degradation of ZEA and its metabolites, and thus reduce the residues of ZEA in liver and muscle tissues. These findings suggest a potential means of mitigating the negative effects of high concentration of ZEA contamination in feed.

Introduction

Zearalenone (ZEA) belongs to a group of mycotoxins with strong estrogenic effects. It is produced as a secondary metabolite by a number of *Fusarium* species, including *Fusarium culmorum* and *Fusarium graminearum* (Hestbjerg *et al*., 2002; Glenn, 2007), and is common in maize and wheat (Yamashita *et al*., 1995; Binder *et al*., 2007; Krska *et al*., 2007). Among domestic animals and poultry, prepubertal female pigs appear to be the most sensitive to ZEA (Fink-Gremmels and Malekinejad, 2007). ZEA is rapidly absorbed after oral administration, and edema and reddening of the vulva are external clinical signs of ZEA intoxication in pigs (Zinedine *et al*., 2007). Malekinejad *et al.* (2006) reported that in pigs ZEA was converted into α-zearalenol (α-ZEA), predominantly catalyzed by 3α-hydroxysteroid dehydrogenase (3α-HSD). Soybean isoflavones (ISOs) are a subgroup of flavonoids found predominantly in soybean (Reinli and Block, 1996) at concentrations ranging from 0.01% to 0.5% (Wang and Murphy, 1994). ISO is primarily active as an estrogen in low-estrogen environments and as an estrogen competitor in high-estrogen environments (Hwang *et al*., 2006). In addition, ISO has been shown to have antioxidant effects (Hsieh *et al*., 2009).

Maize and soybean are two of the principle ingredients in animal feed, and therefore ZEA and ISO often coexist in

† E-mail: qds@mail.hzau.edu.cn

maize–soybean meal-based feed, where they may interact. It is generally believed that ZEA has negative effects on animal production and human health (Zinedine *et al.*, 2007), whereas, conversely, ISO has potentially beneficial effects on animal production(Greiner *et al.*, 2001; Payne *et al.*, 2001). Therefore, the present study focused on whether ISO could increase or reduce ZEA residues in muscle and liver tissues of prepubertal gilts. It was hoped that the study would suggest means of mitigating the negative effects of ZEA contamination of feed.

Material and methods

Animals and experimental design

The experiment was approved by the Institutional Animal Care and Use Committee at Huazhong Agricultural University, Wuhan, China, and conducted in accordance with the National Institute of Health guidelines for the care and use of experimental animals.

Seventy 75-day-old, prepubertal, female pigs (Duroc × Landrace × Yorkshire) with an average body weight of 26.50 ± 0.60 kg were allocated to seven diet treatment groups and treated for 21 days as follows: (1) control group, fed the basal diet, (2) control+0.5 mg ZEA/kg, (3) control+0.5 mg ZEA/kg+300 mg/kg ISO, (4) control+0.5 mg ZEA/kg+600 mg/kg ISO, (5) control+2.0 mg/kg ZEA, (6) control+2.0 mg/kg ZEA+300 mg/kg ISO and (7) control+2.0 mg/kg ZEA+600 mg/kg ISO. The concentration of ISO (300 or 600 mg/kg) corresponds to either 15% or 30% soybean in the diet, respectively. The concentration of ZEA (0.5 or 2.0 mg/kg) corresponds to either a low or high degree of ZEA contamination in the feed. Each treatment was replicated five times with two pigs per replicate. The pigs were housed in concrete floored indoor pens (two pigs of one replicate per pen), and all pigs had free access to feed and water throughout the 21-day study.

Diet preparation

Soybean ISO (extracted from soybean, purity >99%) was obtained from Chengdu Tianyuan Natural Product Co., Ltd (Chengdu, China), and its composition was as follows: daidzein 531.1 mg/g, genistein 321 mg/g, daidzin 90.1 mg/g, glycitin 45.2 mg/g, genistin 17.1 mg/g and glycitein 2.1 mg/g, as determined according to Heimler *et al.* (2004). ZEA (purity >99%), obtained from Sigma-Aldrich (St. Louis, MO, USA) was diluted gradually to 1 mg/g before use with defatted rice bran. The basal diet was formulated to meet National Research Council (NRC, 1998) nutrient requirements, and did not contain soybean meal. The concentration of ISO (300 or 600 mg/kg) corresponds to 15% or 30% soybean in the diet, respectively. The ingredients and chemical composition of the basal diets fed to the control group is listed in Table 1. The concentration of mycotoxin in basal diet was determined according to Gimeno (1979) and Xu *et al.* (1988).

