

Article

Organic ACIDS Treatment Attenuates Cecal Microbiota Dysbiosis During *Salmonella* Minnesota Challenge in Broilers

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Abstract

In this study, we evaluated the effects of a commercial blend of organic acids (formic acid and propionic acid) added to drinking water on the microbiota of broilers experimentally challenged with *Salmonella enterica* serovar Minnesota. A total of 108 broilers were randomly assigned to three groups, each with 36 birds: the Treatment group (treated with organic acids + challenge), a Positive Control group (challenge only), and a Negative Control group (no challenge). At seven days of age, broilers in the Treatment group received the organic acid blend through their drinking water (0.2 mL/L/drinking water). At nine days of age, broilers from the challenged groups were inoculated via feed with *S. Minnesota* (Treatment group—mean: 1.45×10^4 CFU/g; Positive Control group—mean: 3.47×10^4 CFU/g). Swab samples were collected for *Salmonella* enumeration and cecal content for bacterial community profiling using 16S-rDNA gene sequencing. Our results demonstrated that supplementation with organic acids positively modulated the cecal microbiota of broilers under *S. Minnesota* challenge. Broilers receiving the organic acid blend showed improvements in microbial community composition, including an increased abundance of bacterial genera associated with butyrate production, which are recognized for their beneficial effects on intestinal health. Overall, our findings suggest that the blend of organic acids may serve as a nutritional strategy for supporting intestinal health and microbiota stability in broilers challenged with *S. Minnesota*.

Keywords: acid formic; acid propionic; dysbiosis; cecal microbiota



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1. Introduction

Antibiotic use in broiler production has been prohibited in many countries worldwide due to the accelerating spread of antimicrobial resistance and the risks to human health [1,2]. In this context, alternatives to antibiotics have increasingly been explored, including organic acids, probiotics, prebiotics, symbiotics, enzymes, phytogenics, antimicrobial peptides, hyperimmune egg antibodies, bacteriophages, clay, and metals. These additives have been reported to improve intestinal health through different mechanisms that include modulating the gut microbiota, enhancing intestinal barrier function, stimulating the immune response, and reducing pathogen colonization [3].

Among these alternatives, organic acids have received considerable attention due to their broad antimicrobial activity and their ability to promote intestinal health. Organic acids, such as formic, propionic, lactic, and citric acids, reduce the pH of the gastrointestinal tract, creating an unfavorable environment for acid-sensitive pathogens such as *Salmonella* spp. Furthermore, the undissociated forms of these acids can diffuse across bacterial cell membranes, disrupting intracellular pH homeostasis, enzyme activity, and essential metabolic pathways, thereby inhibiting bacterial growth [4,5].

Previous studies have shown that supplementation with organic acids in poultry feed or water can enhance growth performance and meat quality [6,7]. In addition, the ability of OAs to reduce the pH of the upper gastrointestinal tract improves nutrient digestion and inhibits pathogenic bacteria [8].

Among pathogenic bacteria, serovars of *Salmonella enterica* are significant in poultry production, as this microorganism can have effects ranging from asymptomatic infections to acute systemic disease in broilers, leading to economic losses for producers due to mortality and reduced weight gain [9]. In addition, the vast majority of *Salmonella* serovars are zoonotic bacteria, which pose a significant risk to the human population [9]. *Salmonella* is among the most common enteric bacterial pathogens, accounting for 5.2% of pediatric cases diagnosed with acute gastroenteritis [10].

Organic acids added to poultry feed can reduce *Salmonella* spp. growth [11] and colonization in the crop and ceca of broilers [12]. Some studies have used *Salmonella Enteritidis* or *Salmonella* Typhimurium as marker strains [12], leaving the effect of organic acids poorly understood for serovars such as *Salmonella* Minnesota, an important pathogen for humans and animals [13]. Despite the high importance and wide use of metagenomic approaches, the use of deep sequencing to determine the performance of the bacteriome of broilers after experimental challenge remains unexplored.

Thus, in this study, the effect of a commercial blend of organic acids (formic acid and propionic acid) added to drinking water on the cecal microbiota of broilers experimentally challenged with *S. Minnesota* was determined.

2. Materials and Methods

2.1. Preparation of *Salmonella Minnesota* Inoculum

A strain of *Salmonella Minnesota* previously isolated from broilers and resistant to nalidixic acid and spectinomycin (SM^{NalSpc}) was used. The strain was inoculated into Luria–Bertani (LB) broth and incubated at 37 °C for 18 h under agitation. The resulting culture contained approximately 10⁸ to 10⁹ colony-forming units per milliliter (CFU/mL).

2.2. Organic Acid Products

Axeed[®] Liquid (Salmix, Piedade, SP, Brazil) is a commercial drinking water additive composed of a blend of formic and propionic acids, indicated as an alternative to antibiotic growth promoters in poultry production. According to the manufacturer, the same proportions of both formic and propionic acids compose the commercial product.

