A meta-analysis of Lactobacillus-based probiotics for growth performance and intestinal morphology in piglets

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Antibiotics are widely used as growth promoters (AGPs) in livestock production to improve animal performance and health. However, pig producers today face the prohibition of in-feed antimicrobials and have to find safe and effective alternatives. Lactobacillus species are active microorganisms that convey multiple beneficial effects to the host and are one of the most promising AGPs replacements. Here, we aim to comprehensively assess the effects of Lactobacillus spp. supplementation on growth performance and intestinal morphology (villus height [VH], crypt depth [CD], and the V/C ratio) of piglets. Among the 196 identified studies, 20 met the criteria and were included in the meta-analysis. The effects of Lactobacillus-based probiotics supplementation on growth performance and intestinal morphology were analyzed using a random-effects model. And the publication bias was evaluated by funnel plots. Our results revealed that Lactobacillus spp. supplementation significantly improved the growth performance, including average daily feed intake (ADFI), average daily gain (ADG), and the gain-to-feed ratio (G/F) in piglets (P < 0.05). Meanwhile, Lactobacillus spp. remarkably increased VH and the V/C ratio (P < 0.05) in the small intestine, including the duodenum, jejunum, and ileum, which might contribute to an improved digestive capacity of these animals. In conclusion, our findings provide concrete evidence of the growth-promoting effects of Lactobacillus spp. supplementation in piglets and a better understanding of the potential of Lactobacillus-based probiotics as AGPs alternatives in pig production.

KEYWORDS
Lactobacillus, probiotics, piglets, antibiotic alternatives, intestinal morphology, growth performance

Introduction

Antibiotics used as growth promoters at sub-therapeutic doses to animals are an integral part of livestock production. Large-scale addition of antibiotic growth promoters (AGPs) to animal feed can help to improve production efficiency by improving animal performance represented by average daily feed intake (ADFI), average daily gain (ADG),
and gain-to-feed ratio (G/F) (1–3). Moreover, AGPs may alter intestinal morphology and promote digestion and absorption of nutrients in the intestine, which also contribute to animal growth and development (4). In pigs, the improved performance attributed to AGPs addition was between 4 to 8% (3, 5). However, the overuse of antibiotics has induced the development of multi-drug-resistant microorganisms in farm animals. It could seriously endanger animal production and public health (6, 7). Due to this concern, antibiotics used as growth promoters in livestock have been banned in the European Union since 2006 (8). As of 2017, the US has banned the use of medically-important antimicrobials for preventative and growth-promotion purposes the in the livestock sector (9). In addition, China prohibited in-feed antimicrobials in animal production in 2020 (10). Several antibiotic alternatives have been developed, studied, and tested in livestock to face the increasing global restrictions on antibiotic usage while maintaining animal health and performance.

Probiotics are live microbial supplements in adequacy or components of bacteria that confer beneficial effects on the intestinal health of the host (11) through occupying binding sites of the intestinal mucosa or competing for nutrients and niches with pathogenic bacteria (12, 13). Numerous studies have revealed that Lactobacillus species improve the growth performance and decrease the diarrhea ratio of piglets by enhancing nutrition digestibility and intestinal barrier function (14–19). Among the tested probiotics, Lactobacillus species are considered one of the most promising replacements and therefore represent a safe opportunity to substitute AGPs in pigs (18, 20). However, the wide variety of Lactobacillus species and different experimental designs make it difficult to comprehensively understand and further evaluate the effects of Lactobacillus species on swine and finally use them to replace AGPs at large in production. In this regard, meta-analysis constitutes a method integrating and analyzing numerous independent studies on the same subjects and makes the most representative conclusions (21). More importantly, in a meta-analysis, as the amount of data used increases, the precision of estimates can be improved on separate studies with different results (22). It justifies our attempt to employ this approach to determine the effects of Lactobacillus-based probiotics in pigs in the background of the emerging antibiotics alternative research. To provide a mechanism for estimating the effect degree, a strict design and clear selection criteria of studies, and the measurement index are necessary (22).

It is worth mentioning that the gut development of piglets is sensitive to alterations of feed components, reflected by their morphological changes (23). Histologically, the porcine intestine follows the general structure throughout its length and is similar to other monogastric animals and humans: the mucosa surface is covered by a monolayer of epithelium including absorptive enterocytes, goblet, and endocrine cells, etc. (23). The surface lining of epithelium quickly renews themselves and contributes to the absorptive surface and capacity of the intestine. It is commonly accepted to determine villus height, crypt depth, and the V/C ratio as “gold standards” of intestinal morphology, while these histological parameters could be used as a tool to evaluate gut function and responses toward feed ingredients (24). In the current study, we therefore performed a set of meta-analyses to delineate the effects of Lactobacillus species on pig growth performance and intestinal morphology.

