Zearalenone induced oxidative stress in the jejunum in postweaning gilts through modulation of the Keap1–Nrf2 signaling pathway and relevant genes¹

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ABSTRACT: Researches have shown that dietary zearalenone (ZEA) caused oxidative stress in the liver and reproductive organs of postweaning gilts. However, information on the effects of ZEA on oxidative stress of the small intestine in the piglets is limited. The objective of this study was to determine the effects of ZEA exposure on oxidative stress, the Kelch-like erythroid cell-derived protein with CNC homology (ECH)-associated protein 1 (Keap1)–nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway and on immunohistochemistry of the jejunum in postweaning gilts. A 35-d feeding experiment using 40 postweaning gilts (Landrace × Yorkshire × Duroc) with an average BW of 14.01 ± 0.86 kg in 4 groups fed corn–soybean meal-based diets containing 0, 0.5, 1.0, and 1.5 mg ZEA/kg was conducted. The jejunum was obtained at the end of the experiment and used for analyses. The results showed that the activities of total superoxide dismutase and glutathione peroxidase and the relative expressions of Keap1 mRNA and protein in

the jejunum linearly and quadratically decreased ($P < 0.05$) with increasing concentrations of ZEA in the diets. The malondialdehyde content, the integrated optical density of Nrf2 and glutathione peroxidase 1 (GPX1), and the relative expressions of Nrf2, GPX1, quinone oxidoreductase 1 (NQO1), and modifier subunit of glutamate-cysteine ligase (GCLM) mRNA and proteins linearly and quadratically increased ($P < 0.05$) with increasing levels of ZEA. Immunohistochemical analysis showed that Nrf2 and GPX1 immunoreactivity was enhanced by the ZEA treatments, and block localization of yellow and brown immunoreactive substances in the jejunum was observed with increasing levels of ZEA. The results suggest that ingested ZEA induced oxidative stress in the jejunum in postweaning gilts through upregulation of the Keap1–Nrf2 signaling pathway and downstream target genes *NQO1*, *HO1*, and *GCLM*, indicating the important role of the Keap1–Nrf2 signaling pathway in oxidative stress induced by ZEA in the jejunum of the postweaning piglets.

Key words: jejunum, Keap1–Nrf2 pathway, oxidative stress, postweaning gilts, zearalenone

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INTRODUCTION

Zearalenone (ZEA) is a nonsteroidal estrogen mycotoxin produced by *Fusarium* fungi (Zinedine et al., 2007), and the absorbed ZEA is mainly metabolized by hepatic hydroxysteroid dehydrogenase into α -zearalenol and β -zearalenol (Malekinejad et al., 2006; Warth et al., 2013). Ingested ZEA could cause various toxic effects on

animal and human. Studies have shown that swine is the species most sensitive to ZEA and that ZEA causes millions of dollars of losses in pig farms globally each year (Hussein et al., 2001; Jo et al., 2016).

The small intestine is the most important site for nutrient absorption and is also the first physical barrier to prevent ingested toxins from absorption (Oswald, 2006), and intestinal epithelial cells were an important first target site for the dietary toxins (Wan et al., 2013a,b; Fan et al., 2017).

It has been reported that ingested ZEA induced cytotoxicity and oxidative damage in swine intestinal tract cells, exerted toxic effects on intestinal barrier function, and caused immunological and morphological changes in rats (Marin et al., 2013b; Liu et al., 2014; Fan et al., 2017). It was postulated that ZEA induced cytotoxicity and immunotoxicity through ZEA-induced intracellular oxidative stress that led to DNA oxidative damage and apoptosis (Hassen et al., 2007; Marin et al., 2011; Liu et al., 2014).

The Kelch-like erythroid cell-derived protein with CNC homology (ECH)-associated protein 1 (Keap1)-nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway is considered one of the most important mechanisms to protect cells from oxidative stress (Jaiswal, 2004; Copple et al., 2008; Jaramillo and Zhang, 2013). However, there is little information on the changes in mRNA and protein expression of the Keap1-Nrf2 signaling pathway and antioxidant genes in the small intestine of piglets caused by ZEA.

The objective of this study was to assess the effects of dietary ZEA on the antioxidant enzyme activity and on the Keap1-Nrf2 signaling pathway in the jejunum of postweaning piglets. The jejunum was selected because it is one of the sites that are mostly experienced oxidative stress (Gu et al., 2012; Khan et al., 2012).

MATERIALS AND METHODS

Animals used in all experiments were cared for in accordance with the guidelines for the care and use of laboratory animals set by the Animal Nutrition Research Institute of Shandong Agricultural University (Tai'an, Shandong, P.R. China) and the Ministry of Agriculture of China (Beijing, P.R. China).

Preparation of ZEA-Contaminated Diets

Purified ZEA (Fermentek Ltd., Jerusalem, Israel) was first dissolved in acetic ether and then

poured onto talcum powder. The ZEA premix was prepared by blending ZEA-contaminated talcum powder with ZEA-free corn, which was subsequently mixed with a corn-soybean meal to make the ZEA-contaminated diets 0 (Control), 0.5 (ZEA0.5), 1.0 (ZEA1.0), and 1.5 (ZEA1.5) mg/kg. The selection of 0.5 to 1.5 mg ZEA/kg in this study was based on the results of Jiang et al. (2010, 2011) and consideration of the feeding situation in China. The maximum allowable ZEA concentration in the diet of postweaning gilts is 0.5 mg/kg in Chinese feeding standard. However, swine diets occasionally contain 1 to 1.5 mg/kg of ZEA (Zinedine et al., 2007). All diets were prepared in one batch and then stored in covered containers before feeding. A composite sample of each experimental diet was prepared for analysis of ZEA and other mycotoxins before and at the end of the feeding experiment. Zearalenone was analyzed using immunoaffinity column chromatography purification. Aflatoxin (AFL) was measured using liquid chromatography fluorescence detection, whereas deoxynivalenol (DON) was determined using liquid chromatography combined with UV detection. The detection limits were 1.0 µg/kg for AFL, 0.1 mg/kg for ZEA, and 0.1 mg/kg for DON (sum of 3-acetyl DON, 15-acetyl DON, and nivalenol). Analyzed ZEA concentrations were 0, 0.52 ± 0.07 , 1.04 ± 0.03 , and 1.51 ± 0.13 mg/kg in the control, ZEA0.5, ZEA1.0, and ZEA1.5, respectively. Aflatoxin and DON were not detected in any of the diets.