2.3. Experimental Design

Before starting the experiment, samples from the transport boxes of one-day-old broilers were collected to provide information about *Salmonella* spp. circulation in the flock. In summary, sterile swabs with Buffered Peptone Water were rubbed over the meconium present in the transport boxes. Swabs were then placed into a flask containing 50 mL of Selenite broth supplemented with novobiocin (4 mg/mL) (SN broth), and samples were incubated at 37 °C for 24 h. Subsequently, aliquots from SN broth were streaked onto Brilliant Green agar (Himedia, Mumbai, MS, India) and MacConkey agar (Himedia,

Mumbai, MS, India). The plates were incubated at 37 °C for 24 h and then examined for confirmation of the *Salmonella* spp. suggestive colonies’ absence.

Figure 1 summarizes the experimental design. In this experiment, one-day-old broiler chickens (52.7 g ± 0.8 g) were randomly allocated into three groups (comprising 36 poultry; 18 poultry/m²) housed in metal battery cages in a climate-controlled and negative pressure room, where they were kept until the end of the experiment. The metal battery vertical cages include metal trays for fecal collection, which were sanitized twice daily, preventing reinfection of the animals during the experimental period. Chicks were acquired from a commercial hatchery (JBS Food®—Nuporanga, SP, Brazil), while birds from the Cobb lineage were obtained at one day old directly from the hatchery to the research facility, carried in craft boxes (with up to 50 chicks in each) under temperature-controlled conditions.

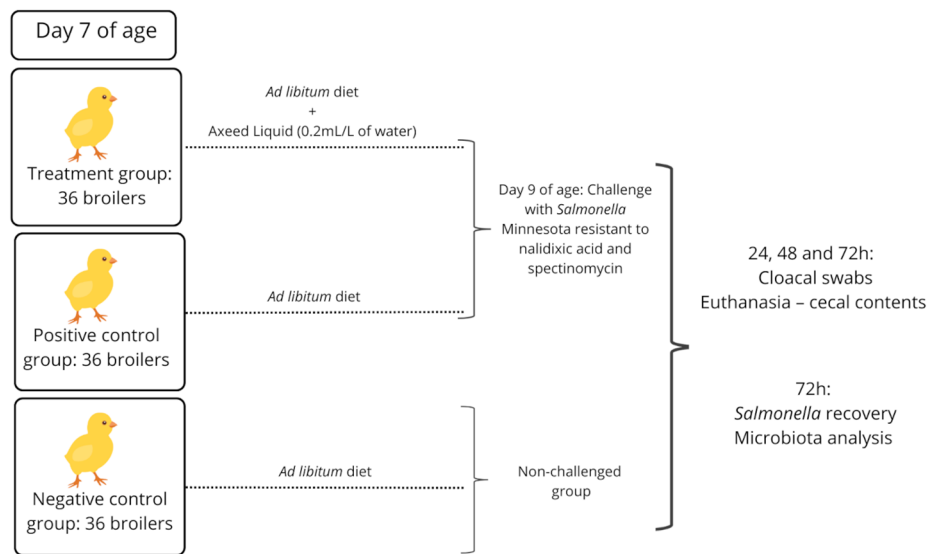


Figure 1. Overview of the experimental design.

Each group was further subdivided into three subgroups with 12 animals each (Table 1). The Treatment group encompassed animals that were challenged with *S. Minnesota* and that received a commercial blend of organic acids composed of formic acid and propionic acid (Axeed® Liquid—Salmix); the Positive Control group included animals that were challenged with *S. Minnesota* and that did not receive any treatment; and the Negative Control group included animals that were neither challenged nor treated. The sample size was determined in accordance with ARRIVE guidelines ($\beta = 90\%$; $\alpha = 5\%$; detection limit: 100 CFU/g).

Table 1. Overview of the experimental design and treatments.

Experimental Group	Dosage (Axeed® Liquid)	N° Animals
Treatment	0.2 mL/L/drinking water	36 broilers divided into three subgroups
Positive control	Not treated	36 broilers divided into three subgroups
Negative control	Not treated	36 broilers divided into three subgroups

Animals received both water and feed ad libitum during the full experimental period. A corn–soybean meal diet was formulated for the initial phase with the following levels: 22% crude protein, 2950 kcal of metabolizable energy/kg of diet, 1.2% digestible lysine, 0.94% digestible methionine + cystine, and 0.85% digestible threonine (Table 2). No antimicrobial agents, anticoccidials, animal-derived by-products, or oils were used.

Table 2. Guaranteed nutrient composition of the corn–soybean meal starter diet for broilers.

