Effects of Bacillus subtilis on jejunal integrity, redox status and microbial composition of intrauterine growth restriction suckling piglets

Article *in* Journal of Animal Science · September 2021

DOI: 10.1093/jas/skab255

CITATIONS
0

READS
25

9 authors, including:

Yang Yun
Nanjing Agricultural University
4 PUBLICATIONS **0** CITATIONS
SEE PROFILE

Shuli Ji
Nanjing Agricultural University
13 PUBLICATIONS **49** CITATIONS
SEE PROFILE

Zhang Xin
Nanjing Agricultural University
1 PUBLICATION **0** CITATIONS
SEE PROFILE

Tian Wang
Nanjing Agricultural University
134 PUBLICATIONS **2,462** CITATIONS
SEE PROFILE

Effects of *Bacillus subtilis* on jejunal integrity, redox status and microbial composition of intrauterine growth restriction suckling piglets[1]

Yang Yun [*], Shuli Ji [*], Ge Yu [*], Peilu Jia [*], Yu Niu [*], Hao Zhang [*], Xin Zhang [*], Tian Wang [*], Lili Zhang [*2]

[*] College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu, China, 210095.

[1] This work was supported by the National Natural Science Foundation of China (No. 31802101) and the Fundamental Research Funds for the Central Universities (No. KJQN201935).

[2] Corresponding author: Lili Zhang, Email: zhanglili@njau.edu.cn.

Abstract

The present study used intrauterine growth restriction (**IUGR**) piglets as an animal model to determine the effect of *Bacillus subtilis* on intestinal integrity, antioxidant capacity, and microbiota in the jejunum of suckling piglets. In total, 8 normal birth weight (**NBW**) newborn piglets (1.62 ± 0.10 kg) and 16 newborn IUGR piglets (0.90 ± 0.08 kg) were selected and assigned to 3 groups. Piglets were orally gavaged with 10 mL sterile saline (**NBW** and **IUGR** groups), IUGR piglets were orally gavaged with 10 mL/d bacterial fluid (*Bacillus subtilis* diluted in sterile saline, gavage in the dose of 2×10^9 colony-forming units per kg of body weight) (**IBS** group) (n = 8). IUGR induced jejunal barrier dysfunction and redox status imbalance of piglets, and changed the abundances of bacteria in the jejunum. Treatment with *Bacillus subtilis* increased ($P < 0.05$) the ratio of villus height to crypt depth (**VH/CD**) in the jejunum, decreased ($P < 0.05$) the plasma diamine oxidase (**DAO**) activity, and enhanced ($P < 0.05$) the gene expressions of zonula occludens-1 (**ZO-1**), Occludin and Claudin-1 in the jejunum of IUGR piglets. Treatment with *Bacillus subtilis* decreased ($P < 0.05$) the concentration of protein carbonyl (**PC**), and increased ($P < 0.05$) the activities of catalase (**CAT**) and total superoxide dismutase (**T-SOD**) in the jejunum of IUGR piglets. Treatment with *Bacillus subtilis* also increased ($P < 0.05$) gene expressions of superoxide dismutase 1 (***SOD1***), *CAT*, and nuclear factor erythroid 2-related factor (***Nrf2***), as well as the protein expressions of heme oxygenase-1 (**HO-1**), SOD1, and Nrf2 in the jejunum of IUGR piglets. Treatment with *Bacillus subtilis* also improved the abundances and the community structure of bacteria in the jejunum of IUGR piglets. These results suggested that IUGR damaged the jejunal barrier function and antioxidant capacity of suckling piglets, altered the

abundances of bacteria in the jejunum. Treatment with *Bacillus subtilis* improved the intestinal integrity and antioxidant capacity, while also improved the abundances and structure of bacteria in the jejunum of suckling piglets.

Key words: *Bacillus subtilis*, gut microbiota, intestinal integrity, intrauterine growth restriction, redox status, suckling piglets

Abbreviations

B. *subtilis*, *Bacillus subtilis*; IUGR, intrauterine growth restriction; NBW, normal birth weight; CFU, colony-forming units; ADG, average daily gain; VH/CD, villus height to crypt depth ratio; ZO-1, zonula occludens-1; PC, protein carbonyl; T-SOD, total superoxide dismutase; CAT, catalase; *Nrf2*, nuclear factor erythroid 2-related factor; *SOD1*, superoxide dismutase 1; *SOD2*, superoxide dismutase 2; HO-1, heme oxygenase-1; VH, villus height; CD, crypt depth; DAO, diamine oxidase; T-AOC, total antioxidant capacity; GSH-Px, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde; 8-OHdG, 8-hydroxy-2-deoxyguanosine; cDNA, complementary DNA; *GPx1*, glutathione peroxidase 1; PMSF, phenylmethylsulfonyl fluoride; BCA bicinchoninic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride; OTUs, Operational taxonomic units; FDR, false discovery rate; PCoA, Principal coordinate analysis; LDA, Linear discriminant analysis; IBW, initial body weight; FBW, final body weight.

INTRODUCTION

Intrauterine growth restriction (**IUGR**) is a specific syndrome of restricted growth and development of the fetus or its organs during gestation (Wu et al., 2006). A neonate with IUGR has a high perinatal mortality and morbidity, as well as increased susceptibility to disease (Pallotto and Kilbride, 2006). Previous studies have shown that the growth of key organs (e.g., brain and heart) of an IUGR fetus is prioritized for protection over that of organs (e.g., intestines) due to metabolic priority (Hu et al., 2015; Monaghan, 2008). Hence, IUGR can cause adverse effects on intestinal development and function.

A high occurrence rate of IUGR occurs in pigs, which are multifetal animals that have been deemed an ideal model for clinical study (Sangild, 2006). Compared to normal body weight (**NBW**) newborns, IUGR piglets show a reduced small intestinal weight and length, as well as a smaller the ratio of small intestine weight to length (D'Inca et al., 2010), Which are indicative of a thinner intestinal wall (Li et al., 2017). Previous studies have indicated that IUGR damages the intestinal morphology of piglets, as indicated by the decreased intestinal villus height and the ratio of villus height to crypt depth in IUGR piglets, and ultimately affects digestion and absorption functions in the small intestine (Huang et al., 2019; Su et al., 2018; Zhang et al., 2020). The developmental defects observed in the intestines of IUGR piglets indicate that homeostasis of the intestinal epithelium is easily disrupted. Huang et al. (2017) found that IUGR caused intestinal oxidative stress and impaired antioxidant capacity. In addition, IUGR can damage intestinal immune function by promoting an imbalance of T lymphocyte sub-populations and poor cytokine secretion ability in the small intestine (Dong et al., 2014; Dong et al., 2015).

Improved intestinal health has been shown in pigs through the regulation of intestinal morphology and functions by probiotics such as *Bacillus subtilis* (B. *subtilis*). B. *subtilis* is a

kind of gram-positive facultative aerobic beneficial species. B. *subtilis* can create an anaerobic environment that supports the growth of beneficial bacteria such as *lactobacillus* and *bifidobacteria* by consuming oxygen in the intestine (Han et al., 2012). B. *subtilis* can also produce vitamins, enzymes, and short-chain fatty acids, that are beneficial to the organism, Moreover, B. *subtilis* shows high resistance to harsh environmental conditions by virtue of its spores, and it can protect live beneficial bacteria as they pass through the acidic environment of the stomach into the gut (Setlow, 2014). It has been suggested that *B. subtilis* improved intestinal health of pigs through the regulation of immune function (Hu et al., 2017). In pigs, B. *subtilis* can alter the gut bacterial diversity by decreasing harmful bacteria and increasing beneficial bacteria (Hu et al., 2014). In addition, B. *subtilis* shows beneficial effects on the digestion, antioxidant capacity, and the growth performance of these animals (Aliakbarpour et al., 2012; Hu et al., 2014; Zhang et al., 2017).

One key period in the growth and development of piglets is the suckling stage. The suckling piglet is in a rapid stage of development; however, the development of organs and their physiological functions are still immature. In particular, the structure and various functions of the intestine have not reached adult levels, leaving the piglet susceptible to alternations in the environment of the intestinal lumen. Most studies have focused on weaning piglets, but little is known about suckling piglets. The aim of the present study was to investigate whether *B. subtilis* would have beneficial effects in this very early life stage of IUGR piglets. The overall goal was to study the effect of B. *subtilis* on the intestinal integrity, barrier function, redox status, and gut microbiota in IUGR suckling piglets.

MATERIALS AND METHODS

Animal experiment

The experiment was approved by the Animal Care and Use Committee of Nanjing Agricultural University (Permit number SYXK-2017–0027), and was carried out in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals of China.

