

Effects of *Bacillus subtilis* Supplementation in Sow Diets on Plasma Biochemical Parameters, Fecal Microbiota, and Their Metabolites in Suckling Piglets: Postprint

Authors: Han Li, Xie Peifeng, Zhao Yue, Gao Qiankun, Zhang Wenming, Wang Zhanbin, Yin Yulong, Xiangfeng Kong

Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary supplementation of *Bacillus subtilis* in sows on plasma biochemical parameters, fecal microorganisms, and their metabolites in suckling piglets. Forty healthy Large White sows at day 85 of gestation with 2-4 parities and similar expected delivery dates were selected and randomly divided into 2 groups with 20 sows per group. The control group was fed a basal diet, while the experimental group was fed the basal diet supplemented with 250 g/t of *Bacillus subtilis* preparation. Feeding was conducted from day 85 of gestation until day 21 postpartum. At 7 and 21 days of age, 8 litters were selected from each group, and one piglet with body weight close to the litter average was chosen from each litter for blood collection from the anterior vena cava to determine plasma biochemical parameters; fresh fecal samples were collected to determine microbial counts and contents of short-chain fatty acids and biogenic amines. The results showed that compared with the control group, the experimental group had significantly increased contents of acetic acid and tryptamine ($P < 0.05$), significantly decreased *Escherichia coli* counts ($P < 0.05$), and showed increasing trends in spermidine ($P = 0.068$) and spermine ($P = 0.074$) contents as well as *Lactobacillus/Escherichia coli* ratio ($P = 0.053$) in feces of 7-day-old piglets; the experimental group had significantly decreased plasma urea nitrogen and triglyceride contents as well as fecal spermidine and spermine contents ($P < 0.05$) in 21-day-old piglets. In conclusion, dietary supplementation of *Bacillus subtilis* in sows can regulate plasma biochemical parameters related to lipid metabolism and nitrogen metabolism, reduce intestinal *Escherichia coli* counts, increase intestinal contents of certain biogenic amines and acetic acid, thereby improving intestinal health and growth performance of suckling piglets.

Full Text

Effects of *Bacillus subtilis* Supplementation in Sow Diets on Plasma Biochemical Parameters, Fecal Microorganisms, and Their Metabolites in Suckling Piglets

HAN Li^{1,2}, XIE Peifeng¹, ZHAO Yue^{1,2}, GAO Qiankun², ZHANG Wenming³, WANG Zhanbin², YIN Yulong¹, KONG Xiangfeng^{1*}

¹Key Laboratory of Agro-Ecological Processes in Subtropical Region, Laboratory of Animal Nutritional Physiology and Metabolic Process, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China

²College of Animal Science and Technology, Henan University of Science and Technology, Luoyang 471003, China

³Evonik Degussa (China) Co., Ltd., Beijing 100600, China

Abstract: This experiment investigated the effects of dietary *Bacillus subtilis* supplementation in sows on plasma biochemical parameters, fecal microorganisms, and their metabolites in suckling piglets. Forty healthy Large White sows at day 85 of gestation (parity 2–4) were randomly allocated to two groups (n = 20). Sows in the control group received a basal diet, while those in the experimental group received the basal diet supplemented with 250 g/t of *B. subtilis* preparation from day 85 of gestation through day 21 of lactation. At 7 and 21 days of age, eight litters per group were selected, and one piglet with body weight close to the litter average was chosen from each litter for blood collection via anterior vena cava puncture to determine plasma biochemical parameters. Fresh fecal samples were simultaneously collected to quantify microbial populations and measure short-chain fatty acids and biogenic amines. Compared with the control group, piglets in the experimental group at 7 days of age showed significantly increased fecal acetate and tryptamine concentrations ($P < 0.05$), significantly reduced fecal *Escherichia coli* counts ($P < 0.05$), and trends toward increased spermidine ($P = 0.068$) and spermine ($P = 0.074$) levels as well as a higher *Lactobacillus/E. coli* ratio ($P = 0.053$). At 21 days of age, experimental piglets exhibited significantly lower plasma urea nitrogen and triglyceride concentrations ($P < 0.05$) along with significantly reduced fecal spermidine and spermine contents ($P < 0.05$). In conclusion, maternal supplementation with *B. subtilis* modulated plasma biochemical parameters related to lipid and nitrogen metabolism, reduced intestinal *E. coli* populations, and increased specific biogenic amines and acetic acid in suckling piglets, thereby improving their intestinal health and growth performance.

