

Effects of silymarin supplementation during late gestation on reproductive performance, haematological parameters, antioxidant status, and gut microbiota in sows

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Abstract: This study aimed to evaluate the effects of silymarin supplementation during late gestation on reproductive performance, haematological parameters, antioxidant capacity, and gut microbiota composition in sows. Twenty parity-4 crossbred sows (Landrace × Yorkshire) were enrolled and randomly allocated in parity blocks to either a control group (CG; $n = 10$, basal diet) or a silymarin-supplemented group (SIL; $n = 10$, basal diet + 200 mg/kg silymarin). The experimental period extended from day 85 of gestation to the completion of farrowing. The results demonstrated that dietary silymarin significantly reduced the number of stillbirths ($P < 0.05$), without exerting a significant effect on the total number and proportion of live-born piglets ($P > 0.05$). No notable differences were observed in haematological parameters between the two groups ($P > 0.05$). However, catalase (CAT) activity and total antioxidant capacity (T-AOC) were significantly elevated in the silymarin group ($P < 0.05$), and superoxide dismutase (SOD) showed a tendency to increase ($P = 0.078$). High-throughput 16S rRNA sequencing revealed 1 671 unique feature sequences in the silymarin group and 1 073 in the control group, with 1 600 sequences shared between the two groups. A trend towards increased dominance was observed in the silymarin group ($P = 0.082$), while both the Shannon and Simpson indices tended to decline ($P = 0.087$; $P = 0.082$), suggesting a possible reduction in microbial diversity. Principal coordinate analysis (PCoA) of β -diversity revealed significant structural differences in gut microbiota between the two groups. SIMPER analysis identified *Terrisporobacter* as the principal genus contributing to these differences. In conclusion, silymarin supplementation during late gestation may enhance reproductive outcomes in sows, potentially through modulation of gut microbial composition and enhancement of systemic antioxidant status.

Keywords: catalase; oxidative stress; silymarin; sow gut microbiota; stillbirth reduction; *Terrisporobacter*

In modern pig production, advances in breeding have increased litter sizes, thereby placing greater demands on maternal lactation capacity. Insufficient feed intake during early lactation often

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limits milk production, adversely affecting growth and increasing mortality among low-birth-weight piglets (Guillemet et al. 2007). High metabolic demands during late gestation and lactation further exacerbate oxidative stress, which has been implicated in stillbirth, intrauterine growth restriction, and reduced reproductive performance (Gupta et al. 2007; Berchieri-Ronchi et al. 2011; Kim et al. 2013).

Milk thistle (*Silybum marianum*) is an herbaceous plant rich in flavonolignans, including silybin A and B, isosilybin A and B, silydianin, and silychristin, which are collectively referred to as silymarin (Choe et al. 2020; Wei et al. 2021). Silymarin exhibits hepatoprotective, antioxidant, and immunomodulatory properties, and has recently gained increasing attention in livestock production. In pigs, dietary supplementation with silymarin or its derivatives has been reported to improve growth performance and feed efficiency. It also enhances antioxidant status and intestinal health, and modulates gut microbiota composition. Moreover, silymarin supplementation helps alleviate oxidative and inflammatory responses (Grela et al. 2020; Koo et al. 2022; Cai et al. 2023; Hossain et al. 2024). In sows, supplementation during late gestation and lactation has been associated with higher milk yield and litter weight gain. It also improves antioxidant capacity and reduces maternal weight loss as well as the levels of pro-inflammatory cytokines (Jiang et al. 2020; Zhang et al. 2021).

Despite these findings, comprehensive studies on the effects of silymarin on reproductive performance, haematological parameters, and gut microbiota in peripartum sows remain limited. The present study therefore aimed to evaluate the impact of dietary silymarin supplementation during late gestation on sow reproductive performance, antioxidant capacity and gut microbiota composition. The findings are intended to provide a scientific basis for its application in commercial pig production.

MATERIAL AND METHODS

The experiment was conducted at a commercial swine research facility in China. The institutional Animal Ethics Committee approved all procedures involving animals in accordance with applicable national guidelines (Approval No.: ANS-CEUA-PJT/PL/202404/192).

