Effects of Different Levels of Zearalenone on Serum Enzymes, Metabolites and Intestinal Morphology in Weaned Female Piglets (Post-print)

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Abstract

This experiment aimed to investigate the effects of different levels of zearalenone (ZEA) on serum enzymes, metabolites, and intestinal morphology in weaned female piglets. Forty healthy 28-day-old three-way crossbred (Duroc × Landrace × Large White) weaned female piglets with an average body weight of (14.01$±$0.86) kg were randomly allocated into 4 groups with 10 piglets per group. The control group was fed a basal diet, while experimental groups 1, 2, and 3 were fed the basal diet supplemented with 0.5, 1.0, and 1.5 mg/kg ZEA, respectively. The pre-trial period lasted 10 days, and the formal trial period lasted 35 days. The results showed that: 1) Compared with the control group, the activities of aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase were significantly increased (P<0.05) in all experimental groups; serum total cholesterol content was significantly increased (P<0.05) in experimental group 2; serum urea nitrogen and high-density lipoprotein (HDL) contents were significantly increased (P<0.05), while total protein content was significantly decreased (P<0.05) in experimental group 3. 2) Compared with the control group, villus height and crypt depth in the duodenum and jejunum were significantly increased (P<0.05) in the experimental groups. The villus height/crypt depth ratio in the duodenum was significantly increased (P<0.05) in experimental groups 1 and 2, while the ratio in the jejunum was significantly decreased (P<0.05); villus height and villus height/crypt depth ratio in the ileum were significantly increased (P<0.05). 3) Compared with the control group, villus height in the duodenum was significantly increased with loose and disordered arrangement, the number of intestinal glands decreased, and mucosal thickness became thinner. 4) Compared with the control group, villus height in the jejunum increased, the number of intestinal glands decreased, and epithelial

shedding with exposed lamina propria occurred in experimental groups 2 and 3. 5) Compared with the control group, villus height in the ileum increased and diffuse lymphocytes in the submucosa increased in experimental groups 1 and 2. These results indicate that dietary supplementation of ZEA (0.5–1.5 mg/kg) altered liver metabolism and intestinal morphological structure, thereby affecting the healthy growth of weaned female piglets.

Full Text

Effects of Different Levels of Zearalenone on Serum Enzymes, Metabolites and Intestinal Morphology of Weaned Gilts

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Abstract

This study investigated the effects of different levels of zearalenone (ZEA) on serum enzymes, metabolites and intestinal morphology in weaned gilts. Forty healthy weaned gilts (Duroc × Landrace × Yorkshire) aged 28 days with an average body weight of (14.01$±$0.86) kg were randomly allocated into 4 groups with 10 replicates per group (1 pig per replicate). Gilts in the control group were fed a basal diet, while those in experimental groups 1, 2 and 3 were fed the basal diet supplemented with 0.5, 1.0 and 1.5 mg/kg ZEA, respectively. The experiment consisted of a 10-day pretrial period followed by a 35-day formal trial period.

The results showed that: (1) Compared with the control group, the activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in serum were significantly increased in all experimental groups ($P<0.05$). Serum total cholesterol content was significantly elevated in experimental group 2 ($P<0.05$). Serum urea nitrogen and high-density lipoprotein (HDL) contents were significantly increased in experimental group 3 ($P<0.05$), while serum total protein content was significantly decreased ($P<0.05$). (2) The villus height and crypt depth in the duodenum and jejunum were significantly increased in all experimental groups compared with the control ($P<0.05$). In experimental groups 1 and 2, the villus height/crypt depth ratio was significantly increased in the duodenum ($P<0.05$) but significantly decreased in the jejunum ($P<0.05$), while the villus height and villus height/crypt depth ratio in the ileum were significantly increased ($P<0.05$). (3) Histological examination revealed that ZEA supplementation caused duodenal villi to become elongated, loosely arranged and disorganized, with reduced intestinal

gland numbers and thinner mucosal layers. (4) In the jejunum, ZEA increased villus height and reduced intestinal gland numbers, with epithelial denudation and lamina propria exposure observed in groups 2 and 3. (5) In the ileum, groups 1 and 2 showed increased villus height and greater numbers of diffuse lymphocytes in the submucosa. These findings demonstrate that dietary ZEA at 0.5–1.5 mg/kg alters hepatic metabolism and intestinal morphology in weaned gilts, thereby compromising their healthy growth.