Crossbred neonatal piglets (Duroc × [Landrace × Yorkshire]) were chosen from sows with similar parity and litter size. A NBW piglet was defined as having a BW within 0.5 standard deviation (**SD**) of the mean BW of the herd, while a piglet with a BW 2 SD lower than the mean herd BW was defined as IUGR (Zhang et al., 2020; Zhang et al., 2021). At birth, 8 male NBW and 16 same-sex IUGR littermates were selected from 8 litters (one NBW piglet and two IUGR piglets per litter). In this study, piglets with birth weights of 1.62 ± 0.10 kg and 0.90 ± 0.08 kg (mean ± SD) were defined as NBW and IUGR piglets, respectively. After sucking colostrum, all piglets were fostered to 3 new sows with good body condition and equivalent milk yields; each sow adopted 8 newborn piglets. The experiment was divided into three treatments consisting of 8 replicates (pens) of 1 pig each. The groups were as follows: NBW piglets were orally gavaged with 10 mL/d sterile saline (**NBW group**), the IUGR piglets were orally gavaged with 10 mL/d sterile saline (**IUGR group**), and the IUGR piglets were orally gavaged with 10 mL/d bacterial fluid (*Bacillus subtilis* diluted in sterile saline, the dose of 2×10^9 colony-forming units (CFU) per kg of body weight) (**IBS group**). Piglet was weighed at 3, 7, 14, and 21 days of age to determine the gavage doses of *Bacillus subtilis*. All piglets only had free access to the sows' breast milk. The trial lasted for 21 days from 3 to 24 days of age.

The *Bacillus subtilis* was provided by Qingdao Vland Biological Technology Co., Ltd. (Qingdao, China). The spore count of *Bacillus subtilis* was 20×10^9 CFU per g. The detailed information of *Bacillus subtilis* was outside the scope of the paper and cannot be described because of commercial sensitivity.

Sample collection

At 24 days of age, all piglets were weighed as the final body weight (FBW) before blood collection. The initial body weight (IBW) and FBW of piglet were used to calculate average daily gain (**ADG**) from 3 days to 24 days of age. Then blood samples were taken from the precaval vein of each piglet. Plasma was obtained by centrifugation at 3000 × *g* for 15 min at 4°C, and stored at -20°C for subsequent analysis. After blood sampling, all selected piglets were killed by exsanguination following an electrical stunning and the jejunum of piglet was immediately collected. The lumen of jejunum was flushed with saline, and approximately 2-centimeter-long jejunum sample was fixed in 4 % paraformaldehyde solution for histological analyses. Mucosa of jejunum was obtained through scraping luminal surface with glass slide, and frozen in liquid nitrogen until further analysis. Jejunal digesta was collected into the sterile freezing tube and stored at -80°C until subsequent analysis.

Intestinal morphology observation

Jejunal segments were dried up using a graded series of xylene and ethano after fixation in paraformaldehyde solution for 24 h. After that, they were embedded in paraffin, then specimens were sliced into 5 micron-thick sections and dyed with hematoxylin and eosin for morphological evaluation. The intestinal morphology images were obtained using a virtual microscope (Nikon 80i, Tokyo, Japan). Intestinal villus height (**VH**), which means from the tip of the villus to the villus-crypt junction, and crypt depth (**CD**) was measured from crypt opening to crypt base (Woyengo et al., 2011). All data were measured by Image-Pro Plus

software. Finally to calculate the ratio of villus height to crypt was defined as VH/CD. Fifteen intact villi and adjacent crypts were randomly selected from each section for the evaluation of jejunal morphology.

Plasma parameter

The activity of plasma diamine oxidase (**DAO**) was detected using a colorimetric assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) based on the spectrophotometry method (Zhang et al., 2020). All procedures strictly followed the manufacturer's instructions.

Jejunal mucosal redox status

The levels of total antioxidant capacity (**T-AOC**), the activities of total superoxide dismutase (**T-SOD**), glutathione peroxidase (**GSH-Px**), and catalase (**CAT**), the concentrations of malondialdehyde (**MDA**) and protein carbonyl (**PC**) in the jejunum were determined using kits which obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) (Ahmad et al., 2012). The concentration of 8-hydroxy-2-deoxyguanosine (**8-OHdG**) in the jejunum were detected by porcine-specific ELISA kits bought from Quanzhou Ruixin Biological Technological Co., Ltd. (China). All procedures strictly followed the manufacturer's instructions.

RNA extraction and quantitative real-time polymerase chain reaction

Total RNA from the jejunum mucosal samples was extracted using the Total RNA Extraction Reagent (Vazyme Biotechnology, Nanjing, Jiangsu, China). Then the concentration and purity of isolated RNA samples were assessed using a Nanodrop-ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). After that, total RNA was reverse-transcribed into complementary DNA (**cDNA**) using a HiScript III RT SuperMix

Reagent (Vazyme Biotechnology, Nanjing, Jiangsu, China). Quantitative real-time polymerase chain reaction was carried out on a StepOne Plus real-time PCR system (Applied Biosystems, Foster City, CA, USA) using ChamQ SYBR qPCR Master Mix (Vazyme Biotechnology, Nanjing, Jiangsu, China), according to the manufacturer's instructions. The primer sequences for the target and housekeeping genes (*β-actin*, *Nrf2*, *CAT*, superoxide dismutase2 (***Sod2***), glutathione peroxidase1 (***GPx1***), *Sod1*, *HO-1*, zonula occludens-1 (***ZO-1***), *Occludin*, *Claudin-1*) were listed in Table 1. The specific mRNAs expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008), and normalized by using gene *β-actin* which is defined as an internal standard.

Western blotting

The mucosa of jejunum was lysed with Cell lysis buffer for Western and IP assay reagent (Beyotime Institute of Biotechnology, Jiangsu, China) with 1 mmol/L phenylmethylsulfonyl fluoride (**PMSF**) (Beyotime Institute of Biotechnology, Jiangsu, China). The lysates were centrifuged at 14000 × *g* for 5 min at 4 °C after homogenate. Then, the concentration of protein in supernatant was determined by using the bicinchoninic acid (**BCA**) assay method (Beyotime Institute of Biotechnology, Jiangsu, China) and the concentration of protein was unified to 8 μg/μL, then mixed with 2 × sodium dodecyl sulfate-polyacrylamide gel electrophoresis (**SDS-PAGE**) sample loading buffer (Beyotime Institute of Biotechnology, Shanghai, China). The mixture was boiled at 99°C for 10 min, before performing gel electrophoresis.

Extracted protein was electrophoresed in SDS-PAGE and transferred onto polyvinylidene fluoride (**PVDF**) membrane (Millipore, Bedford, MA, USA), The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline combined with 0.2% Tween-20 (**TBST**) at room temperature for 2h. After washing membranes with TBST, the membranes

were incubated with primary antibodies at 4°C overnight with shaking on the shaker. Primary antibodies included nuclear transcription factor erythroid 2-related factor (**Nrf2**, Abcam, Cambridge, MA, USA), heme oxygenase 1 **(HO-1**, Proteintech Group, Inc., Wuhan, China), superoxide dismutase 1 (**SOD1**, Proteintech Group, Inc., Wuhan, China), superoxide dismutase 2 (**SOD2**, Proteintech Group, Inc., Wuhan, China) and beta actin (**β-actin**, Proteintech Group, Inc., Wuhan, China). After being washed with TBST, membranes were incubated with appropriate secondary antibody (horseradish peroxidase conjugated Goat Anti-Rabbit IgG, Sigma-Aldrich, USA) at room temperature for 1 hour. Finally, the membranes were used for Western blotting analysis after washing with TBST. The blots were developed using an ECL (biosharp, Hefei, China). The image of membranes was snapped with ChemiDocTM Imaging System (BIO-RAD, Hercules, CA, USA) and the grayscale value of each membrane was measured using Image-Pro Plus 6.0 (Media Cybernetics, Inc., Rockville, USA).

16S rRNA analysis of bacteria

The microbial genomic DNA of jejunal digesta was extracted using MagPure Stool DNA KF kit B (Magen, China) following the manufacturer's instructions (n = 6 for each group). The amount and quality of DNA was determined using Qubit dsDNA BR Assay kit (Invitrogen, USA) and 1% agarose gel electrophoresis, respectively.

The V3–V4 region of bacterial 16S rRNA gene was amplified using the specific primer pair 341F/806R, (341F: 5'- ACTCCTACGGGAGGCAGCAG-3') and (806R: 5'-GGACTACHVGGGTWTCTAAT-3'). The PCR reaction was a thermal cycle with initial denaturation at 94°C for 3 minutes, 30 cycles of 94°C for 30 seconds, 56°C for 45 seconds, 72°C for 45 seconds, and final extension for 10 minutes at 72°C. PCR enrichment was performed in a 50 μ L reaction containing 30ng template, fusion PCR primer and PCR master

mix. The PCR products were purified with AmpureXP beads and eluted in Elution buffer. Libraries were qualified by the Agilent 2100 bioanalyzer (Agilent, USA). The validated libraries were used for sequencing on Illumina MiSeq platform (BGI, Shenzhen, China) following the standard pipelines of Illumina, and generating 2 × 300 bp paired-end reads.