Materials and methods

Study search and inclusion criteria

The protocols used were following the MOOSE guidelines (25). This study aimed to analyze the effects of Lactobacillus species supplementation in piglets with or without E. coli/LPS challenges on growth performance and intestinal morphological parameters. We have identified studies using Lactobacillus spp. including Lactobacillus delbrueckii, Lactobacillus reuteri, Lactobacillus plantarum and Lactobacillus acidophilus etc. published in English from June 2010 to June 2022. The search strategy consisted of a search of English databases, including PubMed, Google scholar, Cochrane library, semantic scholar Embased and Clinical Trials, and a search of Chinese databases, including VIP, CNKI, and WANGANG Data. The search terms included: (Lactobacillus OR Lactobacilli OR Lactic acid bacteria) AND (piglets OR piggy OR pigling) AND (growth performance OR average daily gain OR average daily feed intake OR feed efficiency) AND (duodenal villus height, crypt depth, and villus height to crypt depth OR jejunal villus height, crypt depth and villus height to crypt depth OR ileal villus height, crypt depth and villus height to crypt depth) in titles or abstracts.

The eligibility for inclusion of all studies identified from the searches was independently assessed and compared by the authors where the inclusion/exclusion criteria described previously were also considered (26). Manual selections were conducted on all returned publications based on the relevance of the titles and/or abstracts of the publications to Lactobacillus. Prerequisites of the selected publications were a downloadable full text and available data in English regarding Lactobacillus-based probiotics for growth performance and intestinal morphology in piglets.

Exclusion criteria

Studies excluded from this systematic review and meta-analysis were those that met the following criteria. Firstly, non-experimental articles (review articles); Secondly, articles with incorrect or incomplete data; Thirdly, articles without a control group; Finally, non-probiotics added or combined with other drugs and preparations in the experimental groups. A flow
A flow diagram of studies included in the meta-analysis Lactobacillus-based probiotics for growth performance and intestinal physiology in piglets.

196 articles filtered by keywords, including
140 PubMed
20 Semantic Scholar
5 Clinical Trials
7 CNKI
24 Embase

86 deviating articles were removed

90 articles excluded
Exclusion criteria:
1. Non-experimental articles (18)
2. Articles with incomplete data (12)
3. Articles without a control group (6)
4. Unable to extract data (24)
5. No matching outcome (18)
6. Study subjects were not weaned piglets (10)

110 articles retained (title and abstract)

20 articles selected for data extraction

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Data extraction

Data relating to the effects of Lactobacillus spp. supplementation with or without E. coli/LPS challenges on the growth performance and intestinal physiology in piglets were collected from each selected article using a custom-tailored excel form that included detailed information as follows: animals (breed, sex distribution, and age), sample size, experimental design including the control group set up, target Lactobacillus strains, the amount (dose) of probiotic supplementation, administration methods and duration, authors. When results were available only in graphical format, data were extracted using Review Manager (Version 5.4 provided by Cochrane Training). Graph digitization has been previously shown to be a valid method for extracting study data (27). If the data were unclear or some key data were missing, we attempted to contact the corresponding authors through email to obtain further information. The outcomes were as follows: ADG (average daily gain); ADFI (average daily feed intake); G/F (gain-to-feed ratio); diarrhea rate; Duodenum VH (villus height), CD (crypt depth) and V/C (villus height:crypt depth); Jejunum VH, CD and V/C; and Ileum VH, CD and V/C.

In our initial search, 2123 English language records were hit which, after screening, revealed 196 unique articles. A total of 20 papers that fulfilled the selection criteria were included in the quantitative meta-analysis, including Suo et al. (28); Sayan et al. (14); Chen et al. (29); Liu et al. (30); Yi et al. (15); Yang et al. (31); Wang et al. (32); Qiao et al. (33); Lan et al. (34); Lee et al. (35); Li et al. (36); Tang et al. (37); Tian et al. (38); Sonia et al. (39); Eliette et al. (40); Liu et al. (41); Moturi et al. (42); Lee et al. (43); Cao et al. (44); and Jeong et al. (45). The characteristics of each study are shown in Table 1. The median initial body weight (BW) of the piglet was 6.50 kg (from 1.50 kg to 15.6 kg). In total, 6 studies included used antibiotics as the control group, and 8 studies included had piglets challenged with E. coli or LPS.