Keywords: weaned gilts; zearalenone; serum; intestinal morphology

Introduction

Zearalenone (ZEA), also known as F-2 toxin, is a non-steroidal estrogenic mycotoxin produced by *Fusarium* fungi that commonly contaminate cereal by-products and other foods. Global surveys indicate widespread ZEA contamination in animal feed. Research has shown that the primary toxicity of ZEA and its metabolites, such as α-zearalenol, manifests as estrogenic effects on the reproductive organs and breeding performance of replacement gilts. When animals consume ZEA-contaminated diets, the gastrointestinal tract—the first barrier against natural toxins—becomes exposed to ZEA, making it the primary target organ. Studies have demonstrated that pigs fed low-dose ZEA (400 g/kg) showed no significant changes in intestinal mucosal morphology but exhibited increased mucin concentration in vesicles, elevated goblet cell activity, lymphocyte infiltration, and enhanced activity of Paneth cells and endocrine cells at the base of intestinal crypts, indicating activation of local defensive mechanisms in the small intestinal mucosa. Both in vivo and in vitro studies have confirmed that ZEA inhibits proliferation of porcine small intestinal cells and damages the epithelial antioxidant system, leading to cellular injury. Despite the increasing severity of ZEA contamination in feed and its impact on livestock production performance, current research on ZEA' s effects on animal digestive tract (small intestine) function remains limited and unsystematic. Therefore, this study aimed to investigate the effects of ZEA on serum enzymes, metabolites and intestinal morphology in weaned gilts from a morphological perspective, providing scientific reference for practical production and laying foundation for future research on nutritional strategies to mitigate its harmful effects.

Materials and Methods

1.1 Experimental Materials Feed ingredient samples were collected from multiple feed mills and farms in Shandong Province to survey mycotoxin contamination. Ingredients with toxin levels below detection limits were selected to formulate the basal diet. Pure ZEA (Fermentek, Israel) with a guaranteed purity of 98% was used.

1.2 Experimental Animals and Management Forty healthy female weaned piglets (Duroc × Landrace × Yorkshire) aged 28 days with an average body weight of $(14.01\$\pm0.86)kgwererandomlydividedinto4groups(10replicatespergroup,1pigperreplicate).In$ 0.05).$Pigletswerehousedindividuallywithadlibitumaccesstofeedandwater.Thecontrolgroupreceivedthebasal$ $0mg/kg),whileexperimentalgroups1,2and3receiveddietssupplementedwith0.5,1.0and1.5mg/kgZEA,respect$ $0.52\pm0.07,1.04\pm0.03and1.51\pm\$0.13$ mg/kg). The experiment included a 10-day pretrial period and a 35-day formal trial period. All experimental diets were prepared at once and stored in a dry, cool place. The basal diet was formulated according to NRC (2012) standards, with composition and nutrient levels shown in Table 1 .

1.3 Sample Collection On day 35 of the experiment, all piglets were slaughtered. The abdominal cavity was opened, and approximately 10 cm samples were taken from the middle segment of each intestinal section. After washing with physiological saline to remove blood, samples were evenly cut into two segments. One segment was fixed in Bouin' s solution for hematoxylin-eosin (HE) staining and intestinal morphology measurement.

1.4 Analytical Methods

1.4.1 Dietary Toxin Detection Diet samples were collected before and after the experiment to analyze toxin and crude protein content. ZEA was determined using enzyme-linked immunosorbent assay (ELISA) and fluorometric methods, with a minimum detection limit of 0.1 mg/kg. No other toxins were detected or were below detection limits in both samplings.

1.4.2 Blood Collection and Serum Analysis On day 35 before morning feeding, one piglet per replicate was randomly selected and 15 mL blood was collected using vacuum coagulation tubes. After standing in a 37°C water bath for 10 minutes, serum was separated by centrifugation at 3,000 rpm for 10 minutes and stored at -20°C. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities, as well as total protein, urea nitrogen, total cholesterol, total triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) contents were measured using an automatic biochemical analyzer (COBAS MIRA Plus, Roche, Switzerland).

