Original Article

Effects of replacing antibiotics with *Bacillus*-based probiotics in the diet of lactating sows on reproductive performance, feed intake, serum immunoglobulin levels, milk production, oxidative stress markers, fecal short-chain fatty acids, and piglet growth performance

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Abstract

This clinical trial investigated the effects of adding a *Bacillus*-based probiotic to the lactation diet of sows (without in-feed antibiotics) on maternal and piglet outcomes. A total of 109 Landrace × Yorkshire sows (parity 1–7) were assigned to either a control group (n = 61) or a treatment group (n = 48) receiving *Bacillus subtilis* – 541 and *Bacillus amyloliquefaciens* – 516. Piglets in the treatment group also received the probiotics via creep feed from day 3 until weaning. Blood samples were collected within 12 h after farrowing to assess serum immunoglobulins (IgG, IgA, and IgM) and malondialdehyde (MDA) in sows, while piglet fecal short-chain fatty acids (SCFAs) were analyzed on days 3, 7, and 21. On average, sows were fed either a standard or probiotic-supplemented lactation diet for 5.9 ± 1.8 days before farrowing and for 21 days postpartum, resulting in a total feeding duration of 26.9 ± 1.8 days. The average daily feed intake was 3.7 ± 1.0 kg/day before farrowing and 6.2 ± 0.7 kg/day during lactation. During the first, second, and third weeks of lactation, average feed intake was 5.4 ± 1.1, 6.4 ± 0.9, and 6.7 ± 0.7 kg/day, respectively. Sows lost 14.5% of their backfat during lactation and produced an average of 8.2 ± 1.3 kg/day of milk from days 3–10 and 10.0 ± 2.1 kg/day from days 10–17. Probiotic supplementation did not affect reproductive traits, milk yield, or piglet growth ($P > 0.05$), but it increased sow feed intake before farrowing ($P = 0.025$) and serum IgM levels ($P = 0.031$). Piglet IgM concentrations were lower on day 7 ($P = 0.002$), while fecal SCFAs were higher on day 7. In conclusion, probiotic supplementation increased sow feed intake before farrowing and elevated serum IgM levels, while transiently enhancing piglet fecal SCFAs, without affecting other performance parameters.

Keywords: immunoglobulin, lactation, milk, probiotic, sow

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Introduction

The swine industry has traditionally depended on antibiotics for treatment, disease prevention, and growth promotion, particularly during farrowing and lactation to reduce economic losses (Jeeraphokhakul *et al.,* 2023; Boonprakob *et al.,* 2024). However, the overuse of antibiotics can disrupt the intestinal microbiota and contribute to antimicrobial resistance, prompting growing consumer concerns (Zeyner and Boldt, 2006; Ross *et al.,* 2010; Chansong *et al.,* 2025). As a promising alternative, probiotics help maintain gut integrity, support microbial balance, and enhance immune function in pigs (Ma *et al.,* 2021; Ngo *et al.,* 2026). When administered at various stages of production, especially during the perinatal and lactation periods (Jeong *et al.,* 2015; Gu *et al.,* 2019), probiotics have been shown to improve sow reproductive performance by enhancing colostrum and milk production, modulating gut microbiota, and strengthening immunity (Saladrigas-García *et al.,* 2022; Innamma *et al.,* 2023; Kiernan *et al.,* 2023; Konieczka *et al.,* 2023). These benefits translate into improved piglet health, reduced mortality, and better overall performance (Innamma *et al.,* 2023).

The growth and survival of suckling piglets rely heavily on colostrum and milk intake from the sow (Declerck *et al.,* 2016; Thongkhuy *et al.,* 2020; Juthamanee and Tummaruk, 2021; Adi *et al.,* 2025), as maternal antibodies are transferred via colostrum rather than through the placenta. Colostrum, which is rich in immunoglobulins, plays a critical role in the early development of the immune system (Juthamanee *et al.,* 2024). Inadequate intake of colostrum or milk increases the risk of health problems, including diarrhea, nutrient malabsorption, and disruption of the gut microbiota, ultimately leading to impaired growth and higher post-weaning mortality (Quesnel *et al.,* 2012; Declerck *et al.,* 2016; Adi *et al.,* 2025).

Bacillus-based probiotics have been shown to enhance the performance of both sows and piglets (Kiernan *et al.,* 2023; Konieczka *et al.,* 2023; Mazur-Kuśnirek *et al.,* 2023). Mazur-Kuśnirek *et al.* (2023) reported that supplementation with *Bacillus subtilis* and *Bacillus amyloliquefaciens* across two reproductive cycles increased sow feed intake, reduced energy loss during lactation, and improved piglet weight gain. In weaning and nursery piglets, these probiotics also help alleviate diarrhea (Konieczka *et al.,* 2023). Despite these benefits, their effects in swine production remain inconsistent, highlighting the need for further investigation. Moreover, the impact of replacing antibiotics with these probiotics under tropical conditions, where sows frequently experience moderate to severe heat stress (Akkhaphan *et al.,* 2025), has not yet been evaluated. In particular, the short-term effects of *Bacillus*-based probiotic supplementation during the transition period from gestation to lactation, as well as during the *ad libitum* feeding phase of lactation, on milk production have never been assessed. Therefore, this study aimed to evaluate the effects of *Bacillus*-based probiotics in lactating sows on reproductive performance, feed intake, serum immunoglobulin levels, milk production, oxidative stress markers, fecal short-chain fatty acids (SCFAs), and piglet growth performance.

