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Original Research Article

Dietary supplementation of *Bacillus subtilis* PB6 improves sow reproductive performance and reduces piglet birth intervals

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ABSTRACT

We investigated the effects of dietary supplementation with *Bacillus subtilis* PB6 (*B. subtilis* PB6) during late gestation and lactation on sow reproductive performance, antioxidant indices, and gut microbiota. A total of 32 healthy Landrace × Yorkshire sows on d 90 of gestation were randomly assigned to 2 groups, with 16 replicates per group, receiving basal diet (CON) or the basal diet + 0.2% *B. subtilis* PB6, containing 4.0×10^8 CFU/kg of feed (BS). The litter sizes (total born) and numbers of piglets born alive were larger in the BS group ($P < 0.01$), whereas the weights of piglets born alive and the piglet birth intervals were lower in the BS group ($P < 0.05$). Although the litter weights and piglet bodyweights (after cross-fostering) were lower after BS treatment ($P < 0.05$), the litter sizes, litter weights, lactation survival rate, and litter weight gains at weaning were higher in BS group ($P < 0.05$). The concentrations of malondialdehyde (MDA) in the sow sera at parturition were lower in the BS group ($P < 0.01$). The serum total antioxidant capacity (T-AOC) at parturition and the serum catalase (CAT) concentrations on d 21 of lactation were higher in the BS group ($P < 0.05$). Dietary supplementation with *B. subtilis* PB6 ($P < 0.05$) reduced the serum endotoxin concentrations in the sows and the serum cortisol concentrations of the piglets at d 14 of lactation. The α-diversity indices of microbial were higher in the CON group ($P < 0.05$). At the phylum level, *B. subtilis* PB6 supplementation increased the relative abundances of Gemmatimonadete and Acidobacteria (both $P < 0.01$) and reduced those of Proteobacteria, and Actinobacteria (both $P < 0.05$). At the genus level, *B. subtilis* PB6 supplementation increased the relative abundance of Ruminococcaceae_UCG-013 cc ($P < 0.05$) and reduced that of *Streptococcus* ($P < 0.05$). This study demonstrated that adding 4.0×10^8 CFU/kg *B. subtilis* PB6 to sows' feed during late gestation and lactation could shorten piglet birth intervals, enhance the growth performance of suckling piglets, and improve the gut health of sows during late gestation.

1. Introduction

During late gestation and lactation, sows undergo stress from environmental and physical changes, including restricted feeding, housing changes and infections, and so forth (Kranendonk et al., 2007; Oliviero et al., 2010). The internal balance of the body is also broken, such as proinflammatory cytokines increase and anti-inflammatory cytokines decline during late gestation (Cheng et al., 2018). Furthermore, intestinal balance changes due to reduced intestinal bacterial diversity and increased inflammatory bacteria as the gestational age of the sows increases (Kong et al., 2017). The stress that sows experience changes dramatically during pregnancy

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and lactation, and these changes are harmful to the health of the sow. Even a long period of farrowing could reduce the productive performance of sows (Olivier et al., 2013), whereas nutrient absorption and metabolism during gestation and lactation may affect the weights of piglet at birth and weaning (Kranendonk et al., 2007). However, recent studies have indicated that the health and productivity of sows were improved when *Bacillus subtilis* was added to their feed during gestation and lactation.

B. subtilis is used as a growth promoter, enhancing sow reproductive performance and improving the viability of their progeny. Sow (from d 90 of gestation until postpartum d 21) fed *Bacillius*-based direct-fed (3.75×10^8 CFU/kg of feed) diets had more piglets and greater weaning weights of piglets (Baker et al., 2013). Hayakawa et al. (2016) demonstrated that compound probiotics containing a *Bacillius mesentericus* strain (2.0×10^8 CFU/kg of feed) improved the reproductive performance of sows (farrowing) and growth performance of piglets (weaning). However, Rychen et al. (2017) reported that adding *B. subtilis* PB6 (1.0×10^8 CFU/kg of feed) caused no improvement in the productive performance of sows when adding only 3 weeks before parturition.

B. subtilis PB6 used in this study was a natural strain isolated from the intestines of healthy chickens. It produces antimicrobial substances with broad activity against various strains of *Clostridium* sp. in necrotic enteritis in poultry and *Campylobacter* sp. in vitro (Teo and Tan, 2005), and also secretes substances that promote the growth of *Lactobacillus*. Besides, surfactin produced by *B. subtilis* PB6 is a cyclic lipopeptide antibiotic and biosurfactant, which has hemolytic, antibacterial properties (Heerklotz and Seelig, 2001; Jayaraman et al., 2013). *B. subtilis* PB6 has been used in broiler chickens and laying hens, and has improved intestinal health and eggshell quality respectively (Abdelqader et al., 2013; Jayaraman et al., 2013). Based on the effects of *B. subtilis* in various animals and the obvious effects of *B. subtilis* PB6 in broiler chickens and laying hens, we undertook to verify the effects of *B. subtilis* PB6 on sows.

The purpose of this study was to investigate the effects of *B. subtilis* PB6 supplementation during late gestation and lactation on the reproductive performance, antioxidation indices, and intestinal microbial composition on sows.

2. Materials and methods

The protocol of this study was approved by the Animal Care and Use Committee of Animal Nutrition Institute, Sichuan Agricultural University, and the study was performed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

2.1. Experimental design and animals

This experiment was performed at a commercial pig farm in Sichuan Province, China. A total of 32 mixed-parity Landrace × Yorkshire sows with parity of 2.47 ± 0.50 (mean ± SD) and backfat (BF) thickness of 14.72 ± 1.30 mm, which were bred with the semen of a pool of Landrace boars, were selected. On d 90 of gestation, the sows were randomly assigned to 1 of 2 groups according to their parity and BF, with 16 replicates per group. The dietary treatments included a basal gestation and lactation diet (CON; Table 1) and the same basal diet supplemented with 0.2% *B. subtilis* PB6 (BS; containing *B. subtilis* 4.0×10^8 CFU/kg of feed; Table 1) from d 90 of gestation to weaning on d 21 of lactation. The *B. subtilis* PB6 strain was provided by Kemin Industries, Kemin (Zhuhai, China) Technologies Co., Ltd, and contained a *B. subtilis* concentration of 2.0×10^8 CFU/g of product. It had been isolated from the intestines of healthy chickens and was shown to inhibit *Clostridium perfringens*. All the sows were fed 2.80 kg of the experimental diet from d 90 of gestation to parturition. During gestation, all the sows were housed in individual stalls and fed the gestation-period diet twice a day (08:00 and 15:00), with access to water ad libitum throughout the study. On d 110 of gestation, the sows were moved to the farrowing room. The day of parturition was defined as d 0 of lactation, and the piglets were weaned on d 21 of lactation.

Table 1
Ingredients and chemical compositions of basal diets (as-fed basis, %).

