Effects of modified maifanite on zearalenone toxicity in female weaner pigs

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Abstract

The experiment was conducted to investigate alleviative effects of modified maifanite (MMF) on zearalenone (ZEN) toxicity in female weaner pigs. In this experiment, 32 female weaner pigs (Duroc×Landrace×Large white, 10.50±0.07 kg) were divided into 4 groups (8 pigs/group): control group (0.02 mg/kg ZEN); ZEN-treated group (1.11 mg/kg ZEN); MMF-treated group (1% MMF); ZEN+MMF treated group (1.11 mg/kg ZEN and 1% MMF), The trial period lasted for 28 d. Growth performance, vulva size, genital organs, antioxidant enzyme activities, serum metabolites and ZEN residues in female weaner pigs were determined. The results showed that the treatments had no effect on growth performance and length and width of the vulva. However, vulva area (P=0.038) and proges- terone (P=0.022) were affected by the ZEN+MMF interaction. Treatment with ZEN resulted in a significant increase of the genital organ weight (P=0.002) and decrease of serum superoxide dismutase (P=0.017) activity. Feeding of the ZEN diet decreased the number of red blood cells (P=0.009) and platelets (P=0.002). The MMF reduced methane dicarbonyl aldehyde concentration when fed with ZEN diet but not when fed with the basal diet (ZEN+MMF; P=0.018). In the liver, feeding of the ZEN diet with MMF reduced the levels of ZEN residues (P=0.003). Our findings suggest that the addition of MMF to ZEN diet resulted in partial restoration of antioxidant status and reduced ZEN levels in the liver.

Introduction

Zearalenone (ZEN) is a secondary fungal metabolite of fusarium species (Riley and Petska, 2005). It can be found in many cereal crops, such as maize, maize silage, and wheat. Zearalenone is not commonly fatal but can induce abortion and other breeding problems. Studies have demonstrated that ZEN exposure is associated with carcinogenic, genotoxic, and immunotoxic effects in animals and humans (Ouannes et al., 2005; Tomaszewski et al., 1998; Zinedine et al., 2007; Liu et al., 2014). Female pigs have been found to be particularly susceptible to ZEN. The reproductive system and liver are the major targets of ZEN toxicity (Jiang et al., 2010b; Minervini and Dell’Aquila, 2008; Tiemann and Dänicke, 2007; Zhang et al., 2014). Studies have showed that pathologic changes appeared in pigs exposed to 0.42–1.3 mg ZEN/kg diet (Döll et al., 2004; Teixeira et al., 2011; Jiang et al., 2010a). Zearalenone is metabolized in various tissues, with the major metabolites being α-zearalenol and β-zearalenol (Dänicke et al., 2005; Malekinejad et al., 2005)

Controlling the impact of ZEN is critical. One possible way to address this problem is the addition of aluminosilicate nonnutritive adsorptive materials to feed, such as montmorillonite and clinoptilolite, which can adsorb mycotoxins in farm animals (Abdel-Wahhab et al., 2005). Several types of aluminosilicate clays have been examined as feed additives to reduce mycotoxin effects in pigs (Chaytor et al., 2011). Maifanite is mainly composed of aluminosilicate and displays a high porosity and surface area. The adsorptive property makes it useful for purifying water and adsorbing harmful bacteria. Recently, the effectiveness of maifanite in reducing the detrimental effects of cadmium (Du et al., 2013) and aflatoxin B1 (Fu et al., 2013) in pigs have been reported. However, maifanite is always associated with impurities, which hinders its adsorptive effects. Modified maifanite (MMF) is a new adsorptive additive that is soaked in quaternary ammonium salt, washed with distilled water, dried and smashed. The ZEN and MMF were mixed into the diets. All other chemicals and reagents were of analytical grade.

