Animal Nutrition 6 (2020) 372–378

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Short Communication

Bacillus subtilis ANSB01G culture alleviates oxidative stress and cell apoptosis induced by dietary zearalenone in first-parity gestation sows

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ARTICLE INFO

Article history:
Received 6 November 2019
Received in revised form
1 March 2020
Accepted 27 March 2020
Available online 6 July 2020

Keywords:
Zearalenone
Gestation sow
Bacillus subtilis ANSB01G culture
Mycotoxin biodegradation agent
Oxidative stress
Cell apoptosis

ABSTRACT

This study was conducted to evaluate the alleviation of *Bacillus subtilis* ANSB01G culture as zearalenone (ZEA) biodegradation agent on oxidative stress, cell apoptosis and fecal ZEA residue in the first parity gestation sows during the gestation. A total of 80 first-parity gilts (Yorkshire × Landrace) were randomly allocated to 4 dietary treatments with 20 replications per treatment and one gilt per replicate. The dietary treatments were as follows: CO (positive control); MO (negative control, ZEA level at 246 μg/kg diet); COA (CO + *B. subtilis* ANSB01G culture with 2×10^9 CFU/kg diet); MOA (MO + ZEA level at 260 μg/kg diet + *B. subtilis* ANSB01G culture with 2×10^9 CFU/kg diet). The experiment lasted for the whole gestation period of sows. Results showed that feeding the diet naturally contaminated with low-dose ZEA caused an increase of cell apoptosis in organ and the residual ZEA in feces as well as a decrease of antioxidant function in serum. The addition of *B. subtilis* ANSB01G culture in the diets can effectively alleviate the status of oxidative stress and cell apoptosis induced by ZEA in diets of gestation sows, as well as decrease the content of residual ZEA in feces.

1. Introduction

Mycotoxins are the secondary toxic metabolites generated by naturally occurring metabolic processes in the filamentous fungi that belong to the *Alternaria*, *Aspergillus*, *Penicillium* and *Fusarium* species (Antonissen et al., 2014; Tralamazza et al., 2016). Mycotoxins frequently contaminate various feedstuffs and result in a great economic loss to the global animal production (Zinedine et al., 2007). Among the identified mycotoxins, aflatoxin (AF), ochratoxin (OTA), deoxynivalenol (DON), zearalenone (ZEA), T2 toxin and fumonisin have been recognized as the most toxic compounds to animal health (Binder, 2007). ZEA (F-2 toxin), a non-steroid estrogen mycotoxin, is the most important and prevalent *Fusarium* toxin (Hueza et al., 2014). Due to the presence of a phenol ring which is similar to that of 17β-oestradiol, ZEA and its metabolites, especially α-zearalenol (α-ZEL), can compete with endogenous hormones for the binding sites of the estrogen receptors, and activate the transcription of oestrogen-responsive genes (Kuiper-Goodman et al., 1987; Zinedine et al., 2007). Therefore, animals in the period of active fertility such as the gilts and sows are very sensitive to ZEA (Zielonka et al., 2015). In addition to hepatotoxic, haematotoxic, immunotoxic and genotoxic effects, it is well documented that the oestrogenicity of ZEA and its metabolites cause numerous reproductive dysfunctions in gilts and sows (Abbes et al., 2006; Shi et al., 2018; Wang et al., 2012a, 2012b).

Mycotoxin biodegradation refers to the process of transforming mycotoxin into non-toxic or less toxic metabolites, and it is generally more specific, efficient, and environmentally friendly comparing with physical and chemical detoxification methods. Evidences have shown that ZEA can be degraded by some microorganisms, such as *Acinetobacter* sp. SM04 (Yu et al., 2011), *Rhodococcus pyridinivorans* K408 (Kriszt et al., 2012), *Bacillus licheniformis* CK1 (Yi et al., 2010), *Bacillus amyloliquefaciens* ZDS-1

