Effects of Different Levels of Zearalenone on Serum Enzymes, Metabolites, and Intestinal Morphology in Weaned Gilts (Postprint)

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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 three-way crossbred (Duroc × Landrace × Large White) weaned female piglets at 28 days of age with an average body weight of (14.01$±$0.86) kg were selected and randomly divided into 4 groups with 10 piglets per group. The control group was fed a basal diet, while experimental groups 1, 2, and 3 were fed the basal diet supplemented with 0.5, 1.0, and 1.5 mg/kg ZEA, respectively. The preliminary period was 10 days, and the formal experimental period was 35 days. The results showed: 1) Compared with the control group, the activities of aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase were significantly increased in all experimental groups ($P<0.05$); serum total cholesterol content was significantly increased in experimental group 2 ($P<0.05$); serum urea nitrogen and high-density lipoprotein (HDL) contents were significantly increased in experimental group 3 ($P<0.05$), while total protein content was significantly decreased ($P<0.05$). 2) Compared with the control group, the villus height and crypt depth in the duodenum and jejunum were significantly increased in the experimental groups ($P<0.05$). The villus height/crypt depth ratio in the duodenum was significantly increased in experimental groups 1 and 2 ($P<0.05$), while the villus height/crypt depth ratio in the jejunum was significantly decreased ($P<0.05$); both villus height and villus height/crypt depth ratio in the ileum were significantly increased ($P<0.05$). 3) Compared with the control group, the duodenal villus height was significantly increased and arranged loosely and disorderly in the experimental groups, the number of intestinal glands was reduced, and the mucosal thickness was decreased. 4) Compared with the control group, the jejunal villus height was increased and the number of intestinal glands was reduced in the experimental groups, and in experimental groups 2 and 3, the jejunal

epithelium was shed with the lamina propria exposed. 5) Compared with the control group, the ileal villus height was increased and diffuse lymphocytes in the submucosa were increased in experimental groups 1 and 2. These results indicate that dietary supplementation with ZEA (0.5–1.5 mg/kg) altered hepatic metabolism and intestinal morphological structure in weaned female piglets, thereby affecting their healthy growth.

Full Text

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

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Abstract: The aim of this study was to investigate the effects of different levels of zearalenone (ZEA) on serum enzymes and metabolite and intestinal morphology of weaned gilts. Forty healthy weaned gilts (Duroc×Landrace×Yorkshire) aged 28 days with body weight of (14.01$±$0.86) kg were randomly allocated into 4 groups (10 replicates per group, 1 pig per replicate). Weaned gilts in the control group were fed a basal diet, and those in the experiment groups 1, 2 and 3 were fed ZEA-contaminated diet with 0.5, 1.0 and 1.5 mg/kg ZEA supplementation, respectively. The experiment included 10 days pretrial period and then 35 days test period. The results showed as follows: 1) compared with the control group, the activities of aspartate aminotransferase, alkaline phosphatase and lactate dehydrogenase in experimental groups were significantly increased (P<0.05), serum total cholesterol content in experimental group 2 was significantly increased (P<0.05), and the contents of serum urea nitrogen and high-density lipoprotein in experimental group 3 were significantly increased (P<0.05), while the serum total protein content was significantly decreased (P<0.05). 2) Compared with the control group, the villus height and crypt depth of duodenum and jejunum in experimental groups were significantly increased (P<0.05). In the experimental groups 1 and 2, the ratio of villus height to crypt depth in duodenum was significantly increased (P<0.05), the ratio of villus height to crypt depth in jejunum was significantly decreased (P<0.05), and the villus height and the ratio of villus height to crypt depth in jejunum were significantly increased (P<0.05). 3) Compared with the control group, the villus height in duodenum of experimental groups was significantly increased, and the arrangement of them were loose and disordered. The number of small intestinal glands was decreased and the mucosal thickness became thinner. 4) Compared with the control group, the villus height and the number of small intestinal glands in jejunum of experimental groups was decreased, and the je-

junal epithelium of experimental groups 2 and 3 was denuded and the lamina propria was exposed. 5) Compared with the control group, the villus height in ileum of experimetal groups 2 and 3 was increased, and the number of diffuse lymphatic cells in the submucosa was increased. Therefore, dietary ZEA (0.5 to 1.5 mg/kg) can change the liver metabolism and intestinal morphology of weaned gilts, thereby affecting the healthy growth of weaned gilts.