Statistical analyses

Considering that there are contrary data within the range of the research purpose and the heterogeneity between different studies may interfere with the analyzed results, we used a random-effects model to compute the 95% confidence interval (95% CI) of the standardized mean difference (SMD). Heterogeneity was assessed using chi-square test and the I² parameter (30–60% indicating moderate, 50–90% indicating substantial, and 75–100% indicating considerable heterogeneity). For the identification and assessment of reporting bias, we tested funnel plot symmetry by the Begg and Egger method (47). All the above analyses were performed by the Review Manager (Version 5.4 provided by Cochrane Training). P < 0.05 is considered significant.
| Studies       | Period        | Treatment    | Added amount | Control                          | Sample size and sex | BW ranges          | Outcomes                      | Challenge |
|--------------|---------------|--------------|--------------|----------------------------------|---------------------|--------------------|--------------------------------|------------|
| Suo et al.   | post–weaning  | *L. plantarum* | $1 \times 10^9$ CFU/d | Basal diet and antibiotics       | 150 NA              | 7.69–11.59 kg      | ADG, ADFI, G/F, duodenum V/C, ileum V/C, jejunum V/C | NA         |
| Sayan et al. | pre–weaning   | *L. salivarius* | $1 \times 10^8$ CFU/mL | Basal diet                        | 201 NA              | 1.59–6.18 kg       | ADG, ADFI, G/F, ileum V/C     | E. coli    |
| Chen et al.  | post–weaning  | *L. delbrueckii* | $2.01 \times 10^9$ CFU/g | Basal diet                        | 36 average          | 7.79–16.15 kg      | ADG, ADFI, G/F, duodenum V/C, ileum V/C, jejunum V/C | NA         |
| Liu et al.   | pre–weaning   | *L. fermentum* | $6 \times 10^8$ CFU/mL | Basal diet                        | 36 NA               | 2.31–4.73 kg       | ADG, ileum V/C               | NA         |
| Yi et al.    | post–weaning  | *L. reuteri*   | $5 \times 10^{10}$ CFU/kg | Basal diet and antibiotics       | 144 average         | 6.49–9.89 kg       | ADG, ADFI, ileum V/C, jejunum V/C | NA         |
| Wang et al.  | pre–weaning   | *L. plantarum* | $1 \times 10^9$ CFU/d | Basal diet                        | 60 NA               | 1.50–3.56 kg       | ADG, ileum V/C, jejunum V/C   | NA         |
| Qiao et al.  | post–weaning  | *L. acidophilus* | 0.05%, 0.1%, 0.2% | Basal diet and antibiotics       | 150 females         | 7.53–16.31 kg      | ADG, ADFI, G/F               | LPS        |
| Lan et al.   | post–weaning  | *L. acidophilus* | 1.2, 3 g/kg | Basal diet                        | 175 NA              | 7.15 kg–NA         | ADG, ADFI, G/F               | E. coli    |
| Lee et al.   | post–weaning  | *L. plantarum* | $10^5$, $10^7$, $10^9$ CFU/kg | Basal diet and antibiotics | 108 NA              | 8.74–21.84 kg      | ADG, ileum V/C               | NA         |
| Li et al.    | post–weaning  | *L. mucosae*   | $1 \times 10^7$ CFU/mL | Basal diet                        | 104 average         | 5.90–18.82 kg      | ADG, ileum V/C, jejunum V/C   | E. coli, LPS |
| Tang et al.  | post–weaning  | *L. reuteri*   | $5 \times 10^8$ CFU/kg | Basal diet                        | 90 NA               | 6.1–14.9 kg        | ADG, ileum V/C               | NA         |
| Tian et al.  | post–weaning  | *L. reuteri*   | $5 \times 10^{10}$ CFU/kg | Basal diet                        | 144 males           | 6.49 kg–NA         | ADG, ADFI, F/G, ileum V/C, jejunum V/C, duodenum V/C | NA         |
| Tabasum et al.| post–weaning  | *L. reuteri*   | 0.5%, 0.04% | Basal diet and antibiotics       | 96 NA               | 8.00 kg–NA         | ADG, ADFI, G/F               | E. coli    |
| Riboulet-Bisson et al. | pre–weaning | *L. salivarius* | $1 \times 10^8$ CFU/mL | Basal diet                        | 30 NA               | 12.7–34.4 kg       | ADG, ADFI, G/F               | NA         |
| Liu et al.   | post–weaning  | *L. brevis*    | 0.4, 0.8 g/kg | Basal diet                        | 144 NA              | 15.6–24.9 kg       | ADG, ADFI, G/F               | NA         |
| Moturi et al.| nursery       | *L. salivarius* | $1 \times 10^8$ CFU/mL | Basal diet                        | 30 NA               | 1.54–6.22 kg       | ADG, ileum V/C, jejunum V/C, duodenum V/C | NA         |
| Lee et al.   | post–weaning  | *L. acidophilus* | 0.10% | Basal diet                        | 40 NA               | 7.10–11.60 kg      | ADG, ADFI, G/F               | LPS        |
| Cao et al.   | post–weaning  | *L. acidophilus* | $1 \times 10^8$ CFU/mL | Basal diet                        | 180 NA              | 6.2 kg–NA          | ADG, ADFI, ileum V/C, jejunum V/C | NA         |
| Jeong et al. | post–weaning  | *L. casei*     | $1 \times 10^{11}$ CFU/mL | Basal diet                        | 240 NA              | 7.05 kg–NA         | ADG, ADFI, ileum V/C, jejunum V/C | NA         |

*BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G/F, gain: feed ratio; NA, not applicable; LPS, lipopolysaccharide; V/C, villus height, crypt depth ratio.*
### TABLE 2: Effects of Lactobacillus–based probiotics on the average daily gain (ADG) of pigs from included studies.

