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

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Abstract

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

Full Text

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Abstract

This experiment was conducted to investigate the effects of dietary Bacillus subtilis supplementation in sows on plasma biochemical parameters, fecal microbiota, and microbial metabolites in suckling piglets. Forty healthy Large White sows at day 85 of gestation (parity 2–4) with similar expected farrowing dates were randomly allocated into two groups of 20 sows each. 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. The feeding trial lasted from day 85 of gestation to day 21 postpartum. 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 to determine plasma biochemical parameters. Fresh fecal samples were also collected to quantify microbial populations and measure short-chain fatty acids and biogenic amines. The results showed that compared with the control group, the experimental group exhibited significantly increased fecal acetate and tryptamine contents ($P < 0.05$) and significantly decreased Escherichia coli counts ($P < 0.05$) in 7-day-old piglets, along with increasing trends in the Lactobacillus/E. coli ratio ($P = 0.053$) and fecal spermidine ($P = 0.068$) and spermine ($P = 0.074$) contents. In 21-day-old piglets, the experimental group showed significantly reduced plasma urea nitrogen and triglyceride concentrations ($P < 0.05$) as well as significantly lower fecal spermidine and spermine contents ($P < 0.05$). In conclusion, dietary B. subtilis supplementation in sows can modulate plasma biochemical parameters related to lipid and nitrogen metabolism, reduce intestinal E. coli populations, increase certain biogenic amines and acetic acid in the gut, thereby improving intestinal health and growth performance in suckling piglets.

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

Introduction

The health status and growth performance of suckling piglets directly influence the economic efficiency of swine operations. Large-scale pig farms commonly face challenges such as low birth weight, high stress, and difficult weaning transitions, which can disrupt intestinal microbiota balance, impair digestion, cause growth retardation and diarrhea, and severely compromise piglet development. Improving the nutritional status and health of periparturient sows can promote the growth of their offspring, making the development of "mother-offspring integrated" nutritional strategies crucial for enhancing piglet health and growth.

Recent studies have demonstrated that probiotics can improve intestinal microbiota balance and nutrient absorption, playing an important role in animal intestinal health and growth. Commonly used probiotics include Lactobacillus, Bifidobacterium, and Bacillus. Among these, Bacillus can form spores, offering resistance to acid, alkali, and high temperature/pressure, which facilitates production and storage, making it an ideal feed additive. Our previous research found that dietary B. subtilis supplementation in sows improved protein utilization, enhanced immunity, increased beneficial bacterial populations and the contents of biogenic amines and short-chain fatty acids (SCFAs) in feces, and improved intestinal health and nutritional status. Additionally, it significantly reduced offspring diarrhea rates and improved litter birth weight (18.05 vs. 17.07 kg), individual birth weight (1.51 vs. 1.50 kg), litter 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 with the control group.

The digestive and immune systems of newborn piglets are immature, making them susceptible to pathogen invasion and diarrhea. Therefore, ensuring early colonization of beneficial bacteria in newborn piglet intestines is critical. While numerous studies have investigated B. subtilis in piglets and nursery pigs, few have examined its effects on offspring through maternal supplementation. Accordingly, this study evaluated the effects of periparturient dietary B. subtilis supplementation on plasma biochemical parameters, fecal microbiota, and microbial metabolites in offspring piglets to provide a basis for probiotic application in mother-offspring integrated nutrition strategies.

Materials and Methods

Experimental Design and Animal Management

The animal trial was conducted from March to May 2017 at the Yong'an Animal Experimental Base of the Institute of Subtropical Agriculture, Chinese Academy of Sciences. Forty healthy Large White sows at day 85 of gestation (parity 2–4) with similar expected farrowing dates were randomly divided into two groups of 20 sows each. 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 of gestation, then switched to lactation diets until day 21 postpartum. Creep feed without B.

subtilis was provided to piglets from day 8 postpartum. Dietary nutrient levels met or exceeded NRC (2012) requirements for swine. Basal diet composition and nutrient levels were as described previously.

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

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. Blood samples were collected via anterior vena cava using heparinized tubes, centrifuged to separate plasma, and stored at -20°C for biochemical analysis. Fresh fecal samples were collected and stored at -80°C for microbial population and metabolite analysis.

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 the manufacturer' s instructions.

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 the method of Jiao et al., with results expressed as log (copies/g) of feces. Specific primers for microbial quantification were synthesized by Sangon Biotech (Shanghai) Co., Ltd., and primer sequences are listed in .

Fecal Microbial Metabolite Analysis

Fecal contents of acetate, propionate, and butyrate were determined by gas chromatography, while tryptamine, tyramine, spermine, and spermidine were measured by liquid chromatography.

Statistical Analysis

Data were initially processed using Excel 2010 and then analyzed by independent samples t-test using SPSS 18.0 software. Results are presented as means ± standard error. Differences were considered significant at $P < 0.05$ and trends at 0.05 $P < 0.10$.

