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Nutritional application of *Bacillus* spp. in broiler chicken and pig diets: a review

Bacillus spp. na nutrição de aves de corte e suínos: uma revisão

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Abstract: The aim was to determine the performance bases for the use of bacteria of the genus *Bacillus* as probiotics in the feeding of broilers and pig, assessing the ability of microorganisms to provide intestinal health development, productive yield and nutritional management of animals. To this end, a literature review was carried out, investigating specific words on various data platforms, in order to collect relevant studies on the subject. The two *Bacillus* species most commonly used in the nutrition of monogastric animals, such as poultry and pigs, are *B. subtilis* and *B. licheniformis*. However, there are also studies investigating the use of *Bacillus* in other species, such as fish and cattle. Most studies on the use of *Bacillus* in animal feed are carried out in different parts of the world, including countries such as the United States, Brazil, China and European countries. It is important to note that the prevalence and focus of studies also vary according to the specific needs and characteristics of each region. The benefits associated with the use of *Bacillus* in the nutrition of monogastrics include improved digestion of nutrients, especially protein and fiber; stimulation of the immune system, helping resistance to disease; reduction of colonization by pathogens in the gastrointestinal tract, promoting intestinal health and improved production performance, including weight gain and feed conversion. Studies on the use of *Bacillus* in monogastric nutrition can be conducted both nationally and internationally, depending on collaboration between research institutions and companies in the agricultural sector.

Key-words: probiotics; supplementation; zootechnics.

Resumo: Este estudo teve como objetivo avaliar o uso de bactérias do gênero *Bacillus* como probióticos na alimentação de frangos e suínos, com foco na saúde intestinal, no desempenho produtivo e no manejo nutricional dos animais. Foi realizada uma revisão de literatura utilizando palavras-chave específicas em diversas bases de dados para reunir estudos relevantes sobre o tema. As espécies de *Bacillus* mais comumente usadas na nutrição de monogástricos, como aves e suínos, são *B. subtilis* e *B. licheniformis*, mas também há pesquisas sobre o uso de *Bacillus* em outras espécies, como peixes e bovinos. A maior parte dos estudos sobre o uso de *Bacillus* na alimentação animal ocorre em países como Estados Unidos, Brasil, China, e na Europa, com variações nos focos e na prevalência dependendo das necessidades regionais. Entre os principais benefícios associados ao uso de *Bacillus* na nutrição de monogástricos estão a melhoria na digestão de nutrientes, especialmente proteínas e fibras; o estímulo do sistema imunológico, que contribui para a resistência a doenças; a redução da colonização de patógenos no trato gastrointestinal, promovendo a saúde intestinal e a melhoria do desempenho produtivo, incluindo aumento do ganho de peso e melhor conversão alimentar. Os estudos sobre o uso de *Bacillus* na nutrição de monogástricos são conduzidos tanto em nível nacional quanto internacional, frequentemente por meio de colaborações entre instituições de pesquisa e empresas do setor agropecuário. Em conclusão, as cepas de *Bacillus* são uma abordagem promissora para otimizar o desempenho e a saúde de monogástricos na produção animal.

Palavras-chave: probióticos; suplementação; zootécnicos.

1. Introduction

Probiotics are live microorganisms that, when administered at appropriate doses, confer health benefits to production animals. Bacillus species have been extensively studied and applied as probiotics in poultry and swine diets due to their beneficial properties [(1)]. Several Bacillus strains have been selected for their ability to balance intestinal microbiota, enhance nutrient digestibility, improve feed efficiency, and reduce the incidence of gastrointestinal diseases [(1)].

The scientific literature on the use of Bacillus in poultry and swine feed is extensive, with primary focus areas including efficacy, mechanisms of action, safety, viability, and economic impact. Research on Bacillus as a probiotic has gained increasing attention, particularly as a natural alternative to antibiotic growth promoters, especially in light of regulatory restrictions and growing concerns over antimicrobial resistance [(2)].

Recent advances in animal nutrition research have yielded promising results regarding the use of Bacillus as a probiotic in poultry and swine feed. Scientific evidence demonstrates improvements in productive performance, including greater weight gain, enhanced feed conversion efficiency, and reduced mortality rates [(3,4)]. Additionally, benefits to intestinal health have been reported, such as decreased inflammation and improved mucosal integrity [(3)].

