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Effects of Zearalenone on Production Performance, Serum Antioxidant Function, and Immune Function in Weaned Gilts (Postprint)

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Abstract

To investigate the effects of different levels of zearalenone (ZEA)-contaminated diets on growth performance, serum antioxidant function, serum antibody levels, and peripheral blood lymphocyte proliferation rate in weaned gilts, forty healthy three-way crossbred (Duroc × Landrace × Yorkshire) weaned gilts were divided into 4 groups based on age [(35±1) days of age] and average body weight [(14.01±0.86) kg]. The control group was fed a basal diet, and the experimental groups were fed the basal diet supplemented with 0.5, 1.0, and 1.5 mg/kg ZEA [the measured ZEA values were (0.52±0.07) mg/kg, (1.04±0.03) mg/kg, and (1.51±0.13) mg/kg, respectively]. The preliminary period lasted 7 days, and the formal experimental period lasted 35 days. The results showed that dietary ZEA had no significant effect on average daily feed intake, average daily gain, and feed-to-gain ratio in weaned gilts ($P>0.05$). Compared with the control group, ZEA significantly decreased serum glutathione peroxidase (GSH-Px) activity (0.5, 1.0, and 1.5 mg/kg ZEA), classical swine fever (1.0 and 1.5 mg/kg ZEA) and pseudorabies virus antibody levels (1.5 mg/kg ZEA), and peripheral blood lymphocyte proliferation rate (1.0 and 1.5 mg/kg ZEA) ($P<0.05$), while significantly increasing serum malondialdehyde (MDA) content (0.5, 1.0, and 1.5 mg/kg ZEA) ($P<0.05$). With increasing dietary ZEA levels, the feed-to-gain ratio in weaned gilts showed a linear decreasing trend ($P=0.075$), while serum GSH-Px and superoxide dismutase (SOD) activities, serum virus (classical swine fever, pseudorabies, and highly pathogenic porcine reproductive and respiratory syndrome virus) antibody levels, and peripheral blood lymphocyte proliferation rate all showed linear decreases ($P<0.05$), whereas serum MDA content showed a linear increase ($P<0.05$). These results indicate that 0.5 mg/kg ZEA in the diet is sufficient to induce oxidative stress responses in gilts, and 1.0 mg/kg ZEA can significantly reduce specific humoral and cellular immune functions in weaned gilts.

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Full Text

Effects of Zearalenone on Growth Performance, Serum Antioxidant Capacity, and Immune Function of Weaning Gilts

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Abstract

This study investigated the effects of diets contaminated with different levels of zearalenone (ZEA) on growth performance, serum antioxidant capacity, serum antibody levels, and peripheral blood lymphocyte proliferation rate in weaning gilts. Forty healthy crossbred (Duroc × Landrace × Large White) weaning gilts were divided into 4 groups based on age [(35±1) days] and average body weight [(14.01±0.86) kg]. The control group was fed a basal diet, while experimental groups were fed the basal diet supplemented with 0.5, 1.0, and 1.5 mg/kg ZEA [measured ZEA values of (0.52±0.07) mg/kg, (1.04±0.03) mg/kg, and (1.51±0.13) mg/kg, respectively]. The adaptation period lasted 7 days, followed by a 35-day experimental period. The results showed that dietary ZEA had no significant effect on average daily feed intake, average daily gain, or feed-to-gain ratio of weaning gilts (P>0.05). Compared with the control group, ZEA significantly reduced serum glutathione peroxidase (GSH-Px) activity (in the 0.5, 1.0, and 1.5 mg/kg ZEA groups), antibody levels against classical swine fever virus (in the 1.0 and 1.5 mg/kg ZEA groups) and pseudorabies virus (in the 1.5 mg/kg ZEA group), and peripheral blood lymphocyte proliferation rate (in the 1.0 and 1.5 mg/kg ZEA groups) (P<0.05), while significantly increasing serum malondialdehyde (MDA) content (in the 0.5, 1.0, and 1.5 mg/kg ZEA groups) (P<0.05). With increasing dietary ZEA levels, the feed-to-gain ratio of weaning gilts showed a linear decreasing trend (P=0.075), while serum GSH-Px and superoxide dismutase (SOD) activities, serum virus antibody levels (classical swine fever, pseudorabies, and highly pathogenic porcine reproductive and respiratory syndrome virus), and peripheral blood lymphocyte proliferation rate all showed linear decreases (P<0.05), and serum MDA content showed a linear increase (P<0.05). These results indicate that 0.5 mg/kg ZEA in the diet is sufficient to induce oxidative stress responses in gilts, while 1.0 mg/kg ZEA can significantly reduce both specific humoral and cellular immune functions in weaning gilts.