Key words: weaned gilts; zearalenone; serum; intestinal morphology

1. Materials and Methods

1.1 Experimental Animals and Diets

Twenty-eight-day-old healthy weaned gilts (Duroc×Landrace×Yorkshire) with an average body weight of (14.01$±$0.86) kg were selected for this study. The basal diet was formulated according to NRC (2012) standards, and its composition and nutrient levels are shown in . Zearalenone (ZEA) standard was purchased from Fermentek with a purity of 98%.

1.2 Experimental Design and Sample Collection

The forty weaned gilts were randomly divided into 4 groups with 10 replicates per group and 1 pig per replicate. The control group was fed the basal diet (0 mg/kg ZEA), while experimental groups 1, 2, and 3 were fed ZEA-contaminated diets with ZEA levels of 0.5, 1.0, and 1.5 mg/kg, respectively [actual measured ZEA concentrations were $(0.52\$\pm0.07), (1.04\pm0.03), and (1.51\pm\$0.13)$ mg/kg]. The experiment consisted of a 10-day pretrial period followed by a 35-day test period. Pigs were housed individually in metabolic cages with free access to feed and water.

1.3 Sample Collection and Processing

At the end of the 35-day feeding trial, blood samples were collected from the anterior vena cava and centrifuged at 3000 r/min for 10 minutes to separate serum, which was stored at -20°C for later analysis. Intestinal tissue samples (approximately 10 cm in length) were collected from the duodenum, jejunum, and ileum, fixed in Bouin' s solution for 24 hours, then dehydrated, cleared, and embedded in paraffin. Sections of 5 m thickness were prepared using a rotary microtome (LEICA RM2135, Germany) for hematoxylin-eosin (HE) staining and morphological observation.

1.4 Measurement Indicators

1.4.1 ZEA Content in Diets and Serum Diet and serum ZEA concentrations were determined by enzyme-linked immunosorbent assay (ELISA) according to standard methods [9]. The detection limit for ZEA was 0.1 mg/kg, and samples below this limit were considered negative.

1.4.2 Serum Enzyme and Metabolite Measurements Serum samples were analyzed using an automatic biochemical analyzer (COBAS MIRA Plus, Roche, Switzerland) to determine the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), as well as the concentrations of total protein (TP), urea nitrogen (UN), total triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL).

1.4.3 HE Staining and Intestinal Morphology Observation After Bouin' s fixation, intestinal tissue samples were dehydrated through graded ethanol series, cleared in xylene, and embedded in paraffin. Sections of 5 m thickness were cut, deparaffinized, and stained with hematoxylin for 10 minutes, followed by rapid differentiation in 1% acid alcohol for 5 seconds. After washing, sections were stained with eosin for 15 seconds, dehydrated through 95% and 100% ethanol, cleared in xylene, and mounted with neutral resin. Morphological observations were performed under a Nikon Eclipse 80i microscope, and images were captured using Motic Images 2000 1.3 software. Villus height, crypt depth, and villus height/crypt depth ratio were measured in at least 15 well-oriented villi and crypts per sample.

1.5 Statistical Analysis

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

2. Results

2.2 Effects of Different ZEA Levels on Serum Enzymes in Weaned Gilts

The effects of dietary ZEA on serum enzyme activities are presented in . Compared with the control group, AST and LDH activities in experimental group 1 were significantly increased ($P<0.05$). AST and LDH activities in experimental group 2 were significantly higher than those in group 1 ($P<0.05$), and activities in group 3 were significantly higher than those in group 2 ($P<0.05$). ALP activity was significantly increased in all experimental groups compared with the control ($P<0.05$). No significant differences were observed in ALT activity among groups ($P>0.05$).

2.3 Effects of Different ZEA Levels on Serum Metabolites in Weaned Gilts

The effects of dietary ZEA on serum metabolites are shown in . Serum TC content in experimental group 2 was significantly higher than that in the control group ($P<0.05$). Serum UN, TC, and HDL contents in experimental group

3 were significantly increased (P<0.05), while TP content was significantly decreased (P<0.05) compared with the control group.