Animal and experimental design

Twenty parity-4 crossbred sows (Landrace × Yorkshire) that were in good body condition (backfat thickness 18.3 ± 3.0 mm) and clinically healthy were selected from the experimental farm. All sows were vaccinated according to the farm's standard protocols. The sows were randomly assigned within parity blocks to one of two groups: a control group (CG; $n = 10$, basal diet) or a silymarin-supplemented group (SIL; $n = 10$, basal diet + 200 mg/kg silymarin). The experimental period commenced on day 85 of gestation and continued until the completion of farrowing. High-performance liquid chromatography (HPLC) analysis revealed that silybin and isosilybin together constituted 31% of the silymarin preparation used (Panjin Huacheng Pharmaceutical Co., Ltd., Panjin, P.R. China). The composition and nutrient levels of the basal diets were presented in Table 1.

Measurement

At parturition, the body weight of each piglet was measured prior to colostrum intake to determine the average birth weight of live-born piglets. The total number of piglets per litter was recorded and categorised as live-born or stillborn (Cong et al. 2024).

Sample collection

At farrowing, six sows were randomly selected for sample collection. From each selected sow, umbilical vein blood was obtained from six randomly chosen piglets. The samples were centrifuged at 3 000 rpm for 15 min at 4 °C to separate the serum, which was subsequently stored at –80 °C for further analysis. On the final day of the trial, fresh faecal samples were collected from each sow and immediately stored at –80 °C pending analysis.

Haematological analysis

Umbilical cord blood samples collected from sows were analysed for routine haematological parameters using a five-part automated veterinary haematology analyser (Model BH-5160 Vet, URIT, Suzhou, P.R. China).

Table 1. Ingredients and chemical composition of basal diet (air-dried basis)

| Composition (%) | | Chemical composition ² | |
|---------------------|--------|-----------------------------------|-------|
| Corn | 36.40 | NE (MJ/kg) | 9.90 |
| Wheat middling | 15.00 | CP (%) | 14.50 |
| Barley | 9.57 | EE (%) | 5.80 |
| Wheat bran | 15.00 | CF (%) | 5.20 |
| Soybean hulls | 9.50 | Ash (%) | 5.40 |
| Soybean meal, 46% | 7.50 | TP (%) | 0.70 |
| Limestone | 0.90 | Ca (%) | 0.71 |
| CaHPO ₄ | 0.43 | Lys (%) | 0.86 |
| Soya-bean oil | 1.69 | Met (%) | 0.16 |
| Premix ¹ | 4.00 | | |
| Total | 100.00 | | |

¹Premix provided per kilogram of complete diet: vitamin A, 13 000 IU; vitamin D3, 2 020 IU; vitamin E, 40.0 mg; vitamin K3, 3.00 mg; vitamin B1, 3.00 mg; vitamin B2, 3.50 mg; vitamin B6, 2.50 mg; vitamin B12, 0.04 mg; niacin, 30.0 mg; vitamin C, 300 mg; folic acid, 1.50 mg; biotin, 0.30 mg; Fe (FeSO₄·H₂O), 100 mg; Cu (CuSO₄·5H₂O), 12.0 mg; I (KI), 0.300 mg; Se (Na₂SeO₃), 0.20 mg; Zn (ZnSO₄·H₂O), 40.0 mg; Mn (MnSO₄·H₂O), 10.0 mg

²NE is calculated, and other nutrient levels are measured values

Ash = crude ash; Ca = calcium; CF = crude fibre; CP = crude protein; EE = ether extract; Lys = lysine; Met = methionine; NE = net energy; TP = total phosphorus

Evaluation of antioxidant capacity in umbilical cord blood

Antioxidant capacity of umbilical cord blood was evaluated using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, P.R. China) in accordance with the manufacturer's instructions.

Intestinal microbial diversity

For gut microbiota analysis, six sows per group were randomly selected. Total genomic DNA was isolated using a commercial extraction kit (Tiangen, Beijing, P.R. China). DNA concentration and purity were assessed using a NanoDrop ND 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA). The V3–V4 region of the *16S rRNA* gene was amplified by PCR using primers 341F (5'-CCTAYGGGRBGCASCAG-3')

and 806R (5'-GGACTACNNGGGTATCTAAT-3'). Amplicons of 400–450 bp were separated on a 1.5% agarose gel and purified using a gel extraction kit (Thermo Fisher Scientific, Waltham, USA). Sequencing libraries were constructed using the Ion Plus Fragment Library Kit (Thermo Fisher Scientific, Waltham, USA). After quantification and quality assessment with a Qubit fluorometer, equimolar amounts of the libraries were pooled and sequenced on the Ion S5™ XL platform by a commercial provider (Qingdao, P.R. China).