Tissue collection and determination of ZEA

At the end of the experiment, five pigs were selected from each treatment group (one pig at random from each replicate), fasted for 24 h, stunned electrically and exsanguinated. The pigs' bodies were dissected, and the excised liver and *longissimus dorsi* muscle were stored at −20°C for subsequent determination of ZEA concentration. Partial samples of liver were excised, frozen in liquid nitrogen and stored at −70°C for subsequent protein extraction.

The residues of ZEA in liver and muscle tissues were measured using an enzyme immunoassay mix kit (R-Biopharm, R1401, Darmatadt, Germany), according to the manufacturer's protocol.

Table 1 *Ingredients and chemical composition of the basal diet*[a]

Ingredients	g/kg	Calculated composition	g/kg
Maize	723.6	DE, MJ/kg	13.4
Rice protein meal	135.0	Lysine	7.5
Wheat bran	40.0	Methionine	2.5
Rapeseed meal	30.0	Met + Cys[c]	4.0
Fish meal	30.0	CP	176.0
L-Lysine HCl	1.4	Calcium	8.2
Vitamin–mineral premix[b]	40.0	Total phosphorus	5.0

DE = digestible energy.
[a]Mycotoxin levels in the basal diet (per kg feed): zearalenone, 50 μg; aflatoxin B1, 2.5 μg; deoxynivalenol, 200 μg.
[b]The vitamin–mineral premix provided (per kg feed): vitamin A, 5000 IU; vitamin D3, 1000 IU; vitamin E, 10 IU; vitamin K3, 1 mg; vitamin B1, 1.50 mg; vitamin B2, 4.00 mg; vitamin B6, 1 mg; vitamin B12, 0.02 mg; niacin, 20 mg; D-pantothenic acid, 8 mg; biotin, 0.1 mg; folic acid, 0.2 mg; Fe, 120 mg; Cu, 160 mg; Mn, 32 mg; Zn, 200 mg; I, 0.2 mg; Se, 0.15 mg; and chloride choline, 0.2 mg.
[c]Met + Cys stands for the total content of methionine and cystine.

Western blot analysis

For protein extraction, ~50 mg of liver tissue from five pigs from each treatment (one pig at random from each replicated) were homogenized on ice with a cold buffer containing proteinase, sonicated and then centrifuged at 12 000 × **g** for 10 min at 4°C. Supernatant was collected and stored at −20°C. The protein concentration was measured using the Bradford dye-binding procedure (Bradford, 1976) with bovine serum albumin as a standard. Protein lysate samples (50 μg) from the different treatment groups were boiled for 5 min in sample buffer, separated in 10% SDS–PAGE and transferred onto nitrocellulose membrane. After blocking in a 5% nonfat dry milk solution in washing buffer containing 10 mM Tris (pH = 7.5), 150 mM NaCl and 0.05% Tween 20, the membranes were incubated overnight at 4°C with a mouse-derived monoclonal antibody against 3α/3β-HSD (Abcam, USA, ab54606 and ab55268) at a dilution of 1 : 200, washed and then incubated for 2 h with a secondary antibody linked to the horseradish-peroxidase-labeled goat anti-mouse IgG (Wuhan Boster Biological Technology, Ltd, China, BA1050) at room temperature. Reactive protein was detected using an ECL chemiluminescence system (Amersham Pharmacia Biotech, UK). To measure the intensities of the bands, the electrophoresis films were scanned into a computer, and Photoshop software was used to convert the color into black/white image. Gray values of the bands (i.e. light intensities) were then measured using the image analysis software, AlphaEastFC.

Statistical analysis

The ANOVA analyses were performed on the premise of the homogeneities of variance by using SAS software. The results of the six experimental treatments were analyzed as a 2×3 factorial design using the ANOVA procedure of the SAS software (SAS Institute Inc., 1990), with ZEA and ISO supplementation levels and their interactions as main effects. These groups were then compared with each other using Tukey's multiple comparisons from the SAS program. The data are expressed as mean $\pm$ s.e. Differences were considered significant at the $P < 0.05$ level.