Item	Gestation	Lactation
Ingredients		
Yellow corn	33.58	40.08
Wheat	20	28
Soybean meal (43% CP)	14.5	18.2
Fish meal (67% CP)	0	2
Expanded soybean	0	5
Wheat bran	8	0
Soybean hulls	18	0
L-Lys HCl (98%)	0.03	0.28
L-Thr (98.5%)	0	0.1
DL-Met (99%)	0	0.03
Limestone	1.4	1.3
Dicalcium phosphate	1.2	1.2
Choline chloride (50%)	0.15	0.15
Sodium chloride	0.4	0.4
Vitamin-mineral premix[1]	2.74	3.26
Total	100	100
Nutrient composition		
Digestible energy, MJ/kg	11.92	13.39
Crude protein	15.03	18.76
Crude fiber	8.75	2.48
Calcium	1.09	1.08
Total phosphorus	0.63	0.69
Available phosphorus	0.38	0.43
Total lysine	0.73	1.11

[1] The vitamin-mineral premix provided the following per kilogram of basal diet: 8,000 IU vitamin A, 2,000 IU vitamin D_3, 12.5 IU vitamin E, 2.5 mg vitamin K, 0.2 mg biotin, 0.25 mg folic acid, 17.5 mg niacin, 12.5 mg pantothenic acid, 8.0 mg riboflavin, 1.0 mg thiamin, 3.00 mg vitamin B_6, 15 μg vitamin B_{12}, 16 mg copper, 0.3 mg iodine, 165 mg iron, 30 mg manganese, 0.3 mg selenium, and 165 mg zinc. The sources of the trace elements were $CuSO_4 \cdot 5H_2O$, KI, $FeSO_4$, $MnSO_4 \cdot H_2O$, Na_2SeO_3, and $ZnSO_4$.

At farrowing, the numbers of piglets born alive, stillborn, and mummified and the birthweights of the piglets born alive were recorded. Based on the number of effective teats on the sows, the litters were standardized to approximately 12 piglets per sow within 24 h after birth by cross-fostering within the treatment group. The piglets were weighed after the standardization of the litters and at weaning, and underwent routine processing procedures (ear notching, tail docking, castration, and supplemental iron injection) within 3 d of farrowing. The feed allowance was progressively increased stepwise by 1.0 kg/d from 1.0 kg on d 0 of lactation to their maximum feed intake, and then the sows were allowed free access to feed until d 21 of lactation (weaning). The feed allocation and refusals were recorded daily. The sows were fed the lactation period diet 4 times a day (at 08:00, 11:00, 15:00, and 20:00) during lactation. The piglets also had free access to water during the lactation, but had no access to creep feed. The temperature of the environment in the farrowing house was maintained at 20 to 25 °C. The temperature of the insulation boards was maintained at 30 to 32 °C, and was reduced as the neonatal piglet age increased.

2.2. Sample collection

The BF thickness was measured at 65 mm to the left side of the dorsal midline at the last rib level, using ultrasound (Renco Lean-

Meatier; Renco Corp., Minneapolis, MN) and recorded on d 89 of gestation and d 1 and 21 of lactation. The total litter sizes were calculated as the sum of the numbers of live-born piglets, stillborn piglets, and mummified piglets. At farrowing, the birth times of the first and last piglets (born alive, stillborn, or mummified) were recorded, and the difference was defined as the duration of farrowing. Piglet birth interval was calculated as the duration of farrowing divided by the total litter size. Fasting blood samples (10 mL) were collected from the sows via the marginal ear vein at farrowing (d 0 of lactation) and on d 14 and 21 of lactation, before the morning meal. Blood samples were collected from the piglets via the anterior vena cava at 14 and 21 d of age. All blood samples were collected into vacuum tubes (5 mL; Jiangsu Yu Li Medical Instrument Co., Ltd, Jiangsu, China). The samples were immediately placed on ice and then centrifuged at 3,000 × *g* for 10 min at room temperature. The serum was stored at −20 °C.

Colostrum samples (30 mL) were collected from each sow before any piglets had sucked, and milk samples (30 mL) were obtained from each sow on d 14 of lactation. Briefly, the piglets were separated from their dams and the udders were cleaned with water, and then 2 mL of oxytocin was injected into the ear vein of each sow. Each sample was a mixture of milk from the anterior, middle, and posterior functional glands and was collected by hand milking. Six samples were collected in each treatment group. The colostrum and milk samples were centrifuged at 3,000 × *g* for 15 min at room temperature. All samples were refrigerated at −20 °C before subsequent analysis.

Fresh feces samples from the sows were collected into sterile tubes. Five samples were collected in each treatment group, and immediately frozen in liquid nitrogen, then transferred to a freezer at −80 °C on d 110 of gestation.

2.3. Milk composition analysis

The frozen colostrum and milk samples were thawed at 4 °C, and 15 mL of each sample were used for analyzing the milk fat, protein, and lactose content with an ultrasonic milk analyzer (Milkyway-CP2; Hangzhou Simple Technology Co., Ltd, Hangzhou, China).

2.4. Oxidant and antioxidant content analyses

The content of malondialdehyde (MDA), the total antioxidant capacity (T-AOC), and the activities of glutathione peroxidase (GSH-Px) and catalase (CAT) were assessed in the sera of sows with specific assay kits (Catalog, A003-1-2, A015-2-1, A006-2-1, A007-1-1; Nanjing Institute of Jiancheng Biological Engineering, Nanjing, China). MDA was quantified with thiobarbituric acid reactive substances (TBARS). T-AOC and the CAT and GSH-Px activities were measured according to a previous study (Wang et al., 2016; Mou et al., 2017).

2.5. Cortisol and endotoxin assays

The endotoxin concentrations in the sow sera and the cortisol concentrations in the piglet sera were determined with respective commercial ELISA kits (Catalog, NO.H094, H255; Nanjing Institute of Jiancheng Biological Engineering). The limits for the determination of the cortisol and endotoxin concentrations were 5.0 ng/mL and 3 EU/mL, respectively. The intra- and inter-assay coefficients of variation were all <10% and <12% for cortisol and endotoxin assays, respectively.