Raw reads generated from the Ion S5™ XL platform were quality-filtered using FLASH (v1.2.7) to obtain high-quality effective tags. These were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using Uparse (v7.0.1001). Taxonomic annotation was performed against the SSU rRNA database. Alpha and beta diversity analyses were conducted based on OTU-level data (Cong et al. 2024).

Statistical analysis

All data were collated in Microsoft Excel and analysed using SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Data normality and outliers were assessed using the UNIVARIATE procedure. Normally distributed data were compared using independent-samples *t*-tests, with the Satterthwaite correction applied for unequal variances. Non-normal data were analysed using the Mann–Whitney *U* test. Reproductive traits were analysed at the sow and litter levels, while other variables were analysed at the individual level. Results were presented as means ± SEM, with *P* < 0.05 considered statistically significant and 0.05 ≤ *P* ≤ 0.10 considered indicative of a trend.

RESULTS

Reproductive performance

As shown in Figure 1, dietary supplementation with 200 mg/kg silymarin during late gestation significantly decreased the number of stillborn piglets compared with the control group (*P* < 0.05). No significant differences were found between the silymarin and control groups in total litter size or the number of live-born piglets (*P* > 0.05).

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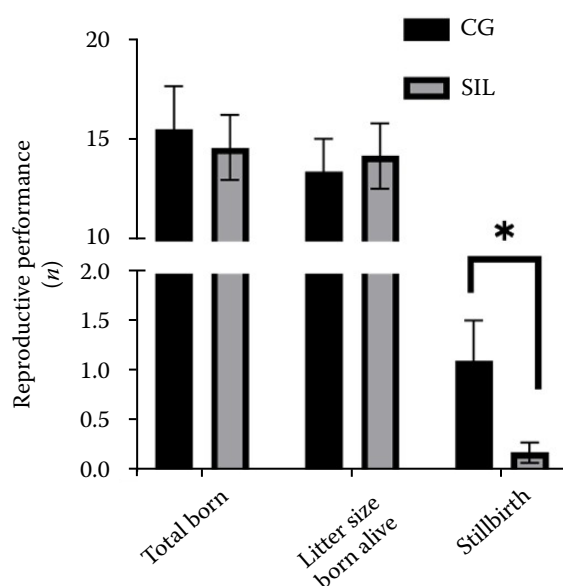


Figure 1. Effects of silymarin supplementation during late gestation on sow reproductive performance
CG = control group; SIL = silymarin supplementation

Umbilical cord blood routine

As shown in Figure 2, dietary supplementation with 200 mg/kg silymarin did not significantly affect lymphocyte (LYM), white blood cell (WBC), red blood cell (RBC), or platelet (PLT) counts in sows compared with the control group ($P > 0.05$).

Antioxidant capacity

As shown in Table 2, sows supplemented with 200 mg/kg silymarin during late gestation had

Table 2. Effects of silymarin supplementation on serum antioxidant capacity in sows

| Item | CG | SIL | <i>P</i> -value |
|---------------|-------------|-------------|-----------------|
| GSH-PX (U/ml) | 465 ± 61.1 | 511 ± 67.2 | 0.246 |
| CAT (U/ml) | 73.8 ± 18.6 | 125 ± 18.3 | 0.001 |
| MDA (nmol/ml) | 4.73 ± 1.41 | 3.92 ± 0.80 | 0.247 |
| SOD (U/ml) | 68.1 ± 9.92 | 77.3 ± 2.71 | 0.078 |
| T-AOC (U/ml) | 248 ± 21.9 | 286 ± 23.5 | 0.022 |

Values are presented as mean ± SEM; $n = 10$ sows per group
CAT = catalase; CG = control group; GSH-PX = glutathione peroxidase; MDA = malondialdehyde; SIL = silymarin supplementation; SOD = superoxide dismutase; T-AOC = total antioxidant capacity

