Effects of purified zearalenone on growth performance, organ size, serum metabolites, and oxidative stress in postweaning gilts

S. Z. Jiang,* Z. B. Yang,*2 W. R. Yang,* J. Gao,† F. X. Liu,* J. Broomhead,‡ and F. Chi‡

*Department of Animal Sciences and Technology, Shandong Agricultural University, Tai’an, Shandong, PR China, 271018; †Tai’an Central Hospital Stomatology, Shandong, PR China, 271000; and ‡Amlan International, Chicago, IL 60611

ABSTRACT: Zearalenone (ZEA), an estrogenic mycotoxin, is produced mainly by Fusarium fungi. Previous studies indicated that acute ZEA exposure induced oxidative stress and damage in multiple organs. Therefore, the present study was designed to investigate the adverse effects of dietary ZEA (1.1 to 3.2 mg/kg of diet) on oxidative stress and organ damage in post-weaning gilts. A total of 20 gilts (Landrace × Yorkshire × Duroc) weaned at d 21 with an average BW of 10.36 ± 1.21 kg was used in the study. Gilts were housed in a temperature-controlled room, divided into 4 treatments, and fed a basal diet only (control) or basal diet supplemented with purified ZEA at a dietary concentration of 1 (ZEA1), 2 (ZEA2), or 3 (ZEA3) mg/kg of diet for 18 d ad libitum. The actual ZEA contents (analyzed) were 0, 1.1 ± 0.02, 2.0 ± 0.01, and 3.2 ± 0.02 mg/kg for control, ZEA1, ZEA2, and ZEA3, respectively. Gilts fed different amounts of dietary ZEA grew similarly with no difference (P > 0.05) in feed intake. Vulva size increased linearly over the 18 d of feeding in gilts fed diets containing 1.1 mg of ZEA/kg or greater (P < 0.001). Relative weight of genital organs, liver, and kidney increased linearly (P < 0.05) in a ZEA-dose-dependent manner. Serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ-glutamate transferase, urea, and creatinine (P < 0.05), and malondialdehyde concentrations in both serum and liver (P < 0.001) were also increased linearly in a ZEA-dose-dependent manner. However, spleen relative weight (P = 0.002) and activities of total superoxide dismutase and glutathione peroxidase (in both serum and liver (P < 0.05) were decreased linearly as dietary ZEA increased. Results showed that besides genital organs, the liver, kidney, and spleen may also be target tissues in young gilts fed diets containing 1.1 to 3.2 mg of ZEA/kg for 18 d. Increased key liver enzymes in the serum suggest progressive liver damage caused by feeding ZEA, and an increase in oxidative stress in gilts is another potential impact of ZEA toxicity in pigs.

Key words: gilt, organ weight, oxidative stress, serum enzyme, vulva size, zearalenone

INTRODUCTION

Zearalenone (ZEA) is produced mainly by Fusarium graminearum growing on grains worldwide (Luo et al., 1990; Schollenberger et al., 2006). The major toxicity of ZEA and its metabolites, such as α-zearalonol (α-ZOL), is attributed to their estrogenic effects on the genital organs and reproduction in gilts (Etienne and Jemmali, 1982; Jiang et al., 2010b). Besides its reproductive effects, ZEA has been shown to be toxic to liver and other tissues in animals, such as causing hepatotoxicity in rabbits (Conková et al., 2001) and piglets (Jiang et al., 2010a), hematotoxicity in rats (Maaroufi et al., 1996), and immunotoxic, oxidative stress and cytotoxicity in mice (Ouanes et al., 2005; Ben Salah-Abbès et al., 2008a, 2009). Zearalenone was also demonstrated to be genotoxic to cells in culture and in mice (Abid-Elseff et al., 2003; Abbès et al., 2007). Although the mechanism of ZEA cytotoxicity is not clear, it is unlikely to be due to its estrogenic effects from binding to cellular estrogen receptors.

Among farm animals, the female pig is the most sensitive species to ZEA (EFSA, 2004). The European Commission recommends limiting dietary ZEA to 0.1

1The authors thank Chia Chung Chen (Department of Applied Chemistry, Chaoyang University of Technology, Taichuang, Taiwan) for his assistance in mycotoxin analysis and general chemistry consultations.