Preparation of zearalenone-contaminated diet

The ZEN (1.00 mg/kg diet) was dissolved in acetic ether solution and sprayed evenly in a small amount of talcum powder. The talcum powder was then left overnight to allow acetic ether evaporation. The ZEN premix was prepared by blending the ZEN-contaminated talcum powder with ZEN-free maize and was subsequently combined with a maize-soybean meal diet at the appropriate level to produce the experimental diet (Jiang et al., 2010a). All diets were prepared in one batch. The diets were sampled for the determination of ZEN levels via enzyme-linked immunosorbent assays. The ZEN contents in the basal diet and ZEN diet were 0.02 mg/kg.
mg/kg and 1.11 mg/kg, respectively.

**Animals, diets and treatments**

Thirty-two post-weaning female pigs (Duroc × Landrace × Large white) with an average body weight of 10.50 ± 0.07 kg were divided into 4 treatments, with 8 replicates per treatment. The treatments were as follows: control diet (0.02 μg ZEN/kg), control + MMF diet (0.02 μg ZEN/kg + 1% MMF), contaminated diet (1.11 μg ZEN/kg) and contaminated + MMF diet (1.11 μg ZEN/kg + 1% MMF). All diets were formulated to meet or exceed all nutrient requirements according to the National Research Council (2012). The percentage composition of the basal diet is shown in Table 1. The pigs were identified with ear tags and individually placed in stainless-steel metabolic cages. The trial period lasted for 28 d. The protocols followed in these experiments were approved by the Northeast Agricultural University Institutional Animal Care and Use Committee.

**Growth performance**

The trial period lasted for 28 d. The feed intake of the female pigs was recorded daily, and their body weights were measured at the end of the period. The ADG, ADFI and FCR were calculated based on these data.

**Vulva measurements and sample collection**

At the end of the study, the length and width of the vulva were measured using electronic vernier caliper (0-200 mm; China Guanglu Electrical Co. Ltd., Zhejiang, China). The area of the vulva was calculated as an approximately diamond shape [(length × width)/2]. Blood samples were obtained by anterior vena cava puncture using 5 mL EDTA2K tubes and 10 mL anticoagulant free vacuum tubes. After samples of anticoagulant free tubes were kept at 37°C for 30 min and centrifuged at 3000 □g for 15 min, serum were collected for subsequent analysis. Then pigs were slaughtered. The genital organs of the female pigs (ovaries + cornu uteri + vagina - vestibule) were isolated and weighed. The liver and the longissimus muscle from the back were collected within 1 h post-mortem. The samples were stored in liquid N2 at -70°C for analysis.

**Blood samples analysis**

The alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), total protein (TP), albumin (ALB), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TG) were determined with an automatic biochemical analyzer (CX4PRO, Beckman Coulter Corp., Miami, FL, USA). Estradiol (E2), progesterone (PG) and testosterone (TS) were determined with a full automatic immune luminescence analyzer (Ala60011, TOSOH, Japan). The blood samples in EDTA2K tubes were sent directly to the laboratory within 3 h to measure white blood cell (WBC), neutrophil (NE), lymphocyte (LY), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), and platelet (PLT) using a blood counter (MEK-7222K, Nihon Kohden, Tokyo, Japan).

**Assessment of antioxidant status**

The activities of superoxide dismutase (SOD) and methane dicarboxylic aldehyde (MDA) in the serum and liver were assayed via colorimetric methods using a spectrophotometer (UV-2401PC, Shimadzu Corp., Kyoto, Japan). The assay kits were purchased from the Nanjing Jiancheng Institute of Bioengineering (Nanjing, Jiangsu, China), and the procedures were performed according to the Institute’s instructions.

**Zearalenone residues**

The residues of ZEN in livers and muscles were determined using ELISA kit for ZEN according to the manufacturer’s instruction (RIDASCREEN® Zearalenone, R1401; R-Biopharm, Darmstadt, Germany).

**Statistical analysis**

Data were analyzed by analysis of variance as a 2×2 factorial arrangement of treatments using the general linear model procedure (SPSS 20.0; IBM-SPSS Inc., Chicago, IL, USA). The results were presented by means and the standard error of the mean. The model accounted for the effect of ZEN, maifanite, and the ZEN×MMF interaction (P=0.018). Adding MMF decreased MDA. The ZEN×MMF interac-

**Results**

**Growth performance**

Throughout the 28 d feeding period, all pigs appeared healthy without mortality. Treatments had no effect on ADG, ADFI or FCR (Table 2).