Raw reads were filtered to remove adaptors and low-quality, ambiguous bases, then paired-end reads were added to tags by the Fast Length Adjustment of Short reads program (FLASH, v1.2.11) to get the tags (Magoc and Salzberg, 2011). The tags were clustered into Operational taxonomic units (OTUs) with a cutoff value of 97% using UPARSE software (v7 .0.1090) (Edgar, 2013) and chimera sequences were compared with the Gold database using UCHIME (v4.2.40) (Edgar et al., 2011) to detect. OTU representative sequences were taxonomically classified using Ribosomal Database Project (**RDP**) Classifier v.2.2 with a minimum confidence threshold of 0.6, and trained on the Greengenes database v201305 by QIIME v1.8.0 (Caporaso et al., 2010). The USEARCH_global (Edgar, 2010) was used to compare all Tags back to OTU to get the OTU abundance statistics table of each sample.

Statistical analysis

All data were analyzed by one-way analysis of variation (ANOVA) using SPSS statistical software (SPSS, ver. 20.0 for Windows, SPSS Inc., Chicago, USA) except the analysis of gut microbiota, multiple comparisons were conducted using the Tukey test. $P < 0.05$ was considered as significant in statistics. Data are presented as the means and SEM.

Differences in the abundances of microbial taxa among three groups were performed using the Kruskal–Wallis test with Benjamini-Hochberg *p*-value correction followed by the post hoc Mann–Whitney U-test in R3.5.0. Alpha and beta diversity were estimated by MOTHUR (v1.31.2) and QIIME (v1.8.0) at the OTU level, respectively in R (version 3.3.1). Principal coordinate analysis (PCoA) was performed based on unweight UniFrac using

QIIME (v1.8.0). The differential bacterial taxa among groups were identified using Linear discriminant analysis (**LDA**) by LEfSe.

RESULTS

Growth performance

Compared with the NBW group, IUGR significantly decreased ($P < 0.05$) the final body weight of piglets, and the ADG of the NBW group was higher than that of the IUGR group (Table 2). Relative to the IUGR group, the final body weight and ADG had the tendencies to be improved in the IBS group.

Jejunal morphology

As shown in Fig. 1, villi with impaired integrity and abnormally obscure appearance of brush border in the jejunum were observed in the IUGR group, compared with the NBW group. However, the integrity of villi and the tight cell junctions in the jejunum were improved in the IBS group, so IUGR piglets had a normally oriented morphology after oral gavage of B. *subtilis*.

As shown in Table 3, no significant difference ($P > 0.05$) in jejunal morphology was observed between the NBW and IUGR groups. Compared with the IUGR group, the VH/CD of the jejunum was significantly increased ($P < 0.05$) in the IBS group.

Intestinal barrier function

Compared with the IUGR group, the activity of plasma DAO was significantly decreased ($P < 0.05$) in the IBS group (Fig. 2A). The gene expression of Claudin-1 was significantly decreased ($P < 0.05$) by IUGR in the jejunum of piglets (Fig. 2B). By contrast, the gene expressions of Occludin, ZO-1, and Claudin-1 were markedly upregulated ($P < 0.05$) in the IBS group, relative to the IUGR group.

Oxidative metabolite levels

As shown in Fig. 3, the effect of oral gavage of B. *subtilis* on the concentrations of PC (A), 8-OHdG (B) and MDA (C) in the jejunum of IUGR piglets. Relative to the IUGR group, the concentration of PC (Fig. 3A) was significantly decreased ($P < 0.05$) in the IBS group.

Jejunal antioxidant activity

As shown in Table 4, the activity of T-SOD was significantly decreased ($P < 0.05$) by IUGR in the jejunum of piglets. However, supplementation with B. *subtilis* increased ($P < 0.05$) the activities of T-SOD and CAT in the jejunum of piglets, relative to the IUGR group.

Compared with the NBW group, the gene expressions of *Nrf2*, *SOD1*, and *SOD2* in the jejunum were significantly decreased ($P < 0.05$) by IUGR (Fig. 4). However, the gene expressions of *Nrf2*, *SOD1*, and *CAT* were significantly upregulated ($P < 0.05$) in the IBS group, compared to the IUGR group.

The protein expression of *Nrf2* were significantly decreased ($P < 0.05$) in the IUGR group, compared with the NBW group (Fig. 5). By contrast, supplementation with B. *subtilis* increased ($P < 0.05$) the protein expressions of *Nrf2*, *SOD1*, and *HO-1* in the jejunum of IUGR piglets.

Microbiota composition of jejunal digesta

As shown in Table 5, the alpha diversity analysis showed that supplementation with B. *subtilis* decreased ($P < 0.05$) the bacterial community richness (Sobs index) in the jejunum of IUGR piglets compared with the IUGR group. There was difference in the structure of the bacterial community between the IUGR and IBS groups by PCoA (Fig. 6A) and ANOSIM (Fig. 6B, $P < 0.05$), and the bacterial composition of these two groups has a distinct clustering property.

At the Phylum level, Firmicutes was the dominant phyla in the jejunum among these groups (Fig. 6C). Compared with the NBW group (98.89%), the relative abundance of Firmicutes was decreased in the IUGR group (95.92%). However, the relative abundance of Firmicutes in the IBS group (99.85%) was higher ($P < 0.05$) than that in the IUGR group (95.92%). Supplementation with B. *subtilis* significantly decreased ($P < 0.05$) the relative abundances of Bacteroidetes (0.01%) and Proteobacteria (0.03%) in the IBS group, compared with the IUGR group (Bacteroidetes, 0.76% and Proteobacteria, 1.66%). At the genus level, we observed that *Lactobacillus*, *Veillonella*, *Clostridium*, and *Streptococcus* were the dominant genera in these groups (Fig. 6D).

To further investigate the differences among each group, we attempted to find the key phylotypes as microbiological markers at the different level in each group using linear discriminant analysis effect size (**LEfSe**). In particular, at the genus level, higher proportions of the genus *Pasteurella* and *Sarcina* were observed in the IUGR group than

those of in the NBW group, and *Bifidobacterium* was significantly expanded in the NBW group (Fig. 7A). Compared with the IUGR group, supplementation with B. *subtilis* increased the proportion of genus *Veillonella*, and decreased proportions of genus *Desulfovibrio*, *Prevotella*, and *Escherichia* in the jejunum of piglets (Fig. 7B).

DISCUSSION

The weight of the IUGR neonate is significantly lower than that of the normal neonate in mammals, and IUGR exerts adverse long-term effects on the growth and development of neonates. In the present study, the ADG were reduced in the IUGR group compared with the NBW group, in agreement with previous studies (Alvarenga et al., 2013; Hu et al., 2017). However, B. *subtilis* had beneficial effects on the ADG of IUGR piglets during the suckling period. A study by Zong et al. (2019) also showed that *Bacillus* treatment improved the ADG, while decreasing the diarrhea rate of weaned piglets by improving the ability to digest and utilize nutrients.

Intestinal morphology is one major indicator reflecting intestinal function and health, and the intestinal villus height and crypt depth are associated with the digestion and absorption of nutrients (Niu et al., 2020). VH/CD is considered an indicator that can be used to evaluate the capacity of nutrient digestion and absorption (Lm et al., 2003). In the present study, no significant difference was observed in VH/CD of suckling piglets between NBW and IUGR groups, consistent with previous study (Hu et al., 2017). B. *subtilis* treatment increased the VH/CD in the jejunum of the IUGR piglets. Previous studies have similarly reported that supplementation with B. *subtilis* increased the small intestinal VH/CD of IUGR suckling piglets (Hu et al., 2017). Therefore, B. *subtilis* treatment might improve the ability of digestion and absorption in IUGR piglets by improving intestinal morphology.

The gut is not only the main place where digestion and absorption occur, it also serves as an important barrier to the invasion of pathogens. The tight junction proteins such as ZO-1, Occludin, and Claudin-1 are vital components required for the integrity of the intestinal barrier (Anderson and Van Itallie, 2009; Xue et al., 2021). Additionally, DAO is an intracellular enzyme that is mainly located in the top of the intestinal villi, but it can be released from the cells into the circulatory system following the impairment of intestinal integrity (Miyoshi et al., 2015). The present study indicated an increase in plasma DAO activity and a decrease in the mRNA expressions of jejunal tight junction proteins in the IUGR group, as well as the impaired integrity of jejunal villi was observed form the sections. Substantial evidence supports the impairment of the intestinal mucosal barrier and an increased permeability of the intestine in IUGR piglets (Zhang et al., 2019; Zhang et al., 2020). In the present study, treatment with B. *subtilis* decreased plasma DAO activity and increased the gene expressions of jejunal tight junction proteins. Similar studies have reported that a B. *subtilis* probiotic strain increased the gene expressions of Claudin-1 and ZO-1 in the intestine of pigs (He et al., 2020; Kim et al., 2019). Taken together, B. *subtilis* supplementation improved the integrity of the intestinal barrier by upregulating the expressions of tight junction proteins.