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2020.03.011

(Xu et al., 2016), *Bacillus pumilus* ES-21 (Wang et al., 2017), and *Bacillus velezensis* A2 (Wang et al., 2018) etc. Results from our previous studies also demonstrated that the *Bacillus subtilis* ANSB01G could degrade ZEA efficiently by 84.6%, 66.3% and 83.0% in naturally contaminated corn, distiller's dried grains with soluble and commercial pig feed, respectively (Lei et al., 2014). Furthermore, we found that *B. subtilis* ANSB01G culture could ameliorate the negative effects of ZEA on reproductive performance in the sexually-immature or pre-pubertal gilts (Shi et al., 2018; Zhao et al., 2015), suggesting that *B. subtilis* ANSB01G might be a potential mycotoxin biodegradation agent (MBA) for detoxifying ZEA. However, whether a low dose of dietary ZEA (a little higher than the Chinese limited value of ZEA, 100 μg/kg) presents a toxic damage to gestation gilts, and the effect of *B. subtilis* ANSB01G culture on the oxidative stress and cell apoptosis responses induced by low-dose ZEA exposure have never been studied in first-parity gestation sows.

In the current study, the effects of *B. subtilis* ANSB01G culture on the serum antioxidant status, metabolic and reproductive organ histopathology, cell apoptosis and the fecal residual ZEA will be tested in first-parity gestation sows fed diets formulated with corn naturally contaminated with ZEA in the whole gestation period.

2. Materials and methods

This experimental procedures and swine management were in accordance with guidelines of the care and use of laboratory animals issued by the Animal Care Committee of China Agricultural University.

2.1. Determination of dietary mycotoxin

The concentrations of ZEA, AF, DON and OTA in the corn and feed samples were determined using the HPLC method as described previously (Zhao et al., 2015). The detection limits were 1.5 μg/kg for ZEA, 0.1 μg/kg for AF (aflatoxin B1 [AFB1], AFB2, aflatoxin G1 [AFG1], and AFG2), 0.02 mg/kg for DON and 0.5 μg/kg for OTA, respectively.

2.2. Mycotoxin biodegradation agent (*B. subtilis ANSB01G culture*)

The mycotoxin biodegradation agent was composed of 40% *B. subtilis* ANSB01G culture and 60% carrier (rice husk meal) by adopting industrial fermentation and dry-processing technologies. The *B. subtilis* ANSB01G, possessing the ability to degrade ZEA, was fermented in a Luria–Bertani medium at 37 °C for 24 h and dried at 65 °C (Zhao et al., 2015). The total viable counts of fermented-dried *B. subtilis* ANSB01G was 1×10^9 CFU/g.

2.3. Animals, housing, and treatments

A total of 80 first-parity gilts (Landrace × Yorkshire) with an average initial BW of 141 ± 5.5 kg were randomly allocated to 4 dietary treatments following a 2 × 2 factorial arrangement, i.e., a normal or ZEA moldy diet, and with or without dietary supplementation of MBA. Each treatment had 20 replicates with one gilt per replicate. The diets for the treatments were: 1) a basal control diet contained 60.52% normal corn (CO), 2) a basal diet contained 60.52% ZEA moldy corn (MO), 3) the CO diet was supplemented with MBA at 2 g/kg (*B. subtilis* ANSB01G count 2×10^9 CFU/kg diet), and 4) the MO diet was supplemented with MBA at 2 g/kg (*B. subtilis* ANSB01G count 2×10^9 CFU/kg diet). All diets used in the study were formulated to be isocaloric and isonitrogenous. The diets (Table 1) were formulated to meet or exceed NRC requirements (NRC, 2012). The experiment was lasted for a whole gestation period of sows from mating to farrowing.

Immediately after the first estrus and mating, all the pregnant gilts were penned (pen size, 0.60 m × 2.2 m) individually with ad libitum access to water in an environmentally controlled facility with slatted plastic flooring and a mechanical ventilation system. All gilts were fed individually and allowed restricted access to diet throughout the gestation period. Then, all the gilts were transferred to farrowing crates (2.13 m × 0.66 m) on d 108 and were fed separately until farrowing. The feeding program for the pregnant gilts was as follows:2 kg of diet per day from d 1 to 4, then 2.4 kg of diet per day from d 5 to 49, then 2.3 kg of diet per day from d 50 to 90, then 3.2 kg of diet per day from d 90 to 107, then 3.5 kg of diet per day from d 108 to 111 of gestation, then 2.5 kg of diet on d 112 and 1.5 kg of diet on d 113, and then 1.0 kg of diet on d 114 of gestation. On farrowing, all gilts received 1.0 kg of feed, which was followed by 1.5 kg of feed on d 1 post-farrowing.

