

Effects of *Bacillus subtilis* on Reproductive Performance of Perinatal Sows and Offspring Growth: Postprint

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Abstract

This study was conducted to investigate the effects of *Bacillus subtilis* on the reproductive performance of peripartum sows and the growth of their offspring. Forty healthy Large White sows at gestation day 85 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. The experimental period was from gestation day 85 to postpartum day 21. Sow backfat thickness was measured at gestation days 85 and 112 and postpartum day 21, and backfat loss was calculated. Blood samples were collected from the ear marginal vein at postpartum days 1 and 21, anticoagulated with heparin, centrifuged to separate plasma, and plasma biochemical indices were determined. At postpartum day 1, the total number of piglets born and number of live-born piglets were recorded, and the birth litter weight of piglets was measured. At postpartum day 21, the weaning litter weight of piglets was measured and the daily weight gain of the weaning litter was calculated; diarrhea conditions in each litter were observed and recorded daily, and the diarrhea rate was calculated. The results showed that compared with the control group, dietary supplementation with *Bacillus subtilis* preparation had no significant effect on sow reproductive performance ($P > 0.05$), but significantly reduced the diarrhea rate of piglets at 1-7 d and 1-21 d ($P < 0.05$), significantly increased the plasma immunoglobulin M (IgM) content in sows at postpartum day 21 ($P < 0.05$), and the plasma albumin ($P = 0.087$) and IgM ($P = 0.096$) contents in sows at postpartum day 1 and backfat thickness at postpartum day 21 ($P = 0.096$) all showed an increasing trend. In conclusion, although dietary supplementation with *Bacillus subtilis* preparation did not affect sow reproductive performance, it could enhance protein utilization and immunity in the body and reduce the diarrhea rate in piglets.

Full Text

Effects of *Bacillus subtilis* on Reproductive Performance of Sows and Growth of Their Offspring during Perinatal Period

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Abstract

This study investigated the effects of dietary *Bacillus subtilis* supplementation on reproductive performance of sows and growth of their offspring during the perinatal period. Forty healthy Large White sows (parity 2-4) at day 85 of gestation were randomly allocated into two groups (n=20): a control group fed a basal diet and a treatment group fed the basal diet supplemented with 250 g/t *Bacillus subtilis* preparation. The trial period spanned from day 85 of gestation to day 21 postpartum. Backfat thickness was measured on days 85 and 112 of gestation and on day 21 postpartum to calculate backfat loss. Blood samples were collected from ear veins on days 1 and 21 postpartum using heparin-coated tubes, and plasma was separated by centrifugation for biochemical analysis. Litter size and number of live-born piglets were recorded on day 1 postpartum, along with litter birth weight. On day 21 postpartum, litter weaning weight was measured to calculate daily litter weight gain, and diarrhea incidence was observed daily to determine diarrhea rate. The results showed that compared with the control group, *Bacillus subtilis* supplementation did not significantly affect sow reproductive performance ($P>0.05$) but significantly reduced diarrhea rates in piglets during days 1-7 and 1-21 ($P<0.05$). Additionally, plasma immunoglobulin M (IgM) content in sows on day 21 postpartum was significantly increased ($P<0.05$), while plasma albumin (ALB) ($P=0.087$) and IgM ($P=0.096$) contents on day 1 postpartum and backfat thickness on day 21 postpartum ($P=0.096$) showed increasing trends. In conclusion, dietary *Bacillus subtilis* supplementation, while not affecting sow reproductive performance, enhanced protein utilization and immunity while reducing piglet diarrhea rates.

Keywords: *Bacillus subtilis*; pregnant sows; suckling piglets; reproductive performance; biochemical parameters

Sow production represents a critical component of pig farming, where sow health

and reproductive performance directly influence farm economic efficiency [?]. However, intensive production systems face numerous challenges including poor reproductive performance, inadequate milk quality or agalactia, postpartum anestrus, inflammatory conditions, compromised immunity, reduced feed intake, and shortened productive lifespan, which indirectly lead to low birth and weaning weights, high diarrhea rates, and poor piglet survival [?]. Research indicates that non-infectious factors such as dietary nutrition are primary causes of sub-optimal reproductive performance [?]. While improving dietary nutrition can enhance sow reproductive performance [?], increased feeding costs, low management standards, and improper feed ingredient ratios often limit these benefits. Consequently, nutritional modulation to improve sow reproductive performance has become a research priority.