2Corresponding author: yzb204@163.com
Received November 2, 2010.
Accepted April 27, 2011.

©2011 American Society of Animal Science. All rights reserved.

3008
mg/kg in piglet and gilt diets (EC, 2006). Ingested ZEA is mainly metabolized in the liver, with its major metabolites being α-ZOL and β-ZOL (Zollner et al., 2002; Malekinjad et al., 2006), suggesting that the liver may be a target organ. Amounts of ZEA used in the present study were based on our investigations in the Shandong province of China from June 2007 to May 2008, and the recent literature (Binder et al., 2007; Zinedine et al., 2007; Jiang et al., 2010a,b). Larger doses of ZEA (40 or 500 mg/kg of BW) significantly increased degenerative changes in the hepatic and renal tissues of mice (Abbès et al., 2006). Evidence of oxidative damage induced by ZEA was obtained from in vitro studies (Abid-essefi et al., 2004; Ouanes et al., 2008). Detrimental effects of ZEA reported on liver enzymes and oxidative stress of multiple organs were observed mostly in studies using large ZEA concentrations (Abbès et al., 2006; Salah-Abbès et al., 2007, 2008b, 2009). Such increased concentrations, however, are not commonly found in animal feedstuffs (Zinedine et al., 2007). Moreover, studies regarding effects of ZEA (1.1 to 3.2 mg/kg) on pigs are fragmented and controversial (Cheng et al., 2004; Ouanes et al., 2008). Detrimental effects of ZEA were obtained from in vitro studies (Abid-essefi et al., 2006). Evidence of oxidative damage induced by ZEA was obtained from in vitro studies (Abid-essefi et al., 2006). Evidence of oxidative damage induced by ZEA was obtained from in vitro studies (Abid-essefi et al., 2006). Evidence of oxidative damage induced by ZEA was obtained from in vitro studies (Abid-essefi et al., 2006). Evidence of oxidative damage induced by ZEA was obtained from in vitro studies (Abid-essefi et al., 2006). Evidence of oxidative damage induced by ZEA was obtained from in vitro studies (Abid-essefi et al., 2006).

**Materials and Methods**

Animals used for all experiments were cared for in accordance with guidelines of the Animal Nutrition Research Institute of Shandong Agricultural University and the Ministry of Agriculture of China for the care and use of laboratory animals.

**Preparation of ZEA-Contaminated Diet**

Purified ZEA (Fermentek, Jerusalem, Israel) was dissolved in acetic ether and then poured onto talcum powder. A ZEA premix was prepared by blending ZEA-contaminated talcum powder with ZEA-free corn, which was subsequently mixed at the appropriate amounts with a corn-soybean meal diet to make the experimental diets. All diets were prepared in 1 batch and then stored in covered containers before feeding. A composite sample of each experimental diet was sampled for analysis of ZEA and other mycotoxins by the Asia Mycotoxin Analysis Center (Chaoyang University of Technology, Taichung, Taiwan), before and at the end of the feeding experiment. Deoxynivalenol (DON) was analyzed using HPLC. Fluorometry and ELISA were used to measure ZEA, fumonisins (FUM), and aflatoxin (AFL) concentrations. The detection limit for these mycotoxins was 1 µg/kg for AFL, 0.1 mg/kg for ZEA, 0.1 mg/kg for the DON, including 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol, and nivalenol, and 0.25 mg/kg for FUM.

**Experimental Design, Animals, and Management**

A total of 20 postweaning female piglets (Landrace × Yorkshire × Duroc) weaned at d 21 with an average BW of 10.36 ± 1.21 (mean ± SD) kg were used in the study. Gilts were randomly allocated to 4 treatments according to BW after 7 d of adaptation. Basal diet (Table 1) was prepared from corn meal, wheat middling, whey powder, soybean oil, soybean meal, fish meal, AA, calcium phosphate, limestone, sodium chloride, and vitamin and mineral premix to meet or exceed minimal requirements according to the NRC (1998). Diets used in the study were isocaloric and isonitrogenous, with differences existing only in ZEA concentration. Pigs were fed a basal diet only (control) or basal diet supplemented with purified ZEA targeting a dietary concentration of 1 (ZEA1), 2 (ZEA2), or 3 (ZEA3) mg/kg of diet fed for 18 d ad libitum. The actual ZEA contents (analyzed) were 0, 1.1 ± 0.02, 2.0 ± 0.01, and 3.2 ± 0.02 (mean ± SD) mg/kg for control, ZEA1, ZEA2, and ZEA3, respectively. Aflatoxin, DON, and FUM were undetectable in all diets (Jiang et al., 2010a).