Nutrient/Component	Specification
Folic acid	Minimum: 0.63 mg/kg
BHT-antioxidant	Minimum: 100.00 mg/kg
Biotin	Minimum: 0.12 mg/kg
Calcium	Minimum: 9.20 g/kg; Maximum: 11.00 g/kg
Copper	Minimum: 10.00 mg/kg
Choline	Minimum: 400.00 mg/kg
Ether extract	Minimum: 30.00 g/kg
Iron	Minimum: 56.00 mg/kg
Phosphorus	Minimum: 7000.00 mg/kg
Iodine	Minimum: 0.62 mg/kg
Lysine	Minimum: 12.00 g/kg
Manganese	Minimum: 75.00 mg/kg
Crude fiber	Maximum: 50.00 g/kg
Ash	Maximum: 100.00 g/kg
Methionine	Minimum: 5200.00 mg/kg
Calcium pantothenate	Minimum: 15.00 mg/kg
Crude protein	Minimum: 220.00 g/kg
Selenium	Minimum: 0.20 mg/kg
Sodium	Minimum: 1800.00 mg/kg
Moisture	Maximum: 130.00 g/kg
Vitamin A	Minimum: 7000.00 IU/kg
Vitamin B1	Minimum: 1.80 mg/kg
Vitamin B12	Minimum: 15.00 mcg/kg
Vitamin B2	Minimum: 4.00 mg/kg
Vitamin B6	Minimum: 3.50 mg/kg
Vitamin D3	Minimum: 2000.00 IU/kg
Vitamin E	Minimum: 16.00 IU/kg
Vitamin K3	Minimum: 3.50 mg/kg
Zinc	Minimum: 50.00 mg/kg

At seven days of age, the animals from the Treatment group started receiving organic acids in water, which persisted until the end of the assay (Figure 1). The organic acid treatment was prepared daily with 0.2 mL/L/drinking water, and the mean water pH was 4.52 after product addition. At this age, the broilers had an average body weight of 177.3 ± 2.0 g.

At nine days of age, broilers were challenged via feed with *Salmonella* Minnesota (SM^{NalSpc}). Feed contamination was achieved with 2 mL of LB broth containing 10^8 and 10^9 CFU/mL added to 10 g of feed. After homogenization for approximately 5 min, small portions were transferred to a plastic bag and mixed with additional feed to a total of 2 kg. Samples from different parts of the feed were analyzed to ensure SM^{NalSpc} homogeneity in the feed. The SM^{NalSpc} counts in the feed on the day of challenge are presented in Table 3.

Table 3. *Salmonella* Minnesota (SM^{NalSpc}) in the prepared feed immediately after contamination.

Subgroups	Treatment Group (log10-CFU/g)	Positive Control (log10-CFU/g)
1	4.36	5.04
2	4.00	3.93
3	4.11	4.64
Mean	4.16	4.54

SM^{NalSpc}: *Salmonella* Minnesota resistant to nalidixic acid and spectinomycin isolated from broilers.

2.4. *Salmonella* Minnesota Excretion and Cecal Colonization

Two kinds of samples were collected from each animal: cloacal swabs and cecal content. Four animals from each subgroup (12 animals per experimental group) were euthanized at 24 h, 48 h, and 72 h post-infection to collect cecal contents. Prior to the euthanasian procedure, cloacal swabs from each animal were also collected (Figure 1).

Swabs were placed into tubes containing 3 mL of SN broth. After homogenization, samples were plated onto Brilliant Green agar supplemented with nalidixic acid and spectinomycin (VBNal/Spc) and incubated at 37 °C for 24 h. Enumeration of SM^{NalSpc} followed the previously described method [14].

Cecal contents were diluted in phosphate-buffered saline (PBS, pH 7.4) at a 1:10 ratio, followed by serial tenfold dilutions in PBS, and were plated onto VBNal/Spc agar (Kasvi, Pinhais, PR, Brazil) at a concentration of 100 µg/mL for SM^{NalSpc} enumeration. Plates were read after 24 h of incubation at 37 °C. Colony-forming units per gram (CFU/g) were determined, and values were log10-transformed for data analysis and interpretation. Bacterial counts less than 10² CFU/g (limit of detection) of the cecal content were considered to be negative results.

2.5. Statistical Analysis of SM^{NalSpc} Excretion and Cecal Counts

The differential excretion of SM^{NalSpc} among groups was evaluated with cloacal swab counts using a non-parametric chi-square test, with a significance level of 5%. Cecal contents were compared using the *t*-test at a 5% significance level.

2.6. Microbial Community Profiling Through 16S-rDNA Gene Sequencing

Fresh cecal contents from animals euthanized at 72 h post-infection were subjected to metagenomic DNA extraction using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden Germany) according to the manufacturer's protocol. DNA quantity and quality were assessed through fluorometry using QubitTM (Thermo Fisher Scientific, Waltham, MA, USA) and spectrophotometry using the NanoDrop OneC (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the V4 region of the 16S-rDNA gene was sequenced using the Illumina MiSeq platform (Illumina, San Diego, CA, USA) with paired-end reads (2 × 300 bp).

Microbiome analysis was performed only at 72 h post-infection, representing the acute phase of infection [15]. Therefore, our results reflect the microbial community at this specific time point and should not be interpreted as the peak of *Salmonella*-induced dysbiosis, which may vary according to the experimental model.

