Postprint: Effects of Bacillus subtilis on Fecal Microbiota and Metabolites in Perinatal Sows

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

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Abstract

This experiment aimed to investigate the effects of Bacillus subtilis on fecal microorganisms and their metabolites in perinatal sows. Forty healthy Large White sows with 2-4 parities, similar expected farrowing dates, and at day 85 of gestation were 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 initiated on day 85 of gestation and terminated on day 21 postpartum. Fresh fecal samples were collected from 8 sows per group on days 100 and 112 of gestation and on days 7, 14, and 21 postpartum to determine the counts of microorganisms, short-chain fatty acids (SCFA), and biogenic amines in the feces. The results showed that, compared with the control group: 1) Dietary supplementation of Bacillus subtilis significantly increased the number of lactobacilli, the lactobacilli/E. coli ratio in sow feces on day 7 postpartum, and the number of Firmicutes in feces on day 21 postpartum ($P<0.05$). 2) Dietary supplementation of Bacillus subtilis significantly increased the contents of acetate, isobutyrate, branched-chain fatty acids, and total SCFA in sow feces on day 100 of gestation ($P<0.05$), the contents of valerate, straight-chain fatty acids, isobutyrate, isovalerate, branched-chain fatty acids, and total SCFA in feces on day 7 postpartum ($P<0.05$), and the contents of propionate, butyrate, and straight-chain fatty acids in feces on day 21 postpartum ($P<0.05$); and significantly decreased the content of isovalerate in feces on day 14 postpartum ($P<0.05$). 3) Dietary supplementation of Bacillus subtilis significantly increased the content of cadaverine in sow feces on day 100 of gestation ($P<0.05$), the contents of spermidine and spermine in feces on day 112 of gestation ($P<0.05$), and the contents of cadaverine, tyramine, spermidine, and spermine in feces on day 14 postpartum ($P<0.05$). In conclusion, dietary supplementation of Bacillus subtilis in perinatal sows can increase the number of beneficial bacteria, and the contents of some biogenic amines and SCFA in the intestine, which has beneficial effects on

improving intestinal health and nutritional status of sows.

Full Text

Title and Abstract

Effects of Bacillus subtilis on Fecal Microbes and Their Metabolites in Peripartum Sows

Han Li[1,2], Xie Peifeng[1], Zhao Yue[1,2], Gao Qiankun[2], Zhang Wenming[3], Wang Zhanbin[2], Yin Yulong[1], Kong Xiangfeng[1]*

[1]Laboratory of Animal Nutritional Physiology and Metabolic Process, Key Laboratory of Agro-Ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China
[2]College of Animal Science and Technology, Henan University of Science and Technology, Luoyang 471003, China
[3]Evonik Degussa (China) Co., Ltd., Beijing 100600, China

Abstract: This study aimed to investigate the effects of Bacillus subtilis on fecal microbes and their metabolites in peripartum sows. Forty healthy Large White sows at day 85 of gestation, with 2–4 parities and similar expected farrowing dates, were randomly allocated into two groups of 20 sows each. 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. Fresh fecal samples were collected from eight sows per group on days 100 and 112 of gestation and on days 7, 14, and 21 postpartum to determine microbial populations and the contents of short-chain fatty acids (SCFA) and bioamines. The results showed that, compared with the control group: (1) dietary Bacillus subtilis significantly increased fecal Lactobacillus counts and the Lactobacillus/Escherichia coli ratio on day 7 postpartum, as well as fecal Firmicutes counts on day 21 postpartum ($P<0.05$); (2) it significantly increased fecal acetate, isobutyrate, branched-chain fatty acid, and total SCFA contents on day 100 of gestation ($P<0.05$), valerate, straight-chain fatty acids, isobutyrate, isovalerate, branched-chain fatty acid, and total SCFA contents on day 7 postpartum ($P<0.05$), and propionate, butyrate, and straight-chain fatty acid contents on day 21 postpartum ($P<0.05$), while significantly decreasing fecal isovalerate content on day 14 postpartum ($P<0.05$); and (3) it significantly increased fecal cadaverine content on day 100 of gestation ($P<0.05$), spermidine and spermine contents on day 112 of gestation ($P<0.05$), and cadaverine, tyramine, spermidine, and spermine contents on day 14 postpartum ($P<0.05$). In conclusion, dietary supplementation with Bacillus subtilis during the peripartum period can increase beneficial bacterial populations and the contents of certain bioamines and SCFA in the sow intestine, thereby exerting beneficial effects on intestinal health and nutritional status.