**Table 1. Ingredients and compositions of the basal diet (control)**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, %</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>53.00</td>
</tr>
<tr>
<td>Wheat middling</td>
<td>5.00</td>
</tr>
<tr>
<td>Whey powder</td>
<td>6.50</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>2.50</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>24.76</td>
</tr>
<tr>
<td>Fish meal</td>
<td>5.50</td>
</tr>
<tr>
<td>L-Lysine HCl</td>
<td>0.30</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.10</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.04</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>0.80</td>
</tr>
<tr>
<td>Limestone, pulverized</td>
<td>0.30</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.20</td>
</tr>
<tr>
<td>Premix2</td>
<td>1.00</td>
</tr>
</tbody>
</table>

**Notes:**
1. Treatments were basal diet supplemented with zearalenone targeting 0, 1, 2, or 3 mg/kg, with analyzed Zearalenone concentrations of 0, 1.1, 2.0, and 3.2 mg/kg, respectively.
2. Supplied per kilogram of diet: vitamin A, 3,300 IU; vitamin D₃, 330 IU; vitamin E, 21 IU; vitamin K₃, 0.75 mg; vitamin B₁, 1.50 mg; vitamin B₂, 5.25 mg; vitamin B₆, 2.25 mg; vitamin B₁₂, 0.02625 mg; pantothenic acid, 13.00 mg; niacin, 22.5 mg; biotin, 0.075 mg; folic acid, 0.45 mg; Mn, 0.00 mg; Fe, 150 mg; Zn, 150 mg; Cu, 9.00 mg; I, 0.21 mg; Se, 0.45 mg.
The experiment was arranged as a randomized design, and individual gilt was used as the replicate unit. Pigs were housed in a cage equipped with a nipple drinker and feeder in a temperature-controlled room at Jinzhuyuan Farm (Yinan, Shandong, China). During the experimental period, the temperature in the nursery room was maintained between 26 and 28°C. Mean relative humidity was approximately 65%. Gilts were fed ad libitum and allowed free access to water throughout the experimental period.

Body weights were measured weekly and at the end of the test. Feed intake and feed refusal of each treatment were recorded daily. Vulva length, width, and height were measured at 4-d intervals to determine the dietary ZEA estrogenic effects, and vulva area was calculated approximately as a diamond shape \[
\text{[(vulva length \times vulva width)/2]}
\] according to Jiang et al. (2010b).

Sample Collection

Pigs were fasted for 12 h at the end of the experimental period. Blood samples of approximately 10 mL were collected from a jugular vein of all animals into nonheparinized tubes, incubated at 37°C for 2 h, centrifuged at 1,500 \( \times \) g for 10 min at room temperature, and the serum separated and stored in 1.5-mL Eppendorf tubes at −20°C for biochemical analyses described below. After the collection of blood samples, piglets were immediately euthanized and genital organs (ovary + cornu uteri + vagina-vestibule), liver, kidney, and spleen were isolated, weighed, and examined for gross lesions. Samples of liver tissue were kept at −80°C until antioxidative enzyme analysis. Organ weights were expressed on a relative BW basis (g/kg).

Preparation of Liver

Samples of liver were weighed, thawed, rinsed with ice-cold deionized water, and dried with filter paper. The samples were then homogenized (20 KHz ultrasonic frequency) at 4°C for 5 min with a Potter (glass-Teflon) in ice-cold buffer (10 mM Tris-HCl, pH 7.4), and centrifuged at 1,800 \( \times \) g for 30 min at room temperature. The supernatant was collected for total superoxide dismutase (TSOD), glutathione peroxidase (GSHPx), and malondialdehyde (MDA) analyses. Liver protein concentration was determined by the method of Bradford (1976) using a protein assay kit A045 (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

TSOD Activity

The method described by Oyanagui (1984) was used for the assay of TSOD activity using a TSOD assay kit A001 (Nanjing Jiancheng Bioengineering Institute). The method is based on the fact that superoxide dismutase (SOD) inhibits the generation of nitrite from oxidation of hydroxylamine by superoxide anion \((O_2^-)\) that is produced by the xanthine/xanthine oxidase system. The activity of TSOD was expressed as units per milliliter (for serum) or units per milligram of protein (for liver homogenate) and determined by measuring the reduction of optical density (OD) of the reaction solution at 550 nm with a spectrophotometer (UV-2000, UNICO Instruments Co. Ltd., Shanghai, China). One unit of SOD was defined as the amount of SOD required to produce 50% inhibition of the rate of nitrite production at 37°C.