Raw reads were assessed using FastQC (v0.11.9) [16], and low-quality sequences were removed using Trimmomatic (v0.39) [17]. Clean reads were imported into QIIME 2 (2025.10) [18] and processed using DADA2 [19], and taxonomic assignment was performed against the SILVA database (release 138) [20]. Additional analyses were conducted in R (version 4.5.2) using the packages microbiome (v1.32.0) [21], microbiomeutilities (v1.00.17) [22], phyloseq (v1.54.0) [23], and ranacapa (v0.1.0) [24]. The package microDecon (v1.0.2) [25] was used to remove contaminant reads based on a blank control community library. Reads assigned to eukaryotes, archaea, chloroplasts, mitochondria, unclassified taxa, and taxa with confidence scores < 0.8 were removed from the dataset.

Alpha diversity (Shannon index) and beta diversity (Bray–Curtis dissimilarity) analyses were performed, including principal coordinates analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA), with pairwise comparisons conducted using the pairwiseAdonis package (v0.4.1) [26]. The microeco package (v1.16.1) [27] was used to calculate Linear Discriminant Analysis (LDA) Effect Size. For exploratory dysbiosis analysis, the dysbiosisR package (v1.0.4) [28] was applied using a binary approach, contrasting the Negative Control group with the other two groups, which were classified as

dysbiotic. Group comparisons were performed using the Wilcoxon rank-sum test, with a significance level of $p < 0.05$.

2.7. Data Availability

All sequencing data were submitted to the NCBI Sequence Read Archive (SRA) database under the BIOPROJECT Accession PRJNA1464506.

2.8. Ethical Statement

In vivo experiments were performed according to the Ethical Principles on Animal Experimentation of the National Council for the Control of Animal Experimentation (CON-CEA). The protocol was approved by the Ethical Committee on Animal Experimentation (CEUA) of the School of Agriculture and Veterinary Sciences (FCAV) on 19 November 2025 (protocol number: 9877460435/2025). Experimental assays were carried out in the Department of Pathology, Reproduction, and One Health at São Paulo State University (FCAV/Unesp).

Figure 6 was generated with the assistance of ChatGPT (OpenAI, version 5.2) to support the initial design and visual organization of the figure. The authors subsequently refined the figure, verified its scientific accuracy, and approved the final version.

3. Results

Although the use of organic acids in broiler chicken production is a widespread practice, its effectiveness against *S. Minnesota* and its impacts on the cecal microbiota remain unclear. In this study, the proportion of animals excreting *S. Minnesota* after treatment with a blend of formic acid and propionic acid in liquid form was lower in the Treatment group than in the Positive Control group ($p < 0.05$) (Table 4). In contrast, the SM^{NalSpc} counts in cecal contents did not differ significantly among the evaluated groups (Supplementary Figure S1). To exclude the possibility of external contamination in the studied animals, transport box swabs were screened for *Salmonella* prior to the initiation of the experiment, with all samples testing negative.

Table 4. Challenged broilers excreting SM^{NalSpc} .

hpi (h)	Treatment Group	Positive Control
24	5	4
48	2	8
72	6	10
Total	13 b	22 a

SM^{NalSpc} : *Salmonella Minnesota* from cloacal swab samples. Proportions followed by different letters differ statistically from each other (χ^2 test, $p < 0.05$). hpi = hours post-infection.

In relation to the bacteriome, the total output reads obtained are described in Supplementary Table S1. Rarefaction curves reached a plateau, indicating that the sequencing depth was sufficient to capture the taxonomic richness of the samples (Supplementary Figure S2). The alpha diversity analysis showed that both the Negative Control group and the Treatment group had higher and significantly different diversity values compared to the Positive Control group (Figure 2A), while the beta diversity analysis was consistent with the patterns previously described. Samples exhibited spatial clustering according to group distribution ($p < 0.05$) (Figure 2B), with clear differences in microbiota composition between pairwise groups ($p < 0.05$) (Table 5).