Results

The homogeneity of variance test showed no significant differences among the overall variances in any of the following ANOVA analyses. As shown in Figure 1, pigs fed on a diet supplemented with ZEA showed significantly increased ZEA residues in liver tissue ($P < 0.05$). ZEA levels in the liver tissue of pigs fed on diets supplemented with 0.5 or 2.0 mg/kg ZEA reached 291.14 and 1352.06 ng/kg, respectively, compared with the control group levels of 26.31 ng/kg. Simultaneous addition of 600 mg/kg ISO to the 2.0 mg/kg ZEA-contaminated diet decreased the ZEA level in liver tissue compared with the diet with 2.0 mg/kg ZEA alone ($P < 0.05$).

As shown in Figure 2, pigs fed on a diet supplemented with 2.0 mg/kg ZEA showed increased ZEA residue levels in muscle tissue ($P < 0.05$). Levels of ZEA in the muscle tissue of pigs fed the diet with 0.5 or 2.0 mg/kg ZEA added reached 9.71 ng/kg and 31.30 ng/kg, respectively, compared with the control group level of 8.51 ng/kg. Addition of 300 or 600 mg/kg ISO to 2.0 mg/kg ZEA-contaminated diet decreased residue level of ZEA in muscle tissue compared with the diet with 2.0 mg/kg ZEA added alone ($P < 0.05$).

Figures 3 and 4 demonstrated that the diet supplemented with ZEA increased the protein expression of 3α/3β-HSD in liver tissue compared with the control group ($P < 0.05$). Diets with both 0.5 mg/kg ZEA and either 300 or 600 mg/kg ISO had no significant effect on the expression of 3α/3β-HSD compared with the diets containing 0.5 mg/kg ZEA alone ($P > 0.05$). Pigs offered ISO and 2.0 mg/kg ZEA showed significantly lower protein expression of 3α/3β-HSD than those offered diets with 2.0 mg/kg ZEA alone ($P < 0.05$).

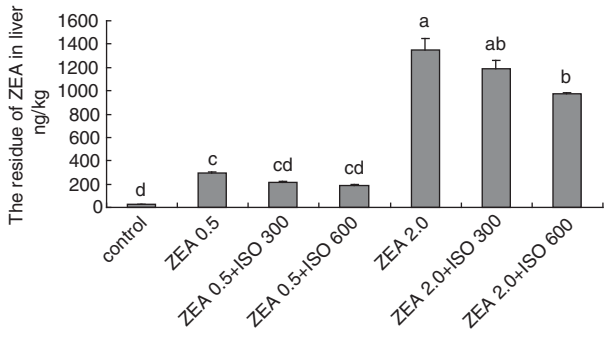

Figure 1 ZEA residue levels in liver tissue of prepubertal gilts. The data are expressed as means $\pm$ s.e., $n = 5$. *P*-values of the main effects among the six experimental groups are: ZEA, $P < 0.0001$; ISO, $P = 0.0014$; ZEA $\times$ ISO, $P = 0.0437$. Mean values with different letter superscripts are significantly different from each other ($P < 0.05$). ZEA = zearalenone; ISO = isoflavone.

Discussion

ZEA is absorbed rapidly after oral administration, and the degree of absorption is difficult to measure owing to extensive biliary excretion. The uptake by a pig after a single

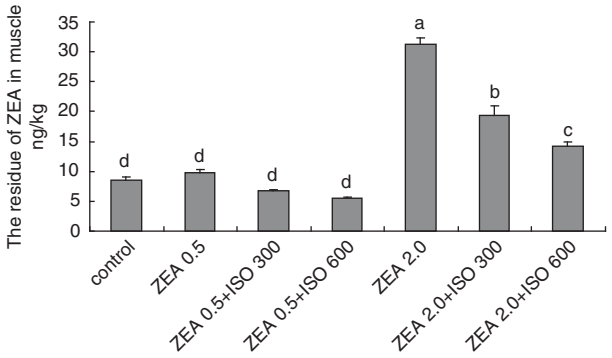

Figure 2 ZEA residue levels in muscle tissue of prepubertal gilts. The data are expressed as means $\pm$ s.e., $n = 5$. *P*-values of the main effects among the six experimental groups are: ZEA, $P < 0.0001$; ISO, $P < 0.0001$; ZEA $\times$ ISO, $P < 0.0001$. Mean values with different letter superscripts are significantly different from each other ($P < 0.05$). ZEA = zearalenone; ISO = isoflavone.