| Studies             | Treatment     | Added amount | Experimental | Control | Weight | Std. mean difference |
|---------------------|---------------|--------------|--------------|---------|--------|----------------------|
| Suo et al. (28)     | L. plantarum  | $1 \times 10^9$ CFU/d | 469 | 57.44 | 30 | 4.30% | 0.17 $[−0.34, 0.68]$ |
| Riboulet-Bisson et al. (40) | L. salivarius | $1 \times 10^9$ CFU/mL | 748 | 55.03 | 8 | 2.00% | $−0.70 [−1.72, 0.32]$ |
| Liu et al. (46)     | L. brevis     | 0.4 g/kg     | 361 | 116.92 | 48 | 4.90% | 0.98 $[0.55, 1.40]$ |
| Liu et al. (46)     | L. brevis     | 0.8 g/kg     | 318 | 116.92 | 48 | 5.00% | 0.61 $[0.20, 1.02]$ |
| Sayan et al. (14)   | L. salivarius | $1 \times 10^9$ CFU/mL | 191 | 64.26 | 87 | 5.90% | 0.40 $[0.12, 0.68]$ |
| Yi et al. (15)      | L. reuteri     | $5 \times 10^{10}$ CFU/kg | 243 | 56.01 | 48 | 5.00% | 0.80 $[0.38, 1.21]$ |
| Liu et al. (46)     | L. brevis     | 0.4 g/kg     | 314 | 74.31 | 30 | 4.30% | 0.37 $[−0.14, 0.88]$ |
| Qiao et al. (33)    | L. acidophilus | 0.05%       | 312 | 74.31 | 30 | 4.30% | 0.60 $[0.08, 1.12]$ |
| Qiao et al. (33)    | L. acidophilus | 0.10%       | 314 | 74.31 | 30 | 4.30% | 0.62 $[0.11, 1.14]$ |
| Moturi et al. (42)  | L. salivarius | 144 $1 \times 10^8$ CFU/mL | 223 | 12.83 | 10 | 2.50% | 0.15 $[−0.73, 1.03]$ |
| Moturi et al. (42)  | L. salivarius | 160 $1 \times 10^8$ CFU/mL | 224 | 12.83 | 10 | 2.40% | 0.22 $[−0.66, 1.10]$ |
| Yang et al. (31)    | L. plantarum  | $5 \times 10^8$ CFU/kg | 270 | 65.48 | 18 | 3.40% | 0.25 $[−0.40, 0.94]$ |
| Tang et al. (37)    | L. plantarum  | $5 \times 10^8$ CFU/kg | 244 | 57.38 | 30 | 4.30% | 0.64 $[0.12, 1.16]$ |
| Tang et al. (37)    | L. reuteri     | $5 \times 10^8$ CFU/kg | 274 | 57.38 | 30 | 4.10% | 1.15 $[0.60, 1.70]$ |
| Lan et al. (34)     | L. acidophilus | 1g/kg       | 488 | 60.02 | 35 | 4.50% | 0.71 $[0.22, 1.19]$ |
| Lan et al. (34)     | L. acidophilus | 2g/kg       | 490 | 60.02 | 35 | 4.50% | 0.74 $[0.26, 1.23]$ |
| Lan et al. (34)     | L. acidophilus | 3g/kg       | 492 | 60.02 | 35 | 4.50% | 0.77 $[0.29, 1.26]$ |
| Lee et al. (34)     | L. acidophilus | 0.10%       | 328 | 12.11 | 20 | 3.40% | 0.97 $[0.31, 1.63]$ |
| Cao et al. (44)     | L. acidophilus | $1 \times 10^8$ CFU/mL | 275 | 40.71 | 36 | 4.60% | 0.15 $[−0.32, 0.61]$ |
| Tabasum et al. (39) | Lactobacillus | 0.50%       | 390 | 66.86 | 24 | 3.10% | 2.16 $[1.44, 2.89]$ |
| Jeong et al. (45)   | L. casei      | 0.10%       | 322 | 47.65 | 60 | 5.30% | 0.65 $[0.28, 1.01]$ |
| Jeong et al. (45)   | L. casei      | 0.20%       | 325 | 47.65 | 60 | 5.40% | 0.29 $[−0.07, 0.65]$ |
| Tian et al. (38)    | L. reuteri     | $5 \times 10^8$ CFU/kg | 675 | 15.87 | 48 | 4.70% | 1.44 $[0.99, 1.90]$ |
| Total (95% CI)      |               |             | 828 |       | 855 | 100.00% | 0.65 $[0.48, 0.82]$ |

Heterogeneity: $\tau^2 = 0.11$; $Chi^2 = 62.39$; $df = 23$ ($P < 0.0001$), $I^2 = 63\%$.

Test for overall effect, $Z = 7.45$ ($P < 0.00001$).
TABLE 3  Effects of *Lactobacillus*-based probiotics on the average daily feed intake (ADFI) pigs from included studies.

