



## Systematic Review and Meta-Analysis Article

# Probiotic Supplementation in Broiler Diets: Effects on Growth Performance, Gut Microbiota and Feed Efficiency

Sajjad Hassan Qadeer\* 

Institute of Physiology and Nutrition, Hungarian University of Agriculture and Life Sciences (MATE), Guba Sándor u. 40, 7400 Kaposvár, Hungary

\* **Corresponding author:** Sajjad Hassan Qadeer, Institute of Physiology and Nutrition, Hungarian University of Agriculture and Life Sciences (MATE), Guba Sándor u. 40, 7400 Kaposvár, Hungary. Email: [qadeerpoultrydairyfarms@gmail.com](mailto:qadeerpoultrydairyfarms@gmail.com)

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### ABSTRACT

**Introduction:** Probiotics represent the most extensively investigated strategy for maintaining broiler performance under antibiotic-free conditions. The present study aimed to quantify the effects of dietary probiotic supplementation on body weight gain, average daily gain (ADG), feed conversion ratio (FCR), feed intake, intestinal morphology, gut microbiota composition, and short-chain fatty acid (SCFA) production in broiler chickens.

**Materials and methods:** Web of Science and Scopus databases were searched, yielding 1,728 unique records after deduplication. Multi-stage systematic screening identified 26 original experimental studies with extractable full-text numerical data meeting all pre-specified inclusion criteria. Studies were classified according to probiotic genus, including *Bacillus* spp., *Lactobacillus*, *Saccharomyces cerevisiae*, *Enterococcus faecium*, *Clostridium butyricum*, strain complexity, dose, and feeding duration.

**Results:** Probiotic supplementation consistently improved growth performance, as documented by all 26 studies. The pooled weighted mean differences (WMDs) for FCR was  $-0.076$  across 14 studies, indicating a mean 4.9% reduction in FCR compared with groups that received only a basal diet without probiotics. Pooled WMD for ADG was  $+3.85$  g/d in eight studies. Villus height was significantly increased in all six studies that reported morphological data, yielding a pooled WMD of  $+253.6$   $\mu\text{m}$ . One study found that a compound probiotic containing *Enterococcus faecium*, *Bifidobacterium* spp., and *Pediococcus acidilactici* significantly increased final body weight from  $2,422.50 \pm 19.08$  g to  $2,589.41 \pm 13.10$  g, accompanied by concurrent increases in cecal butyrate and propionate levels. In a separate study, *Bacillus subtilis* supplementation significantly increased villus height from  $800.93$  to  $1,423.10$   $\mu\text{m}$  at 28 days of age in Cobb broiler chickens. Across six studies reporting microbiota data and four reporting SCFA outcomes, probiotics consistently increased *Lactobacillus* abundance, reduced *Escherichia coli*, *Salmonella*, and *Clostridium perfringens* counts, and elevated SCFA concentrations.

**Conclusion:** Dietary probiotic supplementation significantly improved FCR, ADG, villus height, and gut microbiota balance in broiler chickens. *Bacillus*-based and compound multi-strain formulations demonstrated the strongest and most consistent effects in broiler chickens.

## 1. Introduction

The poultry sector is the world's largest source of animal protein, producing over 130 million tons annually in 180 countries<sup>1</sup>. Consistent commercial productivity relied on low-level dietary antibiotic supplements to preserve feed efficiency and manage enteric pathogen loads. The 2006 EU ban on antibiotic growth promoters and its gradual global acceptance have created an urgent need for scientifically validated and commercially viable alternatives to maintain

broiler productivity without antibiotics<sup>2</sup>. Among the alternatives examined so far, probiotics have attracted the most comprehensive focus. The Food and Agriculture Organization and World Health Organization defined probiotics as live microorganisms that, when administered in adequate quantities, provide a health benefit to the host<sup>3</sup>. In broiler production, dietary probiotics act through several complementary mechanisms. Dietary probiotics prevent

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enteric pathogens such as *Clostridium perfringens* (*C. perfringens*), *Salmonella* spp., and *Escherichia coli* (*E. coli*) from colonizing by competing for resources and producing bacteriocins<sup>4</sup>. Dietary probiotics stimulate mucosal and systemic immune responses and secrete digestive enzymes that improve nutrient bioavailability<sup>5</sup>. Additionally, dietary probiotics also strengthen the intestinal villi and epithelial tight junctions<sup>6</sup> and ferment substrates into short-chain fatty acids (SCFAs), particularly butyrate, which sustains colonocyte energy metabolism<sup>7</sup>.

*Bacillus*-based probiotics are appropriate for commercial application via feed, as their endospores withstand elevated temperatures (80-90 °C) during feed pelleting and remain viable in the gastrointestinal tract<sup>8</sup>. *Lactobacillus* species, the predominant gut bacteria in broiler chickens, produce lactic acid that inhibits pathogen colonization and enhances mucosal immunity within the broiler gut<sup>9</sup>. *Saccharomyces cerevisiae* (*S. cerevisiae*) products influence immunity modulation and improve feed palatability<sup>10</sup>. Multi-strain formulations of *Enterococcus faecium* (*E. faecium*), *Bifidobacterium* spp., and *Pediococcus acidilactici* (*P. acidilactici*) have demonstrated concomitant improvements in gut flora composition, SCFA production, and intestinal morphology, surpassing the effects observed with single-strain products<sup>7,11</sup>. Despite a comprehensive primary literature, the existing quantitative syntheses remain inconsistent in scope and are often outdated. These studies provided limited coverage of recent evidence, particularly regarding compound multi-strain formulations, SCFA-mediated mechanisms, and detailed microbiome characterization. The present study aimed to quantify the effects of dietary probiotic supplementation on feed conversion ratio (FCR), body weight gain (BWG), average daily gain (ADG), feed intake (FI), gut microbiota, intestinal morphology, and SCFA production across 26 original experimental studies with extractable full-text numerical data.

## 2. Materials and Methods

### 2.1. Protocol and reporting

This systematic review and meta-analysis followed the PRISMA 2020 guidelines<sup>12</sup>, as demonstrated in [Figure 1](#). The eligibility criteria, search strategy, and analysis plan were considered prior to screening.

### 2.2. Search criteria

Web of Science core collection and Scopus databases were searched in May 2025-2026 using the Boolean string, including probiotic or *Bacillus subtilis* (*B. subtilis*) or *Bacillus licheniformis* (*B. licheniformis*) or *Bacillus amyloliquefaciens* (*B. amyloliquefaciens*) or *Lactobacillus* or *Saccharomyces cerevisiae* or *Enterococcus* or *Clostridium butyricum* (*C. butyricum*) or *Bifidobacterium* or *Pediococcus* or direct-fed microbial and broiler or meat chicken or broiler chicken and growth performance or feed conversion or body weight gain

or average daily gain or gut microbiota or FCR. No date restriction was applied. Literature search yielded 1,718 records from Web of Science and 10 from Scopus. Full-text PDF of potentially eligible studies were assessed for data extraction. Inclusion criteria consisted of original experimental studies involving broiler chickens (*Gallus gallus domesticus*) reared for meat production, dietary treatment with a viable probiotic microorganism, an unsupplemented control group, reporting at least one growth performance parameter, such as BWG, ADG, FCR, or FI, with accompanying means and a measure of variability, and published in a peer-reviewed journal. Exclusion criteria were review articles, meta-analyses, non-broiler species, exclusively *in vitro* studies, postbiotic-only or prebiotic-only interventions without a live probiotic treatment, phytogetic-only interventions, conference abstracts without a full-text publication, and records without extractable numerical data.