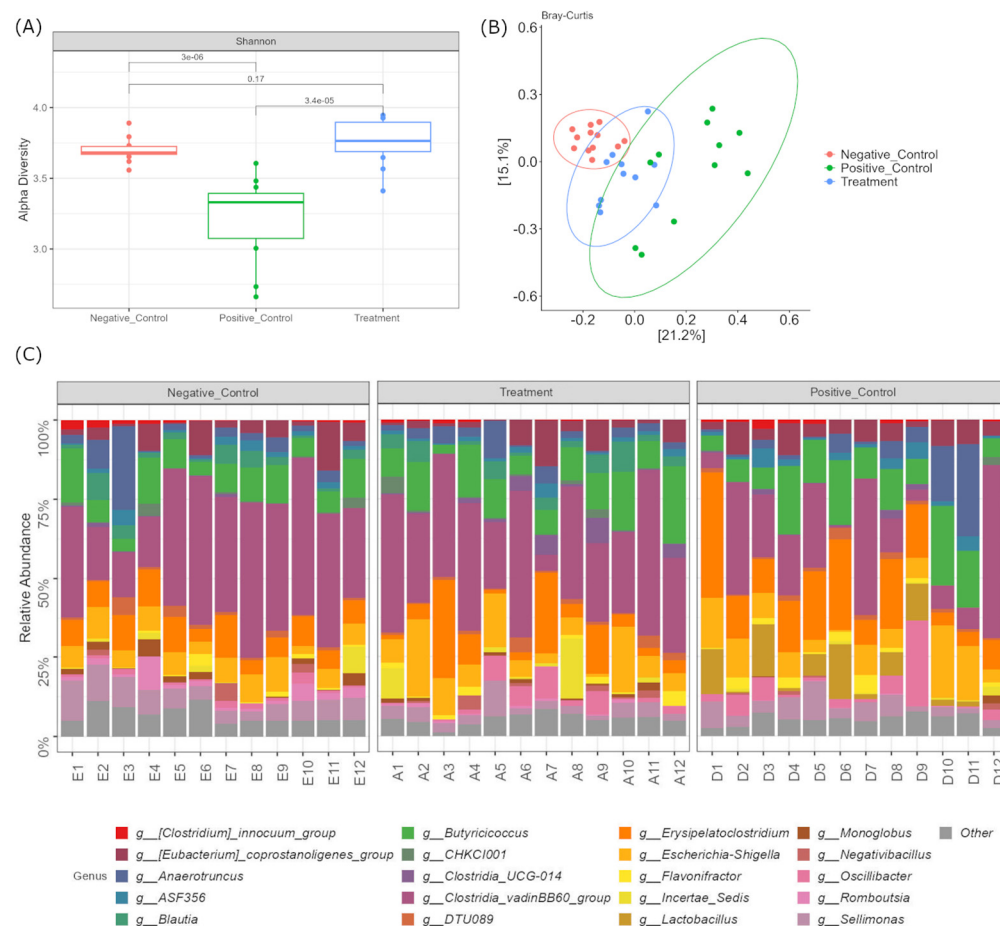


Figure 2. Bacterial composition following challenge with *Salmonella enterica* subsp. *enterica* serovar Minnesota and treatment with Axeed® Liquid. (A) Shannon alpha diversity analysis. For this analysis, outliers (A3 and D7) were removed. The Wilcoxon rank-sum test was used for statistical analysis, with $p < 0.05$ considered statistically significant. (B) Bray–Curtis beta diversity analysis (PERMANOVA: $R^2 = 0.25845$, $p < 0.05$). For this analysis, outliers (A3 and D7) were removed. (C) Relative abundance at the bacterial genus level.

Table 5. Comparisons of microbiota composition between groups.

Groups	R^2	p
Treatment_vs._Positive_Control	0.34202	0.001
Treatment_vs._Negative_Control	0.26567	0.001
Positive_Control_vs._Negative_Control	0.44575	0.001

Permutational multivariate analysis of variance (PERMANOVA) with pairwise comparisons.

The relative abundance at the bacterial genus level highlights the microbial profile within each experimental group (Figure 2C). A marked shift in the abundance of bacterial genera was observed in the group challenged with *S. Minnesota* without treatment (Positive Control group). In contrast, in the challenged group treated with formic acid and propionic acid, the bacterial abundance profile tended to resemble that of the Negative Control group. Additionally, in the Treatment group, certain bacterial genera became highly prominent, exhibiting higher relative abundance (Figure 2C).

As shown in Figure 3, which presents the bacterial genera with the highest relative abundance, *Blautia*, an uncultured group within the class *Clostridia* (*Clostridia_vadinBB60_group*), and *Butyricicoccus* stand out as the most abundant genera within the cecal content. *Blau-*

tia and *Clostridia_vadinBB60_group* showed higher abundances in the Treatment and the Negative Control groups compared to the Positive Control group ($p < 0.05$) (Figure 3).