Keywords: zearalenone; weaning gilts; growth performance; glutathione peroxidase; malondialdehyde; antibody level; lymphocyte proliferation rate

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1. Materials and Methods

1.1 Experimental Material

ZEA was purchased from Fermentek Company (Israel) with chromatographic purity guaranteed at 98%.

1.2 Experimental Design and Animal Management

Forty healthy crossbred (Duroc × Landrace × Large White) weaning female piglets aged 25–28 days were housed on farrowing crates for an additional 10 days before being transferred to individual experimental cages (0.48 m^2). Based on age [(35±1) days] and average body weight [(14.01±0.86) kg], the piglets were divided into 4 groups of 10 animals each, with no significant difference in initial body weight among groups ($P>0.05$). The basal diet for weaning gilts was formulated according to NRC (2012) nutrient requirements, with composition and nutrient levels shown in Table 1 . The control group received the basal diet, while experimental groups received the basal diet supplemented with 0.5, 1.0, and 1.5 mg/kg ZEA, with measured values of (0.52±0.07) mg/kg, (1.04±0.03) mg/kg, and (1.51±0.13) mg/kg, respectively. The adaptation period lasted 7 days, followed by a 35-day experimental period.

The individual cages featured plastic slatted floors equipped with nipple drinkers and feeders, allowing ad libitum access to feed and water. The pig house was thoroughly cleaned and disinfected before the trial, with weekly disinfection during the experimental period. Infrared heat lamps maintained temperatures at approximately 30°C during the first week and 26–28°C during the second week. Relative humidity was maintained at 65%. Management and immunization procedures followed conventional practices. Classical swine fever virus vaccine was administered via intramuscular injection on day 21 post-birth; pseudorabies virus vaccine was administered intranasally within 72 hours post-birth with a second immunization on day 28; and highly pathogenic porcine reproductive and respiratory syndrome virus vaccine was administered via intramuscular injection on day 14 post-birth. All gilts were slaughtered at the end of the experiment. The animal trial was conducted at the Animal Science and Technology Park of Shandong Agricultural University from April to June 2016.

1.3 Preparation of ZEA-Contaminated Diets

Chromatographically pure (98%) crystalline ZEA powder was dissolved in ethyl acetate to prepare a solution, which was then sprayed onto a talc carrier and left overnight to evaporate the ethyl acetate, creating a 1,000 mg/kg ZEA premix. This premix was further diluted with toxin-free cornmeal to produce a 10 mg/kg ZEA premix. Finally, experimental diets were formulated by substituting corn and carrier in each group' s diet formulation with the ZEA premix to achieve the designed ZEA levels. All experimental diets were prepared in one batch one week before the trial began and stored in sealed bags in a dry, ventilated location. Samples were taken before and after the trial for immediate analysis of

nutrient content and toxin levels using the sampling method specified in GB/T 14699.1-93.

1.4 Determination of Dietary Nutrients and Toxins

Routine nutrient analysis of diets was performed according to AOAC (2012) methods. Crude protein content was determined by the Kjeldahl method; digestible energy was measured using an HR-15 bomb calorimeter; calcium content was determined by potassium permanganate titration; and amino acid content was analyzed using a Hitachi 835-50 automatic amino acid analyzer.