2.4. Experimental procedures, sampling, and analysis

Eight pregnant gilts were randomly selected from each treatment, and blood samples collected from the jugular vein into a sterile syringe and centrifuged at 3,000 × *g* for 15 min for serum on d 60 ± 2 of gestation. The serum was stored at 4 °C for further analysis. Besides, fresh fecal samples (at least 0.5 kg) were collected from the same 8 gilts per treatment on d 60 ± 2 of gestation. All the fecal samples were stored at −20 °C until further analysis for residual ZEA using HPLC method according to our previous study. The

Table 1
Dietary composition and nutrient levels of the basal diets (as-fed basis).

Item	Content, %
Ingredients	
Corn	60.52
Soybean meal (43%)	16.00
Soybean oil	1.00
Apple pomace	10.50
Soybean hull	8.50
Calcium phosphate	1.70
Limestone	0.94
Lysine (70%)	0.01
Threonine (99%)	0.02
Vitamin premix[1]	0.03
Mineral premix[2]	0.03
Sodium chloride	0.50
Choline (60%)	0.20
Ethoxyquin (66%)	0.05
Total	100.00
Analyzed value	
CP	13.02
ME, kcal/kg	2,700
SID Lys	0.57
Lys	0.66
Met + Cys	0.42
Thr	0.51
Trp	0.13
Ca	0.90
P	0.55

SID = standardized ileal digestible.
[1] Provided the following per kilogram of diet: vitamin A, 17,500 IU; vitamin D_3, 5,000 IU; vitamin E, 40 IU; vitamin K_3, 5 mg; vitamin B_1, 5 mg; vitamin B_2, 12.5 mg; vitamin B_6, 7.5 mg; vitamin B_{12}, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 30 mg; D-calcium pantothenate, 25 mg.
[2] Provided the following per kilogram of diet: Fe, 100 mg as ferrous sulfate; Cu, 17 mg as copper sulfate; Mn, 40 mg as manganese oxide; Zn, 100 mg as zinc oxide; I, 0.25 mg as potassium iodide; and Se, 0.25 mg as sodium selenite.

activities of glutathione peroxidase (GSH-Px) and total superoxide dismutase (SOD) as well as the concentrations of malondialdehyde (MDA) in the serum were measured using the commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the kit instructions.

After farrowing, 6 sows were randomly selected from each treatment and sacrificed by the approved electrical stunning and exsanguinations. The left liver, kidney, spleen, uterus, ovary, and mammary gland were collected by trained personnel. The DNA fragmentation indicative of apoptosis for the above tissues was examined using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling method (TUNEL). TUNEL assay was performed using In Situ Cell Death Detection Kit (Cat. NO. 11684817910, Roche Molecular Biochemicals, CA, USA) according to the manufacturer's instructions. The tissue samples were fixed in 10% neutral formalin. The fixed tissues were trimmed, embedded in paraffin. Thin sections (5 μm) were sliced and mounted onto slides, and then stained with Hematoxylin and Eosin (H&E) for histopathological examination using an Olympus optical microscope (Olympus microscope, Olympus Corporation, Tokyo, Japan).

Zearalenone residues in the feces were analyzed using the methods of Duca et al. (2009) and Guo et al. (2019) with some modifications. A quantity of 10 g of feces samples were blended in 50 mL of buffer solution of acetic acid-ammonium acetate (pH 4.8). The solution was incubated at 37 °C for 15 h with 1 mL of glucuronidase with a pH 4.0 adjusted with glacial acetic acid. Then, the mixture was extracted with 50 mL of acetonitrile and 1 mL of NaOH (1 mol/L) with mixing at 200 r/min for 60 min. After centrifugation at 5,000 × *g* for 10 min, the supernatant (50 mL) was collected and mixed with buffer phosphate solution (200 mL, pH 7.4). Subsequently, the solution was filtered through a glass fiber filter paper and the filtrate (40 mL) was loaded onto an immune-affinity column, followed by washes with PBS (10 mL) and double distilled water (10 mL), respectively. The retained ZEA was eluted with 1 mL of methanol and the eluent was evaporated to dryness at 40 °C under a stream of nitrogen. The residues were dissolved in 100 μL of mobile phase for HPLC analysis.

