Effects of zearalenone on vulva area, liver function, serum immunoglobulin, antioxidant capability and sex hormone secretion of prepubertal gilts

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ABSTRACT

The study aimed to examine the multi-organs toxicity of zearalenone on prepubertal gilts. A total of 48 Landrace × Yorkshire prepubertal gilts were randomly divided into 4 groups with 12 replicates in each group. The control group were fed with basal diet, the experimental groups were fed test diets supplemented with 0, 200, 800 and 1600 µg/kg zearalenone in the basal diet. The experiment lasted for 28 days. The results showed that the average daily feed intake of prepubertal gilts in each group had no significant change (p > 0.05). Diets supplemented with zearalenone significantly reduced the serum immunoglobulin G, immunoglobulin M, follicle stimulating hormone concentrations and total antioxidant capacity activity of prepubertal gilts (p < 0.05). Diets supplemented with zearalenone significantly increased the vulva area, the serum concentration of interleukin-4 and activities of alanine aminotransferase and alkaline phosphatase of prepubertal gilts (p < 0.05). In conclusion, dietary supplementation of zearalenone has no obvious effect on the average daily feed intake of prepubertal gilts. However, it can increase its vulva area, produce reproductive toxicity, cause liver damage, reduce the serum immunoglobulin concentrations and antioxidant capability and disrupt the secretion of sex hormones.

HIGHLIGHTS

- Dietary supplementation of zearalenone significantly increases the vulva area of prepuber
tal gilts.
- Dietary supplementation of zearalenone significantly increases serum ALT and ALP activities of prepubertal gilts.
- Dietary supplementation of zearalenone reduce serum immunoglobulin levels and antioxi
dant capability of prepubertal gilts.
- Dietary supplementation of zearalenone decreased the secretion of FSH of prepubertal gilts.
- Zearalenone exerts its toxic effects in multiple ways simultaneously.

Introduction

Zearalenone (ZEA) is a secondary metabolite, which is mainly produced by Fusarium spp. and has immunotoxicity (Cai et al. 2019), cytotoxicity (Belgacem et al. 2019), reproductive toxicity (Grenier et al. 2019) and genotoxicity (Yang et al. 2018) effects. ZEA has a wide range of contamination. Gruber-Dorninger et al. (2019) reported that ZEA was detected in 88% of 61,413 feed ingredients and formula feed samples collected from different countries around the world. In the same study, the median value of the pollution was 55 µg/kg, and the highest was 105 mg/kg. China has a complex and diverse climate and vast territory. Raw feed materials are susceptible to ZEA in growing, harvesting, processing, transportation and storage (the worst-hit area of ZEA pollution) (Sun et al. 2017).

ZEA is one of the most common mycotoxins in pig diets. In recent years, immunity and productivity of sows declined due to ZEA pollution, which damages the economic benefits of the pig industry and becomes potentially harmful to food safety. Diets of gestation sows supplemented with ZEA can cause oxidative stress and cell apoptosis in spleen, breast, uterus and ovary, reducing the reproductive performance of first-parity gestation sows (Zhou et al. 2020). The mutagenicity, teratogenicity, carcinogenicity, immunotoxicity and genetic toxicity of ZEA have toxic effects on human and animal health (Caglayan et al. 2020).
However, most studies on the toxicity of ZEA on sows focus on one type of toxicity. There are a few reports on the multi-organs’ toxicity of ZEA to gilts, limiting the development of more effective programs to prevent mycotoxin contamination. Therefore, the prepubertal gilts, which are more sensitive to ZEA, were selected in this study to examine the effects of ZEA on vulva area, liver function, serum immunoglobulin, antioxidant capability and sex hormone secretion of prepubertal gilts.

Materials and methods

Feed preparation

ZEA (purity ≥98%) was purchased from Triplebond (Guelph Ontario, Canada). The basic diet had no anti-biotic and mildew repellent. The prepubertal gilts were fed with the diet according to the standards of the National Research Council (NRC) (2012). The feed composition has been provided in Table 1.