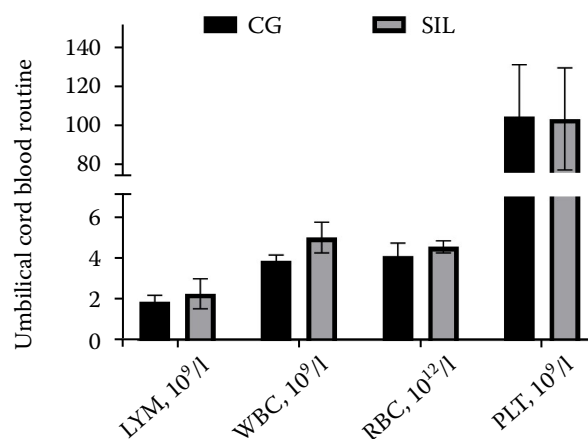


Figure 2. Effects of silymarin supplementation during late gestation on haematological parameters in sow umbilical cord blood

CG = control group; LYM = lymphocyte; PLT = platelet; RBC = red blood cell; SIL = silymarin supplementation; WBC = white blood cell

significantly higher serum catalase (CAT) activity compared with the control group ($P < 0.05$). Total antioxidant capacity (T-AOC) was also significantly higher in the silymarin group ($P < 0.05$). Serum superoxide dismutase (SOD) activity tended to increase in the silymarin group, although the difference was not statistically significant ($P = 0.078$).

Intestinal microbial diversity

As shown in Figure 3, the control group (CG) had 1 073 unique feature sequences, whereas the

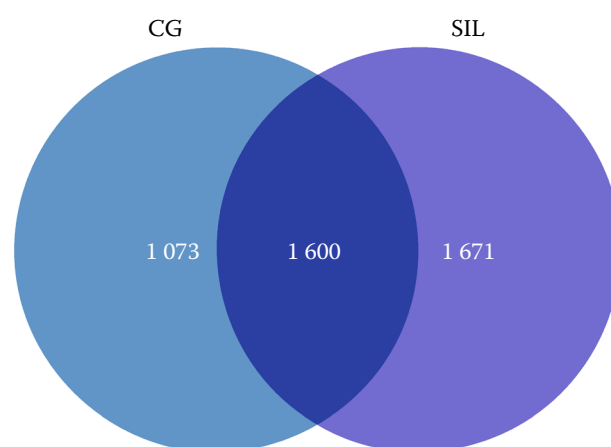


Figure 3. Venn diagram illustrating the effects of silymarin supplementation on sow gut microbial communities
CG = control group; SIL = silymarin supplementation

Table 3. Effects of silymarin supplementation on α -diversity indices of sow gut microbiota

| Item | CG | SIL | <i>P</i> -value |
|----------------|-------------------|-------------------|-----------------|
| chao1 | 1 042 \pm 59.5 | 1 086 \pm 83.2 | 0.687 |
| Dominance | 0.014 \pm 0.003 | 0.031 \pm 0.008 | 0.082 |
| goods-coverage | 1.00 \pm 0.000 | 1.00 \pm 0.003 | 0.291 |
| observed-otus | 982 \pm 53.4 | 1 010 \pm 71.2 | 0.764 |
| Shannon | 7.99 \pm 0.18 | 7.45 \pm 0.22 | 0.087 |
| Simpson | 0.99 \pm 0.003 | 0.97 \pm 0.008 | 0.082 |

Values are presented as mean \pm SEM; *n* = 6 sows per group
CG = control group; SIL = silymarin supplementation