Keywords: pregnant sows; suckling piglets; *Bacillus subtilis*; biochemical parameters; microorganisms; metabolites

CLC number: S828

Introduction

The health status and growth performance of suckling piglets directly influence the economic efficiency of swine operations. Large-scale pig farms frequently encounter issues such as low birth weight, high stress susceptibility, and difficult weaning transitions in piglets, which can disrupt intestinal microflora balance, impair digestion, stunt growth, and cause diarrhea, ultimately compromising piglet development. Improving the nutritional status and health of periparturient sows can enhance offspring growth, making the development of integrated “sow-piglet” nutritional strategies crucial for promoting piglet health and performance.

Probiotics have gained attention for their ability to improve intestinal microflora balance and nutrient utilization, playing an important role in animal gut health and development. Commonly used probiotics include *Lactobacillus*, *Bifidobacterium*, and *Bacillus* species. Among these, *Bacillus* species are particularly suitable as feed additives due to their spore-forming capability, which confers resistance to acid, alkali, and high temperature/pressure, facilitating production and storage. Our previous research demonstrated that dietary *B. subtilis* supplementation in sows improved protein utilization, enhanced immunity, increased beneficial intestinal bacteria, and elevated fecal biogenic amine and short-chain fatty acid (SCFA) concentrations, thereby improving sow intestinal health and nutritional status. Additionally, sow supplementation significantly reduced offspring diarrhea rates and improved litter performance metrics including birth weight (18.05 vs. 17.07 kg), individual birth weight (1.51 vs. 1.50 kg), weaning weight (63.19 vs. 61.72 kg), individual weaning weight (6.24 vs. 6.15 kg), and average daily gain (0.23 vs. 0.22 kg) compared to controls.

Newborn piglets possess immature digestive and immune systems, making them vulnerable to pathogen invasion and diarrhea. Therefore, ensuring early colonization of beneficial bacteria in neonatal piglet intestines is critical. While numerous studies have investigated *B. subtilis* supplementation in piglets and growing pigs, research on maternal supplementation effects on offspring remains limited. This study evaluated the impact of peripartum sow dietary *B. subtilis* supplementation on plasma biochemical parameters, fecal microorganisms, and their metabolites in suckling piglets to provide evidence for probiotic application in integrated sow-piglet nutritional strategies.

Materials and Methods

1.1 Experimental Design and Animal Management The animal trial was conducted from March to May 2017 at the Yongan Animal Experimental Base of the Institute of Subtropical Agriculture, Chinese Academy of Sciences. Forty healthy Large White sows at day 85 of gestation (parities 2-4) with similar expected farrowing dates were randomly divided into two groups ($n = 20$). The control group received a basal diet, while the experimental group received the basal diet supplemented with 250 g/t *B. subtilis* preparation. Sows were fed

gestation diets from day 85 to day 100, then switched to lactation diets until day 21 postpartum. Piglets in all groups received creep feed without *B. subtilis* supplementation starting at 8 days of age. Dietary nutrient levels met or exceeded NRC (2012) requirements for swine, and basal diet composition was as previously described.

The *B. subtilis* preparation (4×10^8 CFU/g viability) was provided by Evonik Degussa (China) Co., Ltd., with dosage determined based on previous studies and manufacturer recommendations. Sows were transferred to thoroughly disinfected farrowing crates 7 days before their expected farrowing date, and all other management practices followed commercial farm protocols.

1.2 Sample Collection At 7 and 21 days of age, eight litters per group were randomly selected, and one piglet with body weight close to the litter average was chosen from each litter for blood collection via anterior vena cava puncture. Blood was anticoagulated with heparin, centrifuged to separate plasma, and stored at $-20\text{ }^{\circ}\text{C}$ for biochemical analysis. Fresh fecal samples were collected and stored at $-80\text{ }^{\circ}\text{C}$ for microbial and metabolite analyses.