2.6. Microbial analyses

The total bacterial DNA in each fecal sample from the CON ($n = 5$) and BS groups ($n = 5$) was extracted on d 110 of gestation with the MO BIO Power Fecal DNA Isolation Kit (MO BIO Laboratories, Inc.) according to the manufacturer's protocol. Before sequencing, the concentration and purity of the extracted genomic DNA were measured. The integrity of the extracted genomic DNA was determined by electrophoresis on a 1% (wt/vol) agarose gel. The DNA was diluted to 1 ng/μL with sterile water. The extracted fecal DNA samples were sent to Novogene Bioinformatics Technology (Beijing, China) for amplicon pyrosequencing on the Illumina HiSeq PE250 platforms. The V4 hypervariable region of the 16S rRNA gene was amplified with the 515F and 806R primers (5′-GTGCCAGCMGCCGCGGTAA-3′ and 5′-GGACTACHVGGGTWTCTAAT-3′). The raw paired-end reads obtained with Illumina HiSeq sequencing were spliced. The spliced sequences were called "raw tags." The raw tags were quality filtered under specific filtering conditions to obtain high-quality clean tags (Bergmark et al., 2012), according to the QIIME (V1.7.0, http://qiime.org/index.html; Caporaso et al., 2010) quality-controlled process. Chimeric filtering was then performed to obtain the effective tags (Fig. 1A). The effective tags were assigned to operational taxonomic units (OTU) using the Uparse software (v7.0.1001 http://drive5.com/uparse/) with 97% sequence similarity. A representative sequence of each OTU was screened for further annotation. The Ribosomal Database Project Classifier version 2.2 used to assign a taxonomic rank to each representative sequence. OTU abundance information was normalized with a standard sequence number corresponding to the sample with the least sequences. Subsequent analysis of α-diversity and β-diversity was based on these normalized output data. The relative abundance of each OTU was examined at different taxonomic levels. At the phylum level, because the sum of the 10 phyla with the greatest relative abundances exceeded 98%, we selected these top 10 phyla for statistical analysis, using the CON group as a reference. At the genus level, we selected those genera with relative abundances ≥0.1% in any samples for statistical analysis.

2.7. Statistical analysis

The original data were checked with Grubbs' test. If $|Xp - X| > \lambda(\alpha, n)$ S, Xp was considered an outlier. The data were tested for homogeneity of variance and a normal distribution with the Shapiro–Wilk method in SAS 9.4 (SAS Institute Inc., Cary, NC) before the parametric analyses. Statistical analyses were performed with the *t*-test procedure in SAS 9.4. Data on the relative abundances of the gut microbiota were analyzed with the Glimmix procedure in SAS 9.4. Differences between means in all statistical analyses were considered statistically significant at $P < 0.05$, and tended to be significant at $0.05 \leq t$, $P < 0.10$.

3. Results

3.1. Reproductive performance of sows at farrowing

The effects of *B. subtilis* PB6 on the reproductive performance of the sows are presented in Table 2. Litter sizes (total born) and numbers of piglets born alive were highly significant greater in the BS group than those in the CON group ($P < 0.01$), whereas the weight of per piglet born alive ($P < 0.01$) and the piglet birth interval ($P = 0.022$) were lower in the BS group than those in the CON group. The duration of farrowing tended to be shorter after *B. subtilis* PB6 supplementation ($P = 0.092$).

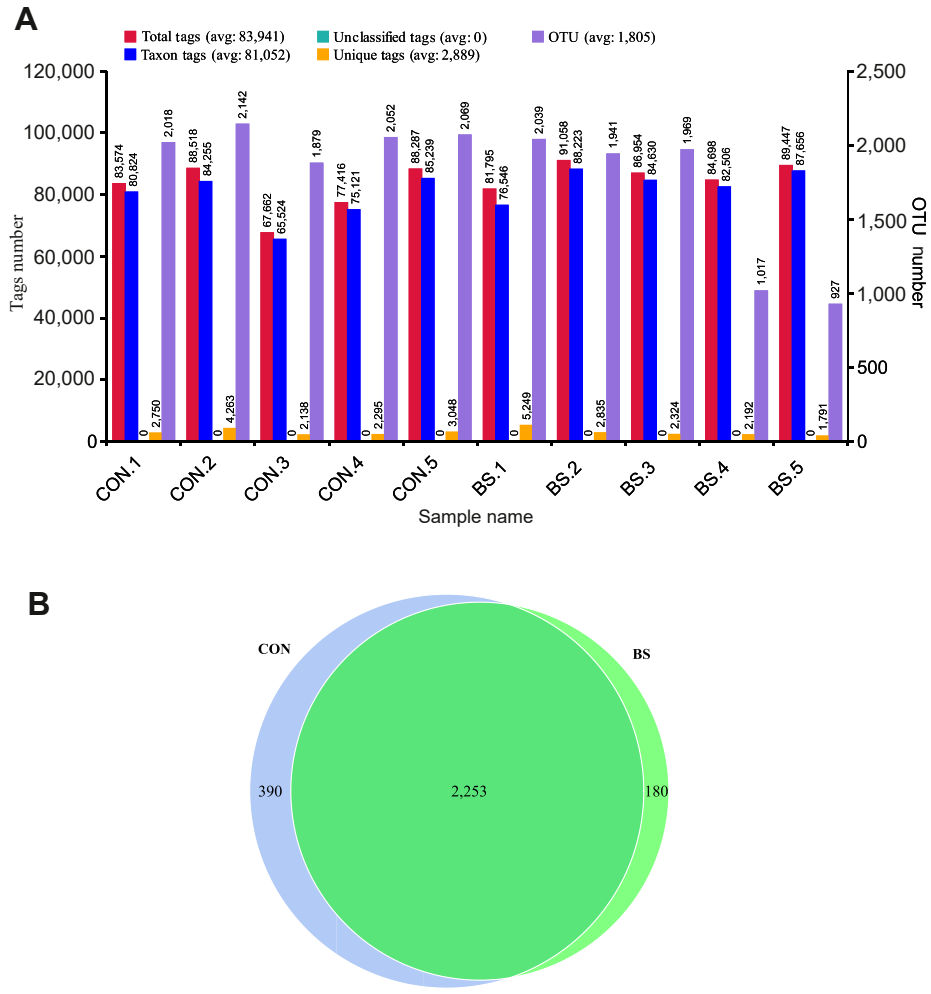

Fig. 1. Effect of *Bacillus subtilis* PB6 supplementation of sows on the fecal microbiota on d 110 of gestation. (A) operational taxonomic units (OTU) clustering and annotation per sample; (B) Venn diagram of OTU. CON, basal diet treatment during gestation; BS, basal diet + 0.2% *B. subtilis* PB6 treatment during gestation.

Table 2
Reproductive performance of sows at farrowing[1].

Item	CON[2]	BS[2]	*P*-value
Parity	2.50 ± 0.13	2.44 ± 0.13	0.734
Litter size (total born)	13.13 ± 0.53	14.94 ± 0.36	<0.01
Number of piglets born alive	11.63 ± 0.64	14.00 ± 0.32	<0.01
Born alive rate, %	88.29 ± 2.89	93.97 ± 1.66	0.098
Number of stillborn piglets	0.81 ± 0.29	0.63 ± 0.21	0.601
Stillborn rate, %	6.17 ± 8.75	4.04 ± 5.13	0.409
Number of mummies	0.69 ± 0.22	0.31 ± 0.12	0.142
Mummies rate, %	5.57 ± 1.92	2.01 ± 0.78	0.101
Litter weight at parturition, kg	18.16 ± 1.12	19.00 ± 0.74	0.539
Born alive weight per piglet, kg	1.56 ± 0.05	1.35 ± 0.04	<0.01
Duration of farrowing, min	255.60 ± 18.98	218.81 ± 8.54	0.092
Piglet birth interval, min	21.65 ± 2.55	15.03 ± 0.58	0.022

[1] Values are means ± SEM.
[2] CON, basal diet treatment; BS, basal diet + 0.2% *B. subtilis* PB6 treatment.