These effects support animal well-being and health while contributing to the productivity and profitability of livestock systems. This review, therefore, aims to consolidate current knowledge on the use of Bacillus species as probiotics in monogastric diets, with a particular focus on zootechnical performance.

2. Material and methods

2.1. Research

Two databases: Web of Science (https://www.webofscience.com/wos) and Scopus (https://www.scopus.com/sources), were used to identify relevant articles, with no restrictions on language or publication date. A Boolean search strategy was applied using the keywords: monogastric OR non-ruminant AND nutrition OR feed OR supplementation OR supply, AND Bacillus.

Articles were screened by title, abstract, and keywords. Only studies meeting the following inclusion criteria were selected: (1) Bacillus was supplemented in the diet, and (2) performance outcomes were reported. Duplicate articles were removed, and abstracts and methodologies were reviewed to exclude studies that did not meet the eligibility criteria (Figure 1).

An analysis of global annual scientific output and publication impact was subsequently performed. Metadata was exported in BibTeX, plain text, and CSV formats, and analyzed using R software with the Bibliometrix package [(5)], as well as VOSviewer software [(6)].

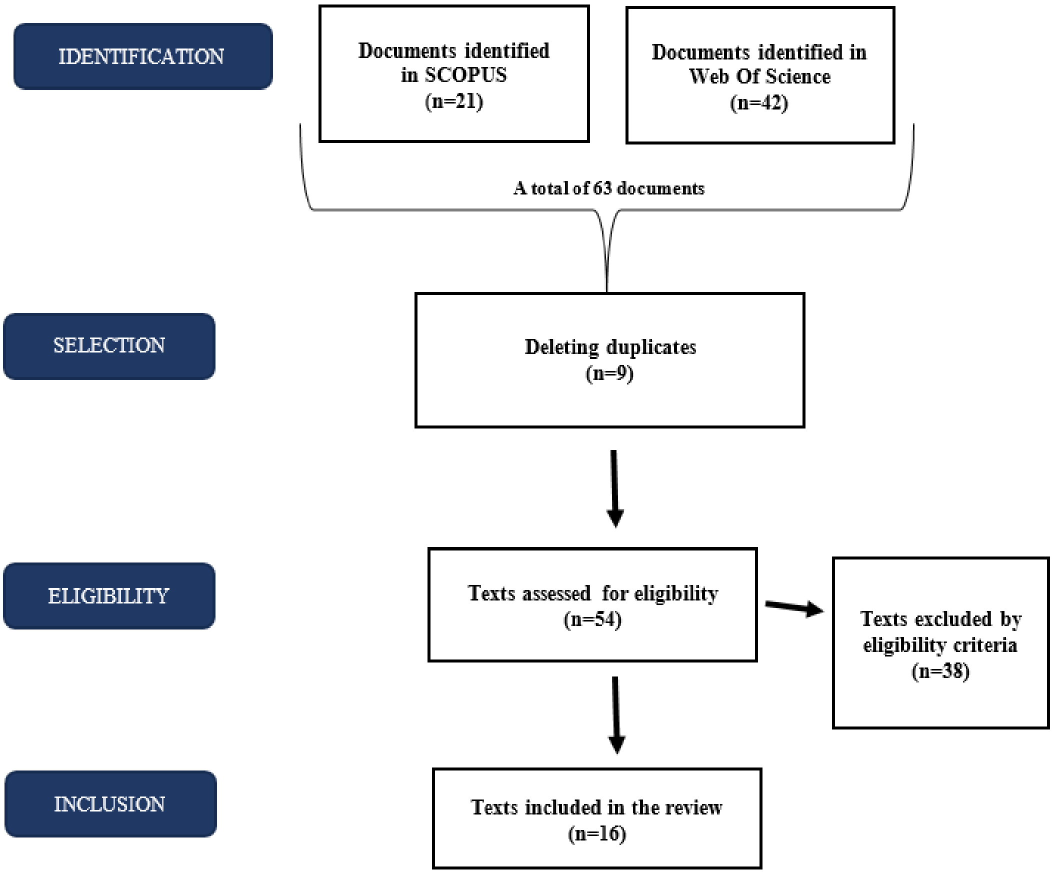

Figure 1. Flowchart illustrating the article selection process.