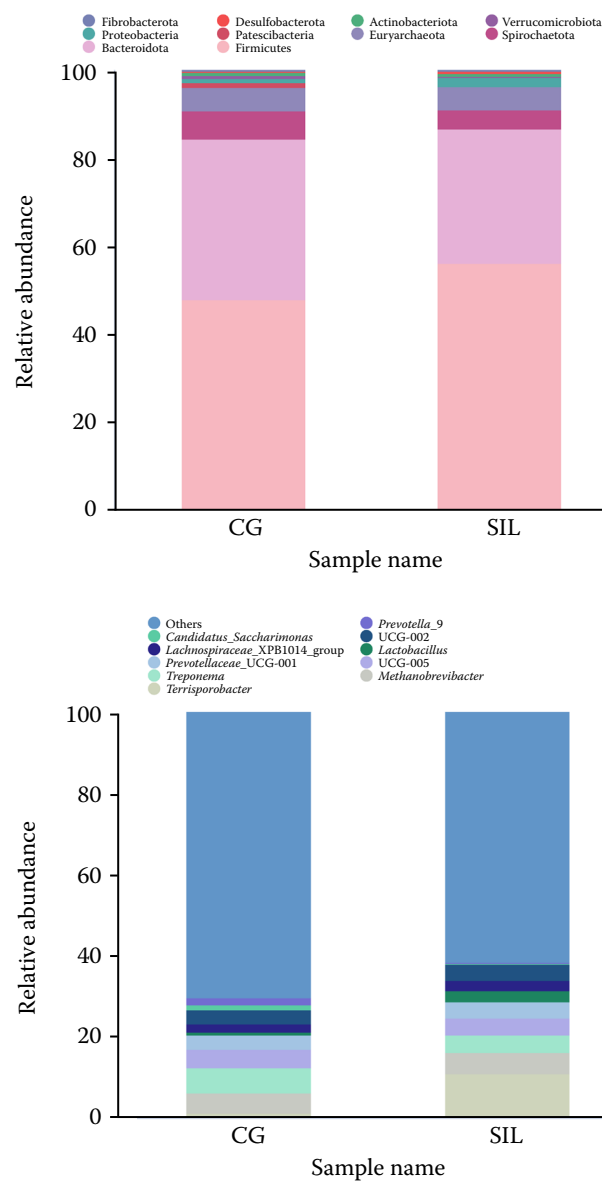


Figure 4. Effects of silymarin supplementation on the relative abundance of gut microbiota taxa in sows
CG = control group; SIL = silymarin supplementation

silymarin-supplemented group (SIL) contained 1 671 unique feature sequences. A total of 1 600 sequences were shared by both groups.

Dietary silymarin supplementation tended to increase the dominance index of the gut microbiota in sows ($P = 0.082$; Table 3). Both the Shannon and Simpson diversity indices tended to decrease in the SIL group compared with the control group ($P = 0.087$ and $P = 0.082$, respectively).

At the phylum level, sows in the SIL group had higher relative abundances of Firmicutes and Proteobacteria, and lower relative abundances of Bacteroidota, Spirochaetota, and Patescibacteria compared with the CG group (Figure 4). The Firmicutes-to-Bacteroidota (F : B) ratio increased from 1.31 in the CG group to 1.83 in the SIL group. At the genus level, the relative abundances of *Terrisporobacter* and *Lactobacillus* were higher in the SIL group, whereas *Treponema* and *Prevotella_9* were lower.

Principal coordinate analysis (PCoA) revealed a clear separation in gut microbial community structure between the SIL and CG groups (Figure 5). SIMPER analysis identified *Terrisporobacter* as the genus that contributed most to the observed microbial differences, showing higher abundance in the SIL group (Figure 6).

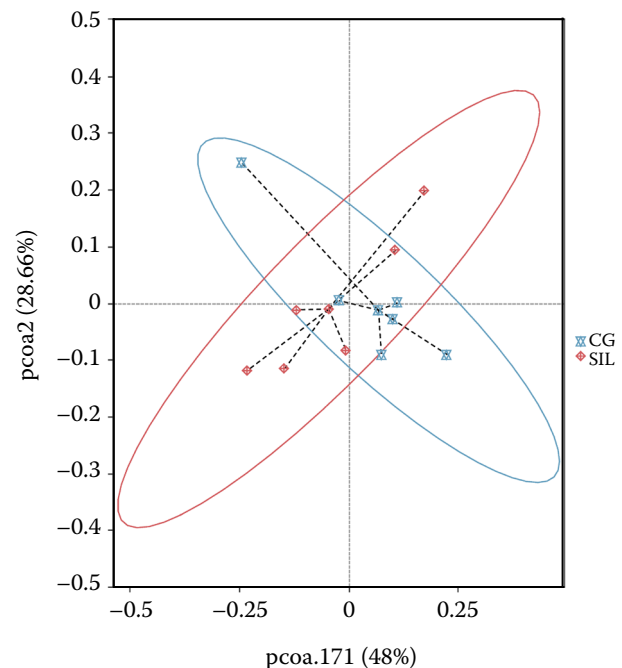


Figure 5. Principal coordinate analysis (PCoA) of gut microbiota in sows supplemented with silymarin
CG = control group; SIL = silymarin supplementation

