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Effects of dietary supplementation with *Latilactobacillus curvatus* CACC879 on faecal organic acid profiles and sulphur-related malodour reduction with maintained growth performance in finishing pigs

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ABSTRACT. This study evaluated the effects of dietary supplementation with *Latilactobacillus curvatus* CACC879 on growth performance, nutrient digestibility, faecal microbiota, faecal organic acid profiles, and malodour compounds in faecal slurry from finishing pigs. Sixty finishing pigs [(Landrace × Yorkshire) × Duroc; 30 males and 30 females; initial body weight, 54.98 ± 3.38 kg] were randomly assigned to one of two dietary treatments for 10 weeks: a basal diet (CON) or the basal diet supplemented with 0.25% *L. curvatus* CACC879 (1.2×10^{10} CFU/g; CACC879). Growth performance, nutrient digestibility, and faecal score were evaluated.

Faecal samples collected from a subset of pigs on day 0 (D0) and day 70 (D70) were subjected to 16S rRNA gene sequencing and organic acid analysis. Hydrogen sulphide and methyl mercaptan concentrations in faecal slurry were measured using standard methods for offensive odour compounds. Dietary supplementation with *L. curvatus* CACC879 did not affect final body weight, average daily gain, average daily feed intake, feed efficiency, or faecal score, although energy digestibility tended to increase. On D70, CACC879 supplementation was associated with altered faecal microbial community structure and nominal differences in selected bacterial genera. In addition, the CACC879 group showed higher concentrations of selected faecal organic acids, including 2-methylbutyric, butyric, isobutyric, isovaleric, propionic, and valeric acids, while hydrogen sulphide and methyl mercaptan concentrations were reduced. These findings suggest that *L. curvatus* CACC879 may be a promising microbial feed additive for modifying faecal fermentation profiles and reducing sulphur-related odour compounds in finishing pigs without impairing growth performance.

Key words: faecal microbiota, feed additive, livestock odour, probiotics, sulphur compounds

INTRODUCTION

Growing concerns regarding antimicrobial resistance have restricted the use of in-feed antibiotic growth promoters in animal production, including swine farming, and have increased the need for effective alternatives. Probiotics are considered a promising strategy for supporting gut health, sustaining microbial balance, and maintaining productive performance under commercial production conditions (European Parliament and Council, 2003; European Commission, 2005; Dowarah et al., 2017; Patience and Ramirez, 2022). Their beneficial effects

are generally attributed to multiple mechanisms, including competitive exclusion of pathogens, stabilisation of the intestinal microbial ecosystem, improved nutrient utilisation, and regulation of host physiological responses (FAO/WHO, 2002; Barba-Vidal et al., 2019; Cao et al., 2025). However, probiotic efficacy is strongly strain-dependent and may vary with dose, pig age, diet composition, and rearing environment (Cutting, 2011; Zhang et al., 2022; Cao et al., 2025).

Lactic acid bacteria, including species formerly classified within the genus *Lactobacillus*, are among the most widely studied probiotic candidates in swine nutrition. Previous studies have shown that selected lactobacilli can improve nutrient digestibility, affect the gut microbiota, maintain intestinal barrier function, and influence immune responses in pigs (Yi et al., 2018; Zhang et al., 2022). These bacteria may also affect short-chain fatty acid (SCFA) production, which represents an important outcome of microbial fermentation that contributes to intestinal homeostasis, epithelial energy supply, and host–microbe interactions (Ríos-Covián et al., 2016; Louis and Flint, 2017; Zhang et al., 2022). Nevertheless, probiotic effects vary between strains, and each candidate requires separate *in vivo* validation.

Besides animal performance and gut health, odour emissions from pig production have become an increasingly important research topic. Pig manure contains several classes of malodorous compounds, including volatile fatty acids, phenols, indoles, ammonia, and volatile sulphur compounds. Among these, hydrogen sulphide is particularly problematic because of its characteristic rotten egg odour and toxicity to animals and farm workers at higher concentrations (Guarrasi et al., 2015; Jo et al., 2015; Kim et al., 2026). Recent studies indicate that manure physicochemical properties, substrate availability, and microbiota composition are closely related to the production of these noxious gases, suggesting that biological or dietary interventions may

help reduce odour emissions from pig production (Lan et al., 2016; Hossain et al., 2024; Kim et al., 2026).

Probiotic supplementation has been proposed as one such strategy because it can affect gut microbial communities and fermentation products, potentially reducing the formation of odour precursors and noxious gas emissions. *Bacillus*-based or multispecies probiotic supplementation has been reported to improve feed efficiency and reduce faecal noxious gas emissions in pigs, while interventions utilising lactobacilli have been associated with changes in the gut microbiota and altered SCFA profiles (Lan et al., 2016; Oh et al., 2021; Cao et al., 2025). These findings suggest that dietary probiotics may provide dual benefits in pig farming by maintaining productivity while reducing faecal noxious gas and odour emissions. However, strain-specific effects on fermentation metabolites and sulphur-related malodour compounds in finishing pigs remain poorly characterised.

Latilactobacillus curvatus CACC879 is a strain isolated from swine faeces for which complete genome information and probiotic potential have been previously reported. Genomic analysis identified multiple traits associated with probiotic functionality, including genes related to acid and bile tolerance, lactate synthesis, antioxidant activity, bacteriocin production, vitamin biosynthesis, and CRISPR/Cas systems. These features suggest that the strain may remain metabolically active in the porcine gastrointestinal system and interact with the resident microbiota (Park et al., 2024). However, whether dietary inclusion of *L. curvatus* CACC879 produces measurable effects in finishing pigs consistent with its genomic and *in vitro* characteristics needs to be investigated.

Accordingly, this study examined whether dietary supplementation with *L. curvatus* CACC879 would affect growth performance, nutrient digestibility, faecal microbiota, faecal organic acid concentrations, and malodorous compounds in faecal slurry from finishing pigs.

MATERIAL AND METHODS

Ethics statement

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of CIALM (Approval No. CIALM 2024-02).

Probiotic strain and *in vitro* characterisation

L. curvatus CACC879 was previously isolated from swine faeces in Korea and characterised as a probiotic candidate. Its complete genome sequence and probiotic-related properties have been reported previously (Park et al., 2024), and additional *in vitro* characteristics are summarised in Supplementary Table S1 and Supplementary Figure S1.

Animals, diets, and experimental design

Sixty finishing pigs [(Landrace × Yorkshire) × Duroc; 30 males and 30 females; initial body weight, 54.98 ± 3.38 kg] were used in a 10-week (70 days) feeding trial. Pigs were randomly assigned to one of two dietary treatments: CON, basal diet; and CACC879, basal diet supplemented with 0.25% *L. curvatus* CACC879 (1.2×10^{10} CFU/g). Each treatment included six replicate pens with five pigs per pen (n = 30 pigs/treatment).

The basal diet was formulated as a practical maize–soybean meal-based diet to meet or exceed the nutrient requirements for finishing pigs according to NRC (2012). The formulation was based on calculated metabolizable energy, crude protein, minerals, and total amino acid contents. Similar basal diet formulations have been used in previous growing-finishing pig studies (Jo et al., 2021; Sampath et al., 2025). The ingredient composition and calculated nutrient contents are presented in Table 1. Feed and water were provided *ad libitum* throughout the experimental period.

Growth performance, nutrient digestibility, and faecal score measurements

Body weight was recorded at the beginning of the trial and at week 10, and average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F) were calculated based on these measurements. The pen was used as the experimental unit for growth performance analysis. Apparent total tract digestibility (ATTD) of dry matter, nitrogen, and energy was determined at week 10 using chromic oxide (Cr_2O_3) as an indigestible marker. Faecal samples collected at week 10 were stored at -20°C until analysis. Before chemical analysis, faecal samples were lyophilised, ground to pass through a 1-mm screen, and analysed for dry matter, nitrogen, gross energy, and chromium concentrations as described by Zhao and Kim (2015). Dry matter content was determined from the difference in sample weight before and after lyophilization. Chromium concentrations in the diet and faecal samples were measured using a UV–visible spectrophotometer (Optizen POP, Seoul, Republic of Korea). Gross energy was determined using a bomb calorimeter (Parr 6100, Moline, IL, USA), and nitrogen content was analysed using a Kjeldahl system (Kjeltec 2300, Foss Tecator AB, Hillerød, Denmark).