2.2. Conceptual framework

The conceptual framework is a visual or analytical representation of the relationships among key terms or concepts within a given topic, field of study, or dataset [(7,8)]. It aims to identify and illustrate semantic connections between essential elements of the domain [(9)]. By collecting keywords and mapping them as a co-occurrence network, the framework expresses the integrated knowledge base within the analyzed body of literature [(8)]. These frameworks were generated using the Biblioshiny platform from Bibliometrix [(5)] and VOSviewer software by Van Eck and Waltman [(6)].

Using metadata exported from Bibliometrix, a new file was created to identify the main Bacillus species used as probiotics in monogastric animal diets, as well as recommended usage, study authors, target animal species, and reported phenotypic outcomes. These data were then used to generate correlation tables.

3. Results

The keyword-based database search yielded 63 documents from 44 sources, 21 from Scopus and 42 from Web of Science. Among these, 9 duplicate records were excluded, resulting in 54 documents that met the initial eligibility criteria. Of these, 38 were later removed due to not meeting the specific inclusion parameters or being review articles. Ultimately, 16 documents were selected for analysis.

3.1. Overview of scientific production

Data analysis indicates a growing trend in publications, particularly from 2015 onward (Figure 2). Between 2012 and 2015, only one publication was recorded annually, suggesting a period of limited research activity or interest in the use of Bacillus spp. in monogastric animal nutrition.

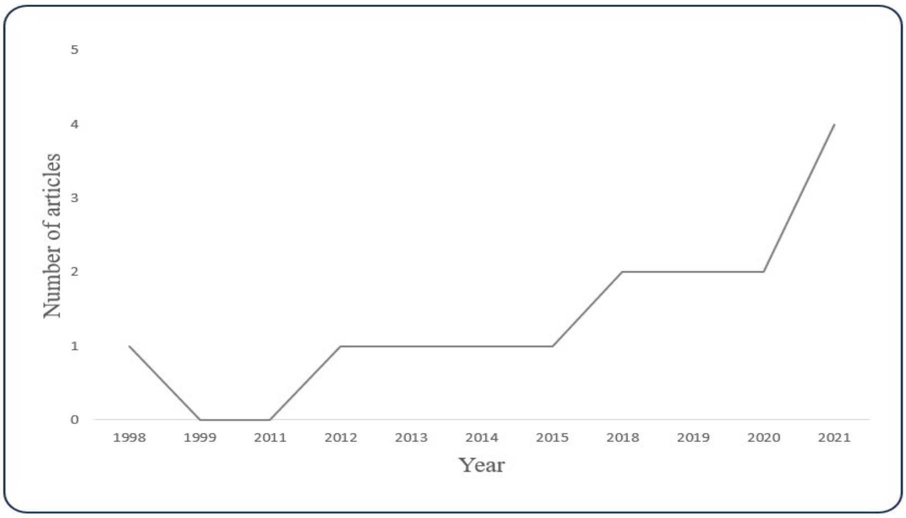

Figure 2. Bibliometric overview of global scientific production on the use of Bacillus spp. in monogastric nutrition.

A temporary stagnation in publication output occurred between 2018 and 2020. In 2018 and 2019, only two studies were published each year. This was followed by a gradual increase, with three publications in 2020 and four in 2021. Production is expected to continue rising through 2024.

During the study period, publications originated from seven countries (Figure 3). The highest number of articles was published in China (n = 6), followed by the United States (n = 2), Brazil (n = 2), and Taiwan (n = 2). Other contributing countries included the Netherlands (n = 1), Germany (n = 1), Korea (n = 1), and Mexico (n = 1).