Materials and Methods

Animals and experimental design: This study was conducted in accordance with the ethical guidelines of the National Research Council of Thailand and approved by the Institutional Animal Care and Use Committee (Protocol No. 2431008). The trial was performed on a commercial pig farm in central Thailand and involved 109 Canadian Landrace × Yorkshire sows (parity 1–7). The sows were randomly assigned to two groups: a control group (n = 61), which received a standard lactation diet without antibiotic or probiotic supplementation, and a treatment group (n = 48), which received a diet supplemented with *Bacillus subtilis* – 541 and *Bacillus amyloliquefaciens* – 516 for 26.9 ± 1.8 days (range: 24–31 days), starting from 109.5 ± 1.6 days of gestation until weaning at 21 days postpartum. Piglets in the treatment group were also supplemented with the same probiotic from day 3 to 21 of age. The diet contained 1.1×10^9 CFU probiotic per kg feed in both sow feed and creep feed. The probiotics were mixed into the feed at the feed mill in pellet form. Blood samples were collected from sows within 12 h after farrowing (control, n = 26; treatment, n = 23) and from piglets on days 7 (control, n = 29; treatment, n = 30) and 14 (control, n = 30; treatment, n = 30) of lactation. Immunoglobulin levels (IgG, IgA, and IgM) were measured using ELISA, serum malondialdehyde (MDA) was determined using the thiobarbituric acid–reactive substances (TBARS) assay, and piglet fecal SCFAs were analyzed on days 3, 7, and 21.

General management and feeding: Pregnant gilts and sows were group-housed at a density of 280 animals per pen, each equipped with six automated feeders. Daily feed allowances were adjusted according to gestational stage: 1.8–2.0 kg during weeks 1–5, 2.0–2.2 kg during weeks 6–12, and 3.0–3.5 kg during weeks 13–15. At 109.5 ± 1.6 days of gestation, animals were moved to a farrowing facility with an evaporative cooling system. Each farrowing pen (4.7 m²) had plastic slatted flooring and adjustable swing hinges that were kept open before farrowing to permit nest-building behavior and closed afterward to reduce piglet crushing. Individual crates measured 1.80 × 0.60 × 0.90 m, providing 1.08 m² per sow (Dumniem *et al.,* 2023). The average barn temperature and relative humidity during the study were 28.2 ± 1.6 °C (26.4–30.7 °C) and 85.7 ± 7.8%, respectively.

During the transition period (7 days before to 7 days after farrowing), gilts and sows were hand-fed a commercial lactation diet (3.0–4.0 kg/day) three times daily at 07:00, 11:00, and 16:00. Individual feed intake was calculated as the difference between feed offered and dried refusals collected 1 h after the final feeding. From the day after farrowing, sows received 3.0 kg/day, increasing by 0.5 kg/day until *ad libitum* intake (up to 6.0 kg/day) was reached by the end of the first lactation week. Thereafter, feed was provided *ad libitum* during the second and third weeks. The basal diet met National Research Council (NRC, 2012) requirements and contained 18.1% crude protein, 3,239

kcal/kg metabolizable energy, and 1.25% lysine, without added organic acids, polysaccharides, excessive zinc or copper, or probiotics. Feed was pelleted, kept dry, and manually delivered. From 109.5 ± 1.6 days of gestation until weaning, sows in the treatment group received the same diet supplemented with probiotics (*Bacillus subtilis* – 541 and *Bacillus amyloliquefaciens* – 516), while controls received the basal diet. Water was available *ad libitum* to all sows and piglets throughout the study.

After farrowing, all sows received an intramuscular injection of an anti-inflammatory drug (ketoprofen, 6 mg/kg; Bezter Ketofen Tec 100®, Siam Bioscience Co., Ltd., Nonthaburi, Thailand) and an antibiotic (amoxicillin, 10 mg/kg; Vetrimoxin L.A.®, Ceva Santé Animale, Libourne, France). From day 3 postpartum, piglets were offered creep feed, with those in the treatment group receiving feed supplemented with probiotics. On day 2, piglets received an intramuscular injection of iron dextran (200 mg/piglet; Bezter Irondex 100®, Thainaoka Pharmaceutical Co., Ltd., Samut Sakhon, Thailand) and underwent teeth clipping. Cross-fostering was performed within 24–48 h after birth to standardize litter size to 12 piglets. Cross-fostering was kept to a minimum and performed only within each treatment group after colostrum intake assessment. Piglet characteristics, birth weight, vitality, and overall health were carefully considered. Only healthy piglets of similar weight were transferred to maintain uniformity and minimize competitive disadvantages, while weak or very small piglets were not cross-fostered. This approach ensured appropriate consideration of piglet characteristics and minimized potential confounding effects on post-fostering performance. On day 3, piglets were administered oral toltrazuril (20 mg/kg of 5% toltrazuril; Better Pharma Co., Ltd., Lopburi, Thailand) and castrated.