Keywords: peripartum sows; Bacillus subtilis; feces; microbes; metabolites

Introduction

Sow health and reproductive performance directly influence the economic efficiency of pig farms. With increasing intensification, pig farms face issues such as unreasonable feed ingredient combinations, lack of specific nutrients, and feed spoilage [1], which disrupt the gut microbiota balance of sows and reduce nutrient utilization efficiency [2], leading to constipation and reproductive disorders that shorten sow longevity and severely impact the development of the pig industry [3]. Consequently, nutritional strategies to improve sow intestinal health have become a research priority. As live microbial preparations, probiotics can improve intestinal microbiota balance. Commonly used probiotics mainly include Bacillus, Lactobacillus, and yeast [4]. Due to their ability to form spores, Bacillus species are resistant to high temperature, pressure, acids, and alkalis, making them convenient for production and storage [5], and thus the most widely used microbial feed additives. Studies have confirmed that Bacillus can lower intestinal pH in pigs, promote beneficial bacterial proliferation, and prevent intestinal diseases [6]; improve intestinal mucosal morphology in piglets [7]; enhance nutrient utilization and immunity; and consequently promote piglet growth and development [8]. Our previous research found that dietary Bacillus subtilis supplementation increased backfat thickness of sows during gestation days 85-112 and day 21 postpartum, and enhanced immune function [9]; meanwhile, it reduced piglet diarrhea rates and improved litter size, number of liveborn piglets, birth weight, individual birth weight, weaning weight, individual weaning weight, and average daily gain. Peripartum sows are susceptible to various stressors that cause gut microbiota imbalance, adversely affecting piglet birth weight, weaning weight, and litter uniformity. Therefore, maintaining gut microbiota balance is crucial for improving sow reproductive performance. However, few studies have reported on the effects of Bacillus subtilis on fecal microbes and their metabolites in sows. Accordingly, this trial evaluated the effects of dietary Bacillus subtilis supplementation on sow fecal microbes and their metabolites to provide a basis for its application in sow production.

Materials and Methods

1.1 Experimental Animals, Grouping, and Management

The animal feeding 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, with 2-4 parities and similar expected farrowing dates, were randomly allocated into two groups of 20 sows each. 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. Sows were fed gestation diets starting from day 85 of gestation, which were switched to lactation diets on day 100 of gestation until day 21 postpartum. The nutritional levels of the basal diets were formulated according to the NRC (2012) nutrient requirements for swine. The composition and nutrient levels of the basal diets are presented in Table 1 . The Bacillus subtilis preparation used in the trial was provided by Evonik Degussa (China) Co., Ltd. (viable count 4×10 CFU/g), and the supplementation dose was determined based on previous studies [7-8,10] and manufacturer recommendations. During the trial, feed allowance for sows at different gestational stages was adjusted according to body condition to prevent excessive leanness or fatness. The temperature in the sow house was maintained at 16–18°C during gestation days 85–110. Sows were transferred to the farrowing house seven days before the expected farrowing date, where temperature was controlled at 20–24°C and humidity at 60%–80%. Other management practices followed commercial pig farm standards.

1.2 Fecal Microbial Quantification

Fresh fecal samples were randomly collected from eight sows per group on days 100 and 112 of gestation and on days 7, 14, and 21 postpartum, and stored at −80°C. Fecal microbial DNA was extracted using the QIAamp DNA Stool Mini Kit (QIAgen, Germany). Microbial quantitative PCR analysis was performed following the method of Jiao et al. [11] to calculate microbial populations, with results expressed as the logarithm of microbial copy numbers per gram of feces [lg(copies/g)]. Specific primers for absolute quantitative PCR of microbes were synthesized by Shanghai Sangon Biotech Co., Ltd. (Table 2).

1.3 Determination of Fecal Microbial Metabolite Contents

The content of short-chain fatty acids (SCFA) in feces was determined by gas chromatography, while bioamine content was detected by liquid chromatography [12].

1.4 Statistical Analysis

Experimental data were analyzed by independent samples t-test using SPSS 18.0 software and expressed as "mean ± standard error." $P<0.05$ indicated significant difference, while 0.05 $P<0.10$ indicated a trend toward significance.

Results

2.1 Effects of Dietary Bacillus subtilis Preparation on Fecal Microbial Populations in Sows

As shown in Table 3 , compared with the control group, the experimental group exhibited significantly increased fecal Lactobacillus counts and Lactobacillus/Escherichia coli ratio on day 7 postpartum ($P<0.05$). Fecal Firmicutes counts were significantly increased on day 21 postpartum ($P<0.05$), while Bifidobacterium counts showed an increasing trend ($P=0.097$).

2.2 Effects of Dietary Bacillus subtilis Preparation on Fecal SCFA Contents in Sows

As shown in Table 4 , compared with the control group, the experimental group showed significantly increased fecal acetate, isobutyrate, branched-chain fatty acid, and total SCFA contents on day 100 of gestation ($P<0.05$); valerate, straight-chain fatty acids, isobutyrate, isovalerate, branched-chain fatty acid, and total SCFA contents on day 7 postpartum ($P<0.05$); and propionate, butyrate, and straight-chain fatty acid contents on day 21 postpartum ($P<0.05$). Additionally, valerate ($P=0.071$) and isovalerate ($P=0.064$) contents on day 100 of gestation, acetate content on day 7 postpartum ($P=0.051$), and total SCFA content on day 21 postpartum ($P=0.057$) showed increasing trends. In contrast, isovalerate content on day 14 postpartum was significantly decreased ($P<0.05$), while branched-chain fatty acid content showed a decreasing trend ($P=0.066$).