Experimental design and animal grouping

A total of 48 Landrace × Yorkshire prepubertal gilts (aged 65 ± 3 days, initial body weight 23.20 ± 0.68 kg) were randomly divided into three treatment (T1, T2 and T3) groups and a control group consisting of 12 replicates with 1 gilt per replicate, and each group received one of the following dietary treatments: 0, 200, 800 and 1600 µg/kg ZEA diet, which lasted for 28 d. The average daily feed intake (ADFI) was counted, and the vulvar area was measured. The blood samples were collected from the anterior vena cava of 6 prepubertal gilts in each group, and immunoglobulins, antioxidant indexes, inflammatory cytokines, reproductive hormones and biochemical indexes were analysed by enzyme-linked immunosorbent assay (ELISA). Before the experiments, the piggery was cleaned and disinfected, and the experimental prepubertal gilts were placed in separate pens.

Dietary preparation was done before the experiments, and the concentrations of ZEA, vomitoxin and aflatoxin B1 in each group were detected by ELISA on 1 and 14 days. The two test results of vomitoxin and aflatoxin B1 met the requirements of China Hygiene Standard for Feeds (GB13078-2017), and the ZEA, vomitoxin and aflatoxin B1 test results were 52.37, 241.60, 825.20, 1634.46 µg/kg and 68.61, 255.26, 837.65, 1652.39 µg/kg; 260.37, 265.11, 246.83, 251.09 µg/kg and 281.40, 276.53, 264.79, 268.21 µg/kg; 3.82, 3.75, 3.68, 3.72 µg/kg and 3.89, 3.82, 3.74, 3.76 µg/kg, respectively.

The experimental protocols were approved by the Animal Care and Use Committee of Hebei Agriculture University (Baoding, China).

All animal experiments complied with the ARRIVE guidelines were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

Determination indexes and methods

Vernier calliper was used to measure the vulva area of prepubertal gilts in each group on 1 and 28 days of the trial period. The vulva area of prepubertal gilts in each group was regarded as a rhombus. The area was calculated as follows: length (long diagonal length) × width (short diagonal length)/2.

On 28 days of the trial, six prepubertal gilts in each group were randomly selected for blood collection of anterior vena cava after the prepubertal gilts were fasted for 12 h. Blood (10 mL) was collected from prepubertal gilts. The serum samples were centrifuged at 3000 g at 4°C for 10 min to separate the serum, and the serum samples were taken and stored at −80°C. The concentrations of antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and alanine aminotransferase (ALT), malondialdehyde (MDA), tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-10 (IL-10), luteinizing hormone (LH), follicle-stimulating

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

| Items           | Content (%) |
|-----------------|-------------|
| Ingredients     |             |
| Corn            | 65.00       |
| Soybean meal    | 19.00       |
| Wheat bran      | 12.00       |
| Premix          | 4.00        |
| Total           | 100.00      |

| Nutrient levelsb | Content (µg/kg) |
|------------------|-----------------|
| Digestible energy | 14.06           |
| Crude protein    | 20.34           |
| Crude fibre      | 2.18            |
| Calcium          | 0.88            |
| Phosphorus       | 0.64            |
| Available phosphorus | 0.46   |
| Lysine           | 1.22            |
| Methionine       | 0.41            |
| Threonine        | 0.81            |

*Premix provides the following (per kg of the diet): VA 360,000 IU, VD 360,000 IU, VE 375 mg, VK3 120 mg, VB1 50 mg, VB2 180 mg, VB6 90 mg, VB12 0.63 mg, niacin1000 mg, pantothenic acid 630 mg, biotin 12 mg, choline19 g, folic acid 100 mg, salt 75 g, lysine 15 g, Fe (as ferrous sulphate) 3 g, Cu (as copper sulphate) 0.375 g, Mn (as manganese sulphate) 1.047 g, Zn (as zinc sulphate), I (as potassium iodide), Se (as sodium selenite), Cr 6 mg, Ga 155 g, P 35 g.

bDE was a calculated value, while the others were measured values.
hormone (FSH), oestradiol (E2), progesterone (P) and total bile acid (TBA) in the serum were quantified using ELISA kits for porcine T-AOC, GSH-Px, SOD, MDA, TNF-α, IL-1β, IL-10, LH, FSH, E2 and P (Beijing Hairuxiangtian Biotechnology Co., Ltd, Beijing, China) and AST, ALP, ALT and TBA (Nanjing Jiancheng 122 Bioengineering Institute, Nanjing, China). The correlation coefficient R value between the sample linear regression and the expected concentration was greater than 0.990. The coefficient of variation within and between plates were less than 10%.