GSHPx Activity

Glutathione peroxidase activity was determined using a GSHPx assay kit A005 (Nanjing Jiancheng Bioengineering Institute) by the method of Maral et al. (1977). Glutathione peroxidase is an enzyme that catalyzes glutathione oxidation by oxidizing the reduced tripeptide glutathione (GSH) into oxidized glutathione. Hydrogen peroxide was used as a substrate of glutathione. Absorbance was recorded at 412 nm. The GSHPx activity was expressed as units per milliliter (for serum) or units per milligram of protein (for liver homogenate), and 1 unit was defined as a decrease in GSH of 1 mM/min after the decrease in GSH per minute of the nonenzymatic reaction was subtracted.

Table 2. Growth performance of gilts (n = 5 per treatment) fed diets with or without zearalenone (ZEA) supplementation

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>ZEA1</th>
<th>ZEA2</th>
<th>ZEA3</th>
<th>SEM</th>
<th>Effect (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW, kg</td>
<td>10.67</td>
<td>10.30</td>
<td>10.53</td>
<td>9.73</td>
<td>0.158</td>
<td>0.548 0.224</td>
</tr>
<tr>
<td>Final BW, kg</td>
<td>19.36</td>
<td>18.76</td>
<td>19.00</td>
<td>18.48</td>
<td>0.206</td>
<td>0.825 0.406</td>
</tr>
<tr>
<td>ADG, g/d</td>
<td>482.3</td>
<td>470.1</td>
<td>470.3</td>
<td>486.0</td>
<td>2.337</td>
<td>0.421 0.155</td>
</tr>
<tr>
<td>ADFI, g/d</td>
<td>787.0</td>
<td>751.2</td>
<td>740.1</td>
<td>736.4</td>
<td>4.704</td>
<td>0.249 0.771</td>
</tr>
<tr>
<td>FE2</td>
<td>0.61</td>
<td>0.63</td>
<td>0.64</td>
<td>0.66</td>
<td>0.005</td>
<td>0.309 0.049</td>
</tr>
</tbody>
</table>

1Analyzed concentrations of ZEA in the diets (mg/kg of diet) were 0 (control), 1.1 (ZEA1), 2.0 (ZEA2), and 3.2 (ZEA3).

2FE = feed efficiency is grams of gain/grams of feed intake.
**MDA Assay**

Malondialdehyde was determined according to Placer et al. (1966) with an MDA assay kit A003 (Nanjing Jiancheng Bioengineering Institute). It was analyzed with the thiobarbituric acid method, measuring MDA-reactive products spectrophotometrically, and results used for lipid peroxidation and oxidative stress in both serum and liver. Absorbance of samples was determined at 532 nm. Results were expressed as nanomoles of MDA per milliliter of serum or nanomoles of MDA per milligram of liver protein.

**Biochemical Variable Determination**

Enzyme activities in serum including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), urea, and creatinine (CRE) were determined using an automatic clinical chemistry analyzer (Roche, Cobus-Mira-Plus, Roche Diagnostic System Inc., Basel, Switzerland).

**Data Calculation and Statistical Analyses**

All data were subjected to ANOVA using the GLM procedure (SAS Inst. Inc., Cary, NC). The data were first analyzed as a completely randomized design with individual piglet as the random factor to examine the overall effect of treatments. Orthogonal polynomial contrasts were then used to determine linear responses to ZEA treatments. Differences among treatments were tested using Duncan’s multiple range test. The common intercept multiple linear regression of each treatment was conducted over 5 time points (d 1, 5, 9, 13, and 17), and a t-test was used to determine the significance of the linear slope. All statements of significance were based on the probability of \( P < 0.05 \).