| Studies               | Treatment      | Added amount | Experimental | Control     | Weight | Std. mean difference |
|-----------------------|----------------|--------------|--------------|-------------|--------|----------------------|
|                       |                |              | Mean  SD  Total | Mean  SD  Total |        |                      |
| Riboulet-Bisson et al. (40) | *L. salivarius* | $1 \times 10^8$ CFU/mL | 962  105.82  8 | 973  91.01  8 | 3.50% | 0.11 [−1.09, 0.88]   |
| Liu et al. (46)        | *L. brevis*    | 0.4 g/kg     | 679  163.13  48 | 591  163.13  48 | 5.80% | 0.54 [0.13, 0.94]    |
| Liu et al. (46)        | *L. brevis*    | 0.8 g/kg     | 678  163.13  48 | 591  163.13  48 | 5.80% | 0.53 [0.12, 0.94]    |
| Yi et al. (15)         | *L. reuteri*   | $5 \times 10^{10}$ CFU/kg | 358  77.02  48 | 310  77.02  48 | 5.80% | 0.62 [0.21, 1.03]    |
| Qiao et al. (33)       | *L. acidphilus* | 0.05%        | 530  76.89  30 | 519  76.89  30 | 5.40% | 0.14 [−0.37, 0.65]   |
| Qiao et al. (33)       | *L. acidphilus* | 0.10%        | 536  76.89  30 | 519  76.89  30 | 5.40% | 0.22 [−0.29, 0.73]   |
| Qiao et al. (33)       | *L. acidphilus* | 0.20%        | 541  76.89  30 | 519  76.89  30 | 5.40% | 0.28 [−0.23, 0.79]   |
| Yang et al. (31)       | *L. plantarum* | $5 \times 10^9$ CFU/kg | 234  21.83  18 | 226  21.83  18 | 4.80% | 0.36 [−0.30, 1.02]   |
| Tang et al. (37)       | *L. plantarum* | $5 \times 10^9$ CFU/kg | 358  74.65  30 | 321  74.65  30 | 5.40% | 0.49 [−0.02, 1.00]   |
| Tang et al. (37)       | *L. reuteri*   | $5 \times 10^9$ CFU/kg | 384  74.65  30 | 321  74.65  30 | 5.30% | 0.83 [0.30, 1.36]    |
| Lan et al. (34)        | *L. acidphilus* | 1g/kg        | 682  48.02  35 | 663  48.02  35 | 5.60% | 0.39 [−0.08, 0.86]   |
| Lan et al. (34)        | *L. acidphilus* | 2g/kg        | 690  48.02  35 | 663  48.02  35 | 5.50% | 0.56 [0.08, 1.03]    |
| Lan et al. (34)        | *L. acidphilus* | 3g/kg        | 691  48.02  35 | 663  48.02  35 | 5.50% | 0.58 [0.10, 1.06]    |
| Lee et al. (43)        | *L. acidphilus* | 0.10%        | 405  14.82  20 | 390  14.82  20 | 4.80% | 0.99 [0.33, 1.65]    |
| Cao et al. (44)        | *L. acidphilus* | $1 \times 10^8$ CFU/mL | 369  51.66  36 | 368  51.66  36 | 5.60% | 0.02 [−0.44, 0.48]   |
| Tabasum et al. (39)    | *Lactobacillus* | 0.50%        | 695  40.09  24 | 484  40.09  24 | 2.80% | 5.18 [3.96, 6.40]    |
| Jeong et al. (45)      | *L. casei*     | 0.10%        | 461  93.74  60 | 433  93.74  60 | 6.00% | 0.30 [−0.06, 0.66]   |
| Jeong et al. (45)      | *L. casei*     | 0.20%        | 453  93.74  60 | 439  93.74  60 | 6.00% | 0.15 [−0.21, 0.51]   |
| Tian et al. (38)       | *L. reuteri*   | $5 \times 10^9$ CFU/kg | 1770  50.25  48 | 1685  50.22  48 | 5.60% | 1.68 [1.21, 2.15]    |
| Total (95% CI)         |                |              | 673  673  100.00% | 673  673  100.00% | 0.61 [0.35, 0.88] |

Heterogeneity, $\text{I}^2 = 82\%$. Test for overall effect, $Z = 4.49$ ($P < 0.00001$).
TABLE 4 Effects of Lactobacillus–based probiotics on the gain to feed (G/F) ratio of pigs from included studies.