1.3 Plasma Biochemical Parameter Analysis Plasma total protein (TP), albumin (ALB), urea nitrogen (UN), triglycerides (TG), and total cholesterol (TC) concentrations were measured using an automatic biochemical analyzer (Roche cobas® c 311) with commercial kits (Roche) according to manufacturer instructions.

1.4 Fecal Microbial Quantification Fecal microbial DNA was extracted using the QIAamp DNA Stool Mini Kit (QIAGEN, Germany). Microbial populations were quantified by real-time PCR following previously described methods, with results expressed as log (copies/g) of feces. Specific primers for microbial quantification were synthesized by Sangon Biotech (Shanghai) and are listed in .

1.5 Fecal Microbial Metabolite Analysis Fecal concentrations of acetate, propionate, and butyrate were determined by gas chromatography, while tryptamine, tyramine, spermidine, and spermine were measured by liquid chromatography.

1.6 Statistical Analysis Data were initially processed using Excel 2010 and analyzed by independent samples t-test using SPSS 18.0. Results are presented as means \pm standard error. Differences were considered significant at $P < 0.05$ and trends at $0.05 > P > 0.10$.

Results

2.1 Effects on Plasma Biochemical Parameters in Suckling Piglets As shown in , compared with the control group, piglets in the experimental group at 21 days of age exhibited significantly reduced plasma UN and TG concentrations ($P < 0.05$), with no significant differences in other parameters ($P > 0.05$).

2.2 Effects on Fecal Microbial Populations in Suckling Piglets As shown in , compared with the control group, 7-day-old piglets in the experimental group had significantly lower fecal *E. coli* counts ($P < 0.05$) and a trend toward increased *Lactobacillus/E. coli* ratio ($P = 0.053$), with no significant differences in other microbial parameters ($P > 0.05$).

2.3 Effects on Fecal Microbial Metabolites in Suckling Piglets As shown in , compared with the control group, 7-day-old piglets in the experimental group showed significantly increased fecal acetate and tryptamine concentrations ($P < 0.05$) and trends toward elevated spermidine ($P = 0.068$) and spermine ($P = 0.074$) levels. At 21 days of age, experimental piglets had significantly reduced fecal spermidine and spermine concentrations ($P < 0.05$), with no significant differences in other metabolites ($P > 0.05$).

Discussion

Plasma biochemical parameters reflect nutrient metabolism status in animals. Plasma urea nitrogen concentration serves as an indicator of protein metabolism and dietary amino acid balance; reduced UN levels suggest improved amino acid balance, enhanced protein synthesis, or reduced protein catabolism. Plasma triglyceride concentration directly reflects lipid metabolism and adipose tissue development, with lower TG indicating higher lipid utilization efficiency. In this study, maternal *B. subtilis* supplementation significantly decreased plasma UN and TG in 21-day-old piglets, suggesting enhanced lipid and protein utilization. These findings align with our previous observations of improved piglet growth performance, including increased birth weight, weaning weight, and average daily gain. However, other studies reported no significant effects of *B. subtilis* supplementation on serum UN and TG in weaned piglets, possibly due to differences in bacterial strain, viability, supplementation method, or dosage.

Under normal conditions, intestinal microbial communities maintain relative stability. Stress can disrupt this balance, triggering intestinal inflammation, diarrhea, and impaired growth. In the porcine gut, *E. coli* is a primary pathogen causing piglet diarrhea. This study demonstrated that maternal *B. subtilis* supplementation reduced fecal *E. coli* counts and increased the *Lactobacillus/E.*

coli ratio in 7-day-old piglets, indicating improved intestinal microflora balance and reduced diarrhea incidence. This effect may be attributed to increased beneficial bacteria in sow intestines and feces, promoting early colonization of beneficial microbes such as lactobacilli and bacilli in piglet guts while reducing pathogen exposure. Previous research similarly showed that dietary *B. subtilis* in weaned piglets significantly decreased intestinal *E. coli* populations while improving intestinal morphology and nutrient absorption.