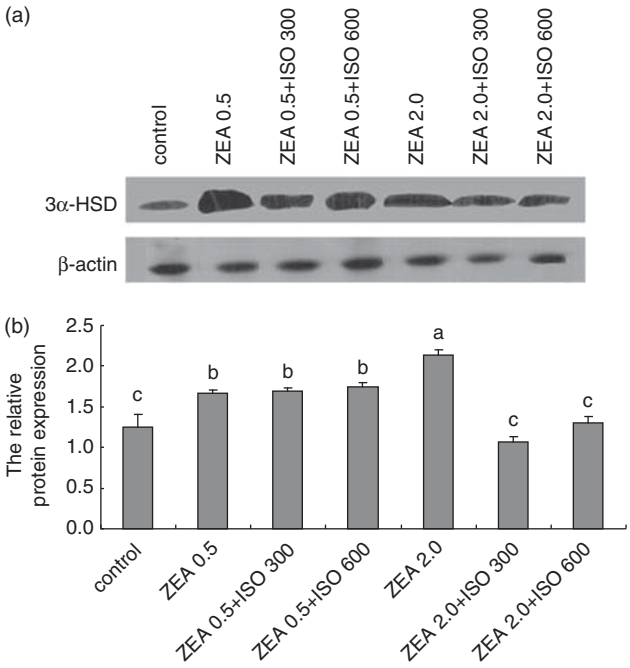

Figure 3 Western blot analysis of the expression of 3α-HSD protein in liver tissue of prepubertal gilts. (a) Western blot analysis of 3α-HSD. (b) The data show the relative levels of 3α-HSD protein in liver tissue of prepubertal gilts. Protein expression was expressed relative to β-actin bands. The data are expressed as means $\pm$ s.e., $n = 5$. *P*-values of the main effects among the six experimental groups are: ZEA, $P = 0.0074$; ISO, $P < 0.0001$; ZEA $\times$ ISO, $P < 0.0001$. Mean values with different letter superscripts are significantly different from each other ($P < 0.05$). HSD = hydroxysteroid dehydrogenase; ZEA = zearalenone; ISO = isoflavone.

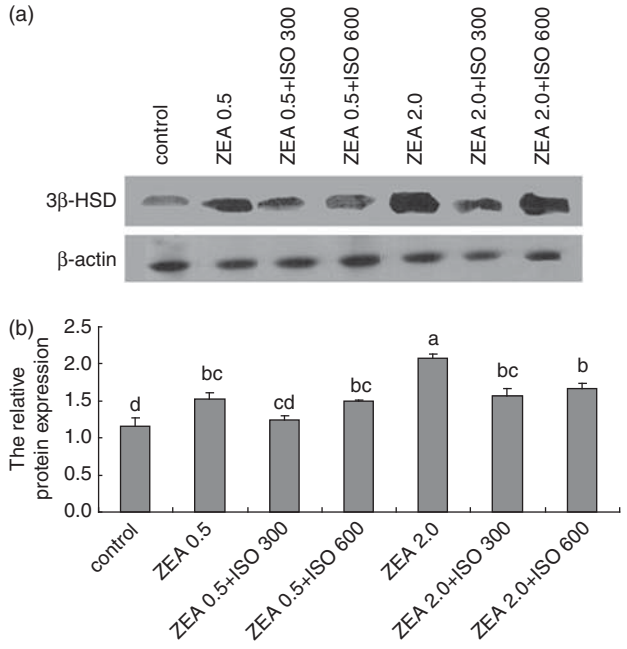

Figure 4 Western blot analysis of the expression of 3β-HSD protein in liver tissue of prepubertal gilts. (a) Western blot analysis of 3β-HSD. (b) The data show the relative level of 3β-HSD protein in liver tissue of prepubertal gilts. Protein expression was expressed relative to β-actin bands. The data are expressed as means ± s.e., $n = 5$. *P*-values of the main effects among the six experimental groups are: ZEA, $P = 0.0006$; ISO, $P = 0.0038$; ZEA × ISO, $P = 0.1334$. Mean values with different letter superscripts are significantly different from each other ($P < 0.05$). HSD = hydroxysteroid dehydrogenase; ZEA = zearalenone; ISO = isoflavone.