**Vulva size and genital organ weight**

As shown in Table 3, there were no effects of ZEN, MMF or the ZEN×MMF interaction on the length and width of the vulva. However, an effect of the ZEN×MMF interaction on vulva area (P=0.038) was observed. The ZEN×MMF interaction indicated that the MMF reduced vulva area when fed with ZEN-contaminated diet but not when fed with the basal diet. Genital organ weight was also affected by ZEN (P=0.002). The genital organ weight of female pigs supplemented with the ZEN diet was greater than that of pigs fed the basal diet. However, there was no effect of the ZEN×MMF interaction on the genital organ weight.

**Antioxidant enzyme activity**

The SOD activity and MDA concentration recorded in the serum and liver of female pigs are shown in Table 4. In the serum, the SOD activity was reduced by feeding ZEN (P=0.017), though there was no observed effect of ZEN on MDA (P=0.714). Additionally, no effect of MMF or the ZEN×MMF interaction on SOD or MDA was observed. In the liver, there was no effect of ZEN, MMF or the ZEN×MMF interaction on SOD activity. The MDA concentration was also not affected by feeding ZEN (P=0.436). However, MDA was impacted by both MMF (P=0.041) and the ZEN×MMF interaction (P=0.018).

**Table 1. Ingredients and chemical composition of the basal diet.**

| Items          | Basal diet |
|----------------|------------|
| Ingredients, % |            |
| Corn           | 62.93      |
| Extruded soybean | 8.00      |
| Soybean meal   | 18.30      |
| Fish meal      | 4.50       |
| Whey powder    | 4.00       |
| Limestone      | 0.83       |
| Dicalcium phosphate | 0.62 |
| Salt           | 0.20       |
| L-Lys+HCl (%)  | 0.10       |
| DL-Met (%)     | 0.02       |
| Vitamin and mineral premix° | 0.50 |
| Calculated chemical composition (on dry matter)° |  |
| Digestible energy, MJ/kg | 14.09 |
| Metabolizable energy, MJ/kg | 12.98 |
| Crude protein, % | 19.03    |
| Calcium, %     | 0.74       |
| Available phosphorus, % | 0.40 |
| Lys, %         | 1.16       |
| Trp, %         | 0.76       |
| Arg, %         | 1.24       |

Lys, lysine; Met, methionine; Cys, cysteine; Thr, threonine; Trp, tryptophan; Arg, arginine. °Vitamin-mineral premix supplied per kg of diet: vitamin A, 15,000 IU; vitamin D3, 3000 U; vitamin E, 40 U; vitamin K3, 2 mg; vitamin B1, 1.50 mg; vitamin B2, 5.25 mg; vitamin B6, 2.25 mg; vitamin B12, 0.02 mg; nicotinic acid, 40 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.075 mg; choline chloride, 0.50 g; Mn, 41.34 mg; Zn, 120.00 mg; Cu, 20.00 mg; Fe, 120.00 mg; Se, 0.30 mg; I, 0.30 mg. °Values in this study were calculated following tables containing data on feed composition and nutritive values in China (Xiong et al., 2008).
tion indicated that the MMF reduced MDA concentration when fed with ZEN diet but not when fed with the basal diet.

**Serum metabolites**

As shown in Table 5, E2 and TS were not affected by ZEN, MMF or the ZEN×MMF interaction. The PG was affected by the ZEN×MMF interaction (P=0.022), as MMF reduced PG when fed with ZEN diet but increased PG when fed with the basal diet. No effects of ZEN, MMF or the ZEN×MMF interaction were observed on ALT, LDH, TP, ALB, HDL, LDL and TG. The WBC, NE, IY, HGB and HCT were also not affected by ZEN, MMF or the ZEN×MMF interaction. However, effects of ZEN on RBC (P=0.009) and PLT (P=0.002) were observed. The RBC and PLT levels in pigs fed ZEN were decreased. Moreover, PLT was affected by the ZEN×MMF interaction (P=0.033).