ATTD was calculated as follows:

$$\text{ATTD (\%)} = [1 - \{(\text{Nf} \times \text{Cd}) / (\text{Nd} \times \text{Cf})\}] \times 100,$$

where: Nf – nutrient concentration in faeces (% dry matter (DM)), Cd – chromium concentration in the diet (% DM), Nd – nutrient concentration in the diet (% DM), and Cf – chromium concentration in faeces (% DM).

Faecal consistency was assessed weekly using a 5-point scoring system: 1 – hard, dry pellet; 2 – firm, formed faeces; 3 – soft, moist faeces that retain shape; 4 – loose, unformed faeces that assume the shape of the container; and 5 – watery faeces.

Faecal organic acid quantification using UPLC-MS/MS

Faecal organic acids were quantified using UPLC-MS/MS following 3-nitrophenylhydrazine (3-NPH) derivatisation. Briefly, 50 mg of faeces was extracted with 10 ml of 50% acetonitrile, centrifuged, and the supernatant was derivatised with 200 mM 3-NPH and 120 mM EDC at 40 °C for 30 min. The reaction was quenched with 0.1% formic acid, and samples were diluted with 10% acetonitrile and analysed using an ACQUITY UPLC I-Class/Xevo TQ-S micro system (Waters, Milford, MA, USA) equipped with an ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm).

The mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The flow rate was 0.5 ml/min, and the injection volume was 5 µl. Quantification was performed in negative electrospray ionisation mode using multiple reaction monitoring for acetic, propionic, butyric, isobutyric, lactic, valeric, isovaleric, and 2-methylbutyric acids.

Faecal organic acid concentrations are reported in ppm based on measurements in the faecal extract. All samples were prepared using the same faecal sample weight, extraction volume, derivatisation procedure, and dilution conditions; therefore, ppm-based values were used to compare treatments between the CON and CACC879 groups.

Analysis of faecal slurry malodour compounds

Faecal slurry malodour compounds, including hydrogen sulphide and methyl mercaptan, were analysed using samples collected at D70 from three individual pigs per treatment (CON, n = 3; CACC879, n = 3). Each individual pig sample was considered an experimental replicate. The analysis was performed by the Siheung Green Environment Center (SHGEC) in accordance with the official National Institute of Environmental Research (NIER) standard methods for offensive odour compounds (NIER Notification No. 2024-65; NIER, 2024), following the Korean standard analytical framework for sulphur-containing designated odourants as summarised in the technical review by Lee (2007). Hydrogen sulphide and methyl mercaptan were quantified as sulphur-containing designated odour compounds using the validated SHGEC sulphur compound analysis system. The method detection limits were 0.008 ppb for hydrogen sulphide and 0.015 ppb for methyl mercaptan.

Faecal microbiota profiling by 16S rRNA gene sequencing

For microbiota analysis and faecal organic acid quantification, 10 pigs per treatment were randomly selected at each sampling time point. Fresh faecal samples were collected directly from the rectum on D0 and D70, and aliquots were stored at -80°C until analysis. Faecal slurry malodour compounds were analysed using a separate subset of D70 samples, as described above. Sequencing libraries were prepared by an external sequencing service provider and sequenced using an MGISEQ platform with paired-end 300-cycle reads. Raw FASTQ reads were quality-checked using FastQC v0.11.5 (Andrews, 2010) and processed in QIIME2 (Bolyen et al., 2019) with the DADA2 plugin (Callahan et al., 2016) for denoising, paired-read merging, and chimera

removal. Taxonomic classification was performed using the QIIME2 feature-classifier plugin against the SILVA database (release 132; Quast et al., 2013). For alpha diversity analysis, the samples were rarefied to a uniform sequencing depth before calculating the Chao1, Shannon, and Simpson indices. Principal coordinate analysis (PCoA) based on Bray-Curtis distances was used to visualise microbial community structure. Beta diversity was visualised separately for D0 and D70 to distinguish treatment-related differences at each sampling time point and avoid the influence of differences between the two time points. PERMANOVA was then performed for each time point to compare the CON and CACC879 groups.

Genus-level comparison, correlation, and functional prediction analyses

Relative abundance at the genus level on D70 was visualised for selected genera showing nominal differences between the CON and CACC879 groups. These genera were compared between the two groups using the Wilcoxon rank-sum test, followed by Benjamini-Hochberg false discovery rate (FDR) correction. Genera showing nominal differences in the Wilcoxon rank-sum test are presented in Figure 4, and the corresponding FDR-adjusted q -values are shown in the panel labels. These results were considered exploratory unless the differences remained significant after FDR correction. Microbial functional potential was predicted using PICRUSt2 (Douglas et al., 2020). Based on the *a priori* hypothesis that CACC879 supplementation would affect predicted microbial pathways related to faecal fermentation and sulphur-containing amino acid metabolism, MetaCyc pathways meeting an exploratory FDR threshold of $q < 0.1$ between the CON and CACC879 groups at D70 were summarised for biological interpretation.

Statistical analysis

All statistical analyses were performed using R software (version 4.4.1; R Foundation for Statistical Computing, Vienna, Austria). Growth performance, nutrient digestibility, faecal score, faecal organic acid concentrations, and malodour compound concentrations were compared between the CON and CACC879 groups using independent two-sample *t*-tests. The pen was used as the experimental unit for growth performance variables, whereas individual pig samples were used for nutrient digestibility, faecal organic acids, malodour compounds, and microbiota-derived variables, where appropriate. Data for growth performance, nutrient digestibility, faecal score, malodour compounds, and organic acids are presented as means with SEM. For microbiota analyses, Bray–Curtis distances were used for PCoA, and PERMANOVA was applied to test treatment-related differences in beta diversity. Associations between bacterial genera and faecal organic acids were evaluated using Spearman correlation analysis with FDR adjustment. Predicted functional pathways were filtered using an exploratory FDR threshold of $q < 0.1$ and interpreted as hypothesis-generating predicted functional features rather than confirmatory functional evidence.

RESULTS

Effects of dietary *L. curvatus* CACC879 supplementation on growth performance, nutrient digestibility, and faecal score in finishing pigs

Dietary supplementation with *L. curvatus* CACC879 did not significantly affect final body weight, average daily gain, average daily feed intake, or feed efficiency during the 10-week feeding trial (Table 2).

Nutrient digestibility and faecal score results are presented in Tables 3 and 4. Apparent total tract digestibility of dry matter and nitrogen was not affected by dietary treatment, whereas

energy digestibility tended to be higher in pigs fed the CACC879-supplemented diet. Faecal scores remained unaffected throughout the experimental period. Overall, CACC879 supplementation did not impair growth performance or faecal consistency and tended to improve energy digestibility.

Effect of dietary *L. curvatus* CACC879 supplementation on faecal slurry malodour compounds

Concentrations of sulphur-containing compounds in faecal slurry are shown in Figure 1. Compared to the CON group, pigs fed the CACC879-supplemented diet showed lower concentrations of hydrogen sulphide and methyl mercaptan. These reductions were statistically significant for both compounds, with a stronger treatment effect observed for methyl mercaptan.

Alpha and beta diversity of the faecal microbiota

Alpha diversity indices of the faecal microbiota are presented in Figure 2. D0 data were used as the baseline reference, whereas the primary interpretation focused on the treatment comparison on D70. At baseline, the CON and CACC879 groups showed comparable distributions of Shannon, Simpson, and Chao1 values. On D70, these alpha diversity indices remained broadly similar between treatments, indicating that CACC879 supplementation did not markedly affect overall faecal microbial richness or diversity.

Beta diversity was assessed using PCoA based on Bray-Curtis distances. At D0, PERMANOVA detected no significant difference in faecal microbial community structure between the CON and CACC879 groups ($R^2 = 0.068$, $P = 0.684$), indicating comparable baseline microbial profiles. On D70, however, microbial community structure differed significantly between treatments ($R^2 = 0.141$, $P = 0.002$). These results indicate that dietary supplementation

with CACC879 was associated with differences in overall faecal microbial community structure after 10 weeks of supplementation.