| Studies | Treatment | Added amount | Experimental | Control | Weight | Std. mean difference |
|---------|-----------|--------------|--------------|---------|--------|----------------------|
|         |           |              | Mean | SD | Total | Mean | SD | Total | IV, Random, 95% CI |
| Riboulet-Bisson et al. (40) | *L. salivarius* | 1 x 10⁸ CFU/mL | 0.78 | 0.09 | 8 | 0.82 | 0.05 | 8 | 1.20% | ~0.52 [−1.52, 0.48] |
| Liu et al. (46) | *L. brevis* | 0.4 g/kg | 0.53 | 0.29 | 48 | 0.417 | 0.29 | 48 | 7.30% | 0.39 [−0.01, 0.80] |
| Liu et al. (46) | *L. brevis* | 0.8 g/kg | 0.47 | 0.29 | 48 | 0.417 | 0.29 | 48 | 7.50% | 0.18 [−0.22, 0.58] |
| Yi et al. (15) | *L. reuteri* | 5 x 10⁸ CFU/kg | 0.68 | 0.07 | 48 | 0.64 | 0.07 | 48 | 7.20% | 0.57 [0.16, 0.98] |
| Qiao et al. (46) | *L. brevis* | 0.4 g/kg | 0.53 | 0.29 | 48 | 0.417 | 0.29 | 48 | 7.30% | 0.39 [−0.01, 0.80] |
| Qiao et al. (46) | *L. brevis* | 0.8 g/kg | 0.47 | 0.29 | 48 | 0.417 | 0.29 | 48 | 7.50% | 0.18 [−0.22, 0.58] |
| Yi et al. (15) | *L. reuteri* | 5 x 10¹⁰ CFU/kg | 0.56 | 0.18 | 30 | 0.518 | 0.18 | 30 | 4.60% | 0.36 [−0.15, 0.87] |
| Qiao et al. (33) | *L. acidophilus* | 0.10% | 0.59 | 0.18 | 30 | 0.518 | 0.18 | 30 | 4.60% | 0.34 [−0.17, 0.85] |
| Qiao et al. (33) | *L. acidophilus* | 0.20% | 0.58 | 0.18 | 30 | 0.518 | 0.18 | 30 | 4.60% | 0.34 [−0.17, 0.85] |
| Yang et al. (31) | *L. plantarum* | 1.16 | 0.09 | 18 | 1.12 | 0.09 | 18 | 2.70% | 0.45 [−0.21, 1.11] |
| Tang et al. (37) | *L. plantarum* | 5 x 10⁸ CFU/kg | 0.68 | 0.12 | 30 | 0.664 | 0.12 | 30 | 4.70% | 0.14 [−0.37, 0.64] |
| Tang et al. (37) | *L. reuteri* | 5 x 10⁸ CFU/kg | 0.71 | 0.12 | 30 | 0.664 | 0.12 | 30 | 4.60% | 0.40 [−0.11, 0.92] |
| Lan et al. (34) | *L. acidophilus* | 1g/kg | 0.72 | 0.12 | 35 | 0.671 | 0.12 | 35 | 5.40% | 0.37 [−0.10, 0.84] |
| Lan et al. (34) | *L. acidophilus* | 2g/kg | 0.71 | 0.12 | 35 | 0.671 | 0.12 | 35 | 5.40% | 0.34 [−0.13, 0.81] |
| Lan et al. (34) | *L. acidophilus* | 3g/kg | 0.71 | 0.12 | 35 | 0.671 | 0.12 | 35 | 5.40% | 0.33 [−0.14, 0.80] |
| Lee et al. (43) | *L. acidophilus* | 0.10% | 0.81 | 0.05 | 20 | 0.81 | 0.05 | 20 | 3.10% | 0.00 [−0.62, 0.62] |
| Cao et al. (44) | *L. casei* | 1 x 10⁸ CFU/mL | 0.75 | 0.30 | 36 | 0.73 | 0.30 | 36 | 5.60% | 0.07 [−0.40, 0.53] |
| Jeong et al. (45) | *L. casei* | 0.10% | 0.7 | 0.31 | 60 | 0.67 | 0.31 | 60 | 9.30% | 0.10 [−0.26, 0.45] |
| Jeong et al. (45) | *L. casei* | 0.20% | 0.72 | 0.31 | 60 | 0.71 | 0.31 | 60 | 9.40% | 0.03 [−0.33, 0.39] |
| Tian et al. (38) | *L. reuteri* | 5 x 10⁸ CFU/kg | 0.38 | 0.14 | 48 | 0.386 | 0.28 | 48 | 7.50% | −0.02 [−0.42, 0.38] |

Total (95% CI) 649 649 100.00% 0.23 [0.12, 0.34]

Heterogeneity: Tau² = 0.00; Chi² = 11.79, df = 17 (P = 0.81); I² = 0%.
Test for overall effect: Z = 4.05 (P < 0.0001).
Results

Forest plots and sensitivity analysis of Lactobacillus species to growth performance in piglets

A meta-analysis was performed to examine the effect of probiotic Lactobacillus spp. on ADG (24 trials, \( n = 1683 \) subjects). The summarized results of standardized mean difference (SMD) and 95% confidence interval (CI) for each study are shown in Table 2. There was a significant positive correlation between ADG and the Lactobacillus spp. addition (SMD = 0.65; 95% CI: 0.48–0.82; \( I^2 = 63\% \); \( P < 0.001 \)), and Lactobacillus spp. supplementation improved the ADG of piglets. Next, the effects of Lactobacillus spp. supplementation on ADFI in piglets were determined by forest plots and sensitivity analysis (19 trials, \( n = 1346 \) subjects). The summarized results of SMD and 95% CI are shown in Table 3. Lactobacillus spp. remarkably increased the ADFI of piglets compared to the control (SMD = 0.61; 95% CI: 0.35–0.88; \( I^2 = 63\% \); \( P < 0.001 \)), but showed a high heterogeneity (\( I^2 = 82\% \)). In the duodenum of piglets, the analysis showed a positive correlation between the V/C ratio and Lactobacillus spp. supplementation (SMD = 0.41; 95% CI: 0.17–0.65; \( I^2 = 65\% \); \( P = 0.002 \)).

Forest plots and sensitivity analysis of Lactobacillus species to the duodenal morphology in piglets

The Lactobacillus-promoted growth performance of piglets identified by our analysis prompted us to further study the effects of Lactobacillus spp. on parameters like villus height (VH), crypt depth (CD), and the V/C ratio, representing changes in the small intestinal morphology of piglets (Table 5, Figure 2). First, we found that porcine duodenal VH (12 trials, \( n = 877 \) subjects) was significantly increased by Lactobacillus spp. supplementation compared to the control (SMD = 0.64; 95% CI: 0.32–0.92; \( I^2 = 79\% \); \( P < 0.001 \)). In contrast, no significant correlation between the addition of Lactobacillus spp. and the duodenal CD was observed (12 trials, \( n = 877 \) subjects; SMD = −0.03; 95% CI: −0.24–0.19; \( I^2 = 54\% \); \( P = 0.81 \)). In the duodenum of piglets, the analysis showed a positive correlation between the V/C ratio and Lactobacillus spp. supplementation (SMD = 0.41; 95% CI: 0.17–0.65; \( I^2 = 65\% \); \( P = 0.002 \)).