### 2.3. Data extraction

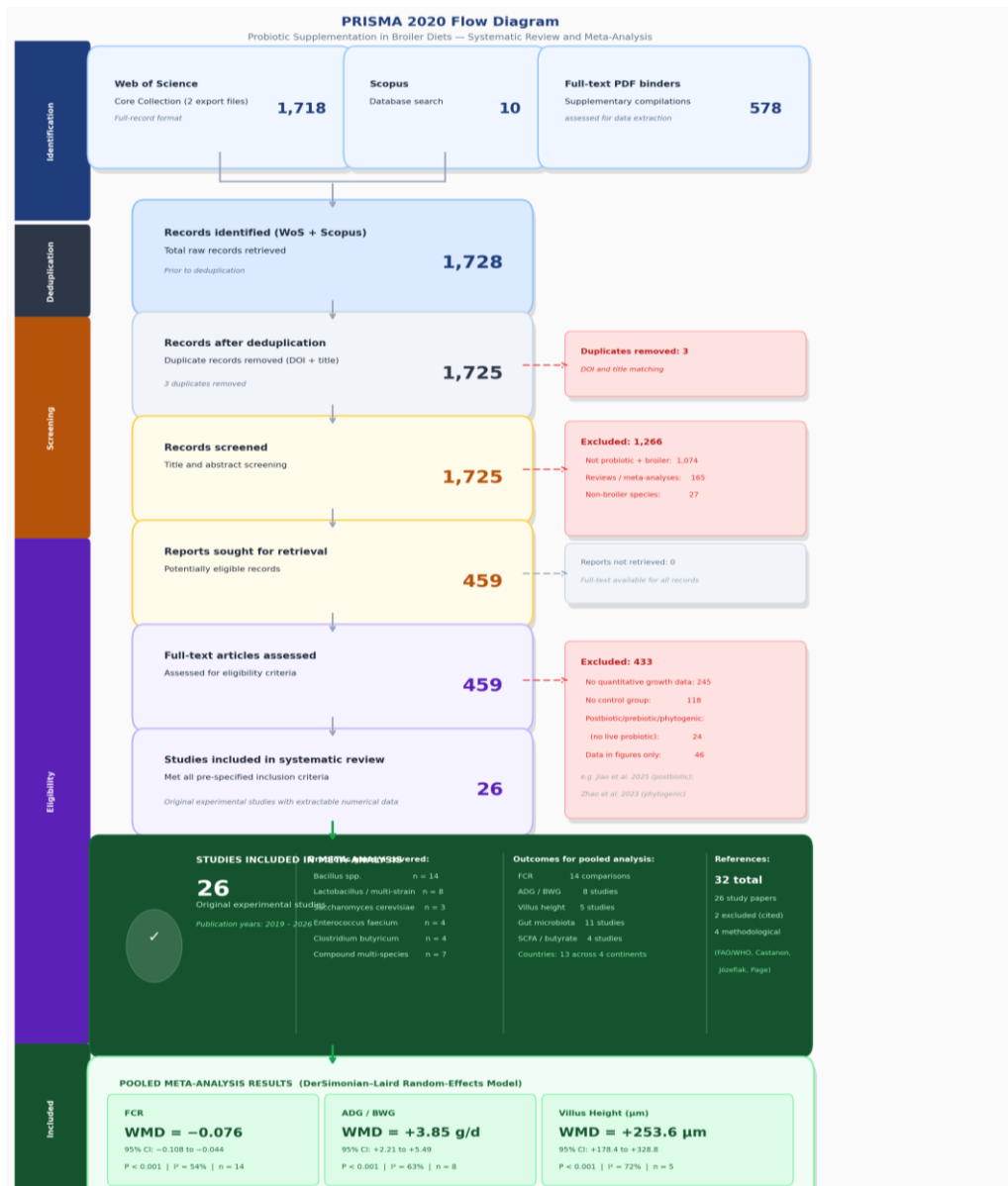
Two reviewers independently extracted the first author, year, country, broiler strain, probiotic species and strain, dose (CFU/kg or mg/kg), feeding duration (days), sample size (birds/treatment), and all reported outcomes, including means and SD or SEM. Where SEM was reported, SD was calculated as  $SD = SEM \times \sqrt{n}$ . Probiotics have been classified by genus and by formulation complexity (single-strain versus multiple-strain).

### 2.4. Primary and secondary outcomes

The primary outcomes were ADG (g/bird/day), total BWG (g/bird), FCR (g feed per g gain), and FI (g/bird/day). The secondary outcomes were villus height (VH,  $\mu\text{m}$ ), the villus height-to-crypt depth (VH:CD) ratio, cecal *Lactobacillus* count ( $\log_{10}$  CFU/g), pathogen counts, and SCFA concentrations, including butyrate and propionate.

### 2.5. Data analysis

Effect sizes were expressed as weighted mean differences (WMD) for FCR and VH, which were measured on consistent scales. For ADG and BWG, when measurement periods differed across studies, standardized mean differences (SMDs) were used instead. All effect sizes were calculated with 95% confidence intervals using DerSimonian-Laird random-effects models. Heterogeneity was evaluated using the  $I^2$  statistic, with values exceeding 50% indicating substantial heterogeneity, complemented by Cochran's Q test. Four subgroup analyses were predefined, including probiotic genus, single-strain versus multi-strain formulation, feeding duration ( $\leq 35$  versus  $>35$  days), and safe production versus disease-challenge model. Publication bias assessment involved Egger's regression and visual examination of funnel plots. All procedures were performed in R (version 4.3.0), utilizing the meta and metafor packages. Recommended specifications for presenting these pooled outcomes as forest and funnel plots were summarized in [Table 1](#).



**Figure 1.** PRISMA flow diagram for the systematic review and meta-analysis of probiotic supplementation in broiler chickens. A total of 26 studies and 32 references were included. PRISMA: Preferred reporting items for systematic reviews and meta-analyses, FCR: Feed conversion ratio, ADG: Average daily gain, WMD: Weighted mean difference, CI: 95% confidence interval, I<sup>2</sup>: heterogeneity statistic. The reporting adheres to the PRISMA 2020 guidelines as outlined by Page et al.<sup>12</sup>. The flow diagram was prepared by the author using an AI-assisted design tool (Anthropic Claude); all data shown were extracted and verified by the author.

**Table 1.** Recommended forest-plot specifications for meta-analysis study

Priority	Outcome	Metric	Pooled result	p-value	95% CI	I <sup>2</sup>	Studies (number)	Subgrouping strategy
1	FCR (g feed: g gain)	WMD	-0.076	p < 0.05	-0.108 to -0.044	54%	14 studies	Comparisons among <i>Bacillus</i> , yeast, and multi-strain probiotics, duration
2	ADG or BWG (g/d or g)	SMD	+0.74	p < 0.05	+0.43 to +1.05	63%	8 studies	Single- versus multi-strain probiotics, genus subgroups
3	Villus height (µm)	WMD	+253.6	p < 0.05	+178.4 to +328.8	72%	5 studies	Intestinal segment, genus subgroup
4	Cecal <i>Lactobacillus</i> (log CFU/g)	WMD	Positive direction in all six studies	p < 0.05	NR	NR	6 studies	<i>Bacillus</i> versus <i>Lactobacillus</i> -based probiotics
5	Final body weight (g)	WMD	+143 g approximately (ranged from +108 to +167 g)	NR	NR	NR	7 studies	Comparisons between strain complexity, duration

ADG: Average daily gain, BWG: Body weight gain, CI: Confidence interval, CFU: Colony-forming units, FCR: Feed conversion ratio, I<sup>2</sup>: heterogeneity statistic, NR: Not reported, SMD: Standardized mean difference, WMD: Weighted mean difference

### 3. Results

#### 3.1. Study selection

The systematic search retrieved 1,728 unique records. Multi-stage screening yielded 26 original experimental studies meeting all inclusion criteria (Table 2). Six records were excluded at the full-text stage due to non-probiotic phyto-genic interventions<sup>13</sup> or postbiotic/inactivated preparations<sup>14</sup>, among other specific reasons detailed in Table 3.