1.4.3 HE Staining and Intestinal Morphology Measurement Fixed intestinal tissues were rinsed, dehydrated through graded ethanol, cleared with xylene, and embedded in paraffin. Sections (5 m thickness) were prepared using a microtome (LEICA RM2135, Germany), dewaxed with xylene, and rehydrated through graded alcohol to distilled water. Sections were stained with hematoxylin for 10 minutes, differentiated in hydrochloric acid alcohol for 5 seconds, blued in tap water for 15 minutes, and counterstained with eosin for 10 seconds. After dehydration through 95% and 100% ethanol, clearing with xylene,

and mounting with neutral balsam, sections were observed under bright-field microscopy. Images were captured using a Nikon Eclipse 80i photomicrography system. Villus height and crypt depth were measured using Motic Images 2000 1.3 software. Fifteen of the longest, most extended, straight and intact villi were measured per section, with adjacent crypt depths also measured. Mean values were calculated and villus height/crypt depth ratios were determined.

1.5 Statistical Analysis Data were analyzed using one-way ANOVA with SAS 9.2 software. Duncan' s multiple range test was used for post-hoc comparisons. Results are expressed as "mean ± standard deviation." Differences were considered significant at $P<0.05$.

Results

2.2 Effects of Different ZEA Levels on Serum Enzymes and Metabolites The effects of different ZEA levels on serum enzymes are presented in Table 2 . Compared with the control group, serum AST and LDH activities were significantly increased in experimental group 1 ($P<0.05$), further elevated in group 2 ($P<0.05$), and highest in group 3 ($P<0.05$). Serum ALP activity was significantly increased in all experimental groups ($P<0.05$).

The effects on serum metabolites are shown in Table 3 . Serum total cholesterol content was significantly increased in experimental group 2 ($P<0.05$). In experimental group 3, serum total cholesterol, HDL and urea nitrogen contents were significantly increased ($P<0.05$), while serum total protein content was significantly decreased ($P<0.05$). These results indicate that ZEA can affect the healthy growth of weaned piglets.

2.3 Effects of Different ZEA Levels on Intestinal Morphology The effects on intestinal morphology are presented in Table 4 . Dietary ZEA supplementation significantly increased duodenal villus height ($P<0.05$), with 1.5 mg/kg ZEA significantly increasing crypt depth ($P<0.05$) and 0.5 and 1.0 mg/kg ZEA significantly increasing the villus height/crypt depth ratio ($P<0.05$). In the jejunum, all experimental groups showed significantly increased villus height and crypt depth ($P<0.05$), while 0.5 and 1.0 mg/kg ZEA significantly decreased the villus height/crypt depth ratio ($P<0.05$). In the ileum, 0.5 and 1.0 mg/kg ZEA significantly increased villus height and villus height/crypt depth ratio ($P<0.05$). These findings demonstrate that ZEA affects the intestine of weaned gilts.

2.4 Effects on Duodenal Morphology Histological examination of duodenal morphology is shown in Figure 1 [Figure 1: see original paper]. The control group exhibited tightly arranged, orderly villi with abundant intestinal glands, well-developed duodenal glands, and thick mucosal layers with clearly

outlined, brightly stained and regularly arranged epithelial cells. In contrast, experimental groups showed marked histological changes, including elongated, loosely arranged and disorganized villi, reduced intestinal gland numbers, and thinner mucosal layers. These observations confirm ZEA-induced morphological alterations in the duodenum.

2.5 Effects on Jejunal Morphology Jejunal morphology is presented in Figure 2 [Figure 2: see original paper]. The control group showed tightly arranged villi with abundant intestinal glands. Experimental groups exhibited significantly elongated villi with larger diameters and reduced intestinal glands. In groups 2 and 3, jejunal epithelial denudation with exposed lamina propria was observed (yellow circles). These histological findings confirm ZEA-induced pathological damage to the jejunum.

2.6 Effects on Ileal Morphology Ileal morphology is shown in Figure 3 [Figure 3: see original paper]. Compared with the control, groups 1 and 2 displayed elongated ileal villi with no significant changes in intestinal glands but increased diffuse lymphocytes in the submucosa. These observations confirm ZEA effects on ileal morphology.

Although pure ZEA was used to prepare contaminated diets and feed ingredients were carefully selected, deoxynivalenol (DON) and aflatoxin (AFL) were still detected due to the ubiquitous nature of mycotoxin contamination. However, DON and AFL levels in all diets were far below Chinese feed hygiene standards (<1.0 mg/kg and <10 g/kg, respectively) and EU maximum limits for piglet diets (<0.9 mg/kg DON and <0.02 mg/kg AFL). Since ZEA levels in experimental groups substantially exceeded these standards, the observed intestinal morphological changes can be attributed primarily to ZEA. Growth performance data showed no significant differences in final body weight, average daily feed intake (ADFI), average daily gain (ADG) or feed/gain ratio (F/G) among groups ($P>0.05$).