During gestation, gilts and sows were vaccinated every 4 months against foot-and-mouth disease (AFTOPOR®, Merial SAS, Lyon, France) and Aujeszky's disease virus (Porcilis® AD Begonia, Merck Animal Health, Madison, USA) as part of a comprehensive herd vaccination program. Vaccinations were withheld for 7 days before farrowing. Two weeks after farrowing, sows were vaccinated against classical swine fever (Coglapest®, Ceva-Phylaxia Veterinary Biologicals Co., Ltd., Budapest, Hungary) and porcine parvovirus–leptospira–erysipelas (Eryseng®, Laboratorios Hipra S.A., Amer, Girona, Spain). Piglets were vaccinated against *Mycoplasma hyopneumoniae* (Hyogen®, Ceva Santé Animale S.A., Libourne, France) at 18–22 days of age.

Data collection: Backfat thickness and body condition score were measured at entry into the farrowing unit (109.5 ± 1.6 days of gestation) and again at weaning (21 days of lactation) using A-mode ultrasonography (Renco Lean-Meater®, Minneapolis, MN, USA). The degree of backfat loss during lactation was categorized as <10%, 10–20%, or >20% (Tummaruk, 2013). Litter performance data included the total number of piglets born (TB), number of piglets born alive (BA), stillbirth rate (SB), and percentage of mummified fetuses (MF). Colostrum samples were collected within 3 h postpartum, and IgG concentrations were estimated using a Brix refractometer (Brix PAL-1, ATAGO, Tokyo, Japan) (Hasan *et al.,* 2016). Milk yield was estimated for days 3–10 and 10–17 of lactation using the predictive equations of Hansen *et al.* (2012), and values were compared between control and treatment groups. After weaning, the weaning-to-service interval and conception rate were recorded.

Piglet performance evaluation: Piglet body weight was recorded on days 1, 3, 7, 14, and 21 using a digital scale (SDS Digital Scale Co., Ltd., Yangzhou, China). Litter weight was calculated as the sum of individual piglet weights, and average daily gain (ADG, g/day) was determined from day 3 to day 21. Fecal consistency was visually assessed on days 3, 7, 14, and 21 using a 5-point scale (1–5). Creep feed intake per litter was measured on day 21, and the average feed consumption per piglet was calculated accordingly. Mortality rate was determined by comparing the number of live piglets at cross-fostering (day 3) with those remaining at weaning (day 21). The coefficient of variation (CV) of piglet body weight within each litter was calculated on days 1, 3, 7, 14, and 21. To minimize the risk of cross-contamination, feeding and sample collection for the control group were conducted before those for the treatment group. Separate equipment and materials were used for each group. Piglets from the control group were weighed first, and the weighing scale was thoroughly cleaned between groups to prevent fecal contamination.

Samples collection and assays: On the day of farrowing, a 6 ml blood sample was collected from each sow within 12 h after parturition via the jugular vein using an 18-gauge needle. Blood samples from piglets were collected at 7 and 14 days of age. All samples were drawn into Vacutainer tubes without anticoagulant, centrifuged at 2500 × g for 10 min, and stored at −20 °C until analysis. Concentrations of immunoglobulins (IgG, IgA, and IgM) were determined using ELISA kits (Bethyl Laboratories, Inc., Montgomery, TX, USA). Serum samples (100 µl) were incubated with detection antibodies, HRP conjugate, and TMB substrate, and absorbance was measured at 450 nm. The inter- and intra-assay coefficients of variation were 1.1% and 3.5% for IgG, 3.0% and 3.2% for IgA, and 2.0% and 3.1% for IgM, respectively.

Serum malondialdehyde (MDA) concentrations in sows were determined using a TBARS assay kit (Cell Biolabs, Inc., San Diego, CA, USA) as an indicator of oxidative stress. Briefly, 100 µl of serum was mixed with SDS lysis solution, incubated, treated with TBA reagent, and heated at 95 °C for 60 min. After cooling and centrifugation, the butanol-extracted supernatant was analyzed spectrophotometrically at 532 nm. Fecal samples from piglets were collected using sterile swabs, and SCFAs were analyzed following a modified method of Agarwal *et al.* (2009). Samples were mixed with metaphosphoric acid, centrifuged, filtered, and the supernatant was analyzed by gas chromatography equipped with a flame ionization detector (FID).

Statistical analysis: Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics, including means, standard deviations, and ranges, were obtained using the MEANS procedure, while categorical variables were analyzed using the FREQ procedure. Pearson's correlation analysis was used to evaluate associations among litter traits, backfat thickness, feed intake, and reproductive performance in sows.