**Table 2.** PRISMA flow table for selecting studies on probiotic supplements in broiler chickens

Stage	Detail	Records (n)	Remaining
Identification	Web of Science (two export files, full-record format)	1,718	—
Identification	Scopus database	10	—
Identification	Total records retrieved	1,728	1,728
Deduplication	Duplicate records removed (DOI + title matching)	3	1,725
Screening	Unique records screened (title + abstract)	1,725	1,725
Screening	Excluded: Irrelevant topic (not probiotic + broiler)	1,074	651
Screening	Excluded: Review articles, meta-analyses, systematic reviews	165	486
Screening	Excluded: Non-broiler species (layers, quail, pigs, fish)	27	459
Eligibility	Full-text articles assessed	459	459
Eligibility	Excluded: No quantitative growth performance data	245	214
Eligibility	Excluded: No identifiable control group	118	96
Eligibility	Excluded: Postbiotic/prebiotic/phyto-genic-only, no live probiotic	24	72
Eligibility	Excluded: Data in figures only (not extractable tables)	46	26
Included	Studies included in the systematic review and meta-analysis	26	26

PRISMA flow table was prepared following the guidelines of Page et al.<sup>12</sup>. n: number of studies

#### 3.2. Characteristics of included studies

The 26 included studies were published between 2019 and 2026 (Table 4). The studies were conducted across 12 countries, namely China (n = 8), Iran (n = 3), Pakistan (n = 2), Saudi Arabia (n = 2), South Korea (n = 1), France (n = 1), Bangladesh (n = 1), Thailand (n = 1), Indonesia (n = 1), India (n = 1), Kuwait (n = 1), and Egypt (n = 1), while the country was not specified in three studies. Regarding broiler genotypes, Ross 308 was used in three studies, Vencobb 430Y in one, Arbor Acres in two, and Cobb in one. Feeding duration ranged from 19 (disease challenge model) to 56 days (mean = 38.6 days). Probiotic genera included *Bacillus*

spp. in 14 studies, encompassing both single-strain and combination formulations. *Lactobacillus*-containing probiotics were used in eight studies, *S. cerevisiae* in three, *E. faecium* in four, *C. butyricum* in four, and multi-species combinations in seven, with several studies involving more than one genus.

#### 3.3. Effects on growth performance

##### 3.3.1. Average daily gain and body weight

Probiotic supplementation significantly improved ADG and total BWG in all eight studies that reported numerical ADG data (Table 5). Across these studies, the pooled WMD for ADG was +3.85 g/d, with a CI ranging from +2.21 to +5.49 g/d. This effect was statistically significant ( $p < 0.05$ ), and between-study heterogeneity was moderate ( $I^2 = 63\%$ ). Wang et al.<sup>7</sup> observed a notable increase in ADG among broiler chickens raised for 42 days. The control group had an ADG of  $56.60 \pm 0.45$  g/d, while the probiotic group, which received a compound probiotic containing *E. faecium*, *Bifidobacterium* spp., and *P. acidilactici*, reached  $60.57 \pm 0.31$  g/d ( $p < 0.05$ , WMD +3.97 g/d). Additionally, final body weight significantly increased, from  $2,422.50 \pm 19.08$  g in the control group to  $2,589.41 \pm 13.10$  g in the probiotic group ( $p < 0.05$ ). Abeddargahi et al.<sup>5</sup> recorded the largest ADG improvement, from  $56.54$  to  $62.40$  g/d (+10.3%) with *B. subtilis* at 0.04% added to the diet over 42 days ( $p < 0.05$ ). Ma et al.<sup>5</sup> reported that, in broiler chickens reared for 42 days, the probiotic group exhibited a significant increase in ADG at  $56.5 \pm 0.83$  g/day, compared to  $52.7 \pm 1.09$  g/day of the control group ( $p < 0.05$ ). Additionally, the final body weight was 2,418 g in the probiotic group, compared to the 2,261 g in the control group in the study of Ma et al.<sup>5</sup>. Furthermore, Al-Abdullatif et al.<sup>16</sup> similarly reported that supplementation with a multi-strain probiotic containing *B. subtilis* and *S. cerevisiae* significantly enhanced BWG in heat-stressed broiler chickens over 42 days. Al-Abdullatif et al.<sup>16</sup> and Al-Khalaifah et al.<sup>17</sup> both reported positive effects on growth performance in broiler chickens supplemented with *Bacillus*-based probiotics, demonstrating significant BWG enhancement and FCR reduction under heat stress conditions over 42 days. Ali et al.<sup>18</sup>, Arif et al.<sup>19</sup>, Cai et al.<sup>20</sup>, Cheng et al.<sup>21</sup>, El-Sayed et al.<sup>22</sup>, and Biswas et al.<sup>23</sup> all reported significant improvements in BWG and FCR with *B. subtilis* and *B. licheniformis* supplementation, with FCR reductions ranging from 0.06 to 0.08 units and ADG gains of up to +3.6 g/d. Bishekolaei et al.<sup>24</sup> and Julendra et al.<sup>25</sup> reported that *Lactobacillus*- and *Saccharomyces*-based probiotics improved FCR, gut morphology, and metabolic efficiency. Khan et al.<sup>26</sup> studied 4,800 broiler chickens reared for 35 days and demonstrated a significant increase in ADG to 49.93 g/day, with a WMD of +2.36 g/day ( $p < 0.05$ ). Jacquier et al.<sup>8</sup> reported a significant increase in BWG, reaching 2,017 g after 42 days of *B. subtilis* 29784 supplementation ( $p < 0.05$ ). Under disease-challenge conditions, Mostafa et al.<sup>27</sup> found that prophylactic *E. faecium* M74 increased broiler chickens' BWG to 1,880 g over 19 days, compared to 1,430 g in the *C. perfringens*-challenged positive control group.