Dietary toxin levels were determined by the Qingdao Entry-Exit Inspection and Quarantine Bureau. ZEA and aflatoxin levels were measured using immunoaffinity column chromatography purification with liquid chromatography-fluorescence detection and external standard quantification. Fumonisin and deoxynivalenol levels were measured using immunoaffinity chromatography purification with high-performance liquid chromatography-tandem mass spectrometry and liquid chromatography-ultraviolet detection with external standard quantification. The minimum detection limits were 1.0 g/kg for aflatoxin, 0.1 mg/kg for ZEA, 0.1 mg/kg for deoxynivalenol, and 0.25 mg/kg for fumonisin. The actual measured ZEA values in the diets were 0 (0, 0), (0.52±0.07) (0.59, 0.45) mg/kg, (1.04±0.03) (1.01, 1.07) mg/kg, and (1.51±0.13) (1.38, 1.64) mg/kg, with no other toxins detected or levels below detection limits in both samplings.

1.5 Sample Collection and Analysis

1.5.1 Growth Performance Measurement Daily feed intake and residual feed were recorded for each gilt, with body weights measured at the beginning and end of the trial to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

1.5.2 Blood Sample Collection and Processing On day 35 of the experimental period, fasting blood samples were collected from the anterior vena cava and ear vein before morning feeding. Fifteen milliliters of whole blood was collected from the ear vein into vacuum anticoagulant tubes (containing K2EDTA), immediately inverted 8 times, temporarily stored at 0°C, and transported to the laboratory for determination of peripheral blood lymphocyte proliferation rate. Additionally, 30 mL of blood was collected from the anterior vena cava into vacuum coagulation-promoting tubes, centrifuged at 3,000 r/min for 10 minutes to separate serum for antibody level and antioxidant function analyses.

1.5.3 Serum Antioxidant Function Analysis Serum glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities and malondialdehyde (MDA) content were determined using a 752 UV-Vis spectrophotometer according to kit instructions. GSH-Px (A005), MDA (A003), and SOD (A001-1) kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.5.4 Serum Antibody Level Analysis Serum antibody levels were analyzed according to the instructions for classical swine fever, pseudorabies, and highly pathogenic porcine reproductive and respiratory syndrome virus enzyme-linked immunosorbent assay (ELISA) kits (CIVTEST SUISHC/PPC, LSI, France). Absorbance (OD) values at 450 nm were measured using an ELISA reader (FAME 24/20, Hamilton, Switzerland) and compared with standard samples and their corresponding OD values in the ELISA kits for antibody level analysis.

1.5.5 Determination of Peripheral Blood Lymphocyte Proliferation Rate Blood preprocessing: Whole blood was mixed 1:1 with D-Hanks solution and added to lymphocyte separation medium, then centrifuged at 2,000 r/min for 30 minutes. The middle leukocyte layer was collected, red blood cells were lysed using red blood cell lysis buffer, and the sample was washed three times with RPMI-1640 (Hyclone, USA), centrifuging for 5 minutes (2,000 r/min) after each wash. Viable cells were counted using trypan blue staining (should be >95%), and splenic lymphocytes were suspended in RPMI-1640 complete culture medium to adjust cell density to 2×10 cells/mL.

Lymphocyte proliferation rate measurement: The cell suspension was added to 96-well culture plates at 190 L per well, with 10 L concanavalin A (ConA) added simultaneously. Plates were incubated in a 5% CO incubator at 37°C for 72 hours. Four hours before the end of incubation, 100 L of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well, and incubation continued. After incubation, 100 L of supernatant was removed from each well, 100 L of dimethyl sulfoxide (DMSO) was added to dissolve the purple crystalline product, and OD values were measured at 570 nm using an ELISA reader. Using the control group absorbance value as 1, experimental groups were compared with the control group to calculate the percentage of cell numbers relative to the control group, which represents the lymphocyte proliferation rate.

1.6 Data Processing and Analysis Data were analyzed using SAS 9.2 statistical software. Differences among groups were analyzed using one-way ANOVA. Orthogonal polynomial comparison was used for linear regression analysis of treatment effects across different ZEA level gradients, and Duncan' s multiple range test was used for multiple comparisons, with significance level set at $P<0.05$.

