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Use of modified halloysite nanotubes in the feed reduces the toxic effects of zearalenone on sow reproduction and piglet development

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ABSTRACT

A study was conducted to determine the effects of feeding a blend of corn contaminated with *Fusarium* mycotoxins on the physical condition of pregnant and suckling sows and the development of their offspring. Halloysite nanotubes modified using the surfactant, stearyldimethylbenzylammonium chloride, were tested for its efficacy in protecting against the detrimental effects of zearalenone (ZEN) exposure. A total of 18 pregnant second parity Yorkshire sows (six per treatment) were fed control diet, contaminated grain diet (ZEN, 2.77 mg/kg), and contaminated grain + 1% modified halloysite nanotube (MHNT) diet (ZEN, 2.76 mg/kg) from 35 to 70 days in pregnancy (DIP), which is the critical period in development of fetuses. The results show that consumption of ZEN led to a reduction in sow's mass gain during 35 to 70 DIP and mass at 110 DIP, backfat at 70 DIP and weaning, placenta weight at 70 DIP and farrowing, the lactation average daily feed intake, and an increase in the weight of ovary at 70 DIP of sows ($P < 0.05$). The total number and average body weight (BW) of fetuses at 70 DIP, the number of piglets born, the litter birth weight, the average BW of piglet at birth, the number of piglets born alive, the born alive litter weight, and born alive piglet BW at farrowing were also decreased by ZEN exposure ($P < 0.05$). The increased expressions of P53, Bax, Cyto C, caspase 9, and caspase 3 and decreased expression of Bcl-2 were observed in the uterus and placenta of sows at 70 DIP, the placenta and fetal uterus at farrowing, and the piglet uterus at weaning ($P < 0.05$). Adding 1% MHNTs decreased the residue of ZEN in maternal and fetal tissues. The number of fetuses and the average fetus BW at 70 DIP, the total number of piglets born, the litter birth weight, the born alive piglet BW at farrowing, the average piglet BW, the litter weaned weight, and the average day gain at weaning were increased by adding 1% MHNTs, compared with the ZEN-treated group ($P < 0.05$). The MHNTs significantly reduced the damage to the fat in the colostrum and the protein and lactose in the milk induced by the ZEN-contaminated feed ($P < 0.05$). Modified halloysite nanotubes could be used as adsorbent in the feed to reduce the toxic effects of ZEN.

1. Introduction

Fusarium mycotoxins are likely the most common mycotoxins globally [1]. They are frequently found in foods predominantly cereals and forages [2,3]. Both the risk of substantial economic losses and the threat to human health could result from exposure to the toxins [4]. One of the

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Fusarium toxins, zearalenone (ZEN), could occur in concentrations that cause adverse effects in farm animals, especially in pigs [5].

Zearalenone is particularly toxic to the reproductive system, resulting in reduced litter size, increased embryolethal resorption, decreased fertility, and changes in the progesterone and estradiol plasma levels in laboratory animals [6,7]. Zearalenone frequently occurs in piglets under natural conditions, which are caused by ZEN exposure in the uterus and placental transfer from the exposed sow to her piglets or by the stored ZEN during gestation release *via* suckling the piglets [8]. Exposure to ZEN during gestation can delay the fetal development and lead to premature oocyte depletion in adulthood of the offspring [9,10]. At the beginning of gestation in pigs, the maternal and fetal blood is separated by the endothelial cells of the maternal blood vessels, maternal connective tissue, and endometrial epithelial cells (epitheliochorial placenta), which affects the direct contact of the maternal blood with the fetal chorion. However, during the mid-gestation, the fetuses with rapidly developing organ system absorb largely maternal nutrition through placental transfer [11]. Because of the large contact surface and the intensive nutrient exchange in this period, a placental transfer of ZEN appeared to be likely in middle or late pregnancy in swine [8,12]. The adverse effects of ZEN may be even more pronounced during pregnancy, as the fetuses are susceptible to toxins because of their fragile developmental state and inadequate defense mechanism. So the organogenesis period of 35 to 70 days in pregnancy (DIP) was selected for this study as the fetuses might show possible teratogenic effects. In addition to the reproductive disorders, ZEN is frequently implicated in several genotoxic effects [13,14]. Zearalenone has been reported to induce apoptosis *in vivo* and *in vitro* [15–18]. However, available results concerning the induction of apoptosis after ZEN exposure are controversial [19,20]. The inconclusive results on the induction of apoptosis by the mycoytoxin ZEN prompted us to investigate its effects on the apoptotic patterns in the pregnant sows and its offspring.