2.5. Statistical analysis

The data were analyzed as a 2 × 2 factorial design using the MIXED procedure of SAS 9.1 (SAS Inst., Inc., Cary, NC, USA); the toxins level, MBA level, and their interaction were fixed factors, and the experimental period and animal were random factors. When there was an interaction, post hoc Duncan's multiple range tests were used to analyze differences among treatment means. Variability in the data was expressed as the standard error means (SEM), and a probability level of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Dietary mycotoxin concentrations

ZEA and other mycotoxins in the normal corn were not detectable. The concentration of ZEA in the moldy corn was 467 μg/kg. The concentrations of ZEA measured in CO, COA, MO and MOA diets were 10, 14, 246 and 260 μg/kg, respectively. The AF concentrations measured in MO and MOA diets were 2.0 and 2.2 μg/kg, respectively. No other mycotoxin was detected in any of the diets.

3.2. Serum antioxidant indices

Significant interactions ($P < 0.05$) between toxin and MBA were observed from the serum SOD activity and MDA content of pregnant gilts (Table 2), suggesting dietary MBA effectively alleviated the negative effects of toxin on serum antioxidant indices and protected animals from the oxidative stress. Meanwhile, the serum SOD activity was decreased ($P < 0.05$) and serum MDA level was increased ($P < 0.05$) in pregnant gilts fed with toxin diets compared with the pregnant gilts fed with no toxin diets. The addition of MBA had no effects ($P > 0.05$) on serum GSH-Px, SOD, and MDA.

3.3. Histopathology in organs and cell apoptosis

All measured organs (liver, kidney, spleen, uterus, ovary and mammary gland) in CO and COA groups had normal histological morphology with little lesions (Fig. 1). The liver, kidney, uterus, ovary, and mammary gland in MO group had moderate lesions, such as different degrees of swelling degeneration, inflammatory cell infiltration, bleeding, and necrosis. All the organs in MOA group showed lighter lesions compared with those in MO group. The total TUNNEL positive cells in spleen, breast, uterus, and ovary were increased ($P < 0.05$) in MO group compared with CO and MOA groups (Table 3), but no difference ($P > 0.05$) was observed in liver or kidney.

3.4. Residual ZEA in feces

No interactions ($P > 0.05$) were observed in the fecal residual ZEA among the dietary treatments. The residual ZEA content in feces of gilts fed ZEA-contaminated diets supplemented with MBA (MOA group) was lower ($P < 0.05$) than that of gilts fed ZEA contaminated diets (MO group), as shown in Table 4.

Table 2
Effect of *Bacillus subtilis* ANSB01G culture on serum antioxidant index of first-parity gilts when exposed to ZEA.[1]

Item	Dietary treatment[2]				Pooled SEM[3]	Source of variation (*P*-value)		
	CO	COA	MO	MOA		Main effect of toxins diets	Main effect of MBA level	Toxins diets × MBA level
GSH-Px, U/mL	2,077	2,023	1,948	2,123	84.32	0.36	0.35	0.15
SOD, U/mL	52.02^{b}	49.03^{b}	33.89^{a}	45.08^{b}	2.82	<0.01	0.16	<0.01
MDA, nmol/mL	1.83^{a}	1.80^{a}	2.95^{b}	2.40^{ab}	0.28	0.01	0.26	<0.01

ZEA = zearalenone; MBA = mycotoxin biodegradation agent; GSH-Px = glutathione peroxidase; SOD = superoxide dismutase; MDA = malondialdehyde. $^{a, b}$ Mean values within a row without a common superscript differ significantly ($P < 0.05$).

[1] Means represent 8 replications per treatment ($n = 8$/group).

[2] CO, a positive basal control diet contained 60.52% normal corn; COA, CO + MBA at 2 g/kg diet; MO, a negative basal control diet containing 60.52% moldy corn; MOA, MO + MBA at 2 g/kg diet.

[3] Pooled standard error of the means.