3.2. Reproductive performance of sows during lactation

As shown in Table 3, whereas litter weights ($P = 0.035$) and piglet bodyweights ($P = 0.026$) by cross-fostering were lower in the BS group than those in the CON group (litters were standardized to approximately 12 piglets per sow by cross-fostering within the treatment groups), the litter sizes, litter weights, lactation survival rate, and litter weight gains at weaning were significantly increased by supplementation with *B. subtilis* PB6 ($P < 0.05$).

3.3. Composition of colostrum and milk

As shown in Table 4, the fat content of the colostrum tended to be higher ($P = 0.090$) after *B. subtilis* PB6 supplementation, whereas the lactose and protein content of the milk did not differ between the 2 groups (both $P > 0.05$).

3.4. Oxidative and antioxidative indicators in the sera of sows

As shown in Table 5, the MDA concentrations of the sow sera were highly significant lower in the BS group than those in the CON group at parturition ($P = 0.004$).

As shown in Table 6, T-AOC of the sow sera at parturition ($P = 0.044$) and the CAT activities in the sow serum on d 21 of lactation ($P = 0.014$) were higher in the BS group than those in the CON group. The activity of GSH-Px did not differ between the 2 groups ($P > 0.05$).

3.5. Endotoxin and cortisol in the sera of sows and piglets

As shown in Fig. 2A, the endotoxin concentrations in the sow sera on d 14 of lactation were highly significant lower in the BS

Table 3
Growth performance of suckling piglets, feed intake, backfat (BF) thickness, and BF loss in sows during lactation[1].

Item	CON[2]	BS[2]	*P*-value
Litter size by cross-fostering	12.31 ± 0.33	12.31 ± 0.22	1.000
Litter size at weaning	9.56 ± 0.39	10.88 ± 0.20	<0.01
Lactation survival rate, %	78.13 ± 3.12	88.63 ± 1.99	<0.01
Litter weight by cross-fostering, kg	19.03 ± 0.75	17.09 ± 0.43	0.035
Litter weight at weaning, kg	57.92 ± 4.07	69.04 ± 1.48	0.019
Litter weight gain, kg	38.89 ± 4.17	51.95 ± 1.68	<0.01
Piglet body weight by cross-fostering, kg	1.55 ± 0.05	1.40 ± 0.04	0.026
Piglet body weight at weaning, kg	6.03 ± 0.30	6.37 ± 0.15	0.331
ADG, g/d	213.6 ± 15	236.8 ± 6.9	0.174
Feed intake during lactation, kg/d	5.77 ± 0.15	5.76 ± 0.10	0.956
BF thickness at d 89, mm	15.32 ± 0.37	14.63 ± 0.26	0.139
BF thickness at farrowing, mm	16.06 ± 0.47	15.44 ± 0.22	0.242
BF thickness at weaning, mm	13.13 ± 0.50	12.50 ± 0.20	0.260
BF loss during lactation, mm	2.94 ± 0.23	2.94 ± 0.19	1.000

[1] Values are means ± SEM.
[2] CON, basal diet treatment; BS, basal diet + 0.2% *B. subtilis* PB6 treatment.

Table 4
Effect of *Bacillus subtilis* PB6 supplementation during gestation and lactation on compositions of sows' colostrum and milk[1].

Item	CON[2]	BS[2]	*P*-value
Colostrum, g/kg			
Fat	50.93 ± 2.43	66.15 ± 7.15	0.090
Protein	96.58 ± 5.63	95.50 ± 5.71	0.895
Lactose	34.98 ± 3.89	34.52 ± 3.71	0.933
Milk, g/kg			
Fat	62.83 ± 7.39	70.28 ± 6.89	0.478
Protein	53.30 ± 1.76	53.07 ± 1.66	0.925
Lactose	49.00 ± 2.82	46.72 ± 2.91	0.544

[1] Values are means ± SEM, $n = 6$ per treatment.
[2] CON, basal diet treatment; BS, basal diet + 0.2% *B. subtilis* PB6 treatment.

group than those in the CON group ($P = 0.007$), and the endotoxin concentrations in the sow sera on d 21 of lactation tended to be lower in the BS group ($P = 0.059$). In Fig. 2B, the cortisol concentrations in the piglet sera were significantly lower at 14 d of age in the BS group than those in the CON group ($P = 0.042$).

3.6. Fecal microbiota

As shown in Fig. 1A, a total of 839,409 effective tags were obtained from all the feces samples, ranging from 67,662 to 91,058 per sample. In total, 810,524 OTU (at the 97% identity level) were detected in all the samples, with an average of 1,805.3 ± 445.6 per sample. A Venn diagram was used for evaluating the distributions of the OTU in the 2 groups. Based on this analysis, a total of 2,253 OTU were shown in both groups (Fig. 1B). The α-diversity and β-diversity of a microbial community reflected its richness and diversity, respectively. The α-diversity indices investigated were the numbers of Observed species, Shannon's index, and the abundance-based coverage estimator (ACE) (Fig. 3). The magnitude of the change in β-diversity was compared with weighted and unweighted UniFrac distances (Fig. 4) (Lozupone et al., 2006).

As shown in Fig. 3, Shannon's index was lower in the BS group than in the CON group ($P = 0.036$). The number of Observed species also tended to be higher in the CON group than in the BS group ($P = 0.099$).

The relative abundances of the 10 most abundant phyla are shown in Fig. 5. These results suggested that at the phylum level, the major proportion of sequences attributable to the phyla were Firmicutes (64.74%) and Bacteroidetes (16.22%), followed by Proteobacteria (4.64%), Spirochaetes (3.89%), and Tenericutes (3.41%). The relative abundances of Gemmatimonadete ($P = 0.001$) and Acidobacteria ($P = 0.003$) were significantly increased in the BS group, whereas those of Proteobacteria ($P = 0.017$) and Actinobacteria ($P = 0.004$) were significantly reduced. At the genus level, the relative abundances of 32 genera were ≥0.1% in any samples. *B. subtilis* PB6 increased the relative abundance of Ruminococcaceae_UCG-013 cc ($P = 0.040$) and reduced that of *Streptococcus* ($P = 0.030$) (Table 7).

Table 5
Effect of *Bacillus subtilis* PB6 supplementation during late gestation and lactation on MDA concentrations in sera of sows (nmol/mL)[1].