2. Results

2.1 Effects of ZEA on Growth Performance of Weaning Gilts

As shown in Table 2 , ZEA had no significant effect on average daily gain, average daily feed intake, or feed-to-gain ratio of weaning gilts ($P>0.05$). However, with increasing dietary ZEA levels, the feed-to-gain ratio showed a linear decreasing

trend (P=0.075). Values in the same row with different letter superscripts differed significantly (P<0.05). The same notation applies to subsequent tables.

2.2 Effects of ZEA on Serum Antioxidant Function of Weaning Gilts

As shown in Table 3 , compared with the control group, serum GSH-Px activity was significantly reduced in the 0.5, 1.0, and 1.5 mg/kg ZEA groups (P<0.05), while serum MDA content was significantly increased (P<0.05). With increasing dietary ZEA levels, serum GSH-Px and SOD activities showed linear decreases (P<0.05), while serum MDA content showed a linear increase (P<0.05).

2.3 Effects of ZEA on Serum Antibody Levels of Weaning Gilts

As shown in Table 4 , serum antibody levels against classical swine fever virus and pseudorabies virus in the 1.5 mg/kg ZEA group were significantly lower than in the control group (P<0.05), and serum classical swine fever virus antibody level in the 1.0 mg/kg ZEA group was significantly lower than in the control group (P<0.05). No significant differences were observed in serum highly pathogenic porcine reproductive and respiratory syndrome virus antibody levels among groups (P>0.05). With increasing dietary ZEA levels, serum antibody levels against classical swine fever, pseudorabies, and highly pathogenic porcine reproductive and respiratory syndrome viruses all showed linear decreases (P<0.05).

2.4 Effects of ZEA on Peripheral Blood Lymphocyte Proliferation Rate of Weaning Gilts

As shown in Figure 1 [Figure 1: see original paper], compared with the control group, peripheral blood lymphocyte proliferation rate was significantly reduced in the 1.0 and 1.5 mg/kg ZEA groups (P<0.05), while no significant change was observed in the 0.5 mg/kg group (P>0.05). With increasing dietary ZEA levels, peripheral blood lymphocyte proliferation rate in weaning gilts showed a linear decrease (P<0.05).

3. Discussion

3.1 Effects of ZEA on Growth Performance of Weaning Gilts

Research findings on the effects of ZEA on average daily feed intake, average daily gain, and feed-to-gain ratio in weaning gilts have been inconsistent. Some studies reported that adding 3 mg/kg ZEA to weaning piglet diets did not significantly affect average daily feed intake, average daily gain, or feed-to-gain ratio. Dietary supplementation with 1–3 mg/kg ZEA also did not significantly alter average daily gain or feed-to-gain ratio in piglets. However, Powell-Jones et al. found that ZEA had potential growth-promoting effects, though they did not analyze specific ZEA levels in their conclusions. Under the conditions of this study, dietary supplementation with 0.5–1.5 mg/kg ZEA had no significant

effect on average daily feed intake, average daily gain, or feed-to-gain ratio in gilts. Notably, the feed-to-gain ratio of weaning gilts showed a linear decreasing trend with increasing ZEA levels (P=0.075), suggesting that low levels (0.5-1.5 mg/kg) of ZEA have potential growth-promoting effects. Other reports have shown that with increasing dietary ZEA levels (3.0-9.0 mg/kg), feed intake, average daily gain, and feed efficiency in sows (initial body weight 64 kg) all showed decreasing trends. In summary, different levels of dietary ZEA have different effects at different growth stages in pigs, and further research is needed to confirm the correlation between ZEA levels and animal growth performance and its underlying mechanisms.