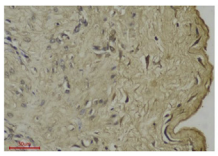
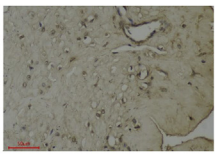
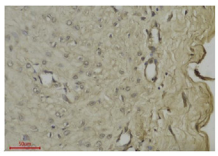
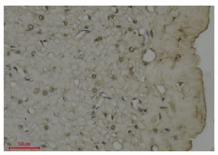
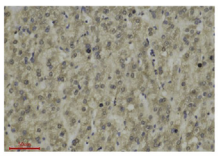
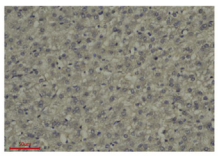
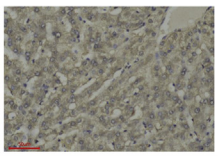
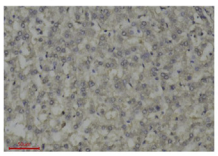
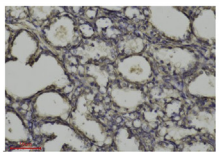
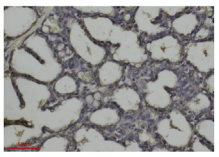
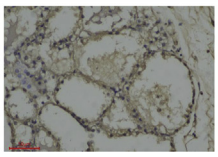
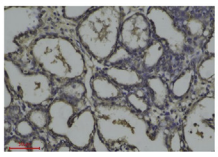
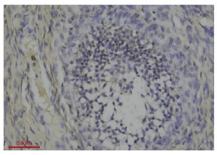
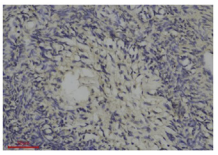
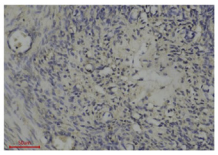
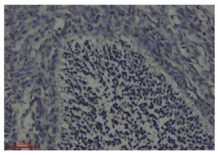
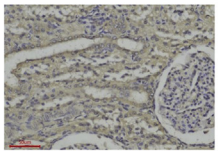
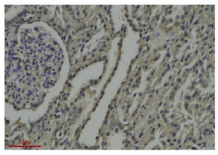
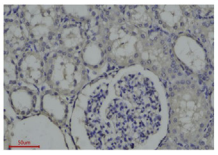
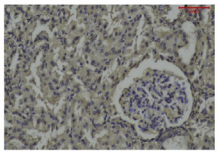
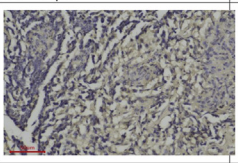
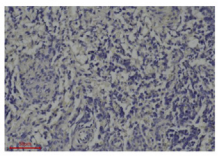
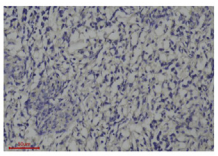
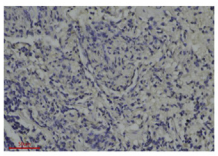
CO[1]	COA[1]	MO[1]	MOA[1]
			
Uterus: a small amount of inflammatory cell infiltration in the inner membrane; capillaries are mildly hyperemic or stagnant. Bar = 50 μm	Uterus: a small amount of inflammatory cell infiltration in the inner membrane; capillaries are mildly hyperemic or stagnant. Bar = 50 μm	Uterus: a large amount of inflammatory cell infiltration in the inner membrane; severe bleeding in lamina propria. Bar = 50 μm	Uterus: a small amount of inflammatory cell infiltration in the inner membrane; capillaries are mildly hyperemic or stagnant. Bar = 50 μm
			
Liver: cellular swelling like a balloon transformation. Bar = 50 μm	Liver: cellular swelling like a balloon transformation. Bar = 50 μm	Liver: cellular swelling like a balloon transformation; hepatic sinus. congestion. Bar = 50 μm	Liver: cellular swelling like a granular transformation. Bar = 50 μm
			
Mammary gland: a small amount of reticular secretion in the acinar cavity; glandular epithelial cells swell and become fatty; occasionally, glandular epithelial cells exfoliate and become necrotic. Bar = 50 μm	Mammary gland: a small amount of reticular secretion in the acinar cavity; glandular epithelial cells swell and become fatty. Bar = 50 μm	Mammary gland: a small amount of reticular or mass secretion in the acinar cavity; glandular epithelial cells swell and become fatty; many glandular epithelial cells disintegrate from myoepithelial cell; the glandular cavity contains exfoliated and necrotic glandular epithelial cells. Bar = 50 μm	Mammary gland: no secretions found in the acinar cavity; glandular epithelial cells swell slightly; a small amount of steatosis occurs. Bar = 50 μm
			