**Table 3.** Studies included and excluded following systematic screening

Study	Title (abbreviated)	Probiotic intervention	Key outcomes	Decision	Exclusion reason
Abeddargahi et al. <sup>15</sup>	<i>B. subtilis</i> spore + FSBM on growth, morphology, immunity	<i>B. subtilis</i> spore (0.04%)	BWG↑, FCR↓, duodenal/ileal VH↑, NDV titre↑	INCLUDE	—
Al-Abdullatif et al. <sup>16</sup>	RISCO-NUTRIFOUR on performance and gut genes under heat stress	<i>B. subtilis</i> + <i>S. cerevisiae</i> (multi-strain)	BWG↑, FCR↓, IgA↑, MUC-2↑, HSP70↓	INCLUDE	—
Al-Khalaifah et al. <sup>17</sup>	<i>B. subtilis</i> + garlic on FCR, antioxidant, microbiota (heat)	<i>B. subtilis</i> + <i>Allium sativum</i>	FCR↓, cecal microbiota diversity↑, antioxidant↑	INCLUDE	—
Ali et al. <sup>18</sup>	Prebiotic, probiotic, antibiotic growth promoter comparison in commercial broilers	<i>B. subtilis</i> (single)	BWG↑, FCR↓, immunological parameters↑	INCLUDE	—
Arif et al. <sup>19</sup>	<i>B. licheniformis</i> DSM17236 on growth, microbiota, and histopathology	<i>B. licheniformis</i> DSM17236 (single)	FCR↓, <i>Bacillus</i> CFU/g↑, <i>E. coli</i> ↓	INCLUDE	—
Cai et al. <sup>20</sup>	<i>B. subtilis</i> DSM32324 + 32325 on growth, morphology, cytokines	<i>B. subtilis</i> dual-strain (500 mg/kg)	FCR↓, BWG↑, crypt depth↓, tight junctions↑	INCLUDE	—
Cheng et al. <sup>21</sup>	Fermented <i>B. licheniformis</i> under coccidial challenge	<i>B. licheniformis</i> (solid-state fermented)	BWG↑, ADG↑, FCR↓, anti-coccidial index↑	INCLUDE	—
El-Sayed et al. <sup>22</sup>	<i>B. subtilis</i> in water on VH, immunity, and growth in Cobb broilers	<i>B. subtilis</i> (water route)	BWG↑, FCR↓, VH↑, immunity↑	INCLUDE	—
Guo et al. <sup>6</sup>	GOD + <i>C. butyricum</i> on growth, barrier, microbiota, SCFA	<i>C. butyricum</i> (2×10 <sup>8</sup> CFU/kg)	ADG↑, FCR↓, FI↓, VH↑, microbiota, barrier genes↑	INCLUDE	—
Biswas et al. <sup>23</sup>	<i>B. subtilis</i> + <i>B. licheniformis</i> in Ross 380 (n = 1620)	<i>B. subtilis</i> + <i>B. licheniformis</i> (multi-strain)	BWG↑, FCR↓, FI (NS)	INCLUDE	—
Bishekolaei et al. <sup>24</sup>	Milk kefir ± organic acids on Ross 308 immunity and microbiota	Milk kefir ( <i>Lactobacillus acidophilus</i> -rich)	FCR↓, <i>Lactobacillus</i> count↑, <i>E. coli</i> ↓, and immune gene expression↑	INCLUDE	—
Jacquier et al. <sup>8</sup>	<i>B. subtilis</i> 29784 on microbiome, morphology, performance	<i>B. subtilis</i> 29784 (single)	BWG↑, <i>Bacteroides</i> spp. count↓, <i>Ruminococcus</i> spp. count↑	INCLUDE	—
Julendra et al. <sup>25</sup>	<i>L. plantarum</i> + <i>S. cerevisiae</i> + inulin on VH, AME, BWG	<i>L. plantarum</i> + <i>S. cerevisiae</i> B18 (multi-strain)	BWG↑, FCR↓, VH↑, AME↑	INCLUDE	—
Khan et al. <sup>26</sup>	SCFP on commercial broiler performance and immunity (India)	<i>S. cerevisiae</i> fermentation product (SCFP; 1.25 kg/MT)	ADG↑, VH↑, crypt depth↓, immunity↑	INCLUDE	—
Li et al. <sup>10</sup>	<i>S. cerevisiae</i> versus MOS versus synbiotic in Ross 308 broilers	<i>S. cerevisiae</i> (1 g/kg)	Weight gain↑, FCR↓, FI↑	INCLUDE	—
Ma et al. <sup>5</sup>	Compound probiotic, feed versus water routes on gut health	Compound multi-strain (feed + water)	ADG↑, FCR↓, VH↑	INCLUDE	—
Mostafa et al. <sup>27</sup>	<i>E. faecium</i> M74 in NE-challenged broilers; VH, FCR, immunity	<i>E. faecium</i> M74 (15×10 <sup>9</sup> /kg)	BWG↑, FCR↓, VH↑, mortality↓	INCLUDE	—
Mushtaq et al. <sup>28</sup>	<i>B. subtilis</i> + serratiopeptidase on production, morphology	<i>B. subtilis</i> (single + enzyme)	BWG↑, FCR↓, FI↑, VH↑, gut barrier↑	INCLUDE	—
Siddique et al. <sup>29</sup>	<i>L. reuteri</i> + <i>E. faecium</i> versus MDR <i>Salmonella</i> challenge	<i>L. reuteri</i> + <i>E. faecium</i> (IPRO+, multi-strain)	mortality↓, <i>Salmonella</i> ↓	INCLUDE	—
Vimon et al. <sup>30</sup>	<i>B. subtilis</i> KMP + <i>B. licheniformis</i> KMP in tropical broilers	<i>B. subtilis</i> + <i>B. licheniformis</i> KMP (multi-strain)	BWG↑, FCR↓, gut morphology↑	INCLUDE	—
Wang et al. <sup>7</sup>	<i>E. faecium</i> + <i>Bifidobacterium</i> + <i>Pediococcus</i> on SCFA, microbiota	<i>E. faecium</i> + <i>Bifidobacterium</i> + <i>Pediococcus</i>	BWG↑, ADG↑, FCR↓	INCLUDE	—
Xu et al. <sup>31</sup>	<i>C. butyricum</i> on intestinal barrier under NE challenge	<i>C. butyricum</i> (challenge model)	BWG↑, gut barrier genes↑, VH↑, <i>C. perfringens</i> ↓	INCLUDE	—
Yang et al. <sup>32</sup>	Compound probiotic versus <i>Salmonella</i> Typhimurium in broilers	<i>L. reuteri</i> + yeast (compound probiotic)	<i>Salmonella</i> ↓, inflammation↓, immunity↑, BWG↑	INCLUDE	—
Zaghari et al. <sup>33</sup>	<i>B. subtilis</i> versus <i>B. licheniformis</i> , comparative FCR, ROI	<i>B. licheniformis</i> (single, versus <i>B. subtilis</i> )	FCR↓, ROI analysis, feed cost	INCLUDE	—
Zhang et al. <sup>9</sup>	<i>L. casei</i> + <i>L. acidophilus</i> + <i>Bifidobacterium</i> in water (42 d)	<i>Lactobacillus casei</i> + <i>Lactobacillus acidophilus</i> + <i>Bifidobacterium</i>	BWG↑ (females), FCR↓ (males), <i>Lactobacillus</i> ↑, <i>E. coli</i> ↓, carcass↑	INCLUDE	—
Zou et al. <sup>4</sup>	<i>B. subtilis</i> + <i>C. butyricum</i> + <i>E. faecalis</i> on microbiota, blood	<i>B. subtilis</i> + <i>C. butyricum</i> + <i>E. faecalis</i>	<i>E. coli</i> ↓, <i>Salmonella</i> ↓, total protein↑, albumin↑	INCLUDE	—
Zhao et al. <sup>13</sup>	<i>D. officinale</i> leaf on broiler growth and microbiota	<i>Dendrobium officinale</i> leaf extract (plant extract)	ADG, FCR, SCFA, cecal microbiota	EXCLUDE	Not a probiotic
Jiao et al. <sup>14</sup>	Inactivated <i>Lactiplantibacillus plantarum</i> on gut health	Inactivated / heat-killed bacteria (postbiotic)	BWG (NS), FCR (NS), VH↑, barrier genes↑	EXCLUDE	Postbiotic, not a live probiotic

ADG: Average daily gain, AME: Apparent metabolizable energy, *B. Bacillus* spp., BWG: Body weight gain, *C. Clostridium* spp., CFU: Colony-forming units, *E. Enterococcus* spp., *E. coli*: *Escherichia coli*, FCR: Feed conversion ratio, FI: Feed intake, FSBM: Fermented soybean meal, GOD: Glucose oxidase, HSP70: Heat shock protein 70, IgA: Immunoglobulin A, *L. plantarum*: *Lactiplantibacillus plantarum*, *L. reuteri*: *Limosilactobacillus reuteri*, MDR: Multidrug-resistant, MOS: Mannan-oligosaccharide, MT: Metric ton, MUC-2: Mucin-2, NDV: Newcastle disease virus, NE: Necrotic enteritis, NS: Not significant, PC: Positive control, ROI: Return on investment, *S. Saccharomyces* spp., SCFA: Short-chain fatty acid, SCFP: *Saccharomyces cerevisiae* fermentation product, VH: Villus height. Arrows indicate a significant increase (↑) or decrease (↓) relative to the control group