Experimental Design, Animals, and Management

A total of 40 postweaning gilts (Landrace × Yorkshire × Duroc) with an average BW of 14.01 ± 0.86 kg were randomly allocated to 4 experimental groups after 10 d of adaptation. The 4 groups of piglets were then randomly assigned to the 4 experimental diets as described above and fed the respective diet for 35 d. The treatments were arranged as a completely randomized design. All piglets were individually housed in cages in a temperature-controlled room at Animal Husbandry Science and Technology Park of Shandong Agricultural University. The diets (Table 1) used in the study were isocaloric and isonitrogenous, with the only difference being ZEA concentration. All nutrient concentrations met or exceeded the minimum requirements established by the NRC (2012). Representative samples for each diet were collected at the beginning and at the end of the experiment for nutrient analyses using AOAC methods (AOAC, 2012).

Table 1. Ingredients and nutrient levels of the basal diet (air-dry basis; %)

Ingredient	Content, %	Nutrient ¹	Analyzed value
Corn	64.5	DE, MJ/kg	13.81
Whey powder	5.0	CP, %	19.82
Soybean meal	23.0	Calcium, %	0.70
Fish meal	5.0	Total phosphorus, %	0.64
L-Lysine HCl	0.2	Lysine, %	1.22
CaHPO ₄	0.7	Sulfur AA, %	0.65
Pulverized limestone	0.3	Threonine, %	0.75
NaCl	0.3	Tryptophan, %	0.22
Premix ²	1.0		

¹Digestible energy was obtained from digestion experiment (Yang et al., 2017), whereas the other nutrient contents were calculated values.

²Premix provided the following per kilogram of diet: 3,300 IU vitamin A, 330 IU vitamin D₃, 24 IU vitamin E, 0.75 mg vitamin K₃, 1.50 mg vitamin B₁, 5.25 mg vitamin B₂, 0.02625 mg vitamin B₁₂, 15.00 mg pantothenate, 22.50 mg niacin, 0.075 mg biotin, 0.45 mg folic acid, 6.00 mg Mn, 150 mg Fe, 150 mg Zn, 9.00 mg Cu, 0.21 mg I, and 0.45 mg Se.

Sample Collection and Preparation

On the last day of the feeding experiment, piglets were euthanized after fasting for 12 h. The jejunum was rapidly isolated and removed from the body after the death of piglet. The jejunum was defined as the section of the small intestine, and the Treitz ligament is an important marker for confirming the beginning of the jejunum during surgery. The removed jejunum was then immediately dissected into 4 portions with 3 of them being immediately frozen in liquid nitrogen and stored at -80°C for the subsequent analyses of antioxidant enzyme activity, gene expression, and western blotting. The fourth sample was being quickly fixed in Bouin's solution for 24 to 48 h used graded alcohol concentrations for hematoxylin and eosin staining and immunohistochemical analysis.

Determination of Antioxidant Enzyme Activity

Sample of jejunum tissue was thawed, rinsed with ice-cold deionized water, and dried with filter paper. The sample was then homogenized with 0.02 mmol/L Tris-HCl (pH 7.4) at a ratio of 1:10 (mg/mL) followed by centrifugation ($10,000 \times g$) at 4°C for 15 min. The supernatant was collected for analyses of total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) using methods described by Jiang et al. (2012) with SOD A001-1, GSH-Px A005, and MDA A003 assay kits, respectively (Nanjing Jiancheng Bioengineering Institute). Protein concentration was also determined using the method of Bradford (1976) with a protein assay kit (A045; Nanjing Jian Cheng Bioengineering Institute, Nanjing, P.R. China).

Immunohistochemical Analysis for Integrated Optical Density of Nrf2 and Glutathione Peroxidase 1

For this analysis, samples stored in Bouin's solution sliced into 5- μm sections using a Leica microtome (RM 2235; Leica Biosystems Nussloch GmbH, Nussloch, Germany). The sections were then processed in the order of immobilization on poly-L-lysine-coated glass slides drying overnight at 37°C , dewaxing, rehydration, and antigen retrieval that was performed in sodium citrate buffer (0.01 mol/L, pH 6.0) using a microwave unit for 20 min at full power, which was followed by washing 3 times (5 min each time) with PBS (0.01 mol/L, pH 7.2). The subsequent sample processing for immunohistochemical analysis was the same as that described by Zhou et al. (2018).

The distributions of Nrf2 and glutathione peroxidase 1 (GPX1) cells in above prepared samples were observed under a microscope (ELIPSE 80i; Nikon Corp., Tokyo, Japan) at $100\times$ magnification using a bright field of view, and the Nrf2 and GPX1 cells were distinguished from others by their brownish yellow color.

To evaluate the extent of staining and quantity of the target antigen of the Nrf2 and GPX1 cells, images were randomly photographed with a microscopic camera system at $100\times$ magnification, which were then analyzed by an image analysis software (Image-Pro Plus 6.0; Media Cybernetics, Inc., Rockville, MD) to obtain the total cross-sectional integrated optical density (IOD; Rivera et al. 2006). A total of 6 stained samples randomly selected from the 10 piglets in each treatment were used in this analysis.

Quantification of *Keap1*, *Nrf2*, *GPX1*, *NQO1*, *HO1*, *GCLM*, and *GAPDH* mRNA Expression Using Quantitative Real-Time PCR

Total RNA was extracted from the jejunum sample stored at -80°C with RNAiso Plus (D9108B; Applied TaKaRa, DaLian, P.R. China) according to the manufacturer's instructions. The purity and concentration of the RNA was assessed using an Eppendorf Biophotometer (RS323C; Eppendorf Aktien Gesellschaft, Hamburg, Germany) at an absorbance ratio of 260:280 nm (a range of 1.8 to 2.0 indicates a pure RNA sample). The RNA integrity was verified using agarose gel electrophoresis. Total RNA was reverse transcribed to cDNA using a Reverse Transcription System kit (Prime-Script RT Master Mix, RR036A; Applied TaKaRa).

A total volume of 20 μL of the PCR mixture containing 10- μL SYBRY Premix Ex Taq II, 0.4- μL DyeII (SYBRY Premix Ex Taq-TII RNaseH Plus, DRR420A; Applied TaKaRa), 0.4 μL of both forward and reverse primers, and 2- μL cDNA (<100 ng) was used for the quantitative real-time PCR (qRT-PCR) analysis. The optimized qRT-PCR protocol included an initial denaturation step at 95°C for 30 s followed by 43 cycles at 95°C for 5 s, 60°C for 34 s, 95°C for 15 s, 60°C for 60 s, and 95°C for 15 s. The qRT-PCR reactions were conducted in an AB 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). The amounts of relative expression of *Keap1*, *Nrf2*, *GPX1*, *NQO1*, *HO1*, *GCLM*, and *GAPDH* mRNA were expressed and calculated as being equal to $2^{-\Delta\Delta\text{Ct}}$ (Livak and Schmittgen, 2001). The analysis

was repeated 3 times for each sample. The primer sequences and production lengths are presented in Table 2.

