



13-14 November 2025

PROBIOTIC SUPPLEMENTATION AS AN ALTERNATIVE STRATEGY FOR THE
PREVENTION OF SALMONELLA SPP. INFECTIONS IN LAYING HENS: A
COMPREHENSIVE REVIEW

Alexandru Udrea*, Alexandra Grigoreanu, David Purec, Cristina Gaspar, Viorel Herman

University of Life Sciences "King Mihai I" from Timișoara, Faculty of Veterinary Medicine,
300645, Calea Aradului No. 119, Timișoara, Romania
*Correspondence: Alexandru.Udrea.IOSUD@usvt.ro

Abstract: The need of intensification in poultry industry has led to extensive antibiotic use strictly for disease prevention, leading to the emergence of multidrug resistance *Salmonella* spp. strains, prompting the research for alternative control strategies. The aim of this review is to evaluate recent evidence in the use of probiotics in preventing *Salmonella* Enteritidis, *Salmonella* Gallinarum, and *Salmonella* Typhimurium infections in laying hens. In order to achieve this literature search covering identified studies meeting specific criteria was conducted. Overall, supplementing the birds with probiotic products showed preponderantly positive effects, acting through modulation of microbiota, immune modulation and inhibition of pathogenic bacteria colonization. Proving to be most effective were species of *Bacillus* and *Lactobacillus*, managing to reduce both cecal and fecal *Salmonella* loads, while also improving microbial balance and production traits. Prebiotic and symbiotic combinations produced transient or variable results. Collectively, the prelevated data point towards the fact that probiotics can restore microbial homeostasis, promote intestinal health, and aim to serve as a sustainable alternative to antibiotic usage in the control and prevention of *Salmonella* infections in layers, supporting antimicrobial stewardship and improved standards of food safety in poultry industry.

• Introduction

The intensification of poultry production has boosted global food supply through major advances in genetics, nutrition, and management, but excessive antibiotic use—especially in feed—has accelerated antimicrobial resistance, notably in *Salmonella* spp. [Kleyn et al. 2021; Mottet et al. 2017; Castro-Vargas et al. 2020; Velge et al. 2005]. As Salmonellosis poses serious economic and zoonotic risks, attention has shifted from antibiotic prophylaxis toward probiotics and prebiotics to limit pathogen colonization in poultry microbiota [Eng et al. 2015; Vandeplas et al. 2017]. Probiotics, beneficial nonpathogenic microorganisms, have demonstrated potential in enhancing gut health and reducing *Salmonella* prevalence, offering a sustainable alternative to antibiotics. This review compiles recent research supporting probiotic efficacy in mitigating disease and addressing the global rise of multidrug-resistant *Salmonella* strains [Krisiak et al. 2021; Naeem et al. 2024; Ruvalcaba-Gómez et al. 2022].