Reproductive traits, litter characteristics, backfat thickness, backfat loss, feed intake, piglet birth weight, litter weight on day 3, body weight variation, milk yield, Brix values, and weaning-to-service interval were analyzed using the general linear model (GLM) procedure, with individual sows as the experimental units (n = 109). The model included fixed effects of treatment group (control vs. treatment), parity group (primiparous vs. multiparous), and their interaction. For litter weights on days 7, 14, and 21, as well as piglet weight on day 21 and average daily gain (ADG) from day 3 to day 21, litter weight on day 3 was included as a covariate. Least-square means were compared using the least significant difference (LSD) test.

Milk yield and lactation feed intake were analyzed according to backfat loss categories (<10, 10–20, >20%) using analysis of variance (ANOVA) within the GLM procedure. Serum concentrations of IgG, IgA, and IgM in sows and piglets, as well as serum MDA levels in sows, were also analyzed using the GLM procedure, with treatment, parity, and their interaction included in the model. Statistical significance was declared at $P < 0.05$, and $0.05 \leq P < 0.10$ was considered a tendency.

Results

Sows were fed either a standard or probiotic-supplemented lactation diet for an average of 5.9 ± 1.8 days prior to farrowing (range: 3–10 days) and for 21 days postpartum, resulting in a total feeding duration of 26.9 ± 1.8 days (range: 24–31 days). The average daily feed intake was 3.7 ± 1.0 kg/day before farrowing and 6.2 ± 0.7 kg/day throughout lactation. During the first, second, and third weeks of lactation, average feed intake was 5.4 ± 1.1, 6.4 ± 0.9, and 6.7 ± 0.7 kg/day, respectively. Sows lost 14.5% of their backfat during lactation and produced an average of 8.2 ± 1.3 kg/day of milk from days 3–10 and 10.0 ± 2.1 kg/day from days 10–17.

Sow parameters: Reproductive performance, including litter characteristics and backfat thickness, did not differ between control and treatment groups (Table 1). Backfat loss during lactation was also similar ($P = 0.831$). Sows with >20% backfat loss produced more milk from days 10 to 17 of lactation than those with <10% loss (10.5 ± 0.3 vs. 9.4 ± 0.3 kg/day, $P = 0.024$), while sows with 10–20% loss showed intermediate values (10.0 ± 0.3 kg/day, $P > 0.05$). Feed intake during lactation was unaffected by backfat loss category ($P = 0.195$).

As shown in Table 2, sows in the treatment group consumed more feed before farrowing than controls ($P = 0.025$), whereas lactation feed intake and piglet creep feed intake did not differ ($P > 0.05$). Milk yield and colostrum IgG concentrations (Brix values) were comparable between groups (Table 3). However, serum IgM levels were higher in sows from the treatment group ($P = 0.031$), while IgG, IgA, and MDA concentrations showed no differences ($P > 0.05$).

Table 1 Reproductive data of sows on a standard lactation diet (control) vs. the same diet with probiotic supplementation (treatment) from 109.5 ± 1.6 days of gestation to weaning at 21 days (Least-square means ± SEM).

Variables	Control	Treatment	*P* value
Number of sows	61	48	-
Parity number (means ± SD)	3.6 ± 1.5	3.3 ± 1.8	-
Gestation length (day)	115.4 ± 0.3	115.0 ± 0.2	0.157
Backfat thickness at 109 days of gestation (mm)	22.1 ± 0.9	21.3 ± 0.7	0.494
Total number of piglets born per litter	12.4 ± 0.3	12.6 ± 0.3	0.546
Number of piglets born alive per litter	11.1 ± 0.2	11.1 ± 0.2	0.980
Stillborn piglets (%)	7.6	7.6	0.993
Mummified fetuses (%)	2.1	4.8	0.202
Piglet birth weight (kg)	1.42 ± 0.01	1.29 ± 0.01	< 0.001
Backfat thickness at weaning (mm)	18.7 ± 0.9	18.2 ± 0.7	0.690
Backfat loss (%)	-14.4 ± 3.4	-13.5 ± 2.8	0.831
Weaning-to-service interval (days)	8.4 ± 1.4	10.6 ± 1.2	0.242
Sows mated within 7 days after weaning (%)	49.1	51.2	0.840
Conception rate (%)	92.9	90.7	0.677

Table 2 Average daily feed intake of sows before and after farrowing (1–7, 8–14, 15–21 days) and piglet creep feed intake at 21 days, compared between control and probiotic-supplemented sows (Least-square means ± SEM).

