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The detoxification effect of vitamin C on zearalenone toxicity in piglets

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ABSTRACT

Zearalenone (ZEN), one of the more virulent mycotoxins occurred in various cereals and feed during recent decades and made serious health hazards to plants, animals and humans. Vitamin C (Vc) has been shown to be an effective antidote to zearalenone. In this paper, the effects of diets containing zearalenone on the growth performance, genital organ and immunoglobulin of weaning piglets and the toxicity alleviation of vitamin C were studied. Piglets were weaned at 21 days of age and 32 healthy female hybrid weaning piglets (Duroc × Landrace × Large white) with a mean weight of 12.27 ± 0.30 kg were randomly selected. The thirty-two female weaning piglets were divided into four treatment groups according to body weight: control; basal diet + vitamin C (150 mg/kg); basal diet + 1.0 mg/kg ZEN; basal diet + 1 mg/kg ZEN + vitamin C (150 mg/kg). There were eight replicates in each group. The test period was twenty-eight days. The results demonstrated that dietary zearalenone could significantly increase the length, width and area of vulva ($P < 0.05$), the genital organ coefficient ($P < 0.05$), the level of IgA, IgG and IgM ($P < 0.05$), the level of BUN, CRE, AST and TBIL ($P < 0.05$), and significantly decrease the level of E2, PROG, LH and FSH ($P < 0.05$). However, the addition of 150 mg/kg vitamin C to dietary zearalenone prevented deformities in the vulva, decrease in immune response capacity, changes in serum biochemical indicators and disorders in hormones level of the piglets that received the diet containing only zearalenone. In conclusion, feeding ZEN of 1.0 mg/kg can result in a deleterious effect on piglets, which was totally or partly ameliorated by dietary supplementation of vitamin C at concentrations about 150 mg/kg diet. This study systematically investigated the inhibition mechanism of vitamin C on ZEN-induced reproductive toxicity, immunotoxicity and hematological toxicity of piglets, and which provided new ideas for reducing the harm of mycotoxins to the animals through means of nutrition regulation.

1. Introduction

Zearalenone (ZEN) is a mycotoxin produced mainly by fungi belonging to the genus *Fusarium* and is universally existed in mildewed corn, wheat, other grains and food (Luo et al., 1990; Schollenberger et al., 2006). Zearalenone is not only a common but dangerous secondary fungal metabolite that can contaminate animal feed at every stage of the food chain (Richard, 2007). Molds and mold spores are usually found in soil and are therefore very susceptible to feed ingredients. Mycotoxins in feed ingredients are produced in pre-harvest fields or under improper storage conditions after harvest (Tiemann and Danicke, 2007). Mycotoxins usually reduce the animal's performance, inhibit the immune function, cause the animals to get sick, and then enter the human food chain through the animal's meat milk and offal, endangering human health (Milićević et al., 2014). It can be seen that mycotoxins can cause damage of different levels to plants, animals and humans in nature, which pose a great threat to environmental safety.

Zearalenone can have many adverse effects on animals, for example, resulting in reduced productivity of animals, chronic injury to organs and tissues, resulting in immunosuppression leading to disease, affecting the reproductive performance of animals, and may even lead to the acute death (Sirot et al., 2012). Studies reported that ZEN is more sensitive to female animals, whereas in all species animals are most susceptible to ZEN, especially pubertal pigs (Shi et al., 2017; Zinedine et al., 2007). Research reported that Speranda added 3 mg/kg of pure ZEN to diets, and the average daily feed intake, average daily gain and feed compensation of weaning piglets were not significantly affected (Šperanda et al., 2006). The 21-day-old weaned pigs were tested for 28

Abbreviations: Vc, vitamin C; ZEN, zearalenone; AFB1, aflatoxins B1; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; BUN, blood urea nitrogen; CRE, creatinine; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, glutamyl transpeptidase; ALP, alkaline phosphatase; TP, total protein; ALB, albumin; GLB, globulin; TBIL, total bilirubin; IgA, immune globulin A; IgG, immune globulin G; IgM, immune globulin M; E_2, estradiol; PROG, progesterone; LH, luteinizing hormone; FSH, follicle stimulating hormone

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days with 1, 2 and 3 mg/kg of ZEN added to the basal diet, and no effect of ZEN on pig performance was also found. ZEN was also found to have no effect on piglet production performance (Zhao et al., 2008). However, the addition of an appropriate amount of vitamin C in the diet can increase the average daily gain of weaned piglets (Liu, 1994).