**Statistical analysis of data**

The statistical data analysis was done using Excel 2016 and SPSS 20.0 software (SPSS, Chicago, IL). One-way analysis of variance (ANOVA) was used to test the significant differences between each group data, while the Duncan method was used for multiple comparisons. A p-value less than .05 (p < .05) showed a significant difference between the groups.

**Results**

**Average daily feed intake and vulva area**

The results of the ADFI and vulva area are shown in Table 2. There were no significant differences in ADFI among the groups (p > .05). Compared with the control group, length, width, vulva area and increment of the vulva area were significantly increased in the T2 and T3 groups (p < .05).

**Serum immunoglobulins**

The results of serum immunoglobulin concentrations are shown in Table 3. Compared with the control group, the serum immunoglobulin G (IgG) and immunoglobulin M (IgM) concentrations in the T2 group and serum IgM concentration in the T3 group were significantly reduced (p < .05) by 13.18%, 17.86% and 15.48%, respectively. However, there was no significant difference in serum IgA concentration among the groups (p > .05).

**Serum antioxidant indexes**

The results of serum antioxidant indexes are shown in Table 4. Compared with the control group, the T-AOC activity in the T3 group was significantly decreased by 59.10% (p < .05). However, there were no significant differences in GSH-Px and SOD activities and MDA concentration in serum among the groups (p > .05).

**Serum concentrations of inflammatory cytokines**

The results of the serum concentrations of inflammatory cytokines are shown in Table 5. Compared with the control group, the concentrations of serum interleukin-4 (IL-4) in T1, T2 and T3 groups were significantly increased (p < .05) by 61.76%, 97.78% and 75.20%, respectively. However, there were no significant differences in TNF-α, IL-1β and IL-10 concentrations among the groups (p > .05).

### Table 2. Effects of zearalenone on average daily feed intake (ADFI) and vulva area of prepubertal gilts (n = 12).

| Items       | Control | T1      | T2      | T3      | SEM  | p-Value |
|-------------|---------|---------|---------|---------|------|---------|
| ADFI (kg)   | 1.33    | 1.29    | 1.28    | 1.38    | 0.028| .792    |
| Vulvar length (mm) | 22.21a  | 27.92ab | 32.34b  | 32.41b  | 1.754| .024    |
| Vulvar width (mm) | 18.45a  | 20.89   | 27.43   | 26.89b  | 1.561| .036    |
| Vulvar area (mm²) | 204.79a | 291.35b | 440.77c | 437.01c | 28.623| <.001   |
| Increment of vulva area (mm²) | 72.81a  | 137.42b | 277.72c | 283.91c | 15.949| <.001   |

ADFI: average daily feed intake; SEM: standard error of the mean.

### Table 3. Effects of zearalenone on serum immunoglobulin concentrations of prepubertal gilts (ng/mL) (n = 6).

| Items         | Control | T1     | T2     | T3     | SEM   | p-Value |
|---------------|---------|--------|--------|--------|-------|---------|
| IgA           | 71.59   | 68.66  | 77.76  | 75.64  | 1.803 | .305    |
| IgG           | 363.60b | 352.81ab| 332.00ab| 315.68a| 7.943 | .034    |
| IgM           | 127.68b | 122.54a| 104.88a| 107.91a| 3.768 | .046    |

IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; SEM: standard error of the mean.

### Table 4. Effects of zearalenone on serum antioxidant indexes of prepubertal gilts (n = 6).

| Items         | Control | T1     | T2     | T3     | SEM   | p-Value |
|---------------|---------|--------|--------|--------|-------|---------|
| T-AOC (U/mL)  | 2.86b   | 2.12ab | 1.96ab | 1.17a  | 0.281 | .046    |
| GSH-Px (pg/mL)| 123.70  | 123.77 | 96.87  | 87.48  | 7.801 | .244    |
| SOD (ng/mL)   | 342.50  | 320.94 | 273.86 | 292.34 | 11.035| .302    |
| MDA (nmol/mL)| 4.46    | 5.13   | 6.65   | 6.105  | 0.396 | .220    |

T-AOC: total antioxidant power; GSH-Px: glutathione peroxidase; SOD: superoxide dismutase; MDA: malondialdehyde; SEM: standard error of the mean.