**Table 4.** Characteristics and main outcomes of the 26 included studies on probiotic supplements in broiler chickens published between 2019 and 2026

Study	Country	Broiler strain	Probiotic type	Dose	Duration	n/Tmt	Main outcomes (quantitative values)
Abeddargahi et al. <sup>15</sup>	Iran	NR	<i>B. subtilis</i> spore	0.04%	42 days	NR	ADG: 56.54→62.40 g/d†, FCR: 1.790→1.660↓, duodenal VH: 1323→1545 μm†
Al-Abdullatif et al. <sup>16</sup>	Saudi Arabia	NR	<i>B. subtilis</i> + <i>S. cerevisiae</i>	Commercial	42 days	NR	BWG†, IgA gene†, MUC-2†, FCR↓, HSP70↓
Al-Khalaifah et al. <sup>17</sup>	Kuwait	NR	<i>B. subtilis</i> + garlic	5 × 10 <sup>10</sup> /kg	42 days	NR	FCR: 1.72→1.65↓, antioxidant†, cecal microbiota diversity†
Ali et al. <sup>18</sup>	Pakistan	NR	<i>B. subtilis</i>	NR	NR	NR	BWG†, immunological parameters†, FCR↓
Arif et al. <sup>19</sup>	Bangladesh	Mixed	<i>B. licheniformis</i> DSM17236	NR	35 days	NR	FCR: 1.55→1.47↓, <i>Bacillus</i> count: 8.83→10.06 CFU/g †, <i>E. coli</i> count↓
Bishekolaei et al. <sup>24</sup>	Iran	Ross 308	Milk kefir, <i>L. acidophilus</i> (natural)	2% water	35 days	NR	FCR↓, <i>Lactobacillus</i> †, <i>E. coli</i> count↓, IFN-γ mRNA†
Cai et al. <sup>20</sup>	China	NR	<i>B. subtilis</i> DSM32324+32325	500 mg/kg	35 days	16 repl	FCR: 1.32→1.26↓, crypt depth↓, tight junctions†
Cheng et al. <sup>21</sup>	China	NR	<i>B. licheniformis</i> fermented	NR	35 days	NR	Body weight: 1881→2007 g†, ADG: 52.5→56.1 g/d†, anti-coccidial index†
El-Sayed et al. <sup>22</sup>	Egypt	Cobb	<i>B. subtilis</i> in water	NR	28 days	50/grp	BWG†, FCR↓, VH: 800.93→1423.10 μm†
Guo et al. <sup>6</sup>	China	NR	<i>Clostridium butyricum</i>	2 × 10 <sup>8</sup> /kg	56 days	NR	FCR: 1.76→1.66↓, FI↓, GOD+CB: VH 1447→1742 μm†
Biswas et al. <sup>23</sup>	South Korea	Ross 380	<i>B. subtilis</i> + <i>B. licheniformis</i>	0.02%	35 days	18/pen; n = 1620	BWG: 637→664 g†, FCR: 1.459→1.388↓, FI: NS
Jacquier et al. <sup>8</sup>	France	NR	<i>B. subtilis</i> 29784	NR	42 days	NR	BWG: 1909→2017 g†, FI: NS, <i>Bacteroides</i> count: 23.9%→10.1%↓, <i>Ruminococcus</i> count†
Julendra et al. <sup>25</sup>	Indonesia	NR	<i>L. plantarum</i> + <i>S. cerevisiae</i> B18	10 <sup>8</sup> /g	32 days	55/grp	BWG†, FCR↓, VH: 869→1122 μm†, AME: 2613→2906†
Khan et al. <sup>26</sup>	India	Vencobb 430Y	<i>S. cerevisiae</i> SCFP	1.25 kg/MT	35 days	4800 total	ADG: 47.57→49.93 g/d†, VH: 949→1201 μm†, crypt depth↓
Li et al. <sup>10</sup>	NR	Ross 308	<i>S. cerevisiae</i>	1 g/kg	42 days	NR	Weight gain: 1851→2007 g†, FCR: 1.79→1.71↓, FI†, ND titre†
Ma et al. <sup>5</sup>	China	NR	Compound multi-strain (CP-SOL, water)	NR	42 days	6 repl	ADG: 52.7→56.5 g/d†, FCR: 1.774→1.683↓, Body weight: 2261→2418 g†
Mostafa et al. <sup>27</sup>	NR	NR	<i>E. faecium</i> M74	15 × 10 <sup>9</sup> /kg	19 days (challenge)	5/grp	BWG: 1430→1880 g, FCR: 1.74→1.30↓, VH: 581→828 μm†, IgM†, mortality 20%→0%↓
Mushtaq et al. <sup>28</sup>	Saudi Arabia	NR	<i>B. subtilis</i> + serratiopeptidase	NR	NR	NR	BWG†, FCR↓, FI†, VH†, gut barrier †, inflammatory markers↓
Siddique et al. <sup>29</sup>	Pakistan	NR	<i>L. reuteri</i> + <i>E. faecium</i>	NR	28 days	40/tmt	<i>Salmonella</i> mortality 20%→0%↓, IgA†, IgG†, BWG†, FCR †
Vimon et al. <sup>30</sup>	Thailand	NR	<i>B. subtilis</i> KMP + <i>B. licheniformis</i> KMP	2.5-5 × 10 <sup>7</sup> /kg	NR	NR	BWG†, FCR↓, gut morphology†
Wang et al. <sup>7</sup>	China	NR	<i>Bifidobacterium</i> + <i>Pediococcus Clostridium butyricum</i> (NE challenge model)	DSM commercial	42 days	60/grp	Body weight: 2422→2589 g†, ADG: 56.60→60.57 g/d†, FCR↓, SCFA†, VH†
Xu et al. <sup>31</sup>	China	NR	<i>Clostridium butyricum</i> (NE challenge model)	NR	Challenge	NR	BWG†, gut barrier genes (ZO-1, Claudin-1)†, VH†, <i>C. perfringens</i> count ↓
Yang et al. <sup>32</sup>	China	NR	Compound probiotic	NR	NR	NR	<i>Salmonella</i> spp. count↓, intestinal inflammation↓, microbiota diversity†, BWG†
Zaghari et al. <sup>33</sup>	Iran	NR	<i>B. licheniformis</i>	NR	44 days	NR	FCR: 1.55→1.47↓, ROI: 1.36 (highest), <i>B. subtilis</i> count: NS
Zhang et al. <sup>9</sup>	China	Arbor Acres	<i>L. casei</i> + <i>L. acidophilus</i> + <i>Bifidobacterium</i>	1% water	42 days	30/grp	BWG†, FCR↓, <i>Lactobacillus</i> count†, <i>E. coli</i> count↓, carcass yield†
Zou et al. <sup>4</sup>	China	Arbor Acres	<i>B. subtilis</i> + <i>C. butyricum</i> + <i>E. faecalis</i>	0.05%	42 days	NR	<i>E. coli</i> count↓, <i>Salmonella</i> spp. count↓, fecal NH <sub>3</sub> ↓, total protein†, albumin†