Nuclear Protein Extraction and Western Blot Analysis

The total protein of the jejunum was extracted from the sample stored at -80°C using radioimmunoprecipitation assay lysis buffer supplemented with phenylmethanesulfonyl fluoride (Beyotime Biotechnology, Shanghai, P.R. China) according to manufacturer's instructions. The samples were incubated on ice for 30 min, and the supernatant was collected using centrifugation ($12,500 \times g$) at 4°C for 10 min. After protein quantification with a bicinchoninic acid protein assay kit [Tiangen Biotech (Beijing) Co., Ltd.], the solution containing 50 μg of total protein was loaded onto an SDS-PAGE gel and subjected to electrophoresis. The proteins were transferred to 0.22- μm polyvinylidene difluoride (PVDF) membranes. After blocking the PVDF membranes in 5% skim milk for 2 h at room temperature, blots were washed for 30 min with Tris-buffered saline containing 0.1% Tween 20 (TBST; 20 mM Tris, pH 7.5; 150 mM NaCl; and 0.1% Tween-20) and incubated overnight at 4°C with the following primary antibodies: *Nrf2* (1:1,000), *GPX1* (1:1,000), *Keap1* (1:500), hemeoxygenase 1 (**HO1**; 1:10,000), quinone oxidoreductase 1 (**NQO1**; 1:10,000), glutamate-cysteine ligase (**GCLM**; 1:5,000), and β -actin (1:2,000). The membranes were then washed in TBST for 30 min and incubated again at 37°C

Table 2. Sequence of primers for real-time PCR

Target gene ¹	Accession no.	Primer sequence (5' to 3') ²	Product size, bp
<i>Nrf2</i>	RA_011763	F: GAAAGCCAGTCTTCATTGC R: TTGGAACCGTGCTAGTCTCA	190
<i>GPX1</i>	NC_010444.3	F: GGCACAACGGTGC GG GACTA R: AGGCGAAGAGCGGGTGAGCA	159
<i>Keap1</i>	NM_001114671.1	F: GCCTCATCGAGTTCGCTTAC R: CACGGACCACTGTCAATC	105
<i>HO1</i>	NM_001004027.1	F: GCTGAGAATGCCGAGTTCAT R: TGTAGACCGGGTTCTCCTTG	142
<i>NQO1</i>	NM_001159613.1	F: TGAATTACATCTCTGTGGTTTA R: AGAATGACACTCATATTAGGCG	171
<i>GCLM</i>	CV868255.1	F: ACAATACAACGGTTCAGGTGAGT R: GCCTGTAAAATGTGTCATTGAGG	122
<i>GAPDH</i>	NM_001206359.1	F: ATGGTGAAGGTCGGAGTGAA R: CGTGGGTGGAATCATACTGG	154

¹*Nrf2* = nuclear factor erythroid 2-related factor 2; *Keap1* = Kelch-like erythroid cell-derived protein with CNC homology (ECH)-associated protein 1; *GPX1* = glutathione peroxidase 1; *HO1* = hemeoxygenase 1; *NQO1* = quinone oxidoreductase 1; *GCLM* = modifier subunit of glutamate-cysteine ligase; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase.

²F = forward primer; R = reverse primer.

with anti-rabbit IgG antibody (1:5,000; Beyotime Biotechnology; CoWin Biosciences, Beijing, P.R. China) and anti-mouse IgG (1:5,000; Beyotime Biotechnology) for 1.5 h. Following washing with TBST for 30 min, membranes were immersed in a high-sensitivity luminescence reagent (BeyoECL Plus; Beyotime Biotechnology) and then exposed to film using a Fusion FX imaging system and FusionCapt Advance FX7 software (Beijing Oriental Science and Technology Development Co., Ltd., Beijing, P.R. China). Protein concentrations were determined using Image-Pro Plus 6.0 (Media Cybernetics, Inc.).

Statistical Analysis

All data were subjected to 1-way ANOVA analysis using the generalized linear model procedure of SAS 9.2 (SAS Inst. Inc., Cary, NC). Data were initially analyzed as a completely randomized design, with treatment as fix effect and individual piglet as random factor. Orthogonal polynomial contrasts were used to determine linear and quadratic responses to the dietary ZEA concentrations. The significance of differences among treatments was tested using Duncan's multiple range tests and significance was declared at $P < 0.05$.

RESULTS

Effects of ZEA on Antioxidant Enzyme Activity of Jejunum

Activities of T-SOD and GSH-Px in the jejunum tissue decreased linearly ($P < 0.01$) and quadratically ($P < 0.01$), whereas MDA concentration increased quadratically ($P < 0.05$) as the dietary ZEA concentrations increased from 0 to 1.5 mg/kg (Table 3). All concentrations of ZEA used in this study decreased activities of T-SOD and GSH-Px and increased MDA concentration with the lowest ($P < 0.01$) T-SOD activity and highest MDA

concentration ($P < 0.01$) being observed for piglets in ZEA1.0.

Effects of ZEA on Nrf2 and GPX1 Immunoreactivities in Jejunum

Immunohistochemical analysis showed that Nrf2 and GPX1 immunoreactivities were mainly localized in the lamina propria around the intestine gland, whereas negative or faint Nrf2 and GPX1 immunoreactivities were observed in most of the intestinal villus epithelium in the jejunum of postweaning gilts (Figs. 1 and 2). A light yellow immunoreactive substance of Nrf2 and GPX1 was observed in the control. The localization pattern of immunoreactivity in the ZEA-treated piglets was essentially the same as that in the control. However, compared with the control, the immunoreactivities of Nrf2 and GPX1, as indicated by the IOD values, were linearly ($P < 0.01$) and quadratically ($P < 0.001$) enhanced with increasing levels of ZEA (Table 4). Consistent with the results of the antioxidant enzyme activity, the jejunum of piglets fed ZEA-containing diets had greater ($P < 0.01$) immunoreactivities of Nrf2 and GPX1 than control, with the greatest ($P < 0.01$) immunoreactivities of Nrf2 and GPX1 being observed for ZEA1.0 piglets.