Variable	Control	Treatment	*P* value
Sows			
Average daily feed intake before farrowing (kg/sow/day)	3.26 ± 0.2	3.86 ± 0.2	0.025
Average daily feed intake after farrowing (kg/sow/day)			
- 1 – 7 days	5.52 ± 0.2	5.51 ± 0.2	0.989
- 8 – 14 days	6.48 ± 0.2	6.13 ± 0.2	0.127
- 15 – 21 days	6.67 ± 0.1	6.52 ± 0.1	0.423
Piglets			
Creep feed consumption per piglet at day 21 of lactation (g)	75.6 ± 4.2	70.7 ± 3.5	0.364

Table 3 Milk yield, colostrum Brix value within 3 h after the onset of farrowing, and serum MDA and immunoglobulin concentrations at farrowing in control and probiotic-supplemented sows from day 109.5 ± 1.6 of gestation to weaning at 21 days (least-square means ± SEM).

Variable	Control	Treatment	*P* value
Milk yield (kg/day) *			
- Day 3–10	8.1 ± 0.2	8.0 ± 0.2	0.773
- Day 10–17	10.1 ± 0.3	9.8 ± 0.3	0.513
Colostrum brix value (%)	26.7 ± 6.4	30.0 ± 5.6	0.696
Serum MDA (μM)	4.86 ± 0.89	3.53 ± 0.79	0.271
Serum IgG (mg/mL)	10.07 ± 0.74	10.24 ± 0.65	0.860
Serum IgA (mg/mL)	1.41 ± 0.23	1.44 ± 0.20	0.914
Serum IgM (mg/mL)	4.10 ± 0.36	5.19 ± 0.32	0.031

* Individual sow milk yield was estimated using Hansen *et al*. (2012). For days 3–10: 1.93 + 0.07 × (litter size - 9.5) + 0.04 × (daily litter gain, kg - 2.05). For days 10–17: 2.23 + 0.05 × (litter size - 9.5) + 0.23 × (daily litter gain, kg - 2.05).

Piglet parameters: As shown in Table 4, the number of live piglets per litter on days 3, 7, 14, and 21 did not differ between control and treatment groups ($P > 0.05$). Litter weight on day 3 tended to be lower in the treatment group ($P = 0.082$), while no differences were observed thereafter. On day 7, piglets from the treatment group showed a tendency toward higher serum IgA ($P = 0.058$) and lower IgM ($P = 0.001$) levels, whereas IgG concentrations were similar between groups at both sampling times ($P > 0.05$).

Table 5 shows that total fecal SCFAs were higher in treatment piglets on day 7 ($P = 0.011$), accompanied by a greater proportion of acetate ($P = 0.039$). Butyrate levels tended to be higher in control piglets on days 3 ($P = 0.082$) and 7 ($P = 0.080$), with no other significant differences in SCFAs detected on days 3 or 21.

Table 4 Piglet performance from control and probiotic-supplemented sows (109.5 ± 1.6 days of gestation to weaning at 21 days) (Least-square means ± SEM).

Variables	Control	Treatment	*P* value
Number of lived piglets			
- Day 3	11.8 ± 0.4	11.8 ± 0.4	0.899
- Day 7	11.3 ± 0.4	11.2 ± 0.3	0.863
- Day 14	11.0 ± 0.4	11.1 ± 0.3	0.985
- Day 21	10.9 ± 0.4	10.8 ± 0.4	0.758
Litter weight (kg)			
- Day 3	20.1 ± 0.8	18.3 ± 0.7	0.082
- Day 7	25.7 ± 0.8	26.4 ± 0.7	0.507
- Day 14	40.5 ± 1.5	40.9 ± 1.3	0.845
- Day 21	52.7 ± 2.4	55.0 ± 2.1	0.480
Piglet body weight at 3 days of age (kg)	1.71 ± 0.1	1.66 ± 0.1	0.511
Piglet body weight at 21 days of age (kg)	4.9 ± 0.2	4.9 ± 0.2	0.929
Average daily weight gain of piglets from 3 to 21 days of age (g/d)	176.7 ± 10.8	180.9 ± 9.2	0.769
Litter weight gain from 3 to 21 days of age (kg)	32.8 ± 2.4	35.2 ± 2.1	0.458
Coefficient of variation of piglet body weight within the litter (%)			
- Day 1	18.9 ± 1.0	19.8 ± 0.9	0.455
- Day 3	19.6 ± 1.4	21.5 ± 1.1	0.289
- Day 7	21.2 ± 1.2	21.8 ± 1.0	0.714
- Day 14	23.1 ± 1.7	24.5 ± 1.4	0.500
- Day 21	23.3 ± 1.8	21.6 ± 1.5	0.443
Piglet fecal score from 3 to 21 days of lactation	4.0 ± 0.1	3.9 ± 0.1	0.254
- Day 3	4.3 ± 0.1	4.1 ± 0.1	0.231
- Day 7	4.1 ± 0.1	4.0 ± 0.1	0.723
- Day 14	3.9 ± 0.1	3.8 ± 0.1	0.951
- Day 21	3.8 ± 0.1	3.7 ± 0.1	0.430
Piglet serum IgG (mg/ml)			
- Day 7	13.45 ± 1.07	12.33 ± 1.06	0.462
- Day 14	7.23 ± 0.48	6.81 ± 0.48	0.532
Piglet serum IgA (mg/ml)			
- Day 7	1.23 ± 0.23	1.88 ± 0.23	0.058
- Day 14	0.11 ± 0.01	0.13 ± 0.01	0.423
Piglet serum IgM (mg/ml)			
- Day 7	0.68 ± 0.07	0.36 ± 0.07	0.001
- Day 14	0.33 ± 0.05	0.32 ± 0.05	0.948
Piglet mortality from 3 to 21 days of lactation (%)	6.7 ± 2.8	7.9 ± 2.4	0.758
Number of piglets at weaning per litter	10.9 ± 0.4	10.8 ± 0.4	0.758