Probiotics have been shown to improve intestinal microecology, enhance nutrient digestion and metabolism, boost immunity, and promote animal growth [?], leading to widespread application as microecological feed additives in livestock production. Common probiotics include *Bacillus*, lactic acid bacteria, *Bifidobacterium*, enterococci, and yeasts. Among these, *Bacillus* forms spores with strong resistance to heat, pressure, acids, and alkalis, facilitating production and storage, making it one of the most widely applied probiotics [?]. Studies demonstrate that *Bacillus* supplementation in sow or piglet diets increases beneficial gut bacteria, reduces pathogenic bacteria [?], improves piglet exposure to beneficial microbes, decreases diarrhea rates, and enhances litter size, birth weight, and weaning weight [?] while increasing serum immunoglobulin levels [?]. These effects are crucial for improving sow reproductive performance and piglet development.

Most research on *Bacillus subtilis* has focused on nursing and weaning piglets, with limited studies on gestating and lactating sows. Therefore, this trial evaluated the effects of *Bacillus subtilis* supplementation in perinatal sow diets on reproductive performance and offspring growth, while examining metabolic regulatory mechanisms through plasma biochemical parameters to provide evidence for “mother-offspring integrated” nutritional strategies using probiotics in sow production.

1.1 Experimental Animals, Grouping, and 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 (parity 2-4) at day 85 of gestation with similar expected farrowing dates were randomly divided into two groups (n=20). The control group received a basal diet, while the treatment group received the basal diet supplemented with 250 g/t *Bacillus subtilis* preparation. Gestating sow diet was fed from day 85 to 100 of gestation, after which it was replaced with lactating sow diet until day 21 postpartum. Dietary nutrient levels were formulated according to NRC (2012) standards. The *Bacillus subtilis* preparation (provided by Evonik Degussa (China) Co., Ltd.) contained

4.0×10^9 CFU/g, with dosage determined based on previous studies [?] and manufacturer recommendations. The trial period ran from day 85 of gestation to day 21 postpartum. Sows were housed in gestation crates from day 85 to 107, then transferred to farrowing crates 7 days before expected parturition. All other management practices followed commercial pig farm standards.

1.2 Measurement of Sow Reproductive Performance and Backfat Thickness

Backfat thickness was measured using a Renco ultrasound device on days 85 and 112 of gestation and day 21 postpartum to calculate backfat changes before and after farrowing. On day 1 postpartum, total litter size, number of stillbirths, number of live-born piglets, and litter birth weight were recorded to calculate average individual birth weight. On day 21 postpartum, number of weaned piglets and litter weaning weight were recorded to calculate litter weaning weight, litter weight gain, daily litter weight gain, average daily gain, and average individual weaning weight [?].

1.3 Measurement of Piglet Diarrhea Rate

Daily observations and recordings of diarrhea incidence were conducted for each litter to calculate diarrhea rate [?].

1.4 Determination of Sow Plasma Biochemical Parameters

On days 1 and 21 postpartum, blood samples were collected from ear veins of 8 randomly selected sows per group into heparin-coated tubes, and plasma was separated by centrifugation. Plasma total protein (TP), albumin (ALB), blood ammonia (AMM), triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), immunoglobulin G (IgG), and immunoglobulin M (IgM) concentrations, as well as alkaline phosphatase (ALP) activity, were measured using an automatic biochemical analyzer (Roche Cobas® c311) with Roche reagent kits according to manufacturer instructions [?].

1.5 Data Processing and Analysis

Data were initially processed using Excel 2010 and then analyzed using SPSS 18.0 software via independent samples t-test. Results are expressed as “mean \pm standard error.” $P < 0.05$ was considered statistically significant, while $0.05 \leq P < 0.10$ indicated a trend.

2.1 Effects of *Bacillus subtilis* on Sow Reproductive Performance

As shown in Table 2, compared with the control group, the treatment group showed numerical increases in litter size, live-born piglets, litter birth weight, average individual birth weight, litter weaning weight, litter weight gain, average

daily gain, and average individual weaning weight, though differences were not statistically significant ($P>0.05$).

2.2 Effects of *Bacillus subtilis* on Sow Backfat Thickness

Table 3 shows that compared with the control group, the treatment group exhibited increasing trends in backfat thickness on day 21 postpartum ($P=0.096$) and backfat thickness change during days 85-112 of gestation ($P=0.092$).

2.3 Effects of *Bacillus subtilis* on Piglet Diarrhea Rate

Table 4 demonstrates that compared with the control group, the treatment group significantly reduced diarrhea rates during days 1-7 and 1-21 ($P<0.05$), with a decreasing trend during days 15-21 ($P=0.097$).