Effects of ZEA on Relative Expression of Keap1, Nrf2, GPX1, NQO1, HO1 and GCLM mRNA and Expression of Nuclear Keap1, Nrf2, GPX1, NQO1, HO1 and GCLM Proteins

Relative expressions of *Nrf2*, *GPX1*, *NQO1* and *GCLM* mRNA in the jejunum of postweaning gilts increased linearly ($P < 0.01$) and quadratically ($P < 0.01$) whereas relative expression of *Keap1* decreased linearly ($P < 0.001$) and quadratically ($P < 0.001$) as the dietary ZEA concentrations increased (Table 5). Relative expression of *HO1* mRNA in the jejunum was increased quadratically ($P < 0.001$) only with the increasing concentrations of ZEA in the diet. Compared

Table 3. Effects of zearalenone (ZEA) on antioxidant capacity in the jejunum of postweaning gilts

Item ¹	Control	Treatment ²			SEM	P		
		ZEA0.5	ZEA1.0	ZEA1.5		Treatment	Linear	Quadratic
T-SOD, active units/mg protein	18.67 ^a	14.27 ^b	9.46 ^c	13.87 ^b	0.081	<0.001	0.006	0.002
GSH-Px, active units/mL	181.07 ^a	156.33 ^b	110.41 ^c	108.53 ^c	0.993	<0.001	<0.001	<0.001
MDA, nmol/mg protein	3.49 ^c	4.41 ^b	6.51 ^a	4.29 ^b	0.046	<0.001	0.085	0.016

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹T-SOD = total superoxide dismutase; GSH-Px = glutathione peroxidase; MDA = malondialdehyde.

²Control, ZEA0.5, ZEA1.0, and ZEA1.5 represent the basal diet with an additions of 0, 0.5, 1.0, and 1.5 mg/kg ZEA, respectively, with analyzed ZEA concentrations being 0, 0.52 ± 0.07 , 1.04 ± 0.03 , and 1.51 ± 0.13 mg/kg, respectively.

Figure 1. Effects of zearalenone (ZEA) on *nuclear factor erythroid 2-related factor 2* (*Nrf2*) localization in the jejunum of postweaning gilts in treatments of Control (A1, A2, A3), ZEA0.5 (B1, B2, B3), ZEA1.0 (C1, C2, C3), and ZEA (D1, D2, D3). Control, ZEA0.5, ZEA1.0, and ZEA1.5 represent the basal diet with an additions of 0, 0.5, 1.0, and 1.5 mg/kg ZEA, respectively, with analyzed ZEA concentrations being 0 , 0.52 ± 0.07 , 1.04 ± 0.03 , and 1.51 ± 0.13 mg/kg, respectively. LE = intestinal villus epithelium; G = intestinal gland; S = lamina propria. Red arrows show immunoreactive cells of *Nrf2* and blue arrows show immune-negative cells of *Nrf2*. Scale bars: approximately 20 μ m for A2, B2, C2, D2, A3, B3, C3, and D3, and 100 μ m for A1, B1, C1, and D1.

with control, piglets fed diets containing 0.5, 1.0, and 1.5 mg/kg of ZEA had lower ($P < 0.01$) relative expression of *Keap1* mRNA and higher ($P < 0.05$) relative expressions of *Nrf2*, *GPX1*, *NQO1*, and *GCLM* mRNA except for the relative expression of *GCLM* mRNA that was higher ($P < 0.05$) at the ZEA concentrations of 1.0 and 1.5 mg/kg diet only.

Western blot analysis revealed positive bands of appropriate sizes for genes *Keap1*, *Nrf2*, *GPX1*,

NQO1, *HO1*, *GCLM*, and β -actin (Fig. 3). Similar to mRNA relative expression, relative expressions of *Nrf2*, *GPX1*, *NQO1*, *HO1*, and *GCLM* proteins in the jejunum also linearly ($P < 0.05$) and quadratically ($P < 0.05$) increased, but relative expression of *Keap1* protein linearly and quadratically decreased ($P < 0.05$) with increasing levels of ZEA. However, it appeared that individual protein expression responded to dietary ZEA concentrations differently. Although relative expression of

Figure 2. Effects of zearalenone (ZEA) on *glutathione peroxidase 1 (GPX1)* localization in the jejunum of postweaning gilts in treatments of Control (A1, A2, A3), ZEA0.5 (B1, B2, B3), ZEA1.0 (C1, C2, C3), and ZEA (D1, D2, D3). Control, ZEA0.5, ZEA1.0, and ZEA1.5 represent the basal diet with an additions of 0, 0.5, 1.0, and 1.5 mg/kg ZEA, respectively, with analyzed ZEA concentrations being $0, 0.52 \pm 0.07, 1.04 \pm 0.03$, and 1.51 ± 0.13 mg/kg, respectively. LE = intestinal villus epithelium; G = intestinal gland; S = lamina propria. Red arrows show immunoreactive cells of *GPX1* and blue arrows show immune-negative cells of *GPX1*. Scale bars: approximately 20 μ m for A2, B2, C2, D2, A3, B3, C3, and D3 and 100 μ m for A1, B1, C1, and D1.

protein for *Keap1* gene decreased ($P < 0.05$) and for *GPX1* and *NQO1* gene increased ($P < 0.05$) by 0.5, 1.0, and 1.5 mg ZEA/kg diet, relative expressions of *Nrf2*, *HO1*, and *GCLM* proteins were increased ($P < 0.05$) only with dietary ZEA concentrations at 1.0 and 1.5 mg/kg.

DISCUSSION

Total superoxide dismutase and GSH-Px are important components of the body's antioxidant defense system. They can effectively block the chain reaction induced by the free radicals and decrease

Table 4. Effects of zearalenone (ZEA) on the immunoreactive integrated optic density of *Nrf2* and *GPX1* in the jejunum of postweaning gilts

Item ¹	Control	Treatment ²			SEM	P		
		ZEA0.5	ZEA1.0	ZEA1.5		Treatment	Linear	Quadratic
<i>Nrf2</i>	948 ^d	7,001 ^c	9,599 ^a	8,913 ^b	0.082	<0.001	0.004	<0.001
<i>GPX1</i>	1,141 ^c	5,091 ^b	8,436 ^a	5,031 ^b	0.069	<0.001	0.002	<0.001

^{a-d}Means within a row with different superscripts differ ($P < 0.05$).

¹*Nrf2* = nuclear factor erythroid 2-related factor 2; *GPX1* = glutathione peroxidase 1.

²Control, ZEA0.5, ZEA1.0, and ZEA1.5 represent the basal diet with an additions of 0, 0.5, 1.0, and 1.5 mg/kg ZEA, respectively, with analyzed ZEA concentrations being 0, 0.52 ± 0.07 , 1.04 ± 0.03 , and 1.51 ± 0.13 mg/kg, respectively.