2.4 Effects of Different ZEA Levels on Duodenal Morphology in Weaned Gilts

The effects of ZEA on duodenal morphology are shown in [Figure 1: see original paper]. Compared with the control group, villus height and crypt depth in the duodenum were significantly increased in all experimental groups (P<0.05). The villus height/crypt depth ratio was significantly increased in experimental groups 1 and 2 (P<0.05) but decreased in group 3. Histological examination revealed that villi in the duodenum of experimental groups were loosely arranged and disorganized, with reduced numbers of intestinal glands and thinner mucosal layers. The duodenal morphology showed dose-dependent damage with increasing ZEA levels.

2.5 Effects of Different ZEA Levels on Jejunal Morphology in Weaned Gilts

The effects of ZEA on jejunal morphology are presented in [Figure 2: see original paper]. Villus height in the jejunum was significantly increased in experimental groups 1 and 2 (P<0.05) but decreased in group 3 compared with the control. The villus height/crypt depth ratio was significantly decreased in all experimental groups (P<0.05). Histological observation showed that villus height and the number of intestinal glands were reduced in experimental groups. The jejunal epithelium in groups 2 and 3 exhibited denudation with exposed lamina propria, indicating severe damage.

2.6 Effects of Different ZEA Levels on Ileal Morphology in Weaned Gilts

The effects of ZEA on ileal morphology are illustrated in [Figure 3: see original paper]. Compared with the control group, villus height in the ileum was significantly increased in experimental groups 1 and 2 (P<0.05) but showed no significant difference in group 3. The number of diffuse lymphoid cells in the submucosa was increased in experimental groups, particularly in groups 2 and 3, where lymphoid follicles were more prominent.

3. Discussion

Serum enzymes (ALT, AST, ALP, and LDH) are important indicators of liver function and cellular integrity [16-17]. ALT primarily reflects hepatocellular membrane integrity, AST indicates mitochondrial function and hepatocellular damage, while ALP is associated with bile duct epithelial cell function and can reflect lipid and protein metabolism status [18-19]. In this study, dietary ZEA significantly increased serum AST, ALP, and LDH activities, consistent with previous reports that ZEA at 40 mg/kg BW significantly elevated serum ALT,

AST, and ALP activities [20]. The increased serum enzyme activities suggest that ZEA causes hepatocellular damage and metabolic dysfunction.

Serum metabolites reflect the overall nutritional and metabolic status of animals. Elevated serum TC, UN, and HDL levels observed in this study indicate altered protein and lipid metabolism due to ZEA toxicity. Previous studies have shown that ZEA exposure increases serum cholesterol levels while decreasing total protein concentrations [22]. ZEA and its metabolites can disrupt protein synthesis and DNA replication, leading to metabolic disorders [23]. The significant changes in serum metabolites observed in this study demonstrate that ZEA at concentrations of 0.5-1.5 mg/kg can cause metabolic disturbances in weaned gilts.

ZEA primarily affects the reproductive system but also causes significant damage to the gastrointestinal tract. The intestinal mucosa serves as the first barrier against ingested toxins, and ZEA can be metabolized in the intestinal epithelium to form α-ZEA, β-ZEA, α-ZEL, and β-ZEL [25]. The present study revealed that ZEA exposure significantly altered intestinal morphology, including increased villus height and crypt depth in the duodenum and jejunum, but decreased villus height/crypt depth ratio, particularly in the jejunum. These changes are consistent with previous findings that ZEA at 1.04 mg/kg causes significant damage to intestinal villi and crypts [27].

The intestinal morphology changes induced by ZEA include villus atrophy, epithelial cell denudation, and lymphoid tissue hyperplasia. The increased crypt depth may reflect enhanced cell proliferation to compensate for villus epithelial damage. However, the decreased villus height/crypt depth ratio indicates impaired intestinal function. The histological observations of loose villus arrangement, reduced gland numbers, and epithelial denudation in the jejunum demonstrate the progressive nature of ZEA-induced intestinal damage. The increased number of lymphoid cells in the ileal submucosa suggests an inflammatory response to ZEA exposure.

In conclusion, dietary ZEA at concentrations of 0.5, 1.0, and 1.5 mg/kg significantly affected serum enzyme activities, metabolite concentrations, and intestinal morphology in weaned gilts. The damage showed a dose-dependent pattern, with the most severe effects observed at 1.5 mg/kg ZEA. These findings indicate that ZEA can impair liver function and intestinal integrity, thereby compromising the health and growth performance of weaned gilts.

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