oral dose of 10 mg/kg BW has been estimated at 80% to 85%, and significant serum levels have been measured in pigs within 30 min of feeding (Biehl *et al*., 1993). After absorption, ZEA is converted into α-ZEA or β-ZEA in the liver, predominantly by 3α/3β-HSD (Olsen *et al*., 1981). Malekinejad *et al.* (2006) reported differences among species in the hepatic biotransformation of ZEA, with α-ZEA being the main metabolite in the liver of the pig. In the present study, we determined ZEA residue levels in muscle tissue as representative of the edible organs of a pig. In addition, we measured ZEA residue levels in liver tissue, where ZEA is converted. In pigs fed the diet containing ZEA at 2.0 mg/kg for 21 days, residue levels of ZEA in liver and muscle tissues reached 1.35 and 0.031 μg/kg, respectively. James and Smith (1982) found that, after 4 weeks of feeding ZEA at 40 mg/kg of feed, residues in the liver varied between 78 and 128 μg/kg. Chickens fed with contaminated feed also showed ZEA residues in muscle and liver tissues (Mirocha *et al*., 1982). ZEA was detected at a mean concentration of 2.1 μg/kg BW in the livers of hens fed contaminated maize (1580 μg ZEA/kg maize; Danicke *et al*., 2002). All these studies demonstrate that ZEA residues accumulate in muscle and liver tissue when animals are fed diets contaminated with high levels of ZEA, and that these concentrations of ZEA increased with increasing amounts of ZEA in the feed.

ISO may act primarily as an estrogen in low-estrogen environments and as an estrogen competitor in high-estrogen environments (Hwang *et al*., 2006). In addition, ISO has been shown to elicit antioxidant effects (Hsieh *et al*., 2009). We show that pigs offered ISO and 2.0 mg/kg ZEA simultaneously exhibited significantly lower protein expression of 3α/3β-HSD than those offered diets with 2.0 mg/kg ZEA alone. Zollner *et al.* (2002) reported finding large amounts of α-ZEA but only trace amounts of ZEA in muscle tissues of pigs fed on a diet of ZEA-contaminated oats. α-ZEA displays a stronger estrogenic effect than ZEA (Kuiper-Goodman *et al*., 1987). Olsen *et al.* (1981) reported another biotransformation pathway for ZEA in animals, whereby ZEA and its reduced metabolites can conjugate with glucuronic acid, which is catalyzed by uridine diphosphate glucuronyl transferases (UDPGT). Following metabolism, these conjugates are excreted in the urine and bile fluid (Mirocha *et al*., 1981). Omoni and Aluko (2005) reported that UDPGT activity increased in various tissues in rats fed a high-ISO diet (0.81 mg/g) for 2 weeks. Our prior study indicated that ISO can counteract the estrogenic influence of a high dose of ZEA (2.0 mg/kg) in the diet of prepubertal gilts (Wang *et al*., 2010). Metabolism may accelerate the biotransformation and degradation of ZEA and its metabolites, and then decrease the estrogenic toxicity of ZEA and its metabolites.

In conclusion, we demonstrated here that muscle and liver tissues retained ZEA residues when pigs are fed on a diet contaminated with high concentrations of ZEA, and that the concentrations of ZEA in muscle and liver tissue increase with increasing amounts of ZEA in the feed. When diets are contaminated with high levels of ZEA, the addition of ISO may accelerate the biotransformation and degradation of ZEA and its metabolites, and reduce the levels of ZEA residues in liver and muscle tissue of prepubertal gilts.

Acknowledgments

This work was supported by the National Natural Science Foundation (Grant No.30771561/C120104), China, and by a National Nonprofit Institute Research Grant from CATAS-TCGRI (Grant No. 1630032012021).

References

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

Binder EM, Tan LM, Chin LJ, Handl J and Richard J 2007. Worldwide occurrence of mycotoxins in commodities, animal feed and feed ingredients. Animal Feed Science and Technology 137, 265–282.

Bradford MM 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Analytical Biochemistry 72, 248–254.

Danicke S, Ueberschar KH, Halle I, Matthes S, Valenta H and Flachowsky G 2002. Effect of addition of a detoxifying agent to laying hen diets containing uncontaminated or Fusarium toxin-contaminated maize on performance of hens and on carryover of zearalenone. Poultry Science 81, 1671–1680.

Fink-Gremmels J and Malekinejad H 2007. Clinical effects and biochemical mechanisms associated with exposure to the mycoestrogen zearalenone. Animal Feed Science and Technology 137, 326–341.

Gimeno A 1979. Thin layer chromatographic determination of aflatoxins, ochratoxins, sterigmatocystin, zearalenone, citrinin, T-2 toxin, diacetoxyscirpenol, penicillic acid, patulin, and penitrem A. Journal – Association of Official Analytical Chemists 62, 579–585.

Glenn AE 2007. Mycotoxigenic Fusarium species in animal feed. Animal Feed Science and Technology 137, 213–240.