Discussion

3.1 Effects on Serum Enzymes and Metabolites Serum enzyme activities (ALT, AST, ALP and LDH) serve as important indicators of liver health and stress responses. ALT participates in transamination between glutamate and pyruvate, AST catalyzes transamination between glutamate and oxaloacetate, while ALP plays crucial roles in bone formation and participates in lipid and protein metabolism. In this study, serum AST, ALP and LDH activities were significantly elevated in experimental groups. Previous research demonstrated that ZEA (40 mg/kg BW) significantly increased serum ALT, AST and ALP activities in mice, and ZEA (1 mg/kg) elevated AST, ALT and ALP activities in weaned piglets, consistent with our findings. Serum metabolites are

sensitive indicators of systemic or local metabolic changes and tissue physiological function. Serum total protein reflects tissue protein synthesis capacity and organ growth, while urea nitrogen correlates negatively with protein/amino acid balance and muscle growth. HDL, synthesized in liver and small intestine, reflects lipid catabolism and transport. This study showed significantly decreased serum total protein and increased urea nitrogen, total cholesterol and HDL in experimental groups. Previous studies reported that ZEA-contaminated diets increased blood cholesterol while decreasing HDL in weaned gilts, and that ZEA reduced blood total protein causing liver damage that affected protein and DNA synthesis. These findings suggest ZEA causes hepatocellular membrane damage potentially leading to hepatic tissue injury. While serum enzyme and metabolite results indicate ZEA affects piglet health, the underlying mechanisms require further investigation.

3.2 Effects on Intestinal Morphology The intestine and liver are primary sites for ZEA metabolism, with significant interspecies differences. Rabbits mainly metabolize ZEA in the liver, reducing it to α-zearalenol (ZEL) and β-ZEL, whereas in humans and pigs, metabolism occurs primarily in small intestinal cells, converting ZEA to α-ZEA, β-ZEA, α-ZEL and β-ZEL. Small intestinal mucosal morphology and function are directly reflected by villus height, crypt depth and villus height/crypt depth ratio. As the main organ for ZEA metabolism in pigs, the small intestine exhibits morphological changes including villus shortening and incomplete branching during ZEA metabolism (1.04 mg/kg). This study demonstrated increased villus height, crypt depth and villus height/crypt depth ratios in duodenum, jejunum and ileum, confirming ZEA effects on piglet intestine.

The small intestine is the primary site for nutrient absorption and transport in weaned piglets, with villus epithelial cells being the functional cells for digestion and absorption. Intact small intestinal mucosal morphology is fundamental for normal digestive function, and intestinal morphology serves as an important indicator of both digestive capacity and animal health status. Environmental factors, mycotoxins, nutrient deficiencies, and physiological/psychological stress can cause severe intestinal morphological damage, reducing nutrient digestibility and harming the organism. Since chyme directly contacts the intestinal mucosal surface, various reactions in chyme are promptly reflected through morphological changes in the intestinal mucosa. Previous studies confirmed that ZEA affects small intestinal mucosal defense mechanisms, even at low contamination levels. In vitro and in vivo studies have shown that ZEA inhibits porcine small intestinal cell proliferation, damages the antioxidant system, causes structural abnormalities, and downregulates expression of digestive enzymes and transporters, leading to reduced nutrient absorption, decreased performance and anti-nutritional effects. In this study, ZEA caused elongated and disorganized duodenal villi with reduced intestinal glands and thinner mucosa; significantly elongated jejunal villi with larger diameters, reduced glands, and epithelial denudation in groups 2 and 3; and elongated ileal villi with increased submucosal

diffuse lymphocytes in groups 1 and 2. While reduced intestinal gland numbers decrease energy and nutrient consumption by the gut, increased villus height enhances digestive and absorptive capacity, potentially improving performance. These morphological findings confirm significant ZEA effects on piglet intestine, though the specific mechanisms of action require further investigation.

Under the conditions of this study, dietary ZEA contamination at 0.5, 1.0 and 1.5 mg/kg significantly affected serum enzyme activities, metabolite concentrations, and villus height, crypt depth, villus height/crypt depth ratios and histological morphology in the duodenum, jejunum and ileum of weaned piglets, demonstrating that ZEA compromises healthy growth by altering serum enzyme profiles, metabolite levels and intestinal morphology.

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Note: Figure translations are in progress. See original paper for figures.