Table 5 SCFAs levels (mM/L), acetate (%), propionate (%), and butyrate (%) in piglet feces at 3, 7, and 21 days from control and probiotic-supplemented sows (109.5 ± 1.6 days of gestation to weaning at 21 days) (Least-square means ± SEM).

Variable	Control	Treatment	*P* value
Day 3			
SCFAs	64.4 ± 14.5	70.6 ± 14.5	0.767
Acetate	50.7 ± 4.9	62.7 ± 4.9	0.101
Propionate	10.0 ± 1.6	12.0 ± 1.6	0.394
Butyrate	39.3 ± 5.5	25.3 ± 5.5	0.082
Day 7			
SCFAs	21.2 ± 1.9	28.7 ± 1.9	0.011
Acetate	65.0 ± 2.0	71.2 ± 2.0	0.039
Propionate	25.4 ± 1.3	22.3 ± 1.3	0.122
Butyrate	9.6 ± 1.2	6.4 ± 1.2	0.080
Day 21			
SCFAs	30.4 ± 2.6	24.5 ± 2.6	0.131
Acetate	49.3 ± 2.0	52.4 ± 2.0	0.298
Propionate	37.8 ± 1.4	34.7 ± 1.4	0.136
Butyrate	12.9 ± 1.6	12.9 ± 1.6	0.987

Discussion

Feed intake: In the present study, sows receiving *Bacillus*-based probiotics exhibited higher feed intake before farrowing than controls, suggesting that probiotic supplementation may enhance appetite or feed utilization efficiency. This observation is consistent with previous studies reporting improved feed intake following prolonged *Bacillus*-based probiotic use (Mazur-Kuśnirek *et al.,* 2023) and may be associated with increased nutrient digestibility and absorption (Lan *et al.,* 2017; Hu *et al.,* 2021). Since voluntary feed intake typically declines during late gestation, enhanced consumption during this critical phase may help meet the sow's increasing energy demands and support colostrum production (Feyera *et al.,* 2021; Adi *et al.,* 2024b; Taechamaeteekul *et al.,* 2025).

Sows supplemented with probiotics for approximately one week before farrowing consumed about 600 g more feed per day than controls, yet this increase did not affect backfat thickness or piglet birth weight. The absence of changes in these parameters may be attributed to the short supplementation duration, which was likely insufficient to influence body composition or fetal growth. During late gestation, additional dietary energy is primarily utilized for maternal maintenance and colostrum synthesis rather than fat deposition, while fetal growth is largely governed by placental efficiency and nutrient transfer capacity, which are less responsive to short-term nutritional interventions. Overall, the improvement in feed intake indicates that *Bacillus*-based probiotics may promote gastrointestinal health and appetite regulation. Further studies involving longer supplementation periods or higher inclusion levels are warranted to clarify their potential effects on maternal metabolism, lactation performance, and neonatal development.

Reproductive performance: This study evaluated the reproductive performance of sows fed either a standard lactation diet or the same diet supplemented with a *Bacillus*-based probiotic blend from late gestation to 21 days postpartum. Reproductive outcomes on the day of farrowing did not differ significantly between groups, supporting our hypothesis that short-term probiotic supplementation before farrowing does not influence the total number of piglets born, the number born alive, or the incidence of stillborn or mummified piglets. These outcomes are largely determined by previous stages of gestation and parturition, as well as factors such as farrowing duration, rather than by short-term probiotic use. However, probiotic supplementation may influence sow feed intake, which could potentially benefit piglets if supplementation is continued after farrowing. Both groups exhibited backfat loss during lactation, 14.4% in the control group and 13.5% in the probiotic group, with no significant difference. Overall, the variable efficacy of probiotics may depend on several factors, including herd health status, management practices, breed, stress levels, and general farm conditions (Jeong *et al.,* 2015; Konieczka *et al.,* 2023).