Greiner LL, Stahly TS and Stabel TJ 2001. The effect of dietary soy genistein on pig growth and viral replication during a viral challenge. Journal of Animal Science 79, 1272–1279.

Heimler D, Vignolini P, Galardi C, Pinelli P and Romani A 2004. Simple extraction and rapid quantitative analysis of isoflavones in soybean seeds. Chromatographia 59, 361–365.

Hestbjerg H, Nielsen KF, Thrane U and Elmholt S 2002. Production of trichothecenes and other secondary metabolites by Fusarium culmorum and Fusarium equiseti on common laboratory media and a soil organic matter agar: an ecological interpretation. Journal of Agricultural and Food Chemistry 50, 7593–7599.

Hsieh HM, Wu WM and Hu ML 2009. Soy isoflavones attenuate oxidative stress and improve parameters related to aging and Alzheimer's disease in C57BL/6J mice treated with D-galactose. Food and Chemical Toxicology 47, 625–632.

Hwang CS, Kwak HS, Lim HJ, Lee SH, Kang YS, Choe TB, Hur HG and Han KO 2006. Isoflavone metabolites and their *in vitro* dual functions: they can act as an estrogenic agonist or antagonist depending on the estrogen concentration. Journal of Steroid Biochemistry and Molecular Biology 101, 246–253.

James LJ and Smith TK 1982. Effect of dietary alfalfa on zearalenone toxicity and metabolism in rats and swine. Journal of Animal Science 55, 110–118.

Krska R, Welzig E and Boudra H 2007. Analysis of Fusarium toxins in feed. Animal Feed Science and Technology 137, 241–264.

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

Malekinejad H, Maas-Bakker R and Fink-Gremmels J 2006. Species differences in the hepatic biotransformation of zearalenone. Veterinary Journal 172, 96–102.

Mirocha CJ, Pathre SV and Robison TS 1981. Comparative metabolism of zearalenone and transmission into bovine milk. Food and Cosmetics Toxicology 19, 25–30.

Mirocha CJ, Robison TS, Pawlosky RJ and Allen NK 1982. Distribution and residue determination of [3H]zearalenone in broilers. Toxicology and Applied Pharmacology 66, 77–87.

NRC 1998. NRC, nutrient requirements of swine, 10th revised edition. National Academy Press, Washington, DC, USA.

Olsen M, Pettersson H and Kiessling KH 1981. Reduction of zearalenone to zearalenol in female rat liver by 3 alpha-hydroxysteroid dehydrogenase. Acta Pharmacological Toxicology 48, 157–161.

Omoni AO and Aluko RE 2005. Soybean foods and their benefits: potential mechanisms of action. Nutrition Reviews 63, 272–283.

Payne RL, Bidner TD, Southern LL and Geaghan JP 2001. Effects of dietary soy isoflavones on growth, carcass traits, and meat quality in growing-finishing pigs. Journal of Animal Science 79, 1230–1239.

Reinli K and Block G 1996. Phytoestrogen content of foods – a compendium of literature values. Nutrition and Cancer – An International Journal 26, 123–148.

SAS Institute 1990. SAS/STAT® user's guide, Version 6, 4th edition. SAS Institute, Cary, NC, USA.

Wang DF, Zhang NY, Peng YZ and Qi DS 2010. Interaction of zearalenone and soybean isoflavone on the development of reproductive organs, reproductive hormones and estrogen receptors expression in prepubertal gilts. Animal Reproduction Science 122, 317–323.

Wang HJ and Murphy PA 1994. Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year, and location. Journal of Agricultural and Food Chemistry 42, 1674–1677.

Xu YC, Zhang GS and Chu FS 1988. Enzyme-linked immunosorbent assay for deoxynivalenol in corn and wheat. Journal – Association of Official Analytical Chemists 71, 945–949.

Yamashita A, Yoshizawa T, Aiura Y, Sanchez PC, Dizon EI, Arim RH and Sardjono 1995. Fusarium mycotoxins (fumonisins, nivalenol, and zearalenone) and aflatoxins in corn from Southeast Asia. Bioscience Biotechnology and Biochemistry 59, 1804–1807.

Zinedine A, Soriano JM, Molto JC and Manes J 2007. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. Food Chemistry and Toxicology 45, 1–18.

Zollner P, Jodlbaure J, Kleinova M, Kahlbacher H, Kuhn T, Hochsteiner W and 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.