Taxonomic composition of the faecal microbiota

The taxonomic composition of the faecal microbiota at the phylum, family, and genus levels is shown in Figure 3. D0 profiles were included as baseline reference data, while the main interpretation focused on the comparison between the CON and CACC879 groups on D70.

At the phylum level, the faecal microbiota in both groups was mainly composed of Firmicutes and Proteobacteria. At the family level, Enterobacteriaceae and Clostridiales vadinBB60 group accounted for relatively large proportions of the community in both treatments. At the genus level, *Escherichia-Shigella*, Rikenellaceae RC9 gut group, and Christensenellaceae R-7 group were the dominant genera observed at D70. Overall, selected taxa showed differences in relative abundance between treatments on D70, whereas D0 profiles served as baseline reference data.

Selected genera showing nominal differences between treatments on D70

Relative abundance at the genus level on D70 was compared between the CON and CACC879 groups using the Wilcoxon rank-sum test followed by Benjamini-Hochberg FDR correction (Figure 4). Several genera showed nominal differences between treatments based on the Wilcoxon rank-sum test; however, none remained significant after FDR correction. Therefore, these genus-level differences were interpreted as nominal taxonomic patterns rather than confirmatory evidence of differential abundance.

Genera showing higher relative proportion in CACC879 than in CON included *Allisonella*, *Ruminococcus gnavreai* group, *Enterococcus*, *Syntrophococcus*, *Rummeliibacillus*, *Megasphaera*, *Shuttleworthia*, *Bacteroides*, *Prevotella* 7, *Lachnospiraceae* NK3A20 group, and *Oribacterium*. In contrast, *Oscillibacter*, *Parabacteroides*, *Candidatus Soleaferrea*, and *Ruminococcaceae* UCG-014 had a lower relative abundance in CACC879 than in CON.

Odour-related genera were also reviewed using the same genus-level dataset. *Bilophila* did not differ between treatments, whereas *Fusobacterium* was detected in the CON group but not in the CACC879 group. However, these observations were not statistically significant after FDR correction and were therefore interpreted cautiously as exploratory odour-related differences in the faecal microbiota.

Effect of dietary *L. curvatus* CACC879 supplementation on faecal organic acid concentrations in finishing pigs on D70

The concentrations of faecal organic acids on D70 are presented in Figure 5. Compared to the CON group, pigs fed the CACC879-supplemented diet showed significantly higher concentrations of 2-methylbutyric acid ($P = 0.0253$), butyric acid ($P = 0.0495$), isobutyric acid ($P = 0.0198$), isovaleric acid ($P = 0.0101$), propionic acid ($P = 0.0279$), and valeric acid ($P = 0.0092$). In contrast, acetic acid and lactic acid did not differ significantly between treatments on D70.

Overall, dietary supplementation with *L. curvatus* CACC879 was associated with increased concentrations of selected faecal organic acids on D70.

Spearman correlation analysis between faecal organic acids and bacterial genera on D70

Figure 6 shows the Spearman correlation analysis with FDR correction examining associations between faecal organic acid concentrations and major bacterial genera on D70. The organic acids showed similar correlation patterns as bacterial genera. After FDR correction, significant correlations were observed mainly for butyric acid, propionic acid, and valeric acid.

Butyric acid was positively correlated with several genera, including *Dialister*, *Megasphaera*, *Shuttleworthia*, *Bacillus*, Erysipelotrichaceae UCG-004, *Prevotella* 9, *Prevotella* 2, and *Subdoligranulum*. Propionic acid showed a similar trend, with positive correlations with *Dialister*, *Bacillus*, Erysipelotrichaceae UCG-004, *Prevotella* 9, *Prevotella* 2, and *Subdoligranulum*. Valeric acid was positively correlated with *Prevotella* 7, *Dialister*, *Megasphaera*, and *Shuttleworthia*. Together, faecal organic acid concentrations at D70 were correlated with the relative abundance of specific bacterial genera.

Differences in predicted functional profiles of the faecal microbiota on D70

Predicted functional pathway profiles of the faecal microbiota on D70 were compared between the CON and CACC879 groups. MetaCyc pathways meeting the exploratory FDR threshold of $q < 0.1$ are presented in Figure 7. Hierarchical clustering showed differences in pathway profiles between treatments, with some pathways having higher predicted values in CACC879 and others in CON.

Pathways with relatively higher predicted abundance in CACC879 included the superpathway of (R,R)-butanediol biosynthesis, the superpathway of 2,3-butanediol biosynthesis, pyruvate fermentation to propanoate II (acrylate pathway), the superpathway of glycerol degradation to 1,3-propanediol, nitrate reduction VII denitrification, and several additional metabolic pathways. In contrast, pathways showing relatively higher predicted abundance in CON

included the superpathway of glycolysis and the Entner-Doudoroff pathway, the superpathway of L-cysteine biosynthesis, L-cysteine biosynthesis IV, L-homocysteine and L-cysteine interconversion, phospholipid biosynthesis-related pathways, and tryptophan-related metabolism. Overall, the predicted functional profiles differed between treatments on D70.

DISCUSSION

The present study showed that dietary supplementation with *L. curvatus* CACC879 was associated with changes in faecal microbial community structure, faecal organic acid profiles, and sulphur-related malodour compounds in finishing pigs. On day 70, CACC879 supplementation increased the concentrations of selected faecal organic acids and reduced hydrogen sulphide and methyl mercaptan concentrations without negatively affecting growth performance or faecal consistency. These findings suggest that *L. curvatus* CACC879 may be useful as a microbial feed additive for modifying faecal fermentation and reducing sulphur-related malodour compounds in finishing pigs.

The characteristics of *L. curvatus* CACC879 support the rationale for evaluating its *in vivo* effects. The complete genome sequence of *L. curvatus* CACC879 has already been reported, together with acid and bile tolerance, lactate production, adhesion-associated functions, bacteriocin-related traits, antioxidant potential, and vitamin biosynthesis capacity (Park et al., 2024). In the present study, supplementary *in vitro* data further demonstrated acid and bile tolerance, heat tolerance, cell adhesion ability, and immunomodulatory responses in a cell-based assay, supporting the potential application of *L. curvatus* CACC879 as a microbial feed additive.

Several previous studies have evaluated the effects of probiotics on growth performance, nutrient digestibility, gut microbiota, fermentation metabolites, and odour-related parameters in

pigs (Yi et al., 2018; Wang and Kim, 2021; Zhang et al., 2022; Cao et al., 2025). Therefore, the main contribution of the current study is in evaluating *L. curvatus* CACC879 rather than in the novelty of the measured endpoints. Under the conditions of the present finishing-pig trial, dietary supplementation with CACC879 did not impair productive performance and was associated with changes in faecal organic acid profiles and reduced concentrations of sulphur-related malodorous compounds.

The inclusion level of 0.25% was selected as a practical supplementation dose for evaluating *L. curvatus* CACC879 as a microbial feed additive in commercial-type feeding conditions. Based on the inclusion level and viable cell count of the product, this dose supplied approximately 3.0×10^{10} CFU/kg feed, equivalent to 3.0×10^7 CFU/g feed. At this dose, CACC879 supplementation increased concentrations of several faecal organic acids and reduced sulphur-related malodour compounds without adverse effects on growth performance or faecal consistency. However, because only one inclusion level was tested, the present study could not determine whether the observed responses were dose-dependent or whether a higher dose would produce stronger effects. Further dose-response studies are required to establish the optimal supplementation level of *L. curvatus* CACC879 for finishing pigs.

The beta diversity results support an effect of CACC879 supplementation on the faecal microbiota at the end of the trial. PCoA and PERMANOVA were performed separately for each sampling time point. No significant difference was observed between the CON and CACC879 groups on D0, whereas the overall faecal microbial community structure differed significantly on D70. These findings suggest that CACC879 supplementation was associated with differences in faecal microbial community structure after 10 weeks of feeding (Dowarah et al., 2017; Zhang et al., 2022). However, beta diversity did not identify the specific taxa or functions responsible for

these differences. Therefore, this result should be interpreted in conjunction with the genus-level comparison, faecal organic acid profiles, and predicted functional pathway analyses.