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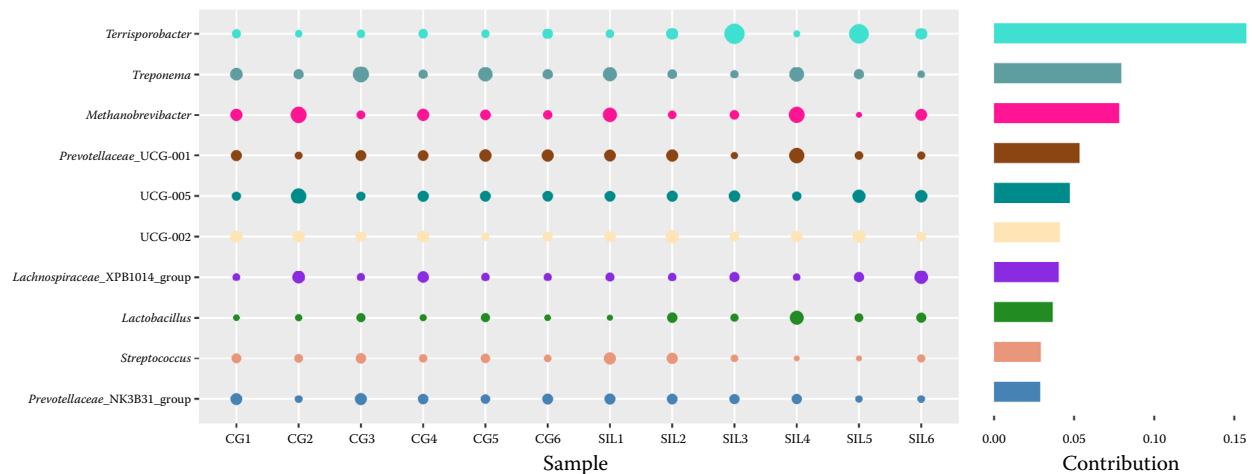


Figure 6. SIMPER analysis of differential gut microbiota between sows in the silymarin (SIL) and control (CG) groups

DISCUSSION

The reproductive effects of silymarin supplementation in sows may vary according to the silybin concentration, dosage, and duration of administration. Previous studies have reported inconsistent outcomes. Dietary supplementation with silymarin during late gestation had no significant effects on total litter size, the number of live-born piglets, or the stillbirth rate (Jiang et al. 2020). Similarly, no significant differences in litter size or piglet birth weight were observed when sows received 4 g/day of silymarin during late gestation (Farmer et al. 2014). In contrast, supplementation with 0.05%, 0.1%, or 0.2% silymarin during the perinatal period did not change total litter size, but it significantly reduced the stillbirth rate (Zhang et al. 2021). Other studies have also reported that silymarin supplementation can improve reproductive performance in sows (Cong et al. 2025). These results are consistent with those of the present study, in which silymarin supplementation markedly reduced the number and proportion of stillbirths. Collectively, these findings highlight the potential of silymarin to enhance reproductive outcomes in sows. No abnormal changes were observed in routine haematological parameters of umbilical cord blood, including red and white blood cell counts, haemoglobin concentration, and platelet count. All values in the silymarin-supplemented group were within the normal range, indicating that at the tested supplementation level, silymarin was safe for both sows and their foetuses.

As gestation progresses, the increasing metabolic demands of sows lead to greater production of reac-

tive oxygen species (ROS), which further intensifies during lactation due to enhanced mammary activity (Berchieri-Ronchi et al. 2011; Kim et al. 2013). Progressive oxidative stress is recognised as a major factor that impairs reproductive performance through mechanisms such as embryonic loss and intrauterine growth restriction (Gupta et al. 2007; Zhao and Kim 2020). In the present study, supplementation with 200 mg/kg silymarin during late gestation significantly increased serum CAT activity and T-AOC, with a tendency to elevate SOD activity, indicating an enhanced systemic antioxidant defence. These findings are in line with previous reports that silymarin can directly scavenge free radicals (Kiruthiga et al. 2007), inhibit NADPH oxidase-mediated ROS production (Jung et al. 2013), and activate endogenous antioxidant systems to maintain redox balance (Detaille et al. 2008; Boudierba et al. 2014). Similar increases in serum CAT and GSH-Px activities have been reported in sows receiving silymarin (Jiang 2022), and improved lactational performance has been associated with enhanced antioxidant status (Li et al. 2022). Consistent results have also been reported under commercial farm conditions, where supplementation with silymarin-containing feed additives reduced oxidative damage, improved reproductive outcomes in sows (Papatsiros et al. 2023) and enhanced growth and antioxidant biomarkers in weaned piglets (Papatsiros et al. 2024; Zhang et al. 2024). Collectively, these findings indicate that silymarin supplementation enhances systemic antioxidant defences in sows, thereby mitigating oxidative stress and supporting reproductive performance under the high metabolic load of late gestation and lactation.