Item	CON[2]	BS[2]	*P*-value
At parturition	7.16 ± 0.77	4.07 ± 0.27	0.004
Day 14 of lactation	6.05 ± 0.62	4.72 ± 0.50	0.128
Day 21 of lactation	4.66 ± 0.46	4.94 ± 0.60	0.721

[1] Values are means ± SEM, $n = 6$ per treatment.
[2] CON, basal diet treatment; BS, basal diet + 0.2% *B. subtilis* PB6 treatment.

4. Discussion

In this study, we concentrated on the effects of *B. subtilis* PB6 supplementation to sow diets in late gestation and lactation on their reproductive performance.

Table 6
Effect of *Bacillus subtilis* PB6 supplementation during late gestation and lactation on the antioxidant capacity in the sera of sows[1].

Item	CON[2]	BS[2]	*P*-value
T-AOC, active U/mL			
At parturition	8.65 ± 1.51	15.68 ± 2.65	0.044
Day 14 of lactation	10.16 ± 1.55	15.39 ± 4.29	0.292
Day 21 of lactation	7.08 ± 1.93	10.92 ± 2.38	0.238
CAT, active U/mL			
At parturition	1.95 ± 0.31	2.24 ± 0.28	0.509
Day 14 of lactation	2.02 ± 0.29	1.96 ± 0.31	0.884
Day 21 of lactation	1.27 ± 0.18	2.36 ± 0.31	0.014
GSH-Px, active U/mL			
At parturition	763.67 ± 32.89	780.17 ± 49.47	0.787
Day 14 of lactation	1,000.17 ± 33.15	924.33 ± 38.68	0.168
Day 21 of lactation	1,003.17 ± 50.00	1,024.33 ± 40.81	0.750

[1] Values are means ± SEM, $n = 6$ per treatment.
[2] CON, basal diet treatment; BS, basal diet + 0.2% *B. subtilis* PB6 treatment.

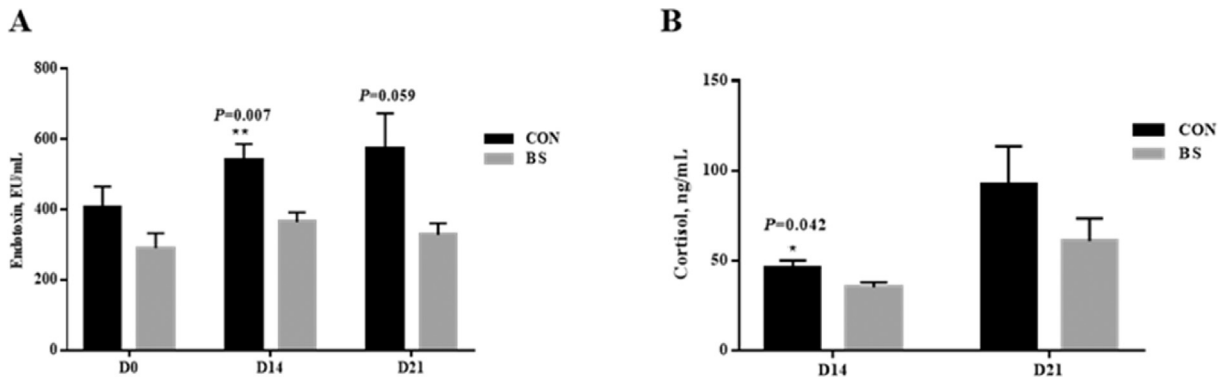

Fig. 2. Effect of *Bacillus subtilis* PB6 supplementation during late gestation and lactation on endotoxin concentrations in the sera of sows and the cortisol concentrations in the sera of piglets. (A) Endotoxin concentrations in sow serum. (B) Cortisol concentrations in piglet sera. CON, basal diet treatment during lactation; BS, basal diet + 0.2% *B. subtilis* PB6 treatment during lactation. D 0 = at parturition; D 14 = d 14 of lactation; D 21 = d 21 of lactation. Data are expressed as means ± SEM; $n = 6$ for each treatment. *, $P < 0.05$; **, $P < 0.01$.

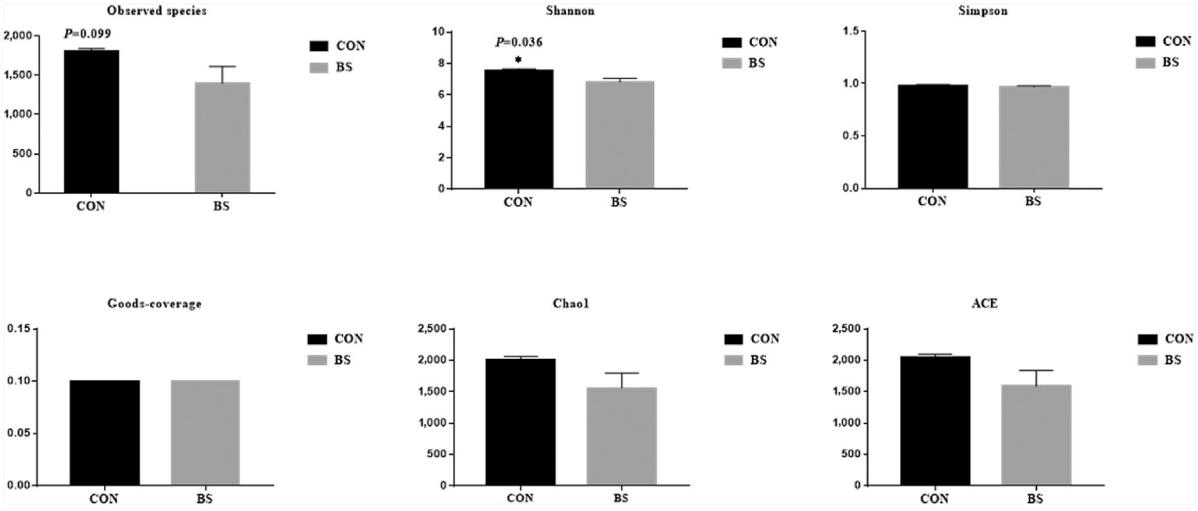

Fig. 3. Effect of *Bacillus subtilis* PB6 supplementation of sows on the α-diversity of their gut microbial communities on d 110 of gestation. CON, basal diet treatment during gestation; BS, basal diet + 0.2% *B. subtilis* PB6 treatment during gestation. Data are expressed as means ± SEM; $n = 5$ for each treatment. *, $P < 0.05$.

4.1. Piglets status at farrowing

Although the litter sizes (total born) were larger in the BS group, this difference was not attributable to *B. subtilis* PB6 supplementation, since litter sizes were determined by conception rate in early pregnancy that occurred before treatment because the *B. subtilis* PB6 started adding from d 90 of gestation (Böhmer et al., 2006; Baker et al., 2013). It was likely that random errors caused such a result when we selected the sows.