The comparison of individual genera identified nominal differences in selected bacterial taxa on D70, although none remained significant after Benjamini-Hochberg FDR correction. Hence, these results were interpreted cautiously as exploratory taxonomic findings rather than as evidence of differential abundance. This conservative assumption is appropriate because individual taxon-level comparisons in microbiome studies can be affected by multiple testing, sample size, inter-individual variation, and taxonomic resolution.

Nevertheless, the differences in selected bacterial genera may be relevant when considered alongside the other microbiota and metabolite results. The beta diversity analysis on D70 showed a significant difference in overall faecal microbial community structure between treatments, while faecal organic acid profiles and PICRUSt2-predicted functional pathways also differed between groups. Therefore, the differences observed at the genus level were not considered as independent evidence but were interpreted alongside the beta diversity, faecal organic acids, and predicted functional pathway results. Similar approaches combining microbiota composition with metabolite profiles have been used in animal microbiome studies (Lee et al., 2022; Dai et al., 2025). Thus, these findings indicate that CACC879 supplementation was associated with differences in faecal microbial community structure and fermentation-related profiles.

The faecal organic acid results provide functional support for the observed differences in the faecal microbiota. Increased concentrations of butyric, propionic, valeric, isobutyric, isovaleric, and 2-methylbutyric acids indicate that CACC879 supplementation altered faecal fermentation. Short-chain fatty acids and related organic acids are major microbial fermentation products and their composition is closely related to substrate availability and microbial community

structure (Ríos-Covián et al., 2016; Louis and Flint, 2017). In the present study, several genera involved in fermentation or organic acid production, including *Megasphaera*, *Prevotella* 7, *Bacteroides*, *Shuttleworthia*, and Lachnospiraceae NK3A20 group, showed nominal differences between treatments on D70. In addition, butyric, propionic, and valeric acids were positively correlated with genera such as *Dialister*, *Megasphaera*, *Shuttleworthia*, *Prevotella*, and *Subdoligranulum*. These findings suggest that the higher faecal organic acid concentrations were related to changes in microbial fermentation rather than to FDR-confirmed differences in individual genera. Consistent with these findings, PICRUSt2 analysis showed a higher predicted abundance of propanoate production via pyruvate fermentation in the CACC879 group. However, the organic acid-producing capacity of CACC879 itself was not directly evaluated. Therefore, these results do not demonstrate that the supplemented strain directly produced the measured metabolites.

The reductions in hydrogen sulphide and methyl mercaptan were interpreted in relation to sulphur-associated microbial functions and selected odour-related taxa. PICRUSt2 analysis showed lower predicted abundance of cysteine-related pathways, including L-cysteine biosynthesis and L-homocysteine/L-cysteine interconversion in the CACC879 group than in the CON group. Since hydrogen sulphide production by bacteria can involve the metabolism of sulphur-containing amino acids, the lower predicted abundance of these cysteine-related pathways may partly explain the lower concentrations of sulphur-related malodour compounds observed in the CACC879 group. The odour-related genera *Bilophila* and *Fusobacterium* were also analysed because of their potential relevance to odour emission linked to sulphur. *Bilophila* did not differ between treatments, whereas *Fusobacterium* was detected only in the CON group. Although this difference was not statistically significant after FDR correction, it may help explain the lower

hydrogen sulphide concentration observed in the CACC879 group. However, this finding does not indicate that CACC879 reduced the abundance of sulphur-metabolising bacteria. Moreover, targeted qPCR or shotgun metagenomic analysis of sulphur-metabolising taxa and functional genes was not performed in the present study. Therefore, these findings point to potential associations but do not provide direct evidence of the underlying mechanisms.

From a practical perspective, the reduction in hydrogen sulphide and methyl mercaptan is an important outcome of the present study. These sulphur-containing compounds are major contributors to swine manure odour and remain important targets in odour-control strategies (Lee, 2007; Jo et al., 2015). Recent reviews have shown that feed additives and microbial interventions can affect noxious gas emissions by changing intestinal fermentation and manure characteristics (Hossain et al., 2024). However, the increase in faecal organic acid concentrations observed in the CACC879 group should not be interpreted as an unequivocally beneficial effect on odour. Volatile fatty acids and other organic acids can also contribute to manure odour emission, depending on their chemical composition, volatility, faecal pH, and manure storage conditions. Therefore, the higher concentrations of selected faecal organic acids observed after CACC879 supplementation indicate changes in faecal fermentation, but do not by themselves demonstrate an overall reduction in odour. In the present study, hydrogen sulphide and methyl mercaptan concentrations were reduced, but total odour intensity, volatile fatty acid emissions, slurry pH, and a broader range of volatile organic compound profiles were not analysed. Consequently, the present results cannot determine whether CACC879 supplementation reduces overall odour emissions from swine manure. Further studies should therefore evaluate faecal organic acid profiles together with volatile organic compound emissions, slurry pH, and instrumental or sensory measurements of odour to determine the practical and environmental effects of CACC879 supplementation.

The lack of significant improvement in growth performance may be related to the physiological stage of the animals and the experimental conditions. The pigs used in this study were in the finishing stage with an initial body weight of approximately 55 kg and were fed a nutritionally adequate maize-soybean meal diet under commercial-type feeding conditions. Growth responses to probiotic supplementation may be less pronounced in finishing pigs than in younger animals or in pigs exposed to greater nutritional, microbial, or environmental challenges. Probiotic responses can also vary with strain characteristics, dose, pig age, diet composition, health status, and environmental conditions (Liao and Nyachoti, 2017; Barba-Vidal et al., 2019). In the current study, CACC879 supplementation did not adversely affect final body weight, average daily gain, average daily feed intake, feed efficiency, or faecal consistency, while energy digestibility tended to increase. These results suggest that the effects of CACC879 were more evident in faecal fermentation and odour measurements than in conventional growth-performance variables. This interpretation is consistent with previous probiotic studies showing that probiotic supplementation can affect gut microbiota, nutrient utilisation, or noxious gas emissions without consistently improving growth performance (Zhang et al., 2022; Cao et al., 2025). Therefore, under the conditions of this study, CACC879 may have greater potential for modifying faecal fermentation and odour than for directly promoting growth. Further studies using different inclusion levels, longer feeding periods, or combinations with other probiotic strains are needed to determine whether these approaches can produce measurable improvements in production performance.

This study has certain limitations. First, only one supplementation level of *L. curvatus* CACC879 was evaluated, so dose-dependent responses and the optimal inclusion level could not be determined. Second, the comparison of bacterial genera was exploratory. Although several genera showed nominal differences between treatments, none remained significant after

Benjamini-Hochberg FDR correction. Therefore, these taxa should not be interpreted as differentially abundant. Third, the predicted functional pathways were inferred using PICRUSt2 rather than measured directly by metagenomic or metatranscriptomic methods. Pathways meeting the exploratory threshold of $q < 0.1$ should therefore be interpreted cautiously, and the predicted differences in fermentation- and sulphur-containing amino acid metabolism require confirmation using shotgun metagenomic, metatranscriptomic, or targeted functional gene analyses. Fourth, sulphur-metabolising populations, genes involved in sulphur metabolism, sulphur intermediates in manure, slurry pH, total odour intensity, volatile fatty acid emissions, and a broader range of volatile organic compound profiles were not directly quantified. Therefore, the relationship between changes in the microbiota, predicted functional pathways, organic acid profiles, and lower concentrations of sulphur compounds cannot be directly established from the present data. The possible effects of increased faecal organic acid concentrations on odour emissions also require further evaluation under manure storage and emission conditions. In addition, carcass characteristics and meat and fat quality traits were not assessed. Future studies should therefore include dose-response designs and direct measurements of microbial functional genes, sulphur metabolites, manure odour, and carcass and meat quality to confirm the proposed mechanisms and assess the practical application of CACC879.

Overall, the present study supports *L. curvatus* CACC879 as a candidate microbial feed additive for finishing pigs, particularly for improving faecal fermentation profiles and reducing the production of sulphur-related malodour compounds under the conditions tested.

