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Response of the nuclear receptors PXR and CAR and their target gene mRNA expression in female piglets exposed to zearalenone

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ABSTRACT

A study was conducted to determine the effects of zearalenone (ZEN) on the mRNA expression of pregnane X receptor (PXR), constitutive and rostane receptor (CAR), and phase I and II enzymes as well as the toxicity in the liver of female weanling piglets. Thirty-two female weanling piglets (Duroc × Landrace × Large white, 12.27 ± 0.30 kg)were divided into four groups (n = 8 piglets/group) that were supplemented with 0 (control), 0.5, 1 or 2 mg/kg ZEN. The trial period lasted for 28 d. The results showed that the ZEN supplementation in the diets (0.5–2 mg/kg) had no effect on growth performance but dose-dependently increased serum aspartate aminotransferase, alanineaminotransferase, alkaline phosphatase, and γ-glutamyltransferase activities ($P < 0.05$). The ZEN residue in the liver ($P < 0.01$) was also linearly and dose-dependently increased. Furthermore, the mRNA expression of PXR, CAR, phase I enzymes (i.e., cyp2e1, cyp3a5, cyp2a6, cyp1a1, and cyp1a2), and phase II enzymes (i.e., gsta1, gsta2, ugt1a3) significantly increased linearly in a dose-dependent manner ($P < 0.05$). However, the spleen relative weight and the glutathione peroxidase activity in the liver ($P < 0.05$) linearly decreased as the dietary ZEN concentration increased; the mRNA expression of the nuclear receptors PXR and CAR is responsive to ZEN in female piglets, and ZEN increases the mRNA expression of their target genes. This finding shows that the nuclear receptor signaling system plays an important role in the defense against ZEN.

1. Introduction

Mycotoxins are toxic metabolites of various fungi and are commonly observed in food and foodstuffs, which can cause serious health problems in both animals and humans (Mccormick, 2013; Reddy et al., 2010). Fusarium mycotoxins are likely the most common mycotoxins globally (Placinta et al., 1999). Exposure to these toxins could result in both substantial economic losses and threats to human health (Kolosova et al., 2007). One of the Fusarium toxins, i.e., zearalenone (ZEN), also known as F-2 toxin, is a secondary metabolite produced by Fusarium species (Bennett and Klich, 2003; Richard, 2007; Zinedine et al., 2007). ZEN widely grows in maize, barley, wheat, and other commodities used as primary ingredients in many foodstuffs intended for human or animal consumption (Zinedine et al., 2007). Investigations have revealed that animal feed was seriously contaminated by ZEN worldwide (Zinedine et al., 2007). Animals that consume feed contaminated by ZEN can suffer serious health, growth, and reproductive performance defects. ZEN exerts harmful health effects via its strong estrogenic activities. ZEN is particularly toxic to the reproductive system, resulting in a reduced litter size, induced central precocious puberty, increased embryo-lethal resorption, decreased fertility, and changes in the plasma levels of progesterone and estradiol in animals (Escrivá et al., 2015; Yang et al., 2016). In addition, ZEN plays an increasingly recognized role as a mammalianendocrine disrupter with demonstrated effects in both males and females across different species (Green et al., 1990; Turcotte et al., 2005). ZEN make oxidative damage to goats, reducing their immunity and lower milk production(Huang et al., 2017). ZEN causes brain damage, liver and kidney damage, impaired male reproductive capacity in rats(Long et al., 2016; Ren et al., 2016a). ZEN also displays hepatotoxic, hematotoxic, immunotoxic and genotoxic properties (Ren et al., 2016b; Turcotte et al., 2005). ZEN may also pose a risk to wild fish in their natural habitat due to its overall oestrogenic activity in the environment (Schwartz et al., 2010).

ZEN not only causes serious harm to livestock, poultry and fish, but also has a strong toxic effect on the human body. ZEN Residues for Animal Products is absorbed by the human body, ZEN can induce cancers, such as oesophageal cancer (Luo et al., 1990) and breast cancer (Belhassen et al., 2015; Kakeya et al., 2002), and at low doses, has

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proproliferative properties in prostate cancer cells (Kowalska et al., 2017). ZEN can also lead to precocious puberty, affect male reproductive health, cause testicular cancer, cryptorchidism, hypospadias and poor semen quality (Gajecki et al., 2004). ZEN has generated significant negative effects on animal reproduction and human health and has thus become an issue of global concern; As the zearalenone pollution is not easy to avoid, in recent years, many scientists at home and abroad mainly The zearalenone detoxification technology has been studied in depth. El-Nekeety and Bai application of suction additives detoxification technology (Bai et al., 2018; El-Nekeety et al., 2017), Fu and He application of microbial biodegradation technology to solve the feed corn red Moldy ketone pollution problems have made gratifying achievements (Fu et al., 2016; He et al., 2016). In general, these measures have slowed down zearalenone to a certain extent Ketones, but there are still some unavoidable limitations. The ZEN adsorption effect is not complete, and at the same time also have nutrients on the adsorption. Micro- The effect of biodegradable agents is greatly affected by the environment inside and outside the body. In fact, the use of adsorbents or bio-degraded The agent does not yet allow mycotoxins in animals to completely disappear. Once mycotoxins enter the blood, they will still Cause animal health and meat, eggs, milk residues of mycotoxins.