Forest plots, sensitivity analysis, and funnel plots of Lactobacillus species to jejunal morphology in piglets

In the porcine jejunum, the effects of probiotics on VH, CD, and the V/C ratio were evaluated (Table 5, Figure 2). In the jejunum of piglets (14 trials, \( n = 880 \) subjects), the analysis showed a positive effect with the addition of Lactobacillus spp.
Funnel plots of the publication bias analysis of included outcomes on pigs. (A) Average daily gain, (B) Average daily feed intake, (C) Gain: feed ratio, (D) Duodenal VH, (E) Duodenal CD, (F) duodenal V/C, (G) Jejunal VH, (H) Jejunal CD, (I) Jejunal, (J) Ileal VH, (K) Ileal CD, (L) Ileal V/C. n = 12; CD, crypt depth; SE, standard error; SMD, standard mean difference; V/C, villus height: crypt depth; VH, villus height; V/C, villus height: crypt depth.

on the VH values (SMD = 0.45; 95% CI: 0.21~0.70; I² = 65%; P = 0.005). In addition, the effect of Lactobacillus spp. addition on the jejunal CD was also examined (14 trials, n = 880 subjects), and Lactobacillus supplementation significantly decreased CD, compared to the control (SMD = −0.15; 95% CI: −0.44~0.13; I² = 75%; P < 0.001). The changes of the V/C ratio between the control and the addition of Lactobacillus spp. were analyzed (13 trials, n = 844 subjects), and found that it was significantly
TABLE 5  Effects of Lactobacillus–based probiotics on small intestinal morphology of pigs from included studies.

| Outcomes       | No. of trials | Effect size | P-value | Heterogeneity | P-value |
|----------------|---------------|-------------|---------|---------------|---------|
|                |               | SMD 95%CI   |         | I^2  | Tau^2 | Chi^2 |
| Duodenum        |               |             |         |     |       |       |
| Villus height (VH) | 12            | 0.64 [0.32, 0.96] | <0.001  | 79%  | 0.23  | 52.18 | <0.001 |
| Crypt depth (CD) | 12            | −0.03 [−0.24, 0.19] | 0.810   | 54%  | 0.07  | 23.83 | 0.010  |
| VH/CD           | 12            | 0.41 [0.17, 0.65] | <0.001  | 63%  | 0.10  | 30.04 | 0.002  |
| Jejunum         |               |             |         |     |       |       |
| Villus height (VH) | 14            | 0.45 [0.21, 0.70] | <0.001  | 66%  | 0.13  | 38.07 | <0.001 |
| Crypt depth (CD) | 14            | −0.15 [−0.44, 0.13] | 0.300   | 75%  | 0.21  | 53.03 | <0.001 |
| VH/CD           | 13            | 0.58 [0.28, 0.88] | <0.001  | 76%  | 0.22  | 50.84 | <0.001 |
| Ileum           |               |             |         |     |       |       |
| Villus height (VH) | 13            | 0.42 [0.19, 0.64] | <0.001  | 62%  | 0.10  | 31.95 | 0.001  |
| Crypt depth (CD) | 13            | −0.27 [−0.46, −0.09] | 0.004   | 47%  | 0.05  | 22.77 | 0.030  |
| VH/CD           | 13            | 0.60 [0.33, 0.87] | <0.001  | 73%  | 0.16  | 44.85 | <0.001 |

increased by the addition of Lactobacillus spp. (SMD = 0.58; 95% CI: 0.28~0.88; I^2 = 76%; P < 0.001).

Forest plots and sensitivity analysis of Lactobacillus species to ileum morphology in piglets

Finally, we analyzed the effects of probiotic Lactobacillus spp. supplementation on the ileal morphology of piglets (13 trials, n = 973 subjects; Table 5, Figure 2). The ileal VH was strongly positively correlated with Lactobacillus spp. addition (SMD = 0.42; 95% CI: 0.19~0.64; I^2 = 62%; P = 0.001). Next, the effects of Lactobacillus spp. addition on the ileal CD were determined (13 trials, n = 973 subjects). Lactobacillus spp. was associated with a significant reduction of the ileal CD (SMD = −0.27; 95% CI: −0.46~−0.09; I^2 = 47%; P =0.03). For the ileal V/C ratio (13 trials, n = 973 subjects), the addition of probiotic Lactobacillus spp. significantly increased this value when compared to the control (SMD = 0.60; 95% CI: 0.33~0.87; I^2 = 73%; P < 0.001).

Discussion

Antibiotics are widely used as growth promoters in livestock to improve animal growth performance and health. Several hypotheses on the AGPs’ mode of action have been proposed, including reducing pathogenic load and toxin production and inhibiting gut disorders while improving intestinal physiology (48, 49). However, due to the side effects (e.g., antibiotic resistance and environmental pollutants of its residues), the use of antibiotics has been restricted worldwide, which results in the urgent need to find alternative routes to manage animal health and maintain production efficiency (50, 51). Lactobacillus species are one of the most commonly used probiotic agents in swine and are considered one such potent AGPs replacement (18, 52, 53). Therefore, this study systematically reviewed and performed a set of meta-analyses to determine the effects of probiotic Lactobacillus spp. on porcine growth performance and intestinal morphology. The main findings were as follows: (1) Lactobacillus spp. supplementation can improve piglets’ performance including ADFI, ADG and the G/F ratio, and is superior to antibiotics in growth promotion; (2) Lactobacillus spp. supplementation substantially modified the small intestinal morphology, increased VH and the V/C ratio of piglets in all segments, whereas decreased the jejunal and ileal CD.