IUGR has been shown to cause an imbalance of redox in the small intestine of piglets, because intestine with the impaired integrity is more susceptible to oxidative damage (Wang et al., 2010). Oxidative stress can cause damage to cells by promoting lipid peroxidation, oxidation and inactivation of proteins, and harmful DNA modifications (Ott et al., 2007). The concentration of protein carbonyl (**PC**) can be used to assess the level of protein oxidative damage (Hauck et al., 2019). In the present study, the concentration of PC in the jejunum was increased in the IUGR group, but treatment of IUGR piglets with B. *subtilis* significantly decreased the concentration of PC. Growing evidence has shown that probiotics have

antioxidant abilities, as reflected by improving the activity of antioxidant enzymes and scavenging excessive ROS in vivo or vitro (Wang et al., 2017). Previous studies also have shown that B. *subtilis* can inhibit the production of reactive oxygen metabolites, such as MDA, PC, and 8-OHdG (Zhang et al., 2017).

The body has a natural antioxidant system which can clear harmful metabolites generated by oxidative stress. This system mainly includes enzymatic antioxidants such as SOD, CAT, and GPx (Mates et al., 1999). In the present study, we found that the jejunal SOD activity was significantly decreased in the IUGR group. B. *subtilis* treatment increased the SOD and CAT activity in the jejunum of IUGR piglets. SOD is a major antioxidant enzyme that catalyzes the conversion of the highly reactive superoxide anion to the less reactive species H_2O_2, H_2O_2 is further detoxified into harmless molecules by CAT (Mates et al., 1999). Similarly, previous studies have indicated that IUGR reduced the antioxidant enzymes activity and damaged the antioxidant system in the jejunum of piglets (Huang et al., 2017; Zhang et al., 2020). Previous evidence has shown that B. *subtilis* treatment could enhance the activity of antioxidant enzymes in different tissues (Bai et al., 2017; Zhang et al., 2017). Therefore, B. *subtilis* treatment appeared to promote antioxidant ability by increasing the antioxidant enzyme activity, thereby decreasing the level of oxidative stress products.

We further investigated the molecule mechanism of antioxidant capacity of B. *subtilis* by studying the nuclear factor erythroid 2-related factor 2 (**Nrf2**) signaling pathway. Nrf2 plays a vital role in the defense responses to oxidative stress, it can induce the expressions of downstream genes such as *SOD*, *HO-1* and *CAT* (Bellezza et al., 2018). In the present study, the expressions of *Nrf2* and its downstream genes in the jejunum was decreased in the IUGR group. However, B. *subtilis* treatment enhanced the expressions of *SOD*, *HO-1* and *CAT* by inducing *Nrf2* expression in the jejunum of IUGR piglets. Recent evidence has demonstrated that probiotic *Bacillus spp*. can alleviate the H_2O_2-induced IPEC-1 oxidative stress by

suppressing ROS levels and inducing *Nrf2* expression (Wang et al., 2017). The combined data from this study indicated that B. *subtilis* might improve jejunal antioxidant ability by activating *Nrf2* signaling pathway. A recent research found that probiotics could activate *Nrf2* signaling pathway by secreting small molecule such as 5-methoxyindoleacetic acid (Saeedi et al, 2020). In the future research, therefore, it will be critical to investigate which a small molecule produced by B. *subtilis* activates *Nrf2* signaling pathway.

Growing evidence suggests that the composition of gut microbiota is closely related to the host health (Shreiner et al., 2015; Saffouri et al., 2019). Therefore, we investigated whether B. *subtilis* altered the composition of microbiota in the jejunum to affect the growth and development of IUGR piglets. In the present study, no difference in the microbial α-diversity was found between the NBW and IUGR groups, but B. *subtilis* decreased the microbial richness in the jejunum of IUGR piglets. Analogously, a recent report indicated no significant difference in the microbial richness of NBW and IUGR piglets at 21 days of age (Zhang et al., 2019). In the current study, no difference was apparent in the bacterial community structure of the NBW and IUGR piglets, in agreement with a previous study carried out by Zhang et al. (2019). However, we observed a significant separation of the bacterial community structures in the jejunum between the IUGR and IBS groups, which might be related to the reduction in microbial richness by B. *subtilis* treatment.

The dominant phylum in the jejunum was Firmicutes. The IUGR piglets showed a decrease in the abundance of Firmicutes and the value of Firmicutes/Bacteroidetes ratio compared to the NBW piglets. Previous studies have shown that the Firmicutes/Bacteroidetes ratio is positively correlated with an increased ADG (Ding et al., 2021). We also observed that B. *subtilis* treatment increased the abundance of Firmicutes, while decreasing the abundances of Bacteroidetes and Proteobacteria in IUGR piglets. The ratio of Firmicutes to Bacteroidetes was also markedly increased in the IBS group. Therefore, we speculated that a

high Firmicutes/Bacteroidetes ratio might be associated with the improvement of ADG in the IBS group. The Proteobacteria include a variety of pathogens such as *Escherichia* and *Salmonella*, and a previous study revealed an increase in the abundance of Proteobacteria in association with dysbiosis of the gut microbial community (Shin et al., 2015). Therefore, in the present study, the improvement of gut microbiota in IBS group might be related to the low abundance of Proteobacteria.

Lactobacillus was the most abundant genus in the jejunum of suckling piglets. LEfSe analysis revealed a high proportion of *Pasteurella* in the jejunum of IUGR piglets, in agreement with results reported by Zhang (2019). Many *Pasteurella* species are opportunistic pathogens for animals (Wilson and Ho, 2013), they will increase the susceptibility of IUGR piglets to disease. We also observed a low proportion of *Bifidobacterium* in the IUGR group, in agreement with previous study (Zhang et al., 2019). *Bifidobacterium* is a commonly used probiotic for the treatment of certain diseases by regulating the gut microbiota, and it also plays important roles in nutrient metabolism and immune homeostasis of the host (Azad et al., 2018). The present study also indicated an increased abundance of *Veillonella* and decreased abundances of *Desulfovibrio*, *Prevotella*, and *Escherichia* in the jejunum of IBS group. *Veillonella* could metabolize lactate to produce acetate and propionate, which are absorbed by intestinal epithelial cells and used for synthesis and metabolism by the host (Kastl et al., 2020).

Previous data also confirmed that *Escherichia* had negative correlations with ADG and carcass weight, and it was reported that the abundance of *Desulfovibrio* was negatively associated with body mass index (Fu et al., 2015; Torres-Pitarch et al., 2020). As pointed out above, the improvement of ADG in the IBS group might be related to the increased abundance of *Veillonella*, but it may also be attributed to the decreased abundances of *Escherichia* and *Desulfovibrio*. Furthermore, a recent study has shown that pigs with a high

abundance of *Prevotella* tended to have poorer feed efficiency; therefore, *Prevotella* could be considered a potential biomarker for lower feed efficiency (Niu et al., 2015).

In conclusion, IUGR delayed growth, damaged the intestinal barrier function and antioxidant capacity, and altered the abundance of bacteria in the jejunum of suckling piglets. However, oral gavage of B. *subtilis* improved the growth performance and the jejunal integrity, and appeared to increase jejunal antioxidant capacity by activating the Nrf2 signaling pathway in IUGR piglets. B. *subtilis* treatment also altered the structure of the bacterial community by increasing the abundance of beneficial bacteria and decreasing the abundance of detrimental bacteria in the jejunum of IUGR suckling piglets. Therefore, B. *subtilis* appears to be beneficial to the intestinal health and functions of piglets, and would be expected to regulate the growth and development of IUGR piglets in pig production. These findings also suggested that piglets could be supplement with B. *subtilis* before weaning to prevent the oxidative stress caused by the weaning process.

Disclosures

There are no relevant conflicts of interest relevant to this article.

LITERATURE CITED

Aliakbarpour H. R., M. Chamani, G. Rahimi, A. A. Sadeghi, and D. Qujeq. 2012. The Bacillus subtilis and Lactic Acid Bacteria Probiotics Influences Intestinal Mucin Gene Expression, Histomorphology and Growth Performance in Broilers. Asian-Australas J. Anim. Sci. 25(9):1285-1293. doi：10.5713/ajas.2012.12110.