Table 5. Effects of zearalenone (ZEA) on the relative expression of *Keap1*, *Nrf2*, *GPX1*, *NQO1*, *HO1*, and *GCLM* mRNA in the jejunum of postweaning gilts

Item ¹	Control	Treatment ²			SEM	P		
		ZEA0.5	ZEA1.0	ZEA1.5		Treatment	Linear	Quadratic
<i>Keap1</i>	1.00 ^a	0.58 ^b	0.57 ^b	0.59 ^b	0.010	<0.001	<0.001	<0.001
<i>Nrf2</i>	1.00 ^c	3.45 ^b	4.08 ^a	3.90 ^a	0.080	<0.001	0.005	<0.001
<i>GPX1</i>	1.04 ^c	1.45 ^b	1.82 ^a	1.53 ^b	0.018	<0.001	0.002	0.002
<i>NQO1</i>	1.01 ^d	1.49 ^c	2.07 ^b	3.16 ^a	0.065	<0.001	<0.001	<0.001
<i>HO1</i>	1.00 ^d	1.56 ^c	2.09 ^b	2.64 ^a	0.061	<0.001	0.0802	<0.001
<i>GCLM</i>	1.00 ^b	1.18 ^b	2.29 ^a	2.35 ^a	0.057	<0.001	<0.001	<0.001

^{a-d}Means within a row with different superscripts differ ($P < 0.05$).

¹*Nrf2* = nuclear factor erythroid 2-related factor 2; *Keap1* = Kelch-like erythroid cell-derived protein with CNC homology (ECH)-associated protein 1; *GPX1* = glutathione peroxidase 1; *HO1* = hemoxygenase 1; *NQO1* = quinone oxidoreductase 1; *GCLM* = modifier subunit of glutamate-cysteine ligase.

²Control, ZEA0.5, ZEA1.0, and ZEA1.5 represent the basal diet with an additions of 0, 0.5, 1.0, and 1.5 mg/kg ZEA, respectively, with analyzed ZEA concentrations being 0, 0.52 ± 0.07 , 1.04 ± 0.03 , and 1.51 ± 0.13 mg/kg, respectively.

oxidative stress (Kullisaar et al., 2002; Yarru et al., 2009). Malondialdehyde is the final product of lipid peroxidation and has cytotoxic and genotoxic effects by reacting with biomolecules (Gil et al., 2006). The observations that T-SOD and GSH-Px activities were decreased, and MDA concentration was increased in the jejunum by ZEA at the concentrations of 0.5 to 1.5 mg/kg diet indicated that ZEA used in this study induced oxidative stress of the piglets. It has been suggested that ingested ZEA could cause oxidative stress thereby change the main metabolic processes in the body or the organisms such as cell membrane metabolism, protein biosynthesis, and glycolysis (Liu et al., 2013). In vitro study has shown that ZEA at the concentrations of 7 and 8 $\mu\text{g/mL}$ decreased T-SOD and GSH-Px enzyme activity and increased the MDA content in swine's small intestine IPEC-J2 cells (Fan et al., 2017). Liu et al. (2014) reported that ZEA at the dietary concentrations of 48.5 to 146 mg/kg induced a significant increase in MDA formation and a significant decrease in T-SOD activity in the jejunum of pregnant rats, leading to oxidative stress (Zhou et al., 2015). The negative effects of ZEA on the activity of antioxidant enzymes were also

observed in the serum and ovarian granulosa cells of pigs (Yin et al., 2014; Qin et al., 2015). Although the exact mechanism by which ZEN induced oxidative stress is not known, research has shown that ZEA enhanced the formation of reactive oxygen species (ROS) leading to oxidative damage (Jia et al., 2014; Qin et al., 2015). To the best of our knowledge, this study was the first to show in vivo that dietary ZEA at the concentrations of 0.5 to 1.5 mg/kg decreased antioxidant enzyme activity in the jejunum of postweaning piglets. Whether the decreases of T-SOD and GSH-Px activity was the direct effect of ZEA or the indirect effect of ZEA via oxidative stress on their gene expressions need to be further investigated.

Glutathione peroxidase 1 is an important selenoprotein in the body that plays an important role in scavenging oxygen free radicals and protecting cell components from oxidative stress and damage (Zachara, 2015). In this study, the increased immunoreactive cells of the lamina propria around the jejunum enteric glands and immunoreactive cells in the villus was consistent with the enhanced expressions of *GPX1* mRNA and protein in the jejunum of ZEA-treated piglets. It is interesting to

Figure 3. Effects of zearalenone (ZEA) on the expression of Keap1, Nrf2, GPX1, NQO1, HO1, and GCLM proteins in the jejunum of post-weaning gilts. ^{a-d}Means with different superscripts differ ($P < 0.05$). Control, ZEA0.5, ZEA1.0, and ZEA1.5 represent the basal diet with an additions of 0, 0.5, 1.0, and 1.5 mg/kg ZEA, respectively, with analyzed ZEA concentrations being 0, 0.52 ± 0.07 , 1.04 ± 0.03 , and 1.51 ± 0.13 mg/kg, respectively. Nrf2 = nuclear factor erythroid 2-related factor 2; Keap1 = Kelch-like erythroid cell-derived protein with CNC homology (ECH)-associated protein 1; GPX1 = glutathione peroxidase 1; HO1 = hemeoxygenase 1; NQO1 = quinone oxidoreductase 1; GCLM = modifier subunit of glutamate-cysteine ligase. Significance differences in linear and quadratic (P -values) when contrasting control vs. ZEA0.5, ZEA1.0, and ZEA1.5 were $P < 0.05$, respectively (Keap1, Nrf2, GPX1, HO1, NQO1, GCLM, linear: $P < 0.05$; quadratic: $P < 0.05$, respectively).

note that GSH-Px activity decreased whereas the expression of GPX1 increased with the increasing concentrations of ZEA in this study. The discrepancy between these 2 results is likely related to the diverse GSH-Px compositions and the role of this polymorphism in relation to the function of GPX1 and its role in protection against oxidative stress (Jefferies et al., 2005; Yin et al., 2013). Glutathione peroxidase is a family of enzymes with peroxidase activity. There are currently 8 GSH-Pxs that have been identified, each encoded by a different gene, and GPX1 is one of them. Glutathione peroxidase 1 is a cytoplasmic GPX, a tetrameric protein composed of 4 identical subunits, which is a stress-type GPX and plays a key role in attenuating and eliminating oxidative stress (Utomo et al., 2004). Although the ingested ZEA caused oxidative stress leading to decreased total GSH-Px activity in the jejunum, it is likely that the oxidative stress induced jejunum epithelial cells to increase the expression of GPX1 protein to resist the oxidative stress. Therefore, it is speculated that the increased expression of *GPX1* plays an important role in resisting the generation of ROS and oxidative stress by ZEA. In contrast, some studies have found that ZEA increased GSH-Px activity in the duodenal mucous membrane of chickens (Grešáková et al., 2012) and the expression of *GPX1* mRNA in granulosa cells of pigs (Qin et al., 2015) and in the liver of piglets (Marin et al., 2013a). Altogether these results indicated that total GSH-Px activity and expressions of different genes of the isozymes might respond to

the ZEA induced oxidative stress differently in different organs or tissues in different animals.