• Material and method

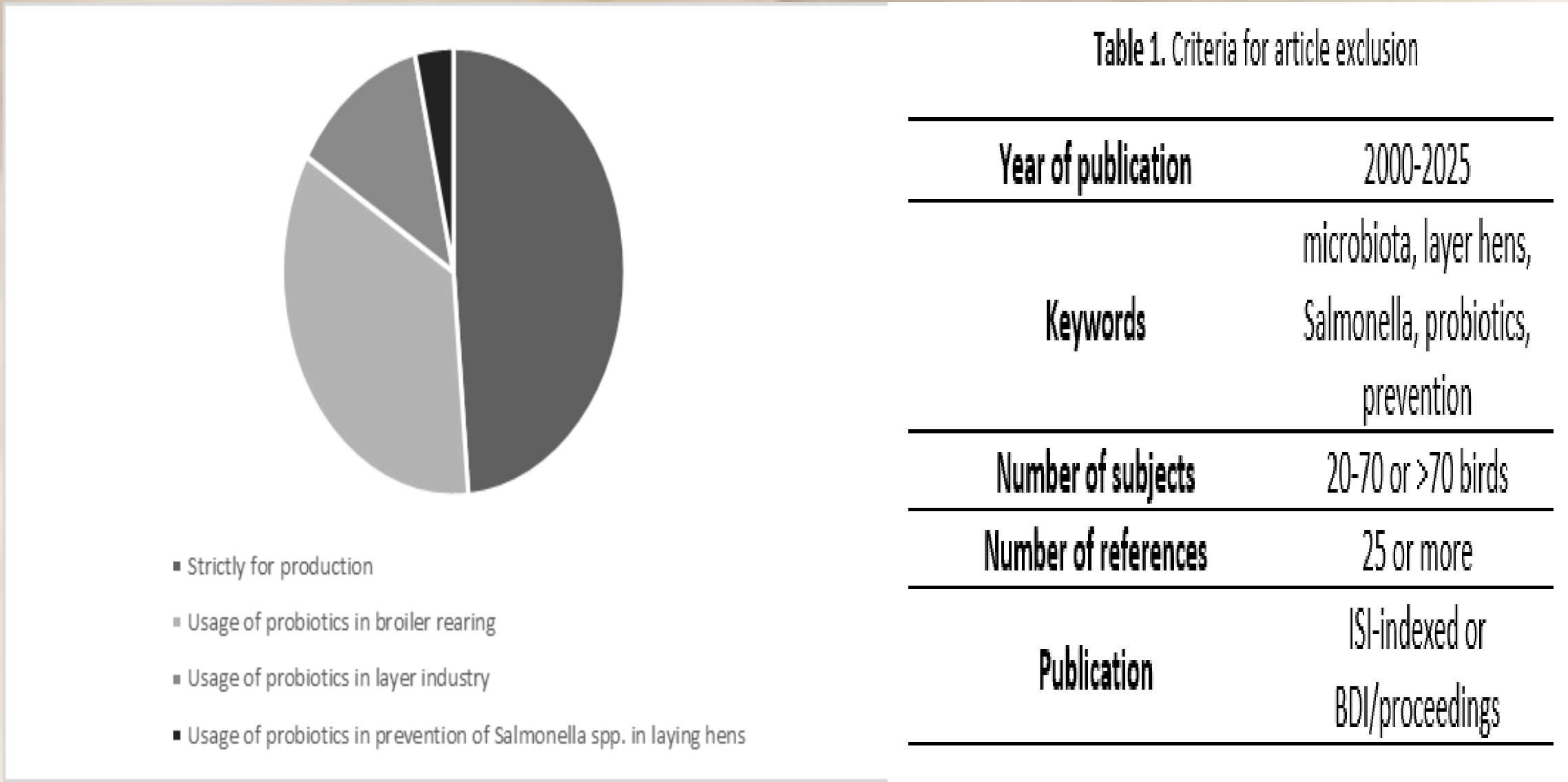


Table 2. Number of subjects tested in the "Usage of probiotics in prevention of Salmonella spp." category				
<10 subjects	10-20 subjects	20-50 subjects	50-70 subjects	>70 subjects
20 articles	50 articles	25 articles	5 articles	10 articles