Alvarenga A. L., H. Chiarini-Garcia, P. C. Cardeal, L. P. Moreira, G. R. Foxcroft, D. O. Fontes, and F. R. Almeida. 2013. Intra-uterine growth retardation affects birthweight and postnatal development in pigs, impairing muscle accretion, duodenal mucosa morphology and carcass traits. Reprod. Fertil. Dev. 25(2):387-395. Doi: 10.1071/RD12021.

Ahmad H., J. Tian, J. Wang, M. A. Khan, Y. Wang, L. Zhang, and T. Wang. 2012. Effect of dietary sodium selenite and selenium yeast on antioxidant enzymes activities and oxidative stability of chicken breast meat. J. Agric. Food Chem. 60:7111-7120. doi: 10.1021/jf3017207.

Anderson J. M., and C. M. Van Itallie. 2009. Physiology and function of the tight junction. Cold Spring Harb. Perspect. Biol. 1(2): 1-16. doi: 10.1101/cshperspect.a002584.

Azad M., M. Sarker, T. Li, and J. Yin. 2018. Probiotic Species in the Modulation of Gut Microbiota: An Overview. Biomed. Res. Int. (2018): 1-8. doi: 10.1155/2018/9478630.

Bai K., Q. Huang, J. Zhang, J. He, L. Zhang, and T. Wang. 2017. Supplemental effects of probiotic Bacillus subtilis fmbJ on growth performance, antioxidant capacity, and meat quality of broiler chickens. Poult. Sci. 96(1):74-82. doi: 10.3382/ps/pew246.

Bellezza I., I. Giambanco, A. Minelli, and R. Donato. 2018. Nrf2-Keap1 signaling in oxidative and reductive stress. Biochim. Biophys. Acta. Mol. Cell Res. 1865(5):721-733. doi: 10.1016/j.bbamcr.2018.02.010.

Caporaso J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich, J. I. Gordon, G. A. Huttley, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods. 7(5):335–336. doi:10.1038/nmeth.f.303.

D'Inca R., M. Kloareg, G. C. Gras-Le, and I. Le Huerou-Luron. 2010. Intrauterine growth restriction modifies the developmental pattern of intestinal structure, transcriptomic profile, and bacterial colonization in neonatal pigs. J. Nutr. 140(5):925-931. doi: 10.3945/jn.109.116822.

Ding H., X. Zhao, C. Ma, Q. Gao, Y. Yin, X. Kong, and J. He. 2021. Dietary supplementation with Bacillus subtilis DSM 32315 alters the intestinal microbiota and metabolites in weaned piglets. J. Appl. Microbiol. 130(1):217-232. doi: 10.1111/jam.14767.

Dong L., X. Zhong, H. Ahmad, W. Li, Y. Wang, L. Zhang, and T. Wang. 2014. Intrauterine Growth Restriction Impairs Small Intestinal Mucosal Immunity in Neonatal Piglets. J. Histochem. Cytochem. 62(7):510-518. doi: 10.1369/0022155414532655.

Dong L., X. Zhong, L. Zhang, L. Kong, Y. Kong, T. Kou, and T. Wang. 2015. Impaired intestinal mucosal immunity is associated with the imbalance of T lymphocyte sub-populations in intrauterine growth-restricted neonatal piglets. Immunobiology. 220(6):775-781. doi: 10.1016/j.imbio.2014.12.017.

Edgar R. C.. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. methods. 10(10):996-998. doi: 10.1038/NMETH.2604.

Edgar R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 27:2194-2200. doi: 10.1093/bioinformatics/btr381.

Edgar R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 26(19):2460-2461. doi: 10.1093/bioinformatics/btq461.

Fu J., M. J. Bonder, M. C. Cenit, E. F. Tigchelaar, A. Maatman, J. A. Dekens, E. Brandsma, J. Marczynska, F. Imhann, and R. K. Weersma 2015. The Gut Microbiome Contributes to a Substantial Proportion of the Variation in Blood Lipids. Circ. Res. 117(9):817-824. doi: 10.1161/CIRCRESAHA.115.306807.

Han G. Q., Z. T. Xiang, B. Yu, D. W. Chen, H. W. Qi, X. B. Mao, H. Chen, Q. Mao, and Z. Q. Huang. 2012. Effects of different starch sources on Bacillus spp. in intestinal tract and expression of intestinal development related genes of weanling piglets. Mol. Biol. Rep. 39(2):1869-1876. doi: 10.1007/s11033-011-0932-x.

Hauck A. K., Y. Huang, A. V. Hertzel, and D. A Bernlohr. 2019. Adipose oxidative stress and protein carbonylation. J. Biol. Chem. 294(4):1083-1088. doi: 10.1074/jbc.R118.003214.

He Y., K. Kim, L. Kovanda, C. Jinno, M. Song, J. Chase, X. Li, B. Tan, and Y. Liu 2020. Bacillus subtilis: a potential growth promoter in weaned pigs in comparison to carbadox. J. Anim. Sci. 98(9). doi: 10.1093/jas/skaa290.

Hu L., Y. Liu, C. Yan, X. Peng, Q. Xu, Y. Xuan, F. Han, G. Tian, Z. Fang, and Y. Lin. 2015. Postnatal nutritional restriction affects growth and immune function of piglets with intra-uterine growth restriction. Br. J. Nutr. 114(1):53-62. doi: 10.1017/S0007114515001579.

Hu L., X. Peng, H. Chen, C. Yan, Y. Liu, Q. Xu, Z. Fang, Y. Lin, S. Xu, and B. Feng 2017. Effects of intrauterine growth retardation and Bacillus subtilis PB6 supplementation on growth performance, intestinal development and immune function of piglets during the suckling period. Eur. J. Nutr. 56(4):1753-1765. doi: 10.1007/s00394-016-1223-z.

Hu Y., Y. Dun, S. Li, S. Zhao, N. Peng, and Y. Liang. 2014. Effects of Bacillus subtilis KN-42 on Growth Performance, Diarrhea and Faecal Bacterial Flora of Weaned Piglets. Asian-Australas J. Anim. Sci. 27(8):1131-1140. doi: 10.5713/ajas.2013.13737.

Huang Q., W. Xu, K. W. Bai, J. T. He, H. Ahmad, L. Zhou, L. L. Zhang, and T. Wang. 2017. Protective effects of leucine on redox status and mitochondrial-related gene abundance in the jejunum of intrauterine growth-retarded piglets during early weaning period. Arch. Anim. Nutr. 71(2):93-107. doi: 10.1080/1745039X.2017.1279712.

Huang S., N. Li, C. Liu, T. Li, W. Wang, L. Jiang, Z. Li, D. Han, S. Tao, and J. Wang. 2019. Characteristics of the gut microbiota colonization, inflammatory profile, and plasma metabolome in intrauterine growth restricted piglets during the first 12 hours after birth. J. Microbiol. 57(9):748-758. doi: 10.1007/s12275-019-8690-x.

Kastl A. J., N. A. Terry, G. D. Wu, and L. G. Albenberg. 2020. The Structure and Function of the Human Small Intestinal Microbiota: Current Understanding and Future Directions. Cell. Mol. Gastroenterol Hepatol. 9(1):33-45. doi: 10.1016/j.jcmgh.2019.07.006.

Kim K., Y. He, X. Xiong, A. Ehrlich, and Y. Liu. 2019. Dietary supplementation of Bacillus subtilis influenced intestinal health of weaned pigs experimentally infected with a

pathogenic E. coli. J. Anim. Sci. Biotechno. 10(1):1-12. doi: 10.1186/s40104-019-0364-3.

Li N., W. Wang, G. Wu, and J. Wang. 2017. Nutritional support for low birth weight infants: insights from animal studies. Br. J. Nutr. 117(10):1390-1402. doi: 10.1017/s000711451700126x.

Lm A., B. Jrp, and B. Djh. 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed Sci. Tech. 108(1–4):95-117. doi: 10.1016/S0377-8401(03)00163-9.

Mates J. M., C. Perez-Gomez, and D. C. I. Nunez. 1999. Antioxidant enzymes and human diseases. Clin. Biochem. 32(8):595-603. doi: 10.1016/S0009-9120(99)00075-2.

Miyoshi J., H. Miyamoto, T. Goji, T. Taniguchi, T. Tomonari, M. Sogabe, T. Kimura, S. Kitamura, K. Okamoto, and Y. Fujino. 2015. Serum diamine oxidase activity as a predictor of gastrointestinal toxicity and malnutrition due to anticancer drugs. J. Gastroen. Hepatol. 30(11):1582-1590. doi: 10.1111/jgh.13004.