It has been suggested that the main mechanism of cell defense against oxidative stress is the activation of the Nrf2-antioxidant response element (ARE) signaling pathway that controls protein products to enhance the antioxidant capacity of cells by detoxifying and eliminating the expression of active oxidants and electrophilic reagents (Nguyen et al., 2009). Nuclear factor erythroid 2-related factor 2 is a receptor for exogenous toxic substances and oxidative stress and plays an important role in the defense against cell oxidative stress and exogenous toxic substances (Wang et al., 2009). Under basal nonactivation conditions, Nrf2 is tightly regulated by the repressor protein Keap1 in the cytoplasm to form the Keap1-Nrf2 complex (Deshmukh et al., 2017). However, Nrf2 and Keap1 in the cytoplasm are uncoupled and transferred to the nucleus and combined with the Maf protein to form a hetero 2 polymer under the oxidative stress. After the combination of the hetero 2 polymer and ARE, the expressions of the phase II detoxification and antioxidant enzymes such as HO1, NQO1, GCLM, and GPX1 are upregulated to enhance the ability of cell in protecting cells from oxidative damage (Ishii et al., 2002; Chen and Shaikh, 2009). These protein products are involved in detoxification and elimination of the expression of genes for active oxidants and electrophiles to enhance the antioxidant capacity of cells (Moffit et al., 2007; Nguyen et al., 2009).

Immunohistochemical method to locate the Nrf2 protein in the jejunum cytoplasm and nucleus of postweaning gilts was utilized in this study in an attempt to elucidating the mechanism by which ZEA affected antioxidant enzyme activities. The results showed that Nrf2-positive reactions were enhanced in the ZEA treatments and that block localizations of yellow and brown immunoreactive substances were observed and the expressions of Nrf2 mRNA and protein were upregulated with increasing levels of ZEA. Increased expressions of Nrf2 mRNA and protein in the jejunum by ZEA were also reported in rate by Liu et al. (2014). In addition, it has been reported that ZEA induced oxidative damage and activated the Keap1–Nrf2 signaling pathway in a cell culture system (Wu et al., 2014). However, there is little information about the relationship between ZEA and expressions of *HO1*, *NQO1*, and *GCLM* in the jejunum of postweaning gilts. The findings of increased relative expressions of *HO1*, *NQO1*, *GCLM*, and *GPX1* mRNA and respective proteins together with the decreased relative expressions of *Keap1* mRNA and Keap1 protein caused by ZEA in this study demonstrated that ZEA activated the Nrf2 gene and the downstream target genes *HO1*, *NQO1*, *GCLM*, and *GPX1* and inhibited the expression of Keap1 in the jejunum of the postweaning piglets. Jeong et al. (2006) have proposed that the dissociated Nrf2 from the Nrf2–Keap1 association in response to the stress signals enters the nucleus where Nrf2 combines with the ARE thereby activates the expression of Nrf2 and its downstream target genes *HO1*, *NQO1*, and *GCLM*. A similar mechanism might also have been involved in the regulations of Nrf2, *HO1*, *NQO1*, *GCLM*, and *GPX1* expression in the jejunum at the presence of ZEA, the oxidative stressor, in this study. In addition to being controlled by the Keap1–Nrf2 signaling pathway, the *HO1*, *NQO1*, and *GCLM* genes are also regulated by other cell signaling pathways, such as nuclear factor kappa B, mitogen-activated protein kinase, and c-Jun N-terminal kinase-p62/sequestosome 1 (JNK-p62/SQSTM1; Shih and Yen, 2007; Zou et al., 2012). Therefore, whether the enhanced expression of the *HO1*, *NQO1*, and *GCLM* genes in the jejunum caused by ZEA in this study regulated by the classical Keap1–Nrf2 signaling pathway alone or by multiple signaling pathways need to be further studied.

In conclusion, dietary ZEA at the concentrations of 0.5 to 1.5 mg/kg in the corn–soybean-based diet induced oxidative stress of the jejunum of postweaning piglets as indicated by the decreased antioxidant enzyme activity and increased MDA

concentration. The increased accumulations of *Nrf2*, *GPX1*, *HO1*, *NQO1*, and *GCLM* in the jejunum in ZEA fed piglets indicated that the Keap1–Nrf2 signaling pathway might be one of the mechanisms involved in protecting the jejunum from oxidative stress induced by ZEA in postweaning gilts. Moreover, the researches in the jejunum also inspire us to understand a complex relationship among the various signaling pathways in modulation of ZEA-induced oxidative stress.

LITERATURE CITED

- AOAC. 2012. Official methods of analysis of the AOAC. AOAC Int., Rockville, MD.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.
- Chen, J., and Z. A. Shaikh. 2009. Activation of Nrf2 by cadmium and its role in protection against cadmium-induced apoptosis in rat kidney cells. *Toxicol. Appl. Pharmacol.* 241:81–89. doi:10.1016/j.taap.2009.07.038
- Copple, I. M., C. E. Goldring, N. R. Kitteringham, and B. K. Park. 2008. The Nrf2–Keap1 defence pathway: Role in protection against drug-induced toxicity. *Toxicology* 246:24–33. doi:10.1016/j.tox.2007.10.029
- Deshmukh, P., S. Unni, G. Krishnappa, and B. Padmanabhan. 2017. The keap1-nrf2 pathway: Promising therapeutic target to counteract ros-mediated damage in cancers and neurodegenerative diseases. *Biophysical Reviews* 9:41–56.
- Fan, W., T. Shen, Q. Ding, Y. Lv, L. Li, K. Huang, L. Yan, and S. Song. 2017. Zearalenone induces ROS-mediated mitochondrial damage in porcine IPEC-J2 cells. *J. Biochem. Mol. Toxicology* 31:e21944.
- Gil, L., W. Siems, B. Mazurek, J. Gross, P. Schroeder, P. Voss, and T. Grune. 2006. Age-associated analysis of oxidative stress parameters in human plasma and erythrocytes. *Free Radic. Res.* 40:495–505. doi:10.1080/10715760600592962
- Grešáková, L., R. Bořutová, S. Faix, I. Plachá, K. Cobanová, B. Košíková, and L. Leng. 2012. Effect of lignin on oxidative stress in chickens fed a diet contaminated with zearalenone. *Acta Vet. Hung.* 60:103–114. doi:10.1556/AVet.2012.009
- Gu, X. H., Y. Hao, and X. L. Wang. 2012. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 2. Intestinal oxidative stress. *Poult. Sci.* 91:790–799. doi:10.3382/ps.2011-01628
- Hassen, W., I. Ayed-Boussema, A. A. Osoz, A. d. e. C. Lopez, and H. Bacha. 2007. The role of oxidative stress in zearalenone-mediated toxicity in Hep G2 cells: Oxidative DNA damage, glutathione depletion and stress proteins induction. *Toxicology* 232:294–302. doi:10.1016/j.tox.2007.01.015
- Hussein, H. S., and J. M. Brasel. 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology* 167:101–134.
- Ishii, T., K. Itoh, and M. Yamamoto. 2002. Roles of Nrf2 in activation of antioxidant enzyme genes via antioxidant responsive elements. *Methods Enzymol.* 348:182–190.
- Jaiswal, A. K. 2004. Nrf2 signaling in coordinated activation of antioxidant gene expression. *Free Radic. Biol. Med.* 36:1199–1207. doi:10.1016/j.freeradbiomed.2004.02.074