**Zearalenone residues**

As reported in Table 6, the levels of ZEN residues were increased in the liver (P<0.001) and muscle (P=0.002) when pigs were fed the ZEN (1.11 mg/kg diet) diet. The addition of MMF to the ZEN diet was effective in reducing the levels of ZEN in the liver (ZEN×MMF, P=0.003). No effect of MMF (P=0.095) or the ZEN×MMF interaction (P=0.081) on the level of ZEN residues in muscle was found.

**Discussion**

In our experiment, growth performance was not found to be affected by ZEN (1.11 mg/kg diet) when pigs were fed the ZEN diet. No effect of MMF (P=0.095) or the ZEN×MMF interaction (P=0.081) on the level of ZEN residues in muscle was found.

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Table 2. Growth performance of pigs fed zearalenone-contaminated diet with or without modified maifanite.

| Items                  | Treatments° | SEM | ZEN | MMF | Interaction |
|------------------------|-------------|-----|-----|-----|-------------|
|                        | 0% MMF      | 1% MMF | 0% MMF | 1% MMF |             |
| Initial body weight, kg| 10.50       | 10.50 | 10.50 | 10.50 | 0.283       | 1.000     | 1.000     | 1.000     |
| Final body weight, kg  | 26.74       | 25.34 | 26.74 | 29.26 | 0.790       | 0.270     | 0.737     | 0.270     |
| ADG, kg/d              | 0.58        | 0.53  | 0.58  | 0.67  | 0.021       | 0.153     | 0.650     | 0.153     |
| ADFI, kg/d             | 1.06        | 0.95  | 1.04  | 1.14  | 0.038       | 0.318     | 0.966     | 0.226     |
| FCR, kg/kg             | 0.55        | 0.56  | 0.56  | 0.59  | 0.011       | 0.408     | 0.371     | 0.534     |

ZEN, zearalenone; MMF, modified maifanite; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio. Values are the means from 8 individual pigs. °Basal diet was contaminated with 0.02 mg/kg of ZEN, while ZEN diet was contaminated with 1.11 mg/kg of ZEN. #The P values represent the main effect of ZEN, the main effect of MMF, and the interaction of ZEN and MMF.

Table 3. Vulva and genital organ weight in pigs fed zearalenone-contaminated feed with or without modified maifanite.

| Items                  | Treatments° | SEM | ZEN | MMF | Interaction |
|------------------------|-------------|-----|-----|-----|-------------|
|                        | 0% MMF      | 1% MMF | 0% MMF | 1% MMF |             |
| Length, mm             | 15.39       | 16.32 | 18.39 | 17.91 | 0.464       | 0.069     | 0.822     | 0.489     |
| Width, mm              | 10.26       | 10.96 | 11.94 | 9.76  | 0.387       | 0.774     | 0.394     | 0.136     |
| Area, mm²              | 78.91       | 89.69 | 108.69 | 87.36 | 2.641       | 0.060     | 0.375     | 0.038     |
| Genital organ, g§       | 11.48       | 10.47 | 19.54 | 17.27 | 0.484       | 0.002     | 0.166     | 0.549     |

ZEN, zearalenone; MMF, modified maifanite. Values are the means from 8 individual pigs. °Basal diet was contaminated with 0.02 mg/kg of ZEN, while ZEN diet was contaminated with 1.11 mg/kg of ZEN. #The P values represent the main effect of ZEN, the main effect of MMF, and the interaction of ZEN and MMF. §Genital organ weight: ovary + corn uteri + vagina + vestibule.