The major toxicity of ZEN 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., 2010a). Reproductive toxicity of ZEN to animals is largely due to its estrogen-like structure and is capable of binding to estrogen receptors in vivo to stimulate the transcription of estrogen-sensitive genes, thereby promoting estrus effects, resulting in a series of wigs for female animals Symptoms (Nikov et al., 2000; Mehmood et al., 2000). ZEN and its metabolites can cause estrogen hyperthyroidism and reproductive disorders in female animals. Studies have shown that diets containing 50–100 mg/kg ZEN in sow diets can result in prolonged ovulation cycles, impaired fetal development, and reduced gestation in piglets (Ruzsas et al., 1979). In addition, large levels of ZEN can cause many symptoms, such as swollen vulva sows, delayed puberty, false estrus, changes in ovarian shape and habitual abortion (Gao et al., 2017).

Besides its reproductive effects, ZEN has been shown to be toxic to liver and other tissues in animals, such as causing hepatotoxicity in weaning piglets (Jiang et al., 2010b), immunotoxic, oxidative stress and cytotoxicity in mice (Ouanes et al., 2005; Ben Salah-Abbes et al., 2008, 2009) and hematotoxicity in rats (Maaroufi et al., 1996). Studies demonstrated that low concentrations of ZEN (10 μM) inhibited the proliferation of Jurkat T cells and noted that this inhibition was due to ZEN-induced apoptosis of T cells (Luongo et al., 2006). Research reported that 30-day-old piglets were fed with diets containing ZEN 1 mg/kg, and female piglets were observed on the 7th day. ZEN has a strong cytotoxicity, not only because ZEN and its metabolites can alter the cell membrane structure, and it can inhibit DNA replication, RNA transcription, and protein synthesis, and thus induce programmed cell death (Rainey et al., 1990). Studies found that ZEN can significantly inhibit the proliferation of mouse lymphocytes and promote lymphocyte apoptosis in ZEN study on lymphocyte proliferation and apoptosis in vitro (Ma et al., 2009).

The sensitivity of different animals to ZEN is different. Rotter reported that pigs are most sensitive to ZEN, followed by mice, rats, rabbits, while poultry and ruminants are relatively insensitive to ZEN (Rotter, 1996). As the main site of ZEN metabolism, the liver is one of the main target organs of ZEN, so the liver and liver cells are vulnerable to ZEN. Sun et al. evaluated the degree of damage of ZEN to rat liver by measuring the levels of lactate dehydrogenase (LDH), albumin and intracellular DNA in rat hepatocyte culture medium. The results showed that ZEN had no significant effect on the secretion of LDH, but contents of albumin (ALB) and DNA showed a downward trend, indicating that ZEN caused damage to rat liver (Sun and Wang, 1997). The ZEN of 1.5 mg/kg BW was injected into the peritoneum of female rats for 48 h, results showed that alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine (CRE), bilirubin (BIL) and other biochemical indicators in the serum have changed (Maaroufi et al., 1996).