Monaghan P.. 2008. Early growth conditions, phenotypic development and environmental change. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363(1497):1635-1645. doi: 10.1098/rstb.2007.0011.

Magoc T. and S. L. Salzberg. 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 27(21):2957-63. doi : 10.1093/bioinformatics/btr507.

Niu Q., P. Li, S. Hao, Y. Zhang, S. W. Kim, H. Li, X. Ma, S. Gao, L. He, and W. Wu. 2015. Dynamic distribution of the gut microbiota and the relationship with apparent crude fiber digestibility and growth stages in pigs. Sci. Rep. 5(9938):1-7. doi: 10.1038/srep09938.

Niu Y., J. T. He, Y. W. Zhao, Z. D. Gan, M. M. Shen, L. L. Zhang, and T. Wang. 2020. Dietary enzymatically treated Artemisia annua L. supplementation improved growth performance and intestinal antioxidant capacity of weaned piglets. Livest. Sci. 232:1-8. doi: 10.1016/j.livsci.2020.103937.

Ott M., V. Gogvadze, S. Orrenius, and B. Zhivotovsky. 2007. Mitochondria, oxidative stress and cell death. Apoptosis. 12(5):913-922. doi: 10.1007/s10495-007-0756-2.

Pallotto E. K. and H. W. Kilbride. 2006. Perinatal outcome and later implications of intrauterine growth restriction. Clin. Obstet. Gynecol. 49(2):257-269. doi: 10.1097/00003081-200606000-00008.

Saffouri G. B., R. R. Shields-Cutler, J. Chen, Y. Yang, H. R. Lekatz, V. L. Hale, J. M. Cho, E. J. Battaglioli, Y. Bhattarai, and K. J. Thompson. 2019. Small intestinal microbial dysbiosis underlies symptoms associated with functional gastrointestinal disorders. Nat. Commun. 10(1):1-11. doi: 10.1038/s41467-019-09964-7.

Sangild P.T.. 2006. Gut responses to enteral nutrition in preterm infants and animals. Exp. Biol. Med. 231(11):1695-1711. doi: 10.1111/j.1365-2362.2006.01736.x.

Setlow P.. 2014. Spore Resistance Properties. Microbiol Spectr. 2(5):1-14. doi: 10.1128/microbiolspec.TBS-0003-2012.

Shin N. R., T. W. Whon, and J. W. Bae. 2015. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 33(9):496-503. doi: 10.1016/j.tibtech.2015.06.011.

Schmittgen, T.D., and K.J. Livak. 2008. Analyzing real-time PCR data by the comparative CT method. Nat. Protoc. 3:1101–1108. doi: 10.1038/nprot.2008.73.

Shreiner A. B., J. Y. Kao, and V. B. Young. 2015. The gut microbiome in health and in disease. Curr. Opin. Gastroenterol. 31(1):69-75. doi: 10.1097/MOG.0000000000000139.

Su W., H. Zhang, Z. Ying, Y. Li, L. Zhou, F. Wang, L. Zhang, and T. Wang. 2018. Effects of dietary L-methionine supplementation on intestinal integrity and oxidative status in intrauterine growth-retarded weanling piglets. Eur. J. Nutr. 57(8):2735-2745. doi: 10.1007/s00394-017-1539-3.

Saeedi B. J., K. H. Liu, J. A. Owens, R. M. Jones, D. P. Jones, and A. S. Neish. 2020. Gut-resident *lactobacilli* activate hepatic Nrf2 and protect against oxidative liver injury. Cell Metab. 31(5):956-968. doi: 10.1016/j.cmet.2020.03.006.

Torres-Pitarch A., G. E. Gardiner, P. Cormican, M. Rea, F. Crispie, J. V. O'Doherty, P. Cozannet, T. Ryan, and P. G. Lawlor. 2020. Effect of cereal soaking and carbohydrase supplementation on growth, nutrient digestibility and intestinal microbiota in liquid-fed grow-finishing pigs. Sci. Rep. 10(1):1023. doi: 10.1038/s41598-020-57668-6.

Woyengo, T. A., J. C. Rodriguez-Lecompte, O. Adeola, and C. M. Nyachoti. 2011. Histomorphology and small intestinal sodium-dependent glucose transporter 1 gene expression in piglets fed phytic acid and phytase-supplemented diets. J. Anim. Sci. 89:2485–2490. doi: 10.2527/jas.2010-3204.

Wang X., W. Wu, G. Lin, D. Li, G. Wu, and J. Wang. 2010. Temporal proteomic analysis reveals continuous impairment of intestinal development in neonatal piglets with intrauterine growth restriction. J. Proteome Res. 9(2):924-935. doi: 10.1021/pr900747d.

Wang Y., Y. Wu, Y. Wang, A. Fu, L. Gong, W. Li, and Y. Li. 2017. Bacillus amyloliquefaciens SC06 alleviates the oxidative stress of IPEC-1 via modulating

Nrf2/Keap1 signaling pathway and decreasing ROS production. Appl. Microbiol Biotechnol. 101(7):3015-3026. doi: 10.1007/s00253-016-8032-4.

Wang Y., Y. Wu, Y. Wang, H. Xu, X. Mei, D. Yu, Y. Wang, and W, Li. 2017. Antioxidant Properties of Probiotic Bacteria. Nutrients. 9(5):1-15. doi: 10.3390/nu9050521.

Wilson B. A. and M. Ho. 2013. Pasteurella multocida: from zoonosis to cellular microbiology. Clin. Microbiol. REV. 26(3):631-655. doi: 10.1128/cmr.00024-13.

Wu G., F. W. Bazer, J. M. Wallace, and T. E. Spencer. 2006. Board-invited review: intrauterine growth retardation: implications for the animal sciences. J. Anim. Sci. 84(9):2316-2337. doi: 10.2527/jas.2006-156.

Xue C., Y. Li, H. Lv, L. Zhang, C. Bi, N. Dong, A. Shan, and J. Wang. 2021. Oleanolic acid targets the gut-liver axis to alleviate metabolic disorders and hepatic steatosis. J. Agric. Food Chem. doi: 10.1021/acs.jafc.1c02257.

Zhang H., Y. Chen, S. Ji, P. Jia, J. Xu, Y. Li, and T. Wang. 2021. Pterostilbene attenuates liver injury and oxidative stress in intrauterine growth-retarded weanling piglets. Nutrition. 81:1-8. doi: 10.1016/j.nut.2020.110940.

Zhang H., Y. Chen, Y. Li, and T. Wang. 2020. Protective effect of polydatin on jejunal mucosal integrity, redox status, inflammatory response, and mitochondrial function in intrauterine growth-retarded weanling piglets. Oxid. Med. Cell Longev. (2-3):1-14. doi: 10.1155/2020/7178123.

Zhang H., Y. Li, Y. Chen, L. Zhang, and T. Wang. 2019. N-Acetylcysteine protects against intrauterine growth retardation-induced intestinal injury via restoring redox status and mitochondrial function in neonatal piglets. Eur. J. Nutr. 58(8):3335-3347. doi: 10.1007/s00394-018-1878-8.

Zhang L., K. Bai, J. Zhang, W. Xu, Q. Huang, and T. Wang. 2017. Dietary effects of Bacillus subtilis fmbj on the antioxidant capacity of broilers at an early age. Poult. Sci. 96(10):3564-3573. doi: 10.3382/ps/pex172.

Zhang W., C. Ma, P. Xie, Q. Zhu, X. Wang, Y. Yin, and X. Kong. 2019. Gut microbiota of newborn piglets with intrauterine growth restriction have lower diversity and different taxonomic abundances. J. Appl. Microbiol. 127(2):354-369. doi::10.1111/jam.14304.

Zong X., T. H. Wang, Z. Q. Lu, D. G. Song, J. Zhao, and Y. Z. Wang. 2019. Effects of Clostridium butyricum or in combination with Bacillus licheniformis on the growth performance, blood indexes, and intestinal barrier function of weanling piglets. Livest. Sci. 220:137-142. doi: 10.1016/j.livsci.2018.12.024.

Figure legend

Fig. 1. Effect of oral gavage of *Bacillus subtilis* on jejunal histomorphology in IUGR suckling piglets. All samples were stained with hematoxylin and eosin (HE). Scale bar represents 100 μm. NBW, normal birth weight; IUGR, intrauterine growth restriction; IBS, IUGR with oral administration of *Bacillus subtilis*.

Fig. 2. Effect of oral gavage of *Bacillus subtilis* on plasma diamine oxidase activity (A) and the mRNA levels of jejunal tight junction protein (B) in IUGR suckling piglets. Data are expressed as mean with their standard errors represented by vertical bars, n = 8. * $P < 0.05$. DAO, plasma diamine oxidase; ZO-1, zonula occludens 1. NBW, normal birth weight; IUGR, intrauterine growth restriction; IBS, IUGR with oral administration of *Bacillus subtilis*.