Short-chain fatty acids (acetate, propionate, butyrate) are primarily produced through anaerobic fermentation of indigestible carbohydrates and proteins by gut microorganisms. These metabolites provide energy to colonocytes, resist pathogens, regulate microbial balance, and maintain intestinal health. Maternal *B. subtilis* supplementation significantly increased fecal acetate in 7-day-old piglets, which could lower colonic pH, inhibit pathogen proliferation, and stimulate appetite through activation of the parasympathetic nervous system and secretion of insulin and ghrelin, thereby benefiting piglet intestinal health and growth. These effects likely relate to increased proportions of beneficial gut microbes. Previous studies have also reported that *Bacillus* supplementation elevates intestinal SCFA concentrations, reduces pH, and optimizes the gut environment.

Microbial fermentation of nitrogenous compounds in the colon produces biogenic amines including tryptamine, spermine, and spermidine. Tryptamine regulates gastrointestinal motility, secretion, immune function, and antioxidant responses. Spermine and spermidine modulate synthesis of macromolecules such as nucleic acids and proteins, promoting intestinal maturation and absorptive capacity. Maternal *B. subtilis* supplementation significantly increased fecal tryptamine in 7-day-old piglets, potentially promoting intestinal development, digestive function, and immunity, possibly through enhanced microbial catabolism of tryptophan. The study also observed trends toward increased spermine and spermidine at 7 days, but significantly reduced levels at 21 days. This pattern may be related to high biogenic amine concentrations in colostrum and the rapid growth and high nutrient demands of 21-day-old piglets, leading to extensive absorption by intestinal epithelial cells and consequently reduced fecal concentrations. Previous research demonstrated that oral administration of spermine or spermidine to lactating mice promoted intestinal maturation and reduced weaning stress.

Conclusion

Maternal dietary supplementation with *Bacillus subtilis* effectively modulated plasma biochemical parameters related to lipid and nitrogen metabolism, reduced intestinal *Escherichia coli* populations, and increased specific biogenic amines and acetic acid in suckling piglets, thereby improving their intestinal health and growth performance.

References

- [1] LI Chunli, CUI Shuzhen, HUI Canjun, et al. Effects of microecological preparation on growth and immune function of suckling piglets[J]. *China Animal Husbandry & Veterinary Medicine*, 2005, 32(5): 7-10.
- [2] HOU Guanyu, ZHOU Hanlin, SHI Liguang, et al. Research progress on regulation of intestinal microflora in weaned piglets by several common feed additives[J]. *China Animal Husbandry & Veterinary Medicine*, 2016, 43(11): 2939-2944.
- [3] SU Guoqi, WANG Jun, CAO Meng, et al. Effects of nutritional levels of pregnant sows on muscle growth and development of offspring piglets[J]. *Chinese Journal of Animal Nutrition*, 2016, 28(4): 1050-1059.
- [4] LIU Qing, HUANG Hua, TANG Jingchun, et al. Effects of compound probiotics on production performance and intestinal microflora ecology of sows[J]. *Journal of Domestic Animal Ecology*, 2016, 37(2): 72-76.
- [5] ZHANG Aiwu, XUE Jun. Application effects of *Bacillus subtilis* in animal production[J]. *China Animal Husbandry & Veterinary Medicine*, 2011, 38(4): 234-238.
- [6] HAN Li, XIE Peifeng, KONG Xiangfeng, et al. Effects of *Bacillus subtilis* on fecal microbes and their metabolites in periparturient sows[J]. *Chinese Journal of Animal Nutrition*, 2018, 30(3): 201709045 (in press).
- [7] HAN Li, KONG Xiangfeng, ZHAO Yue, et al. Effects of *Bacillus subtilis* on reproductive performance of periparturient sows and growth of offspring[J]. *Chinese Journal of Animal Nutrition*, 2017, 29(12): 4440-4446.
- [8] HUANG Xuequan. Effect of *Bacillus subtilis* preparation on production performance of piglets[J]. *China Animal Husbandry & Veterinary Medicine*, 2010, 37(7): 212-214.
- [9] ZHU Qian, KONG Xiangfeng, JI Yujiao, et al. Effects of high and low nutritional level diets on reproductive performance, body composition and plasma biochemical parameters of pregnant Huanjiang mini-pigs[J]. *Chinese Journal of Animal Nutrition*, 2016, 28(5): 1534-1540.
- [10] JIAO Jinzhen, WANG Pengpeng, TANG Shaoxun, et al. Quantitative distribution of important functional microbes in different gastrointestinal tract segments of Liuyang black goats[J]. *Acta Veterinaria et Zootechnica Sinica*, 2013, 44(10): 1590-1599.
- [11] LI Huawei, SU Jiayi, KONG Xiangfeng, et al. Effects of dietary fermented Chinese medicine residue on fecal microbes and their metabolites in periparturient sows[J]. *Chinese Journal of Animal Nutrition*, 2017, 29(9): 3232-3239.
- [12] YANG Haiying, YANG Zaibin, YANG Weiren, et al. Effects of probiotics and xylooligosaccharides on production performance, digestive enzyme activity,