Results

Effects of Maternal B. subtilis Supplementation on Plasma Biochemical Parameters of Suckling Piglets

As shown in , compared with the control group, the experimental group exhibited significantly reduced plasma UN and TG concentrations in 21-day-old piglets ($P < 0.05$). No significant differences were observed for other parameters ($P > 0.05$).

Effects of Maternal B. subtilis Supplementation on Fecal Microbiota of Suckling Piglets

As shown in , maternal B. subtilis supplementation significantly decreased fecal E. coli counts in 7-day-old piglets ($P < 0.05$) and increased the Lactobacillus/E. coli ratio ($P = 0.053$). No significant differences were observed for other parameters ($P > 0.05$).

Effects of Maternal B. subtilis Supplementation on Fecal Microbial Metabolites of Suckling Piglets

As shown in , compared with the control group, the experimental group showed significantly increased fecal acetate and tryptamine contents in 7-day-old piglets ($P < 0.05$), with increasing trends in spermidine ($P = 0.068$) and spermine ($P = 0.074$) contents. In 21-day-old piglets, fecal spermidine and spermine contents were significantly reduced ($P < 0.05$). No significant differences were observed for other parameters ($P > 0.05$).

Discussion

Plasma biochemical parameters reflect nutrient metabolism in animals. Plasma urea nitrogen content can evaluate protein metabolism and dietary amino acid balance; when amino acid balance is optimal, plasma UN decreases, indicating enhanced protein synthesis or reduced protein catabolism. Plasma triglyceride concentration directly reflects lipid metabolism status and determines adipose tissue development and fat deposition capacity, with lower values indicating higher fat utilization efficiency. In this study, maternal B. subtilis supplementation significantly reduced plasma UN and TG concentrations in 21-day-old piglets, suggesting enhanced fat and protein utilization in suckling piglets. These findings align with previous observations of improved growth performance, including increased birth weight, weaning weight, and average daily gain. However, other studies reported no significant effects of B. subtilis on serum UN and TG in weaned piglets, possibly due to differences in bacterial strain, viability, supplementation method, or dosage.

Under normal conditions, intestinal microbiota composition and populations remain relatively stable. Stress can disrupt this balance, leading to intestinal inflammation, diarrhea, and impaired growth. In the intestinal microbiota, E.

coli is a major 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 microbiota balance and reduced diarrhea rates. This may be attributed to increased beneficial bacteria in sow intestines and feces, promoting early colonization of beneficial bacteria such as Lactobacillus and Bacillus in piglet intestines while reducing exposure to pathogens. Lee et al. reported that dietary B. subtilis supplementation in weaned piglets significantly reduced intestinal E. coli populations and improved intestinal morphology, promoting nutrient absorption.

Short-chain fatty acids (acetate, propionate, and butyrate) are primarily produced by anaerobic fermentation of indigestible carbohydrates and proteins by intestinal microbes. They can be absorbed by the colon to provide energy, resist pathogens, regulate microbiota balance, and maintain intestinal health. This study found that maternal B. subtilis supplementation significantly increased fecal acetate content in 7-day-old piglets, which can lower colonic pH, inhibit pathogen proliferation, and stimulate the parasympathetic nervous system to promote insulin and ghrelin secretion, thereby enhancing appetite and benefiting intestinal health and growth. These effects may be related to increased proportions of beneficial bacteria. Xin et al. also reported that Bacillus supplementation increased intestinal SCFA contents and lowered pH, optimizing the intestinal environment.

Microbial fermentation of nitrogenous substances in the colon produces biogenic amines such as tryptamine, spermine, and spermidine. Tryptamine is an important mediator regulating gastrointestinal motility, secretion, immune function, and antioxidant stress. Spermine and spermidine can regulate macromolecular synthesis of nucleic acids and proteins, promoting intestinal maturation and absorptive function. This study showed that maternal B. subtilis supplementation significantly increased fecal tryptamine content in 7-day-old piglets, which could promote intestinal development, digestive function, and immunity, possibly by enhancing tryptophan catabolism by intestinal microbes. Maternal supplementation also increased spermidine and spermine contents in 7-day-old piglets but significantly decreased them in 21-day-old piglets. This may be related to high biogenic amine concentrations in colostrum. Our previous study found that dietary B. subtilis supplementation significantly increased fecal spermine and spermidine contents in pregnant sows. The rapid growth and high nutritional demands of 21-day-old piglets may lead to increased absorption of these amines by intestinal epithelial cells, resulting in reduced fecal concentrations. Tao et al. demonstrated that oral administration of spermine or spermidine to lactating mice promoted intestinal maturation, reduced early weaning stress, and enhanced the intestinal mucus barrier.

Conclusion

Dietary Bacillus subtilis supplementation in sows can regulate plasma biochemical parameters related to lipid and nitrogen metabolism, reduce intestinal Escherichia coli populations, increase certain biogenic amines and acetic acid in the gut, thereby improving intestinal health and growth performance in suckling piglets.

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