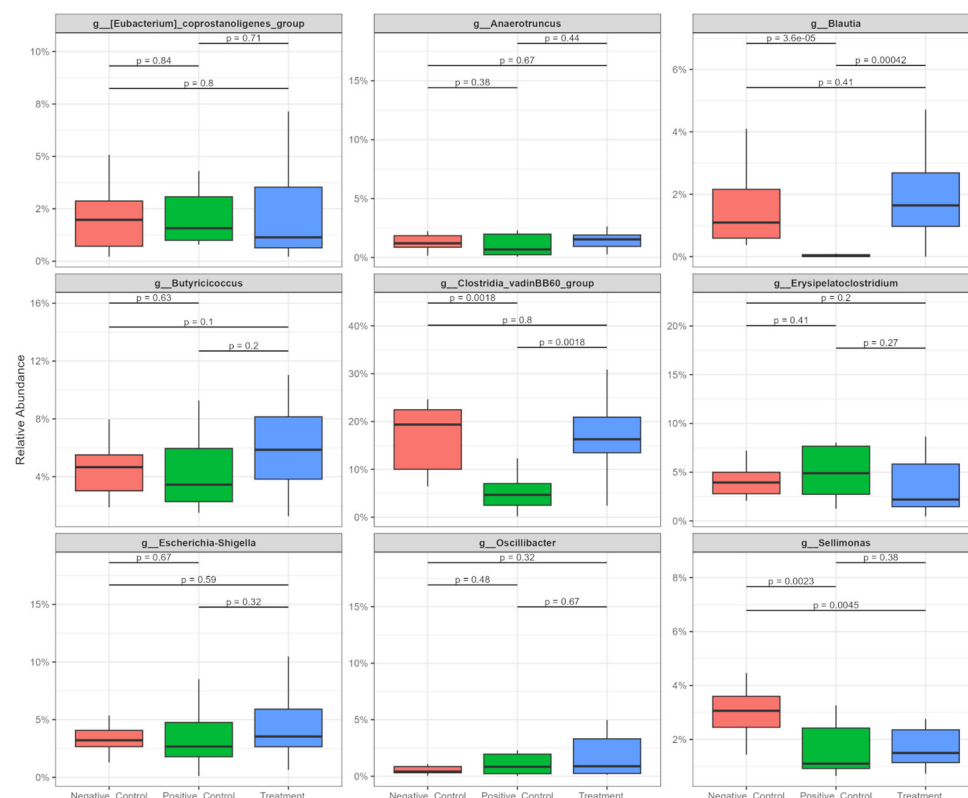


Figure 3. The most abundant bacterial genera identified in the analyzed samples. Statistical significance was assessed using the Wilcoxon rank-sum test, with $p < 0.05$ considered significant.

We have found discriminative bacteria in the group challenged with SM^{NalSpc} and treated with formic acid and propionic acid (Treated group). The genera *Blautia* and *Clostridia_UCG-014* and the family *Lachnospiraceae_CHKCI001* were identified as biomarkers (Figure 4). The Negative Control group exhibited a greater diversity of taxa, while the Positive Control group maintained a lower representation of bacterial genera.

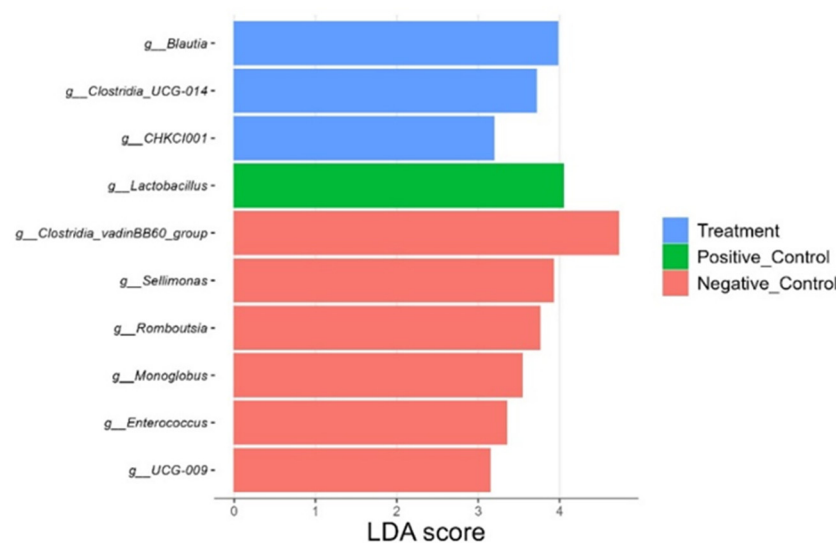


Figure 4. Potential bacteria genera as biomarkers. Identification by linear discriminant analysis (LEfSe).

Following dysbiosis analysis, a separation between the Negative Control and Positive Control groups was observed, while the Treatment group exhibited an intermediate profile, suggesting either a partial disruption of the microbiota or a restoration of homeostasis (Figure 5). Consequently, organic acids seem to drive both the homeostasis and resilience of intestinal microbiota (Figure 6).

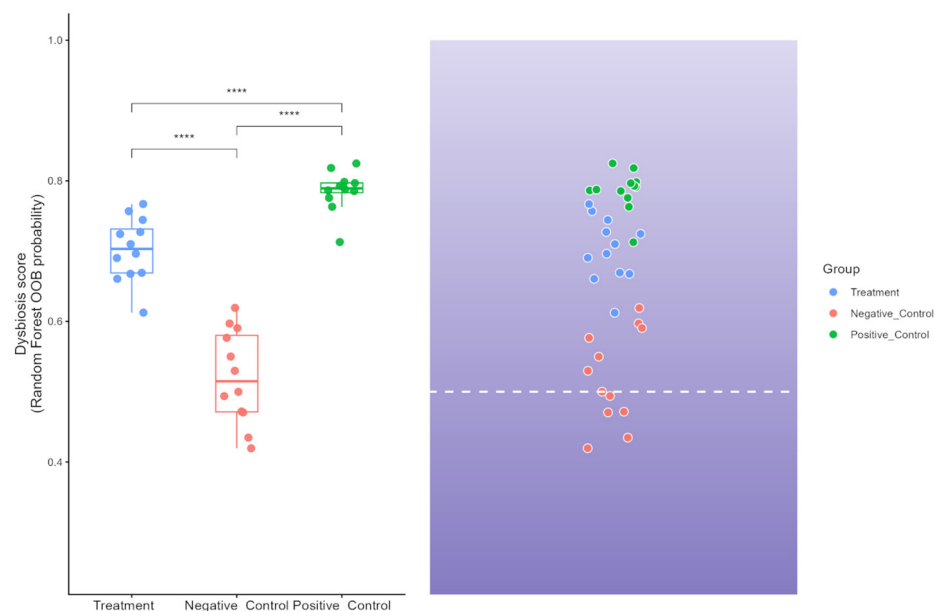


Figure 5. Dysbiosis score derived from a Random Forest model (out-of-bag probability) across experimental groups. Statistical significance was assessed using the Wilcoxon rank-sum test; **** indicates $p < 0.05$. The dashed line represents 0.5.