• Results and discussions

Study	Study design	Probiotic intervention	Target pathogens	Primary outcome measured
L.S. Upadhyay et al. 2015	120 birds 4 groups: control, Probiotic, Prebiotic, Probiotic+Prebiotic	<i>B. subtilis</i> <i>L. acidophilus</i> <i>Enterococcus faecium</i> <i>Bifidobacterium longum</i>	<i>S. enteritidis</i>	Prevalence of pathogen
S.D. Upadhyay et al. 2016	180 birds Negative control Probiotic control T1: <i>B. subtilis</i> T2: <i>L. acidophilus</i>	<i>B. subtilis</i> <i>L. acidophilus</i>	<i>S. enteritidis</i>	Prevalence of <i>Lactobacillus</i> spp. in the gut microbiota
J.K. Chh et al. 2017	24 birds divided in three groups: C0H, S0L, P0D	<i>B. subtilis</i> C0L2	<i>S. enteritidis</i>	Percentage of <i>Firmicutes</i> , <i>Pseudomonas</i> , <i>Salmonella</i> in the microbiota
P. Adhikari et al. 2019	52 white leghorns T1-control T2-5% unchallenged T3-5% challenged-0.2% probiotics T4-5% challenged-0.5% probiotics	<i>Lactobacillus plantarum</i>	<i>S. enteritidis</i>	Immunological regulation
S. Khan et al. 2020	6 groups, with 7 hens each - Neg Control (NC) - Salm challenged (SC) - Continuous probiotic salmonella challenged (CP) - Continuous probiotic supplemented control (CPC) - Intermittent probiotic salmonella challenged (IP) - Intermittent probiotic control (IPC)	<i>B. subtilis</i> DSM 32224/32325 <i>B. pasteurii</i> DSM 10000	<i>S. typhimurium</i>	Microbiota changes during salmonellosis challenge and the effect of probiotics in restoring gut flora and on salmonella load
E.A. Kimminau et al. 2021	144 pullets 3 treatments: a) untreated b) <i>B. subtilis</i> plus depyrimidination c) <i>B. subtilis</i> plus 20% depyrimidination	<i>B. subtilis</i> plus- <i>B. subtilis</i> /lichenformis M05	<i>S. enteritidis</i>	Impact of probiotics on the capacity of pathogens to colonize the gut
P.T. Price et al. 2021	60 hens 56 weeks age, individual cages groups: Control Bacillus spp. Yeast cell wall Combination of probiotics and yeast cell wall	<i>Bacillus</i> spp. Yeast cell wall	<i>S. enteritidis</i>	Changes in the microbiota after inoculation and differences between groups
M. Kalani et al. 2022	120 birds 44 wks old 5 treatments, 5 replicates, 4 birds in each group: - Negative control - Positive control - Antibiotic treatment - Probiotic treatment - Antibiotic+probiotic treatment	<i>Bifidobacterium</i> <i>Bifidobacteris</i>	<i>S. enteritidis</i>	Impact of <i>B. subtilis</i> post-inoculation in each control group
M.K. Sharma et al. 2022	72 hens with 6 replicates, 4 hens for each replicate: - Control - Positive control - Probiotics	<i>Aspergillus niger</i>	<i>S. enteritidis</i>	Proportion of <i>B. subtilis</i> and pathogenic bacteria in each group
I. Poudel et al. 2025	72 Hy-line W36 hens at 24 weeks of age 18 birds/treatment including negative control: - Negative control - Positive control - PBO 1 - PBO 2	<i>B. subtilis</i> <i>B. pasteurii</i>	<i>S. enteritidis</i>	Potential of probiotics to reduce the infection rate

Probiotic supplementation generally benefits laying hens by reducing *Salmonella* infections, though results vary with strain, dose, and timing. Targeted use lowers pathogen colonization and modulates immune responses, as shown by reduced cecal counts and altered cytokine profiles [Kimminau et al. 2021; Adhikari et al. 2019]. Prebiotics offered transient effects [Murate et al. 2015], while *Aspergillus niger* improved gut health [Sharma et al. 2022]. *Bacillus*-based probiotics and yeast derivatives reduced bacterial loads (~1.4 log₁₀ MPN/g) and outperformed antibiotics and prebiotics [Price et al. 2021; Kalani et al. 2022; Poudel et al. 2025]. Across *S. Gallinarum* and *S. Typhimurium* models, *Bacillus* spp. improved egg quality, restored microbial balance, and countered dysbiosis [Upadhyaya et al. 2016; Oh et al. 2017; Khan et al. 2020]. Overall, probiotics—especially *Bacillus* strains—enhance gut health and immunity, offering a sustainable alternative to antibiotics in poultry production.

• Conclusions

Probiotics show consistent benefits in controlling *Salmonella* spp. infections in laying hens by enhancing gut microbiota balance, immune modulation, and pathogen resistance [Huang et al. 2022; Padgett et al. 2021]. Despite strain and dosage variations, *Bacillus*-based supplements notably reduce *S. Enteritidis*, *S. Gallinarum*, and *S. Typhimurium* loads while improving gut integrity and productivity [Kimminau et al. 2021; Upadhyaya et al. 2016; Khan et al. 2020]. Overall, probiotics represent a sustainable alternative to antibiotics for maintaining poultry health and mitigating antimicrobial resistance [Price et al. 2021; Kalani et al. 2022; Poudel et al. 2025].