CONCLUSIONS

Dietary supplementation with *Latilactobacillus curvatus* CACC879 did not impair growth performance or faecal consistency in finishing pigs and was associated with altered faecal fermentation profiles. In particular, *L. curvatus* CACC879 supplementation increased the concentrations of selected faecal organic acids and reduced sulphur-related malodorous compounds, including hydrogen sulphide and methyl mercaptan. These effects were accompanied by an altered faecal microbial community structure, nominal differences between selected bacterial genera, and changes in predicted microbial functions related to fermentation and sulphur-containing amino acid metabolism. Although further studies are needed to confirm the underlying mechanisms and dose-dependent responses, the present findings suggest that *L. curvatus* CACC879 may be a promising microbial feed additive for modifying faecal fermentation and reducing odour in finishing pigs.

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CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

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Table 1. Ingredients and chemical composition of the basal finishing pig feed

Item	Basal diet
Ingredients, %	
maize	77.81
soybean meal	16.90
tallow	0.44
molasses	2.00
MCP (monocalcium phosphate)	0.90
limestone	0.75
lysine (78%)	0.35
methionine (98%)	0.03
threonine (98%)	0.11
tryptophan (98%)	0.01
dalt	0.20
mineral mix ¹	0.20
vitamin mix ²	0.20
choline (25%)	0.10
total	100.00
Calculated value	
crude protein, %	15.00
metabolizable energy, kcal/kg	3300
calcium, %	0.59
phosphorus, %	0.52
total lysine, %	0.97
total methionine, %	0.28
total threonine, %	0.64
total tryptophan, %	0.17
crude fat, %	3.40
crude fibre, %	2.20
crude ash, %	3.82

¹ provided per kg of diet: mg: Fe 100 as ferrous sulphate, Cu 17 as copper sulphate, Mn 17 as manganese oxide, I 0.5 as potassium iodide, Se, 0.3 as sodium selenite; ² provided per kg of diet: IU: vit. A 10,800, vit. D₃ 4,000, vit. E 40; mg: vit. K₃ 4, vit. B₁ 6, vit. B₂ 12, vit. B₆ 6, vit. B₁₂ 0.05, biotin 0.2, folic acid 2, niacin 50, D-calcium pantothenate 25; ³ calculated amino acid values are expressed on a total amino acid basis

Table 2. The effect of dietary *Latilactobacillus curvatus* CACC879 supplementation on growth performance in finishing pigs

Items	CON	CACC879	SEM	P-value
Body weight, kg				
Initial	54.97	54.98	0.02	0.9999
Week 10	112.96	114.93	1.72	0.9104
Overall				
ADG, g	828	856	25	0.8249
ADFI, g	2445	2467	41	0.1919
G:F	0.339	0.348	0.011	0.6401

CON – basal diet, CACC879 – basal diet supplemented with *L. curvatus* CACC879 at 0.25% (1.2×10^{10} CFU/g); ADG – average daily gain, ADFI – average daily feed intake, G:F – feed efficiency; SEM – standard error of the mean; P-values were calculated using an independent two-sample *t*-test; $P > 0.05$ (not statistically significant)

Table 3. The effect of dietary *Latilactobacillus curvatus* CACC879 supplementation on nutrient digestibility in finishing pigs

Items, %	CON	CACC879	SEM	P-value
Week 10				
Dry matter	70.58	71.45	0.79	0.6851
Nitrogen	65.33	66.97	0.66	0.2612
Energy	67.10	69.08	0.64	0.0543

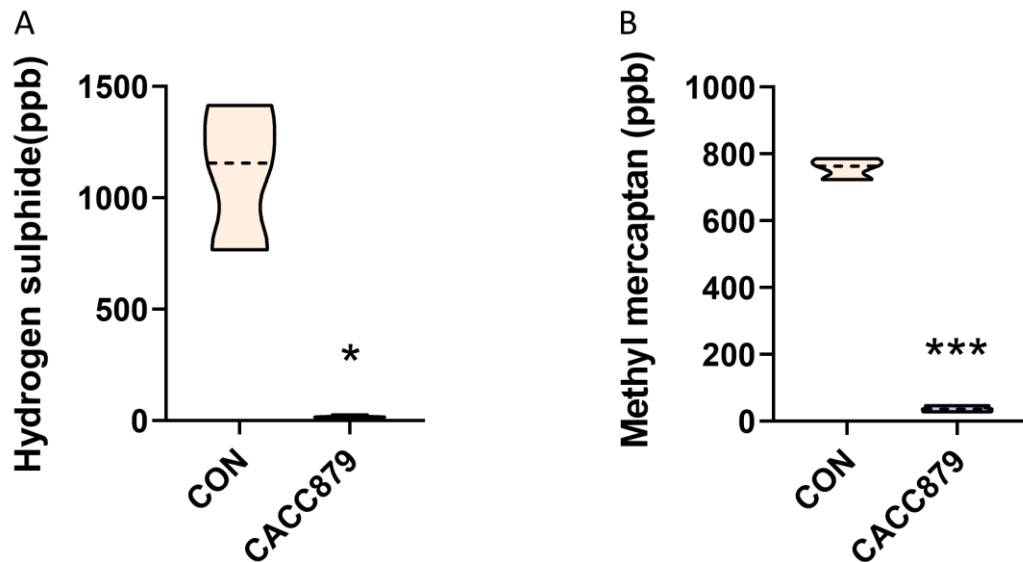
CON – basal diet, CACC879 – basal diet supplemented with *L. curvatus* CACC879 at 0.25% (1.2×10^{10} CFU/g), SEM – standard error of the mean; P-values were calculated using an independent two-sample *t*-test; $P > 0.05$ (not statistically significant)

Table 4. The effect of dietary *Latilactobacillus curvatus* CACC879 supplementation on faecal score in finishing pigs¹

Items	CON	CACC879	SEM	P-value
Faecal score ¹				
Week 1	3.35	3.31	0.10	0.9595
Week 2	3.33	3.31	0.06	0.9794
Week 3	3.31	3.29	0.08	0.9754
Week 4	3.29	3.25	0.08	0.9615
Week 5	3.27	3.23	0.07	0.8857
Week 6	3.24	3.18	0.10	0.9047
Week 7	3.23	3.18	0.08	0.8886
Week 8	3.21	3.15	0.09	0.8887
Week 9	3.20	3.14	0.07	0.7759
Week 10	3.18	3.11	0.05	0.6948

CON – basal diet, CACC879 – basal diet supplemented with *L. curvatus* CACC879 at 0.25% (1.2×10^{10} CFU/g), SEM – standard error of the mean; P-values were calculated using an independent two-sample *t*-test; $P > 0.05$ (not statistically significant)

¹ faecal score = 1 hard, dry pellet; 2 firm, formed stool; 3 soft, moist stool that retains shape; 4 soft, unformed stool that assumes the shape of the container; 5 watery liquid that can be poured

**Figure 1.** Effects of dietary *Latilactobacillus curvatus* CACC879 supplementation on faecal slurry malodour compounds in finishing pigs

(A) Hydrogen sulphide concentration and (B) methyl mercaptan concentration in faecal slurry. CON, basal diet; CACC879, basal diet supplemented with *L. curvatus* CACC879 at 0.25% (1.2×10^{10} CFU/g). Values are presented as means $\pm$ SEM. SEM – standard error of the mean. Asterisks indicate significant differences between treatments (* $P < 0.05$, *** $P < 0.001$)