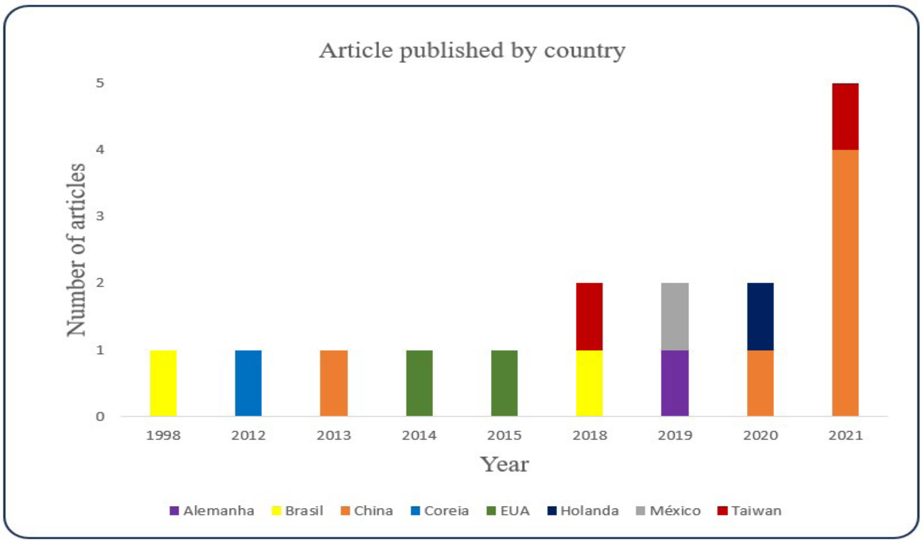

Figure 3. Scientific output by country on the use of Bacillus spp. in monogastric nutrition. Bar colors represent individual countries, corresponding to the legend. Germany (purple) has one publication in 2019. Brazil (yellow) published two articles, one in 1998 and another in 2018. China (orange) contributed one publication in 2013, one in 2020, and four in 2021. Korea (blue) published one production in 2012. The United States of America (green) has two publications, one in 2014 and one in 2015. Mexico (gray) has one publication in 2019. Taiwan (red) produced two studies, one in 2018 and another in 2021.

3.2 Conceptual framework

FA total of 214 keywords were identified across the selected documents and are illustrated in the correlation network shown in Figure 4. This network reveals the relationships and proximity among terms based on co-occurrence. Larger circles indicate higher citation frequency. At the center, Bacillus subtilis appears as the most frequently cited term and is interconnected with other clusters, represented by different colors, starting with "beta-mannanase," "phytase," "fermentation," and "monogastric animal".

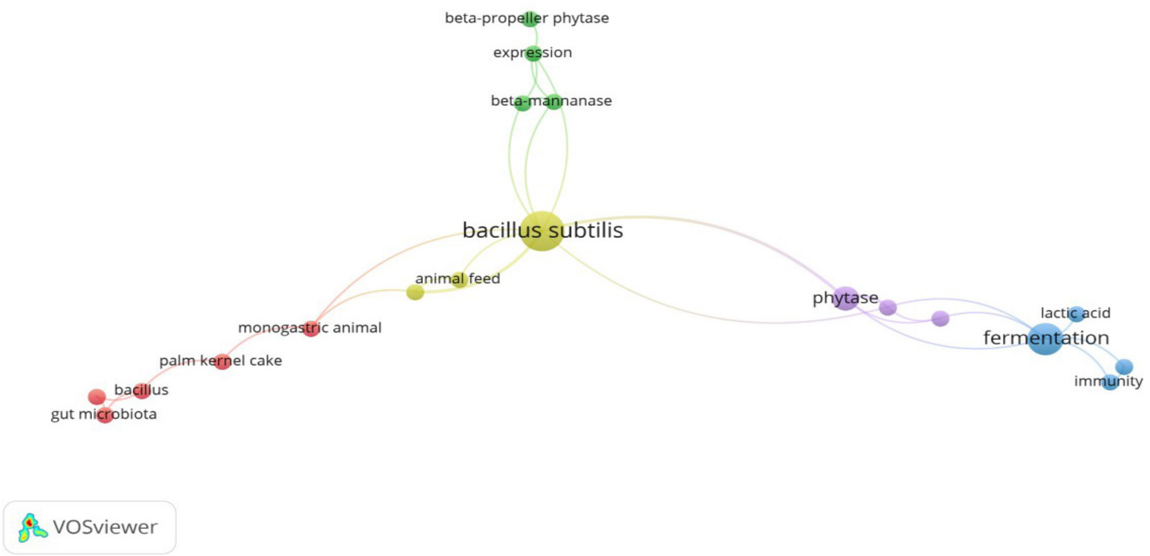

Figure 4. List of keywords found in the analyzed documents.