The weight of each piglet born alive in the BS group was smaller than in the CON group, but the litter sizes in the BS group were higher than those in the CON group. Previous study showed that there was a negative linear correlation between litter sizes and piglet weights due to a uterine constraint on prenatal piglet growth when more embryos competed for uterine resources (Kerr and Cameron, 1995; Wolf et al., 2008). Hence, the smaller weight of each piglet born alive could be explained by the larger litter sizes in BS group, although there could be a little effect when *B. subtilis* PB6 supplied in late gestation owing to restricted feed intake (Wu et al., 2006; Campos et al., 2012). The same result also showed in previous studies. Rychen et al. (2017) reported that the piglet weight at birth in the *B. subtilis* group was smaller than that in the control group when sows were fed 1.0×10^8 CFU/kg *B. subtilis* PB6 in their feed from d 90 of gestation until weaning. The number of born alive was larger in BS group than in CON group, which could be explained that supplementation of *B. subtilis* PB6 declined the transformation of prenatal piglet to mummy in late gestation. This result was consistent with a previous study that supplementation of *B. subtilis* to sows increased the numbers of live births at farrowing (Baker et al., 2013).

4.2. Duration of farrowing and piglet birth interval

An ever-increasing number of studies have shown that a long period of farrowing could affect the health of the sow until early lactation (Martineau et al., 1992; Herpin et al., 1996; Dijk et al., 2005). Previous studies have also indicated that large litters, large numbers of live-born piglets, and high birthweights could increase the duration of farrowing (Rens and Lende, 2004), and that high birthweights could extend the piglet birth interval (Motsi et al., 2006). In this study, the piglet birth interval was reduced by *B. subtilis* PB6 supplementation, and the duration of farrowing tended to be shorter in the BS group than in the CON group, whereas the total piglets born and the numbers born alive were larger in the BS group than in the CON group. This differed, in part, from the results of Van Rens and Van der Lende (2004). *B. subtilis*

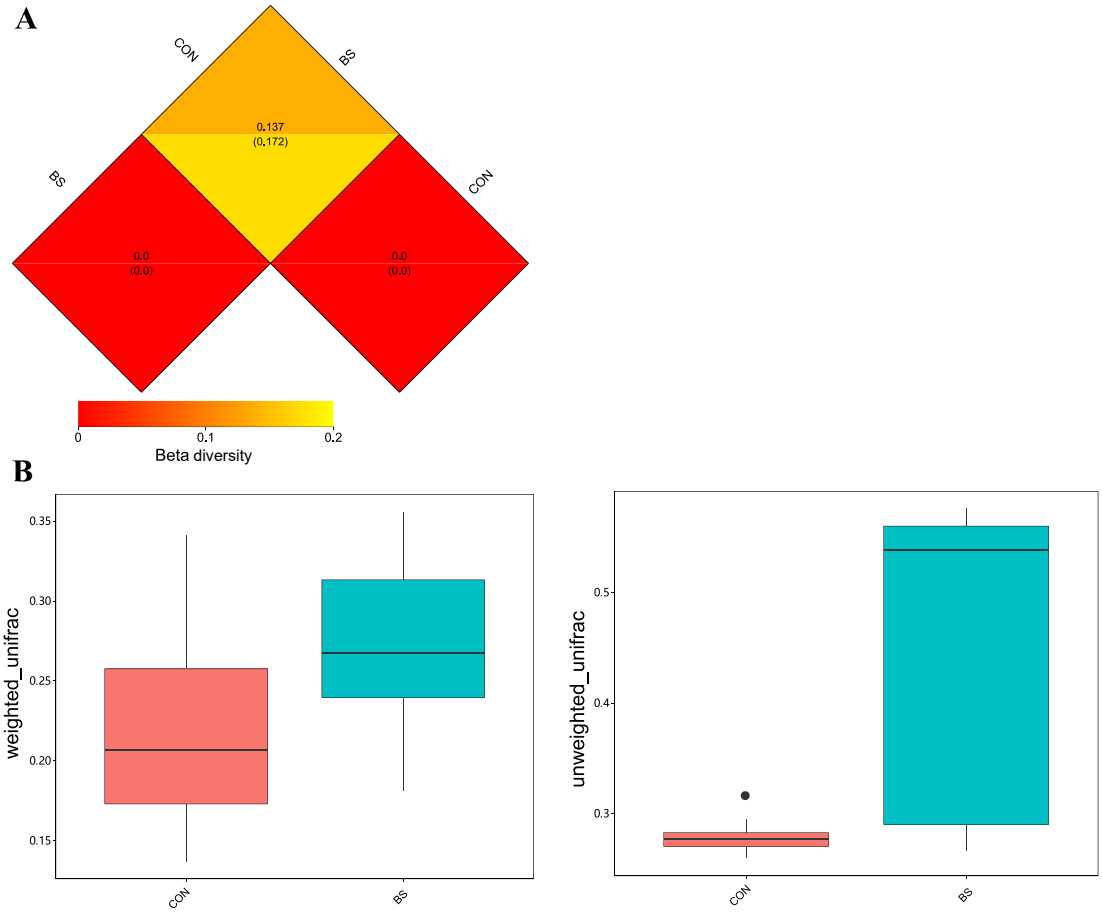

Fig. 4. Effect of *Bacillus subtilis* PB6 supplementation of sows on the β-diversity of their gut microbial communities on d 110 of gestation. (A) Distance matrix heatmap of β-diversity. Upper and lower numbers in the grid represent the weighted UniFrac and unweighted UniFrac distances, respectively. (B) The weighted UniFrac and unweighted UniFrac distances in β-diversity in the 2 groups. Data are expressed as means ± SEM; $n = 5$ for each treatment. CON, the basal diet treatment during gestation; BS, the basal + 0.2% *B. subtilis* PB6 treatment during gestation. The dot on the bar of the control in Fig. 4B (right) represents a outlier in control group.

PB6 may have played an important role. That study showed that the antioxidant capacity of sows was improved by the addition of *B. subtilis* PB6. Moreover, our results (Table 3) indicated that *B. subtilis* PB6 fed to sows increased their capacity for breastfeeding.

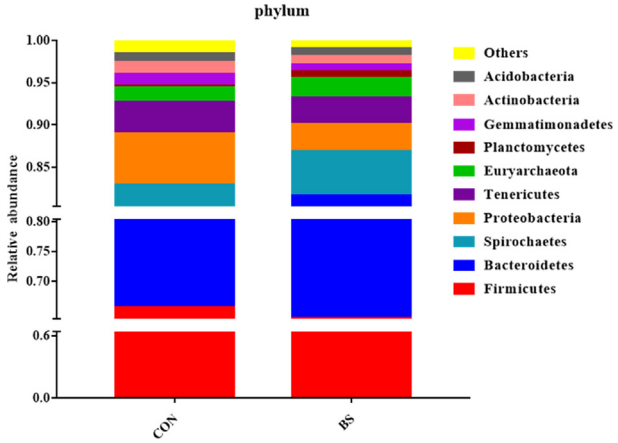

Fig. 5. Relative abundances of the fecal microbiota at the phylum level. This figure shows the relative abundances of the top 10 phyla in the fecal microbiota. Data are expressed as means ± SEM; $n = 5$ for each treatment. CON, the basal diet treatment during gestation; BS, the basal + 0.2% *B. subtilis* PB6 treatment during gestation.