Fig. 3. Effect of oral gavage of *Bacillus subtilis* on the concentration of jejunal PC (A), 8-OHdG (B) and MDA (C) in IUGR suckling piglets. Data are expressed as mean with their standard errors represented by vertical bars, n = 8. * $P < 0.05$. PC, protein carbonyl; 8-OHdG, 8-hydroxy-2-deoxyguanosine; MDA, malondialdehyde. NBW, normal birth weight; IUGR, intrauterine growth restriction; IBS, IUGR with oral administration of *Bacillus subtilis*.

Fig. 4. Effect of oral gavage of *Bacillus subtilis* on the mRNA expressions of jejunal antioxidant related genes in IUGR suckling piglets. Data are expressed as mean with their standard errors represented by vertical bars, n = 8. * $P < 0.05$. *Nrf2*, nuclear factor erythroid 2-related factor 2; *HO-1*, heme oxygenase-1; *SOD1*, superoxide dismutase 1; *SOD2*, superoxide dismutase 2; *Cat*, catalase, *GPx1*, glutathione peroxidase 1. NBW, normal birth weight; IUGR, intrauterine growth restriction; IBS, IUGR with oral administration of *Bacillus subtilis*.

Fig. 5. Effect of oral gavage of *Bacillus subtilis* on the expressions of jejunal related antioxidant protein in IUGR suckling piglets. Data are expressed as mean with their standard errors represented by vertical bars, n = 8. * $P < 0.05$. Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2. NBW, normal birth weight; IUGR, intrauterine growth restriction; IBS, IUGR with oral administration of *Bacillus subtilis*.

Fig. 6. Principal coordinate analysis (PCoA) of all samples (A) and analysis of similarities (ANOSIM) (B) indicate that microbial structure in each group. The percentage of community abundance on Phylum level (C) and Genus level (D) indicate that gut microbiota composition of these groups. Data are shown as means, n = 6. NBW, normal birth weight; IUGR, intrauterine growth restriction; IBS, IUGR with oral administration of *Bacillus subtilis*.

Fig. 7. Different bacterial taxa enriched in each group. Histograms of a linear discriminant analysis (LDA) score (threshold: ≥2) in jejunal samples. n = 6. NBW, normal birth weight; IUGR, intrauterine growth restriction; IBS, IUGR with oral administration of *Bacillus subtilis*.

Table 1. Primer sequences of target gene.

Gene	Accession No.	Sequences
Nrf2	MH_101365.1	F: GCCCAGTCTTCATTGCTCCT
		R: AGCTCCTCCCAAACTTGCTC
HO-1	NM_001004027.1	F:TACCGCTCCCGAATGAACAC
		R: TGGTCCTTAGTGTCCTGGGT
GPx1	NM_214201.1	F: CAAGTCCTTCTACGACCTCA
		R: GAAGCCAAGAACCACCAG
SOD1	NM_001190422.1	F: GTACCAGTGCAGGTCCTCAC
		R: TTTGCCAGCAGTCACATTGC
SOD2	NM_214127	F: GGACAAATCTGAGCCCTAACG
		R: CCTTGTTGAAACCGAGCC
CAT	NM_214301.2	F: CTGTAAGGCTAGTCGGACACC
		R: ATATCAGGTTTCTGCGCGGC
ZO-1	XM_021098896.1	F: TGCCAGCTGGAGCTTAGAAC
		R: AGGCATCAAGAGGGGCTACT
Occludin	NM_001163647.2	F: CAGTGGTAACTTGGAGGCGT
		R: CCGTCGTGTAGTCTGTCTCG
Claudin-1	NM_001244539.1	F: AGAAGATGCGGATGGCTGTC
		R: ACTGGGGTCATGGGGTCATA
β-actin	XM_003124280.5	F: GCCAGAAGGACTCCTACGTG
		R: CATGTCGTCCCAGTTGGTGA

Nrf2, nuclear factor erythroid 2-related factor 2. *HO-1*, heme oxygenase-1. *GPx1*, glutathione peroxidase 1. *SOD1*, superoxide dismutase 1. *SOD2*, superoxide dismutase 2. *CAT*, catalase. *ZO-1*, zonula occludens 1.

Table 2. Effect of oral gavage of *Bacillus subtilis* on the growth performance of IUGR suckling piglets from 3 to 24 days of age[1.]

Items[3]	Groups[2]			SEM	*P* value
	NBW	IUGR	IBS		
IBW (day 3), kg	2.24[a]	1.51[b]	1.53[b]	0.09	< 0.01
FBW (day 24), kg	7.16[a]	5.66[b]	6.73[ab]	0.25	0.04
ADG, g/d	234.10	197.30	247.50	0.01	0.14

[1] Data are expressed as means and SEM, n = 8. Mean values with different letters indicate a significant difference ($P < 0{:}05$).

[2] NBW, normal birth weight; IUGR, intrauterine growth restriction; IBS, IUGR with oral administration of *Bacillus subtilis*.

[3] IBW, initial body weight; FBW, final body weight; ADG, average daily gain.

Table 3. Effect of oral gavage of *Bacillus subtilis* on jejunal morphology of IUGR suckling piglets[1].

Items[3]	Groups[2]			SEM	*P* value
	NBW	IUGR	IBS		
VH, μm	347.53	343.65	389.16	12.18	0.25
CD, μm	200.87	213.55	167.16	9.37	0.11
VH/CD	1.87[b]	1.81[b]	2.43[a]	0.10	< 0.01

[1] Data are expressed as means and SEM, n = 8. Mean values with different letters indicate a significant difference ($P < 0{:}05$).

[2] NBW, normal birth weight; IUGR, intrauterine growth restriction; IBS, IUGR with oral administration of *Bacillus subtilis*.

[3] VH, villus height; CD, crypt depth; VH/CD, the ratio of villus height to crypt depth.

Table 4. Effect of oral gavage of *Bacillus subtilis* on jejunal antioxidant activity in IUGR suckling piglets[1].

Items[3]	Group[2]			SEM	*P* value
	NBW	IUGR	IBS		
T-AOC, U/mg protein	1.39	1.11	1.09	0.06	0.128
T-SOD, U/mg protein	132.80[b]	111.06[c]	167.86[a]	5.75	< 0.01
CAT, U/mg protein	8.22[b]	7.89[b]	9.75[a]	0.22	< 0.01
GSH-Px, U/mg protein	63.99	60.41	58.57	1.05	0.095

[1] Data are expressed as means and SEM, n = 8. Mean values with different letters indicate a significant difference ($P < 0.05$).

[2] NBW, normal birth weight; IUGR, intrauterine growth restriction; IBS, IUGR with oral administration of *Bacillus subtilis*.

[3] T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase.

Table 5. Effect of oral gavage of *Bacillus subtilis* on alpha diversity of jejunal bacteria in IUGR suckling piglets[1].

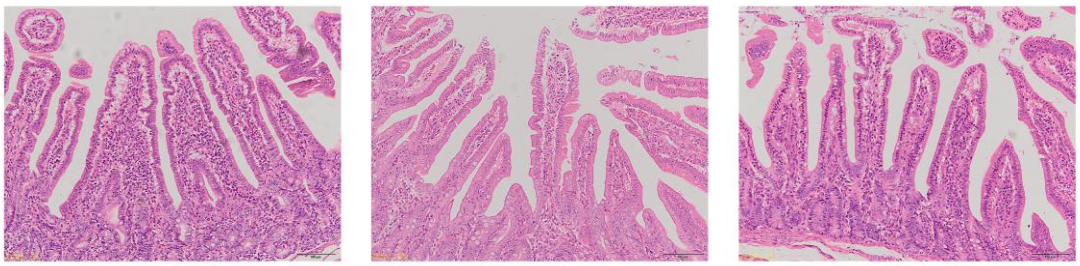
Items[3]	Groups[2]			SEM	*P* value
	NBW	IUGR	IBS		
Sobs index	169.67[a]	163.67[a]	87.33[b]	15.13	0.03
Chao index	253.22	252.79	170.51	22.08	0.24
Ace index	309.43	301.67	292.98	23.52	0.96
Shannon index	0.97	1.31	0.91	0.11	0.37
Simpson index	0.65	0.50	0.64	0.04	0.27

[1] Data are expressed as means and SEM, n = 6. Mean values with different letters indicate a significant difference ($P < 0{:}05$).

[2] NBW, normal birth weight; IUGR, intrauterine growth restriction; IBS, IUGR with oral administration of *Bacillus subtilis*.

[3] Sobs, observed species.