Multiple correspondence analysis produced a two-dimensional plot (Figure 5) that explained 77.11% of the total variability among the most frequently used terms. Dimension 1 (Dim 1) accounted for 66.69% of the variability, while Dimension 2 (Dim 2) accounted for 10.42%. In the scatterplot, each point represents an observation, and the distance between points reflects their degree of association or dissimilarity, with correlations that may be either positive or negative. A positive relationship was observed among the terms Bacillus subtilis subsp., phytic acid, and monogastric.

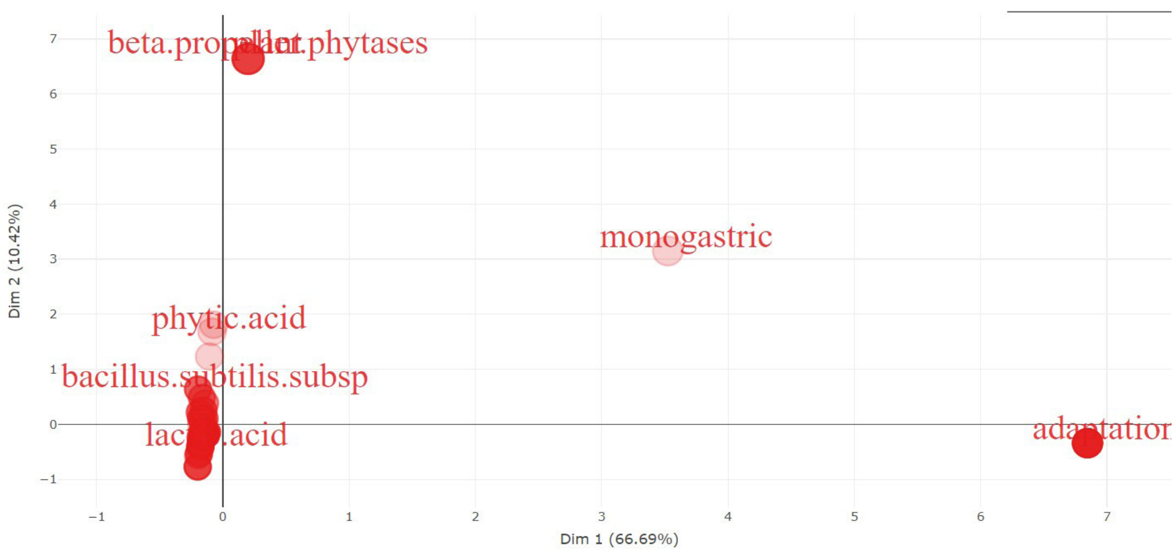

Figure 5. Multiple correspondence analysis (MCA) of the most frequently used terms in the evaluated publications.

Figure 6 presents the thematic map for using Bacillus spp. in monogastric nutrition, highlighting the most frequently used terms across the evaluated publications. The most developed and significant themes relate to the effects of probiotic fermentation on intestinal health and the role of Bacillus as a feed additive for modulating the intestinal microbiota of monogastric animals.

Themes in the upper-right quadrant, characterized by high centrality and density, are identified as "driving themes" [10]. In contrast, "beta-mannanase" and "applications" are classified as basic themes, less developed but essential within the field. Emerging themes, such as "enzyme in the diet of monogastrics" and "expression of beta-helical phytase," are still underexplored but show potential for advancing the understanding of probiotics in monogastric nutrition.