Ovary: normal structure; a small number of eosinophils; no obvious histopathological changes observed. Bar = 50 μm	Ovary: bleeding seen in the follicular membrane; a small number of eosinophils. Bar = 50 μm	Ovary: bleeding seen in the follicular membrane; a small number of eosinophils; necrosis in the follicular granulocytes. Bar = 50 μm	Ovary: bleeding seen in the follicular membrane; a small number of eosinophils. Bar = 50 μm
			
Kidney: renal tubular epithelial cells swell; vacuole denaturation; epithelial cells occasionally detached from the basement membrane; exserted in the renal tubule. Bar = 50 μm	Kidney: renal tubular epithelial cells swell; vacuole denaturation; epithelial cells occasionally detached from the basement membrane; exserted in the renal tubule. Bar = 50 μm	Kidney: severe swelling of renal tubular epithelial cells; steatosis; epithelial cells exfoliate and necrosis; silk reticular protein was found in renal tubules. Bar = 50 μm	Kidney: severe swelling of renal tubular epithelial cells; steatosis; epithelial cells exfoliate and necrosis; silk reticular protein was found in renal tubules. Bar = 50 μm
			
Spleen: the red and white pulp areas were clearly demarcated under the microscope; occasionally, a small amount of plasmocyte infiltration. Bar = 50 μm	Spleen: the red and white pulp areas were clearly demarcated under the microscope; occasionally, a small amount of plasmocyte infiltration. Bar = 50 μm	Spleen: the red and white pulp areas were clearly demarcated under the microscope; decreased lymphocyte number in the red medullary region; the lymphocytes sparsely arranged; occasionally, a small amount of plasmocyte infiltration. Bar = 50 μm	Spleen: the red and white pulp areas were clearly demarcated under the microscope; decreased lymphocyte number in the red medullary region; the lymphocytes sparsely arranged; occasionally, a small amount of plasmocyte infiltration. Bar = 50 μm

Fig. 1. Effect of *Bacillus subtilis* ANSB01G culture on histopathology in organs of first-parity gilts when exposed to zearalenone (ZEA). [1]CO, a positive basal control diet contained 60.52% normal corn; COA, CO + MBA at 2 g/kg diet; MO, a negative basal control diet contained 60.52% moldy corn; MOA, MO + MBA at 2 g/kg diet.

Table 3
Effect of *B. subtilis* ANSB01G culture on the total number of TUNEL positive cells in the image range of 3,072 × 2,048 under H&E 200× magnification.[1]

Item	Dietary treatment[2]				Pooled SEM[3]	Source of variation (*P*-value)		
	CO	COA	MO	MOA		Main effect of toxins diets	Main effect of MBA level	Toxins diets × MBA level
Liver	3.88	3.63	4.63	4.74	1.26	0.39	0.41	0.17
Kidney	5.88	5.50	7.50	6.63	2.64	0.44	0.16	0.26
Spleen	6.50^{a}	6.88^{a}	9.74^{b}	7.50^{a}	1.56	<0.01	0.30	<0.01
Mammary gland	3.50^{a}	3.88^{a}	5.63^{b}	4.00^{a}	1.29	<0.01	0.45	<0.01
Uterus	2.63^{a}	2.88^{a}	5.00^{b}	3.13^{a}	0.75	<0.01	0.12	<0.01
Ovary	3.50^{a}	3.50^{a}	7.00^{b}	4.50^{a}	1.30	<0.01	0.04	<0.01

MBA = mycotoxin biodegradation agent.
[a, b] Mean values within a row without a common superscript differ significantly ($P < 0.05$).
[1] Means represent 6 replications per treatment ($n = 8$/group).
[2] CO, a positive basal control diet contained 60.52% normal corn; COA, CO + MBA at 2 g/kg diet; MO, a negative basal control diet contained 60.52% moldy corn; MOA, MO + MBA at 2 g/kg diet.
[3] Pooled standard error of the means.

Table 4
Effect of *B. subtilis* ANSB01G culture on residual ZEA in feces of first-parity gilts exposed to ZEA.[1]

Item	Dietary treatment[2]				Pooled SEM[3]	Source of variation (*P*-value)		
	CO	COA	MO	MOA		Main effect of toxins diets	Main effect of MBA level	Toxins diets × MBA level
ZEA, μg/kg fresh feces	18.34^{a}	11.38^{a}	372.70^{c}	170.66^{b}	13.03	<0.01	0.16	0.21

ZEA = zearalenone; MBA = mycotoxin biodegradation agent.
[a,b] Mean values within a row without a common superscript differ significantly ($P < 0.05$).
[1] Means represent 8 replications per treatment ($n = 8$/group).
[2] CO, a positive basal control diet contained 60.52% normal corn; COA, CO + MBA at 2 g/kg diet; MO, a negative basal control diet contained 60.52% moldy corn; MOA, MO + MBA at 2 g/kg diet.
[3] Pooled standard error of the means.