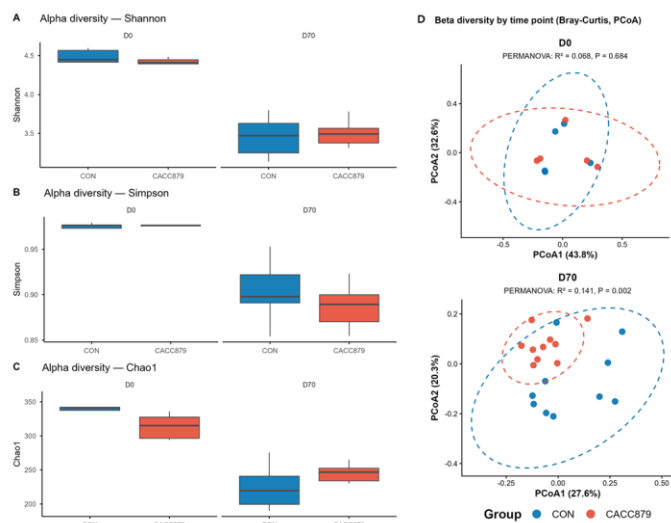


Figure 2. Alpha and beta diversity of faecal microbiota in finishing pigs fed *Latilactobacillus curvatus* CACC879

(A-C) Shannon, Simpson, and Chao1 indices at D0 and D70 in the CON and CACC879 groups. Alpha diversity indices were calculated after rarefying samples to an even sequencing depth. (D) PCoA plots based on Bray-Curtis distances separated by sampling time point. PERMANOVA was performed within each time point to compare the CON and CACC879 groups. D0 – day 0; D70 – day 70; CON, basal diet; CACC879, basal diet supplemented with *L. curvatus* CACC879 at 0.25% (1.2×10^{10} CFU/g)

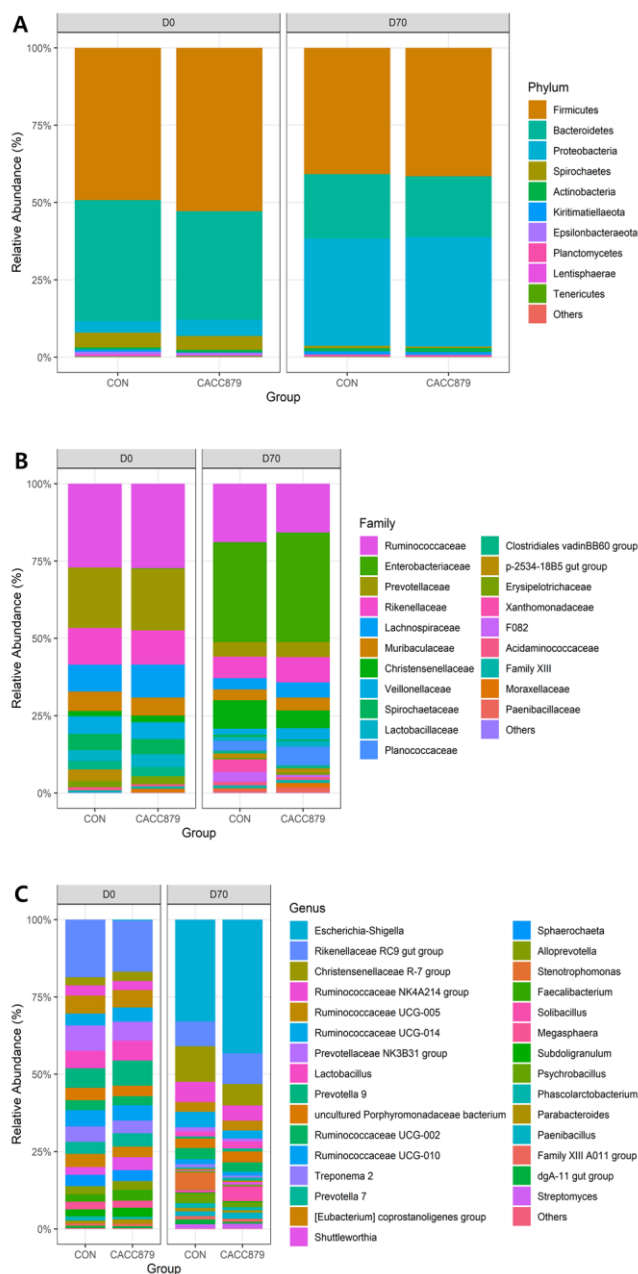


Figure 3. Taxonomic composition of the faecal microbiota at D0 and D70 in the CON and CACC879 groups

Stacked bar plots show the relative abundance (%) of major taxa arranged in decreasing order of abundance. Panel A shows the phylum-level composition, panel B shows the family-level composition, and panel C shows the genus-level composition. Each bar represents the mean relative abundance for each group at each sampling time point, and low-abundance taxa are grouped as 'Others'. D0 – day 0; D70 – day 70; CON, basal diet; CACC879, basal diet supplemented with *Latilactobacillus curvatus* CACC879 at 0.25% (1.2×10^{10} CFU/g)

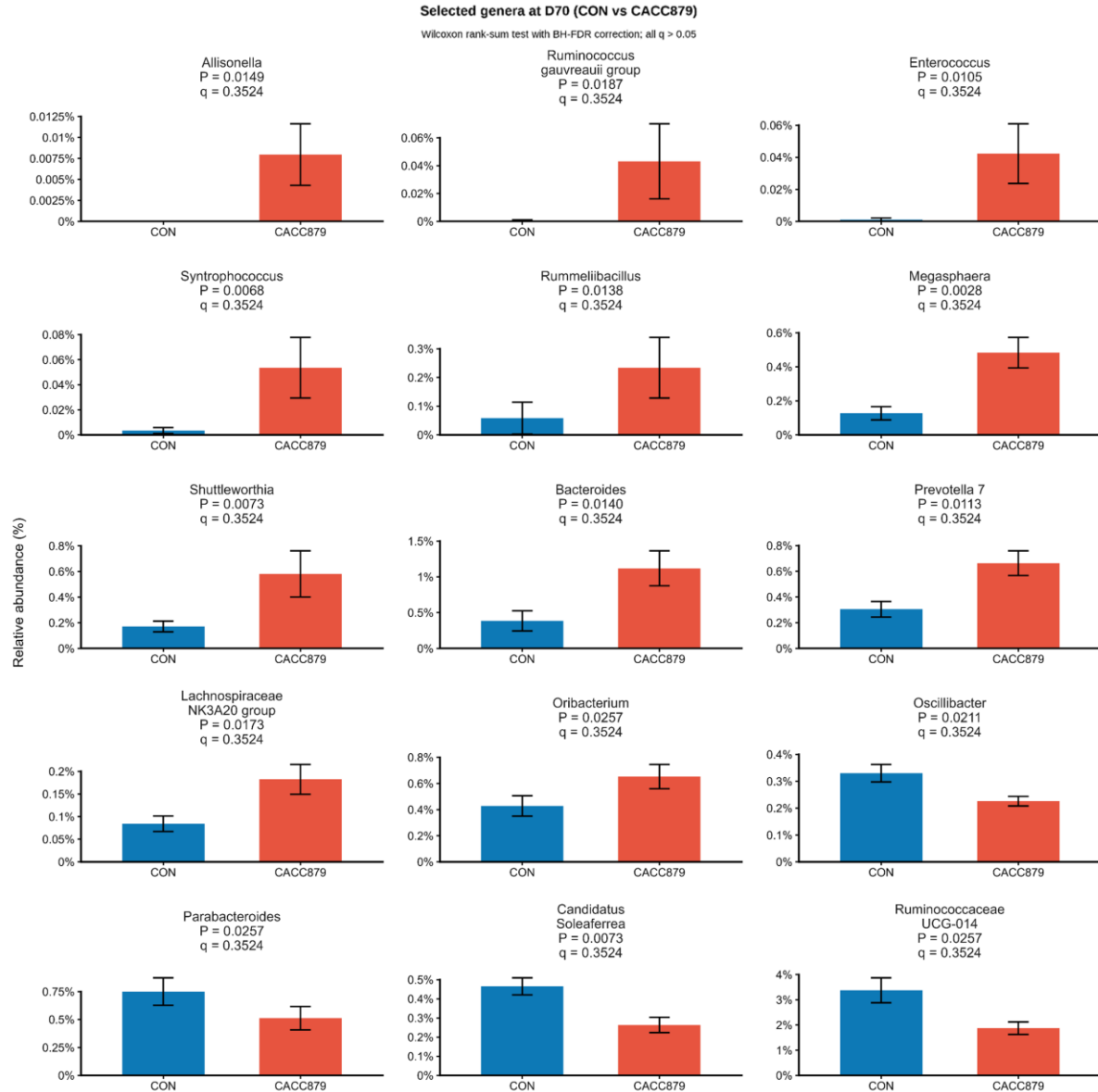


Figure 4. Selected genera showing nominal differences at D70 between the CON and CACC879 groups