Natural materials have become attractive to prevent mycotoxins from animal feed because of their economical adsorption efficiency. From the early 1950s, dietary clay supplements (bentonite and kaolin) have been used as binding and lubricating agents in the production of pelleted feeds, resulting in positive effects in terms of either faster growth or reduced incidence of diseases and death in animals [21]. Halloysite is chemically similar to kaolinite except that its higher hydrated water content results in a tubular morphology [22]. Organomodified mineral adsorbents are more effective for ZEN adsorption compared with their raw analogs [23]. Clay and soils (montmorillonite, illite, muscovite, sepiolite, and palygorskite) modified using stearyldimethylbenzylammonium chloride (SKC), which are nontoxic to humans [24,25], have been used as barriers to prevent pollution from food or feed [26]. Moreover, other studies have proven that the clay minerals can bind ZEN *in vitro* and propose this sorbent as a good candidate for detoxification of ZEN present in foods [27–29]. The modified halloysite nanotube (MHNT) used in this study is an effective adsorbent with high porosity and surface area [27]. However, the efficiency of MHNTs to protect piglets from ZEN-contaminated feeds during gestation has not been reported in the scientific literature.

Many studies have investigated the effects of longer time exposure to lower ZEN concentrations on farm animals than ours [11,30–32]. The aims of this study were as follows: (a) to investigate the physiological responses of sows exposed to mold-contaminated corn with high doses of ZEN (2.76–2.77 mg/kg) in reproductive performance; (b) to determine the toxicologic effects induced by ZEN on the development of the fetus and the piglets during 35 to 70 DIP; (c) to evaluate the correlation between maternal and fetal states through the effects of ZEN exposure on the expression of apoptosis parameters; and (d) to evaluate the effect of MHNTs as ZEN adsorption *in vivo*.

2. Materials and methods

2.1. Sorbent materials and modification

Powder halloysite nanotubes (HNTs, $Al_2Si_2O_5(OH)_4 \cdot nH_2O$) were refined from clay minerals with a purity of 95% from Henan province (China). The powder was prepared according to the method described by previous study [33]. The HNTs were modified using SKC (Jingwei Chemical Co., Ltd., Shanghai, China) according to the methods described previously [34].

2.2. Strain and molding

Fusarium graminearum, the ZEN-producing fungus [35], was obtained from the Agricultural Culture Collection of China (No. ACCC36249). The fungus was cultivated on potato dextrose agar (potato extract 0.4%, glucose 2%, and agar 1.5%, with a pH of 5.6 ± 0.2). All the culture media were obtained from Fluka (Bornem, Belgium) [36].

The corn used for the experiment was provided by the Xiang Fang Experimental Bases of Northeast Agricultural University and milled in a hammer mill with a 40-mesh screen (Trapp, TRF model 90). The molding procedure was approved and supervised by the Institute of Animal Nutrition in Northeast Agricultural University. The ZEN production *in vitro* was conducted according to the procedures outlined by Martins and Martins [37]. The studies of mycotoxin production by *F graminearum* were conducted on trays containing 1000 g of sterilized cracked corn, adjusting the water activity (a_w) to 0.97 by adding 400 mL of distilled water. The autoclaved substrate was inoculated with 40 mL of the spore suspension, according to the following procedure: 100 mL of sterile distilled water was added to each slant for 5-day culture, gently scraping the agar surface to yield a turbid suspension, corresponding to 1×10^{14} spores/mL. Forty milliliters of this suspension was added to the cracked corn. The inoculated flasks were stirred daily for the first 5 days. The culture conditions used in this experiment, 28 °C for 15 days, followed by 12 °C for 20 days, with ZEN concentration reaching peak on the 35th day of incubation are described by Martins and Martins [37]. To equalize the moisture in the samples, each sample was dried at 60 °C for 96 hours and stored in a freezer at −20 °C until analysis [38].