### Table 5. Effects of zearalenone on serum inflammatory factors of prepubertal gilts (ng/L) (n = 6).

| Items         | Control | T1     | T2     | T3     | SEM   | p-Value |
|---------------|---------|--------|--------|--------|-------|---------|
| TNF-α         | 246.45  | 252.24 | 292.39 | 313.63 | 12.936| .284    |
| IL-1β         | 270.31  | 314.85 | 357.83 | 359.22 | 23.446| .187    |
| IL-4          | 39.64a  | 64.12b | 78.40b | 69.45b | 4.708 | .008    |
| IL-10         | 139.80  | 135.08 | 126.49 | 84.77  | 10.560| .274    |

TNF-α: tumour necrosis factor-alpha; IL-1β: interleukin-1 beta; IL-4: interleukin-4; IL-10: interleukin-10; SEM: standard error of the mean.

Means in a row without the same superscript are different significantly (p < .05).
**Table 6.** Effect of zearalenone on blood reproductive hormones of prepubertal gilts (n = 6).

| Items                        | Control | T1    | T2    | T3    | SEM  | p-Value |
|------------------------------|---------|-------|-------|-------|------|---------|
| LH (IU/mL)                   | 13.55   | 13.51 | 13.70 | 13.52 | 0.968| 0.894   |
| FSH (IU/L)                   | 9.72^b  | 9.53^ab| 9.42^a | 9.49^ab| 0.050| 0.035   |
| E2 (pmol/L)                  | 46.75   | 45.95 | 47.90 | 47.72 | 0.359| 0.189   |
| P (pmol/L)                   | 1 619.45| 1 561.46| 1 550.19| 1 610.42| 12.751| 0.131   |

LH: luteinizing hormone; FSH: follicle stimulating hormone; E2: oestradiol; P: progesterone; SEM: standard error of the mean.

^a,b^ Means in a row without the same superscript are different significantly (p < .05).

**Table 7.** Effects of ZEA on serum biochemical indexes of prepubertal gilts (n = 6).

| Items                        | Control | T1    | T2    | T3    | SEM  | p-Value |
|------------------------------|---------|-------|-------|-------|------|---------|
| AST (IU/L)                   | 74.28   | 72.30 | 78.90 | 77.65 | 1.621| 0.512   |
| ALT (IU/L)                   | 50.67^a | 50.24^a| 61.12^b| 63.60^b| 2.185| 0.512   |
| ALP (IU/L)                   | 115.03^a| 121.72^ab| 123.06^ab| 135.29^b| 2.805| 0.044   |
| TBA (µmol/L)                 | 21.42   | 22.10 | 26.38 | 27.17 | 1.064| 0.106   |

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; TBA: total bile acid; SEM: standard error of the mean.

^a,b^ Means in a row without the same superscript are different significantly (p < .05).

**Serum biochemical indexes**

The results of reproductive hormones in the serum are shown in Table 6. Compared with the control group, the FSH concentration in the T2 group was significantly reduced (p < .05) by 3.09%. However, there were no significant differences in E2, LH and P concentrations in serum among the groups (p > .05).

**Serum reproductive hormones**

The results of serum biochemical indexes are shown in Table 7. Compared with the control group, the serum ALT activity in the T2 group and the serum ALT and ALP activities in the T3 group were significantly increased (p < .05) by 20.62%, 25.52% and 17.61%, respectively. However, there were no significant differences in AST activity and TBA concentration in the serum among the groups (p > .05).

**Discussion**

ZEA can cause reproductive disorders, immune suppression and liver damage in pigs (Pierron et al. 2016; Wang et al. 2018; Grenier et al. 2019), which hinders the productivity of the pig industry. The lack of research on the toxic mechanism of ZEA affects the formulation of more effective anti-mildew programs.