- Jaramillo, M. C., and D. D. Zhang. 2013. The emerging role of the Nrf2-Keap1 signaling pathway in cancer. *Genes Dev.* 27:2179–2191. doi:10.1101/gad.225680.113
- Jefferies, S., Z. Kote-Jarai, D. Goldgar, R. Houlston, M. J. Frazer-Williams, R. A'Hern, R. Eeles, J. Henk, M. Gore, P. Rhys-Evans, et al.; MPT Collaborators; MPT Collaborators. 2005. Association between polymorphisms of the GPX1 gene and second primary tumours after index squamous cell cancer of the head and neck. *Oral Oncol.* 41:455–461. doi:10.1016/j.oraloncology.2004.09.012
- Jeong, W. S., M. Jun, and A. N. Kong. 2006. Nrf2: A potential molecular target for cancer chemoprevention by natural compounds. *Antioxid. Redox Signal.* 8:99–106. doi:10.1089/ars.2006.8.99
- Jia, Z., M. Liu, Z. Qu, Y. Zhang, S. Yin, and A. Shan. 2014. Toxic effects of zearalenone on oxidative stress, inflammatory cytokines, biochemical and pathological changes induced by this toxin in the kidney of pregnant rats. *Environ. Toxicol. Pharmacol.* 37:580–591. doi:10.1016/j.etap.2014.01.010
- Jiang, S. Z., Z. B. Yang, W. R. Yang, B. Q. Yao, H. Zhao, F. X. Liu, C. C. Chen, and F. Chi. 2010. Effects of feeding purified zearalenone contaminated diets with or without clay enterosorbent on growth, nutrient availability, and genital organs in post-weaning female pigs. *Asian-Austral. J. Anim. Sci.* 23:74–81.
- Jiang, S. Z., Z. B. Yang, W. R. Yang, J. Gao, F. X. Liu, J. Broomhead, and F. Chi. 2011. Effects of purified zearalenone on growth performance, organ size, serum metabolites, and oxidative stress in postweaning gilts. *J. Anim. Sci.* 89:3008–3015. doi:10.2527/jas.2010-3658
- Jiang, S. Z., Z. B. Yang, W. R. Yang, S. J. Wang, Y. Wang, J. Broomhead, S. L. Johnston, and F. Chi. 2012. Effect on hepatonephric organs, serum metabolites and oxidative stress in post-weaning piglets fed purified zearalenone-contaminated diets with or without calibrin-Z. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 96:1147–1156. doi:10.1111/j.1439-0396.2011.01233.x
- Jo, H., C. Kong, M. Song, and B. G. Kim. 2016. Effects of dietary deoxynivalenol and zearalenone on apparent ileal digestibility of amino acids in growing pigs. *Anim. Feed Sci. Technol.* 219:77–82.
- Khan, R., A. Q. Khan, W. Qamar, A. Lateef, F. Ali, M. U. Rehman, M. Tahir, S. Sharma, and S. Sultana. 2012. Chrysin abrogates cisplatin-induced oxidative stress, p53 expression, goblet cell disintegration and apoptotic responses in the jejunum of wistar rats. *Br. J. Nutr.* 108:1574–1585. doi:10.1017/S0007114511007239
- Kullisaar, T., M. Zilmer, M. Mikelsaar, T. Vihalemm, H. Annuk, C. Kairane, and A. Kilk. 2002. Two antioxidative lactobacilli strains as promising probiotics. *Int. J. Food Microbiol.* 72:215–224.
- Liu, G., T. Yan, J. Wang, Z. Huang, X. Chen, G. Jia, C. Wu, H. Zhao, B. Xue, L. Xiao, et al. 2013. Biological system responses to zearalenone mycotoxin exposure by integrated metabolomic studies. *J. Agric. Food Chem.* 61:11212–11221. doi:10.1021/jf403401v
- Liu, M., R. Gao, Q. Meng, Y. Zhang, C. Bi, and A. Shan. 2014. Toxic effects of maternal zearalenone exposure on intestinal oxidative stress, barrier function, immunological and morphological changes in rats. *PLoS One* 9:e106412. doi:10.1371/journal.pone.0106412
- Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{(-ΔΔC(T))} method. *Methods* 25:402–408. doi:10.1006/meth.2001.1262
- Malekinejad, H., R. Maas-Bakker, and J. Fink-Gremmels. 2006. Species differences in the hepatic biotransformation of zearalenone. *Vet. J.* 172:96–102. doi:10.1016/j.tvjl.2005.03.004
- Marin, D. E., G. C. Pistol, I. V. Neagoe, L. Calin, and I. Taranu. 2013a. Effects of zearalenone on oxidative stress and inflammation in weanling piglets. *Food Chem. Toxicol.* 58:408–415. doi:10.1016/j.fct.2013.05.033
- Marin, D. E., I. Taranu, G. Pistol, and M. Stancu. 2013b. Effects of zearalenone and its metabolites on the swine epithelial intestinal cell line: IPEC 1. *Proc. Nutr. Soc.* 72:85–89.
- Marin, D. E., I. Taranu, R. Burlacu, G. Manda, M. Motiu, I. Neagoe, C. Dragomir, M. Stancu, and L. Calin. 2011. Effects of zearalenone and its derivatives on porcine immune response. *Toxicol. In Vitro* 25:1981–1988. doi:10.1016/j.tiv.2011.06.022
- Moffit, J. S., L. M. Aleksunes, M. J. Kardas, A. L. Slitt, C. D. Klaassen, and J. E. Manautou. 2007. Role of NAD(P)H:quinone oxidoreductase 1 in clofibrate-mediated hepatoprotection from acetaminophen. *Toxicology* 230:197–206. doi:10.1016/j.tox.2006.11.052
- Nguyen, T., P. Nioi, and C. B. Pickett. 2009. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *J. Biol. Chem.* 284:13291–13295. doi:10.1074/jbc.R900010200
- NRC. 2012. Nutrient requirements of swine. 11th ed. Natl. Acad. Press, Washington, DC.
- Oswald, I. P. 2006. Role of intestinal epithelial cells in the innate immune defence of the pig intestine. *Vet. Res.* 37:359–368. doi:10.1051/vetres:2006006
- Qin, X., M. Cao, F. Lai, F. Yang, W. Ge, X. Zhang, S. Cheng, X. Sun, G. Qin, W. Shen, et al. 2015. Oxidative stress induced by zearalenone in porcine granulosa cells and its rescue by curcumin in vitro. *PLoS One* 10:e0127551. doi:10.1371/journal.pone.0127551
- Rivera, A., L. F. Agnati, T. L. Horvath, J. J. Valderrama, L. C. A. De, and K. Fuxe. 2006. Uncoupling protein 2/3 immunoreactivity and the ascending dopaminergic and noradrenergic neuronal systems: Relevance for volume transmission. *Neuroscience* 137:1447–1461.
- Shih, P. H., and G. C. Yen. 2007. Differential expressions of antioxidant status in aging rats: The role of transcriptional factor Nrf2 and MAPK signaling pathway. *Biogerontology* 8:71–80. doi:10.1007/s10522-006-9033-y
- Utomo, A., X. Jiang, S. Furuta, J. Yun, D. S. Levin, Y. C. Wang, K. V. Desai, J. E. Green, P. L. Chen, and W. H. Lee. 2004. Identification of a novel putative non-selenocysteine containing phospholipid hydroperoxide glutathione peroxidase (NPGPx) essential for alleviating oxidative stress generated from polyunsaturated fatty acids in breast cancer cells. *J. Biol. Chem.* 279:43522–43529. doi:10.1074/jbc.M407141200
- Wan, L. Y., C. S. Woo, P. C. Turner, J. M. Wan, and H. El-Nezami. 2013a. Individual and combined effects of *Fusarium* toxins on the mRNA expression of pro-inflammatory cytokines in swine jejunal epithelial cells. *Toxicol. Lett.* 220:238–246. doi:10.1016/j.toxlet.2013.05.003