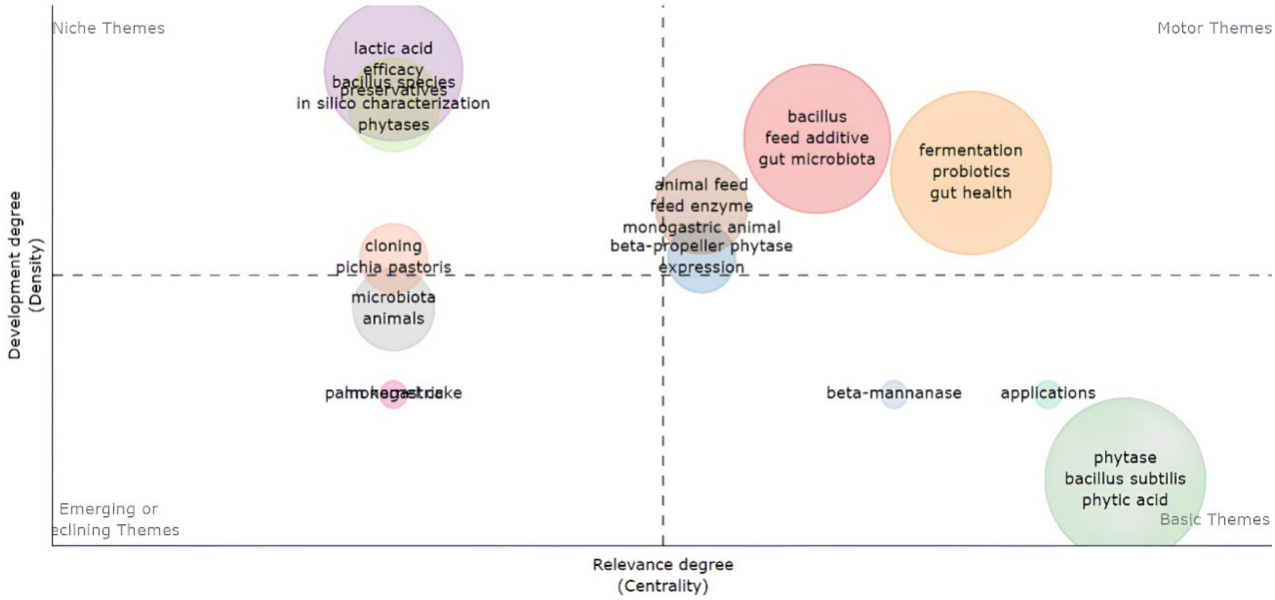

Figure 6. Thematic map of the most frequently used terms in the evaluated publications.

3.3. Action of bacteria of the genus Bacillus in the diet of monogastric animals

Table 1 summarizes the effects of supplementing broiler diets with various Bacillus strains, along with inclusion rates and performance outcomes. Among the reported benefits, the inclusion of B. subtilis LS 1-2 at a concentration of 10^8 CFU/g of feed led to linear improvements in feed intake, weight gain (from 1.639 kg to 1.769 kg), and feed conversion ratio (from 1.8 to 1.7), indicating its potential to enhance growth and feed efficiency in broilers [11]. The B. subtilis strain DSM32315, evaluated by Hernandez-Patlan *et al.* [12] and Whelan *et al.* [13], also proved effective in modulating the intestinal microbiota of chickens by reducing opportunistic pathogens (10^6 spores/g of feed) and controlling the proliferation of Clostridium perfringens (2×10^9 CFU/g).

Supplementation with B. coagulans at 5×10^9 CFU/kg of feed increased body weight (from 1.812 kg to 2.10 kg), average daily gain (from 42 g to 49 g), and antioxidant capacity, suggesting benefits for both growth and oxidative stress resistance in broilers [14]. Additionally, B. licheniformis at 1×10^6 CFU/g improved intestinal morphology and antioxidant status [15]. Strain combinations such as B. subtilis + B. amyloliquefaciens (10^6 spores/g), B. subtilis var. natto N21 (BS) + B. coagulans L12 (10^6 CFU/g), and B. subtilis strains NP122, B2, and AM0904 (Sporulin®, Novus International Inc.) demonstrated enhanced nutrient absorption [16], greater average daily gain [17], and improvements in feed conversion and overall performance [18].

Table 1. *Bacillus spp.* strains used in dietary supplementation, inclusion levels, and effects on zootechnical performance parameters of broiler.