Bar plots show the relative abundance (%) of genera with nominal differences between treatments based on the Wilcoxon rank-sum test. Benjamini-Hochberg FDR-adjusted q -values are indicated in the facet labels. D70 – day 70; CON, basal diet; CACC879, basal diet supplemented with *Lactobacillus curvatus* CACC879 at 0.25% (1.2×10^{10} CFU/g). Data are presented as means $\pm$ SEM. SEM – Standard error of the mean. None of the genera remained significant after FDR correction ($q > 0.05$)

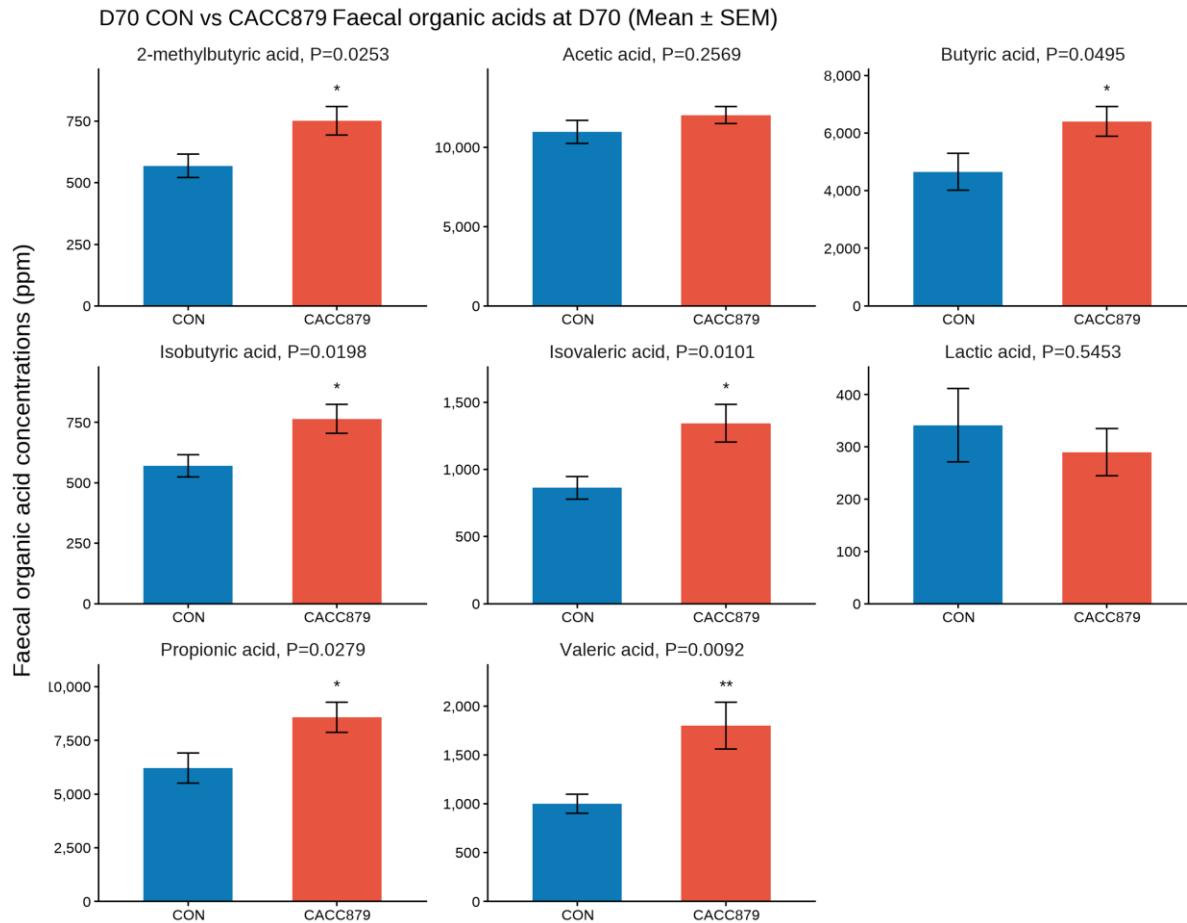


Figure 5. Effects of dietary *Latilactobacillus curvatus* CACC879 supplementation on faecal organic acid concentrations at D70 in finishing pigs

Concentrations of faecal organic acids were measured in prepared faecal extracts from the CON and CACC879 groups and are expressed as ppm. D70 – day 70; CON, basal diet; CACC879, basal diet supplemented with *L. curvatus* CACC879 at 0.25% (1.2×10^{10} CFU/g). Data are presented as means $\pm$ SEM. SEM – Standard error of the mean. Asterisks indicate significant differences between treatments (* $P < 0.05$, ** $P < 0.01$)

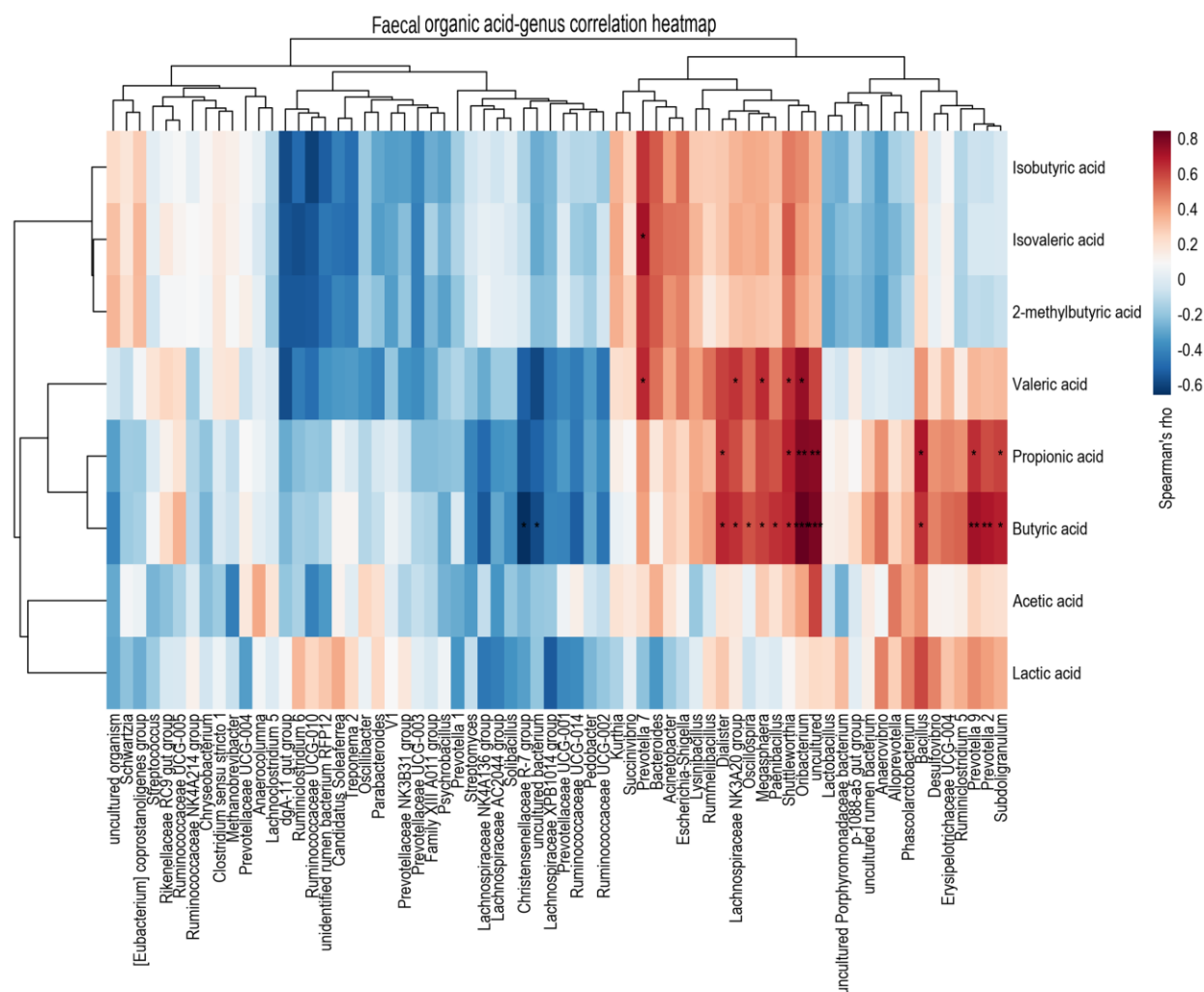


Figure 6. Correlations between faecal organic acids and bacterial genera in finishing pigs