In this study, there was no significant difference in ADFI of prepubertal gilts in each group, which is consistent with our previously published results from the first 14 days of the trial (Wu et al. 2021), indicating that feeding ZEA at this test dose for 28 days had no significant effect on ADFI of gilts. Su et al. (2018) found no significant change in ADFI of weaning gilts after adding 1 mg/kg ZEA to the diets for 28 days. Reddy et al. (2018) who fed the 6 weeks old growing pigs supplemented with 0.8 mg/kg ZEA for 28 days and found that there was no significant difference in terminal weight, ADG and ADFI of growing pigs. Shen et al. (2021) also showed that gilts fed on diets containing ZEA (about 300 µg/kg) for 25 days did not significantly affect on terminal weight, ADG and ADFI of gilts. The results of this experiment showed that ZEA had multi-organs toxicity in gilts, but had no significant effect on ADFI, which may be due to the digestive system is not the main target of the toxic effect of ZEA. The vulva area of prepubertal gilts in each treatment group increased significantly, indicating that the supplementation of 200, 800 and 1600 mg/kg ZEA could show typical symptoms of oestrogenic effects of ZEA, and the oestrogenic effects of ZEA symptoms of the T2 group were the most obvious. The addition of ZEA to the diets of weaning gilts, prepubertal gilts and first-parity gilts significantly increased the vulva area (Zinedine et al. 2007; Su et al. 2018; Reddy et al. 2018a, 2018b; Wu et al. 2021; Zhou et al. 2022).

In this study, the IgM and IgG concentrations in the serum of the prepubertal gilts of the T2 and T3 groups were significantly reduced, indicating that ZEA affected the secretion of immunoglobulins in the prepubertal gilts and the humoral immune function and reduced immunity. This may be due to the ability of ZEA to inhibit proliferation of B and T cells and reduce the number of CD4 cells (Abbès et al. 2006; Vlata et al. 2006). Yang et al. (2016) also found that the IgG and IgM concentrations in the serum of the weaning gilts in the treatment groups decreased significantly after 18 days of feeding diets supplemented with 1, 2 and 3 mg/kg of ZEA. Marin et al. (2011) reported that when the concentration of ZEA, α-zearalanol (α-ZOL) and β-zearalanol (β-ZOL) exceeded 5 µM, the concentrations of IgA, IgM and IgG were significantly reduced. Shi et al. (2018) showed that diet containing about 596.86 µg/kg ZEA and 796 µg/kg deoxynivalenol to pre-pubertal female gilts for 28 days could significantly reduce the concentration of IgG in plasma. Differences in results might be attributed to age differences in animal model and different doses of ZEA contamination. Antioxidative enzymes, such as GSH-Px, SOD and catalase (CAT), comprise the major defence systems designed to combat the deleterious effects of excess reactive oxygen species production and cellular lipid peroxidation (Sies 1991). The activities of T-AOC and SOD in the serum of the prepubertal gilts of the
treatment groups were significantly reduced, in line with previous reports (Wu et al. 2021), indicating that ZEA could reduce the antioxidant performance of the prepubertal gilts by inhibiting the activities of antioxidant enzymes. A previous study also found that the activities of T-AOC, SOD and GSH-Px were in the serum of weaning gilts fed with a diet with 1 mg/kg ZEA were significantly reduced (Shi et al. 2017). Qin et al. (2015) treated porcine ovarian granulosa cells cultured in vitro with 15, 30 and 60 μM ZEA, and found that the internal reactive oxygen levels significantly increased, and the activities of SOD and CAT were significantly reduced. Superoxide dismutase 1 (SOD1) and CAT mRNA expression levels were significantly down-regulated after 60 μM treatment for 16 h. A previous study found that diet containing 246 μg/kg to first-parity gilts could significantly decreased the serum SOD activity and significantly increased the serum MDA level (Zhou et al. 2020). ZEA can inhibit the expression of antioxidant oxidase regulatory genes, which may be the reason for the relevant results. Qin et al. (2015) reported that ZEA can inhibit the mRNA expression of antioxidant enzymes, such as SOD1 and CAT, and reduce the activity of antioxidant enzymes, decepting antioxidant performance.