In addition to its antioxidant activity, silymarin may also exert beneficial effects through modulating the gut microbiota. Being rich in flavonolignans, silymarin has been shown to inhibit gut microbial metabolism at concentrations as low as 200 mg per litre (Valentova et al. 2020). In the present study, silymarin supplementation was associated with a trend towards lower Shannon and Simpson indices in sow gut microbiota, suggesting a decrease in microbial diversity accompanied by observable changes in community composition. These observations are consistent with previous findings showing that dietary silymarin supplementation reduced microbial diversity and richness, as indicated by lower Chao1 and ACE indices (Xu et al. 2022b).

A reduction in microbial diversity may reflect the establishment of a more stable or favourable intestinal environment (Veljovic et al. 2017); however, this interpretation should be treated with caution and warrants further validation in future studies. *In vitro* studies have demonstrated that silymarin can inhibit bacterial growth by altering cell membrane permeability, disrupting surface structures, and interacting with ribosomal subunits, thereby suppressing protein synthesis and impairing bacterial function (Zheng 2017; Xu et al. 2022b). Consistent with these findings, silymarin supplementation in the present study reduced the abundance of certain potentially pathogenic bacteria while increasing the relative abundance of beneficial genera.

Analysis of relative microbial abundance revealed that the Firmicutes-to-Bacteroidota (F : B) ratio in the gut microbiota of sows in the SIL group was 1.83, which was higher than that in the control group. The F : B ratio is closely associated with the maintenance of intestinal homeostasis, and shifts in this ratio have been linked to various pathological conditions. For example, an elevated F : B ratio has been associated with obesity, whereas a reduced ratio has been correlated with intestinal inflammation (Shen et al. 2018; Abenavoli et al. 2019). An increased abundance of Firmicutes may enhance the host's capacity to extract energy from the diet, supporting foetal growth during late gestation and potentially improving milk production during the subsequent lactation period (Jo and Kim 2023).

SIMPER analysis in the present study indicated that the higher relative abundance of *Terrisporobacter*

in the SIL group was the main contributor to differences in gut microbiota composition between sows receiving silymarin supplementation and controls. *Terrisporobacter* is a Gram-positive, spore-forming bacterium that is either aerobic or facultatively anaerobic and is known for its ability to produce butyrate (Xu et al. 2022a). Previous studies have reported a positive association between *Terrisporobacter* abundance and litter size. Increased levels of *Terrisporobacter* have also been found to be negatively correlated with plasma free fatty acid concentrations in sows, suggesting a role in reducing circulating free fatty acids and mitigating inflammatory responses during late gestation (Chen et al. 2021). These findings suggest that silymarin may indirectly enhance reproductive performance and gut health by promoting beneficial bacterial genera and optimising microbial composition.

Taken together, these results have important implications for commercial farm conditions. Dietary silymarin supplementation during late gestation significantly reduced the number and proportion of stillbirths, tended to increase the birth weight of healthy piglets, and enhanced maternal antioxidant capacity without adversely affecting sow or foetal health. The modulation of gut microbiota, including increased abundance of beneficial genera such as *Terrisporobacter* and a higher F : B ratio, may further support foetal development and maternal well-being. Overall, these findings indicate that silymarin could represent a practical nutritional strategy to improve reproductive performance and offspring vitality in commercial sow herds.

CONCLUSION

Silymarin supplementation during late gestation reduced stillbirths, improved maternal antioxidant status, and altered gut microbiota in sows, with *Terrisporobacter* as the key genus associated with these changes.

These results highlight the potential of silymarin to enhance reproductive performance and gut health in gestating sows.

Conflict of interest

The authors declare no conflict of interest.

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