**RESULTS**

**Growth and Vulva Size**

All piglets appeared healthy without mortality throughout the 18-d feeding period. Overall growth and feed intake among treatments were not affected by the additional ZEA in the diet (Table 2). Gilts fed diets containing 1.1 mg of ZEA/kg or greater had increased vulva length, vulva width, vulva height, and vulva area compared with the control (\( P < 0.05 \); Table 3). Increasing dietary ZEA linearly increased (\( P < 0.001 \)) vulva length, width, height, and area. The effects of time and ZEA on vulva development and linear regression of vulva size changes are shown in Figure 1. Slope ratio contrast indicated that the vulva size of ZEA1, ZEA2, and ZEA3 was 277, 643, and 762% greater than the control (\( P < 0.05 \)), respectively.

**Organ Weights**

Relative genital organ weight of piglets supplemented with 3.2 mg of ZEA/kg (ZEA3) was greater than that of controls (\( P < 0.05 \); Table 4), whereas spleen relative weight was reduced (\( P = 0.024 \)) in ZEA3 gilts. Gilts

**Table 3. Vulva size of gilts (n = 5 per treatment) fed diets with or without (ZEA) supplementation**

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>ZEA1</th>
<th>ZEA2</th>
<th>ZEA3</th>
<th>SEM</th>
<th>Effect (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length, mm</td>
<td>17.18a</td>
<td>18.70b</td>
<td>20.57a</td>
<td>21.63a</td>
<td>0.164</td>
<td>0.002 &lt;0.001</td>
</tr>
<tr>
<td>Width, mm</td>
<td>13.72a</td>
<td>15.39b</td>
<td>17.61a</td>
<td>18.69a</td>
<td>0.104</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>Height, mm</td>
<td>13.26a</td>
<td>15.07b</td>
<td>15.87ab</td>
<td>16.83a</td>
<td>0.106</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>Area, mm²</td>
<td>118.0a</td>
<td>145.0b</td>
<td>186.2²</td>
<td>208.1²</td>
<td>1.999</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
</tbody>
</table>

**Figure 1.** Effects of dietary zearalenone (ZEA) on vulva size. Vulva size was calculated by multiplying vulva length by width, then dividing by 2. Data presented are means of 5 replicate pigs per treatment. Zearalenone was not detectable in the control diet; ZEA1, ZEA2, or ZEA3 represent the control diet with the addition of 1.1, 2.0, or 3.2 mg of ZEA/kg, respectively. Slopes of the daily increase in vulva size of the control, ZEA1, ZEA2, and ZEA3 groups were 6.5, 17.9, 41.5, and 49.2, respectively. Significance differences in slopes (P-values) when contrasting controls vs. ZEA1, ZEA2, and ZEA3 were 0.021, 0.006, and 0.002, respectively.
fed the diet containing 2.0 mg of ZEA/kg or greater (ZEA2 and ZEA3) had reduced relative spleen weight \( (P < 0.05) \) compared with controls. The relative genital organ weights increased \( (P < 0.05) \) with increasing dietary ZEA, starting at 1.1 mg of ZEA/kg. The relative genital organs, liver, and kidney weights of piglets were increased linearly as dietary ZEA increased \( (P < 0.05) \). On the contrary, the relative spleen weights decreased linearly with increasing concentrations of dietary ZEA \( (P < 0.05) \).

### Biochemical Variables

Results of serum biochemical analysis revealed that gilts fed ZEA3 for 18 d increased \( (P < 0.05) \) serum concentrations of AST, ALT, ALP, GGT, urea, and CRE compared with controls (Table 5). Serum concentrations of the enzymes, except AST, as well as urea and CRE were increased in gilts fed ZEA2 compared with controls \( (P < 0.05) \). However, in ZEA1 gilts, only serum ALP was increased compared with controls \( (P < 0.05) \). Addition of dietary ZEA linearly increased \( (P < 0.05) \) all tested serum biochemical variables in the study.

### Oxidative Stress

Piglets in the ZEA2 and ZEA3 groups had decreased serum and liver activities of TSOD and GSHPx \( (P < 0.05) \) and greater MDA concentrations \( (P < 0.001) \) than controls (Table 6). Compared with controls, ZEA1 gilts had decreased activity of TSOD and increased MDA concentrations in the serum \( (P < 0.05) \), but no observed differences in TSOD, GSHPx, or MDA in the liver. Increasing dietary ZEA linearly decreased \( (P < 0.001) \) activities of TSOD and GSHPx in both serum and liver, whereas MDA concentrations in both serum and liver were linearly increased \( (P < 0.001) \) as dietary ZEA concentrations increased.