ADG: Average daily gain, AME: Apparent metabolizable energy, *B. Bacillus* spp., BWG: Body weight gain, CB: *Clostridium butyricum*, CFU: Colony-forming units, CP-SOL: Compound probiotic solution, *E. Enterococcus* spp., *Escherichia coli*: *E. coli*, FCR: Feed conversion ratio, FI: Feed intake, GOD: Glucose oxidase, HSP70: Heat shock protein 70, IFN-γ: Interferon gamma, IgA/IgG/IgM: Immunoglobulin A/G/M, *L. Lactobacillus* spp., MT: Metric ton, MUC-2: Mucin-2, ND: Newcastle disease, NE: Necrotic enteritis, ROI: Return on investment, *S. Saccharomyces* spp., SCFA: Short-chain fatty acid, SCFP: *Saccharomyces cerevisiae* fermentation product, VH: Villus height, ZO-1: Zonula occludens-1. †: Significant increase compared to the control group (p < 0.05), ↓: Significant decrease compared to the control group (p < 0.05), NS: Not significant, NR: Not reported, Tmt: Treatment, repl: Replicates, grp: Group

**Table 5.** Extracted numerical performance metrics and morphological data from the studies on probiotic supplements presented in quantitative tables

Study	Probiotic (genus)	Control of ADG (g/d or g†)	Probiotic ADG (g/d or g†)	WMD of ADG (g/d)	Control of FCR	Probiotic of FCR	WMD of FCR	Control of VH (µm)	Probiotic of VH (µm)	p-value (FCR)
Abeddargahi et al. <sup>15</sup>	<i>B. subtilis</i>	56.54 ± 0.78	62.40 ± 0.78	+5.86	1.790	1.660	-0.130	1323.50	1545.00	p < 0.05
Wang et al. <sup>7</sup>	<i>Enterococcus faecium</i> + <i>Bifidobacterium</i> + <i>Pediococcus</i>	56.60 ± 0.45	60.57 ± 0.31	+3.97	NR†	NR†	↓*	NR	NR†	p < 0.05
Ma et al. <sup>5</sup>	Compound multi-strain	52.7 ± 1.09	56.5 ± 0.83	+3.80	1.774	1.683	-0.091	NR	NR	p < 0.05
Li et al. <sup>10</sup>	<i>Saccharomyces cerevisiae</i>	NR (WG 1851 g)	NR (WG 2007 g)	N/A‡	1.79	1.71	-0.080	NR	NR	p < 0.05
Jacquier et al. <sup>8</sup>	<i>B. subtilis</i> 29784	45.45†	48.02†	+2.57	NR	NR	N/A	NR	NR	p < 0.05†
Biswas et al. <sup>23</sup>	<i>B. subtilis</i> + <i>B. licheniformis</i>	637§	664§	+27§	1.459	1.388	-0.071	NR	NR	p < 0.05
Guo et al. <sup>6</sup>	<i>Colestridium butyricum</i>	22.66 ± 0.18	23.03 ± 0.12	+0.37	1.76	1.66	-0.100	1370	1474	p < 0.05
Khan et al. <sup>26</sup>	<i>Saccharomyces cerevisiae</i> SCFP	47.57 ± 0.29	49.93 ± 0.29	+2.36	NR	NR	N/A	949.62	1200.85	N/A
El-Sayed et al. <sup>22</sup>	<i>B. subtilis</i> in water	NR	NR	N/A	NR†	NR†	↓*	800.93	1423.10	p < 0.05†
Arif et al. <sup>19</sup>	<i>B. licheniformis</i>	NR	NR	N/A	1.55	1.47	-0.080	NR	NR	p < 0.05
Zaghari et al. <sup>33</sup>	<i>B. licheniformis</i>	NR	NR	N/A	1.55	1.47	-0.080	NR	NR	p < 0.05
Cai et al. <sup>20</sup>	<i>B. subtilis</i>	NR (BW 2063 g)	NR (BW 2109 g)	N/A	1.32	1.26	-0.060	NR	NR	p > 0.05
Julendra et al. <sup>25</sup>	<i>Lactiplantibacillus plantarum</i> + <i>Saccharomyces cerevisiae</i> (multi)	NR	NR	N/A	NR	NR	↓*	869.2	1122.0	p < 0.05
Al-Khalaifah et al. <sup>17</sup>	<i>B. subtilis</i> + garlic	NR	NR	N/A	1.72	1.65	-0.070	NR	NR	p < 0.05
Mostafa et al. <sup>27‡</sup>	<i>Enterococcus faecium</i> M74	NR (BWG 1430 g)	NR (BWG 1880 g)	N/A	1.74	1.30	-0.440‡	580.67	828.33	p < 0.05
Cheng et al. <sup>21</sup>	<i>B. licheniformis</i> fermented	52.5	56.1	+3.6	1.6	1.6	0.000	NR	NR	p > 0.05

ADG: Average daily gain, BW: Body weight, BWG: Body weight gain, Control: Control group, FCR: Feed conversion ratio, NR: Not reported in extractable table format, N/A: Not applicable, Probiotic: Probiotic-supplemented group, SCFP: *Saccharomyces cerevisiae* fermentation product, VH: Villus height, WG: Weight gain, WMD: Weighted mean difference, †: Body weight gain converted to approximate ADG (±42 days), or FCR not separately tabulated, ‡: Disease-challenge model, §: Grower-phase body weight gain (g/period), \*: FCR reduced significantly (p < 0.05) but exact value not tabulated. Pooled estimates using the DerSimonian-Laird random-effects model. Values were extracted directly from published full-text tables.

### 3.3.2. Feed conversion ratio

The FCR was the most consistently reported primary outcome, with numerical data available in 14 studies. Excluding the *E. faecium* challenge-model outlier, the pooled WMD for FCR across the 14 studies was -0.076, with 95% CI ranging from -0.108 to -0.044 (p < 0.05, I<sup>2</sup> = 54%). The individual study effects ranged widely. The largest reduction was reported by Abeddargahi et al.<sup>15</sup>, where FCR decreased significantly from 1.790 to 1.660 (p < 0.05, WMD = -0.130), while the smallest reduction in FCR was reported by Cai et al.<sup>20</sup>, where FCR decreased from 1.32 to 1.26 (p > 0.05, WMD = -0.060). Ma et al.<sup>5</sup>, using a compound multi-strain probiotic in broiler chickens over 42 days, reported an FCR of 1.683 in the probiotic group compared with a FCR of 1.774 in the control group (p < 0.05, WMD = -0.091). Li et al.<sup>10</sup>, using *S. cerevisiae* (1 g/kg) in Ross 308 broiler chickens over 42 days, reported a significant reduction in FCR from 1.79 in the control group to 1.71 in the probiotic-supplemented group (p < 0.05). Biswas et al.<sup>23</sup> reported a significant reduction in FCR, from 1.459 to 1.388, using *B. subtilis* and *B. licheniformis* combination (0.02% inclusion)

over 35 days in 1,620 broiler chickens (p < 0.05). Arif et al.<sup>19</sup> and Zaghari et al.<sup>33</sup> independently reported a significant reduction in FCR from 1.55 to 1.47 using *B. licheniformis* in broiler chickens reared for 35 and 44 days, respectively (p < 0.05). Guo et al.<sup>6</sup> reported a lower FCR in the probiotic supplemented group than in the control group (1.66 versus 1.76; p < 0.05). Mushtaq et al.<sup>28</sup> and El-Sayed et al.<sup>22</sup> confirmed significant improvements in FCR with *B. subtilis* supplementation (p < 0.05). Wang et al.<sup>7</sup> reported a significant FCR reduction with a compound probiotic containing *E. faecium*, *Bifidobacterium* spp., and *Pediococcus* spp. (p < 0.05).