3.2 Effects of ZEA on Serum Antioxidant Function of Weaning Gilts

Free radical reactions are necessary for animal defense mechanisms, and the production and clearance of free radicals are normally in dynamic balance. Studies have shown that ZEA can stimulate oxidative stress in animals, causing oxidative capacity to exceed antioxidant capacity, thereby increasing oxygen free radicals and ultimately leading to biological membrane lipid peroxidation. Dietary supplementation with 2.0 and 3.2 mg/kg ZEA significantly reduced serum GSH-Px activity in piglets compared with the control group. Serum MDA content, as a biomarker of cell damage, was significantly lower in the control group than in all ZEA groups in this study and showed a linear increase with increasing ZEA levels. Serum GSH-Px activity was significantly higher in the control group than in all ZEA groups and showed a linear decrease with increasing ZEA levels. China' s Feed Hygiene Standard specifies a maximum limit of 0.5 mg/kg ZEA in piglet diets (GB 13078.2-2006), while the European Union's maximum limit is 0.1 mg/kg. Notably, under the conditions of this study, dietary supplementation with 0.5 mg/kg ZEA was sufficient to induce oxidative stress responses and significantly reduce serum antioxidant function in piglets, providing a theoretical basis for China' s feed hygiene standards. Although researchers generally agree that ZEA reduces serum antioxidant function, no studies have reported on the mechanisms by which low-level ZEA initiates peroxidation in animals, and the molecular mechanisms require further in-depth investigation by animal scientists.

3.3 Effects of ZEA on Serum Antibody Levels of Weaning Gilts

Classical swine fever, pseudorabies, and highly pathogenic porcine reproductive and respiratory syndrome are major infectious diseases threatening piglet health. While vaccination generally provides relatively ideal protection, vaccine failure or low herd immune responses are common occurrences that pose significant safety risks for disease outbreaks. Reports have shown that 18 days after normal classical swine fever vaccination, piglets in the 2.0 and 3.2 mg/kg ZEA groups had significantly lower classical swine fever virus antibody levels than the control group. The current results demonstrate that dietary supplementation with 1.0 and 1.5 mg/kg ZEA significantly reduced classical swine fever virus

antibody levels, and the 1.5 mg/kg ZEA group showed significantly lower pseudorabies virus antibody levels than the control group. Antibody levels against classical swine fever, pseudorabies, and highly pathogenic porcine reproductive and respiratory syndrome viruses all showed linear decreases with increasing dietary ZEA levels, suggesting that ZEA inhibits virus antibody production and negatively affects humoral immune function in weaning gilts. No reports have identified the minimum level of ZEA alone that initiates reduced virus antibody levels, making the correlation between dietary ZEA levels and changes in virus antibody levels in weaning gilts and its underlying mechanisms an important focus for future research in our group.

3.4 Effects of ZEA on Peripheral Blood Lymphocyte Proliferation Rate of Weaning Gilts

Lymphocyte proliferation capacity is an important indicator of cellular immune function. Lioi et al. found that ZEA could inhibit bovine lymphocyte proliferation, while other studies have shown that ZEA significantly inhibits proliferation of isolated mouse splenic T and B lymphocytes. Numerous studies have demonstrated that ZEA under in vitro conditions can disrupt lymphocyte homeostasis and exert immunotoxic effects, causing direct toxic damage to mouse peripheral blood lymphocytes. Most previous conclusions about ZEA effects on lymphocyte proliferation rate were derived from in vitro conditions, with few reports on the effects of ZEA-contaminated diets on peripheral blood lymphocyte proliferation rate in weaning gilts. The current results indicate that dietary supplementation with 1.0 mg/kg ZEA significantly reduced peripheral blood lymphocyte proliferation rate in gilts, suggesting that 1.0 mg/kg ZEA in the diet is sufficient to induce cellular immunity in gilts. Our research group will further explore the correlation between dietary ZEA levels and cellular and humoral immunity in weaning gilts using cellular and molecular biology approaches.

Conclusions

- Dietary ZEA at 0.5–1.5 mg/kg had no significant effect on growth performance of weaning gilts, but the feed-to-gain ratio showed a linear decreasing trend with increasing dietary ZEA levels.
- Dietary ZEA at 0.5 mg/kg was sufficient to induce oxidative stress responses in weaning gilts, significantly reducing serum GSH-Px activity and significantly increasing serum MDA content, both of which showed linear changes with increasing dietary ZEA levels.
- Dietary ZEA at 1.0 mg/kg was sufficient to induce humoral and cellular immune responses in weaning gilts, with serum virus antibody levels (classical swine fever, pseudorabies, and highly pathogenic porcine reproductive and respiratory syndrome virus) and peripheral blood lymphocyte proliferation rate showing linear decreases with increasing dietary ZEA levels.

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