Strain	Inclusion	Performance	Reference
B. subtilis LS 1-2	10^8 CFU/g feed	Linear improvement in feed intake, body weight gain, and feed conversion rate	Sen et al. (2012) [11]
B. subtilis *B. amyloliquefaciens*	10^6 spores /g feed	Improvements in intestinal integrity and nutrient absorption	Latorre et al. (2015) [16]
B. subtilis DSM 32315	10^6 spores/g feed	Stabilization of the intestinal microbiota and inhibition of opportunistic pathogens	Hernandez-Patlan et al. (2019) [12]
B. coagulans	5×10^9 CFU /kg feed	Increases in body weight, average daily gain, and increased antioxidant capacity	Zhang et al. (2021) [14]
B. licheniforme strain H2 (CCTCC NO: M2011133)	1×10^6 CFU/g diet	Enhancement of intestinal morphology and antioxidant capacity	Zhao et al. (2020) [15]
B. subtilis DSM32315	2×10^9 CFU /g	Control of *C. perfringens* proliferation in the broiler intestine	Whelan et al. (2019) [13]
B. subtilis	1.5×10^5 CFU /g	Enhanced growth performance, elevated nitric oxide levels, and reduced coccidia-specific antibodies in chickens	Lee et al. (2014) [19]
B. subtilis var. *natto* N21 *B. coagulans* L12	10^6 CFU/g of feed	Improved feed conversion ratio, final carcass weight, and growth performance	Yeh, Hsieh and Chen (2018) [18]
B. subtilis (NP122, B2 and AM0904 Sporulin ®, Novus International Inc.)	250g/ton.	Increased average daily gain and improved feed efficiency	Hayashi et al. (2018) [17]

CFU = colony forming unit

Table 2 presents the effects of Bacillus strains on swine nutrition. Inclusion of B. cereus at 10^{12} spores/kg of feed significantly improved feed conversion in weaned piglets, with values of 1.904 and 2.099 for the probiotic-treated and control groups, respectively. Additionally, the probiotic-treated group consumed 10% less feed to achieve the same weight as the control, indicating its potential to enhance feed efficiency (20). Similar results were reported by Li, Jiang, and Qiao (21), who observed increased average daily gain (from 252 to 285 g/day) and improved feed conversion (from 1.56 to 1.43) with the inclusion of B. subtilis at 10^7 CFU/kg of feed. Likewise, B. subtilis KN-42 at 4×10^9 or 20×10^9 CFU/kg improved average daily gain (from 885 to 897 g/day) and feed efficiency compared to non-supplemented groups (22), underscoring its growth-promoting potential in pigs.

In terms of digestive health, supplementation with B. subtilis DSM32315 at 2×10^9 CFU/g enhanced microbiota diversity, composition, and metabolite production (23). Additionally, B. subtilis ASAG 216 at 1×10^8 CFU/mL improved immune function, antioxidant capacity, and intestinal integrity in piglets (24). Another noteworthy result was achieved with B. amyloliquefaciens SC06, where inclusion at 100 mg/kg or 10^9 CFU/kg significantly reduced the incidence of diarrhea in weaned piglets (25).

The combination of B. subtilis and B. coagulans at 2.5–5% dietary inclusion significantly enhanced growth performance and immunity in finishing pigs (26), indicating its potential to support healthy development and a robust immune response during this critical production phase.

Table 2. *Bacillus spp.* strains used in dietary supplementation, inclusion levels, and effects on zootechnical performance parameters of pigs.

Strain	Inclusion	Performance	Reference
B. cereus	10^{12} spores/kg feed	Improved feed conversion rate and feed intake	Zani et al. (1998) [20]
B. subtilis DSM32315	2×10^{9} CFU /g	Improvements in the intestinal microbiota diversity, composition, and metabolites	Ding et al. (2021) [23]
B. subtilis	10^{7} CFU /kg of feed;	Increases in average weight daily gain and feed conversion rate	Li, Jiang and Qiao (2021) [21]
B. subtilis ASAG 216	1×10^{8} CFU/mL	Improved immune function, antioxidant capacity, and intestinal integrity of piglets	Jia et al. (2021) [24]
B. amyloliquefaciens SC06	100 mg/kg of 10^{9} CFU/kg	Reduced incidence of diarrhea in weaned piglets	Ji et al. (2013) [25]
B. subtilis KN-42	4×10^{9} CFU and $20\text{x}10^{9}$ CFU/kg of feed	Improved average daily gain and feed efficiency compared to the non-supplementation group	Peet-Schwering et al. (2020) [22]
B. subtilis *B. coagulans*	2.5 to 5% inclusion in the diet	Enhanced growth performance and immune response in finishing pigs	Huang et al. (2021) [26]

CFU = colony forming unit

4. Discussion

The presence of Bacillus subtilis as a central node in the methodological research network of this review indicates its predominant role in studies involving monogastric animals, particularly chickens and pigs. All reviewed studies investigated the effects of Bacillus species administered through feed, addressing both their roles in fermentation and their impacts on animal health and performance.