Table 4. Antioxidant enzymatic activity in pigs fed zearalenone-contaminated feed with or without modified maifanite.

| Items                  | Treatments° | SEM | ZEN | MMF | Interaction |
|------------------------|-------------|-----|-----|-----|-------------|
|                        | 0% MMF      | 1% MMF | 0% MMF | 1% MMF |             |
| Serum                  |             |       |     |     |             |
| MDA, nmol/mL           | 3.66        | 3.82  | 4.35 | 3.50  | 0.237       | 0.714     | 0.491     | 0.323     |
| SOD, U/mL              | 89.26       | 88.80 | 60.67 | 74.08 | 3.326       | 0.017     | 0.368     | 0.338     |
| Liver                  |             |       |     |     |             |
| MDA, nmol/mg           | 0.73        | 0.77  | 1.01 | 0.60  | 0.035       | 0.436     | 0.041     | 0.018     |
| SOD, U/mg              | 158.17      | 170.37 | 144.24 | 159.68 | 3.436       | 0.123     | 0.091     | 0.822     |

ZEN, zearalenone; MMF, modified maifanite; MDA, methanediacrylclyc aldehyde; SOD, superoxide dismutase. Values are the means from 8 individual pigs. °Basal diet was contaminated with 0.02 mg/kg of ZEN, while ZEN diet was contaminated with 1.11 mg/kg of ZEN. #The P values represent the main effect of ZEN, the main effect of MMF, and the interaction of ZEN and MMF.
mg/kg diet) or MMF (1% diet). Our results were consistent with those of previous studies, where growth performance among treatments was also not affected by ZEN (Etienne and Jemmal, 1982; Green et al., 1990; James and Smith, 1982; Jiang et al., 2010b; Yang et al., 2008). However, FCR was shown to be decreased when gilts (average weight 64 kg) were fed ZEN (0, 3, 6, or 9 mg/kg diet) (Young et al., 1981). Furthermore, Kalliamurthy et al. (1997) observed a reduction in ADFI when diets containing ZEN (0.25 mg/kg BW/d) were fed to male rats. The difference in the performance of the pigs in the present study might be due to the duration of exposure (28 d) and ZEN levels. The levels used in this experiment may not be sufficiently high to elicit significant changes in growth performance. Most toxicological data on animals has been obtained using medium to high doses of ZEN (2 to 90 mg/kg of feed) (Jiang et al., 2010b). Although such high dosages are not commonly found in animal feeds, the applied dosage in this study does not meet to the amounts of ZEN measured in naturally contaminated feed (EFSA, 2011). Modified maifantite had no adverse or positive effects on growth performance in our experiment. The toxic of ZEN is a kind of estrogen-like hormone with a strong affinity for estradiol receptors (Jiang et al., 2010a), and moderate doses increase vulva size in females (Düll et al., 2003; Kordi et al., 1992).

Table 5. Serum metabolites in pigs fed zearalenone-contaminated feed with or without modified maifantite.

| Items       | Basal diet | ZEN diet | SEM | ZEN | MMF | Interaction |
|-------------|------------|----------|-----|-----|-----|-------------|
|             | 0% MMF     | 1% MMF   |     |     |     |             |
| E2, pg/mL   | 35.55      | 39.31    | 38.82 | 29.48 | 1.252 | 0.260       | 0.328       | 0.059       |
| PG, ng/mL   | 0.74       | 1.37     | 0.85  | 0.62  | 0.059 | 0.052       | 0.167       | 0.022       |
| TS, ng/dL   | 31.00      | 38.10    | 30.20 | 32.95 | 1.832 | 0.462       | 0.250       | 0.585       |
| ALT, U/L    | 52.50      | 50.67    | 55.00 | 61.50 | 2.284 | 0.204       | 0.631       | 0.404       |
| AST, U/L    | 60.00      | 62.67    | 63.50 | 67.50 | 0.929 | 0.075       | 0.133       | 0.734       |
| LDH, U/L    | 850.00     | 1010.00  | 1310.00 | 885.00 | 88.975 | 0.390       | 0.490       | 0.161       |
| TP, g/L     | 57.50      | 62.00    | 56.00 | 54.50 | 0.993 | 0.073       | 0.484       | 0.191       |
| ALB, g/L    | 17.50      | 20.33    | 20.50 | 18.50 | 0.645 | 0.670       | 0.790       | 0.120       |
| HDL, mmol/L | 1.00       | 0.86     | 0.83  | 0.87  | 0.021 | 0.106       | 0.296       | 0.101       |
| LDL, mmol/L | 1.20       | 1.31     | 1.45  | 1.31  | 0.084 | 0.485       | 0.925       | 0.496       |
| TC, mmol/L  | 0.64       | 0.64     | 0.61  | 0.69  | 0.024 | 0.834       | 0.482       | 0.445       |
| WBC, ×10^3/L| 14.00      | 12.40    | 13.40 | 11.55 | 0.761 | 0.659       | 0.320       | 0.938       |
| NE, %       | 1.95       | 2.15     | 2.80  | 2.80  | 0.186 | 0.114       | 0.802       | 0.902       |
| LY, %       | 12.00      | 10.10    | 10.30 | 7.15  | 0.645 | 0.145       | 0.121       | 0.652       |
| RBC, ×10^12/L| 7.09      | 7.13     | 6.57  | 6.72  | 0.049 | 0.009       | 0.398       | 0.588       |
| HGB, g/L    | 113.50     | 123.00   | 113.50 | 109.00 | 2.257 | 0.196       | 0.609       | 0.196       |
| HCT, L/L    | 35.20      | 39.60    | 37.05 | 34.45 | 0.989 | 0.451       | 0.673       | 0.151       |
| PLT, ×10^9/L| 384.00     | 437.00   | 336.50 | 305.00 | 6.631 | 0.002       | 0.453       | 0.033       |