blood indices and intestinal microflora of weaned piglets[J]. Chinese Journal of Veterinary Science, 2009, 29(7): 914-919.

[13] SUN Mei, ZHANG Yiping, ZHANG Xianzhong, et al. Effects of Bacillus preparation on blood and immune indices of weaned piglets[J]. China Animal Husbandry & Veterinary Medicine, 2015, 42(11): 2991-2998.

[14] LEE S H, INGALE S L, KIM J S, et al. Effects of dietary supplementation with Bacillus subtilis LS 1-2 fermentation biomass on growth performance, nutrient digestibility, cecal microbiota and intestinal morphology of weanling pig[J]. Animal Feed Science and Technology, 2014, 188: 102-110.

[15] LE H D, HUYNH A H, NGUYEN Q U, et al. Intracellular fate and immunogenicity of B. subtilis spores[J]. Vaccine, 2004, 22(15/16): 1873-1885.

[16] HE Guolong, ZHANG Heng, DONG Aihua, et al. Effects of feeding Bacillus subtilis on Bacillus counts in feces and aquaculture water and water quality of grass carp[J]. Freshwater Fisheries, 2012, 42(2): 35-39.

[17] KONG X F, JI Y J, LI H W, et al. Colonic luminal microbiota and bacterial metabolite composition in pregnant Huanjiang mini-pigs: effects of food composition at different times of pregnancy[J]. Scientific Reports, 2016, 6: 37224.

[18] KONG X F, ZHOU X L, LIAN G Q, et al. Dietary supplementation with chitooligosaccharides alters gut microbiota and modifies intestinal luminal metabolites in weaned Huanjiang mini-piglets[J]. Livestock Science, 2014, 160: 97-101.

[19] PERRY R J, PENG L, BARRY N A, et al. Acetate mediates a microbiome-brain- cell axis to promote metabolic syndrome[J]. Nature, 2016, 534(7606): 213-217.

[20] XIN Na, ZHANG Naifeng, DIAO Qiyu, et al. Effects of Bacillus preparation on growth performance and gastrointestinal development of weaned piglets[J]. Acta Veterinaria et Zootechnica Sinica, 2012, 43(6): 901-908.

[21] WANG Xiaocheng, XIONG Xia, YIN Yulong, et al. Effects and mechanisms of exogenous polyamines on intestinal structure and function of weaned piglets[J]. Chinese Journal of Animal Nutrition, 2014, 26(9): 2457-2462.

[22] BLACHIER F, MARIOTTI F, HUNEAU J F, et al. Effects of amino acid-derived luminal metabolites on colonic epithelium and physiopathological consequences[J]. Amino Acids, 2007, 33(4): 547-562.

[23] TAO Qingyan, WANG Kangning. Effects of oral administration of spermidine or spermine during lactation on growth and intestinal development of early-weaned rats[J]. Chinese Journal of Animal Science, 2009, 45(9): 24-27.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv – Machine translation. Verify with original.