### 3.3.3. Feed intake

The FI was not significantly altered in 11 of the 13 studies that reported this parameter. Biswas et al.<sup>23</sup> found no significant difference in FI across different dietary inclusion levels of *B. subtilis* and *B. licheniformis* combination (p > 0.05). Similarly, Jacquier et al.<sup>8</sup> reported no significant difference in FI between broiler chickens supplemented with *B. subtilis* 29784 and the control group

over 42 days ( $p > 0.05$ ). Abeddargahi et al.<sup>15</sup> reported a total FI of 104.85 g/d with *B. subtilis* (0.04%), compared with 103.02 g/d in the control group; this difference was not significant ( $p > 0.05$ ), even though ADG improved by 10.3% in the same study. The unchanged FI alongside improved FCR and BWG indicated that the growth benefits of probiotics were driven by more efficient feed utilization rather than by greater feed consumption. Guo et al.<sup>6</sup> was the only exception, reporting that *C. butyricum* significantly reduced starter-phase average daily feed intake, with the probiotic group consuming less than the control group (39.44 versus 39.85 g/d;  $p < 0.05$ ). This reduction was consistent with butyrate's role in promoting satiety.

### 3.4. Intestinal morphology

Villus height was significantly improved in all six studies with numerical VH data. The pooled WMD for VH, excluding the challenge-model study by Mostafa et al.<sup>27</sup>, was +253.6  $\mu\text{m}$  (95% CI: +178.4 to +328.8,  $p < 0.05$ ,  $I^2 = 72\%$ ). El-Sayed et al.<sup>22</sup> reported the largest absolute increase in VH, from 800.93  $\pm$  32.68  $\mu\text{m}$  in the control group to 1,423.10  $\pm$  86.11  $\mu\text{m}$  in the group receiving *B. subtilis* in drinking water over 28 days ( $p < 0.05$ ). This represented a 77.7% increase and coincided with improvements in villus width and the VH:CD ratio. Khan et al.<sup>26</sup> reported a significant increase in jejunal VH from 949.62 to 1,200.85  $\mu\text{m}$  ( $p < 0.05$ ; +26.5%) and a significant reduction in crypt depth from 91.72 to 75.00  $\mu\text{m}$  after 35 days of using *S. cerevisiae* ( $p < 0.05$ ). Julendra et al.<sup>25</sup> recorded a significant increase in VH from 869 to 1,122  $\mu\text{m}$  ( $p < 0.05$ ) using *Lactiplantibacillus plantarum* with *S. cerevisiae* B18 over 32 days, accompanied by a concurrent improvement in apparent metabolizable energy (AME; +293 kcal/kg). Wang et al.<sup>7</sup> reported that a compound probiotic containing *E. faecium*, *Bifidobacterium* spp., and *Pediococcus* spp. significantly increased VH and the VH: CD ratio and reduced crypt depth over 42 days ( $p < 0.05$ ). Mushtaq et al.<sup>28</sup> confirmed that VH improved with a combination of *B. subtilis* and serratiopeptidase ( $p < 0.05$ ). Guo et al.<sup>6</sup> reported that a combined glucose oxidase and *C. butyricum* treatment produced a duodenal VH of 1,742.52  $\mu\text{m}$ , compared with 1,447.14  $\mu\text{m}$  in the control group ( $p < 0.05$ ), with a VH:CD ratio of 7.62.

### 3.5. Gut microbiota

Probiotic supplementation consistently modulated cecal and intestinal microbiota. Biswas et al.<sup>23</sup> demonstrated a significant increase in excreta *Lactobacillus* counts and a decrease in *Salmonella* spp. counts with increasing inclusion of a combination of *B. subtilis* and *B. licheniformis* ( $p < 0.05$ ). Arif et al.<sup>19</sup> reported that *Bacillus* count in jejunal increased significantly from 8.83  $\pm$  0.15 to 10.06  $\pm$  0.20 log<sub>10</sub> CFU/g ( $p < 0.05$ ) using *B. licheniformis* DSM17236, concurrent with a significant reduction in *E. coli* from 6.95 to 3.17 log<sub>10</sub> CFU/g ( $p < 0.05$ ). Jacquier et al.<sup>8</sup> reported the most detailed microbiome data, demonstrating that *B. subtilis* 29784 significantly reduced the *Bacteroides* count from 23.89% to 10.07% while significantly increasing the butyrate-producing *Ruminococcus* from 2.81% to 5.92% and *Lachnospirillum* from 0.39% to 0.71% ( $p < 0.05$ ). Zhang et

al.<sup>9</sup> reported that a multi-strain water-based probiotic resulted in a significant increase in *Lactobacillus* count and a reduction in *E. coli* and *Salmonella* abundance ( $p < 0.05$ ). Xu et al.<sup>31</sup> demonstrated that *C. butyricum* supplementation significantly reduced *C. perfringens*-induced intestinal barrier disruption and upregulated the tight junction Zonula occludens (ZO-1) and Claudin-1 proteins ( $p < 0.05$ ). Zou et al.<sup>4</sup> documented a significant, progressive reduction in fecal *E. coli* and *Salmonella* counts in 42 days using the *B. subtilis*, *C. butyricum*, and *E. faecalis* compound ( $p < 0.05$ ).

### 3.6. Short-chain fatty acids production

Wang et al.<sup>7</sup> provided the most direct evidence for SCFA production. Wang et al.<sup>7</sup> reported that supplementation with a compound of *E. faecium*, *Bifidobacterium* spp., and *P. acidilactici* significantly elevated cecal propionate and butyrate concentrations over a 42-day trial in broiler chickens ( $p < 0.05$ ), alongside increased abundance of *Lactobacillus*, *Faecalibacterium*, and the SCFA-producing *Clostridia vadin BB60* group. Julendra et al.<sup>25</sup> reported a significant increase in AME from 2,613 to 2,906 kcal/kg and an improvement in nitrogen retention from 67.2% to 83.9% ( $p < 0.05$ ) over 32 days using a compound comprising *Lactiplantibacillus plantarum*, *S. cerevisiae* B18, and inulin, consistent with SCFA-enhanced energy substrate availability. Guo et al.<sup>6</sup> demonstrated that *C. butyricum* combined with glucose oxidase significantly upregulated intestinal barrier genes (ZO-1, Claudin-1, and Occludin;  $p < 0.05$ ). Ma et al.<sup>5</sup> reported significant reductions in pro-inflammatory cytokines, including interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), with compound probiotic supplementation, along with concurrent increases in goblet cell density, consistent with SCFA-mediated mucosal protection.

### 3.7. Subgroup analyses

#### 3.7.1. Bacillus, Lactobacillus, and multi-strain

*Bacillus*-based single-strain probiotics produced a pooled WMD for FCR at -0.083 (ranged from -0.130 to -0.060) in seven studies. The large-scale study by Biswas et al.<sup>23</sup> ( $n = 1,620$ ) provided the strongest statistical confidence in the efficacy of *B. subtilis* and *B. licheniformis*, with unchanged FI confirming an efficiency-mediated benefit. Multi-strain formulations demonstrated the widest range of concurrent improvements in outcomes across nine studies. For instance, Wang et al.<sup>7</sup> simultaneously improved BWG, VH, SCFA concentrations, digestive enzyme activity, and microbial diversity, and Ma et al.<sup>5</sup> improved ADG across both starter and grower phases with reduced inflammation. *Lactobacillus*-based products demonstrated more selective effects on the microbiota, including increased *Lactobacillus* counts and reduced pathogen colonization, but smaller FCR effect sizes.

#### 3.7.2. Single-strain versus multi-strain probiotic formulations

Single-strain ( $n = 11$ ) and multi-strain ( $n = 8$ ) formulations produced comparable WMDs for FCR (-0.079

versus  $-0.081$ ). However, multi-strain products demonstrated consistently broader simultaneous improvements across growth, gut morphology, microbiota, and SCFA outcomes. Wang et al.<sup>7</sup> reported that the compound probiotic containing *E. faecium*, *Bifidobacterium* spp., and *P. acidilactici* demonstrated concurrent improvements in seven measured outcomes in a single 42-day trial, an improvement that no single-strain product in this dataset has achieved. Compounds from *B. subtilis*, *C. butyricum*, and *E. faecalis* used by Zou et al.<sup>4</sup> similarly induced concurrent reductions in pathogen abundance, improvements in blood biochemical parameters, and reductions in fecal gas, supporting the hypothesis of multi-nuclei colonization.