4. Discussion

4.1. Serum antioxidant indices

Researches have indicated that oxidative stress plays critical roles in the cytotoxic mechanism of mycotoxins (Hassen et al., 2007). It has been reported that ZEA can induce oxidative stress, which can cause damages to organs including the liver and kidney with subsequent changes in some enzymatic parameters and cells including cleavages of proteins, endogenous DNA lesions and lipid peroxidation in gilts (Shi et al., 2018) and mouse (Hou et al., 2013). SOD and GSH-Px as the key enzymes of antioxidant system can scavenge free radicals generated from oxidant stress, reduce oxidative damage, and maintain cell structure. Under stress, the activity of SOD and GSH-Px was reduced, whereas the level of MDA in the serum was increased. Consequently, the activities of GSH-Px and SOD have been recognized as the leading parameters of anti-oxidative stress. In the current study, the toxin diets reduced serum SOD level and increased MDA level, while the addition of MBA counteracted these negative effects, suggesting that *B. subtilis* ANSB01G effectively relieved the ZEA toxicity on the body cell of pregnant sows. This result agreed with the results from Shi et al. (2018), who reported that the dietary ZEA and DON negatively affected the plasma antioxidant indices, and dietary MBA reduced the body oxidant damage induced by mycotoxin in gilts, but no interaction was found between toxin and MBA on these indices. Wang et al. (2012b) found feeding diets with ZEA (200 to 800 μg/kg) to weanling gilts decreased SOD and increased MDA in serum, while the addition of montmorillonite clay alleviated these adverse effects induced by ZEA. In the present study, significant interactions between toxins and MBA was observed in the serum antioxidant indices of pregnant gilts, besides, the addition of MBA counteracted the negative effects of ZEA on the antioxidant indices, suggesting that the disadvantageous effects of ZEA and the advantageous effects of MBA might be associated with the length of feeding period, the physiological status of animals, and even animal species and age (Ben et al., 2015; Ren et al., 2016).

4.2. Histopathology in organs

ZEA may cause organ and tissue dysfunctions, resulting in a disruption of homeostasis. Numerous studies indicated that ZEA caused the histological changes in the liver, lamina propria, intestine mucosa and ovary (Obremski et al., 2005; Przybylska-Gornowicz et al., 2015; Tiemann et al., 2006; Weaver et al., 2014). In agreement with the results observed in our previous study, we found moderate lesions with the subclinical inflammation, such as different degrees of swelling degeneration, inflammatory cell infiltration, bleeding and necrosis in the liver, kidney, uterus, ovary and mammary gland in gilts from MO group. The results confirmed that long-term feeding diet contaminated with a low level of ZEA damaged the metabolic and reproductive organs (Danicke and Winkler, 2015; Doll et al., 2014). In addition, some studies revealed that ZEA increased the size of the reproductive tract in prepubertal gilts and induced an apparent histological change in the ovary of immature gilts (Shi et al., 2018; Zwierzchowski et al., 2005). However, a study of evaluating the effect of low-dose ZEA on the histological structure of duodenum in gilts found that ZEA did not change the architecture of the duodenum mucosa including the thickness of the mucosa, the length of the villi and the ratio of villus height to crypt depth (Lewczuk et al., 2016). The results from this study showed that long-term feeding diets containing mycotoxins (ZEA level at 260 μg/kg) could cause tissue damages of pregnant gilts, but the damages could be alleviated substantially by adding MBA into diets, which was supported by the lighter lesions observed in the tissues from the gilts fed diets treated with yeast based feed additives (Weaver et al., 2014).