### DISCUSSION

The similar growth rate, feed intake, and feed efficiency of the piglets among all the treatment groups indicated that dietary ZEA concentration in the range of 1.1 to 3.2 mg/kg of diet had no negative effects on growth performance of the gilts fed a corn meal- and soybean meal-based diet. This, combined with the observation that gilts did not sort the diet according to the ZEA supplementation, suggests that gilts within a treatment group likely consumed a similar amount of ZEA and that differences obtained among treatment groups were likely attributable to the different concentrations of dietary ZEA.

Zearalenone induces hyperestrogenosis in pigs, with typical clinical symptoms being swollen vulva, prolapse of the vagina and rectum, and enlargement of the mammary glands (Stob et al., 1962). A previous study in our laboratory revealed that ZEA (1.3 mg/kg) resulted in a hyperestrogenism by increasing both vulva size and

---

**Table 4.** Relative organ weights (g/kg of BW) of gilts \((n = 5\) per treatment\) fed diets with or without zearalenone (ZEA) supplementation\(^1\)

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control</th>
<th>ZEA1</th>
<th>ZEA2</th>
<th>ZEA3</th>
<th>SEM</th>
<th>Treatment</th>
<th>Linear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genital organs</td>
<td>0.33(^{a})</td>
<td>0.53(^{a})</td>
<td>0.74(^{b})</td>
<td>1.16(^{c})</td>
<td>0.009</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Liver</td>
<td>30.2(^{a})</td>
<td>33.4(^{a})</td>
<td>36.7(^{b})</td>
<td>44.3(^{c})</td>
<td>0.096</td>
<td>0.016</td>
<td>0.001</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.18(^{a})</td>
<td>5.61(^{b})</td>
<td>6.04(^{b})</td>
<td>6.53(^{c})</td>
<td>0.093</td>
<td>0.029</td>
<td>0.0006</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.20(^{a})</td>
<td>2.10(^{b})</td>
<td>1.90(^{c})</td>
<td>1.83(^{c})</td>
<td>0.021</td>
<td>0.024</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

\(^{a–d}\)Within a row, means without a common superscript differ \((P < 0.05)\).

\(^1\)Analyzed concentrations of ZEA in the diets (mg/kg of diet) were 0 (control), 1.1 (ZEA1), 2.0 (ZEA2), and 3.2 (ZEA3).

**Table 5.** Serum biochemical blood markers of gilts \((n = 5\) per treatment\) fed diets with or without zearalenone (ZEA) supplementation\(^1,2\)

<table>
<thead>
<tr>
<th>Serum marker</th>
<th>Control</th>
<th>ZEA1</th>
<th>ZEA2</th>
<th>ZEA3</th>
<th>SEM</th>
<th>Treatment</th>
<th>Linear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate aminotransferase, U/L</td>
<td>40.1(^{b})</td>
<td>44.6(^{b})</td>
<td>53.5(^{b})</td>
<td>66.9(^{c})</td>
<td>1.180</td>
<td>0.012</td>
<td>0.001</td>
</tr>
<tr>
<td>Alanine aminotransferase, U/L</td>
<td>49.5(^{a})</td>
<td>55.0(^{b})</td>
<td>62.3(^{a})</td>
<td>66.0(^{a})</td>
<td>0.658</td>
<td>0.005</td>
<td>0.002</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/L</td>
<td>221.0(^{a})</td>
<td>245.6(^{b})</td>
<td>264.7(^{b})</td>
<td>298.4(^{a})</td>
<td>1.475</td>
<td>0.017</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>γ-Glutamyltransferase, U/L</td>
<td>32.2(^{a})</td>
<td>35.8(^{b})</td>
<td>38.0(^{b})</td>
<td>48.7(^{a})</td>
<td>0.292</td>
<td>0.029</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>2.8(^{a})</td>
<td>3.8(^{a})</td>
<td>4.2(^{a})</td>
<td>5.3(^{a})</td>
<td>0.309</td>
<td>0.016</td>
<td>0.006</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>71.2(^{a})</td>
<td>86.1(^{b})</td>
<td>89.7(^{b})</td>
<td>96.3(^{a})</td>
<td>1.011</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^{a–c}\)Within a row, means without a common superscript differ \((P < 0.05)\).