Acids support intestinal homeostasis and resilience in poultry

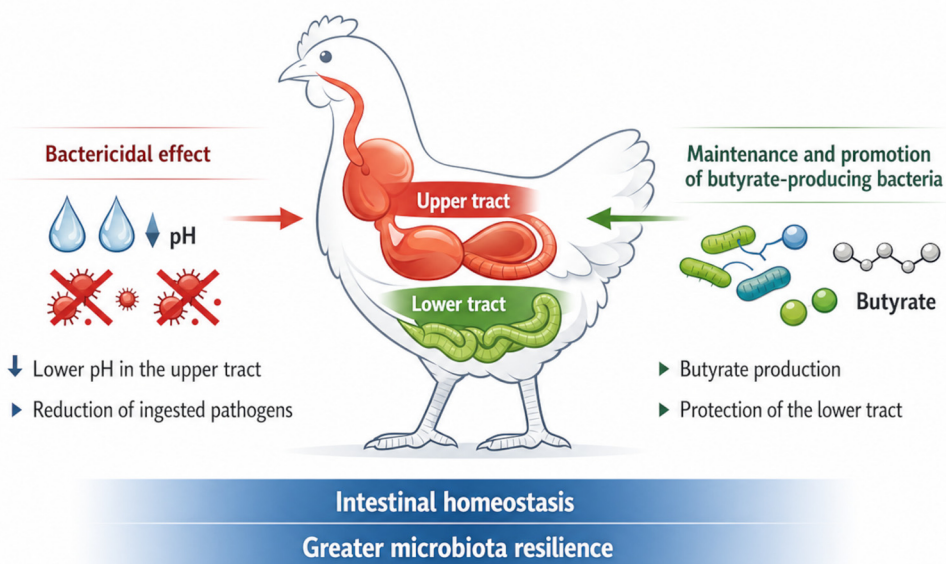


Figure 6. Schematic representation of the proposed role of organic acids in intestinal homeostasis and resilience in poultry. Red and green arrows represent the effect of bactericidal and butyrate in the upper and lower tracts, respectively. The figure was created with the assistance of artificial intelligence (ChatGPT, OpenAI, Version 5.2) and refined by the authors.

4. Discussion

The inflammation induced by *Salmonella* infection stimulates chemical changes in the gut environment, which favor the proliferation of opportunistic bacteria while impairing the growth of health-associated bacterial taxa [29]. Disruptions are characterized by reduced microbial diversity, a loss of beneficial commensals, and increased instability of the microbial ecosystem, reflecting a dysbiotic state [30].

Treatment with the commercial blend of formic acid and propionic acid restored the bacterial diversity to levels comparable to the Negative Control group. These results support the role of organic acids in modulating and stabilizing gut microbial communities, inhibiting pathogenic bacteria [31,32] without the harmful effects of antimicrobial use [33]. Notably, feed supplementation with organic acids has been previously associated with a decrease in *Salmonella* spp. in the intestinal content [34].

In this study, although organic acid supplementation significantly reduced cloacal shedding of *S. Minnesota*, it did not significantly decrease cecal bacterial counts during the experimental period. This discrepancy may reflect differences in the site of action of organic acids. When administered in drinking water, organic acids are expected to exert their greatest antimicrobial activity in the upper gastrointestinal tract [35]. Consequently, their concentration and antimicrobial activity may be reduced before reaching the ceca due to absorption and dissociation along the gastrointestinal tract, limiting their ability to decrease cecal colonization. Furthermore, cecal samples were collected only up to 72 h post-challenge, which may have been insufficient for detecting changes in bacterial persistence at this site, as *Salmonella* colonization dynamics may vary over time [15].

When observing the highest relative abundance of bacterial genera, *Blautia*, class *Clostridia* (*Clostridia_vadinBB60_group*), and *Butyrivibrio* stood out in the Treatment and Negative Control groups. These genera are well-known producers of both butyrate and other short-chain fatty acids (SCFAs) [36–38]. Additionally, *Sellimonas*, frequently identified in the Treatment group, has been associated with butyrate-producing bacterial species, and this genus has been described as a potential marker of microbiota recovery following disturbances in humans [39]. Dietary supplementation with butyrate has been recognized as an effective strategy for improving gut health and performance in broiler chickens [40]. The increase in butyrate-producing bacteria has a high positive impact on intestinal health, strengthening the intestinal barrier and modulating *Salmonella* virulence ability, anti-inflammatory action, and the maintenance of low cecal pH, which reduces pathogenic bacteria adhesion [36–38]. The increased abundance of putative butyrate-producing genera may have contributed to improved intestinal health and increased colonization resistance, as suggested in previous studies. However, since SCFA concentrations were not measured, these functional effects remain speculative.