Spearman correlation coefficients between faecal organic acid concentrations and relative abundances of major bacterial genera at D70 are shown. The color scale indicates Spearman's rho. Asterisks indicate significant correlations after false discovery rate correction. D70 – day 70

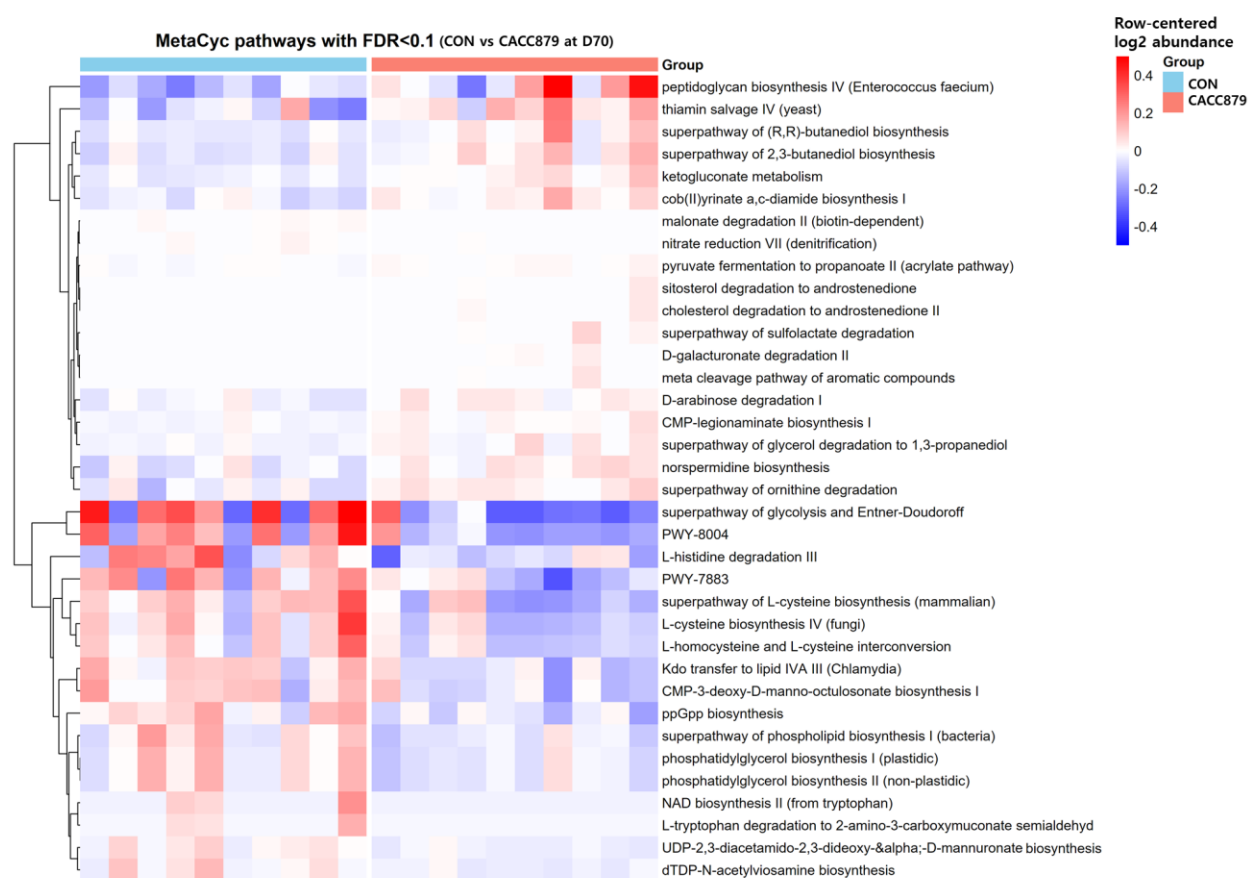
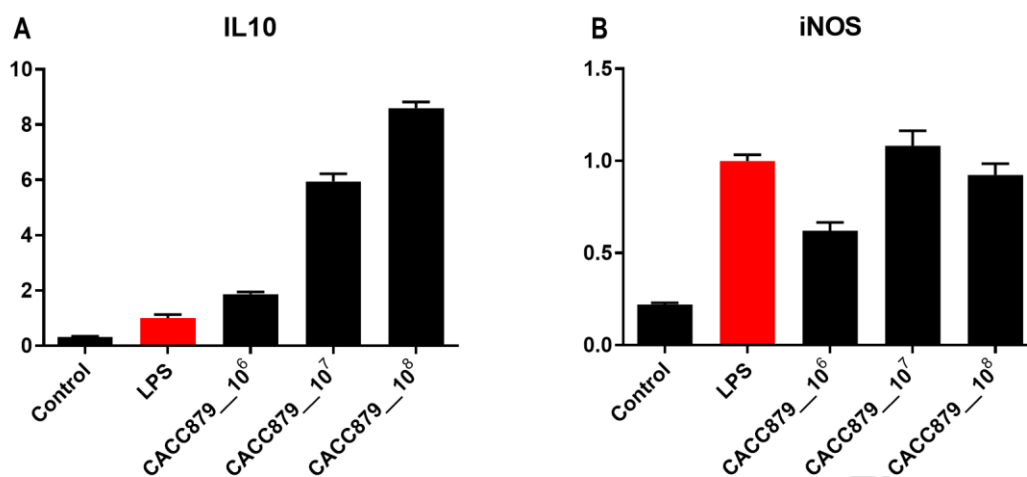


Figure 7. PICRUST2-inferred MetaCyc pathway profiles in faecal microbiota at D70

Predicted MetaCyc pathway abundances were compared between the CON and CACC879 groups, and pathways meeting an exploratory FDR threshold of $q < 0.1$ are shown as a heatmap. Columns represent individual samples and rows represent MetaCyc pathways; the top annotation bar indicates treatment group. The color scale indicates standardized relative pathway abundance. Both pathways and samples were ordered by hierarchical clustering. FDR – Benjamini-Hochberg false discovery rate; D70 – day 70; CON, basal diet; CACC879, basal diet supplemented with *Latilactobacillus curvatus* CACC879 at 0.25% (1.2×10^{10} CFU/g)



Supplementary figure 1. Effects of *Latilactobacillus curvatus* CACC879 treatment on IL10 and iNOS expression

IL10, interleukin-10; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide, CFU – colony-forming units. *L. curvatus* CACC879 was applied at 10^6 , 10^7 , and 10^8 CFU/well

Supplementary Table 1. Acid, bile, and heat tolerance and cell adhesion of *Latilactobacillus curvatus* CACC879

Acid tolerance , log CFU/ml, mean $\pm$ S.D			Bile tolerance, log CFU/ml, mean $\pm$ S.D		
pH 7.0 (control)	pH 2.5	Tolerance, %	control	0.3% oxgall	Tolerance, %
8.31 $\pm$ 0.17	7.91 $\pm$ 0.04	95.2	8.87 $\pm$ 0.03	8.58 $\pm$ 0.15	96.7
Heat tolerance, log CFU/ml, mean $\pm$ S.D			Cell-adhesion ability, log CFU/ml, mean $\pm$ S.D		
Control	Heat	Tolerance, %	0 h (control)	2 h	Tolerance, %
9.00 $\pm$ 0.05	4.91 $\pm$ 0.16	54.6	8.87 $\pm$ 0.11	4.95 $\pm$ 0.08	55.8