\(^1\)Data per piglet were run in duplicate in a single assay to avoid interassay variation.

\(^2\)Analyzed concentrations of ZEA in the diets (mg/kg of diet) were 0 (control), 1.1 (ZEA1), 2.0 (ZEA2), and 3.2 (ZEA3).
Antioxidant enzymatic activity and concentration of malondialdehyde in the serum or liver of gilts (n = 5 per treatment) fed diets with or without zearalenone (ZEA) supplementation. 

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>ZEA1</th>
<th>ZEA2</th>
<th>ZEA3</th>
<th>SEM</th>
<th>Treatment</th>
<th>Linear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione peroxidase, U/mL</td>
<td>111.3^a</td>
<td>105.8^b</td>
<td>89.4^c</td>
<td>84.2^d</td>
<td>0.920</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total superoxide dismutase, U/mL</td>
<td>115.1^a</td>
<td>108.7^b</td>
<td>102.6^c</td>
<td>98.2^d</td>
<td>0.182</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Malondialdehyde, nmol/mL</td>
<td>7.6^a</td>
<td>9.7</td>
<td>12.0^b</td>
<td>14.8^c</td>
<td>0.158</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione peroxidase, U/mg of protein</td>
<td>207.6^a</td>
<td>178.2^b</td>
<td>144.4^c</td>
<td>121.7^d</td>
<td>1.867</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total superoxide dismutase, U/mg of protein</td>
<td>106.0^a</td>
<td>99.3^b</td>
<td>89.2^c</td>
<td>79.8^d</td>
<td>0.730</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Malondialdehyde, nmol/mg of protein</td>
<td>8.6^a</td>
<td>9.9^b</td>
<td>10.7^c</td>
<td>12.9^d</td>
<td>0.117</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

4–6Within a row, means without a common superscript differ (P < 0.05).
1Data per piglet were run in duplicate in a single assay to avoid interassay variation.
2Analyzed concentrations of ZEA in the diets (mg/kg of diet) were 0 (control), 1.1 (ZEA1), 2.0 (ZEA2), and 3.2 (ZEA3).

Note: Table 6. Antioxidant enzymatic activity and concentration of malondialdehyde in the serum or liver of gilts (n = 5 per treatment) fed diets with or without zearalenone (ZEA) supplementation.

Ovary-uterus weights, which was confirmed by proliferative changes of the ovary tissues in gilts (Jiang et al., 2010b). Vulva swelling and reddening in gilts were also seen within 7 d of ZEA (1.5 mg/kg or 2 mg/kg) exposure (Rainey et al., 1990). At the end of an 18-d feeding period, pigs fed the greatest amount of dietary ZEA in the study showed a 25.9, 36.2, 26.9, and 76.3% increase in vulva length, width, height, and area, respectively, compared with the control group. These observations may be useful in the development of carcass indicators of drug contamination.

Detrimental effects of ZEA on reproductive organs have been shown in rodents (Underhill et al., 1995), pigs (Farnworth and Trenholm, 1981; James and Smith, 1982), rabbits, and monkeys (Zinedine et al., 2007). Consistent with previously reported results, the present study shows that continuous feeding of ZEA, as little as 1.1 mg/kg of diet for 18 d, significantly increases relative genital organ weights of gilts. Increased liver weights were found in male and female FDRL Wistar rats after an exposure to ZEA at 3 mg/kg of BW (Becci et al., 1982). Similar changes in relative liver weight, as well as degeneration of the liver tissues, were also found in female piglets fed a 1.3-mg/kg ZEA-contaminated diet (Jiang et al., 2010a). To the knowledge of the authors, there is no literature on the effects of ZEA (alone) on spleen weight of piglets. Tiemann et al. (2006) reported a dose-dependent increase in spleen weight in gilts fed both DON and ZEA at 9.57 and 0.358 mg/kg, respectively. In the present study, both relative liver and kidney weights were increased in piglets fed the 3.2-mg/kg ZEA diet, whereas gilts fed the diet containing 2.0 or 3.2 mg of ZEA/kg had decreased relative spleen weights. However, a significant increase in relative genital organ weights was observed in gilts fed diets containing 1.1, 2.0, or 3.2 mg of ZEA/kg compared with controls. These results suggest that the response of organ weight to ZEA supplementation is dose dependent, and the order of organ weight sensitivity is reproductive organs > spleen > liver > kidney.