2.4 Effects of *Bacillus subtilis* on Sow Plasma Biochemical Parameters

Table 5 reveals that compared with the control group, the treatment group significantly increased plasma TP content on day 1 postpartum ($P<0.05$) and plasma IgM content on day 21 postpartum ($P<0.05$), while plasma ALB ($P=0.087$) and IgM ($P=0.096$) contents on day 1 postpartum showed increasing trends. Compared with day 1 postpartum, both groups exhibited significantly increased plasma TP, TC, LDL-C, and IgG contents on day 21 postpartum ($P<0.05$), with significantly decreased plasma ALP activity and AMM content ($P<0.05$). The control group showed significantly decreased plasma IgM content on day 21 postpartum ($P<0.05$), whereas the treatment group showed significantly increased plasma HDL-C content ($P<0.05$) and decreased plasma TG content ($P<0.05$).

Discussion

Sow management constitutes the core of pig production, with reproductive performance being a key determinant of farm profitability. Previous studies have shown that probiotics such as *Bacillus* and lactic acid bacteria can improve sow productivity [?], making investigation of efficient probiotics for “mother-offspring integrated” management highly significant. In this trial, dietary supplementation with 250 g/t *Bacillus subtilis* preparation, while not significantly affecting sow reproductive performance, numerically increased litter size, live-born piglets, litter birth weight, average individual birth weight, litter weaning weight, litter weight gain, average individual weaning weight, and average daily gain, suggesting that *Bacillus subtilis* promoted fetal and nursing piglet development to some extent. Previous research reported that 100 g/t *Bacillus subtilis* in sow diets significantly increased piglet weaning weight [?], differing from our results—possibly due to variations in viable cell counts per gram or strain activity, indicating that optimal dosage requires further investigation. The reduced diarrhea rates during days 1-7 and 1-21 suggest that *Bacillus subtilis* improved

piglet intestinal health, likely by maintaining and adjusting intestinal microecological balance. *Escherichia coli* is a primary pathogen causing diarrhea in nursing piglets, and newborn piglets acquire their gut microbiota primarily from sow feces. Supplementing sow diets with *Bacillus subtilis* can improve sow intestinal health, maintaining environmental *E. coli* and other pathogens at safer levels and reducing piglet exposure, which is crucial for piglet development—consistent with previous findings [?].

Backfat thickness directly reflects sow body condition and energy reserves across physiological stages. During lactation, sows mobilize substantial body reserves to maintain milk production, but excessive mobilization causes severe body condition loss, compromising offspring growth [?]. This study showed that *Bacillus subtilis* supplementation increased backfat thickness on day 21 postpartum and backfat change during days 85–112 of gestation, likely by improving intestinal environment and enhancing nutrient digestion, metabolism, and utilization to meet both sow and fetal requirements, suggesting that *Bacillus* supplementation may promote postpartum body condition recovery.

Plasma biochemical parameters reflect nutritional status and metabolic conditions, where TP and ALB contents indicate protein absorption and metabolism [?]. In this trial, *Bacillus subtilis* supplementation significantly increased plasma TP content on day 1 postpartum and showed an increasing trend in ALB content, suggesting enhanced protein utilization in late gestation. Probiotics interact complexly with intestinal epithelial cells, indigenous microbes, and immune cells [?]. *Bacillus* primarily activates local and systemic immune function while enhancing host digestive capacity by influencing gut microbiota colonization [?]. This trial showed increased plasma IgM content in postpartum sows, with some increase in IgG content on days 1 and 21 postpartum, indicating enhanced immunity—consistent with Li et al. [?].

During lactation, sows undergo various physiological changes to meet piglet developmental needs [?]. This study found that compared with day 1 postpartum, plasma TP, TC, LDL-C, and IgG contents significantly increased on day 21 postpartum, while plasma ALP activity and AMM content significantly decreased, suggesting enhanced metabolic capacity at day 21 postpartum. Additionally, the control group showed significantly decreased plasma IgM content on day 21 postpartum, whereas the treatment group showed no significant changes, indicating that *Bacillus subtilis* supplementation may alleviate nitrogen metabolism and immunity changes induced by lactation. Plasma TG and TC contents directly reflect lipid metabolism status, with lower values indicating higher fat utilization efficiency; HDL-C transports TC to hepatocytes for oxidation [?]. This trial showed that compared with day 1 postpartum, *Bacillus subtilis* supplementation increased plasma HDL-C content and decreased TG content on day 21 postpartum, effects not observed in the control group, suggesting that *Bacillus subtilis* improved lipid metabolism in sows at day 21 postpartum.

In conclusion, dietary *Bacillus subtilis* supplementation, while not significantly affecting sow reproductive performance, enhanced protein utilization and immu-

nity while reducing piglet diarrhea rates.

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