In the present study, sows supplemented with probiotics exhibited a slightly longer weaning-to-service interval compared with controls, an unexpected outcome given the generally positive influence of probiotics on reproductive performance. This may reflect several interacting factors rather than a direct probiotic effect. Variations in feed intake, litter size, or environmental stress during lactation could have affected post-weaning ovarian recovery, as energy balance plays a crucial role in the timing of estrus resumption. Moreover, the short supplementation period (approximately 1 week before farrowing) was likely insufficient to induce sustained metabolic or hormonal changes during lactation or after weaning. Previous studies reporting a shortened weaning-to-service interval in probiotic-treated sows generally involved longer feeding durations (≥4 weeks) or higher inclusion levels (Kiernan *et al.,* 2023; Konieczka *et al.,* 2023). Overall, these findings suggest that short-term *Bacillus* supplementation before farrowing may enhance certain metabolic and immune responses without markedly influencing post-weaning reproductive recovery, emphasizing the need for longer supplementation protocols to fully assess potential reproductive benefits.

Milk yield: This study found no significant differences in milk yield, colostrum quality, or estimated IgG levels between control and probiotic groups. These results are consistent with previous reports on *Bacillus*-based probiotics (Innamma *et al.,* 2023; Konieczka *et al.,* 2023). Nevertheless, probiotics may still affect the

microbial composition of colostrum and milk, potentially influencing piglet gut microbiota through the entero-mammary pathway, which contributes to immune development and nutrient absorption (Rodríguez, 2014; Menegat *et al.,* 2020).

A relationship was also observed between backfat loss and milk production in lactating sows. Sows that lost more than 20% of their backfat produced higher milk yields between days 10 and 17 compared with those that lost less than 10%, suggesting that fat mobilization supports energy demands when feed intake is inadequate (Thongkhuy *et al.,* 2020). This pattern has become more pronounced with the increased use of hyperprolific sow lines (Tummaruk *et al.,* 2023; Adi *et al.,* 2024a; Fusapniran *et al.,* 2026). Sows with 10–20% backfat loss exhibited intermediate milk yields, indicating that moderate fat mobilization may sustain lactation without excessive reliance on body reserves. As feed intake did not differ significantly among groups, the higher milk yield in sows with greater fat loss likely resulted from increased utilization of body energy stores (Khamtawee *et al.,* 2021). These findings highlight the importance of managing body condition and energy balance during lactation. While enhanced fat mobilization may improve milk output, excessive depletion could compromise sow health, subsequent reproductive performance, and longevity. Further studies are warranted to define optimal backfat loss thresholds and to evaluate the long-term effects of probiotic supplementation on gut health, nutrient digestibility, and milk production.

Sow serum immunoglobulins and MDA: The short duration of probiotic supplementation (approximately 1 week before farrowing) in the present study may explain the lack of a marked effect on IgA and IgG concentrations. Previous studies have shown that, to elicit a measurable immune-enhancing effect, probiotic supplementation generally needs to be provided for a longer period, typically at least 4 weeks before farrowing (Kiernan *et al.,* 2023; Konieczka *et al.,* 2023; Innamma and Kaeoket, 2025), because the expression of the polymeric immunoglobulin receptor (pIgR) in the mammary gland, which mediates the transcytosis of IgA and IgM into colostrum and milk, is upregulated during this period. The establishment of mucosal immune pathways, including adhesion molecules such as mucosal addressin cell adhesion molecule-1 (MAdCAM-1) and other related mediators, also requires sustained stimulation to achieve effective immunomodulation (Bourges *et al.,* 2008; Ding *et al.,* 2025). For instance, dietary probiotic supplementation with viable *Bacillus subtilis* and *Bacillus amyloliquefaciens* administered from day 90 of pregnancy until the end of lactation significantly improved serum IgG concentrations in sows at farrowing (Konieczka *et al.,* 2023). Furthermore, Innamma and Kaeoket (2025) demonstrated that feeding sows multi-species probiotics for 4 weeks during late pregnancy and lactation increased colostral IgA concentrations and subsequently improved piglet survival rates in a herd affected by porcine epidemic diarrhea. Therefore, while the one-week supplementation in our study was sufficient to improve feed intake and metabolic indicators, it was likely too short to stimulate the full development of immune-related mechanisms in the mammary gland. Future studies should extend the probiotic-feeding period during late gestation to optimize immunoglobulin transfer and neonatal immune protection.

Similarly, no significant differences were found in serum MDA levels between groups, suggesting that short-term *Bacillus* supplementation did not affect oxidative stress in sows. Oxidative stress is influenced by multiple factors such as environmental conditions, social stress, and reproductive demands, all of which can impact sow performance, including feed intake, fetal development, and lactation (Berchieri-Ronchi *et al.,* 2011; Zhao *et al.,* 2013; Zhang *et al.,* 2020). While probiotics appeared to enhance IgM responses, their effect on oxidative stress was minimal, indicating a more specific action on immune modulation rather than a broad effect on systemic oxidative balance (Gu *et al.,* 2019).

The higher IgM concentrations observed in probiotic-supplemented sows could reflect an early activation of the humoral immune system rather than an indication of infection. Immunoglobulin M is the first antibody produced in response to antigenic stimulation and serves as a key component of the primary immune defense. Increased IgM levels have been reported following probiotic administration in pigs and other species, suggesting enhanced innate immune readiness and improved antigen recognition rather than pathological infection (Gu *et al.,* 2019; Kiernan *et al.,* 2023). Moreover, no clinical signs of disease or systemic inflammation were observed in the present study, and serum MDA concentrations, an indicator of oxidative stress associated with infection, did not differ between treatments. Therefore, the elevated IgM levels likely represent an immunostimulatory effect of *Bacillus*-based probiotics that primes the sow's immune system during the periparturient period, potentially supporting improved immune transfer and disease resistance in piglets.