- Wan, L. Y., P. C. Turner, and H. El-Nezami. 2013b. Individual and combined cytotoxic effects of *Fusarium* toxins (deoxynivalenol, nivalenol, zearalenone and fumonisins B1) on swine jejunal epithelial cells. *Food Chem. Toxicol.* 57:276–283. doi:10.1016/j.fct.2013.03.034
- Wang, L., H. Jiang, Z. Yin, M. Aschner, and J. Cai. 2009. Methylmercury toxicity and Nrf2-dependent detoxification in astrocytes. *Toxicol. Sci.* 107:135–143. doi:10.1093/toxsci/kfn201
- Warth, B., M. Sulyok, F. Berthiller, R. Schuhmacher, and R. Krska. 2013. New insights into the human metabolism of the *Fusarium* mycotoxins deoxynivalenol and zearalenone. *Toxicol. Lett.* 220:88–94. doi:10.1016/j.toxlet.2013.04.012
- Wu, K., X. Liu, M. Fang, Y. Wu, and Z. Gong. 2014. Zearalenone induces oxidative damage involving Keap1/Nrf2/HO-1 pathway in hepatic L02 cells. *Mol. Cell. Toxicol.* 10:451–457.
- Yang, L. J., S. J. Wang, W. R. Yang, L. B. Huang, F. X. Liu, S. Z. Jiang, and Z. B. Yang. 2017. Effect of zearalenone on production performance, serum antioxidant capacity and immune function of weaning gilts. *Chin. J. Anim. Nutr.* 29:2843–2850.
- Yarru, L. P., R. S. Settivari, E. Antoniou, D. R. Ledoux, and G. E. Rottinghaus. 2009. Toxicological and gene expression analysis of the impact of aflatoxin B1 on hepatic function of male broiler chicks. *Poult. Sci.* 88:360–371. doi:10.3382/ps.2008-00258
- Yin, J., W. Ren, G. Liu, J. Duan, G. Yang, L. Wu, T. Li, and Y. Yin. 2013. Birth oxidative stress and the development of an antioxidant system in newborn piglets. *Free Radic. Res.* 47:1027–1035. doi:10.3109/10715762.2013.848277
- Yin, J., W. Ren, J. Duan, L. Wu, S. Chen, T. Li, Y. Yin, and G. Wu. 2014. Dietary arginine supplementation enhances intestinal expression of SLC7A7 and SLC7A1 and ameliorates growth depression in mycotoxin-challenged pigs. *Amino Acids* 46:883–892. doi:10.1007/s00726-013-1643-5
- Zachara, B. A. 2015. Selenium and selenium-dependent antioxidants in chronic kidney disease. *Adv. Clin. Chem.* 68:131–151. doi:10.1016/bs.acc.2014.11.006
- Zhou, C., Y. Zhang, S. Yin, Z. Jia, and A. Shan. 2015. Biochemical changes and oxidative stress induced by zearalenone in the liver of pregnant rats. *Hum. Exp. Toxicol.* 34:65–73. doi:10.1177/0960327113504972
- Zhou, M., L. J. Yang, M. H. Shao, Y. X. Wang, W. R. Yang, L. B. Huang, X. M. Zhou, S. Z. Jiang, and Z. B. Yang. 2018. Effects of zearalenone exposure on the TGF- β 1/Smad3 signaling pathway and the expression of proliferation or apoptosis-related genes of post-weaning gilts. *Toxins* 10: 49.
- Zinedine, A., J. M. Soriano, J. C. Moltó, and J. Mañes. 2007. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. *Food Chem. Toxicol.* 45:1–18. doi:10.1016/j.fct.2006.07.030
- Zou, X., Z. Feng, Y. Li, Y. Wang, K. Wertz, P. Weber, Y. Fu, and J. Liu. 2012. Stimulation of GSH synthesis to prevent oxidative stress-induced apoptosis by hydroxytyrosol in human retinal pigment epithelial cells: Activation of Nrf2 and JNK-p62/SQSTM1 pathways. *J. Nutr. Biochem.* 23:994–1006. doi:10.1016/j.jnutbio.2011.05.006