Figure 1



NBW IUGR IBS

Figure 2

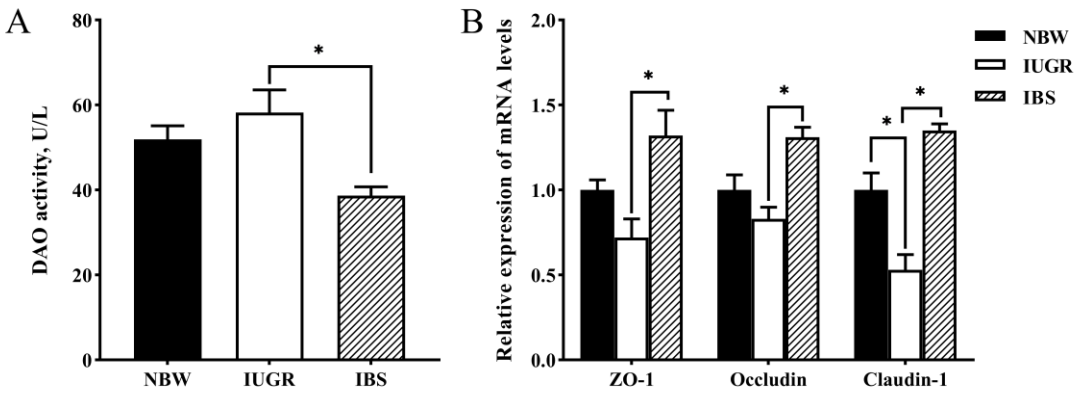


Figure 3

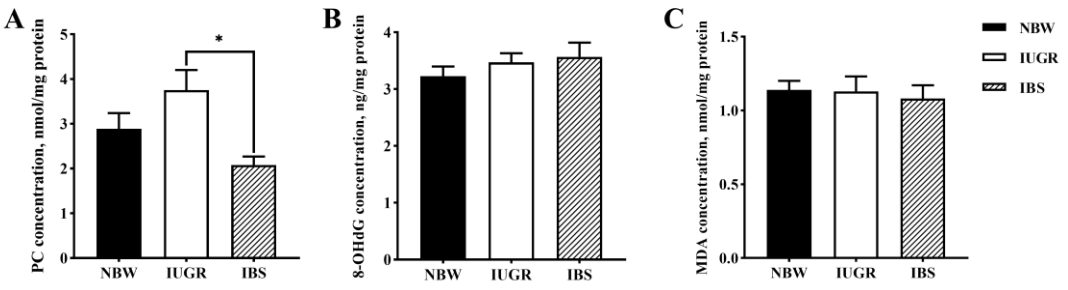


Figure 4

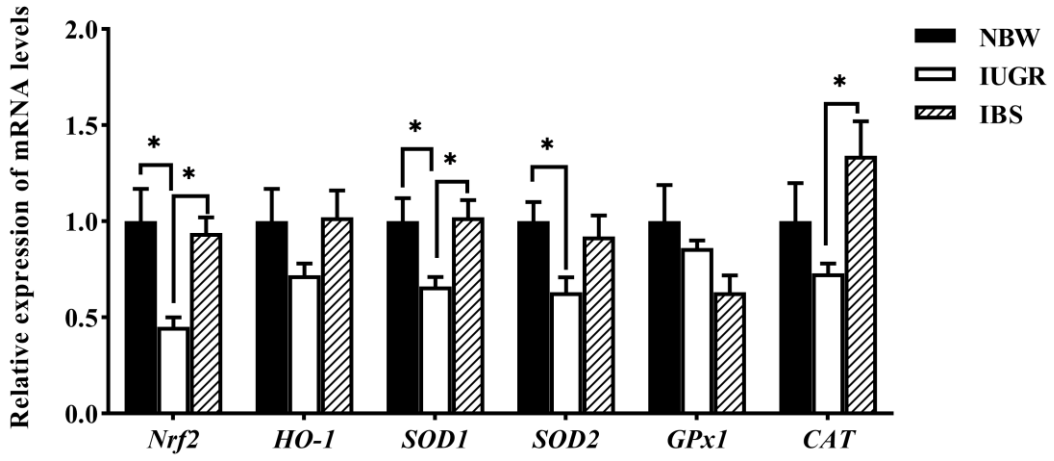


Figure 5

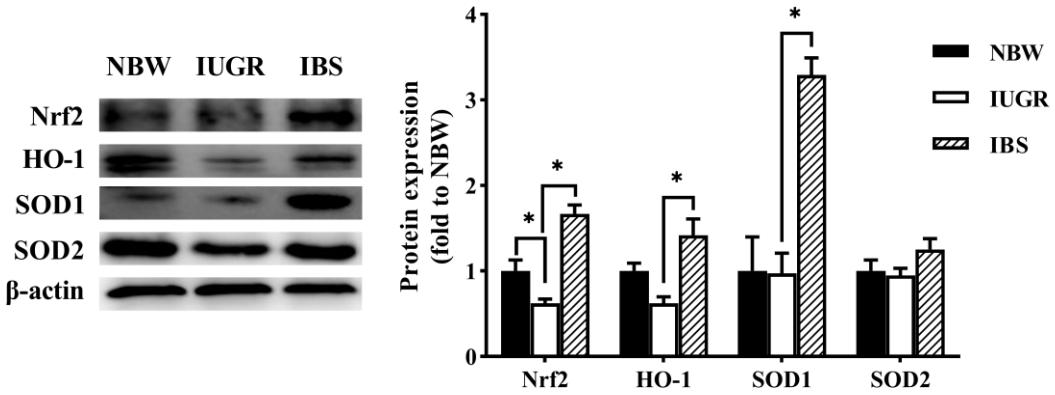


Figure 6

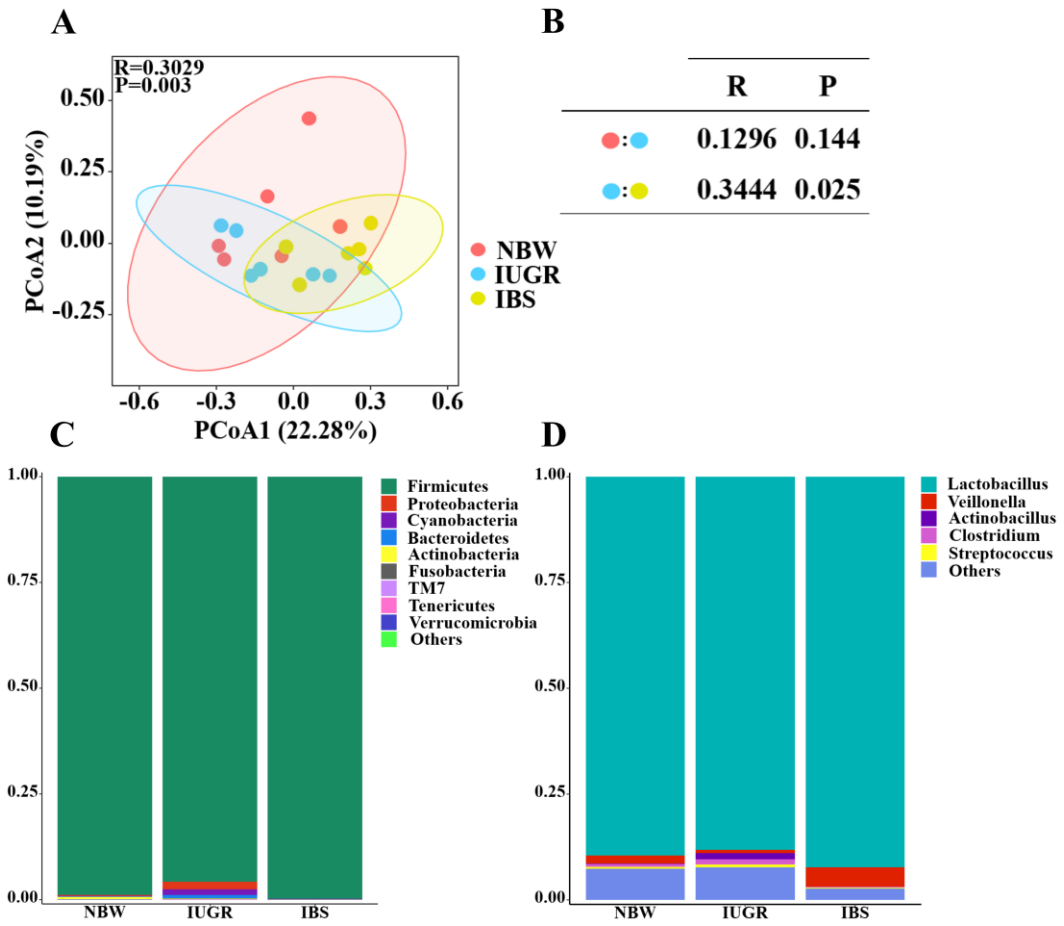


Figure 7