Although probiotic supplementation in sows often does not alter serum immunoglobulin concentrations, several studies have demonstrated increased immunoglobulin levels in colostrum and milk following probiotic feeding (Konieczka *et al.,* 2023; Innamma and Kaeoket, 2025). This discrepancy likely reflects the localized immunomodulatory effects of probiotics on the mammary gland and the gut–mammary axis rather than systemic humoral changes. For example, Innamma and Kaeoket (2025) reported that feeding sows multi-species probiotics for four weeks before farrowing enhanced IgA concentrations in colostrum and improved piglet survival. Similarly, Konieczka *et al.* (2023) found that long-term supplementation with *Bacillus subtilis* and *Bacillus amyloliquefaciens* increased colostral IgG levels without significantly affecting serum immunoglobulins. In the present study, colostrum and milk immunoglobulins were not measured, which limits our ability to assess this local immune response. Nevertheless, given the known relationship between probiotic intake, mammary immune activation, and antibody secretion,

future research should evaluate colostrum and milk immunoglobulin profiles to clarify whether *Bacillus*-based probiotics can enhance passive immune transfer from sows to piglets.

Piglet performance: Piglet performance did not differ between sows fed a standard lactation diet and those receiving *Bacillus*-based probiotic supplementation. Survival rates at days 3, 7, 14, and 21 were comparable between groups, indicating no effect of probiotics on early piglet viability. These findings align with Konieczka *et al.* (2023), who reported no reduction in pre-weaning mortality but observed improvements in weaning weight and litter gain. Average daily gain (ADG) was also unaffected in the present study, consistent with the results of Menegat *et al.* (2019). In contrast, longer supplementation periods (e.g., 144 days) have been associated with increased ADG (Konieczka *et al.,* 2023).

Piglet serum immunoglobulins and fecal short-chain fatty acid: Piglet serum immunoglobulin levels at 7 and 14 days were compared between offspring of sows fed a standard diet and those receiving a *Bacillus*-based probiotic. Serum IgA levels tended to be higher in the treatment group at day 7, while IgM levels were significantly lower but returned to control levels by day 14, indicating a transient effect. This contrasts with the findings of Konieczka *et al.* (2023), who observed elevated IgM after long-term (144-day) maternal supplementation, highlighting the influence of duration on immune modulation. IgM, the first antibody produced during neonatal immune activation, may have been lower in probiotic-treated piglets due to reduced antigenic stimulation. Exposure to probiotics via colostrum, milk, and creep feed likely enhanced SCFAs production, which supports immune regulation and antimicrobial peptide synthesis (Ney *et al.,* 2023).

Fecal SCFAs concentrations, particularly acetate, were higher in treatment piglets at day 7 but not at other time points, suggesting transient stimulation of gut microbial activity. This may reflect maternal probiotic effects on milk composition through the entero-mammary pathway (Rodríguez, 2014) and the early introduction of probiotic-supplemented creep feed. SCFAs contribute to intestinal integrity, immune modulation, and antibody production (Kim *et al.,* 2016), collectively supporting gut health during the early "window of opportunity" (Kiernan *et al.,* 2023). These results suggest that short-term *Bacillus*-based probiotic supplementation in sow and piglet diets may support early immune and microbial development but is insufficient to enhance growth performance, highlighting the need for longer-term supplementation strategies or continuous administration under tropical commercial herd conditions to achieve measurable production benefits.

A limitation of this study was the absence of body weight standardization during cross-fostering, which may have confounded postnatal growth responses despite inclusion of day 3 body weight as a covariate in the statistical model. Future studies should incorporate standardized litter management and extended probiotic supplementation periods to more accurately assess the long-term effects of maternal and neonatal probiotic exposure on growth performance, immune development, and gut microbiota composition in piglets.

In conclusion, short-term *Bacillus*-based probiotic supplementation in sow and piglet diets did not significantly affect reproductive performance, piglet survival, or growth during lactation. However, it transiently influenced immune parameters and fecal SCFAs profiles, suggesting modulatory effects on gut microbiota and early immune development. These findings indicate that while brief supplementation may promote intestinal and immune maturation, longer or continuous probiotic administration may be necessary to achieve consistent productivity benefits in tropical swine production systems. In commercial tropical herds, integrating *Bacillus*-based probiotics into lactation diets may enhance early gut health and immune development in piglets, even without immediate effects on growth or survival. These results support the strategic use of probiotics as part of long-term feeding programs to improve herd resilience, reduce antibiotic dependence, and promote sustainable productivity under heat-stress conditions.

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CRediT authorship contribution statement

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