ZEN has been shown to induce chromosome aberrations in mouse bone marrow (Maaroufi et al., 1996). The reference show that ZEN can pass through human endometrium which illustrates the risk of exposure of the fetus (Tomaszewski et al., 1998). Therefore, it is important to decrease the ZEN exposure to prevent its detrimental effects. It has been reported that mycotoxins such as aflatoxin have epoxidation and that vitamin C can inhibit this effect to a certain extent. The most important thing is that vitamin C can prevent aflatoxin activation (Hussein and Brasel, 2001). Related studies have reported that the addition of adequate amounts of vitamin C and selenium to the basal diet allows the animals to avoid zearalenone poisoning to a certain extent and also to alleviate the organism poisoning (Sahoo and Mukherjee, 2003; Alpsoy and Yalvac, 2011; Lindemann et al., 1993). Studies have also shown that vitamin C can stimulate the body's immune system and promote the synthesis of antibody organisms, thereby preventing aflatoxin-induced immune dysfunction (Hu, 2001). The results show that mycotoxins can cause the body to produce oxidative stress, while vitamin A, vitamin C and vitamin E can reduce this oxidative stress (Shi et al., 2017). However, how vitamin C promotes detoxification of zearalenone in the animal body is unclear (Bennett et al., 1994; Nuryono et al., 2005).

Therefore, the rapid and efficient elimination of residual ZEN in animal feed and food is a problem that we need urgently to solve. Our hypothesis is that the vitamin C used as a nutrient might with improve the growth of piglets. The current experiment was conducted to evaluate the toxicity of 1.0 mg/kg ZEN diet on growth performance, serum metabolites, genital organs and oxidative stress of piglets. Therefore, we study the effect of ZEN on the kidneys of the weaned piglets in this research project, from the blood biochemical indicators, tissue structure and immune indicators. To explore the possible mechanism of ZEN on the damage caused by piglets' blood, immune organs and reproductive organs and the detoxification effect of vitamin C on ZEN, so as to provide a theoretical basis for better solving the problem of mycotoxin contamination, and to reduce ZEN's degree of pollution to the environment.

2. Materials and methods

2.1. Chemicals and vitamin C

ZEN was obtained from Fermentek Ltd. (Jerusalem, Israel) with a purity of more than 98.5% and a shelf life of 8 months at room temperature. Vitamin C was purchased from Harbin Datang Minsheng Feed Co., Ltd. in Heilongjiang Province. The molecular formula is C6H8O6 and the molecular weight is 176.1. ZEN (0 mg/kg, 1.0 mg/kg) and Vitamin C (2 mg/kg, 150 mg/kg) were added to the diet as finished product (Shi et al., 2017).

2.2. Preparation of zearalenone-contaminated diet

The crystalline powder ZEN (1.0 mg/kg) with the chromatographic purity of more than 98.5% was dissolved in methanol. A small amount of the diet without added zearalenone was fetched, and then the ZEN solution in methanol was evenly sprayed onto the diet. The feed was left overnight to evaporate the methanol, then the ZEN-treated diet was placed in a mini blender and mixed with the remaining normal feed in stages (Shi et al., 2017). Enzyme-linked immunosorbent assay was used to detect ZEN levels in the diet (Feng et al., 2003). The ZEN contents in the basal diet and ZEN diet were 0.03 mg/kg and 1.22 mg/kg, respectively.

2.3. Experimental animals and feeding management

Piglets were weaned at 21 days of age and 32 healthy female hybrid weaning piglets (42 ± 1 day of age, Duroc × Landrace × Large white) with a mean weight of 12.27 ± 0.30 kg were randomly selected. Piglets were housed individually in test cages (0.72 m^3). The single cages use plastic slatted floors, nipple drinkers and troughs were installed, and piglets are free to eat and drink. Before the start of the experiment, the pig houses should be thoroughly cleaned and disinfected, and the pigs should be disinfected once a week during the experiment. When infrared-insulated lamps were installed, the temperature of the trial cage was kept at about 30 °C in the first week, and the temperature in the pig house was maintained at 26–28 °C in the first two weeks. The relative humidity of the pig house was 65%. The test period was 28 days. The percentage composition of the basal diet is shown in Table 1 (Shi et al., 2017). Vulva length, width, and height were measured at 7-d intervals to determine the dietary ZEN estrogenic effects, and vulva area was calculated approximately as a diamond