The growth performance is an essential indicator of pig health and the economic benchmark of the production system. Comparable to the AGPs, there are also theories on the mechanism of growth improvement of probiotics in animals, which include improving the gut barrier function, nutrient utilization, gut microbiome, intestinal morphology, and immunity (18). In particular, important indexes like average daily gain, average daily feed intake, and the gain-to-feed ratio reflect nutrient uptake and absorptive capacity, where higher values in the Lactobacillus-treated piglets implied improved nutrient utilization (45). Our meta-analysis revealed that ADG and ADFI were increased by 13.8% and 7.02% on average by Lactobacillus addition. It is suggested that Lactobacillus-based feeding could enhance feed palatability (54), which may explain the increased feed intake. A probiotic-improved G/F ratio was reported by us, suggesting that Lactobacillus supplementation brings about a more cost-effective feeding program than the control piglets. Another mechanism by which Lactobacillus spp. enhance animal performance may be via the promotion of beneficial bacteria and inhibition of harmful microorganisms in the intestinal microenvironment (55).
Growing evidence indicates that gut microbiota plays a crucial role in host metabolic health and fitness. A healthy small intestine has a dominance of Lactobacillus spp., which may be disrupted by perturbations like weaning and changes of feed in piglets (56). By adding a Lactobacilli compound (including L. gasseri, L. reuteri, L. acidophilus and L. fermentum), Huang et al. showed that significantly decreased the E. coli and aerobe counts, and increased Lactobacilli and anaerobe counts in the digesta and mucosa, thereby promoting growth performance of pigs (57). Furthermore, Lactobacillus spp. can increase the levels of microbial metabolites such as butyrate to alleviate piglet diarrhea, which directly and indirectly affects growth performance (58). Although the gut microbiome is not analyzed here, we choose to study the effects of Lactobacillus spp. in piglets at the age of weaning. When antibiotics are often used due to sudden changes in diets and gut microbiota dysbiosis, which further impair pig performance and health. It is noteworthy that at weaning, a reduction of intestinal VH or villus atrophy may occur (56).

In this regard, we found that Lactobacillus spp. supplementation significantly increased VH and the V/C ratio in the small intestine of piglets. It may be partly due to the Lactobacillus-increased daily feed intake, resulting in a trophic action on the development of intestinal epithelium (18). As the main digestive and absorptive site, increases in small intestinal VH and the V/C ratio are directly related to the larger surface area and enhanced epithelium turnover and cell mitosis activation (24, 59). It allows for enhanced uptake of dietary substances while excluding noxious agents in the gut lumen (60). Finally, the improved gut morphology can facilitate digestion, and absorption of nutrients, fluid, and electrolytes for piglets (18, 24, 59, 60), promoting growth performance (61). This was supported by studies we have summarized in our meta-analysis and numerous other studies (14, 18). In addition, in piglets challenged with LPS or E. coli, carbohydrate and fatty acid utilization can be compromised due to inflammation, while the probiotic can alter the villus-crypt architecture and influence the associated enzyme activity and nutrient transport receptor expression (62, 63). Similarly, Zhang et al. demonstrated that Lactobacilli supplementation increases digestive enzyme activities and promotes growth performance (12). Furthermore, the addition of Lactobacillus resulted in the enhancement of genes for the metabolism, transport, and catabolism of vitamins, amino acids, lipids, and polyketides, thus the improvement of growth performance (64). In addition, we also reported a location-specific response of intestinal histology driven by Lactobacillus spp. i.e., Lactobacillus supplementation improved all jejunal and ileal histological parameters, likely related to the gradient distribution of microbiota along the pig small intestine (65, 66). It is also suggested that the probiotics, live bacteria, their signaling or metabolites, must have reached the distal part of the intestine in piglets and become effective. However, care must be taken as the meta-analysis approach has limitations.

For instance, when the number of included studies is small, the number of trial characteristics is large, and the heterogeneity of data becomes large, which was also seen in our studies. And even if the number of studies is increased, Meta-analysis might not fully explain all but kept a residual heterogeneity (21, 22, 25).

Conclusion

In conclusion, our findings indicate that Lactobacillus spp. supplementation plays a crucial role in improving growth performance of piglets by increasing ADF, ADFI and the G/F ratio, in parallel modifying the intestinal morphology, especially in the jejunum and ileum. It suggests that Lactobacillus spp. can be regarded as a promising alternative to replace AGPs usage in pig production. Based on our analysis, we suggest that future studies focus on documenting the effects of Lactobacillus spp. supplementation on the porcine gut microbiome; evaluating probiotics viability in farm conditions and generating protocols and regulations for the application in the industry.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

PH and H-YL: conceptualization, writing, and supervision. CuZ and JY: formal analysis. CuZ, JY, MZ, ChZ, LY, and ZL: investigation. CuZ, JY, PH, and H-YL: writing the original draft. DC and SC: reviewing, revising, and editing the manuscript. DC, PH, and H-YL: funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
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