In this study, compared with the control group, the concentration of IL-4 in the serum of the prepubertal gilts in each treatment group was significantly increased, in line with previous reports (Wu et al. 2021), indicating that ZEA might be causing the inflammatory response in prepubertal gilts. IL-4 is produced by Th2 cells and promotes B cell responses. Obremski (2014) found that ZEA could stimulate Th1 and Th2 lymphocytes to produce IL-4 by shifting the Th1/Th2 balance to the humoral immune response. Previous studies have shown that ZEA can inhibit the expression levels of IL-10 receptors (Reddy et al. 2018a, 2018b) and upregulate the mRNA expression level of hdac11 (IL-10 suppressor gene) (Pistol et al. 2015), which may be one of the reasons why there was no significant difference in serum IL-10 concentration in this study. Inflammatory reactions such as vaginitis, mastitis and metritis are the common symptoms of ZEA poisoning (Zhao et al. 2013). Jia et al. (2020) showed that diet containing 269.1 μg/kg ZEA to piglets for 21 days could significantly increase the levels of TNF-α, IFN-γ, IL-1β and IL-6 in the serum of the piglets. After feeding piglets with 316 g/kg ZEA supplemented diet for 18 d, the concentrations of TNF-α and IL-1β in the serum were significantly increased, and the expression levels of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2) mRNAs in porcine spleen were also increased (Marin et al. 2013). Differences in results might be attributed to age differences in animal model and different doses of ZEA contamination.

In this study, ZEA significantly reduced the FSH concentration in the serum of prepubertal gilts. He et al. (2018a) reported that ZEA inhibited the synthesis and secretion of FSH of pig via the non-classical oestrogen membrane receptor and G protein-coupled receptor 30 (GPR30). MicroRNA-7(MiR-7) mediates the ZEA signalling pathway, which inhibits FSH synthesis and secretion by targeting Finkel–Biskis–Jinkins murine osteosarcoma viral oncogene homolog (FOS) (He et al. 2018b). According to Fu et al. (2018), compared with the control group, the concentrations of FSH and P in the serum of the weaning piglets fed with dietary supplementation of ZEA for 21 days were significantly reduced. Similarly, serum FSH and P concentrations were significantly lower in weaning gilts fed with 1 mg/kg ZEA for 28 days than those of the control group (Su et al. 2018). We previously reported that ZEA significantly reduced the LH and E2 concentrations in the serum of prepubertal gilts (Wu et al. 2021), indicating that ZEA had a persistent disruptive effect on reproductive hormone secretion in prepubertal gilts.

In clinical medicine, the concentration of ALT and AST in blood is usually used to evaluate liver function (He et al. 2022). In the present study, the activities of ALT and ALP in the treatment groups were significantly higher than those in the control group, indicating that ZEA caused liver damage in prepubertal gilts. Jiang et al. (2012) found that the activities of AST, ALT and ALP were significantly increased in piglets after 22 days of feeding 1 mg/kg ZEA supplemented feed. Jiang et al. (2011) observed that AST, ALT and ALP activities were significantly increased in weaning gilts after 18 days of feeding 1.1, 2.0 and 3.2 mg/kg ZEA supplemented feed. ALT exists in the cytoplasm while AST exists in mitochondria. Acute liver injury is generally dominated by elevated ALT, which may be one of the reasons why AST did not show significant difference in this study. ZEA can cause liver damage by causing oxidative stress and inflammation (Wu et al. 2022). Shi et al. (2017) found that weaning gilts were fed with 1 mg/kg ZEA supplemented diet for 28 days and showed lesions in liver tissues. MDA concentration was significantly increased, and total antioxidant power, and SOD and GSH-Px activities were significantly reduced (Shi et al. 2017). ZEA can also affect the expression of genes and proteins in signalling pathways, such as mitogen-activated protein kinase...
(MAPK) (transforming growth factor-β-activated kinase 1) TAK1, JNK, (p38MAPK) p38 and nuclear factor kappa B (NF-xB), leading to immunosuppression in the liver of pigs (Pistol et al. 2015).

Conclusion
In conclusion, dietary supplementation of ZEA for 28 days has no obvious effect on the ADFI of pre-pubertal gilts, but it can increase its vulva area, produce reproductive toxicity, cause liver damage, reduce the serum immunoglobulin concentrations and antioxidant capability and disrupt the secretion of sex hormones.

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Ethical approval
The experimental protocols were approved by the Animal Care and Use Committee of Hebei Agriculture University (Baoding, China). All animal experiments complied with the ARRIVE guidelines were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guide-lines, EU Directive 2010/63/EU for animal experiments.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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