Bacillus species are effective probiotics due to their ability to form spores, which enables them to withstand adverse conditions such as extreme temperatures during feed pelleting, high or low pH, dehydration, and pressure [27, 28, 29]. Additionally, members of the Bacillus genus are prolific producers of bioactive compounds, ranging from extracellular enzymes that enhance nutrient digestibility and absorption to antagonistic substances that inhibit pathogenic bacteria in the gastrointestinal environment [13, 30].

The data in Tables 1 and 2 support the efficacy of Bacillus spp. in enhancing broiler and swine performance. Strains such as B. subtilis var. natto N21 (BS), B. subtilis LS 1-2, and B. amyloliquefaciens DSM 25840 demonstrated significant improvements in weight gain, feed conversion, and overall feed efficiency. These findings align with those of Bahaddad *et al.* [31], who advocate the use of Bacillus strains as beneficial feed supplements for monogastrics, emphasizing their role in increasing digestible amino acid content and supporting healthy intestinal microbiota.

Beyond performance enhancement, Bacillus species contribute to improved immune function, particularly in reducing the incidence of diarrhea. Solitary lymphoid follicles in the intestinal mucosa respond to immunostimulants by increasing in number and enhancing lymphoid tissue development and macrophage function [32]. Additionally, competitive exclusion of pathogenic bacteria by Bacillus strains lowers pathogen colonization, thereby reducing the risk of contamination by foodborne pathogens [33].

Variation in recommended dosages and strain-specific responses was also noted, emphasizing the importance of tailoring probiotic supplementation to animal species, physiological stage, and age, especially during the early life stages, which are critical for all species [34]. These findings underscore the need for further research to clarify the effects of Bacillus strains across different production systems. Supplementation outcomes differ between poultry and swine due to physiological and digestive differences. In poultry, benefits include enhanced growth, feed efficiency, nutrient absorption, improved intestinal morphology, and protection against oxidative stress. In swine, improvements extend beyond weight gain and feed conversion to include greater microbiota stability, reduced intestinal disorders, and strengthened immune responses during critical developmental phases.

Our findings have practical implications for the poultry and swine industries, offering evidence-based guidance for incorporating Bacillus strains into monogastric diets. Such applications can enhance animal performance and production efficiency, supporting more sustainable and economically viable livestock systems while encouraging further research into their use in other animal species

4. Conclusion

Bacillus strains represent a promising strategy for enhancing the performance and health of monogastric animals, supporting their use as effective probiotics in livestock.

Conflicts of interest statement

The authors declare no conflicts of interest.

Data Availability Statement

Data will be provided upon request by the corresponding author.

Author contributions

Conceptualization: J. S. Conceição and N. S. Evangelista-Barreto. Data curation: J. S. Conceição; S. A. Carvalho; C. L. Santos; E. Pereira and M. Melo. Formal analysis: J. S. Conceição; S. A. Carvalho and N. S. Evangelista-Barreto. Investigation: J. S. Conceição; S. A. Carvalho; C. L. Santos; E. Pereira and M. Melo. Methodology: J. S. Conceição and N. S. Evangelista-Barreto. Project administration: J. S. Conceição. Resources: J. S. Conceição; S. A. Carvalho; C. L. Santos; E. Pereira and M. Melo. Software: J. S. Conceição. Supervision: N. S. Evangelista-Barreto. Validation: J. S. Conceição and N. S. Evangelista-Barreto. Visualization: J. S. Conceição and N. S. Evangelista-Barreto. Writing (original draft): J. S. Conceição; S. A. Carvalho; C. L. Santos; E. Pereira; M. Melo and N. S. Evangelista-Barreto.

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