2.3 Effects of Dietary Bacillus subtilis Preparation on Fecal Bioamine Contents in Sows

As shown in Table 5 , compared with the control group, the experimental group exhibited significantly increased fecal cadaverine content on day 100 of gestation ($P<0.05$), with putrescine content showing an increasing trend ($P=0.074$). Spermidine and spermine contents were significantly increased on day 112 of gestation ($P<0.05$). On day 14 postpartum, cadaverine, tyramine, spermidine, and spermine contents were all significantly increased ($P<0.05$).

Discussion

Lactobacillus and Bifidobacterium are dominant bacterial groups in the pig intestine that play important roles in maintaining intestinal health [6]. This study found that Bacillus subtilis increased fecal Lactobacillus, Bifidobacterium, and Firmicutes counts, as well as the Lactobacillus/Escherichia coli ratio in peripartum sows, suggesting improved intestinal microbiota balance and enhanced reproductive performance and offspring intestinal health [9]. These effects may be related to bacteriocins and other antimicrobial substances produced by Bacillus, as well as beneficial microbial metabolites, which is consistent with previous research [10]. Peripartum sows are prone to oxidative stress, which adversely affects litter size and offspring growth [13]. Studies have shown that Bacillus and Lactobacillus possess antioxidant functions that can reduce cellular damage [14-15], suggesting that Bacillus subtilis may improve sow nutritional status and reproductive performance [9], possibly through increased beneficial bacteria and enhanced total antioxidant capacity. Additionally, increased Bifidobacterium counts in sow feces may promote early colonization in newborn piglet intestines, prevent piglet diarrhea, and improve sow constipation and milk quality [16].

Short-chain fatty acids such as acetate, propionate, and butyrate are primarily produced by anaerobic bacteria including Lactobacillus and Bifidobacterium in the colon through fermentation of dietary fiber, resistant starch, and other carbohydrates. They participate in energy supply, regulate intestinal microbiota balance, and improve intestinal function [17]. Specifically, acetate provides energy for intestinal bacteria and mucosa [18]; propionate inhibits cholesterol synthesis and participates in the tricarboxylic acid cycle to supply energy; butyrate is a major metabolite of Firmicutes that provides energy for colonic epithelial cells and promotes cellular metabolism and growth [12]. This study found that Bacillus subtilis increased fecal acetate, propionate, and butyrate contents in peripartum sows, thereby benefiting intestinal health and nutrient metabolism. This may be related to its production of highly active digestive enzymes or its promotion of Lactobacillus and Bifidobacterium proliferation. Research has shown that Lactobacillus can increase SCFA content and butyrate percentage in chicken ceca [19] and promote butyrate production in piglet colon [10]. Duncan et al. [20] reported that Bifidobacterium can promote the conversion of lactate to butyrate. Thus, Bacillus subtilis may improve sow nutritional status and meet energy requirements by increasing beneficial intestinal bacteria, which benefits maternal intestinal health and fetal development. Branched-chain fatty acids are products of leucine, isoleucine, or valine after oxidative deamination and decarboxylation, serving as markers of protein catabolism in the intestinal lumen [21]. This study found that dietary Bacillus subtilis increased branched-chain fatty acid contents in peripartum sow feces, suggesting increased catabolism of these amino acids in the colon, though the physiological significance remains to be elucidated.

Intestinal bioamines are primarily produced by intestinal cells and microbes metabolizing nitrogenous substances. They can alleviate cellular oxidative damage, participate in intestinal mucosal barrier development and maintenance, and play important roles in placental growth and fetal development [22]. Putrescine, cadaverine, spermine, and spermidine are essential components of animal cells that regulate nucleic acid and protein synthesis, while tyramine exhibits significant antioxidant activity [23]. This study demonstrated that dietary Bacillus subtilis increased bioamine contents in peripartum sow feces, possibly because Bacillus, Lactobacillus, or Bifidobacterium can produce amino acid decarboxylases that participate in the catabolism of bioamine precursor amino acids such as lysine, tyrosine, ornithine, and arginine. Lopez-Garcia et al. [24] reported that rat placenta in late gestation requires high concentrations of spermidine, spermine, and putrescine to meet fetal growth demands, and luminal polyamines can be absorbed by the body for cell growth in other organs and tissues [25]. Therefore, we hypothesize that Bacillus subtilis may promote fetal growth and development by increasing bioamine synthesis in the body.

Conclusion

In summary, dietary supplementation with Bacillus subtilis can increase the populations of beneficial bacteria and the contents of certain bioamines and SCFA in the sow intestine, thereby improving intestinal health and nutritional status of peripartum sows.

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