4.3. Cell apoptosis

In the present study, ZEA increased the total TUNNEL positive cells in the spleen, mammary gland, uterus and ovary, implying that the apoptotic process was accelerated in these organs or tissues, which was consistent with the changes in histopathology observed

in the ovary and uterus in the current study. Similarly, dietary ZEA up-regulated the expression of apoptotic protein caspase-3 in the ovary and uterus, but down-regulated the expression of anti-apoptotic protein anti-apoptosis (Bcl-2) in the ovary of gilts in our previous study (Shi et al., 2018). This implied that dietary ZEA could induce the apoptosis in ovary and uterus in gilts because the caspase-3 protein is the key protein in the execution, and the Bcl-2 plays a pivotal role in determining whether cells survive or die. The impacts of ZEA on apoptosis have been conformed in vitro in cell cultures (Kouadio et al., 2007). Furthermore, apoptosis in the ovary and the uterus might be related to hormonal secretion because ZEA in the diets could bind to oestrogen receptors and activate the transcription of oestrogen-responsive genes, thereby affects endocrine function (Brzuzan et al., 2015). Long-term exposure to ZEA in feed led to dysfunction of developing ovarian cells (Skorska-Wyszynska et al., 2004) due to disruptions in the physiological levels of steroid hormones and the activity of hydroxysteroid dehydrogenases (Zielonka et al., 2014). The addition of MBA appeared to reduce the total TUNNEL positive cells in the spleen, mammary gland, uterus and ovary compared with MO group, and alleviated the adverse effects of ZEA on apoptosis, which was in accord with our previous study that adding MBA in the diet contaminated with low levels of ZEA recovered the expression levels of the apoptosis-related proteins in ovaries and uterus in the immature gilts (Shi et al., 2018). Also Western blot analysis demonstrated that feeding ZEA to prepubertal gilts increased their protein expression of 3 alpha- or 3 beta-hydroxysteroid dehydrogenase, and that the addition of isoflavone to the ZEA-contaminated diet decreased the protein expression of 3 alpha- or 3 beta-hydroxysteroid dehydrogenase compared with the control group (Wang et al., 2013).

4.4. Residual ZEA in fresh feces

It was supposed that ZEA can be excreted very fast by urine with low residue in tissue (Obremski et al., 2003). However, several studies indicated that low ZEA residue could be accumulated in the gastrointestinal tissue and liver of gilts after weeks of exposure to a low dose of ZEA in diets (Gajecka et al., 2018; Zielonka et al., 2015). ZEA and its metabolites are predominantly excreted via the fecal route in the form of glucuronides due to an extensive biliary excretion. The occurrence of a remarkable enterohepatic cycling may explain the relatively long persistence of the mycotoxin and its derivatives in the body, particularly in pigs. Therefore, we paid close attention to the residual ZEA in feces in this study. As expected, a high residue of ZEA in feces was found in MO group, and the concentration of ZEA in MOA was decreased by 57% compared with MO group, indicating the biodegradation of ZEA occurred in the intestinal tract of pregnant gilts fed with MBA and the accumulation concentration of ZEA in the chyme and feces were relatively less than that in MO group (Shi et al., 2018; Zhao et al., 2015). The dietary supplementations with ZEA at 0.5 and 2.0 mg/kg caused an increase of ZEA residue level in the muscle and liver tissue of prepubertal gilts, and the addition of isoflavone to the ZEA-contaminated diet may accelerate the biotransformation and degradation of ZEA and its metabolites to reduce the residues of ZEA in the liver and muscle tissue of gilts (Wang et al., 2013).

5. Conclusions

In conclusion, a long-term dietary exposure to low doses of ZEA should be considered an important risk factor for subclinical inflammation in the organ and tissue of gestation gilts. Moreover, the supplementation of the *B. subtilis* ANSB01G culture in the contaminated diet could alleviate the oxidative stress, tissue damage and cell apoptosis induced by ZEA via degrading ZEA in the intestinal tract and decreasing ZEA absorption by intestine epitheliums.

Author contributions

Jianchuan Zhou: methodology, formal analysis, writing - original draft. Xiang Ao: writing - original draft. Yuanpei Lei: formal analysis. Cheng Ji: supervision. Qiugang Ma: conceptualization, validation, writing - review & editing, supervision, project administration.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgements

This study was supported by National Natural Science Foundation of China (Grant No. 31572447), Beijing Municipal Natural Science Foundation (Grant No. 6172017) and a Special Fund for Agro-scientific Research in the Public Interest (201403047). We thank the professional suggestions from Prof. Lihong Zhao during manuscript preparation.

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