ZEN: zearalenone; MMF: modified maifantite; E2: estradiol; PG: progesterone; TS: testosterone; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; TP: total protein; ALB: albumin; HDL: high density lipoprotein; LDL: low density lipoprotein; TG: triglycerides; WBC: white blood cell; NE: neutrophil; LY: lymphocyte; RBC: red blood cell; HGB: hemoglobin; HCT: hematocrit; PLT: platelets. Values are the means from 8 individual pigs. *Basal diet was contaminated with 0.02 mg/kg of ZEN, while ZEN diet was contaminated with 1.11 mg/kg of ZEN. The P values represent the main effect of ZEN, the main effect of MMF, and the interaction of ZEN and MMF.

Table 6. Zearalenone residues in pigs fed zearalenone-contaminated feed with or without modified maifantite.

| Items | Basal diet | ZEN diet | SEM | ZEN | MMF | Interaction |
|-------|------------|----------|-----|-----|-----|-------------|
|       | 0% MMF     | 1% MMF   |     |     |     |             |
| Liver, ng/kg | 13.46   | 17.82    | 856.63 | 385.77 | 19.247 | <0.001       | 0.003       | 0.003       |
| Muscle, ng/kg | 2.78   | 3.21     | 30.60 | 17.62 | 1.444 | 0.002       | 0.095       | 0.081       |

ZEN: zearalenone; MMF: modified maifantite. Values are the means from 8 individual pigs. *Basal diet was contaminated with 0.02 mg/kg of ZEN, while ZEN diet was contaminated with 1.11 mg/kg of ZEN.

The P values represent the main effect of ZEN, the main effect of MMF, and the interaction of ZEN and MMF.
Oxidative damage is one of the toxic effects of ZEN (Yu et al., 2011). Methane dicarboxylic aldehyde is a terminal product of lipid peroxidation, and the MDA can be used to estimate the extent of lipid peroxidation (Han et al., 2006). Superoxide dismutase can clear super oxygen anion free radicals and protect cells from damage. Our results were similar to those of Zourgui et al. (2008) who reported that ZEN (40 mg/kg BW) can increase MDA concentrations in the liver and kidney. Moreover, Salah-Abbès et al. (2008) reported that SOD activity was decreased in rats fed diet containing ZEN (40 mg/kg BW). In general, ZEN induces damage in the liver and reduces its ability to remove oxygen free radicals. Pigs fed diets containing MMF displayed a lower concentration of MDA in the liver compared to those fed diets without MMF. The addition of MMF to ZEN diets can relieve the ZEN-induced changes in antioxidant enzymatic activity observed in pigs to some extent.