We inferred that the sows and piglets had better physical strengths at farrowing due to the improvement digestibility of sow nutrients when the sows were supplied with *B. subtilis* PB6, although we did not measure nutrient digestibility. We made this inference based on the study of Patarapree et al. (2018), who showed that *B. subtilis* improved the digestion and utilization of nutrition in grower period of piglets.

4.3. Growth performance of piglets after cross-fostering

There were initial differences in litter weights and average piglet bodyweights by cross-fostering. This could be explained by the following factors. First, based on the number of effective teats on the sows, the litters were standardized to approximately 12 piglets per sow within 24 h after birth (cross-fostering) within the treatment groups; Second, there were no differences in body-weight per litter within each group, but the bodyweights were smaller in BS group than in the CON group. Finally, we tried to keep the piglets with their maternal sows. Although cross-fostering caused such a different beginning, we still wanted to continue the experiment because we wanted to see if the addition of *B. subtilis* PB6 improved the growth performance of the piglets on the premise.

The weaning weights of the piglet litters and the litter weight gains were higher when sows were supplemented with *B. subtilis*

Table 7
Effect of *Bacillus subtilis* PB6 supplementation of sows on the relative abundances in their microbial communities[1] at the genus levels (≥0.1% in any samples; the raw data have been changed to $\log_{10}$ values) on d 110 of gestation.

Item	CON[2]	BS[2]	*P*-value
Lactobacillus	0.59 ± 0.18	0.86 ± 0.19	0.342
Clostridium_sensu_stricto_1	0.93 ± 0.10	0.80 ± 0.07	0.309
Treponema_2	0.29 ± 0.15	0.57 ± 0.19	0.259
Terrisporobacter	0.80 ± 0.07	0.88 ± 0.06	0.372
Lachnospiraceae_XPB1014_group	0.59 ± 0.08	0.56 ± 0.09	0.802
Streptococcus	0.14 ± 0.23	−0.52 ± 0.11	0.030
Romboutsia	0.29 ± 0.07	0.44 ± 0.09	0.189
Turicibacter	0.32 ± 0.07	0.30 ± 0.10	0.882
Ruminococcaceae_UCG-005	0.42 ± 0.07	0.38 ± 0.06	0.707
Ruminococcaceae_UCG-002	0.47 ± 0.06	0.43 ± 0.08	0.729
Methanobrevibacter	0.00 ± 0.25	0.29 ± 0.17	0.367
Ruminococcaceae_NK4A214_group	0.50 ± 0.04	0.42 ± 0.05	0.256
Sarcina	−0.28 ± 0.16	−0.15 ± 0.22	0.660
Prevotellaceae_NK3B31_group	0.02 ± 0.20	0.29 ± 0.14	0.303
Rikenellaceae_RC9_gut_group	0.21 ± 0.10	0.25 ± 0.06	0.715
Christensenellaceae_R-7_group	0.22 ± 0.03	0.26 ± 0.04	0.448
Ruminococcaceae_UCG-014	0.06 ± 0.06	−0.01 ± 0.11	0.560
Lachnospiraceae_AC2044_group	−0.15 ± 0.09	−0.20 ± 0.14	0.813
[Eubacterium]_coprostanoligenes_group	0.09 ± 0.08	−0.02 ± 0.02	0.230
Prevotellaceae_UCG-003	−0.44 ± 0.14	−0.28 ± 0.13	0.421
Desulfovibrio	−0.13 ± 0.07	−0.36 ± 0.08	0.058
Ruminococcus_1	−0.18 ± 0.05	−0.37 ± 0.11	0.161
Phascolarctobacterium	−0.39 ± 0.11	−0.63 ± 0.02	0.053
Ruminococcaceae_UCG-013 cc	−0.24 ± 0.07	−0.48 ± 0.07	0.040
Parabacteroides	−0.54 ± 0.11	−0.44 ± 0.14	0.600
Family_XIII_AD3011_group	−0.25 ± 0.06	−0.35 ± 0.09	0.433
Oscillospira	−0.39 ± 0.07	−0.58 ± 0.12	0.198
Anaerotruncus	−0.56 ± 0.08	−0.54 ± 0.12	0.926
Ruminococcaceae_UCG-009	−0.53 ± 0.11	−0.59 ± 0.07	0.622
Papillibacter	−0.46 ± 0.10	−0.70 ± 0.06	0.086
dgA-11_gut_group	−0.52 ± 0.11	−0.63 ± 0.08	0.441
Ruminococcaceae_UCG-010	−0.41 ± 0.04	−0.46 ± 0.03	0.366

[1] Values are means ± SEM, $n = 5$ per treatment.
[2] CON, basal diet treatment; BS, basal diet + 0.2% *B. subtilis* PB6 treatment.

PB6, which was consistent with previous studies (Alexopoulos et al., 2004; Stamati et al., 2006; Jeong et al., 2015; Rychen et al., 2017). Kritas et al. (2006) reported higher fat and protein percentages in the milk of ewes treated with *B. subtilis*. The differences between 2 groups may be associated with the higher fat content of the milk from the sows fed *B. subtilis* PB6 in this study. Previous studies have also shown that piglets consumed better-quality milk when sows were fed *B. subtilis* during lactation, which may partly explain the higher weaning weights observed in the present study (Kyriakis et al., 1992; Alexopoulos et al., 2004; Stamati et al., 2006; Zhu et al., 2012; Sun et al., 2013; Kritas et al., 2015). Another reason for these weaning weights and weight gains may be the higher milk yield of the sows supplied with *B. subtilis* PB6. Inatomi et al., (2017) reported that when sows were fed mixed probiotics (15 g/d) containing *B. subtilis*, the milk yield of the sow and the litter weights at farrowing improved. Although we did not measure the milk yield, it has been demonstrated in recent studies that sows and ewes supplemented with a compound probiotic containing *B. subtilis* produced more milk than those without supplementation (Kritas et al., 2006; Inatomi et al., 2017).

Previous studies have shown that *B. subtilis* or mixed probiotics improved the lactation survival rate. The present study also indicated that the lactation survival rate during suckling was higher in the BS group than in the CON group, as in previous studies (Böhmer et al., 2006; Stamati et al., 2006; Liu et al., 2017). The improved lactation survival rate of piglets when sows were supplied with *B. subtilis* PB6 may be partly attributable to the transfer of the *Bacillus* strain from the sows to the piglets or to the reduction of the amount of *Clostridium* shed into the environment by the sows (ME et al., 2008; Baker et al., 2013). Either way, the difference in the numbers of weaned piglets differed significantly between the BS and CON groups.