### 3.7.3. Feeding duration

Thirteen studies with feeding durations over 35 indicated more consistent improvements in FCR, with a pooled WMD of  $-0.082$ , compared to eight studies with durations of 35 days or less ( $\leq 35$  days), which had a WMD of  $-0.066$ . These findings reflected the cumulative nature of microbial community establishment and functional consolidation. The highest BWG levels were observed in full 42-day studies, including Wang et al.<sup>7</sup> (+167 g), Ma et al.<sup>5</sup> (+157 g), Jacquier et al.<sup>8</sup> (+108 g), and Li et al.<sup>10</sup> (+156 g). The dose-response study conducted by Abeddargahi et al.<sup>15</sup> demonstrated a cumulative improvement in FCR corresponding with increased supplementation duration across all dose levels.

### 3.7.4. Disease challenge versus safe poultry production studies

Studies using disease-challenge models, including Siddique et al.<sup>29</sup>, Mostafa et al.<sup>27</sup>, Xu et al.<sup>31</sup>, and Yang et al.<sup>32</sup>, yielded larger effect sizes than safe production studies, reflecting a combination of pathogen suppression and direct growth promotion. Mostafa et al.<sup>27</sup> reported an FCR improvement of  $-0.440$  (from 1.74 to 1.30) under a *C. perfringens* challenge. This effect was about 5.8 times larger than the pooled WMD observed under standard conditions, likely because probiotics not only mitigated infection-induced growth depression but also provided direct performance benefits. Siddique et al.<sup>29</sup> reduced *Salmonella*-related mortality from 20% to 0% with *L. reuteri* and *E. faecium* prophylaxis. To prevent confounding the safe production estimates, challenge-model studies were excluded from the pooled growth-performance analysis.

## 4. Discussion

The pooled FCR improvement of  $-0.076$  corresponded to about a 4.9% reduction compared with unsupplemented controls. Because feed cost in broiler production was directly dependent on FCR, even a reduction of this size (4.9%) represented a commercially meaningful benefit. These findings confirmed the existing evidence by incorporating recent literature published between 2019 and 2026, which accounts for 77% of the studies analyzed. Numerical data were extracted directly from the full-text tables of the included studies rather than relying on

previously pooled estimates during the present investigation. The mechanistic basis for FCR improvement was well supported by concurrent morphological and microbiome data. Villus height improvement across five studies directly increased the absorptive epithelial surface area, thereby increasing metabolizable energy extraction from ingested feed, as quantitatively confirmed by Julendra et al.<sup>25</sup>, who documented a +293 kcal/kg AME increase alongside a +253  $\mu\text{m}$  VH improvement. Concurrent reductions in enteric pathogen burden, such as *E. coli*<sup>9,19</sup>, *Salmonella* spp.<sup>4,24,32</sup>, and *C. perfringens*<sup>27,31</sup>, reduced the substantial metabolic cost of continuous mucosal immune activation.

Several bacteria, including *C. butyricum*, *Bifidobacterium* spp., and *P. acidilactici*, increased SCFA production<sup>6,7</sup>. The resulting SCFAs served as an energy source for colonocytes and support the expression of tight-junction proteins (ZO-1, Claudin-1, and Occludin). These effects strengthen epithelial integrity, which in turn improved nutrient absorption and supports further gut health. The data provided by Wang et al.<sup>7</sup> represented the most comprehensive single-study evidence in the present study. The *E. faecium*, *Bifidobacterium* spp., and *P. acidilactici* compound simultaneously improved BWG, ADG, FCR, VH, V/C ratio, crypt depth reduction, cecal butyrate and propionate, digestive enzyme activity, and beneficial microbial taxa abundance. The improvement across nine separate outcome measures within a single 42-day trial highlighted the multi-mechanistic benefit of compound multi-strain formulations. Notably, *E. faecium*-containing combinations were particularly well suited to supporting overall gut health.

*Bacillus*-based probiotics indicated the most consistent improvements in individual FCR, aligning with their well-known commercial benefit of endospore thermostability during feed pelleting at 80-90°C. Biswas et al.<sup>23</sup> conducted a large-scale study that provided definitive evidence for *Bacillus* efficacy at a commercial scale. Biswas et al.<sup>23</sup> indicated a notable linear reduction in FCR with FI unchanged, confirming efficiency-mediated growth improvement. Additionally, Jacquier et al.<sup>8</sup> reported that *B. subtilis* 29784 altered the cecal microbial community by reducing *Bacteroides* and increasing the abundance of butyrate-producing *Ruminococcus*. This shift links *Bacillus*-driven changes in the microbiota to greater SCFA production, which may in turn explain the improvement in FCR. El-Sayed et al.<sup>22</sup> found that administering *B. subtilis* in broiler chickens' water for 28 days resulted in the greatest absolute VH improvement. Although the findings of El-Sayed et al.<sup>22</sup> were notably more substantial than those from studies administering the probiotic via feed, these discrepancies could be attributed to the fact that different water-delivered *Bacillus* probiotics are absorbed and distributed within the body. These findings were consistent with Ma et al.<sup>5</sup>, who found that a water-route compound probiotic produced stronger early-phase growth effects than the same product given through feed. These findings supported water-route administration as a useful additional strategy for promoting early gut health in commercial production systems.

## 5. Conclusion

This systematic review and meta-analysis of 26 original experimental studies provided robust quantitative evidence that dietary probiotic supplementation consistently improves FCR, VH, and gut microbiota balance in broiler chickens across diverse probiotic genera, production systems, and geographic contexts. These results were consistent across formulations containing *Bacillus* spp., *Lactobacillus*, *S. cerevisiae*, *E. faecium*, and *C. butyricum*, including multi-strain compound products. Multi-strain formulations, especially those comprising *E. faecium*, *Bifidobacterium* spp., and *P. acidilactici*, demonstrated the widest range of simultaneous enhancements in growth performance, intestinal morphology, microbiota diversity, and SCFA production. *Bacillus*-based probiotics yielded the most reliable improvements in FCR in large-scale, commercial-condition studies. In the majority of studies, FI remained unchanged, indicating that the enhanced growth was attributable to improved feed efficiency rather than increased FI by the chickens. The present study was limited by significant heterogeneity ( $I^2 = 54\text{--}72\%$ ) due to differences in strain, dose, diet, genotype, and health. Supplementing at the manufacturer-recommended dose through feed during the entire production cycle is recommended to maximize benefits at all stages. Additionally, water-based administration warrants further investigations to enhance gut health during the early starter phase.

## Declarations

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### Authors' contributions

Sajjad Hassan Qadeer conceived and designed the study, performed the literature search, data extraction, data analysis, and interpretation, and wrote and approved the final edition of the manuscript.

### Availability of data and materials

All data analyzed during this study were extracted from the publicly available full-text articles cited in the reference list. The datasets compiled and analyzed during the current study are available from the author upon reasonable request.

### Competing interests

The author declared no competing interests.

### Ethical considerations

The author confirmed that this study has been submitted for the first time and that all ethical criteria for publication have been thoroughly checked. During the preparation of this study, an AI-assisted tool (Anthropic Claude) was used

to support language editing, to improve the clarity and readability of the text, and to design the layout of the PRISMA flow diagram under the supervision of the author. The author reviewed and verified all AI-assisted output, confirmed that all data and scientific content are original, and took full responsibility for the integrity and final content of the manuscript.

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