Remarkably, *Blautia* was the most discriminative genus in the organic acid blend treatment. *Blautia* has frequently been associated with microbial intestinal stability in swine [41] and gut health in broilers [42]. Although the mechanisms underlying its enrichment remain unclear, the higher representation of this genus in the organic acid treatment may indicate a favorable modulation of the intestinal microbial ecosystem, warranting further investigation into its potential role as a biomarker of gut health in broilers.

In the discriminative analysis, the Negative Control group exhibited a greater diversity of taxa. These findings are consistent with the expected profile of healthy, unchallenged broilers, in which a complex and diverse microbiota is associated with intestinal functional stability [43]. In contrast, the Positive Control group maintained a lower representation of bacterial genera, indicating that *Salmonella* infection promoted microbiota alterations. This pattern has been reported in poultry, where pathogen challenge promoted shifts in microbial composition [44].

The present study has some limitations that should be considered when interpreting the results. The gut microbiota was evaluated only at 72 h post-infection, preventing the assessment of the temporal dynamics of microbial changes following *Salmonella* challenge. In addition, the efficacy of the organic acid blend was not directly compared with other feed additives under identical experimental conditions. However, the microbial profile of the Treatment group suggested a partial preservation of the intestinal bacterial community compared with the Positive Control group. Although the bacterial composition did not completely resemble that of the Negative Control group, the representation of beneficial taxa indicates that formic and propionic acid supplementation in water may have mitigated some of the microbiota disturbances associated with *Salmonella* challenge. These findings support the hypothesis that dietary organic acids contribute to maintaining intestinal microbial balance during enteric pathogen infection, potentially enhancing gut resilience in broiler chickens.

Compared with other alternatives to antimicrobials, organic acids offer practical advantages, including ease of administration through drinking water, broad-spectrum antimicrobial activity against enteric pathogens, and compatibility with commercial poultry production systems [34]. Nevertheless, alternative feed additives are not mutually exclusive, and growing evidence suggests that combinations of organic acids with other compounds may exert complementary or synergistic effects on intestinal health, gut microbiota modulation, and pathogen control [3].

5. Conclusions

The administration of the organic acid blend (formic and propionic acid) in drinking water reduced *Salmonella* Minnesota cloacal shedding and promoted beneficial changes in the cecal microbiota of experimentally challenged broilers, including an increased relative abundance of bacterial genera commonly associated with intestinal health. Our results suggest that the organic acid blend contributed to limiting pathogen dissemination while favorably modulating the intestinal microbial community during the acute phase of infection. These findings support the potential use of organic acids administered through drinking water as a practical complementary strategy for *Salmonella* control in poultry production.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/poultry5040056/s1>, Figure S1: SM^{NalSpc} counts in cecal contents per hours post-infection; Figure S2: Sample-based rarefaction curves of taxonomic richness; Table S1: Read counts after quality filtering.

Author Contributions: Conceptualization, F.M.S., M.d.M.S.S. and R.R.; methodology, F.M.S., M.d.M.S.S. and M.C.T.; formal analysis, F.M.S., M.d.M.S.S. and M.C.T.; investigation, F.M.S., M.d.M.S.S., A.L.C.R., A.M.d.A. and M.C.T.; resources, R.R., C.V.L.R., A.L.C.R. and A.M.d.A.; writing, original draft preparation, M.C.T.; writing, review and editing, F.M.S., M.d.M.S.S. and M.C.T.; supervision, F.M.S.; project administration, F.M.S. and M.d.M.S.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: In vivo experiments were performed according to the Ethical Principles on Animal Experimentation of the National Council for the Control of Animal Experimentation (CONCEA). The protocol was approved by the Ethical Committee on Animal Experimentation (CEUA) of the School of Agriculture and Veterinary Sciences (FCAV) on 19 November 2025 (protocol number: 9877460435/2025). Experimental assays were carried out in the Department of Pathology, Reproduction, and One Health at São Paulo State University (FCAV/Unesp).

Informed Consent Statement: Not applicable.

Data Availability Statement: All sequencing data were submitted to the NCBI Sequence Read Archive (SRA) database under the BIOPROJECT Accession PRJNA1464506.

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Conflicts of Interest: Authors Renato Ravetti and Cyanna Ravetti were employed by the company Salmix Industria e Comercio Ltda. The remaining 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. The company Salmix Industria e Comercio Ltda provides the drinking water additive. The company Salmix Industria e Comercio Ltda had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

SM ^{NalSpc}	<i>Salmonella</i> Minnesota, previously isolated from broilers and resistant to nalidixic acid and spectinomycin
VBNal/Spc	Brilliant Green Agar supplemented with nalidixic acid and spectinomycin
PCoA	Principal coordinates analysis
PERMANOVA	Permutational multivariate analysis of variance
CFU/g	Colony-forming units per gram

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