4.4. Feed intake and BF loss of sows during lactation

Although the growth performance of the offspring in the BS group was significantly better than that in the CON group during lactation, there were no differences in the feed intake or BF loss by sows during lactation between the 2 groups. The most likely explanation was that *B. subtilis* PB6 increased the digestive enzyme activity of sows, improving their digestion and their absorption of the nutrients in their feed during lactation. Hayakawa et al. (2016) reported that compound probiotics containing *B. subtilis* improved the ileal digesta of broilers. Previous studies have shown that *B. subtilis* could secrete exoenzymes, including proteases and amylases (Zokaeifar et al., 2012), and simultaneously improve the activities of host lipases and proteases (Zokaeifar et al., 2012; Li et al., 2012; Liu et al., 2017). Therefore, better nutrient utilization during lactation would result in higher milk quality when *B. subtilis* PB6 was added to sows' feed.

4.5. Antioxidant capacity and endotoxin in sow serum

This study demonstrated that *B. subtilis* PB6 improved the antioxidant capacity of sows. The gestation, parturition, and lactation of sows are associated with oxidative stress, and the excessive free radicals produced by oxidative stress disrupted the balance between the pro-oxidant and antioxidant systems (Castillo et al., 2005; Berchieri-ronchi et al., 2011). CAT, GSH-Px (antioxidative enzymes) and T-AOC play key roles in the self-defense of an organism (Rajput et al., 2013a), removing excess free radicals and preventing lipid peroxidation. Another important index of the body's antioxidant capacity is MDA (Wills, 1966; Coskun et al., 2005; Nawito et al., 2016), a product of lipid peroxidation. In this study, *B. subtilis* PB6 reduced the MDA concentrations and increased the T-AOC at parturition and increased the CAT activities on d 21 of lactation in the sow sera. These results were consistent with the research of Wei-fen, 2015, who demonstrated that *B. subtilis* B10 could protect against oxidative stress by increasing the rate of free radical scavenging by enhancing the enzymatic defense system. However, the average value of MDA concentrations in CON group seemed to decline from parturition to d 21 of lactation but it was stable in BS group, which can be explained by *B. subtilis* PB6 protected lipid from being oxidized during lactation. Several studies have shown that the application of certain *B. subtilis* strains could improve the antioxidant capacity of poultry (Rajput et al., 2013a, 2013b; Zhang et al., 2017). Although farrowing lead to oxidant stress (Szczubia et al., 2013), supplementation with *B. subtilis* PB6 improved the antioxidant capacities of the sows.

The bacterial endotoxin lipopolysaccharide (LPS) causes inflammation (Kauf, 2004). *B. subtilis* PB6 may ease the inflammation of sows during lactation, because in this study, we demonstrated that the endotoxin concentrations in the sow sera (on postnatal d 14 and 21) were reduced by BS supplementation. This reduction in endotoxin was important in accelerating the physical recovery of the sows. This finding confirmed that the sows in the BS group were more capable of breastfeeding than those in the CON group.

4.6. Cortisol in piglet serum

This study indicated that *B. subtilis* PB6 could ease the stress that piglets faced during the suckling period. *Bacillius subtilis* PB6 reduced the cortisol concentrations in the piglet sera on postnatal

d 14. Cortisol is a marker of stress in an organism (Roth, 1985; Limberaki et al., 2011), and there is a positive correlation between endotoxin-induced mastitis, neonatal diarrhea, and the plasma cortisol concentrations in cattle (Paape et al., 1974; Gwazdauskas et al., 1978; Massip, 1979). The reduction in the cortisol concentrations in the piglet sera in the BS group indicated that the piglets may have suffered less diarrhea, which was conducive to growth.

4.7. Intestinal microbes of sow at d 110 of gestation

The gut microbiota plays a key role in maintaining health and regulating pathogenesis in the host (Chassard et al., 2012; Ghoshal et al., 2012; Hayakawa et al., 2016). Pregnancy is associated with immunological and metabolic changes that may be related to the compositional dynamics of the microbiota (Koren et al., 2012; Kong et al., 2017). The composition of the intestinal microbiota is affected by multiple factors (Penders et al., 2006; Wu et al., 2011). This study showed that supplementation with *B. subtilis* PB6 increased the relative abundances of the phyla Gemmatimonadete and Acidobacteria and reduced relative abundances of Actinobacteria, Proteobacteria, and *Streptococcus*. According to the numbers of Observed species and Shannon's index, the α-diversity of the gut microbial community decreased when sows fed *B. subtilis* PB6. Kong et al., (2017) reported that the gut microorganismal diversity decreased and the abundances of Proteobacteria and Actinobacteria increased in late gestation in sows. Despite the reduction in microbial diversity, beneficial microbes, such as Gemmatimonadete and Acidobacteria, were more numerous when *B. subtilis* PB6 was added to the sows' feed. Proteobacteria actively participates in inflammatory bowel disease (Hansen et al., 2012; Koren et al., 2012; Mukhopadhya et al., 2012; Morgan et al., 2012), and a high proportion of Actinobacteria is associated with inflammatory bowel disease and colon cancer (Frank et al., 2007; Brim et al., 2017). *Streptococcus* is always a pathogenic bacterium (Yong et al., 2008). *Bacillus* species have been detected that could colonize the intestinal tract (Barbosa et al., 2005; Guo et al., 2006), and display the features for such colonization, including survival and germination in the gut, biofilm formation, and the secretion of antimicrobial compounds. Therefore, *B. subtilis* PB6 may inhibit the reproduction of harmful bacteria in the intestine in the study. The increase of relative abundance of Ruminococcaceae_UCG-013 cc in BS group may increase the carbohydrate fermentation in the sow gut during lactation (Gosalbes et al., 2011). These results suggested that *B. subtilis* PB6 may inhibit the proliferation of harmful bacteria and promote beneficial microbial growth, facilitating gut health.

5. Conclusions

The present research suggested that dietary supplementation with 4×10^8 CFU/kg *B. subtilis* PB6 in late gestation and lactation periods could reduce the piglet birth interval, and improve the growth performance of the suckling piglets (after cross-fostering) by enhancing their antioxidation capacity and reducing the endotoxin concentrations in the sow sera and the cortisol concentrations in the piglet sera. *B. subtilis* PB6 also could improve the gut health of the sows during late gestation.

Author contributions

De Wu designed the study, Yan Li and Meng Cao performed the research, Yan Li collected the data, Meng Cao and Jian Li analyzed the data, and Qianqian Zhang and Jian LI wrote the manuscript. All authors read and approved the final manuscript.

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.

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