Organisms encounter many xenobiotics with potentially harmful consequences (Wei et al., 2000b). However, organisms are protected against the potentially harmful effects of foreign compounds or xenobiotics by a complex network of drug metabolizing enzymes and regulators (Kodama and Negishi, 2006a; Moreau et al., 2008; Sueyoshi and Negishi, 2001; Tien and Negishi, 2006). Xenobiotic metabolism mostly occurs in the liver. Upon exposure to a xenobiotic, the expression and activity of hepatic enzymes that are involved in the metabolism of the xenobiotic are increased due to the activation of “xenosensors”, such as pregnane X receptor (PXR) and constitutive and rostane receptor (CAR). This organism's own metabolism can make up or cooperate with the above two kinds of means to reduce the ZEN residue.

PXR, which is also known as the steroid and xenobiotic sensor (SXR), and CAR are members of the orphan nuclear receptor subfamily. Both PXR and CAR are activated by xenobiotics and act as master regulators of phase I through III enzymes in the detoxification and elimination of steroids, bile acids, and xenobiotics (Honkakoski et al., 1998; Rosenfeld et al., 2003; Ueda et al., 2002). The purpose of the phase I and II enzymes is to transform compounds into more polar forms that can be transported by phase III proteins across membranes for excretion. The detoxification genes that are induced by PXR and CAR include several phase I cytochrome P450 enzymes (CYPs) (Kliewer et al., 1998; Lemaire et al., 2004; Maglich et al., 2002; Wei et al., 2000a); phase II enzymes, such as uridine di phospho-glucuronosyltransferases (UDPGT),glutathione-S-transferases (GSTs) and sulfotransferases (SULTs) (Huang et al., 2004; Assem et al., 2004; Rosenfeld et al., 2003; Rungemorris et al., 1999; Sugatani et al., 2001); and phase III transporters, such as multidrug resistance-associated protein 2 (MRP2) and multidrugresistance protein 1 (MDR1) (Geick et al., 2001; Kast et al., 2002; Payen et al., 2002).

The regulation of nuclear receptors, including PXR and CAR, and the corresponding phase I and II target genes may play a central role in ZEN biotransformation. Several in vitro studies have demonstrated that the addition of ZEN leads to an up-regulation of the mRNA levels of the phase I and II enzymes and the activation of their transcriptional regulators PXR and CAR in primary hepatocyte cultures. However, in vivo studies investigating the response of PXR, CAR, and the phase I and II enzymes to ZEN are lacking.

The purpose of the present study is to determine the response of the nuclear receptors PXR and CAR and the mRNA expression of their target genes to different concentrations of ZEN in female piglets. To find out animals' ability to detoxify ZEN.

2. Materials and methods

2.1. Preparation of the ZEN-Contaminated diet

Pure crystalline ZEN was dissolved in absolute methanol(1 mg/mL), and the solution was sprayed onto 100 g of the ground control diet. The treated feed was left overnight at room temperature to allow the solvent to evaporate and was later mixed into the basal diet to provide the desired levels of ZEN/kg feed. The treatment diet was prepared once every 7 d and then stored in covered containers before use. The diets were sampled for the determination of ZEN levels via enzyme-linked immunosorbent assays. The actual ZEN contents (analyzed) were 0.02, 0.55 ± 0.01, 1.10 ± 0.02, and 2.11 ± 0.02 mg/kg for control diet and ZEN diet.

2.2. Experimental design, animals, and management

In total, 32 post-weaning female piglets (Landrace × Yorkshire × Duroc) that were weaned at 35 d with an average BW of 12.27 ± 0.30 kg (mean ± SD) were used in this study. The gilts were randomly allocated to 1 of 4 treatments according to their BW after 7 d of adaptation. The 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 a vitamin and mineral premix to meet or exceed the minimal requirements. The diets used in the study were isocaloric and isonitrogenous, and the only difference across the diets was the ZEN concentration. The pigs were fed an either basal diet only (control) or a basal diet supplemented with purified ZEN at a dietary concentration of 0.5, 1, or2 mg/kg of feed for 28 d adlibitum.

The experiment was structured as a randomized design, and individual gilts were used as the replicate units. The pigs were housed in a cage equipped with a nipple drinker and feeder in a temperature-controlled room at the Northeast Agricultural University Experimental Base. During the experimental period, the temperature in the room was maintained between 26 and 28 °C. The mean relative humidity was

Table 1
Composition and nutrient levels of basal diets (air-dry basis).

Items	Basal diet
Ingredients (%)	
Corn	69.56
Peeled soybean meal	17.65
Wheat bran	5.00
Fish meal	3.00
Soybean oil	1.50
Stone powder	0.78
Calcium phosphate	0.80
Salt	0.35
Lysine (98%)	0.26
Choline chloride	0.10
Premix[a]	1.00
Total	100
Nutrient levels [b]	
Digestible energy (Kcal/kg)	3330
Crude protein (%)	16.65
Calcium (%)	0.65
Available phosphor (%)	0.56
Lysine (%)	1.06
Methionine (%)	0.28

[a] Treatments consisted f a basal diet supplemented with Zenralenone targeting 0,0.5, 1, or 2 mg/kg.

[b] Supplied per kilogram of diet: vitamin A, 3300 IU; vitamin D3,330 IU; vitamin E, 24 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, 15.00 mg; niacin, 22.5 mg; biotin, 0.075 mg; folic acid, 0.45 mg; Mn, 6.00 mg; Fe, 150 mg; Zn, 150 mg; Cu, 9.00 mg; I,0.21 mg; Se, 0.45 mg.