Serum enzyme activities are commonly used as a diagnostic tool in animal health (Homolka, 1969). Much attention has been paid to toxins produced from Fusaria fungi including ZEA, which causes adverse effects such as liver and kidney damage, with subsequent changes in some enzymatic variables in rats and rabbits (Maa-ourfi et al., 1996; Conková et al., 2001; Abbès et al., 2006). In the current study, measurements of ALT in pigs fed the 2- and 3.2-mg/kg of ZEA diets were beyond a normal range of ALT (31 to 58 U/L; Kaneko et al., 1997). It has been reported that increased serum ALT may result from acute hepatic necrosis (Abdel-Wahhab et al., 2006; Jiang et al., 2010a). The alterations of ALT activities and urea concentrations observed in pigs fed 2 and 3.2 mg/kg of ZEA-contaminated diets suggests possible ZEA-induced hepatic damage. Indeed, in the present study, we confirmed the histopathological changes of the liver by photomicrographs of hematoxylin- and eosin-stained liver sections, and ultrastructural photos of liver sections. Similar to the present study, greater serum concentrations of ALT, AST, and ALP in ZEA-treated female Wistar rats (1.5, 3, or 5 mg/kg of BW) were reported by Maaroufi et al. (1996). Abbès et al. (2006) also concluded that ZEA at 40 or 500 mg/kg of BW could result in changes of biochemical variables and degeneration of hepatic and renal tissues in mice.

Antioxidant enzymatic and nonenzymatic antioxidants of the body are the major defense system to prevent organ injuries from the excessive quantity of reactive oxygen species that cause cellular lipid peroxidation (Sies, 1991). Research has demonstrated that ZEA may have an important role in cellular oxidative stress (Freeman and Crapo, 1981; Ben Salah-Abbès et al., 2008b, 2009; Zourgui et al., 2008). A study in Vero and caco-2 cell lines showed that ZEA induced oxidative damage by increasing lipid peroxidation (Abid-Essefi et al., 2004). Ben Salah-Abbès et al. (2007) reported that SOD and GSHPx activities decreased with progressive liver and kidney injury in ZEA-treated mice. Ben Salah-Abbès et al. (2009) also observed that ZEA supplementation at 40 mg/kg significantly reduced SOD and GSHPx activity in testes of mice. The activities of SOD and GSHPx are known to serve as protective responses...
to eliminate reactive free radicals (Cheung et al., 2001). Therefore, the decreased TSOD and GSHPx activity in both serum and liver found in the present study suggest that ZEA feeding induced oxidative stress in the liver and subsequently resulted in damage to the liver and potentially other organs. As an end product of lipid peroxidation, MDA is commonly used to monitor lipid oxidation status in the body (Sumida et al., 1989). The increased MDA concentrations in both serum and liver may be a result of reduced antioxidant enzymatic activities (TSOD and GSHPx) associated with ZEA supplementation. Zearalenone, at 40 mg/kg of BW, induced a significant increase in MDA formation compared with control groups in both liver and kidney extracts of mice (Zourgui et al., 2008). Borutova et al. (2008) reported that decreased GSHPx activity could be a result of the conjugation of GSHPx with ZEA or its metabolites. Based on the evidence from this and former research, it is reasonable to propose that oxidative damage may be a secondary effect of ZEA toxicity in pigs and other animals.

This study demonstrated that feeding postweaning gilts with diet containing ZEA at the concentrations of 1.1 to 3.2 mg/kg of diet for 18 d linearly enlarged vulva size, linearly increased relative weights of genital organs, liver, and kidney, but linearly reduced the relative weight of spleen, and linearly decreased the antioxidant status of liver and serum. In addition to the estrogenic effects of ZEA, the observation that ZEA increased oxidative stress of gilts in this study offers another possible explanation for ZEA toxicity in pigs. Further studies are needed to understand the cellular and molecular mechanism of ZEA toxicity in animals.

**LITERATURE CITED**