The ZEN interferes with steroid hormone synthesis and metabolism via interaction with estrogen receptors (LeBlanc et al., 1995). Changes in serum hormones indicate that ZEN acts as an endocrine disruptor (Kalliamurthy et al., 1997; Milano et al., 1995). In our experiment, there were no observed effects of 1.11 mg ZEN/kg diet on E2 or TS. This finding differed from those of Mitak et al. (2001), who reported E2 content was increased in female rats subjected to oral ZEN administration (2.5 mg/kg) during the rutting period. Berger et al. (1981) reported that ZEN consumption (40 mg/kg) results in depressed plasma TS in prepubertal boars. These differences may be due to the impact of ZEN on serum hormones, which is related to the ZEN dosage, and the dose of 1.11 mg ZEN/kg diet used in the present experiment was likely not sufficiently high to effect the biochemical indices. Long and Diekmann (1984) reported PG was decreased in postbreeding gilts fed 60 or 90 mg ZEN/kg diet at 2, 3, and 6 wk. The result show that no effects of MMF on ZEN toxicity in PG. This result may be due to MMF cannot completely absorb ZEN, and very low ZEN doses can act as endocrine disruptors with regard to PG (Gajecka et al., 2013).

In the blood, the observed increases in serum ALT and AST indicate that ZEN may cause liver damage (Horejsi et al., 1989). The ZEN-treated group (40 mg/kg BW) showed a significant increase in ALT (Salah-Abbès et al., 2008). Abbès et al. (2006b) reported that ZEN causes a decrease in TP level and produces unfavorable effects on immune function. When pigs were fed the diet containing 1.3 mg/kg of ZEN 24 d, the serum levels of AST and ALT were increased and the levels of TG and HDL were decreased compared to control (Jiang et al., 2010a). In contrast, in our experiment, there were no observed effects of ZEN, MMF, or the ZEN+MMF interaction on serum biochemical parameters.

The decline of PLT indicated that ZEN might cause damage to the hemostasis blood system. This finding is in accord with the work of Jiang et al. (2010a). They reported that HGB were decreased in piglets fed diets contaminated with 1.3 mg ZEN/kg diet. A higher dose of 40 mg ZEN/kg BW was shown to result in an increase of HGB and a decrease of PLT in rats (Abbès et al., 2006a). The decrease in HGB caused by ZEN might be due to RBC decompensation and inhibition of protein synthesis (Abid-Essefi et al., 2004). The number of RBC and PLT observed in pigs fed the ZEN diet were decreased in our experiment. However, the content of HGB was not affected by ZEN. Interestingly, MMF interacted with ZEN for PLT, exhibiting an opposite trend when ZEN was present or absent. It is unclear if the result produced by the quaternary ammonium salt from modification process or some trace elements in MMF (Wang and Shan, 2004).

Long-term intake of ZEN can lead to the accumulation of ZEN residues in the liver and muscle. The liver is the major organ subjected to ZEN accumulation (Zöllner et al., 2002). When pigs were fed the diet containing 40 mg/kg of ZEN 4 wk, the ZEN concentration of residues reach 78-128 µg/kg (James and Smith, 1982). Our results showed that MMF was effective in reducing the levels of ZEN residues in the liver. ZEN can be metabolized in various tissues, particularly the liver, and the major metabolites of ZEN are α-zearalenol and β-zearalenol (Zinedine et al., 2007). Due to the limit of detection method, the contents of α-zearalenol and β-zearalenol in tissues were not measured in the present study.

Conclusions

No differences in growth performance were observed among the treatments applied in this study. Feeding of ZEN at 1.11 mg/kg diet not only caused reproductive toxicity but also resulted in the accumulation of ZEN residues in liver and muscle. The addition of MMF to ZEN diet resulted in partial restoration of the ZEN-induced changes in genital organ parameters and antioxidant enzyme activities as well as reducing ZEN levels in the liver. Further cellular and molecular studies will be needed to thoroughly understand the in vivo reproductive toxicity of ZEN.

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